DEVELOPMENT, IMPLEMENTATION AND EVALUATION OF CLINICAL AND GENETIC SCREENING PROGRAMS FOR HEREDITARY TUMOUR SYNDROMES

JANE S. GREEN

DEVELOPMENT, IMPLEMENTATION AND EVALUATION OF CLINICAL AND GENETIC SCREENING PROGRAMS FOR HEREDITARY TUMOUR SYNDROMES

by

JANE S. GREEN, BSc, MSc

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ABSTRACT

Families with an autosomal dominant predisposition to benign or malignant tumours were identified in Newfoundland in the 1980s, including families with von Hippel-Lindau disease (VHL), the multiple endocrine neoplasias, type 1 (MEN-1), and type 2 (MEN-2), and the hereditary colon cancers, familial adenomatous polyposis (FAP), and hereditary non-polyposis colon cancer (HNPCC). Each family was identified because of an excess of early deaths and disabilities in family members who had presented with symptomatic disease, and an increased anxiety in affected and unaffected family members.

The medical team recognized that a multidisciplinary approach to the disease in each family was necessary to improve the prognosis, and that a screening program might be central to this type of care.

Successful genetic screening programs had been developed since the 1960s for severe or lethal hereditary diseases with neonatal or early childhood onset: for early identification and treatment of affected infants, or for identification of those at risk of having an affected child with provision of counselling to allow informed reproductive decisions. Subsequently predictive testing for incurable late-onset diseases, such as Huntington disease, was introduced to reduce uncertainty. Many lessons were learned from these early screening programs, about the ethical requirements of screening for hereditary disease, and the need for coordination with pre-screening and post-screening education, counselling, and follow-up.

In the 1980s there were recommendations for screening programs for hereditary tumour syndromes, for early identification of gene carriers to provide reproductive counselling, and for early identification and treatment of tumours to improve the prognosis. At the same time there were concerns that more harm than good might result from this type of program. The large Newfoundland families with VHL, MEN-1, MEN-2, FAP and HNPCC provided an opportunity to develop, implement, and then evaluate clinical and genetic screening programs for these hereditary tumour syndromes.

For each "hereditary cancer" an extended pedigree was documented and medical records reviewed. A clinical screening protocol was developed based on the type of tumours and ages at which they occurred. Educational materials were prepared, counselling was provided, and clinical screening was offered to affected and at-risk family members. Medical and psychosocial results of screening were documented. Genetic testing by linkage analysis or mutation detection was developed in collaboration with molecular geneticists in Newfoundland and elsewhere, and predictive testing was offered to at-risk family members. Affected and unaffected members of the VHL family were interviewed to assess the psychosocial implications of the disease and the screening program, and a formal health care evaluation was completed for the VHL screening program, including a cost-benefit analysis.

For VHL, an earlier age at diagnosis, an improved prognosis (reduced morbidity and mortality), and a better quality of life (reduced anxiety, and more informed reproductive decisions) were demonstrated for those identified by

screening, compared with those presenting symptomatically. The overall cost to society of screening and management of presymptomatic disease was less than the combined costs of treatment of symptomatic disease and the costs related to early deaths and disabilities. Screening programs for the other hereditary tumour syndromes were less formally reviewed, but provided similar results.

As a group these hereditary cancers differed from untreatable adult-onset disorders because the harmful effects of the disease genes could be mitigated. At the same time, important differences were identified within this group of diseases (the age at onset, severity of disease, the spectrum of disease expression, the potential for treatment, and the state of genetic knowledge) which influenced the development of specific screening programs, and the attitudes of family members towards participation in the clinical and genetic components of screening.

It was concluded that screening programs combining clinical and genetic testing methods to identify and provide reproductive counselling for gene carriers, and to identify and treat presymptomatic tumours, provide appropriate management for hereditary tumour syndromes, and that this approach reduces monetary costs to the health care system, as well as monetary and psychosocial costs to individual family members.

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LIST OF ABBREVIATIONS

A	adenosine
ACTH	adrenocorticotropic hormone
AD	autosomal dominant
ADPKD	autosomal dominant polycystic kidney disease
AFP	alpha-fetoprotein
APC	adenomatous polyposis coli (gene)
ASHG	American Society of Human Genetics
asymp	asymptomatic
(AT)rep	adenosine thymidine (di-nucleotide repeat)
ATPase	adenosine triphosphatase
AV	arteriovenous
BamHI	(restriction enzyme)
BglII	(restriction enzyme)
bp	base pair(s)
BRCA1, BRCA2	breast cancer 1, breast cancer 2 (gene)
C	cancer
C	cytidine
ca	carcinoma
CA	cytidine adenosine (di-nucleotide repeat)
CARC	carcinoid tumour
CATT	tetranucleotide repeat
CCH	C-cell hyperplasia
CEREB	cerebellar hemangioblastoma
CF	cystic fibrosis
CFTR	cystic fibrosis transmembrane receptor (gene)
chemo	chemotherapy
chr (p,q)	chromosome (short arm, long arm)
CHRPE	congenital hypertrophy of the retinal pigment epithelium
CIS	carcinoma-in-situ
CIU	Clinical Investigation Unit
coinc	coincidental
CT	computerized tomography
D and C	dilatation and curettage
DCC	deleted in colon cancer (gene)
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
ΔF508	deletion of codon 508 (mutation of CFTR gene)
EcoRI	(restriction enzyme)

F	female
FAP	familial adenomatous polyposis
FMTC	familial medullary carcinoma of the thyroid
G	guanosine
gast	gastrin
GI	gastrointestinal
gluc	glucagon
hcg	human chorionic gonadotropin
HD	Huntington disease
5HIAA	5-hydroxyindoleacetic acid
HLA	human lymphocyte antigens
hMLH1, hPMS1, hPMS2	human homologues of MutL gene (genes for HNPCC)
hMSH2	human homologue of MutS gene (gene for HNPCC)
HNPCC	hereditary non-polyposis colon cancer
HPTH	hyperparathyroidism
I^{131}	iodine-131
ICG-HNPCC	International Collaborative Group on Hereditary Non-Polyposis Colon Cancer
kb	kilobase(s)
km	kilometre
LOD	logarithm of the odds
LOH	loss of hererozygosity
LR	low risk
M	male
MCC	mutated in colon cancer (gene)
MEN-1	multiple endocrine neoplasia, type 1
MEN-2 (MEN-2A, MEN-2B)	multiple endocrine neoplasia, type 2 (type 2A, type 2B)
MIBG	metaiodobenzylguanidine (scan)
μmol/d	micromoles per decilitre
MRI	magnetic resonance imaging
MSAFP	maternal serum alpha-fetoprotein
MSUD	maple syrup urine disease
MspI	(restriction enzyme)
MTC	medullary carcinoma of the thyroid
MutL, MutS	salmonella DNA repair genes
N	normal
NF1	neurofibromatosis type 1
NF2	neurofibromatosis type 2
NFLD (Nfld)	Newfoundland
NIH	National Institutes of Health
nmol/d	nanomoles per decilitre

NT	no investigations
NTD	neural tube defect
OPD	Out-Patient Department
p	probability
P	polyp
PANC	pancreatic islet cell tumour
PCR	polymerase chain reaction
PIT	pituitary adenoma
PKD1, PKD2	polycystic kidney disease (1) and (2) (gene)
PKU	phenylketonuria
pmol/L	picomoles per litre
PP	pancreatic polypeptide
prev	previous
PRL	prolactinoma
PTH	parathyroid hormone
PTT	protein truncation testing
QALY	quality adjusted life years
RA	retinal angioma
rad	radiation treatment
RCC	renal cell carcinoma
RET	Ret proto-oncogene (MEN-2 gene)
RFLP	restriction-fragment length polymorphism
RNase	ribonuclease
SCA1	spinocerebellar ataxia, type 1
SD	standard deviation
SSCP	single strand conformation analysis
STR	short tandem repeat
surg	surgery
symp	symptomatic
T	thymidine
TaqI	(restriction enzyme)
thal (α, β)	thalassemia (α-thalassemia, β-thalassemia)
TSD	Tay Sachs disease
U	uninformative
Untr	untreated
US	ultrasound
USA	United States of America
VHL	von Hippel-Lindau disease
VMA	vanillylmandelic acid
VNTR	variable number tandem repeat
$\bar{x}$	mean
yr	year(s)
Z-E	Zollinger-Ellison syndrome

chromosome 2 (HNPCC)
- D2S119
- D2S123
- D2S136

chromosome 3 (VHL)
- D3S18/cLIB1
- D3S601/cLIB 7.1, LIB 19-63
- D3S1038/C13-946
- D3S1250/cLIB-56

chromosome 5 (FAP)
- D5S346
- pi227
- YN5.48

chromosome 10 (MEN-2)
- FNRB
- D10S34
- D10Z1
- D10S94
- RBP3

chromosome 11 (MEN-1)
- D11S288/p3C7
- D11S971/RC27
- D11S970/RC29
- D11S146/pHB159
- D11S533/4F7
- PGA/PGA101
- PYGM/pMCMP1
- INT2/SS6

CHAPTER 1 — INTRODUCTION, LITERATURE REVIEW, AND RATIONALE AND OBJECTIVES OF THESIS

INTRODUCTION

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RATIONALE AND OBJECTIVES OF THESIS

INTRODUCTION

Screening

i) Definition

Screening has been defined as the investigation of asymptomatic members of a population at risk to identify those who are affected by, or carriers of, a particular disease. Early screening programs were directed at endemic or epidemic infectious disease (Mausner and Kramer 1985). A population or sub-population at risk was screened to provide early treatment for those identified to reduce morbidity and mortality and prevent spread of disease. Screening subsequently was introduced for a wide variety of diseases, including hypertension, diabetes, and cancer, to discover and treat disorders not yet clinically recognizable (Fost 1992).

ii) Rationale

The rationale for development of screening programs as outlined by Wilson and Jungner (1968) is based on the following requirements:

- that there is a recognized burden of disease
- that the population at risk can be identified
- that the natural history of disease is known
- that a presymptomatic stage of disease can be identified by a simple, non-invasive test
- that treatment is available
- and that early treatment improves prognosis and/or quality of life.

The screening test(s) used should be valid and reliable, as well as simple and inexpensive, and should have high sensitivity and specificity (Mausner and Kramer 1985, Report of the Royal College of Physicians 1989, Holtzman 1992). Since the positive predictive value depends on the prevalence of the condition in the population, the population to be screened should be chosen with care, ie, there should be a high prevalence of the condition (Mausner and Kramer 1985, Thompson et al 1991). The screening tests should be part of a complete program (Scriver 1980, Modell and Petrou 1988) with education and counselling about the goals of the screening program before testing, and appropriate follow-up after testing, including confidential reporting of results, and diagnostic investigations to confirm positive screening results. Facilities for treatment should be available without financial restrictions for all those testing positive (Committee for the Study of Inborn Errors of Metabolism 1975, Assessing Genetic Risk 1994).

Although the original aim of screening programs was to permit earlier treatment, screening has also been used for research particularly to determine the frequency or natural history of disease, and to develop new screening programs, and, in the context of screening for carriers of hereditary disease, for reproductive counselling (Scriver 1980, Report of the Royal College of Physicians 1989).

iii) Ethics

Because screening, by definition, is directed at asymptomatic individuals rather than those seeking medical attention for recognized symptoms, there is a responsibility to do more than just practice good medicine by treating those identified (Sackett et al 1991, Fost 1992). Particularly there is a requirement to maximize the benefits and minimize any harm. There is a danger that those identified by screening may be discriminated against socially, or in terms of health or life insurance, or employment (Billings and Beckwith 1992a, Ostrer et al 1993). Screening should, therefore, be offered rather than imposed on society, and informed consent required from each individual to be tested. Results of testing must also be given confidentially only to the person screened (Knoppers and Chadwick 1994).

There is also a risk that when a "well" person is classified as "affected", this labelling (Sackett et al 1991) may precipitate the onset of symptoms. Screening may thus take away health (Fost 1992). This may be acceptable if early identification and treatment ultimately improves the medical outcome. But it is not acceptable if screening only lengthens the illness by advancing the onset of disease (Sackett et al 1991). Screening programs must therefore be evaluated to ensure that the goals are achieved, and that biases (eg, lead-time bias [earlier diagnosis with no change in ultimate survival], and length bias [better prognosis in the screened group because of identification of those with more indolent disease]) do not confound the results (Sackett et al 1991).

Screening programs should also be evaluated economically, to determine whether the costs are appropriate for the benefits (both medical and psychosocial) that are obtained (Drummond 1980).

Genetic Screening

Screening has been applied to genetic disorders since the 1960s when screening programs for neonatal detection and treatment of phenylketonuria (PKU) and other inborn errors of metabolism were introduced (Erbe and Boss 1990). Genetic screening programs have now been developed to detect and treat other hereditary diseases. The goal of many genetic screening programs, however, is to identify those at high risk of having a child with a severe or lethal untreatable disorder, and to provide genetic counselling to assist with reproductive decisions (Cao et al 1981, Kaback et al 1993).

i) Definition

Genetic screening is defined as the search within a population for persons with certain genotypes that cause or predispose to genetic disease, in those persons themselves, or in their descendants (Thompson et al 1991). This screening is aimed at a whole population, or a defined segment of the population, when there is a recognized risk of a particular disease, and there is a simple but effective screening technique to identify those with the specific genotype (Assessing Genetic Risk 1994).

ii) Rationale

The rationale for the first population-based genetic screening programs was that early identification would allow early treatment to prevent the harmful consequences of the particular mutation. Neonatal screening for detection of PKU in the early 1960s was the prototype of this type of program (Fost 1992). Although neonatal screening for PKU and possibly other inborn errors of metabolism is now used in most developed countries, the concept of genetic screening was under intense scrutiny before its value was widely accepted (Committee for the Study of Inborn Errors of Metabolism 1975).

It is now acknowledged that early treatment is not the only acceptable objective of genetic screening. Some screening programs are undertaken to permit informed reproductive decisions (Report of the Royal College of Physicians 1989). This includes prenatal screening for age-related chromosomal disorders by amniocentesis or chorionic villus sampling and cytogenetic analysis (eg, Down syndrome screening which was introduced in the late 1960s), and for neural tube defects, originally by amniotic fluid alpha-fetoprotein measurement, and more recently by maternal serum alpha-fetoprotein (MSAFP) measurement. It also includes carrier detection programs in specific high-risk groups. Screening programs that identify carriers and carrier couples for Tay-Sachs disease in Ashkenazi Jewish populations, or for α or β-thalassemia in southeast Asian or Mediterranean populations, have significantly reduced the incidence of these serious or fatal diseases and have

allowed carrier couples to have the normal children that they desired (Modell and Kuliev 1991). Screening programs for sickle cell anemia carriers in black populations were not originally as well accepted (Committee for the Study of Inborn Errors of Metabolism 1975) (since the programs were less successful in educating the population at risk and their health care workers), and population screening for cystic fibrosis carriers is thought by most to be premature (Biesecker et al 1992, Statement of the American Society of Human Genetics 1992).

Screening programs whose initial objective is enumeration or research (Scriver 1980) may result in other successful population-based screening programs for treatment or reproductive counselling, through:

- identification of other high-risk groups,
- development of effective screening techniques whether biochemical, radiological or molecular, or
- development of treatments for identifiable diseases.

Screening programs for presymptomatic identification of carriers of autosomal dominant (AD) disease, such as Huntington disease (HD), myotonic dystrophy, or polycystic kidney disease (ADPKD), to allow reproductive planning and to reduce uncertainty, have also been suggested (Assessing Genetic Risk 1994, Elias and Annas 1994), but few areas would have a sufficiently high incidence for population-based screening. However, predictive testing or presymptomatic diagnosis in members of identified families could be

considered family screening. The HD predictive testing/presymptomatic diagnosis programs have some lessons for other screening programs (Ball and Harper 1992, Wexler 1992), particularly those for other adult-onset diseases. The HD programs have been highly structured and have emphasized the need for counselling and support before and after testing because of the possible negative psychosocial aspects associated with prediction of both increased and decreased risk of an incurable disease (Wiggins et al 1992).

With the rapid expansion of genetic information resulting from the Human Genome Project, new areas are rapidly opening up where genetic screening may be introduced (Holtzman 1992, Caskey 1993b, Editorial 1994a, Motulsky 1994). It has been stated that "It will soon be considered malpractice if preventable disorders are not discovered early enough by established screening programs" (Bickel 1980). However, even if genetic screening programs are desirable and technically possible, they must be developed and introduced only when the medical and psychosocial implications of the programs have been properly considered and evaluated (Croyle and Lerman 1993b, Assessing Genetic Risk 1994).

Screening for Hereditary Tumour Predisposition

The AD tumour predisposition syndromes such as von Hippel-Lindau disease, the multiple endocrine neoplasias, and the hereditary colon cancers, are characterized by a variable age at onset, and variable type and order of

manifestations. They are potentially lethal but treatable if identified early. In the genetics literature, a program for the identification of genetic disease amongst those known to be at risk because of family history, has been considered testing rather than screening (Scriver and Fujiwara 1992). However, within the medical literature for these conditions, the term "screening" has been used for the identification of gene carriers among patients' relatives, and more particularly, for the identification of individual manifestations in those known (or at risk) to carry the gene (Vasen et al 1989b, Vasen et al 1990a, Fost 1992, Eng et al 1994b).

In the past, patients with hereditary tumours frequently presented symptomatically with advanced disease that was difficult to treat, resulting in significant morbidity and mortality (Bussey 1975, Lamiell et al 1989). Because of the late age of onset, many of these patients had several children before the diagnosis was made and the 50% risk of recurrence to any offspring was recognized. Since those at risk can be identified from the pedigree, screening for these syndromes would seem indicated to facilitate early diagnosis of disease,

a) so that particular manifestations can be identified and treated at an early stage, and

b) so that genetic counselling can be given prior to the reproductive period.

This screening may consist of:

a) clinical testing, to identify an early stage of particular manifestations if the natural history of disease is well characterized, and presymptomatic disease can be identified by acceptable investigations (Skogseid 1991a, Vasen et al 1994), with, or without,

b) genetic testing to identify gene carriers by linkage analysis if informative closely-linked markers have been identified, or by mutation analysis if the gene has been cloned and the mutation identified (Glenn et al 1992, Petersen et al 1993).

Once the components of a screening protocol (the type and timing of investigations) have been established, and the screening program has been instituted, it is necessary to evaluate the program (Shortell and Richardson 1978) to determine whether the objectives have been met (earlier identification and more successful treatment of individual manifestations, identification of gene carriers at a pre-reproductive age, and reduced anxiety and increased understanding about the disease), and whether the benefits of these outcomes outweigh the costs to the individuals in these families and to the medical care system (Drummond et al 1986).

There are a number of large families in Newfoundland with these variable AD diseases, including families with von Hippel-Lindau disease (VHL), the multiple endocrine neoplasias (MEN-1 and MEN-2), and the hereditary colon

cancer syndromes, familial adenomatous polyposis (FAP), and hereditary non-polyposis colon cancer (HNPCC) or Lynch syndrome.

The experience with these families can be used:

i) to investigate the feasibility of instituting a screening program,

ii) to determine the appropriate components of a screening protocol,

iii) to compare the medical and genetic outcome for family members who have been screened with those who were not screened, and

iv) to determine the cost, both financial and non-financial, of maintaining such a screening program.

LITERATURE REVIEW

There is extensive literature on all aspects of screening for hereditary disease, including the rationale for screening, the implementation and results of previous screening programs, and ethical concerns regarding screening. This literature is briefly reviewed for the lessons to be learned and applied to the development of new programs. The emphasis is on the successes and problems of earlier programs, and the ethical issues such as autonomy, confidentiality, equity and discrimination which must be considered.

Screening to Allow Early Identification and Treatment of Genetic Disease

i) Neonatal screening

Genetic screening was first introduced in the early 1960s with programs for neonatal identification and treatment of infants with phenylketonuria (PKU) (Committee for the Study of Inborn Errors of Metabolism 1975, Erbe and Boss 1990). A burden of disease had been recognized (untreated PKU causes severe mental retardation requiring costly institutionalized care [Scriver 1980]), a simple test had been developed (the Guthrie bacterial inhibition assay measures phenylalanine concentration in a dried blood spot on filter paper collected from a neonate [Guthrie 1961]), and a treatment was available (early institution of a phenylalanine restricted diet prevents mental retardation [Committee for the Study of Inborn Errors of Metabolism 1975]).

Although there was opposition from the beginning, including from some who denied that elevated phenylalanine caused mental retardation (Bessman 1966), a strong lobby promoted newborn screening for inborn errors of metabolism, particularly PKU, as an innovation in public health to prevent mental retardation (Scriver 1980, Fost 1992). It became clear later that the clinical and genetic features of PKU, the validity of testing, and the effectiveness of treatment were not as well understood as was thought when the screening program began (Wilfond and Fost 1990). There was also much variation in the implementation of screening and in the integration into an overall program including education, informed consent, and follow-up (Scriver

1980). All features of the PKU screening program, and other screening programs initiated subsequently were extensively reviewed in 1975 by the National Academy of Sciences (Committee for the Study of Inborn Errors of Metabolism 1975), and the lessons learned have been instructive in the development of other programs.

Since a positive screening test is not necessarily diagnostic, screening tests must be followed up by appropriate investigations to confirm or reject a specific diagnosis (Report of the Royal College of Physicians 1989). The failure to appreciate the heterogeneity of the hyperphenylalaninemias in the early days of the PKU screening programs, meant that some infants were incorrectly identified as having PKU and unnecessarily, and sometimes detrimentally, treated (Erbe and Boss 1990, Fost 1992). The Guthrie assay identified transient hyperphenylalaninemia, persistent non-PKU hyperphenylalaninemia (both benign conditions not requiring treatment), and tetrahydrabiopterin deficiency (requiring a different treatment), as well as classic PKU. Even within families with classic PKU, there can be variation in clinical expression so treatment must be monitored closely.

The timing of testing was found to be important (American Academy of Pediatrics 1965). For PKU, a false negative result could occur if the test was done too early (eg, on the first day or two of life before there was sufficient intake of phenylalanine). But hospital stays were short and unless screening

was done before infants left hospital, there was a danger that it would not be done at all, and some affected infants could be missed.

The appropriate duration of dietary treatment for PKU was also not clear in the 1960s when screening began (Committee for the Study of Inborn Errors of Metabolism 1975). Because the diet is quite unpalatable, it was desirable to minimize the time that it was required. However, intellectual deficits have subsequently been identified in some of those whose dietary restrictions were stopped in late childhood or early teens. The most serious unanticipated result of stopping the diet early was the occurrence of maternal PKU: severe mental retardation in the non-PKU offspring of mothers with previously treated PKU (Hansen 1973). It is now recognized that dietary restrictions are necessary during and, preferably, prior to pregnancy for women with treated PKU to allow normal development of the fetus.

Ethical principles require that informed consent be given by those participating in a screening program (or by parents or guardians if below the age of consent) (Fost 1992, Assessing Genetic Risk 1994). Some of the original PKU and other screening programs were mandatory (Farfel and Holtzman 1984) to avoid missing affected individuals. It is now agreed by most that screening should be offered to all, but that participation should be voluntary (Report of Royal College of Physicians 1989, Assessing Genetic Risk 1994). Adequate education and counselling about a program are important for

increasing understanding and compliance (Farfel and Holtzman 1984, Wilfond and Fost 1990).

Guthrie spot tests similar to the test for PKU were developed for neonatal detection of a number of other inborn errors of metabolism, including tyrosinemia, histidinemia, maple syrup urine disease and some forms of homocystinuria (Committee for the Study of Inborn Errors of Metabolism 1975). There was some impetus to use the dried blood spots for as many screening tests as possible, whether or not treatment was available, and whether or not the frequency of the specific disease warranted it (Assessing Genetic Risk 1994). Multiplex testing is now discouraged unless each program is independently evaluated (American Academy of Pediatrics 1965, Fost 1992, Assessing Genetic Risk 1994).

Even when multiplex testing is appropriate, there are differing opinions on how much information should be provided about each test. Elias and Annas (1994) believe that general information about similar tests is adequate because too much detail about individual risks (particularly when these risks are low) would cause unnecessary anxiety. Others (Fost 1992, Assessing Genetic Risk 1994) recommend more detailed counselling about each test. Because of the restrictions on multiplex testing, the number of neonatal screening programs in some countries or states has been reduced. Treatment for maple syrup urine disease (MSUD) is very difficult even when started early, so screening for MSUD, which is very rare, may be inappropriate (Erbe and Boss 1990).

Newborn screening for tyrosinemia is appropriate in certain sub-populations such as the Lac St. Jean region of Quebec where the incidence is high (1/650 births) (Demers et al 1994, Grompe et al 1994), but not generally. Screening for congenital hypothyroidism, a severe but treatable disease, is recommended (American Academy of Pediatrics 1977), although the disorder is not always genetic in etiology.

New neonatal screening programs should be developed rationally, not just because a test is available. Programs are appropriate for severe imminent diseases if diagnostic testing, successful treatment, and follow-up are all available for infants that test positive (Assessing Genetic Risk 1994). Neonatal screening for Duchenne muscular dystrophy has been criticized for providing "unwanted" information on an untreatable disease (Marshall 1993) and therefore causing unnecessary anxiety for the parents, but also recommended on the basis that it provides information for future reproductive decisions (for the parents, or other relatives who might also be at risk of having an affected child) and, therefore, may prevent subsequent affected children (Thompson et al 1991).

Neonatal screening programs for cystic fibrosis (CF), one of the most common autosomal recessive diseases in the white population, have also been considered. When the CF gene was cloned in 1989 it was anticipated that a DNA-based test for CF would soon become available (Goodfellow 1989). The diversity of CF mutations now identified means that this is not feasible;

although the ΔF508 mutation is present on 70% of CF chromosomes, over 300 other mutations have been identified, and there is no simple method to detect all mutations (Scriver and Fujiwara 1992). There is no direct biochemical test for CF, but immunoreactive trypsinogen is secondarily elevated in the blood of CF neonates, and Guthrie cards can be used for this testing (Ranieri et al 1994). The randomized controlled trial in Wisconsin (Fost and Farrell 1989), however, has not established that early diagnosis and treatment reduce morbidity and mortality from CF (Hammond et al 1991, Farrell and Mischler 1992). This should be clarified before neonatal screening for CF is expanded.

Screening to Permit Informed Reproductive Decisions

Screening to permit informed reproductive decisions is offered to individuals in specific populations or sub-populations who are at risk to produce a child with a severe or lethal genetic disorder (Charrow et al 1990, Modell 1990). This includes prenatal screening for Down syndrome or for neural tube defects (NTD), and heterozygote screening in high-risk populations to identify carrier couples for specific autosomal recessive diseases.

i) Prenatal screening

Prenatal screening for Down syndrome (Trisomy 21) and other less common trisomies, by amniocentesis and cytogenetic analysis, was first offered in the early 1970s to older pregnant women because of the increased risk of non-disjunction with late maternal age (Hook et al 1983, D'Alton and

DeCherney 1993): in 1988 in the USA, 25-30% of Down syndrome infants were born to mothers over 35 years of age, although only 8% of births occurred in women of this age group (Haddow et al 1992). Early amniocentesis before 15 weeks gestation, or chorionic villus sampling at 9-10 weeks are increasingly used instead of traditional "late" amniocentesis so that an earlier decision can be made about the outcome of the pregnancy if abnormal cytogenetic results are received (D'Alton and DeCherney 1993). For the majority of women tested, a normal screening result is obtained resulting in reassurance and reduced anxiety (Report of the Royal College of Physicians 1989). For those identified to be carrying an affected fetus, most (approximately 85% [Report of the Royal Commission on New Reproductive Technologies 1994]) choose therapeutic abortion, although others use the information to prepare for the birth and management of an affected child (Charrow et al 1990, Platt and Carlson 1992).

Because screening for Down syndrome in pregnant women over the age of 35 was shown to be cost-effective, for economic reasons this has been established as the cut-off age (Henderson 1991, Hagard and Carter 1976). But, although the highest risk of a Down syndrome child is in women over 35 years of age, the greatest number of Down syndrome infants are born to women less than 35 years of age since the majority of births are in this age group (Platt and Carlson 1992). The standard screening for Down syndrome therefore misses the majority of affected fetuses (D'Alton and DeCherney

1993). Advances in prenatal diagnosis have provided new screening tests for this younger age group. It has been recognized that maternal serum alpha-fetoprotein (MSAFP) and unconjugated estriol are decreased, and human chorionic gonadotropin (hCG) increased in Down pregnancies (all measured at 15-17 weeks of gestation) (Wald et al 1988, Haddow et al 1992). Amniocentesis and cytogenetic analysis can be offered to all women with these abnormal values. When all pregnant women are screened with maternal serum testing, 35% of Down pregnancies can be identified by decreased MSAFP alone, and 60% of Down pregnancies can be identified by the combined results (decreased MSAFP and unconjugated esteriol, and increased hCG) (Haddow et al 1992). Anxiety because of a positive result may be greater in those identified by this population screening, since the result was not anticipated (Editorial 1992b, Croyle and Lerman 1993b, Assessing Genetic Risk 1994), than in those screened for late maternal age where the high risk is recognized.

Since the sensitivity and specificity of the MSAFP, unconjugated estriol and hCG assays are low, both false negative and false positive values occur. Those with a false negative result may have a false sense of security, and those with a false positive result may have significant anxiety that is difficult to resolve (Marteau 1989). Thus, there are trade-offs: screening only the high-risk population reduces false positives but misses many Down syndrome infants, whereas screening the whole population detects most Down

pregnancies but increases the number of false positives because of the number of individuals being screened.

Prenatal screening for NTD (ie, spina bifida and anencephaly) by amniocentesis and measurement of amniotic fluid AFP (increased in the presence of fetal NTD) was first offered to women with a previous affected child; these women having about a 3% recurrence risk (D'Alton and DeCherney 1993). This screening, however, can only detect about 10% of fetuses with NTDs since about 90% occur in women with no previous history. Increasingly, MSAFP screening is offered to all pregnant women. Detection of increased MSAFP followed by prenatal ultrasound will identify 80-90% of all NTD fetuses (D'Alton and DeCherney 1993). As with prenatal screening for Down syndrome, the majority of women will receive a normal screening result (Charrow et al 1990). For those who were screened because of a previously affected child, this will provide reassurance and reduction in anxiety. For those receiving an abnormal result, a decision is made either to arrange for expert care at a high-risk birth, or to have a therapeutic abortion.

ii) Heterozygote screening

Heterozygote screening programs have been employed with varying degrees of success for severe or lethal autosomal recessive disorders. (Tsukahara and Kadota 1977, Mouzouras et al 1980). The goal is to identify carrier couples and provide information relevant to their reproductive decisions (Kaback 1990). These screening programs should only be implemented when

the disease is at high frequency at least in a specific population, there is a sensitive and specific test for mass screening, and education and genetic counselling are available to explain the program and interpret the results (Thompson et al 1991). Prenatal diagnosis should be possible or treatment should be available, and the programs should be voluntary with informed consent obtained prior to testing (Fost 1992).

Mandatory screening for carriers of sickle cell anemia, in the American black population, was introduced in various states of the USA in the early 1970s even though these conditions for development of a screening program did not all apply (Committee for the Study of Inborn Errors of Metabolism 1975, Farfel and Holtzman 1984). Sickle cell anemia is an extremely variable condition but with severe disease and early lethality in many affected. Carriers were identified by hematological testing but prenatal diagnosis was not available, nor was treatment possible. Reproductive options for carrier couples were therefore limited to ignoring the risk, or avoidance of pregnancy (Committee for the Study of Inborn Errors of Metabolism 1975). Since it was the black population that was at risk (carrier frequency of approximately 9%) and therefore screened for sickle cell anemia, these options were seen by some as racial genocide (Fost 1992). Because of poor planning, and poor education of both those being screened and the health care workers, there was confusion about the meaning of carrier status, and stigmatization and frequent discrimination against those identified (Wilfond and Fost 1990). This

emphasizes the need for strict confidentiality in a screening program. Results should not be given to a third party without prior consent, whether this is another family member, an employer, or an insurance company (Knoppers and Chadwick 1994).

A similar attempt at sickle cell anemia screening in Greece in 1973 resulted mainly in social ostracism of the carriers identified (Petrou et al 1992). Many of the problems encountered with sickle cell anemia screening resulted from the fact that it was imposed on a population rather than planned with that population — indicating the harm that can occur even when screening is initiated with the best intentions (Committee for the Study of Inborn Errors of Metabolism 1975).

Carrier screening for Tay Sachs disease (TSD) in those of Ashkenazi-Jewish descent (Kaback et al 1993), and for the thalassemias, particularly in Mediterranean populations (Mouzouras et al 1980, Scriver et al 1984), also introduced in the 1970s, met with much greater success because prenatal diagnosis was available and the programs were planned with leaders of the communities involved. There was extensive education of health care workers and potential participants, and testing of pilot projects (eg, for Tay Sachs disease in the Washington/Baltimore area in 1970-71 [Kaback 1972], and for β-thalassemia in Sardinia in 1976-1978 [Cao et al 1981] and in Montreal in 1978 [Scriver et al 1984]) before implementation of voluntary screening programs. Prenatal diagnosis and therapeutic abortion was considered an

option by many carriers because of the uniformly severe burden of disease (Cao et al 1989, Kaback et al 1993) — (the burden of disease being a subjective measure of the impact of a disease on an individual or family, comprising concepts of degree of risk, and severity of medical and psychosocial effects of the disease). British Pakistani couples at risk for β-thalassemia, however, were much less likely to participate in screening until first trimester diagnosis (by chorionic villus sampling and molecular techniques), and, thus, early therapeutic abortion became possible (Modell and Modell 1990).

While decreased incidence of disease should not be the primary goal of screening, this may be one of the results of such a program. The high uptake of prenatal diagnosis and selective therapeutic abortion for both TSD and β-thalassemia has resulted in a significant decline in births of affected individuals and therefore a reduced burden of disease to the family and to the community. For example, there has been a 90% reduction in incidence of TSD in the Jewish population of the USA and Canada (1970-1993) (Kaback et al 1993), and also a 90% decrease in thalassemia major births in Sardinia where the carrier frequency is 1/9, and 1/80 couples are at risk (Cao et al 1989). Carriers have not only been able to avoid the birth of an affected child, but they (and those identified as non-carriers) have been able to have the normal children that they desired but would previously have been afraid to have (Modell and Kuliev 1993).

Screening for TSD was originally based on an enzymatic assay (measurement of serum hexosaminidase levels) (Kaback et al 1993), a test that required precisely controlled conditions. The genetic defect causing TSD has now been recognized, and three mutations identified which account for approximately 95% of mutant alleles in Ashkenazi-Jewish populations. A more specific DNA-based assay for TSD carrier status in these populations has therefore been developed (Triggs-Raine et al 1990). However, if not all mutations have been identified, or if the clinical and genetic heterogeneity of a particular disease have not been characterized, DNA-based screening is not appropriate (Motulsky 1994).

Recently, voluntary sickle cell anemia screening programs in the United Kingdom have been more acceptable than earlier programs (Petrou et al 1992, Modell and Modell 1990). Midtrimester prenatal diagnosis of sickle cell anemia has been available since 1977 but there was little demand for screening until first trimester prenatal diagnosis and early therapeutic abortion became possible in the early 1980s. The decision for or against prenatal diagnosis and therapeutic abortion can be more difficult for many sickle cell anemia carrier couples than for TSD or thalassemia carrier couples because of the variable severity of the disease. Approximately 50% of all carrier couples, however, request prenatal diagnosis, and this increases to greater than 90% of those with a previously affected child (Petrou et al 1992). If prenatal screening is not desired, neonatal diagnosis and prophylactic treatment of affected infants with

penicillin and folic acid are now recommended to reduce morbidity by preventing infections (Modell and Modell 1990).

Carrier screening for cystic fibrosis, the most common severe, autosomal recessive disease in Europeans, has been extensively discussed, particularly since the cystic fibrosis transmembrane receptor (CFTR) gene was cloned (Rommens et al 1989). However, there still is no direct biochemical or physiological carrier testing available for CF, as was available for TSD and β-thalassemia when those screening programs began (Scriver and Fujiwara 1992). Carrier screening is therefore only possible by mutation analysis, and the sensitivity of this testing is not yet sufficient for population-based carrier screening, except in certain sub-populations such as Ashkenazi Jewish populations where few different mutations occur.

As stated previously, the most common mutation, ΔF508, is present on 70% of CF chromosomes but over 300 other mutations are known, and some mutations have not yet been identified. There is no single test or efficient combined testing to identify all mutations (Serre et al 1991). The cost of population-based screening is also extremely high — an estimated $2.2 million per CF birth prevented (Wilfond and Fost 1990). Until 90-95% of carriers can be accurately identified, CF population-based carrier screening is considered premature (Biesecker et al 1992, Scriver and Fujiwara 1992, Workshop on Population Screening for the Cystic Fibrosis Gene 1990). Meanwhile, the genetic basis for the wide phenotypic variability of CF is being worked out, and

improved therapies for both the pancreatic and pulmonary features of the disorder are being developed, thus increasing the life-span and improving the quality of life of those with CF (Wilfond and Fost 1990, Kaback et al 1993, Statement of the ASHG on Cystic Fibrosis Carrier Screening 1992).

Even when carrier screening for a particular disease is appropriate, the coordination of this screening with educational and genetic counselling components of a program is necessary (Farfel and Holtzman 1984). There is, however, a severe shortage of geneticists and genetic counsellors to provide this service (Ball and Harper 1992, Editorial 1994a). If population screening for CF carriers were introduced, there would be an even greater strain on genetic services.

The fact that heterozygote screening programs until now have been targeted to particular ethnic groups has had both positive and negative consequences (Zeesman et al 1984, Modell and Petrou 1988, Report of the Royal College of Physicians 1989, Holtzman 1992). In some cases this has meant that a screening program has been tailored to the needs of the specific community (Kaback et al 1993), but in other situations, discrimination or stigmatization of participants has been the result (Farfel and Holtzman 1984).

Heterozygote screening frequently takes place early in pregnancy, because it is perceived as a reproduction-related test (Kaback et al 1993). This is not the ideal timing for carrier testing since it reduces the options available to carrier couples identified (Workshop on Population Screening for the Cystic

Fibrosis Gene 1990, Report of the Royal College of Physicians 1989). In order to reach those who could benefit from carrier screening at an earlier stage, improved education and communication is necessary (Modell and Petrou 1988, Williamson 1993). This has been conducted by community or church groups of the relevant populations, or through pilot studies in high schools.

Particularly with regard to screening for CF carriers, several different approaches have been considered, including offering this screening to high school students (Cobb et al 1991, Mitchell et al 1993), to all adults in a family practice clinic (Modell 1993) or to all parents in a pediatric practice (Watson et al 1992), to relatives of known CF carriers in a "cascade" strategy (Super et al 1994), to prenatal couples (Livingstone et al 1994), or to pregnant women followed by the partner only if the woman is a carrier (Mennie et al 1992). Each method has advantages and disadvantages and the choice between them is complex (Editorial 1992a, Editorial 1994b, Raeburn 1994).

Carrier screening for the fragile X syndrome, one of the most common single gene causes of mental retardation, has also been considered, particularly since the mutation was identified to be an expanded trinucleotide repeat (Sutherland and Richards 1993). Carrier females can be identified, and offered genetic counselling and prenatal diagnosis. Currently the diagnostic techniques are used for screening for the fragile X premutation and mutation within families at risk, and pilot projects are evaluating the efficacy of population-based screening programs (Caskey 1993b, Rousseau et al 1994).

It was previously stated that one of the pre-conditions for development of a new genetic screening program was the availability of a reliable and sensitive test to detect individuals with a specific genotype. For many genetic diseases, valid biochemical or physiological tests were not available; it was not until molecular genetic techniques were developed for mapping and cloning genes, and identifying linked markers or specific mutations, that screening became possible. Because of the significance of advances in molecular genetics to the development of new screening programs, I will briefly review this progress before discussing screening programs for late-onset hereditary disease.

Implications of Advances in Molecular Genetics to the Development of Genetic Screening Programs

The rapid advances in molecular genetics in the 1980s, and even more so since the Human Genome Projects were initiated in 1990, have resulted in identification of DNA alterations, whether linked markers or actual mutations of specific genes, that can be used for population and family screening for an increasing number of genetic diseases (Report of the Royal College of Physicians 1989, Collins and Galas 1993, Sutherland and Richards 1994). Restriction fragment length polymorphism (RFLP) markers are heritable changes of DNA base sequence which alter the ability of restriction enzymes to cut DNA (Botstein et al 1980). As such they can be identified by laboratory techniques: the appropriate restriction enzyme is added to a DNA sample, and the

fragments produced then separated by electrophoresis and identified by a specific radioactively-labelled probe.

RFLPs are rarely the cause of a genetic disease but are distributed throughout the genome, and have been used extensively as the markers for linkage mapping of disease genes. After a gene is mapped, these markers can also be used to follow a disease gene through a family, to allow predictive testing for those at risk (Botstein et al 1980). RFLP markers have some disadvantages, however. Their distribution throughout the genome is not random, and they usually have relatively few alleles, therefore, may be uninformative for mapping or predictive testing in a particular family.

Short tandem repeat (STR) markers (di-, tri- or tetra-nucleotide repeat sequences, of which the most commonly used are the "CA" repeat markers) are randomly distributed and are much more numerous than RFLP markers (Litt and Luty 1989, Dausset and Cann 1994). These STR markers have the additional advantage that there are frequently multiple alleles (each with a different number of copies of the repeated unit) which are readily distinguishable on the basis of size (Weber and May 1989, Sutherland and Richards 1994). The use of STR markers is frequently coupled with the technique of polymerase chain reaction (PCR) (Sakai et al 1985) which allows the amplification (production of multiple copies) of only the stretch of DNA of interest, and detection of marker alleles within this segment of DNA. This combination of the use of STR markers with PCR reduces the amount of DNA

required, simplifies the laboratory procedure, and reduces the time required for testing, all of which are important for development of a screening test for a genetic disease (Assessing Genetic Risk 1994).

The number of specifically located genetic markers identified has increased rapidly over the past few years (Ward and Davies 1993, Assessing Genetic Risk 1994). The increased number of markers has in turn resulted in an acceleration of the mapping and cloning of disease genes (Collins and Galas 1993). It was initially expected that there would be relatively few different mutations of a specific disease gene, and, therefore, that cloning such a gene would rapidly lead to direct mutation analysis for those at risk within known families (Goodfellow 1989). For a few conditions this is the case, particularly for the group of diseases caused by expansion of a triplet repeat sequence (eg, Huntington disease, myotonic dystrophy, and spinocerebellar ataxia) where identifying a mutation is based on determination of the size of the repeated sequence (Mahadevan et al 1992, Sutherland and Richards 1993, Benjamin et al 1994). For other conditions such as tyrosinemia in eastern Quebec (Demers et al 1994, Grompe et al 1994), only one or a few mutations occur in a specific population, and in other situations such as for cystic fibrosis (CF), one mutation may be particularly common (the ΔF508 mutation occurring on 70% of CF chromosomes) but many other mutations (over 300) also occur (Verlingue et al 1994).

For most genes the mutations are extremely diverse and many families have unique mutations (eg, VHL [Crossey et al 1994], neurofibromatosis, type 2 (NF2) [MacCollin et al 1994], or FAP [Powell et al 1993]). For some genes (eg, Marfan syndrome [Sutherland and Richard 1994] and neurofibromatosis, type 1 (NF1) [Upadhyaya et al 1994]), mutations are very difficult to identify with the commonly used techniques such as single strand conformation analysis (SSCP), and denaturing gradient gel electrophoresis (DGGE), (Caskey 1993a), or even by sequencing the gene. For these diseases, genetic screening by direct mutation analysis is possible in relatively few families, and predictive testing by linkage analysis is necessary for the majority of families where the mutation has not been identified (Sutherland and Richards 1994).

Thus diagnostic tools are available to detect those who are at high risk for many genetic diseases, whether through family studies or by population screening. This information, however, must be used with care so that more good than harm results (Assessing Genetic Risk 1994, Caskey 1993b).

Screening to Reduce Uncertainty

i) Huntington disease predictive testing

Few of the adult-onset AD diseases are frequent enough in the population as a whole, or in a geographic or ethnic subgroup, for population screening to be appropriate, but because of the AD inheritance pattern there may be many relatives at risk who could benefit from family screening: the families where these AD diseases occur can be considered the "population at

risk". Because the primary defect for most of these diseases is unknown, the identification of gene carriers by biochemical or physiological testing is not possible. The identification of RFLP markers, however, provided the technological basis for DNA-based family screening, the first example of this being the predictive testing programs for Huntington disease (HD) (Harper and Morris 1989).

HD is an incurable autosomal dominant neurodegenerative disorder presenting in adult life. Family members at risk frequently have a great deal of anxiety because of the inevitable physical and psychiatric decline of those who inherit the gene, and the ramifications for close family members (Hayes 1992). Many of those at risk expressed a desire to know their fate so that they could plan for the future with or without the HD gene.

The HD gene was mapped to the short arm of chromosome 4 in 1983 by linkage to an RFLP marker (Gusella et al 1983), and predictive testing became possible after other RFLP, and later, STR markers were identified in the region. There was concern, however, among the professionals involved with HD families about the potential costs and benefits of such testing (Harper 1992).

Previous population or family screening for genetic disease had been directed towards severe diseases that were imminent, either at birth or in early childhood, to permit early identification and treatment of those who were affected; or to provide reproductive options which could prevent, or allow

preparation for, the birth of a child with a severe disorder (Bloch and Hayden 1990).

Even though the technology was available, there were concerns about the appropriateness of predictive testing for an incurable disease such as HD presenting in adult life (Bloch and Hayden 1990). There were fears that the psychosocial costs from increased anxiety or depression, or discrimination regarding insurance or employment would outweigh the benefits (Billings and Beckwith 1992a, Natowicz et al 1992, Ostrer et al 1993). Family members at risk were surveyed to identify the demand for this testing. In different studies, 50% to 70% of those responding expressed an interest in predictive testing, particularly to reduce the uncertainty under which they were living (Hayes 1992, Codori and Brandt 1994a). Up to 65% of those surveyed desired prenatal testing.

Strict guidelines were developed by geneticists, ethicists, and mental health professionals before the first HD predictive testing programs were initiated in the mid-1980s under carefully controlled research protocols (Craufurd et al 1992). The predictive test was only offered to those over 18 years of age, informed consent was required, extensive pre- and post-test counselling were included for all participants, and results were provided confidentially (Bloch and Hayden 1990, Benjamin et al 1994).

Predictive testing for HD by linkage analysis was thus made available, but the uptake was much lower than expected for both adult and prenatal

testing. Only 9.5% of those eligible participated in the Canadian adult predictive testing program (Quaid and Morris 1993), and only 5% worldwide (Harper et al 1993).

The majority of those who declined testing when it was offered, stated that the perceived costs (psychological stress, potential discrimination regarding insurance or employment, "being unable to "undo" the testing") were too great for the possible benefits (Quaid and Morris 1993). Many felt that they would be interested in testing in the future, if treatment became available, if the test were more accurate (ie, if direct testing rather than linkage analysis were possible), or if the testing process was simplified. Recently the gene was cloned (Huntington's Disease Collaborative Research Group 1993) and because of the type of mutation (an expanded triplet repeat) a direct test is now possible for most family members, including those at 25% risk, without the need for samples from other relatives (Simpson et al 1993). Still, less than half of those at risk request direct testing for the HD mutation. Overall, the high psychosocial cost with little or no perceived benefit is the major deterrent to participation (Babul et al 1993).

There have also been fewer requests for prenatal diagnosis than expected from previous questionnaires (18% in the Vancouver program compared with 65% expected [Adam et al 1993]). The main reason given by eligible persons declining prenatal testing was the hope for a cure in time to benefit their children. Underlying this was a strong desire to have children

despite the risk, and the fact that termination of pregnancy was unacceptable to many, particularly for future rather than imminent disease (Babul et al 1993).

The majority of those tested, particularly those with increased risk, have suffered less psychological trauma than expected (Wiggins et al 1992, Codori and Brandt 1994a). Unexpectedly, however, about 10% of those with decreased risk, had difficulty accepting the results (Huggins et al 1992). The fact that the overall response to HD predictive testing was good may be attributable to the extensive pre- and post-test counselling (Wiggins et al 1992, Kessler 1994); or to self-selection of participants (Codori et al 1994b, Kessler 1994, van der Steenstraten et al 1994), so that those perceiving an inability to cope with an increased risk result did not take part in predictive testing. Tibben et al (1993) suggest, however, that the apparent favourable response is in fact denial of the undesirable results.

There is concern that psychosocial responses may not be as favourable in the future if unsuitable candidates are persuaded by well-meaning professionals to take the test, or if the extensive counselling, which supports those being tested, decreases, either because of the good outcomes previously demonstrated, or because demand for all forms of genetic screening increases beyond the capacity of geneticists to provide the necessary counselling (Craufurd et al 1992, Wexler 1992, Codori et al 1994b, Kessler 1994).

The cloning of the gene and possibility of direct testing for the mutation, even for those at 25% risk, facilitates testing for some who could not be tested

previously, but raises new issues such as the conflict of interest between one person's "right to know", and another's "right not to know" (Ball and Harper 1992, MacMillan and Quinn 1993, Simpson et al 1993, Benjamin et al 1994), since identifying the mutation in one person may at the same time identify another individual as an obligate gene carrier.

Screening for Adult-Onset Disease

i) Screening for untreatable adult-onset disease

Genetic testing, or screening, is being recommended for an increasing number of adult-onset hereditary disorders, as more and more genes are mapped and cloned (Editorial 1994a). Some, like HD, are untreatable, for instance, spinocerebellar ataxia, type 1 (SCA-1), another ultimately fatal neurodegenerative disorder generally presenting in adulthood. Here, also, predictive testing is requested to end the uncertainty of being at 50% risk. The desire for predictive testing, and the use of this knowledge, are correlated with the perceived burden of disease — although prenatal diagnosis and termination of pregnancy are generally unacceptable, those who consider SCA-1 to be a severe burden do not plan to reproduce unless found to be non-carriers (Nance et al 1994). Since the SCA-1 mutation is an expanded triplet repeat as in HD, direct mutation testing is possible.

ii) Screening for treatable adult-onset disease

Other late-onset autosomal dominant disorders are treatable, and the results of screening investigations (whether clinical or genetic) are used for clinical management, as well as for reproductive decisions and reduction of uncertainty. For both Marfan disease and NF2, the likelihood of successful treatment is enhanced by early diagnosis of specific manifestations (Bridges et al 1992, MacCollin et al 1993. The age at onset and severity of disease are extremely variable, and for Marfan disease, particularly, includes those severely affected at birth, and adults with mild manifestations unrecognized except by careful examination. Clinical screening of those at risk using specific investigations to detect known manifestations has been used within families to identify and treat pre-symptomatic disease. For these disorders, a major benefit of screening is improved medical outcome (Bridges et al 1992, MacCollin et al 1993). Because of the variable age of onset, however, clinical screening must be continued indefinitely for those with negative investigations. The addition of molecular genetic testing to identify gene carriers could refine the clinical screening programs.

For NF2, no genetic heterogeneity has been recognized, and even though about 50% of NF2 families have a new mutation, the mutation can be identified in the majority of families (MacCollin et al 1994). When this presymptomatic diagnosis is added to a clinical screening program there is minimal additional psychological burden, and costly clinical screening can be restricted to gene

carriers. Because the typical age of onset of acoustic neuromas (the most common manifestation of NF2) is in the twenties or later, genetic testing before age 18 is not recommended.

For Marfan disease, on the other hand, extensive clinical and genetic heterogeneity has been recognized (Franke and Furthmayr 1994) and a mutation of the major gene, fibrillin, can only be identified in a minority of families (even in those linked to the fibrillin locus on chromosome 15) (Sutherland and Richards 1994). Although family members have expressed interest in a definitive molecular screening test for themselves, or for prenatal diagnosis (Bridges et al 1992), predictive testing by mutation detection or linkage analysis is only available for a few families.

Predictive testing for NF1 has also been considered, however the gene is large, and until protein truncation testing (PTT) (Roest et al 1993) was used recently (Heim et al 1994), relatively few mutations were detected. The phenotype is also extremely variable with many gene carriers having skin manifestations only, and the severity of disease cannot be predicted. Prenatal diagnosis is, however, desired by some gene carriers, for preparation for the birth of an affected child rather than for termination (Benjamin et al 1993).

Tuberous sclerosis is another AD disorder with variable age at onset and variable manifestations (Smalley et al 1994). Very mild disease expression or non-penetrance may be seen. Thus, even with comprehensive clinical investigations, it may be difficult to make a diagnosis (Al-Guzali et al 1989).

A DNA diagnostic test to identify gene carriers would, therefore, be desirable; but there are at least two loci for tuberous sclerosis, and frequently the gene involved in a particular family is unknown.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary diseases with an incidence of approximately 1/1,000, therefore, more common than cystic fibrosis or Huntington disease (Elles et al 1994). Although up to 30% of patients with ADPKD have mild or unrecognized disease, 45% develop end-stage renal disease (therefore requiring dialysis or kidney transplant) by age 60 (Gabow 1993).

The PKD1 gene was mapped to chromosome (chr) 16 in 1985 (Reeders et al 1985), and a second locus was subsequently identified on chromosome 4 (Peters et al 1993). Because of this heterogeneity, predictive testing by linkage analysis was only available to families where chr16 linkage could be demonstrated. The request for predictive testing, in any case, was not high. The majority of gene carriers could be identified by symptomatic disease, or positive ultrasound testing by age 30 (Parfrey et al 1990, Bear et al 1992), and many family members did not desire a definitive diagnosis before this age (Gabow 1993). Prenatal diagnosis and termination were rarely requested (Hodgkinson et al 1990) because of the late onset and unpredictable severity of the disease, and because of the availability of treatment, but genetic testing is used to identify non-carriers for potential organ donation (Gabow et al 1993). Although the disease is treatable, there still is no evidence that presymptomatic

diagnosis and treatment delays or prevents end-stage renal disease (Gabow 1993). The demand for predictive testing for ADPKD is unlikely to change dramatically even though the PKD1 gene was recently cloned (European Polycystic Kidney Disease Consortium 1994). Few mutations have so far been identified, and the PKD1 gene is difficult to screen for mutations because of extensive homologies to neighbouring regions.

Screening has also been recommended for early diagnosis and prophylactic treatment of hemochromatosis (Edwards and Kushner 1993), a common autosomal recessive disorder that is associated with significant morbidity and mortality in symptomatic patients. The gene has been mapped to the HLA region of chromosome 6 and, within families, homozygotes can be identified by linkage analysis, but for population screening a transferrin assay is necessary. Prophylactic phlebotomy is then recommended to reduce the excessive iron stores and prevent future disease (Edwards and Kushner 1993, Phatak et al 1994).

iii) Screening for hereditary cancers

The most recent class of diseases where genetic screening has been proposed is the group of hereditary cancers, including hereditary colon and breast cancers (Biesecker et al 1993, King et al 1993, National Advisory Council for Human Genome Research 1994, Evans 1995), and the less common cancer predisposition syndromes such as von Hippel-Lindau disease, Li-Fraumeni syndrome (Li et al 1992), and hereditary retinoblastoma

(Knudson?). DNA-based predictive testing for the hereditary cancers has only been available recently (Lynch et al 1993d, Weber et al 1994, van de Water 1994), since the mapping and cloning of genes, for example, for familial adenomatous polyposis (FAP) (Nishisho et al 1991), hereditary non-polyposis colon cancer (HNPCC) (Leach et al 1993, Bronner et al 1994), and hereditary breast or breast-ovarian cancer (Miki et al 1994), and the identification of mutations in some families. But even if genetic screening is possible in a particular family, there is concern whether predictive testing for hereditary cancer does more harm than good (Statement of the American Society of Human Genetics 1994, Weber et al 1994). The quotation from Sophocles, "It is sorrow to be wise when wisdom profits not" (quoted in Wexler 1992) expresses the sentiment of some opponents of this type of screening. It is not always known whether the medical outcome is improved for those identified presymptomatically to carry the particular mutation (Li et al 1992, King et al 1993), and psychosocial problems including increased anxiety, discrimination, and stigmatization have been documented (Lerman et al 1991, Roberts 1991, Lynch et al 1994a, National Advisory Council for Human Genome Research 1994).

Clinical screening programs for FAP have proceeded successfully for some time (Bussey 1975, Bulow 1991) because there is a consistent premalignant marker (multiple colorectal polyps) to identify gene carriers, and, more important, to identify those requiring prophylactic surgery (Jarvinen

1985). Predictive testing with linked markers or mutation analysis has more recently been available in families at risk to distinguish gene carriers and non-carriers, and therefore to reduce the number requiring clinical screening (Petersen et al 1993). FAP, however, accounts for only a small proportion of hereditary colon cancer. The more common HNPCC does not have a reliable premalignant marker, and colon cancer may not be the first or only cancer. Besides the variable expression of HNPCC, there is genetic heterogeneity with at least four genes predisposing to clinically indistinguishable phenotypes (Nystrom-Lahti et al 1994a, Nicolaides et al 1994); this complicates identification of the gene or mutation in a particular family. The reliability of recommended clinical screening programs for those who are mutation-positive or at high risk, is only now being evaluated (Vasen et al 1994).

Similarly for hereditary breast cancer, two loci, BRCA1 and BRCA2 (which produce phenotypes that are only partially clinically distinguishable) have already been identified (Miki et al 1994, Wooster et al 1994). There is also variable expression of BRCA1 and BRCA2, so that gene carriers may develop other cancers (Biesecker et al 1993, Ford et al 1994) before, or as well as, breast cancer (particularly ovarian cancer in BRCA1 families). To add to the difficulties of predictive testing, age of onset is extremely variable for both hereditary colon and hereditary breast cancer, and non-penetrance occurs in at least 5-10% of gene carriers (National Advisory Council for Human Genome Research 1994, Goldgar et al 1994, Rowell et al 1994).

Despite these problems, there is a demand from the public for screening programs to define cancer risk (Croyle and Lerman 1993a). Some of those requesting predictive testing, however, may be from families with sporadic cancer, where there is not a significant increased risk to relatives (King et al 1993, Rowell et al 1994). Geneticists may have difficulty distinguishing families with hereditary cancer from families with coincidental sporadic cancers, and the public may not recognize the need for this distinction (Motulsky 1994).

Screening programs for hereditary cancers require extensive pre- and post-test education and counselling components (Biesecker et al 1993, Lynch et al 1993d), as in the Huntington disease predictive testing programs, because of the psychosocial implications of predictive testing for cancer (Croyle and Lerman 1993b, Lynch et al 1994a). But, to repeat, there are probably not enough geneticists or genetic counsellors to provide all this counselling (Holtzman 1992, Assessing Genetic Risk 1994, Warner 1994, Weber et al 1994).

It is generally agreed that screening programs for hereditary cancers should be conducted under well-controlled research settings until more information is available on the medical and psychosocial outcomes of screening, and appropriate clinical surveillance for gene carriers is defined (National Advisory Council for Human Genome Research 1994, Statement of the American Society of Human Genetics 1994, NIH Guide 1994). More information is also needed on the spectrum of mutations for each hereditary

cancer, and any differences in the risk of cancer(s) from these different mutations (Statement of the American Society of Human Genetics 1994). Finally, safeguards against discrimination, particularly with regard to insurance, are also necessary for those with a presymptomatic genetic diagnosis (Billings et al 1992b, Caskey 1993b, Ostrer et al 1993), since this may be considered a pre-existing condition.

Health Care Evaluation

Health care programs need to be evaluated in a number of ways to ensure that they do more good than harm to those who require these services, and to ensure that the use of resources is appropriate (Sackett 1980). The first question to be answered is whether the health care program can work in ideal circumstances (a question of efficacy [Stoddart and Drummond 1984a]). The second question is whether the program does work under normal circumstances (a question of effectiveness). This combines efficacy with both the availability and acceptability of the program to those who need it (Sackett 1980). The third question is whether the resources allocated to allow the program to proceed are better used in this way than in some other way. This question of efficiency is assessed by one of the forms of economic evaluation of health care (Drummond et al 1987), usually after the efficacy and effectiveness have been established (Stoddart and Drummond 1984a).

i) Efficacy and effectiveness of health care programs

The first step in evaluation of efficacy and effectiveness of health care programs is to establish the need for the program (Needs Assessment [Scriven 1990]). A health care program should only be developed or implemented after a burden of disability or death from the disease in question has been established. The needs assessment should indicate whether this unmet need is for the population as a whole, or for a specific segment of the population. This needs assessment should initially be done before the program is planned, but should also be repeated subsequently to determine whether the need still exists (Canadian Evaluation Society 1989). The objectives of a health care program should then relate to the established need.

The second step is to determine whether the objectives of the program are being met, and, if so, whether these results, or outcomes, can be attributed to the program rather than to some other variable (Summative Evaluation [Shortell and Richardson 1978, FitzGibbon and Morris 1987]). The third step is to determine whether or not there is a better way to achieve the same results (Formative Evaluation [Shortell and Richardson 1978]). Summative evaluation relates to both the efficacy and effectiveness of the health care program whereas formative evaluation relates more specifically to the effectiveness of the program given that efficacy has been demonstrated.

Ideally, efficacy and effectiveness should be established experimentally through a randomized control trial (Sackett 1980), in which the medical outcome in an experimental group receiving the investigation or treatment is

compared with the outcome in a control group not receiving the intervention, or receiving a different intervention. However there are certain circumstances where randomization is either not possible or unethical (Shortell and Richardson 1978). Determining the appropriate evaluation method therefore includes choices of evaluation design, data type, and data analysis (Patton 1987).

The evaluation design provides the method of gathering comparative information and ruling out the effect of other variables on the outcome, to reduce the chance of alternative interpretations of the results (FitzGibbon and Morris 1987). The evaluation design may be a randomized control trial with equivalent experimental and untreated, or differently treated, control groups; a quasi-experimental design with non-equivalent control (or comparison) groups (Cook and Campbell 1979, FitzGibbon and Morris 1987); or a naturalistic design where the experimental and control groups are chosen for their information content (Patton 1987).

Ideally a randomized control trial is used to insure that the effect seen is due to the treatment and not some other variable, but this is not always possible. It may, for example, be unethical to withhold treatment for a severe or lethal disease by assigning patients to an untreated control group (FitzGibbon and Morris 1987). In other studies the numbers may be too small to allow randomization, particularly when individuals are stratified according to socio-demographic factors or disease severity. This is likely to be the situation when evaluating screening programs for extremely variable tumour predisposition syndromes.

When a quasi-experimental design is used, the differences between the experimental and comparison groups should be documented, and any demonstrated effects considered in light of these differences. The comparison groups may be previous cohorts, or similar groups treated differently at another centre (Cook and Campbell 1979, FitzGibbon and Morris 1987). Threats to internal validity of the results should then be ruled out (Shortell and Richardson 1978), particularly whether there is selection bias in establishing the experimental or comparison groups, and, if historical comparison groups are used, whether the effect is due to improved medical care in general, rather than to the specific treatment. Threats to external validity are also considered to determine the extent to which the results can be generalized to other situations (FitzGibbon and Morris 1987), eg, when evaluating screening programs for hereditary tumour predisposition syndromes, will the results apply to other families, to other locations, and to other similar diseases?

Naturalistic design is particularly suited to evaluation of programs with individualized outcomes, to those dealing with unique situations, and to evaluation of quality of life aspects rather than purely medical effects of programs (Patton 1987). Data (usually qualitative) is collected from "information-rich" cases or situations for comparison of program effects at a personal level.

In general, data to be collected can be quantitative and obtained through valid and reliable measurement of a specific operational indicator of the program objective (Shortell and Richardson 1978); or qualitative, and obtained by

observation, interviews, or questionnaires (Patton 1987). These data are then analyzed either by statistical analysis (usually of quantitative data), or by content or case analysis to search for patterns in in-depth qualitative data. The evaluation design, data type, and data analysis are chosen to suit the specific evaluation, and may be matched in different ways (Patton 1987).

The deductive approach with experimental or quasi-experimental design, qualitative data, and statistical analysis is used to test a pre-formulated hypothesis. An inductive approach with naturalistic design, qualitative data, and content or case analysis, on the other hand, is particularly suited to evaluation of programs where individualized outcomes are important because of variable disease, to evaluation of psychosocial effects of programs, and to formative evaluation of all programs. The inductive approach may also identify unanticipated results and lead to generation of new hypotheses.

The summative evaluation will frequently combine deductive and inductive approaches in order to assess both the specific medical objectives of the health care program, and the related psychosocial outcomes. For the formative evaluation, generally the inductive approach, alone, is used since this evaluation requires a detailed description of the program, including "who does what to whom with what resources" on a day to day basis (Sackett 1980), in order to look for areas for improvement.

ii) Economic evaluation of health care programs

Health care economic evaluation methods have been developed to assist in decision-making because there are insufficient funds for all demands on the

health care budget (Williams 1974, Drummond 1980). Choices, therefore, have to be made (Stoddart and Drummond 1984a, Robinson 1993a). An economic evaluation can help with these decisions by organizing information about costs and benefits in a systematic way (Henderson 1991, Robinson 1993a). The methods used are similar to economic evaluation techniques used in other areas such as education, transportation or town planning (Robinson 1993a). The relevant advantages of a particular course of action or program are weighed against the disadvantages, in such a way that different programs can be compared and prioritised, the overall goal being to use the available resources to obtain maximum benefits in terms of the health of the population as a whole (Williams 1974, Sackett 1980, Henderson 1991).

Health care programs should be evaluated to determine whether to offer a service (Chapple et al 1987), how much of a service to offer (Henderson 1991, Robinson 1993b), or to whom to offer a service (ie, universally, or selectively to a particular high-risk population [Hagard and Carter 1976, Phatak et al 1994]). The economic evaluation is not concerned with the ethics or efficacy of a particular health care program but these should be established before the economic evaluation is undertaken (Stoddart and Drummond 1984a, Chapple et al 1987, Henderson 1991).

Different forms of economic evaluation can be used in different situations (Stoddart and Drummond 1984a, Robinson 1993a, Sackett 1980), depending on whether the goals and outcomes of the programs to be compared are the same or different:

a) cost-minimization analysis, in which the outcomes of the programs being compared are the same and the aim of the analysis is to identify the least costly option (Robinson 1993b),

b) cost-effectiveness analysis, in which the outcomes to be compared, although similar, are different at least in degree, and the outcomes are measured in common (natural) units such as "lives saved" or "pain free days" (Robinson 1993c),

c) cost-utility analysis, in which the outcomes vary and are converted into quality of life units for comparison (Robinson 1993d), and

d) cost-benefit analysis, in which costs and benefits are all converted into monetary units (Hook 1991, Robinson 1993e).

Economic evaluations differ depending on the point of view taken, whether of the patient, the hospital or health care unit, the government, or society in general (Stoddart and Drummond 1984b, Phatak et al 1994), the most appropriate being the assessment of costs and benefits from a societal perspective (Robinson 1993b). However, defining the limits of the effect of the program can be difficult.

Evaluations also differ in the methods used to identify and measure costs and benefits (either in the units used or the values given) (Sackett 1980, Phatak et al 1994). More important, significantly different assumptions can be made about which costs or benefits to include (Hagard and Carter 1976, Stoddart and Drummond 1984a, Modell and Kuliev 1991).

It is important to determine all the consequences of a program and to identify all the potential costs and benefits (direct, indirect, and external) (Drummond et al 1987), even if these costs and benefits are outside the health sector. The costs and benefits will include tangible (or direct) costs (Drummond et al 1987) which are easy to affix a monetary value to (costs for procedures, counselling, patient's travel), and also intangible (or indirect) costs and benefits such as increased or decreased productivity, or quality of life of unaffected family members (Henderson 1991) which are more difficult to quantify (Modell and Kuliev 1993). Intangible costs and benefits also include psychosocial factors: either costs (such as discomfort during a procedure, or anxiety about the risk of disease or the results of testing or treatment), or benefits (such as reassurance about the absence of disease, or reduction of uncertainty) (Stoddart and Drummond 1984b).

One attempt to measure such intangible effects is the use of "quality adjusted life years" (QALYs) (Henderson 1991, Hook 1991) which takes into account the improved quality of life as well as the years of life lost or gained because of an intervention. However, a QALY is more useful for assessing physical symptoms or handicaps, than for assessing psychosocial factors (Henderson 1991).

Not all costs and benefits are in the present. Because it is generally assumed that benefits in the immediate future are of greater value than those in the distant future, and costs are preferably delayed, a method of discounting

may be used to convert future costs and benefits to their present value (Williams 1974, Robinson 1993b, Phatak et al 1994).

The way these different methods of economic evaluation are used can be demonstrated in a series of examples. For cost-minimization analysis, the assumption is that the interventions being compared have identical outcomes, and the analysis is to determine the least expensive option (Robinson 1993b). Outcomes, however, are rarely identical; even if the outcomes differ only in degree of effect, a cost-effectiveness approach should be used in order to compare the consequences as well as the costs.

Walker et al (1991) present their analysis of costs of diagnostic procedures for colorectal cancer detection as a cost-minimization study (the minimization of expected cost per cancer identified), but qualify this because clinical trials have not established the relative effectiveness of colonoscopy and double contrast barium enema in the detection of colon cancer. A cost-effectiveness analysis would be more appropriate for this study.

In cost-effectiveness analysis, outcomes are similar and are measured in natural units such as "lives saved" or "pain free days" (Robinson 1993c). This may be a comparison of two different treatments, or of the proposed treatment and the "status quo option" (either no treatment, or treatment at the time of presentation with symptomatic disease).

Phatak et al (1994) used a decision analysis method for a cost-effectiveness evaluation of early or late treatment of patients with hemochromatosis. The comparison was between a) early identification and

preventive treatment facilitated by screening healthy 30-year-olds, and b) late identification and treatment of symptomatic patients. Information from the literature was used to construct a decision tree to compare the possibility of life-threatening complications and their costs, depending on whether hemochromatosis was identified early or late. In order to prevent bias from incorrect assumptions about several variables (prevalence of disease, probability of different disease manifestations, the cost of the screening test, and the discount rate), a sensitivity analysis was conducted with different values for each of these conditions (Phatak et al 1994, Robinson 1993c). A set of circumstances was thereby determined over which screening 30-year-old males to allow early treatment of hemochromatosis was considered cost-effective.

In cost-utility analysis, outcomes of procedures are measured in units relating to a person's sense of well-being, ie, reflecting their quality of life as well as length of life resulting from the intervention, the most common unit being the QALY (Robinson 1993d). The intention is that by using QALYs, the outcomes of different treatments for one disease, or of treatments of different diseases can be compared. However, QALYs have proven difficult to measure even using health profile questionnaires such as the Rosser index (Rosser et al 1982) which describe health status in terms of "perceived disability and distress".

Cost benefit analysis is the most comprehensive economic analysis but is difficult to apply since human life must be given a monetary value (Williams 1974, Robinson 1993e). Two approaches have been used: a) the human

capital approach where a person's value is related to his or her normal wage (since by death or disability this wage is lost), and b) the stated preference approach where a person's value is based on what he/she is willing to pay for a particular service. The capital approach ignores any costs of "pain and suffering", and both methods give greater value to a person with greater financial resources (Robinson 1993e).

Many economic analyses called "cost-benefit analyses" have been published but the methods used to identify, measure, and value costs and benefits vary greatly (including using non-monetary units for some costs or benefits), making them difficult to compare.

Henderson (1991), and Hagard and Carter (1976) consider both the question of whether prenatal diagnosis is cost-beneficial and the level at which this service should be provided. They demonstrated an economic advantage in providing prenatal screening for Down syndrome at least for women over 35 years of age. However, by expanding the program to individuals below this cut-off risk in terms of maternal age, the costs would outweigh the benefits. The cost of the program included the cost of genetic counselling, and of diagnostic procedures to allow prenatal identification and therapeutic abortion of affected fetuses. The economic benefit is defined as the costs for the care of Down syndrome children avoided, for the family and community, because affected births are prevented. Similarly, Chapple et al (1987) estimate the benefit of DNA diagnostic testing as the costs not incurred for treating the relevant disorders, and Henderson (1991) calculates the benefit of screening

for neural tube defects in specific populations of pregnant women, as the avoided costs of health care, education, and extra family expenses associated with birth of an affected child.

Modell and Kuliev (1991 and 1993) disagree with this approach. In their cost-benefit analysis of a comprehensive program for treatment and prevention of thalassemia, therapeutic abortion of an affected individual is considered a cost of the program not a benefit. Since the goal of at-risk families is to have the desired number of normal children, not to abort affected ones, a non-monetary unit, *genetic fitness*, is used as the unit for benefits of this program or the less comprehensive programs to which it is compared. For each level of genetic services provided, the cost of the program is calculated in terms of both the treatment of the appropriate number of affected children, and the services (education, counselling, prenatal diagnosis and termination) necessary to achieve a healthy family. Highest genetic fitness is achieved with the most comprehensive service. Initially this is the most expensive program, but over a short period of less than 5 years, it becomes the least costly complete program of prevention and treatment.

The dollar value of health care programs can be compared with that of other programs even outside the health care field when monetary units are used for both costs and benefits (Robinson 1993e). This is appropriate since the real cost of a program is the opportunity cost, ie, the achievable outcomes of another (unfunded) program, forgone (Henderson 1991, Williams 1974, Sackett 1980).

Once the evaluation process is complete, it is important to communicate the findings to interested groups, whether the funding agencies, program administrators, participants, or community groups. Although the basic message is the same, the presentation of this message should be tailored to each audience (Morris et al 1987).

RATIONALE AND OBJECTIVES OF THESIS

As stated previously, large families with hereditary tumour syndromes (VHL, MEN-1, MEN-2, FAP, and HNPCC) were identified in Newfoundland in the 1980s, the diagnosis being made when family members were referred for genetic counselling or clinical care. During the initial work-up of each family, a burden of disease was recognized; ie, there were early deaths and disabilities, and increased anxiety in affected and unaffected family members.

The physicians and geneticist (Jane Green) originally involved with each family recognized that family members would benefit from a coordinated approach to their medical management, and that introducing a screening program (for early identification and treatment of individual tumours to reduce morbidity and mortality; for early identification of gene carriers to allow informed reproductive decisions; and for provision of genetic counselling to increase understanding and reduce anxiety) could be central to this type of care. As described above, screening programs had been used successfully for other types of hereditary disease, particularly those with neonatal or early childhood onset, to allow early treatment or to provide information for

reproductive decisions. Predictive testing or presymptomatic diagnosis had also been offered to those at risk for severe, late-onset untreatable disorders such as HD, to reduce uncertainty. The recommendation of clinical and genetic screening programs for hereditary tumour syndromes, to improve the prognosis and quality of life of family members, however, was recent and screening programs had not been fully evaluated.

Because of the large Newfoundland MEN-1, MEN-2, FAP, HNPCC and VHL families identified, there was an opportunity to develop, implement, and evaluate screening programs for these hereditary tumour syndromes. As previously stated, I proposed:

i) to investigate the feasibility of instituting each screening program,

ii) to determine the appropriate components of the screening protocols,

iii) to compare the medical, genetic, and psychosocial outcomes for family members who had been screened with those who had not been screened, and

iv) to determine the cost, both monetary and non-monetary, of maintaining such screening programs.

I could then use this experience to identify and define the factors that must be considered for successful development and implementation of clinical and genetic screening programs for similar late-onset multisystem disorders: for other families, for other locations, and for other diseases.

The same approach was taken for each hereditary tumour syndrome:

a) an extended pedigree was prepared by interviewing family members and searching archival records,

b) the type of tumours, and age at which they occurred were documented by retrospective and prospective studies,

c) a preliminary screening protocol, based on the natural history of the disease thus described, was developed with appropriate medical specialists, and offered to family members,

d) collaborative molecular genetic studies were initiated to map the relevant gene and/or to identify specific mutations,

e) genetic testing by linkage analysis or mutation detection was offered to family members at risk,

f) educational materials about the disease, about the screening program, and about predictive testing were provided for family members and their physicians, and genetic counselling was given when appropriate,

g) the medical and psychosocial outcomes of the clinical and genetic screening programs were reviewed, and

h) the VHL screening program was formally evaluated, including a cost-benefit analysis.

Consent was obtained from family members for participation in each stage of these studies.

Although the objectives of the screening programs were the same, the programs differed in important details because of the differences in the natural history and severity of the five hereditary tumour syndromes, and in the present state of knowledge regarding the medical management, and the molecular genetics of each disorder.

In Chapters 2 to 6, I discuss in detail the development, implementation, and informal evaluation of each of the five clinical and genetic screening programs, and in Chapter 7 present the formal health care evaluation of the VHL screening program. In the final chapter, I summarize the recommendations of this approach to management of hereditary tumour syndromes and other late-onset multisystem diseases; and the benefits to family members, and to society, in general, which can result.

CHAPTER 2 — CLINICAL AND GENETIC SCREENING FOR MULTIPLE ENDOCRINE NEOPLASIA, TYPE 1

INTRODUCTION

Previous studies

Present study

PATIENTS AND METHODS

Ascertainment of Families

Clinical Screening

Predictive Testing

Impact of the Screen Program

RESULTS

Ascertainment of families

Natural history of disease

Morbidity and mortality

i) Hyperparathyroidism
ii) Pituitary tumours
iii) Pancreatic tumours
iv) Carcinoid tumours
v) Adrenal tumours
vi) Other malignancies

Linkage Studies

Impact of the Screening Program

DISCUSSION

Ascertainment of MEN-1(Burin) Families

Clinical Phenotype of MEN-1(Burin)

Mapping of MEN-1(Burin)

Results of Clinical Screening for MEN-1(Burin)

Morbidity and Mortality

Results of Predictive Testing

Costs and Benefits of Screening

Satisfaction with Screening

INTRODUCTION

This chapter describes the identification of a group of Newfoundland families with multiple endocrine neoplasia, type 1 (MEN-1); the documentation of an atypical natural history of disease in these families and comparison with typical MEN-1; and the development, implementation, and review of a clinical and genetic screening program for these families.

Previous Studies

MEN-1 is an autosomal dominant disorder with variable manifestations including hyperparathyroidism, and tumours of the pancreatic islet cells and the anterior pituitary gland (Wermer 1954, Ballard et al 1964, Schimke 1976, Bone 1990). Tumours of the adrenal cortex (Skogseid et al 1992) and thyroid gland (Wermer 1963, Ballard et al 1964), as well as carcinoid tumours of the lung and thymus (Rosai et al 1972, Farid et al 1980, Duh et al 1987) may also occur. Hyperplasia of the parathyroid glands is found in at least 90% of those affected with MEN-1 (Marx et al 1982, Betts et al 1980, Benson et al 1987, Samaan et al 1989) but is not necessarily the initial manifestation (Shepherd et al 1991, Skogseid et al 1991b). Pancreatic tumours (usually gastrinomas, insulinomas, or glucagonomas) have been reported in 32 - 75% of patients in different series (Marx et al 1982, Vasen et al 1989b, Skogseid et al 1991a) and are a major cause of morbidity and mortality in most MEN-1 families (Schimke 1976, Oberg et al 1982, Lips et al 1984, Pipeleers-Marichal et al 1993).

Pituitary tumours (usually prolactin [Carlson et al 1978, Levine et al 1979, Prosser et al 1979, Veldhuis et al 1979] or growth hormone-secreting [Ballard et al 1964], but also including non-functioning adenomas) are less common in most families having been reported in 16 - 40% of patients (Farid et al 1980, Marx et al 1982, Hershon et al 1983). Carcinoid tumours are rare in most series but are a significant cause of morbidity and mortality in some large MEN-1 families (Bear et al 1985, Green et al 1992, Wilkinson et al 1993).

Some of the difference in reported frequency of gland involvement is because of difference in ascertainment (whether autopsy series, or symptomatic or screened patients [Ballard et al 1964, Johnson et al 1967, Majewski and Wilson 1979, Marx et al 1982]). Some, however, apparently reflects a difference in disease expression in certain families (Farid et al 1980, Hershon et al 1983, Brandi et al 1987, Vasen et al 1989b, Skogseid et al 1991a, Shepherd 1991). Whether this difference is because there is more than one gene for MEN-1; because of variable expression of different mutations at one genetic locus, as with the MEN-2A and MEN-2B mutations of the RET proto-oncogene (Mulligan et al 1993, Donis-Keller et al 1993, Eng et al 1994a, Carlson et al 1994); or because of modifying genes, is not yet determined.

The age at onset and severity of disease varies even within families (Schimke 1976, Shepherd 1991, Thakker 1993a), ranging from patients symptomatic in their teens, to those in their 60s or 70s who are asymptomatic and only identified by screening following diagnosis in a younger family

member. In most MEN-1 families the major morbidity and mortality is because of pancreatic islet cell tumours (Majewski and Wilson 1979, Vasen et al 1989b, Skogseid et al 1991a), in the past associated with intractable ulcer disease (Zollinger-Ellison Syndrome [Zollinger and Ellison 1955]) in patients with gastrinomas (Wermer 1963, Van Heerden et al 1986), and more recently associated with pancreatic malignancies (Wolfe and Jensen 1987, Eriksson et al 1990, Pipeleers-Marichal et al 1993). Periodic screening (primarily biochemical screening to detect hyperactivity of the parathyroid, pituitary or pancreas) has been recommended for family members at risk because early identification and treatment of individual manifestations is thought to improve prognosis (Marx et al 1986, Samaan et al 1989, Vasen et al 1989b, Skogseid et al 1991a).

An understanding of the natural history of the disease (particularly the frequency of different manifestations, and the age and order in which they occur) is necessary to plan the appropriate screening protocol for those at risk and for those already known to be affected (Vasen et al 1987). Because some authors suggested that hyperparathyroidism was a prerequisite to the involvement of other glands (Betts et al 1980, Benson et al 1987, Thakker 1993a), screening of those at risk in some families was limited to testing for hyperparathyroidism (Marx et al 1986, Brandi et al 1987, Vasen et al 1989b), with screening for other manifestations concentrated on those identified by this testing. It is now recognized, however, that hyperparathyroidism is not

necessarily the first manifestation (Shepherd 1991, Skogseid et al 1991b), and that the order of gland involvement can be quite variable, so that a more extensive screening protocol may be necessary for all families (Brunt and Wells 1985). However, if clinical heterogeneity can be identified, different screening protocols may be appropriate for different families.

The mapping of the MEN-1 gene to chromosome 11q13 in 1988 (Larsson et al 1988, Bale et al 1989, Thakker et al 1989) and identification of closely-linked informative markers (Nakamura et al 1989, Julier et al 1990, Fujimori et al 1992, Larsson et al 1992b, Thakker et al 1993b) now permits predictive testing in some families.

Present Study

Four Newfoundland families with an atypical presentation of MEN-1 were identified in the late 1970s by Farid (Farid et al 1980). Prolactinomas were more frequent than in most MEN-1 families and were often the presenting feature. Carcinoid tumours of the lung and thymus were also more frequent, whereas pancreatic islet cell tumours were not identified in the first study. Because of the different pattern of manifestations in the Newfoundland kindreds, they were referred to as atypical MEN-1, or MEN-1(Burin) (Bear et al 1985), and, with a similar family from the northwestern USA (Hershon et al 1983), called the prolactinoma variant of MEN-1.

Clinical screening was introduced for some members of the MEN-1(Burin) kindreds in the early 1980s but the full extent of the affected and at-risk members of the families was not yet delineated, and the frequency and age at onset of different manifestations was therefore incomplete. The impetus for further study was the mapping of the MEN-1 gene in 1988.

The following sections describe the results of a retrospective and prospective medical review of the MEN-1(Burin) families, and of genetic linkage studies, carried out to determine whether MEN-1(Burin) could be distinguished from typical MEN-1 by clinical expression of disease or by the genetic locus. This has led to a better understanding of the natural history of the disease which can be used to determine the optimal type and timing of tests for the appropriate clinical screening program for this large group of patients. It has also led to the development of predictive testing by linkage analysis to determine who requires the clinical screening.

PATIENTS AND METHODS

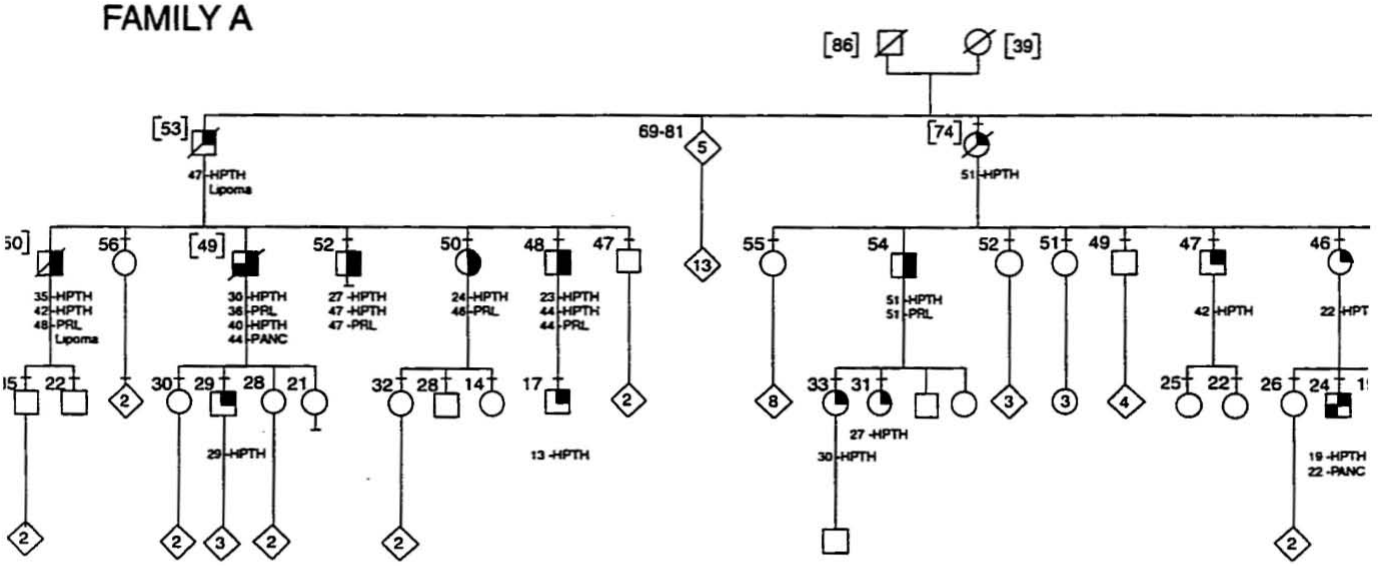
Ascertainment of Families

The four separately-identified families were each from small coastal communities (outports) in the Burin Peninsula/Fortune Bay area of the south coast of Newfoundland. By 1988 there were 42 documented affected individuals, of whom 35 were still living. Family members were interviewed by Jane Green to extend the pedigrees (Figure 2.1) and to search for a connection

Figure 2.1. MEN-1 (Burin) pedigrees with manifestations for each affected member.

Type of manifestation(s) and age at diagnosis are given for each affected family member. Present age or age at death (in brackets) is also indicated.

FAMILY A



hyperparathyroidism (HPTH)

pituitary tumour (PIT)/ prolactinoma (PRL)

pancreatic islet cell tumour ((PANC)

carcinoid tumour of the lung [CARC (L)]
carcinoid tumour of the thymus [CARC(T)]

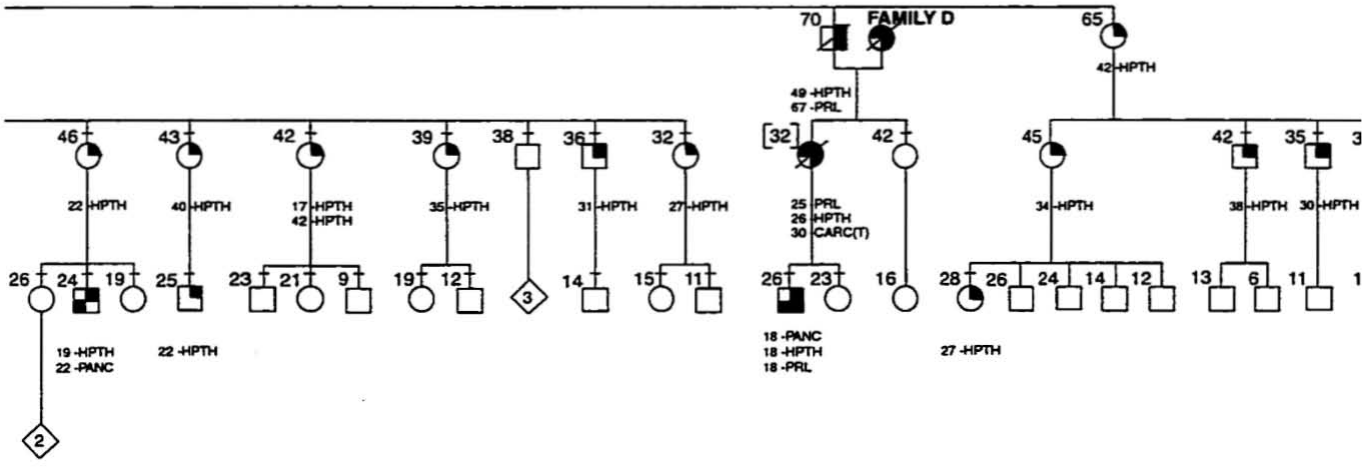
* other malignancy

Screened

69 Age

[31] Age at death

27 -HPTH Age at diagnosis

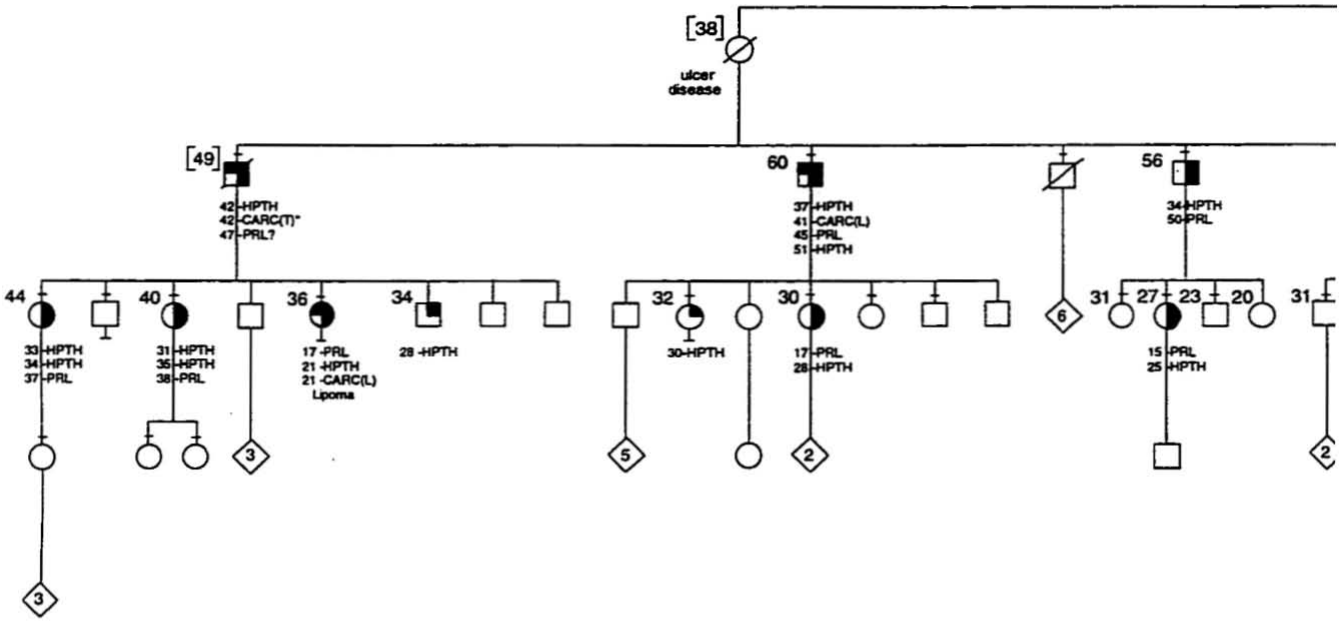


ed

death

diagnosis

FAMILY B



hyperparathyroidism (HPTH)

pituitary tumour (PIT)/ prolactinoma (PRL)

pancreatic islet cell tumour ((PANC)

carcinoid tumour of the lung [CARC (L)]
carcinoid tumour of the thymus [CARC(T)]

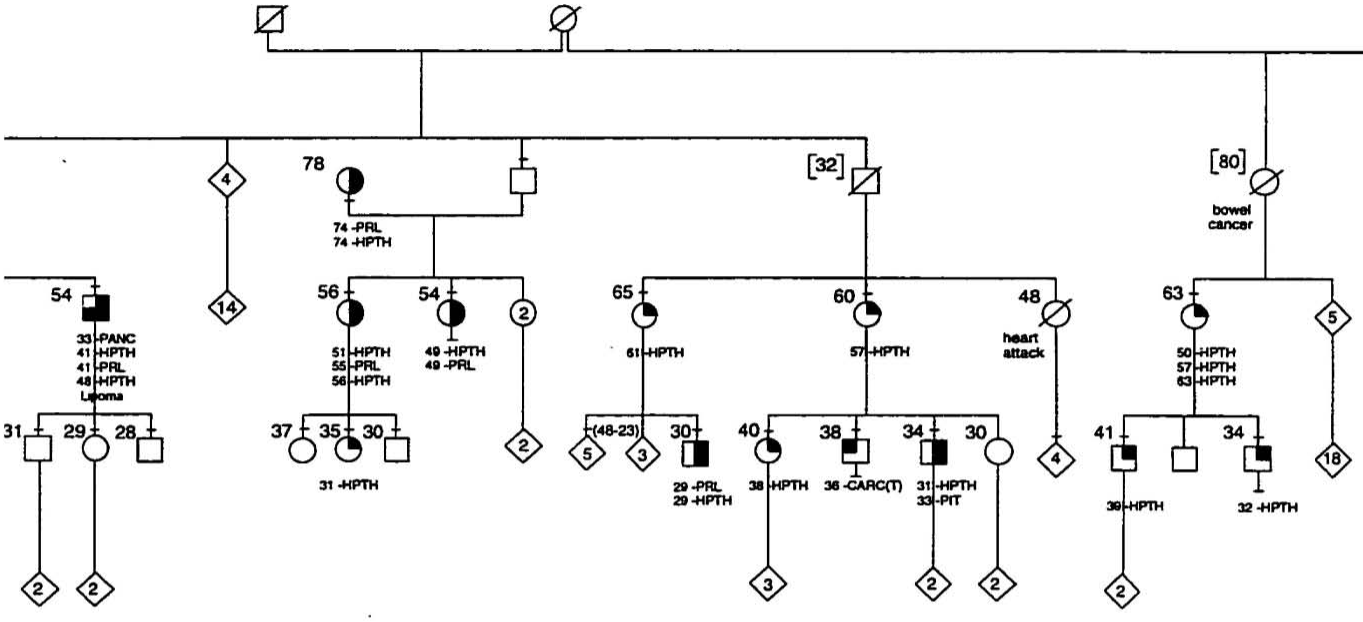
* other malignancy

Screened

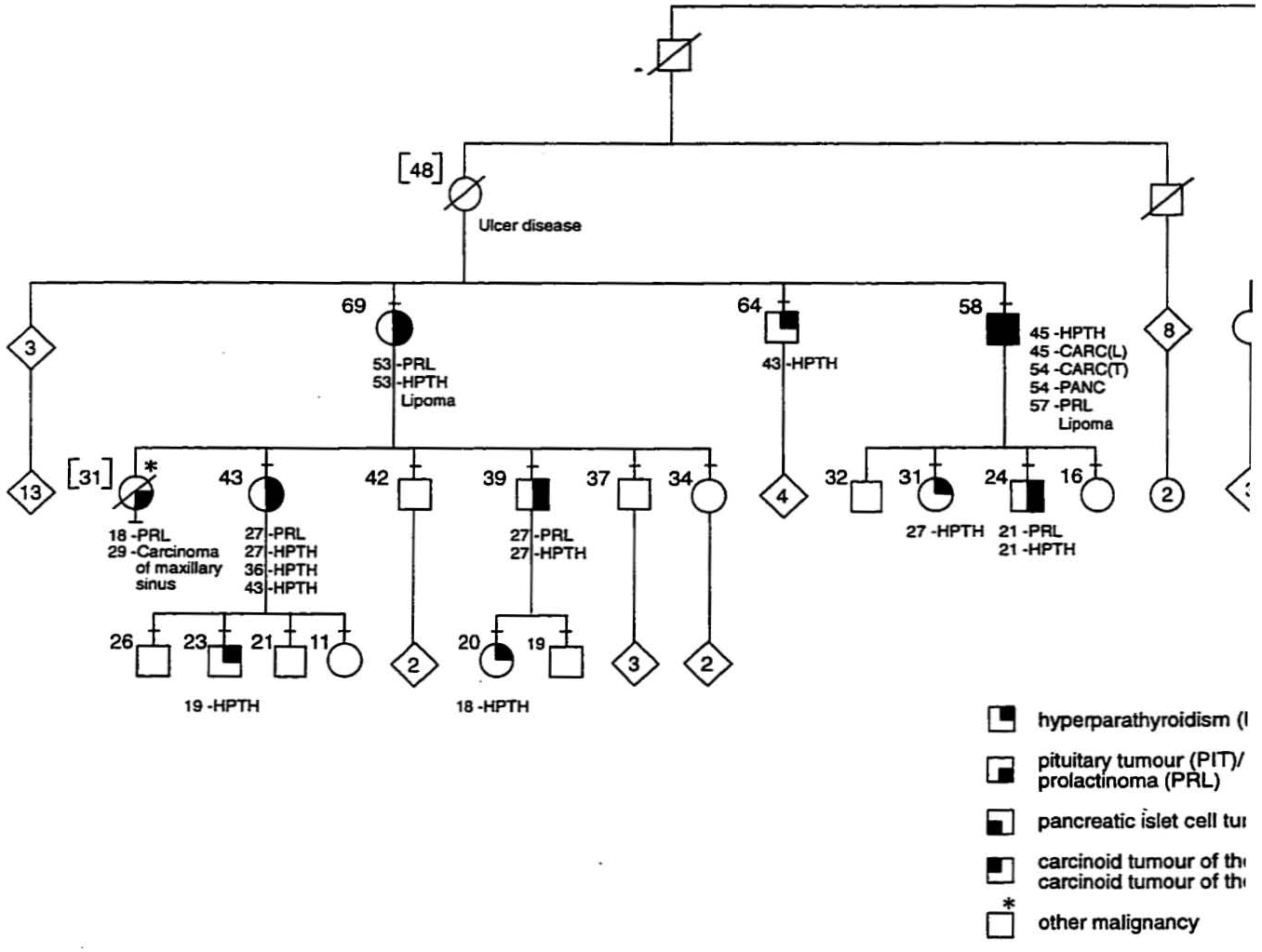
69 Age

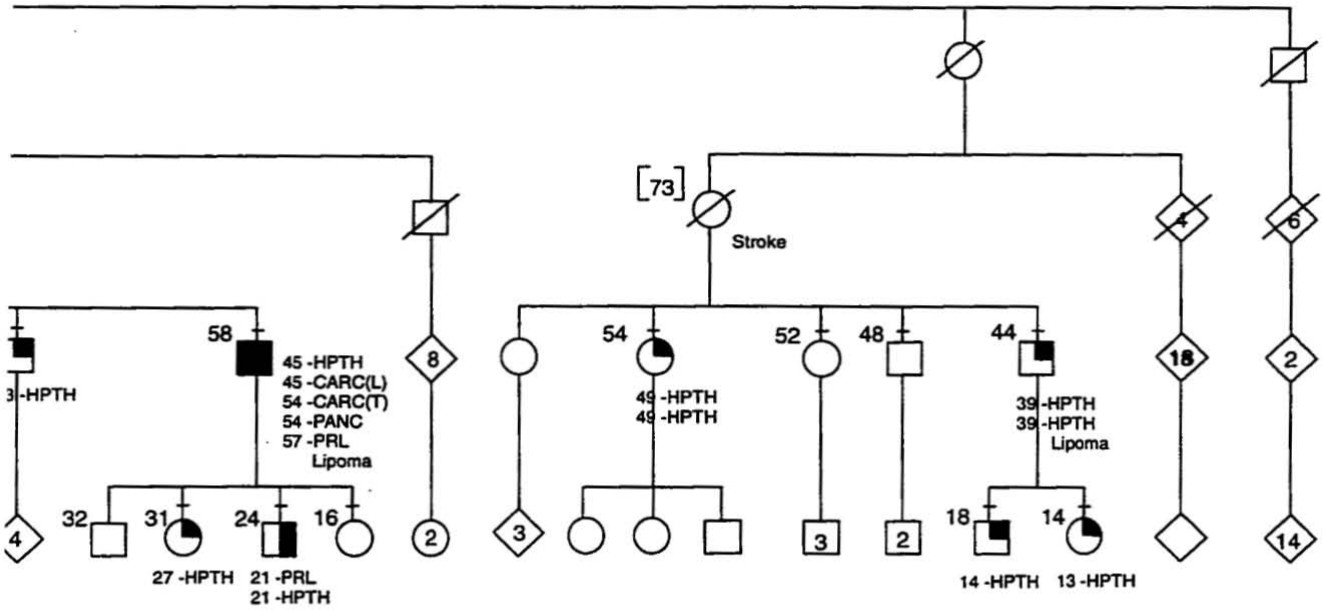
[31] Age at death

27 -HPTH Age at diagnosis


78
74 -PRL
74 -HPTH
[32]
[80]
bowel cancer
54
33 -PANC
41 -HPTH
41 -PRL
48 -HPTH
Lipoma
56
51 -HPTH
55 -PRL
56 -HPTH
54
49 -HPTH
49 -PRL
65
61 -HPTH
60
57 -HPTH
48
heart attack
63
50 -HPTH
57 -HPTH
63 -HPTH
31 -HPTH
29 -PRL
29 -HPTH
38 -HPTH
36 -CARC(T)
31 -HPTH
33 -PIT
39 -HPTH
32 -HPTH

FAMILY C


[48]
Ulcer disease
69
53 -PRL
53 -HPTH
Lipoma
64
43 -HPTH
58
45 -HPTH
45 -CARC(L)
54 -CARC(T)
54 -PANC
57 -PRL
Lipoma
3
8
13
[31]
*
18 -PRL
29 -Carcinoma of maxillary sinus
43
27 -PRL
27 -HPTH
36 -HPTH
43 -HPTH
42
39
27 -PRL
27 -HPTH
37
34
4
32
31
27 -HPTH
24
21 -PRL
21 -HPTH
16
2
26
23
21
11
2
20
19
3
2
19 -HPTH
18 -HPTH
hyperparathyroidism (
pituitary tumour (PIT)/ prolactinoma (PRL)
pancreatic islet cell tu
carcinoid tumour of th
carcinoid tumour of th
*
other malignancy



hyperparathyroidism (HPTH)

pituitary tumour (PIT)/ prolactinoma (PRL)

pancreatic islet cell tumour ((PANC)

carcinoid tumour of the lung [CARC (L)]
carcinoid tumour of the thymus [CARC(T)]

other malignancy

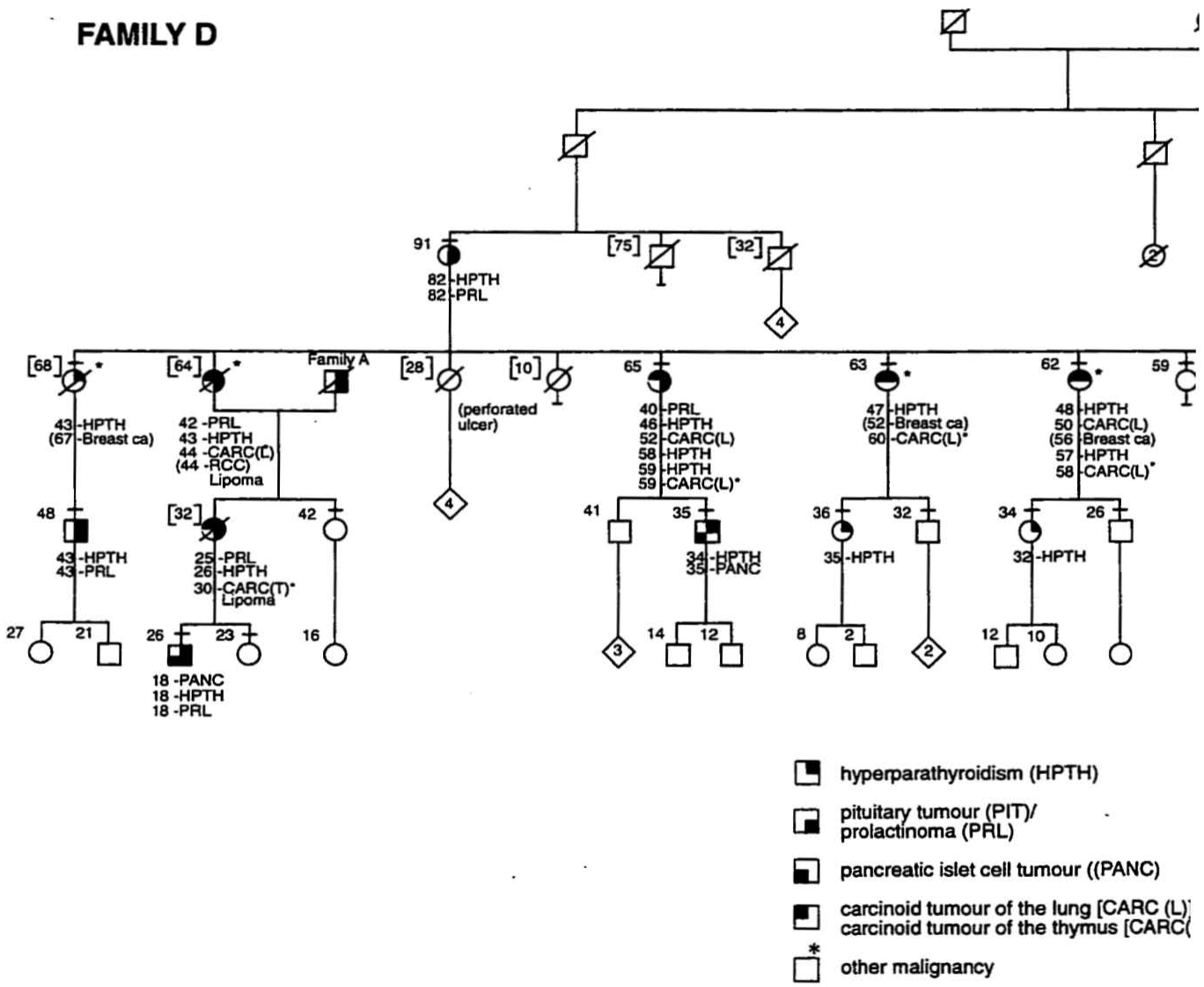
Screened

69 Age

[31] Age at death

27 -HPTH Age at diagnosis

FAMILY D



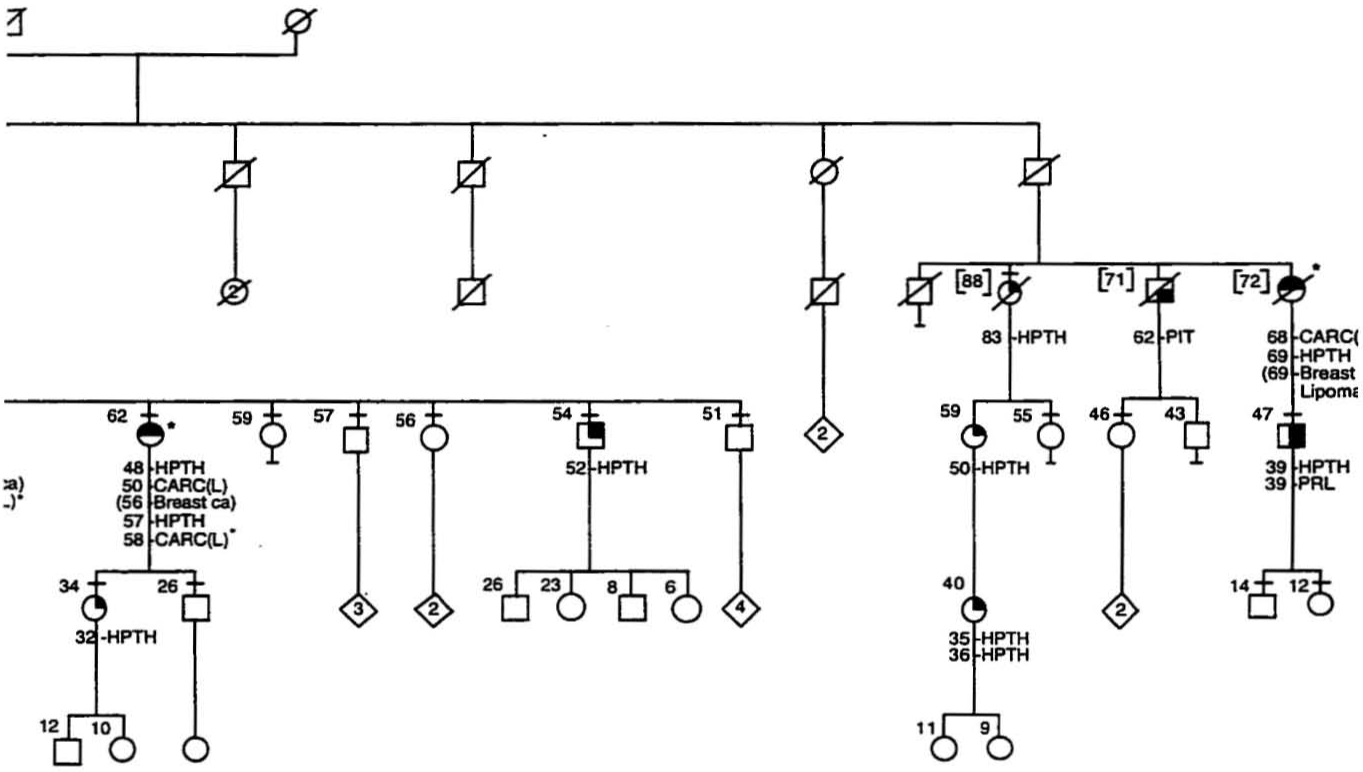
hyperparathyroidism (HPTH)

pituitary tumour (PIT)/
prolactinoma (PRL)

pancreatic islet cell tumour ((PANC)

carcinoid tumour of the lung [CARC (L)]
carcinoid tumour of the thymus [CARC(

* other malignancy



yroidism (HPTH)

iour (PIT)/
a (PRL)

slet cell tumour ((PANC)

mour of the lung [CARC (L)]
mour of the thymus [CARC(T)]

iancy

Screened

69 Age

[31] Age at death

27 -HPTH Age at diagnosis

between the kindreds. After consent was obtained, medical records, including any autopsy records, of affected and apparently unaffected family members were reviewed. Records of some individuals were very limited because of the isolated area, without medical facilities, where these families had previously lived.

Results were recorded for any previous biochemical testing or investigations, and the details and outcome of surgical procedures and pathology were documented. Because there was no established protocol for the screening of MEN-1 families when this program began, knowledge of the presentation of MEN-1(Burin) patients and recommendations from the literature (Oberg et al 1982, Lips et al 1984, Marx et al 1986) were used as guidelines for implementation of a screening protocol which was then modified as new information became available (see later). This screening protocol was offered to known MEN-1 patients and their first degree relatives over 14 years of age. In some circumstances, second degree relatives were also included, particularly when first degree relatives were unavailable or uninterested in participating. Clinics with a physician, geneticist and laboratory personnel in attendance were held in the outport communities where the majority of family members now reside.

Genetic counselling was provided concerning the manifestations of MEN-1(Burin), the inheritance pattern and risk of recurrence of the condition, and the recommended clinical screening. The potential for genetic screening if

the location of the MEN-1(Burin) gene could be identified was also described, and family members were asked about their willingness to participate in linkage studies.

For family members living in other parts of Canada, contact was made through their relatives in Newfoundland. Information on MEN-1 (Burin) and recommendations for screening were sent to those at risk, and to their family doctors, if requested.

Clinical Screening

Participants were examined by the endocrinologist, medical and family histories taken by Jane Green, and blood drawn for measurement of serum calcium, parathyroid hormone (PTH), prolactin and gastrin. Patients with any abnormal screening test results were followed up by investigations at the Clinical Investigation Unit (CIU) of the General Hospital in St. John's. In the CIU, blood work was repeated, and ionized calcium, and fasting gastrin and glucose levels were determined. Other investigations included combined pituitary function testing, CT (computerized tomography) scan of the sella turcica and visual field testing if prolactin was elevated, secretin stimulation test and pancreatic ultrasound if gastrin was elevated, and a chest x-ray, in all family members, for evidence of carcinoid tumours. Until 1993 measurement of glucagon and pancreatic polypeptide were only available under special circumstances but these are now included for patients seen in St. John's. Also,

all known affected persons now have a CT scan or MRI (magnetic resonance imaging) of the sella turcica, and pancreatic ultrasound for baseline studies even if prolactin and gastrin are normal (Figure 2.2).

Results of all screening tests and follow up investigations were recorded, along with the diagnosis, and age of occurrence of each manifestation for each affected individual. The type of treatment required, and the outcome of this treatment were also documented.

In this group of high-risk individuals, the diagnosis of MEN-1 was made if one or more of the following criteria were met (Shepherd 1985, Vasen et al 1989b):

a) confirmed hypercalcemia with elevated or inappropriately normal PTH level,

b) confirmed hyperprolactinemia (> 5X normal) and/or enlarged sella turcica,

c) elevated gastrin level with positive secretin test, elevated glucagon level, or pancreatic mass demonstrated by ultrasound,

d) radiological or histological identification of a carcinoid tumour.

All persons with documented MEN-1 manifestations were screened annually for evidence of subsequent manifestations. Those at risk (at 50% risk or in certain circumstances at 25% risk) were screened every 1 - 2 years with blood work done in their local hospital, this screening being coordinated by the geneticist and the family doctor.

Figure 2.2. Screening protocol for affected and at-risk members of MEN-1 (Burin) families.

SCREENING PROTOCOL FOR AFFECTED AND AT-RISK MEMBERS OF MEN-1(Burin) FAMILIES

	PRIMARY SCREENING*	FOLLOW-UP INVESTIGATIONS
1.	serum total and/or ionized calcium PTH	**repeat ionized calcium with serum PTH and phosphate 24 hr urine calcium parathyroid scan
2.	serum prolactin	combined pituitary function testing visual fields **CT scan/MRI of pituitary fossa
3.	fasting gastrin	secretin stimulation test **fasting glucose **glucagon **pancreatic polypeptide **ultrasound/CTscan of pancreas 72 hour fast
4.	(chest x-ray)	**chest x-ray

* every 1-2 years from age 12
** all patients (other follow-up investigations are performed as indicated by results of primary screening)

PTH - parathyroid hormone

Predictive Testing

At the time of initial screening a blood sample was also taken from consenting family members for DNA extraction by standard procedures (Miller et al 1988) in Dr. Roger Green's laboratory at the Health Sciences Centre in St. John's. In 1991, aliquots of DNA from 171 members of the families (including 65 known to be affected) were sent to Dr. Allen Bale, Department of Genetics, School of Medicine, Yale University, for linkage analysis with a series of markers closely linked to the chromosome 11q13 map location of the gene for typical MEN-1 (Figure 2.3). The markers D11S288, PGA, PYGM, D11S971, D11S970, D11S146, INT2 and D11S533 were used (Table 2.1) (Petty et al 1994), although not all probes were used on every sample. Based on the age at diagnosis in this group of Newfoundland families, family members over 50 years of age were considered unaffected if all screening values were negative. Unscreened family members over 50, or younger at-risk family members, whether screened or not, were categorized as unknown. For each marker at least one blot was run with DNA from members of each kindred. The results were analyzed with the program MLINK from the LINKAGE package to determine the LOD scores for linkage of MEN-1(Burin) to individual markers.

When linkage to chromosome 11q13 markers was demonstrated in the studies with known affected family members (Petty et al 1994), counselling was given to all family members participating in the clinical screening program, and to any others at risk who could be contacted, regarding predictive testing

Figure 2.3. MEN-1/MEN-1 (Burin) region of chromosome 11.

The map location of the MEN-1/MEN-1 (Burin) gene(s) is shown in relation to the position of the markers used for linkage analysis. The percent recombination between markers is also given. This figure is based on published data (Julier et al 1990, Fujimori et al 1992).

MEN 1/MEN-1(BURIN) region of Chromosome 11

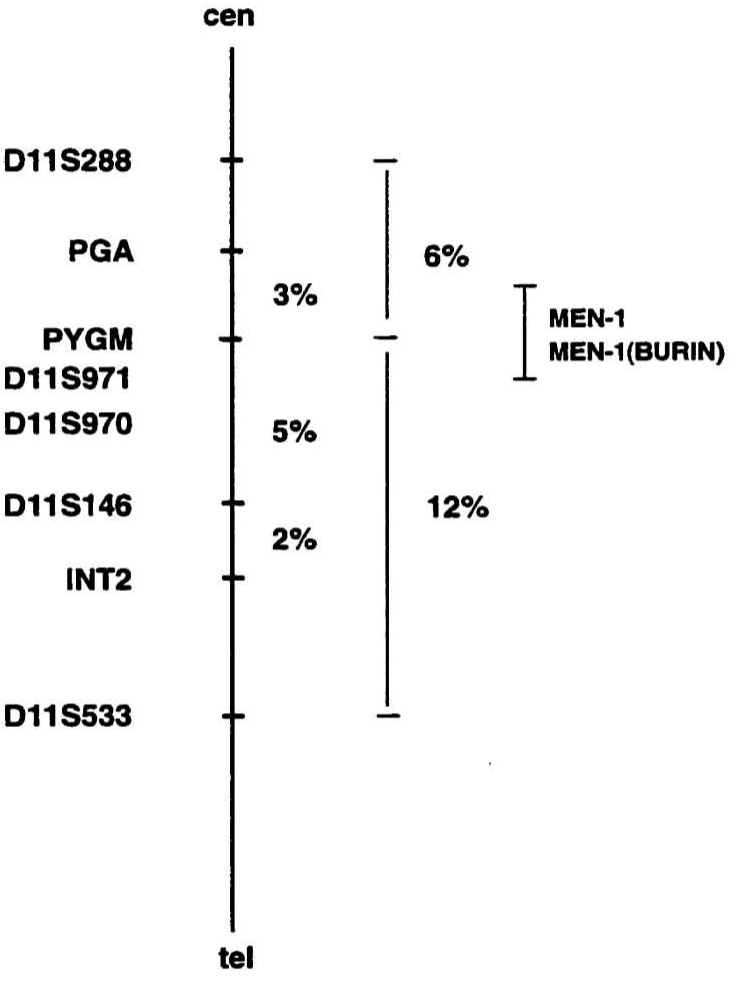


TABLE 2.1. MARKERS AND ALLELES USED FOR LINKAGE ANALYSIS IN MEN-1(Burin) KINDREDS: FOR MAPPING THE GENE AND FOR PREDICTIVE TESTING

POLYMORPHIC LOCI ON CHROMOSOME 11

Location	Locus/Clone	Restriction Enzyme	Alleles
p12-11.2	D11S288/p3C7	MspI	4 alleles
q12-13.2	PGA/PGA101	Bgl II	2 alleles
q12-13.2	PYGM/pMCMP1	MspI	6 alleles
q12-13.2	PYGM/(AT)rep (VNTR)	none*	6 alleles
q13	D11S971/RC27 (CATT)	none*	4 alleles
q13	D11S970/RC29 (CATT)	none*	4 alleles
q12-13.2	D11S146/pHB159	MspI TaqI	4 alleles
q13	INT2/SS6	TaqI BamHI	4 alleles
q13	D11S533/4F7 (VNTR)	PCR	10 alleles

References: Bale et al 1991
Petty et al 1994

PCR - polymerase chain reaction (see, p. 29)
VNTR - variable number tandem repeat
CATT - tetranucleotide repeat

*alleles are detected by PCR sizing

by linkage analysis. The method of linkage analysis was described and also the possible results, including high-risk, low-risk and uninformative status. The implications of these different results, in terms of possible modification of the clinical screening recommendations and of the recurrence risks to children, were also described. It was stressed that linkage analysis gives a probability that the MEN-1 gene was, or was not, inherited, not a certainty because a recombinant event between the gene and the markers used cannot always be detected. Definitive testing by mutation analysis was recommended when the gene and mutation are identified.

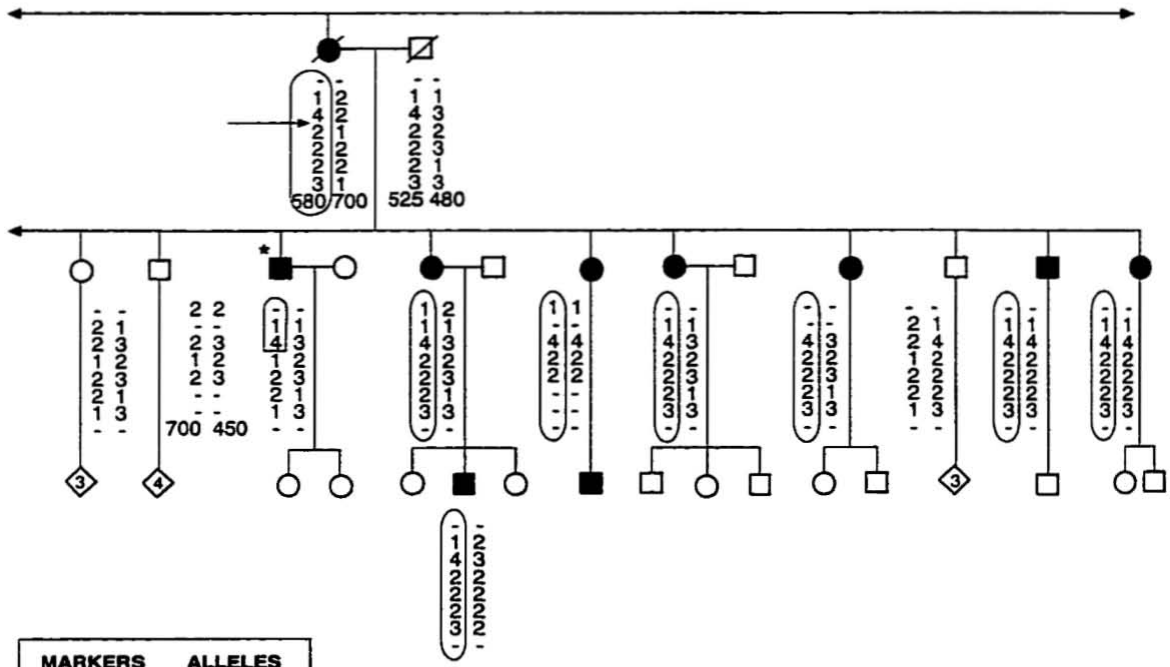
For those wishing to participate in predictive testing (including those who lived in other provinces), consent was obtained, and arrangements were made for the testing to proceed at Dr. Bale's laboratory. Haplotypes for the set of markers used were then determined by Jane Green for all members of each kindred and the haplotype of affected individuals identified (Figure 2.4). The risk status of the first degree relatives was modified according to comparison with this haplotype. All results were reviewed with Dr. Bale and Dr. Elizabeth Petty at Yale University before being delivered to patients.

Results of predictive testing were provided to each at-risk individual in Newfoundland in genetic counselling sessions at clinics held in their own communities. The principles of linkage studies were explained as well as the implications for future medical management based on the specific results obtained. Visual aids were used during the counselling, and a short pamphlet

Figure 2.4. Haplotype of linked markers segregating with MEN-1 (Burin).

A section of the MEN-1 (Burin) Family A pedigree is shown with results of initial linkage analysis to map the MEN-1 (Burin) gene to the MEN-1 region of chromosome 11q13. The haplotype of linked markers segregating with MEN-1 (Burin) in this family is circled. The patient indicated (*) received a recombinant chromosome from his affected mother. The location of the recombination (cross-over) event is marked with an arrow (→).

Haplotype of linked markers segregating with MEN-1 (Burin)



MARKERS		ALLELES
1	D11S288	1,2
2	PGA	1,2
3	PYGM	1-6
4	RC27	1,2
5	RC29	1,2,3
5	D11S146	1,2
7	INT2	1,2,3
8	D11S533	425-700

580 - haplotype inherited with MEN-1 (Burin)

clinically affected

asymptomatic, and normal clinical screening

recombinant chromosome received

location of recombinant event (between PYGM and RC27)

was provided which reinforced the counselling session. When requested, information on the predictive testing process was given to family doctors, and a phone number was provided for any subsequent questions or concerns. Arrangements were made with geneticists in other centres to provide predictive testing results for family members living outside of Newfoundland.

Impact of the Screening Program

There were no formal interviews about the psychosocial implications of MEN-1 and the screening program, but views expressed during counselling sessions and phone calls were documented, and 10 family members were asked informally about the impact of the disease on themselves and their families, and the value of the screening program to individual family members. They were asked, particularly, about their views of their own health before and after the clinical screening program was implemented, their reasons for interest or disinterest in predictive testing, and their use of the information on revised risk status provided by the genetic testing.

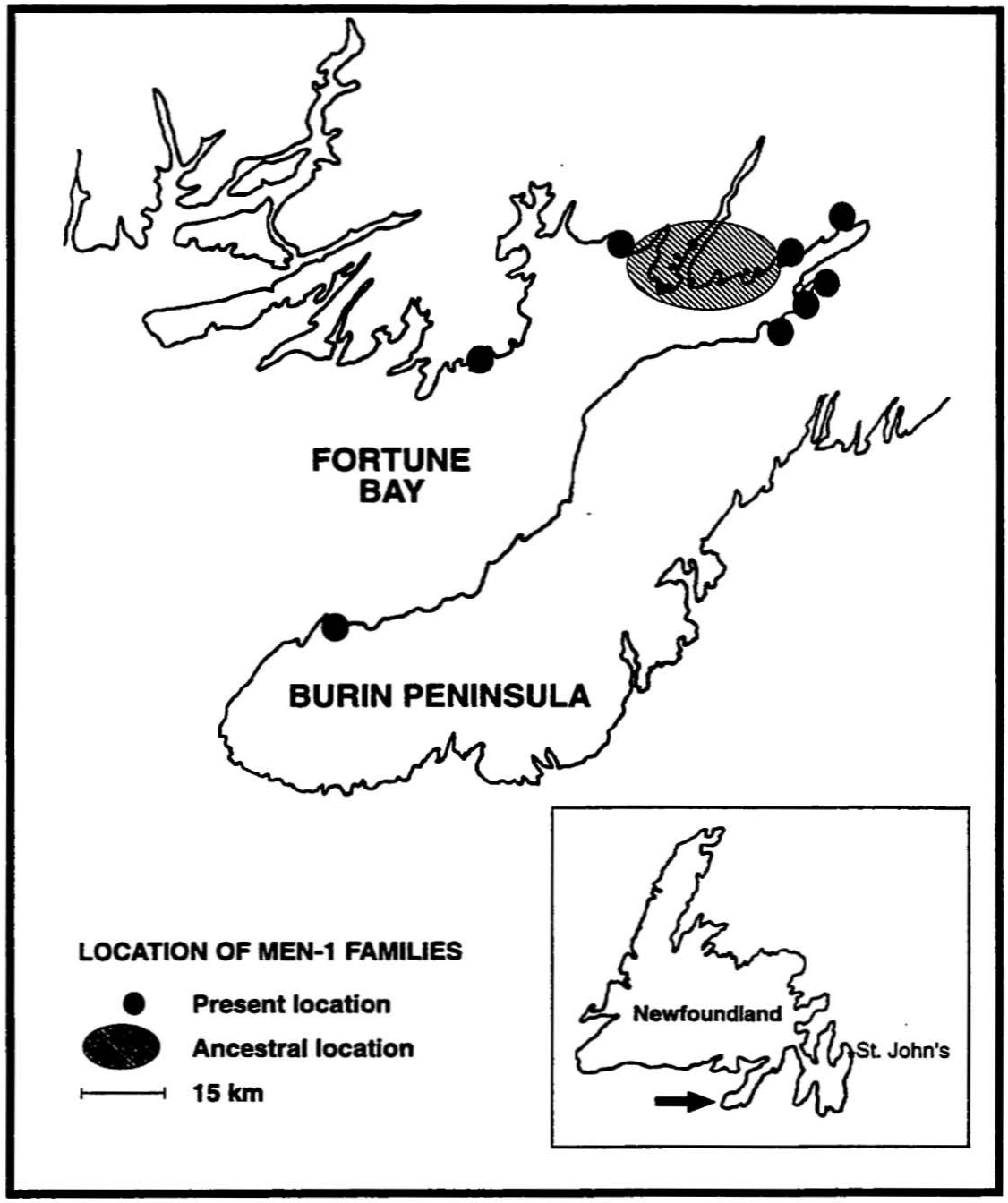
RESULTS

Ascertainment of Families

Ancestors of each of the four kindreds came from a group of very small, abandoned communities on the north shore of Fortune Bay (Figure 2.5). A common ancestor could not be identified but few historical records are

Figure 2.5. Present and ancestral location of MEN-1 (Burin) families.

The isolated communities in which the ancestors of the MEN-1 (Burin) families lived have been resettled. A common ancestor was not identified but review of archival material on births and marriages demonstrated the inter-relationship of families in this cluster of communities on the north shore of Fortune Bay.


FORTUNE
BAY
BURIN PENINSULA
LOCATION OF MEN-1 FAMILIES
Present location
Ancestral location
15 km
Newfoundland
St. John's

available. It is suspected that these families are related because there were very few settlers in this area originally, and three of the four pedigrees consist of two separately identified branches that were only linked by pedigree studies (Figure 2.1, p. 68-71).

Eighty-three members of these families have now been identified as affected; 34(41%) presented symptomatically, and 49(59%) were identified by clinical screening, the majority of these since 1988. One hundred and sixty-nine first degree relatives at 50% risk were also identified in 1989. 108 of these participated in the clinical screening program, and 121 subsequently participated in genetic testing (see later).

Natural History of Disease

Hyperparathyroidism is the most common manifestation, present in 96%, but hypercalcemia was never detected in three affected patients (age 31-71 at the time of death). Pituitary tumours have been identified in 39% (all but 2 of these being prolactinomas), and carcinoid tumours of the lung or thymus are present in 13% of affected. Pancreatic islet cell tumours (either gastrinomas or glucagonomas) are less common but have been identified in six affected (7%) (Table 2.2).

The age at diagnosis was extremely variable, age 15-63 years in symptomatic patients, and age 13-83 years in asymptomatic patients identified

TABLE 2.2. FREQUENCY OF MANIFESTATIONS IN MEN-1(Burin) FAMILIES, AND IN TYPICAL MEN-1 FAMILIES FROM THE LITERATURE

	FREQUENCY OF MANIFESTATIONS			
	Newfoundland MEN-1(Burin)		Literature Typical MEN-1	
	1980	1993	Vasen* (1989)	Skogseid* (1990)
	% (number)		%	
Parathyroid	88%(22)	96%(80)	94%	90%
Pancreas	0% (0)	7% (6)	60%	75%
Pituitary	40%(10)	39%(32)	25%	20%
Carcinoid	16% (4)	13%(12)	-	-
Total Affected	N = 25	N = 83	N = 52	N = 80

* References: Vasen et al 1989b
Skogseid et al 1991a

by screening (Figure 2.6). There has been a shift to an earlier age at diagnosis in the screened group compared to the symptomatic group (Figure 2.7). However, since the majority of the screened group were identified at their initial screening this shift will likely increase as screening for those at risk continues, and more gene carriers are identified prospectively.

Although a number of different glands may be involved, just over half of those with MEN-1 have had only one manifestation of the disease (44/83, or 53%), and the majority of these (41/44) had hyperparathyroidism only. However, 29 patients (35%) had two manifestations and 10 patients (12%) had three or more documented manifestations of MEN-1 (Table 2.3, Figure 2.8).

There have been thirteen deaths (age 31 - 88) of the 83 family members definitely affected with MEN-1 (Table 2.4). Eight of these (age 31 - 73) were directly related to manifestations of MEN-1: two were due to malignant carcinoid tumours of the lung or thymus; two due to malignant carcinoid tumours and another malignancy (breast cancer or renal cell carcinoma); two due to expanding pituitary tumours; one, in a patient with Zollinger-Ellison syndrome, due to sepsis from a perforated ulcer; and one, from renal railure, in a patient with long-standing untreated hyperparathyroidism. Two other MEN-1 patients died of malignancies (one may have had a malignant prolactinoma and the other patient died of breast cancer). Three deaths, in

Figure 2.6. Age at diagnosis of symptomatic, screened, and predicted affected patients in MEN-1 (Burin).

The age at diagnosis, by five year intervals, of symptomatic, screened and predicted affected family members, is illustrated. The range of ages at diagnosis for the three groups overlap; for symptomatic patients (age 15-63), for clinically screened patients (age 13-83), and for predicted affected patients (age 10-54).

Age at diagnosis of symptomatic, screened and predicted affected MEN-1(Burin) patients

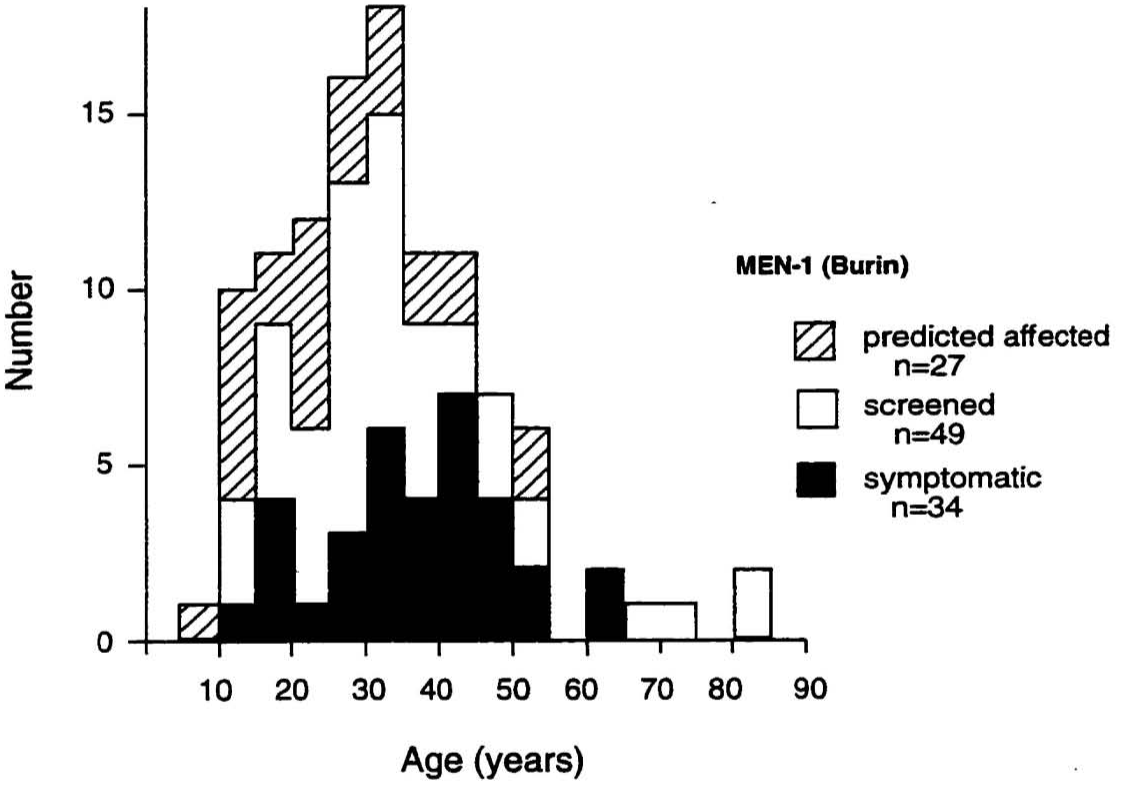


Figure 2.7. Cumulative age at diagnosis for screened and symptomatic MEN-1 (Burin) patients.

The median age at diagnosis of patients detected by screening is 27 years, and of patients identified because of symptoms is 38 years. Because the majority detected by screening were identified at the first set of investigations, the median age at diagnosis for this group is expected to decrease. Ninety percent of each group were identified by 55 years of age.

The youngest patient identified by screening was 12 years of age, and youngest symptomatic patient was 13 years.

Cumulative age at diagnosis (MEN-1)

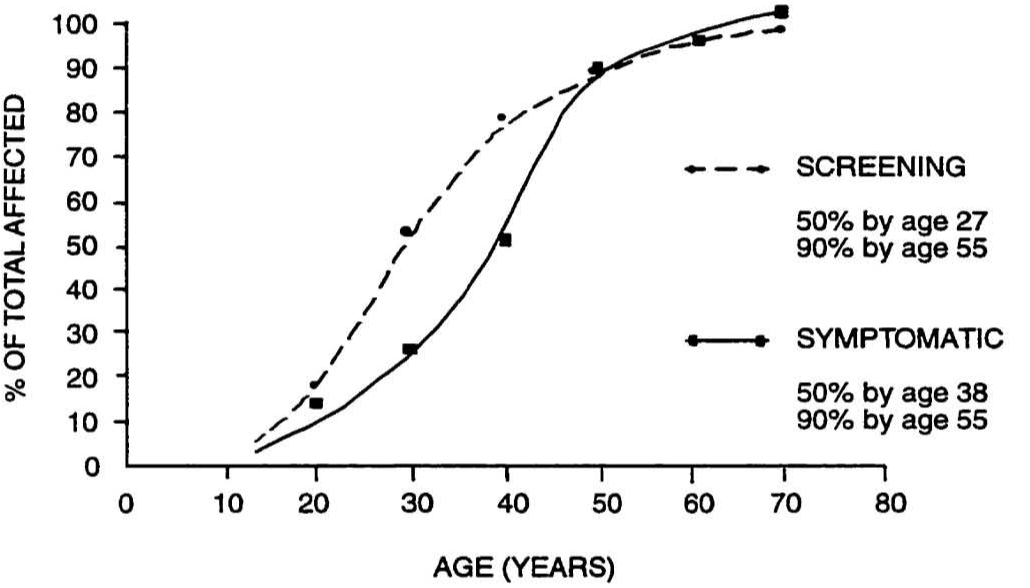


TABLE 2.3. NUMBER AND COMBINATION OF MANIFESTATIONS IN MEN-1(Burin) PATIENTS

Manifestations	Patients
Number/Combination	Number (%)
One	
HPTH	43
PRL	1
PIT*[1]	1
CARC	1
	46 (55.4%)
Two	
HPTH/PRL	21
HPTH/PIT*[2]	1
HPTH/CARC	4
HPTH/PANC	2
	28 (33.7%)
Three	
HPTH/PRL/CARC	5
HPTH/PRL/PANC	3
	8 (9.6%)
Four	
HPTH/PRL/CARC/PANC	1
	1 (1.2%)
TOTAL	83

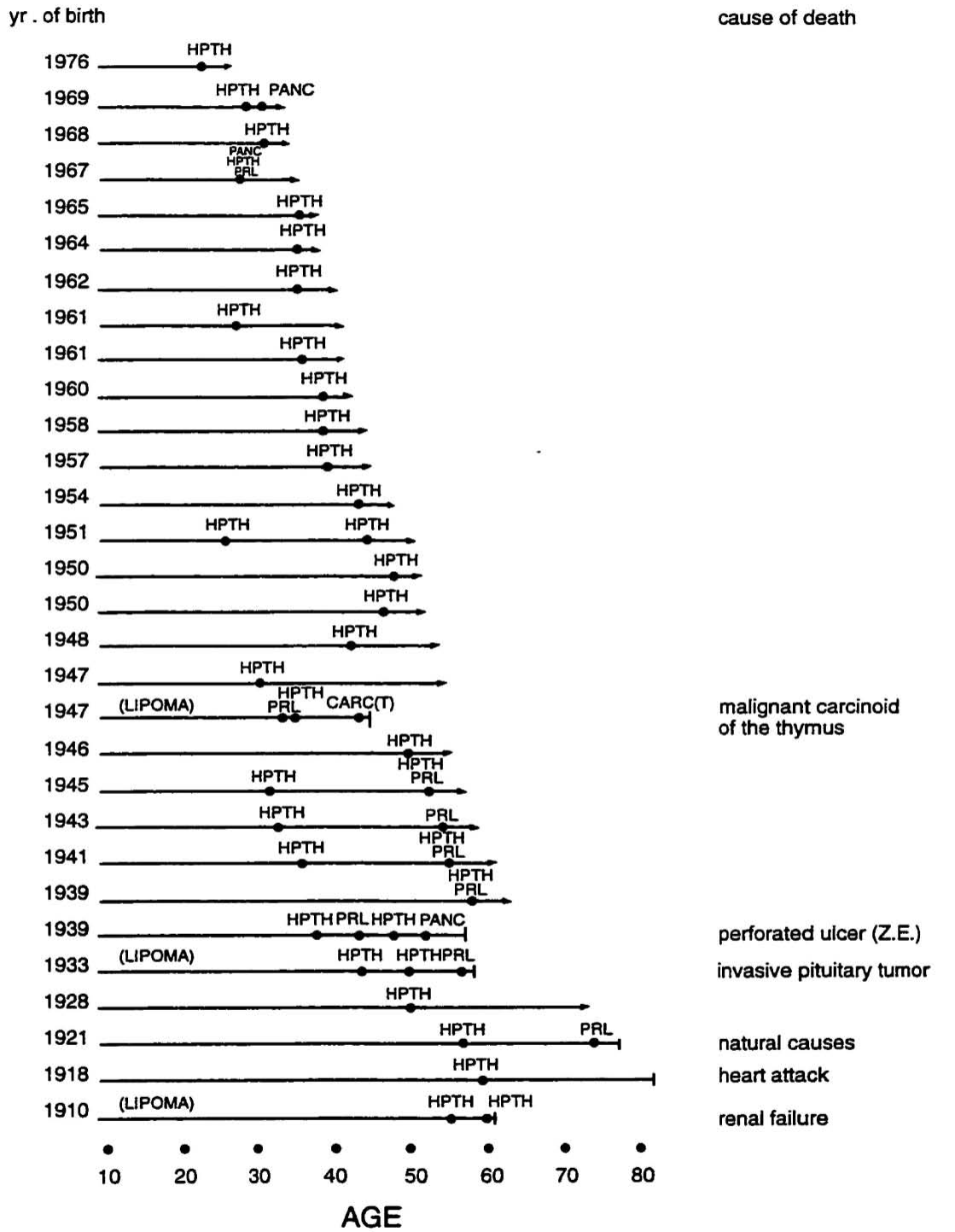
*[1]one patient in 1975 with a "chromophobe adenoma" (prolactin not measured)
*[2]one patient in 1993 with a non-functioning chromophobe adenoma (prolactin normal).
(All other pituitary tumours were prolactinomas.)

HPTH - hyperparathyroidism, PRL - prolactinoma, PIT - pituitary adenoma,
CARC - carcinoid tumour, PANC - pancreatic islet cell tumour

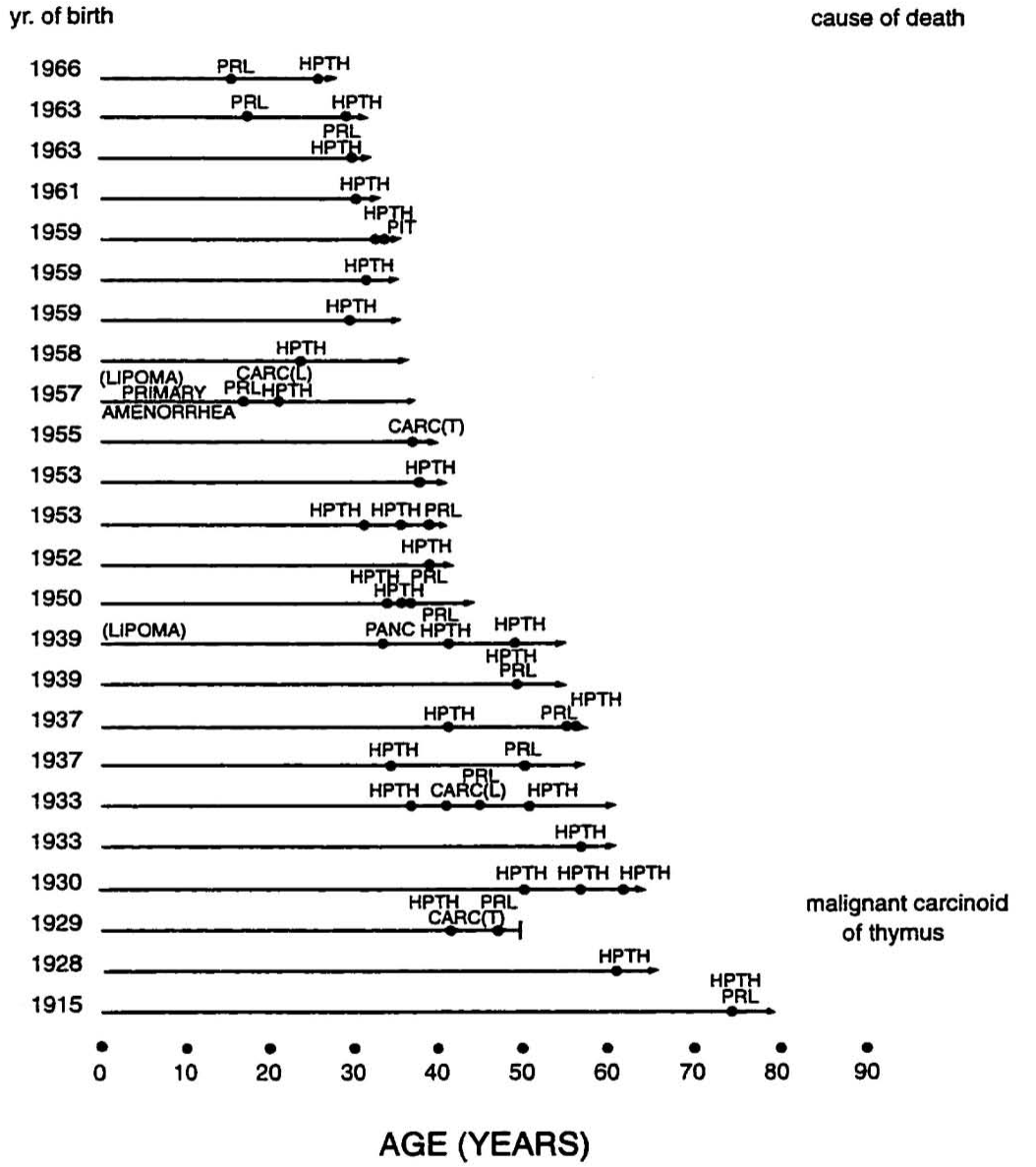
Figure 2.8. Combination and order of occurrence of manifestations in affected MEN-1 (Burin) family members.

The age at diagnosis of individual manifestations is indicated on a timeline for each affected member of the four MEN-1 (Burin) families. Each family is illustrated separately with family members ordered by date of birth. Age and cause of death are given where applicable.

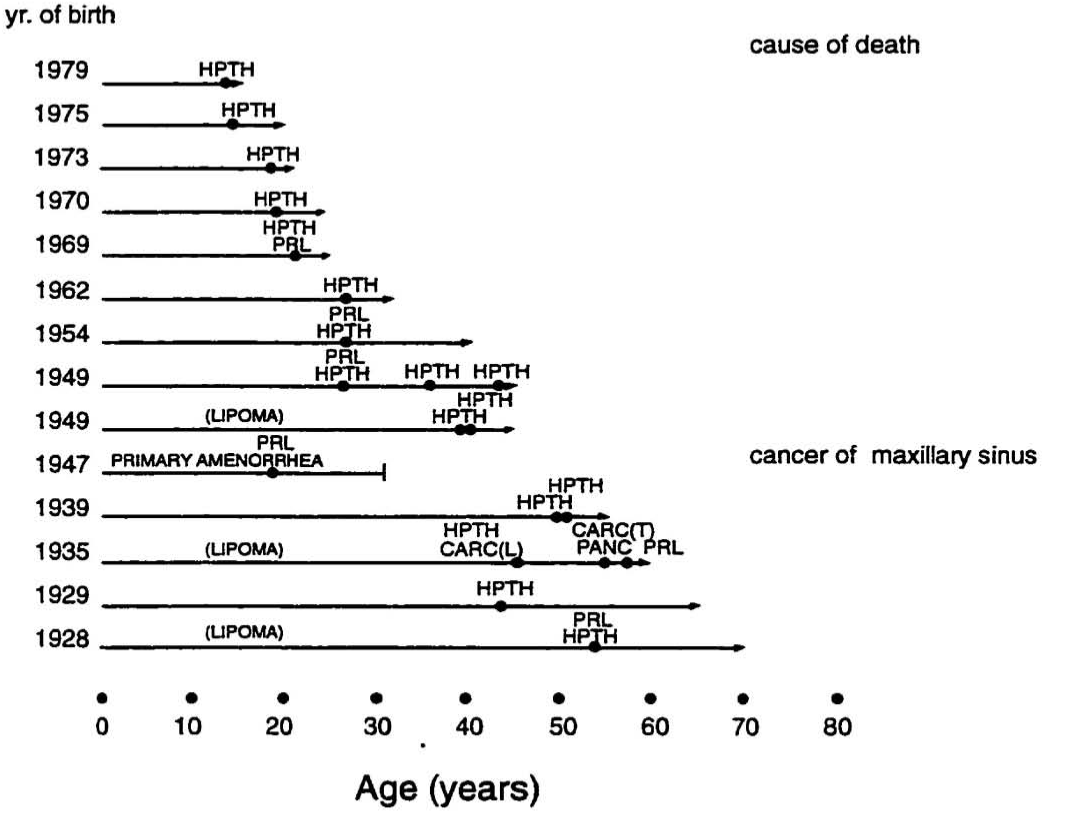
FAMILY A **Combination and order of occurrence of manifestations in affected MEN-1 (Burin) family members**



FAMILY B Combination and order of occurrence of manifestations in affected MEN-1 (Burin) family members



FAMILY C **Combination and order of occurrence of manifestations in affected MEN-1 (Burin) family members**



FAMILY D **Combination and order of occurrence of manifestations in affected MEN-1 (Burin) family members**

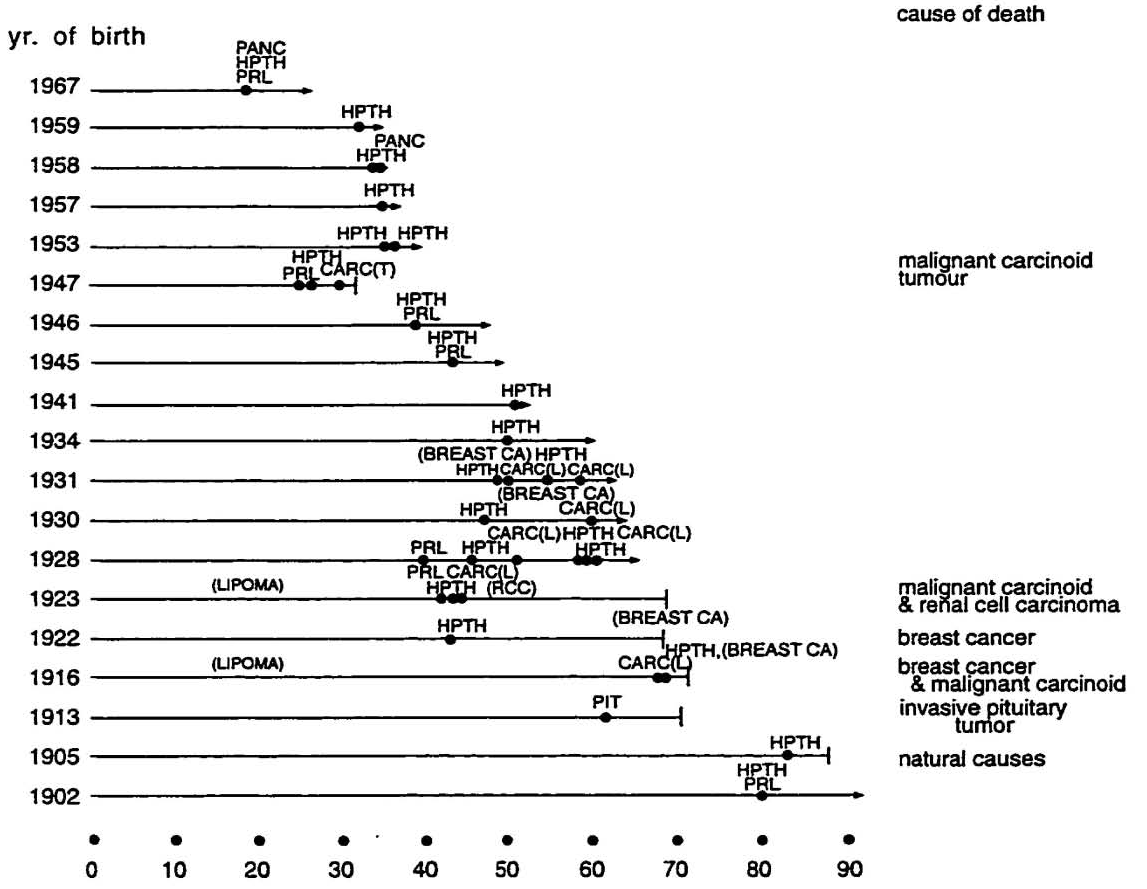


TABLE 2.4. AGE AND CAUSE OF DEATH IN MEN-1(Burin) PATIENTS

Age at Death	Sex	Cause of Death	MEN-1 MANIFESTATIONS			
			HPTH	PRL	PANC	CARC
31	F	Ca maxillary sinus (?malignant prolactinoma)	—	+	NT	NT
32	F	Malignant carcinoid (thymus)	+	+	—	+
49	M	Z-E (Perforation and sepsis)	+	+	+	NT
49	M	Malignant carcinoid (thymus)	+	+	NT	+
50	M	Invasive prolactinoma	+	+	NT	NT
53	M	Renal failure	+	NT	NT	NT
64	F	Malignant carcinoid (lung) and renal cell carcinoma	+	+	—	+
68	F	Breast cancer	+	—	NT	NT
70	M	Unknown	+	+	NT	NT
71	M	Invasive pituitary tumor*	—	+	NT	NT
73	F	Malignant carcinoid (lung) and breast cancer	+	—	—	+
74	F	Heart attack	+	—	—	—
88	F	Natural causes	+	—	—	NT

\+ investigations +ve, or symptomatic disease
— investigations -ve
NT no investigations
* classified as chromophobe adenoma (prolactin not measured)
Z-E Zollinger-Ellison Syndrome
Ca Carcinoma

HPTH - hyperparathyroidism, PRL - prolactinoma, PIT - pituitary adenoma, CARC - carcinoid tumour, PANC - pancreatic islet cell tumour

patients aged 70 - 88, were apparently unrelated to MEN-1. Two obligate carriers from an earlier generation, for whom no medical records are available, died at a young age of "ulcer disease" which may have been MEN-1 related. Seventy of the affected family members are still living (age 14 - 92 years).

Morbidity and Mortality

i) Hyperparathyroidism

Of the 83 affected in the MEN-1(Burin) families, 80(96%) had hyperparathyroidism. Three others had normal calcium levels until the time of death (age 31- 73 yrs). Hyperparathyroidism was present at the time of diagnosis in 69 patients (83%), either alone (62 patients) or in combination with a prolactinoma (7 patients) (Table 2.5). Twenty-seven of these (39%) were investigated because of symptomatic presentation (kidney stones, ulcer disease, or fatigue with muscle and bone pain, alone, or in combination with galactorrhea and amenorrhea). Hypercalcemia with raised or inappropriately normal PTH level was detected by screening of at-risk family members in 42 others (61%), the majority of these being identified during the past five years (Table 2.6).

Hyperparathyroidism developed subsequent to other MEN-1 manifestations in 11 patients (13%). In two of these patients, who presented with amenorrhea and galactorrhea and have been treated for a prolactinoma since their mid-teens, hypercalcemia was only detected after 10 years of regular screening.

TABLE 2.5. INITIAL MANIFESTATION OF MEN-1(Burin)

Glands Involved At Initial Diagnosis		Number (Percent)*
PARATHYROID		69 (83.1%)
- HPTH only	62 (74.7%)	
- HPTH & PRL	7 (8.4%)	
PITUITARY		17 (20.4%)
- PRL only	9 (10.8%)	
- PRL & HPTH	7 (8.4%)	
- PIT only	1 (1.2%)	
PANCREAS		2 (2.4%)
CARCINOID TUMOUR		2 (2.4%)

* This column does not total to 100% because some patients had two glands involved at the time of diagnosis.

HPTH - hyperparathyroidism, PRL - prolactinoma, PIT - pituitary adenoma

N.B. 16.9% of patients did not have hyperparathyroidism at the time of diagnosis.

TABLE 2.6. DETECTION OF HYPERPARATHYROIDISM IN MEN-1(Burin)

Hyperparathyroidism	Number (Percent)	
PRESENT		80 (96%)
At Initial Diagnosis of MEN-1	69 (83%)	
- Symptomatic*1	27	
- Screening*2	42	
Subsequent Manifestation	11 (13%)	
- Symptomatic	0	
- Screening	11	
ABSENT		3 (4%)
TOTAL WITH MEN-1(Burin)		83

*1 Kidney stones, ulcer disease, fatigue, bone and muscle pain
*2 Increased ionized calcium, and increased or inappropriate PTH

PTH - parathyroid hormone

The age at development of hyperparathyroidism is extremely variable. At one extreme, a 14-year-old boy had significant hypercalcemia with raised PTH at his first screening and passed a kidney stone prior to surgery at age 16. At the other extreme, two asymptomatic women in their 80s were each screened when they were recognized to be obligate carriers. Both had mild but confirmed hyperparathyroidism, but because of their age neither had surgery. One died at age 88 of natural causes and the other is now 92-years-old.

Thirty-seven patients (46%) have had a subtotal parathyroidectomy (3½ glands removed) or a total parathyroidectomy with forearm implant. Seven of these required repeat surgery for recurrent hyperparathyroidism 8 - 21 years subsequently. Seventeen patients (21%) who had surgery prior to the recognition of MEN-1, had less than 3 glands removed. Seven in this group had persistence or early recurrence of hypercalcemia post-operatively, and required repeat surgery within two years. Twenty-three patients (29%) with recently identified hyperparathyroidism have not yet had surgery, and three others (4%) died without treatment (Table 2.7).

Two potential complicating factors regarding parathyroidectomy in MEN-1 patients are the presence of more or less than four parathyroid glands in up to 6% of individuals, and the risk of persistent hypocalcemia post-operatively. In this group of families, two patients were documented to have five parathyroid glands, and in two other patients, only three parathyroid glands could be identified. The number of parathyroid glands removed was adjusted

TABLE 2.7. METHOD AND OUTCOME OF TREATMENT OF HYPERPARATHYROIDISM IN MEN-1(Burin)

	Number Treated		No. Requiring Repeat Surgery	
Type of Treatment	#	(%)	#	(%)
Subtotal parathyroidectomy*1 (3½ glands)	37	(46%)	7	(19%)*2
Partial parathyroidectomy (1 or 2 glands)	17	(21%)	7	(41%)*3
Not treated	26	(33%)		
- pending	(23)		—	
- deceased	(3)			
Total with hyperparathyroidism	80			

*1 includes one patient with total parathyroidectomy and forearm implant
*2 recurrent HPTH, 8-21 years post-parathyroidectomy
*3 persistent HPTH or recurrence <2 years post-parathyroidectomy

so that half a gland was left in place. Two patients had persistent post-operative hypocalcemia treated with calcium and vitamin D supplementation. One of these patients had had a total parathyroidectomy with forearm implant, and the other had had 3½ glands removed.

Only one affected family member died of the effects of long-standing hypercalcemia. He presented with kidney stones in 1960 at age 50, and had persistent hypercalcemia after one parathyroid "adenoma" was removed. He declined further surgery and died of renal failure at age 53. Twenty-four patients, however, have had multiple kidney stones (Table 2.8).

ii) Pituitary tumours

The proband in each of the four MEN-1(Burin) families presented in the late 1970s with galactorrhea and amenorrhea, or headaches and visual field defect. Each of the probands had hypercalcemia identified during investigations of the prolactinoma, or had a previous history of kidney stones.

Pituitary tumours were identified in thirty-two (39%) of the 83 affected persons (17 female and 15 male patients) (Table 2.9). Thirty of the pituitary tumours were prolactinomas although three were originally classified in the early 1970s as chromophobe adenomas (these patients had amenorrhea and galactorrhea, and increased prolactin was later measured). Another patient with a chromophobe adenoma, a male who was identified retrospectively as an MEN-1 patient, may also have had a prolactinoma, but he died of invasive pituitary tumour before prolactin assays were available.

TABLE 2.8. MORBIDITY IN AFFECTED MEMBERS OF MEN-1(Burin) KINDREDS

GLAND, TYPE OF MORBIDITY	NUMBER
PARATHYROID	
Multiple kidney stones	24
Ulcers, fatigue, and bone pain	9
Renal failure	1
Multiple surgical procedures	14
Persistant post-operative hypocalcemia	2
PITUITARY	
Primary amenorrhea	2
2°amenorrhea/galactorrhea/infertility	8
Visual field defect	7
Severe headaches, coma	3
Panhypopituitarism	4
Chronic dopamine agonist treatment	15
Surgery/radiation treatment	10
PANCREAS	
Zollinger-Ellison syndrome	2
Metastatic disease	3
Partial pancreatectomy/gastrectomy	6
CARCINOID	
Metastatic disease	10
OTHER MALIGNANCY	
Breast cancer	4
Renal cell carcinoma	1
Ca of maxillary sinus	1

TABLE 2.9. DETECTION OF PITUITARY TUMOURS IN MEN-1(Burin) PATIENTS

Type of Tumour	METHOD OF DETECTION		
	Symptomatic	By Screening	Total
PROLACTINOMA	14[*1]	16[*2]	30
- at initial diagnosis of MEN-1	(9)	(7)	(16)
- subsequent manifestation	(5)	(9)	(14)
CHROMOPHOBE ADENOMA	1	1	2
- at initial diagnosis	(1[*3])	—	(1)
- subsequent manifestation	—	(1[*4])	(1)
TOTAL	15	17	32

[*1] amenorrhea and/or galactorrhea in women; visual field defects, headaches or coma in men

[*2] raised prolactin (≥ 5x normal; all were microadenomas)

[*3] visual field defects; prolactin not tested (1971)

[*4] abnormal CT scan; prolactin normal (1993)

Recently, a macroadenoma of the pituitary was identified by baseline CT scanning of the sella in a 32-year-old male patient with hyperparathyroidism but normal prolactin levels. Following surgery, this tumour was classified as a non-functioning chromophobe adenoma because immunostaining was negative for prolactin, growth hormone, and ACTH (adrenocorticotropic hormone).

Fourteen of the 30 patients with prolactinomas (47%) presented with symptoms before the screening program was introduced. These symptoms included amenorrhea and galactorrhea in nine women (two of these with primary amenorrhea); and visual field defects, severe headaches and coma, or decreased sexual function in five men (Table 2.8). In nine of these patients, prolactinoma was the first symptomatic manifestation of MEN-1. In seven of sixteen others identified by screening, hyperprolactinemia with or without hypercalcemia was present at the initial diagnosis of MEN-1 (Table 2.5). In two women who presented symptomatically with prolactinomas in their mid-teens, hypercalcemia was not identified for 10 years despite annual screening.

Management and outcome differed in patients identified symptomatically or by screening (Table 2.10). Nine patients presenting symptomatically before 1984 with macroprolactinoma or chromophobe adenoma had surgery by either a transfrontal or transphenoidal approach. In all of these, there was an incomplete removal of the tumour, and subsequently radiation therapy and/or bromocryptine treatment was required. Two of these patients died because of the mass effect of the remaining invasive tumour, another died of an

TABLE 2.10. METHOD AND OUTCOME OF TREATMENT FOR PITUITARY TUMOURS IN MEN-1(Burin)

Treatment	PROLACTINOMA		CHROMOPHOBE ADENOMA	
	Symp. n = 14	Asymp. n = 16*1	Symp. n = 1	Asymp. n = 1*1
SURGICAL	9*2	0	1*3	1
MEDICAL				
bromocryptine only	2	7	—	—
bromocryptine and radiation	1	0	—	—
NO TREATMENT				
monitored	0	9	—	—
deceased (other causes)	2	0	—	—
	14	16	1	1

*1 alive and well

*2 all had incomplete removal of tumour and required subsequent medical and/or radiation therapy (2 deaths from pituitary tumour, 4 with panhypopituitarism, 3 alive with severe MEN-1)

*3 died of invasive pituitary tumour, 1971

symp. - symptomatic

asymp. - asymptomatic

"anaplastic carcinoma" of the maxillary sinus which may have been a malignant prolactinoma (see later), and four patients had panhypopituitarism after surgery. Another patient with a macroprolactinoma had bromocryptine treatment and radiation therapy without prior surgery. Two symptomatic patients have been treated successfully with bromocryptine only, and two died of other causes without treatment.

Seven of those with hyperprolactinemia recently identified by screening (prolactin level up to 10x normal) are being treated medically with bromocryptine, and nine others are not yet requiring treatment but are beingclosely monitored. The recent patient with a non-functioning macroadenoma underwent surgery with apparent complete removal of the tumour.

iii) Pancreatic tumours

Pancreatic islet cell tumours were documented in only 6 of the MEN-1(Burin) patients (7%), all male, ages 18-58 at the time of diagnosis (Table 2.11). Although uncommon, the pancreatic tumour was the first manifestation of MEN-1 identified in two of these patients, one patient presenting with severe ulcer disease and the other identified by screening.

As in other MEN-1 families, the pancreatic tumours have been associated with a poor prognosis. Gastrinomas causing Zollinger-Ellison syndrome (Z-E) (intractable ulcer disease) occurred in two men (Table 2.8) with extensive MEN-1 disease including hyperparathyroidism and macroprolactinoma. One of

TABLE 2.11. DETECTION AND TREATMENT OF PANCREATIC TUMOURS IN MEN-1(Burin)

Patient Sex (Age at Diagnosis)	Method of Detection Symptoms/Screening	Peptide Secretion Gast	 Gluc	 PP	Treatment	Outcome Alive/Dead
1. M(33)	Z-E	+			partial & total gastrectomy	+[1]
2. M(36)	Z-E	+			H_2-antagonists	+[1,2]
3. M(18)	U/S		+		partial pancreatectomy	+
4. M(22)	U/S		+		partial pancreatectomy	+
5. M(36)	U/S		+	+	partial pancreatectomy	+
6. M(55)	U/S		unknown		partial pancreatectomy	+

[1] liver metastases; [2] cause of death - perforation and sepsis
Z-E - Zollinger-Ellison Syndrome; U/S - ultrasound screening

Gast - gastrin; Gluc - glucagon; PP - pancreatic polypeptide

these had a partial gastrectomy at age 35 and later a total gastrectomy because of severe ulcer disease. He continues to have very high gastrin levels (>2000 pmol/L; N<43 pmol/L), and has slow growing liver metastases documented with abdominal ultrasound and CT scanning. The second patient with Z-E, age 44 at diagnosis, was treated with H_2-antagonists for 5 years, only partially successfully, and died of sepsis following perforation of a duodenal ulcer. Neither patient had identifiable pancreatic lesions on ultrasound or CT scanning but the first had multiple small nodules seen in the pancreas at the time of gastrectomy; and the second had multiple small gastrinomas of the pancreas, and metastatic tumours in the liver and in peripancreatic lymph nodes identified at autopsy.

Tumours were identified by pancreatic ultrasound screening in four other asymptomatic MEN-1 patients with normal gastrin levels. Each had a partial pancreatectomy. Increased serum glucagon was measured pre-operatively after a pancreatic mass was identified in one patient. In two others, one of whom had had increased serum pancreatic polypeptide pre-operatively, glucagon was the major peptide identified on immunostaining of the pathology specimen. None of these patients, however, had symptoms of the glucagonoma syndrome. Information on the tumour in the fourth patient is unavailable.

Death from ulcer disease was reported in a number of first degree relatives in previous generations for whom no medical records are available.

Although some may have had a gastrinoma with Zollinger-Ellison syndrome, it is more likely that they had untreated hyperparathyroidism. The majority of family members who presented recently with ulcer disease and were investigated, had hypercalcemia with normal gastrin levels, and the symptoms were reversed by parathyroidectomy.

iv) Carcinoid tumours

Carcinoid tumours occurred in eleven patients (13%). Seven of these (1M:6F) had carcinoid tumours of the lung, three (2M:1F) had carcinoid tumours of the thymus, and one patient, a 58 year old male, had carcinoid tumours of both lung and thymus (Table 2.12). All four of the thymic carcinoids were malignant and in two cases this was the cause of death (Table 2.4). Six of the eight lung carcinoids were also malignant (Table 2.8): three patients currently have liver metastases, and two others had metastatic carcinoid of the lung at the time of death as well as another metastatic malignancy (ie, renal cell carcinoma, or breast cancer). Three other MEN-1 patients (all male) have lung lesions seen on chest x-ray, suggestive of carcinoid tumours, but no definite diagnosis has been made.

None of the patients with carcinoid tumours, of the lung or the thymus, have had symptoms of carcinoid syndrome or increased 5-hydroxyindoleacetic acid (5HIAA) (Duh et al 1987). The majority of the carcinoid tumours of the lung, were identified coincidentally and initially thought to be benign. Surgery,

TABLE 2.12. DETECTION AND TREATMENT OF CARCINOID TUMOURS IN MEN-1(Burin)

	Sex	Age at Dx/ Present Age	Location		Detection		Treatment				Outcome	
			Lung	Thymus	Symp.[2]	Coinc.[3]	Untr.	Surg.	Chemo.	Rad.	Alive	Dead
1	F	29/(32)*		+[1]	+			+	+	+		+[4]
2	M	36/38*		+[1]	+				+	+	+	
3	M	42/(49)		+[1]	+			+				+[4]
4	M	45,54/58	+[1]	+[1]	+			+			+	
5	F	21/36	+			+	+				+	
6	M	41/60	+			+	+				+	
7	F	42/(64)	+[1]			+		+		+		+[4]
8	F	50/62	+[1]			+		+			+	
9	F	52/65	+[1]			+		+	+		+	
10	F	60/63	+[1]		+			+	+	+	+	
11	F	68/(72)	+[1]			+		+				+[4]

[1] local or distant metastases; [2]neck mass and/or shortness of breath; [3]coincidental chest x-ray; [4]cause of death;
* 38 - present age; (32) - age at death.
Symp. - symptomatic, Coinc. - coincidental, Untr. - untreated, Surg. - surgery, Chemo. - chemotherapy, Rad. - radiation.

radiation treatment and/or chemotherapy were attempted with little success when rapid enlargement was seen or symptoms developed. A patient with liver metastases documented with somatostatin receptor scanning is being treated with the somatostatin analogue, octreotide (Arnold et al 1993).

The carcinoid tumours of the thymus were identified because of a neck mass or symptoms of shortness of breath. Two patients who presented with a neck mass had incomplete removal of the tumour and died later of widespread metastatic disease. Another patient who presented with shortness of breath has an inoperable thymic carcinoid tumour, treated with radiation and chemotherapy, as his only manifestation of MEN-1(Burin), and the fourth patient has had surgery but has local metastatic disease.

Carcinoid tumours are not only more common in MEN-1(Burin) than in typical MEN-1 families, but have proven difficult to identify by screening and difficult to treat. A chest x-ray is now recommended every two to three years for all known MEN-1(Burin) patients and at least every five years for those at risk, as an attempt to identify the tumours earlier. This may allow earlier and more successful treatment, particularly of carcinoid tumours of the thymus.

v) Adrenal tumours

Only two adrenal gland tumours have been identified in this family. These were coincidentally noted on CT scanning of the abdomen, one in a patient being investigated because of a pancreatic tumour detected on ultrasound screening, and one in a patient with liver metastases from a bronchial carcinoid

tumour. These may be coincidental rather than related to MEN-1 in these patients.

vi) Other malignancies

A 32-year-old member of family C died in 1978 of an invasive tumour of the maxillary sinus, described as a small cell anaplastic carcinoma at biopsy, but which may have been a malignant prolactinoma (personal communication, Dr. Sylvia Asa, Mount Sinai Hospital, Toronto). The patient previously had primary amenorrhea and galactorrhea but did not present to medical care until age 20. She declined surgery until age 29 when a large "chromophobe adenoma" was partially removed. She was also treated with radiation therapy, however she returned 3 years later with an inoperable tumour. This tumour was biopsied, but she died without further treatment or definitive diagnosis.

Breast cancer was identified in four of the affected members of family D (three sisters and their cousin), and another affected sister had renal cell carcinoma. All five of these women also had malignant carcinoid tumours (Table 2.13, Figure 2.9). There are no documented malignancies in non-MEN-1 members of Family D, or non-MEN-1 related malignancies in affected or unaffected members of Families A, B and C.

Linkage Studies

In 1989, when pedigrees were completed and 42 affected family members had been identified, there were 169 first-degree relatives at 50% risk

TABLE 2.13. MALIGNANT TUMOURS IN AFFECTED MEMBERS OF MEN-1(Burin) KINDREDS

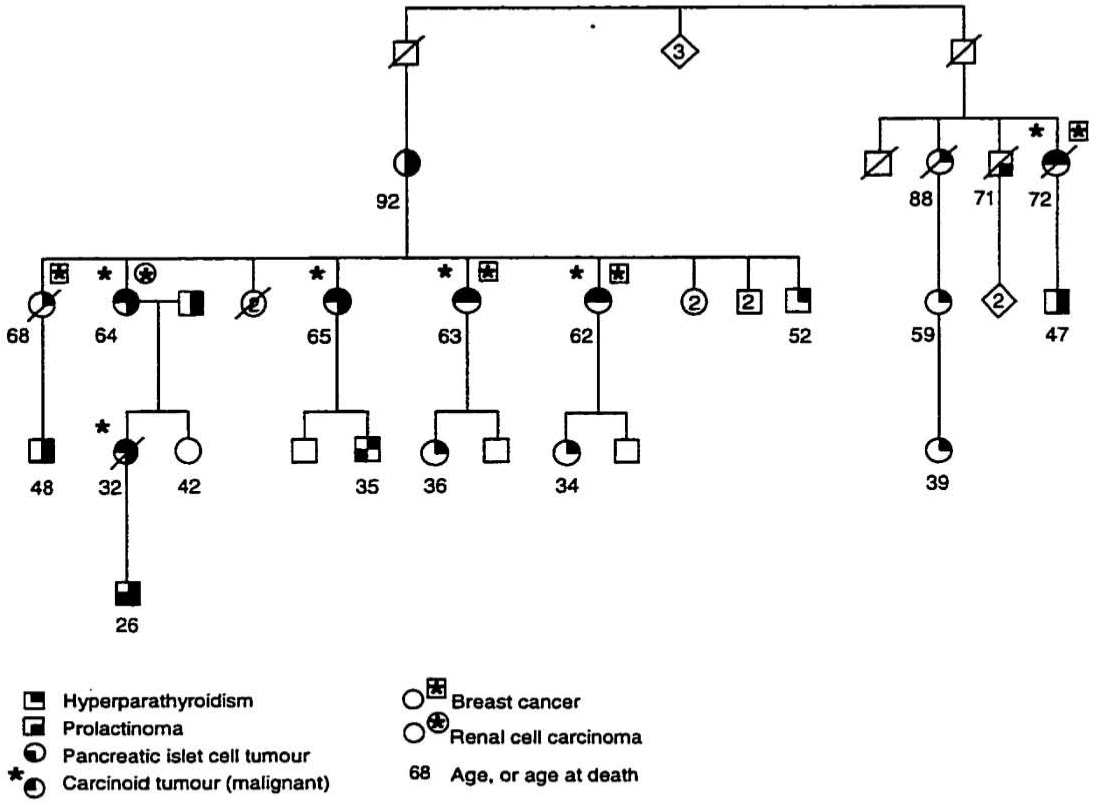
TYPE OF TUMOUR	NUMBER AFFECTED Total (Dead)	
MEN-1 TUMOURS		
Pancreatic Islet Cell		3(1)
- gastrinoma	2(1)	
- glucagonoma	1(0)	
Carcinoid		10(4)
- lung*1*2	6(2)	
- thymus	4(2)	
OTHER (POSSIBLY COINCIDENTAL)		6(4)
- breast cancer*1	4(2)	
- renal cell carcinoma*2	1(1)	
- cancer of maxillary sinus*3	1(1)	
TOTAL MALIGNANCIES		19(7)*1*2

*1 one patient with carcinoid of the lung and breast cancer (dead)
*2 one patient with carcinoid of the lung and renal cell ca (dead)
*3 possible malignant prolactinoma

Figure 2.9. Malignant tumours in affected members of MEN-1 (Burin) Family D.

A simplified pedigree is used to illustrate the malignant tumours identified in Family D. All malignancies, whether characteristic of MEN-1 (eg, carcinoid tumour), or other malignancies (eg, breast cancer, renal cell carcinoma), were in family members affected with MEN-1 (Burin).

Malignant tumours in MEN-1 (Burin) family D



of having inherited MEN-1. On questioning, 143 of these (85%) stated that they were interested in participating in a linkage study, and were also interested in predictive testing if this became available. After appropriate consent forms were signed, DNA samples were obtained from those interested and from relevant affected relatives.

Initially, proximal chromosome 11p and 11q markers linked to the gene for typical MEN-1 were used to determine whether the gene for MEN-1(Burin) also mapped to this region. All of the markers used (D11S288, PGA, D11S971, D11S970, D11S146, INT-2, D11S533) (Table 2.1) gave positive LOD scores (Petty et al 1994), and no cross-overs were seen with PYGM a marker tightly-linked to typical MEN-1. Using the MLINK program a LOD score of 13.65 at $\theta = 0.0$ was obtained for linkage of MEN-1(Burin) to PYGM. Thus the differences between the MEN-1(Burin) and typical MEN-1 phenotype are not likely to be due to mutations of different genes. All affected individuals in the four kindreds shared the same uncommon PYGM allele, and other closely-linked markers were in linkage disequilibrium with MEN-1(Burin) (Figure 2.10), supporting the geographic evidence for a common ancestor (Petty et al 1994).

Predictive testing could then proceed and is now complete for 121 of those at risk (Figure 2.11, Table 2.14). Several family members had blood collected for DNA extraction at the same time as their first clinical screening tests, so for 23 (19%) of those tested (age 13-52), the high-risk predictive testing results and positive clinical screening results were obtained at the same

Figure 2.10. Haplotypes of affected members of individual MEN-1 (Burin) families.

All haplotypes for the set of linked markers that are found in affected members of the four MEN-1 (Burin) kindreds are shown. The most common haplotype in each family is indicated as subgroup (i), and haplotypes generated by cross-overs are indicated as subgroups (ii) or (iii). In one branch of Family B, designated B*, the MEN-1 (Burin) gene is now known to be inherited from an unrelated spouse who is from the same ancestral community as Family D. All affected individuals share the same uncommon PYGM allele.

Haplotypes of affected members of individual MEN-1 (Burin) families

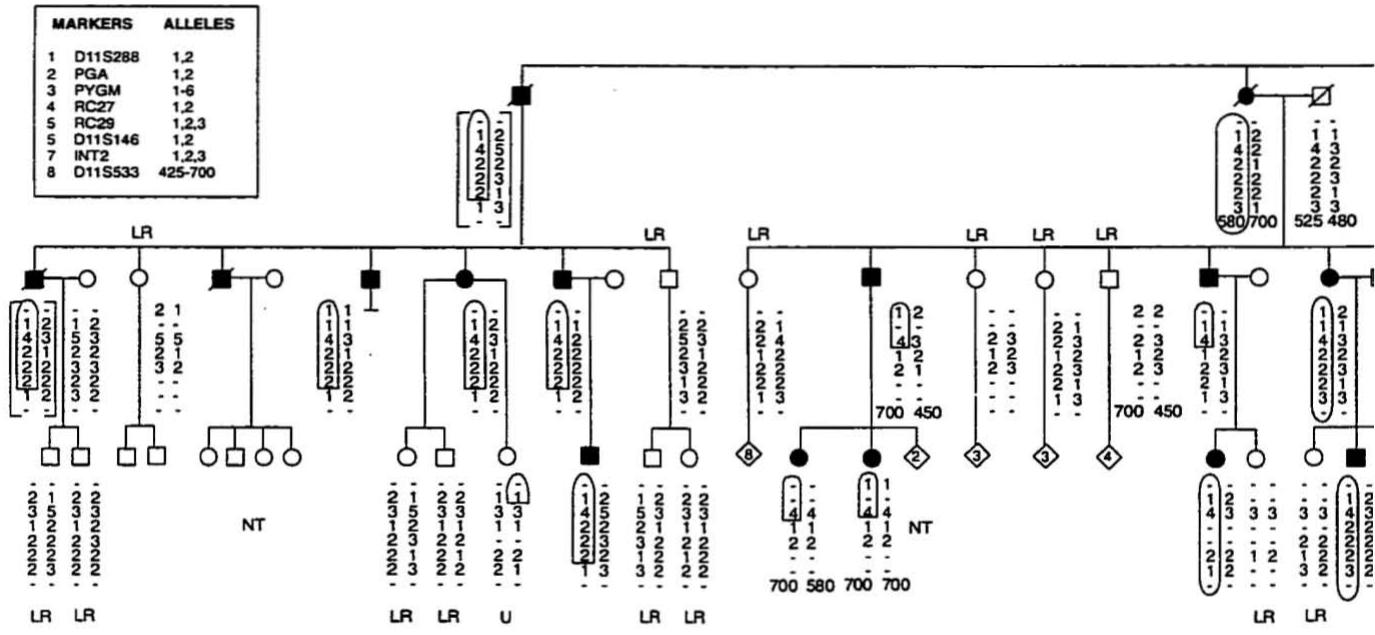
	MARKER ALLELES									
	Families									
	A			B		B*		C	D	
Loci	i	ii	iii	i	ii	i	ii	ii	i	ii
D11S288	1	1	1	1	1	2	2	2	2	2
PGA	1	1	1	-	-	-	-	-	-	-
PYGM	4	4	4	4	4	4	4	4	4	4
D11S971	2	2	1	2	2	2	2	2	2	2
D11S970	2	2	2	2	2	2	2	2	2	2
D11S146	2	2	2	1	1	2	1	2	2	1
INT2	1	3	1	-	-	-	-	-	-	-
D11S533	450	580	700	480	580	470	-	470	"4"	"3"

Figure 2.11. Results of predictive testing for at-risk members of MEN-1 (Burin) families.

The marker data from linkage analysis is given for each family member who requested predictive testing, and also for relevant affected relatives. The haplotype associated with MEN-1 (Burin) is circled. Family members with high-risk results are indicated (*). Those with low risk (LR), or uninformative (U) results are also indicated. When haplotypes could not be determined, the alleles of a particular marker are separated by a comma (ie, 1,2).

FAMILY A

MULTIPLE ENDOCRINE NEOPLASIA,

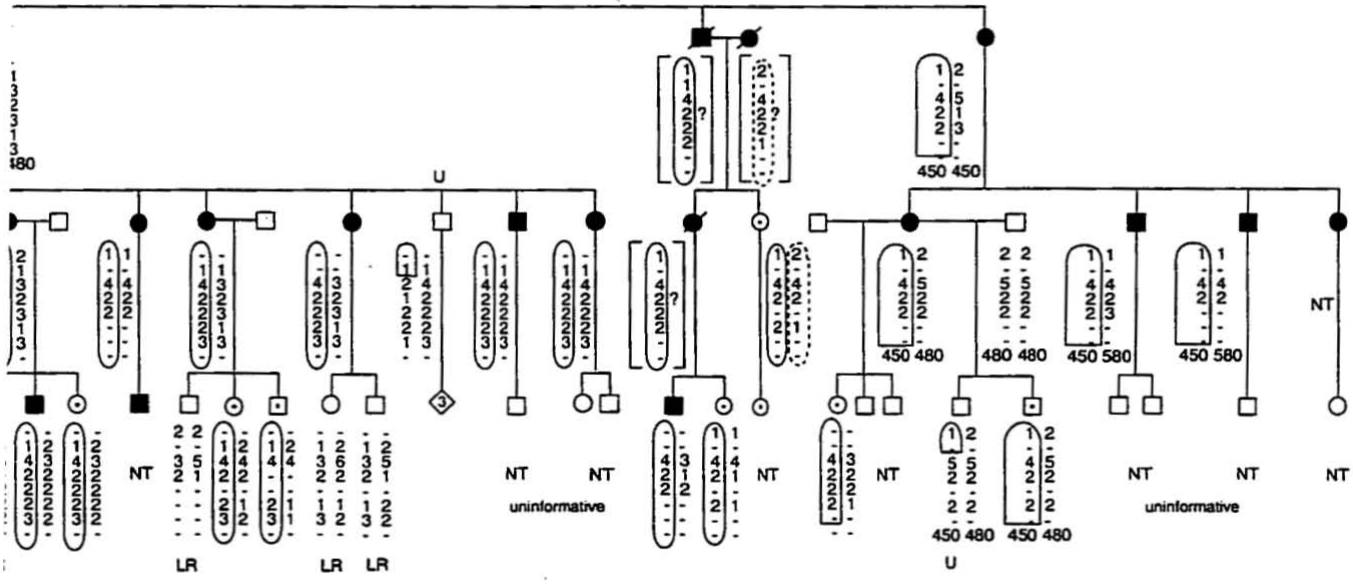


⊙ HIGH RISK

LR LOW RISK

U UNINFORMATIVE

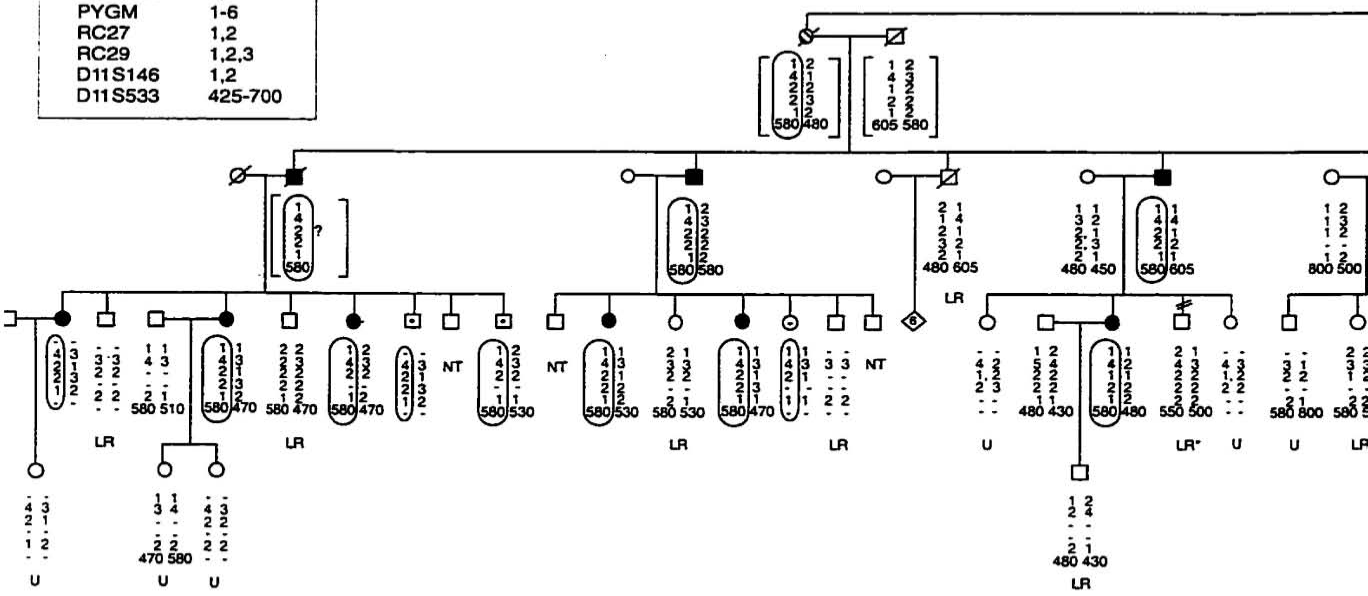
ʌ, TYPE 1


U
450 450
450 480
480 480
450 580
450 580
NT
NT
NT
NT
NT
NT
NT
NT
NT
NT
NT
uninformative
uninformative
LR
LR
LR
U
450 480
450 480

FAMILY B

MULTIPLE ENDOCRINE NEOPLASIA, TY

MARKERS	ALLELES
D11S288	1,2
PYGM	1-6
RC27	1,2
RC29	1,2,3
D11S146	1,2
D11S533	425-700



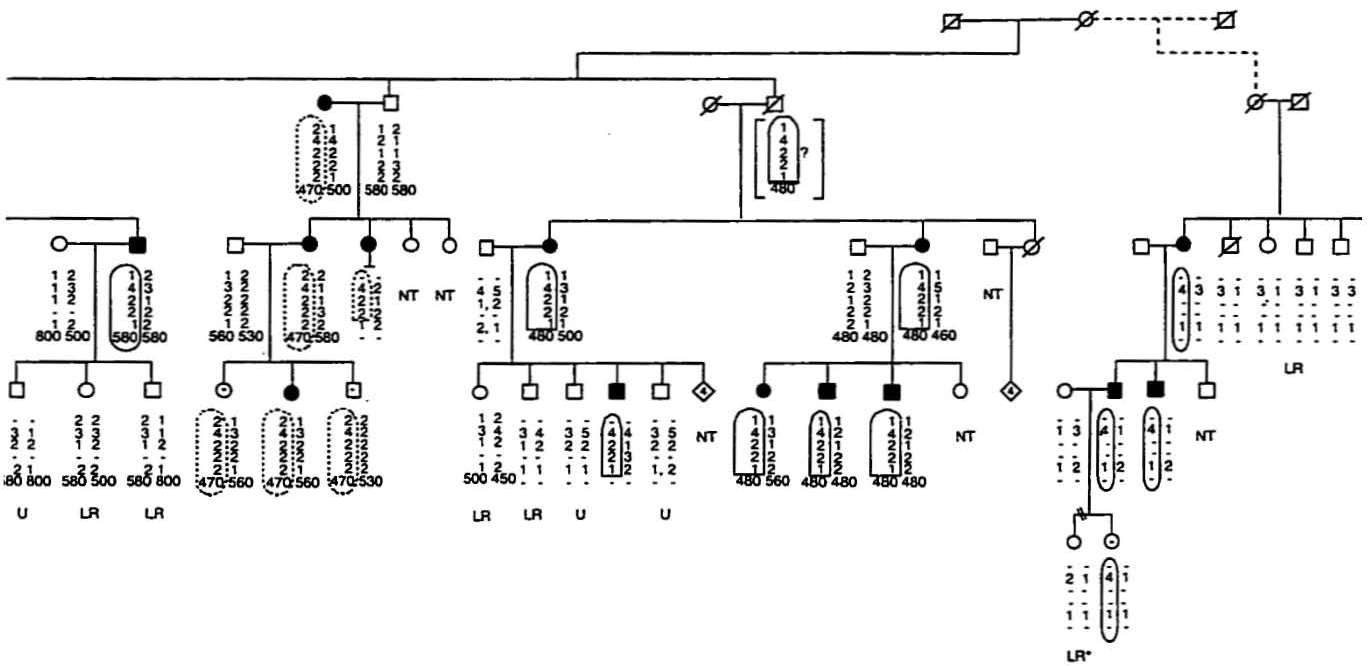
⊙ HIGH RISK

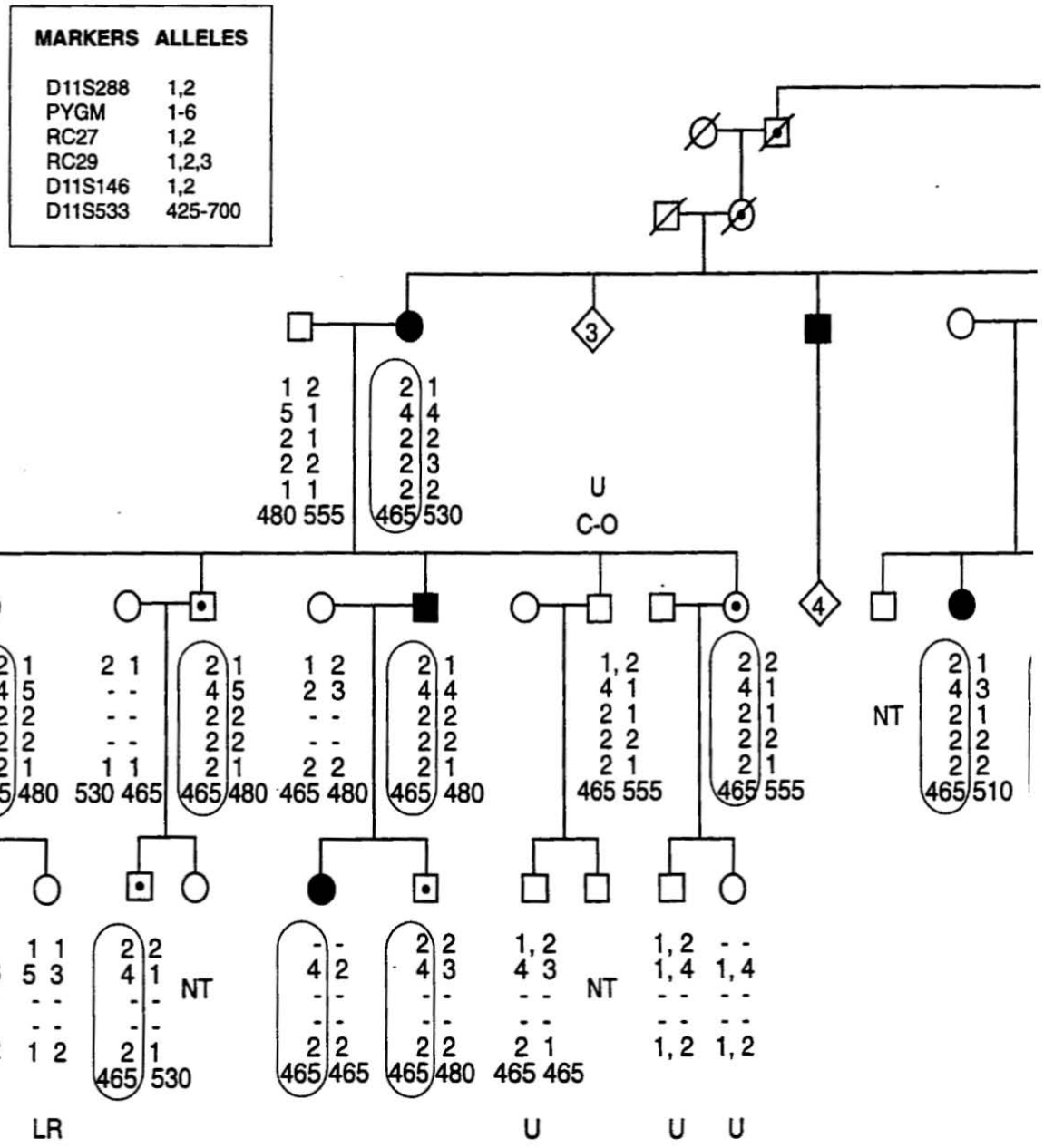
LR LOW RISK

U UNINFORMATIVE

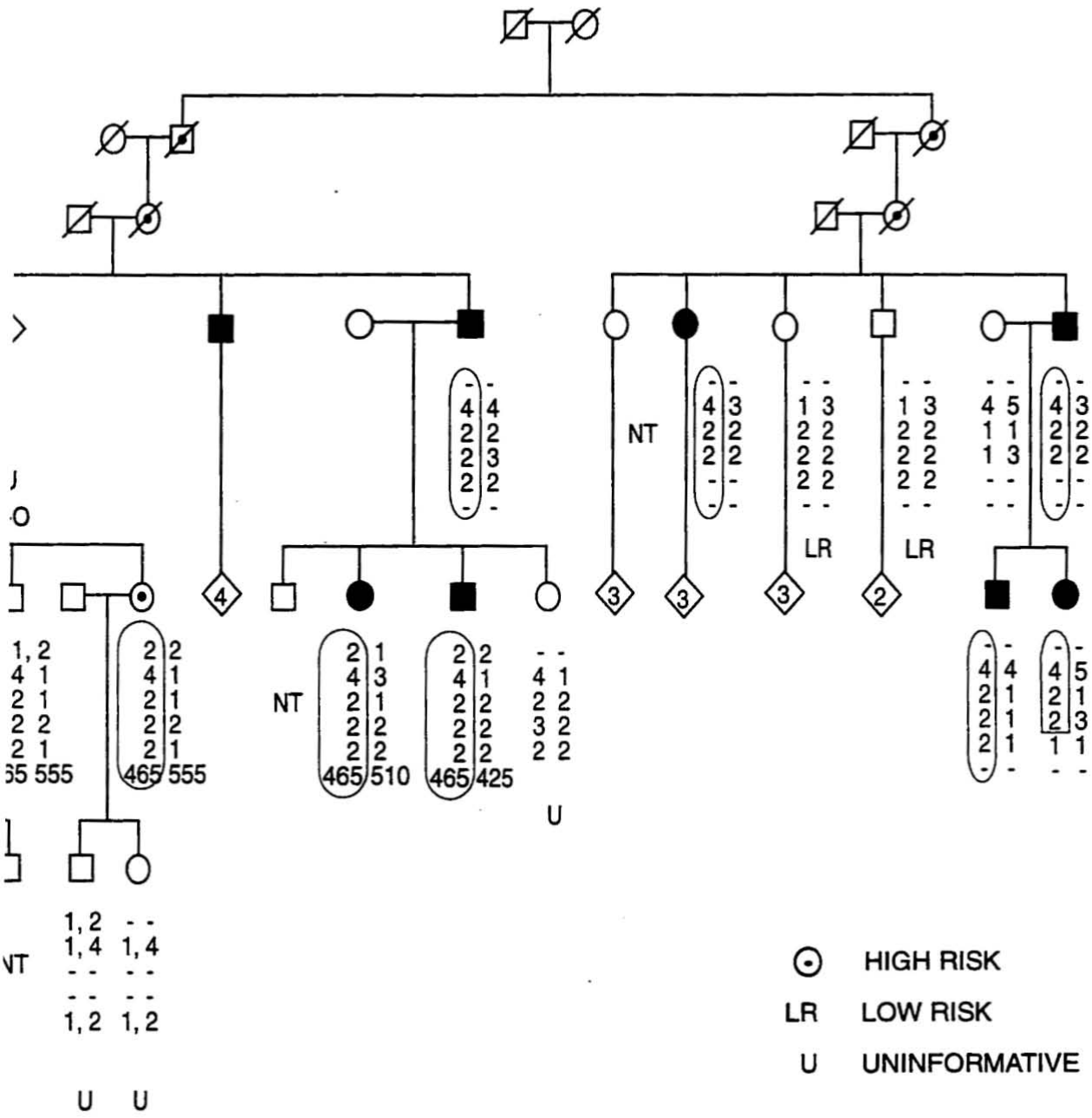
LR* POSSIBLE NON-PATERNITY

A, TYPE 1 (MEN-1)



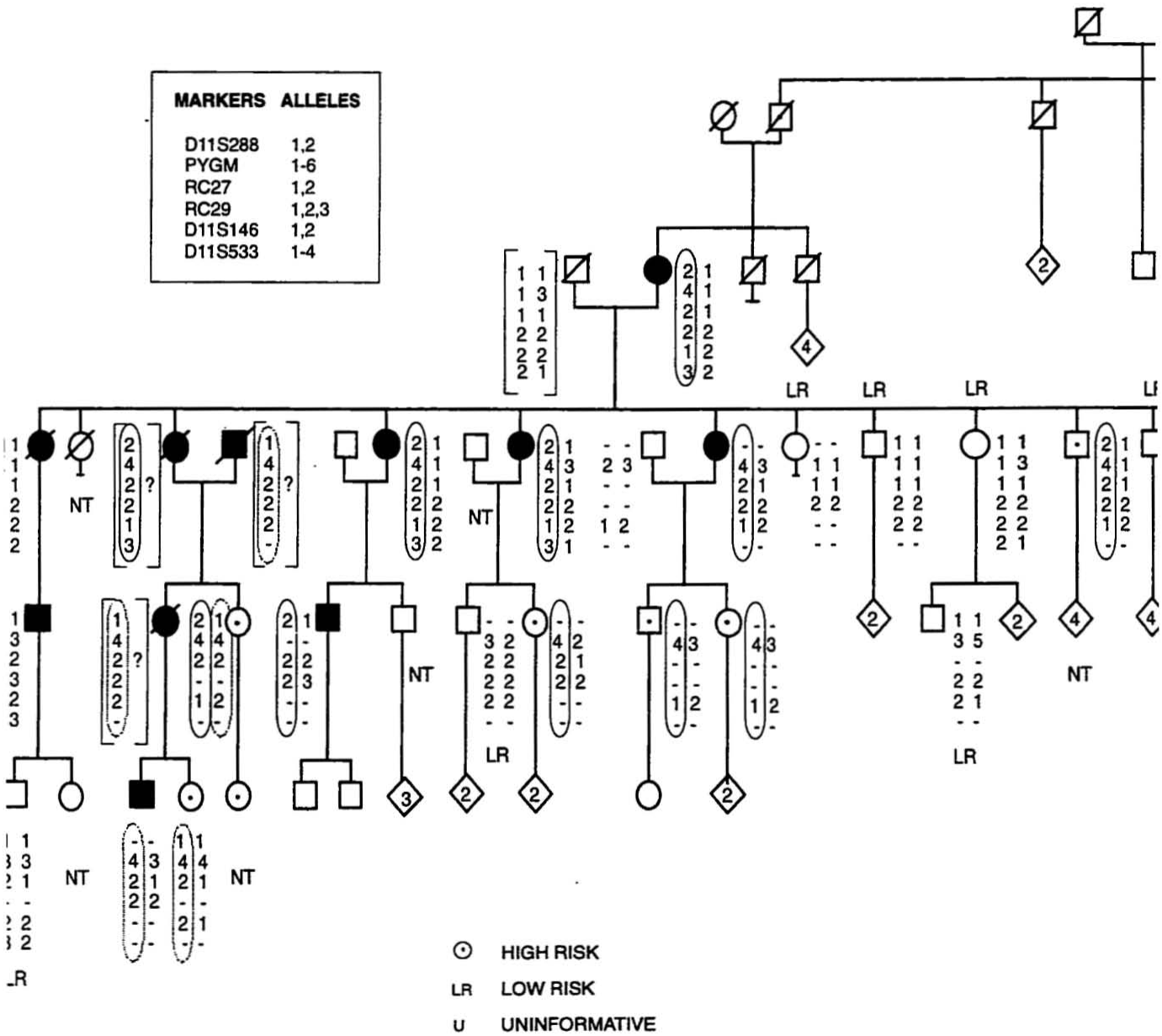

MARKERS ALLELES
D11S288 1,2
PYGM 1-6
RC27 1,2
RC29 1,2,3
D11S146 1,2
D11S533 425-700
3
4
U
C-O
NT
LR
LR
U
U
U
NT
NT

ILTIPLE ENDOCRINE NEOPLASIA, TYPE 1 (MEN-1)



FAMILY D

MULTIPLE ENDOCRINE NEOPLASIA, TYPE



IPLE ENDOCRINE NEOPLASIA, TYPE 1 (MEN-1)

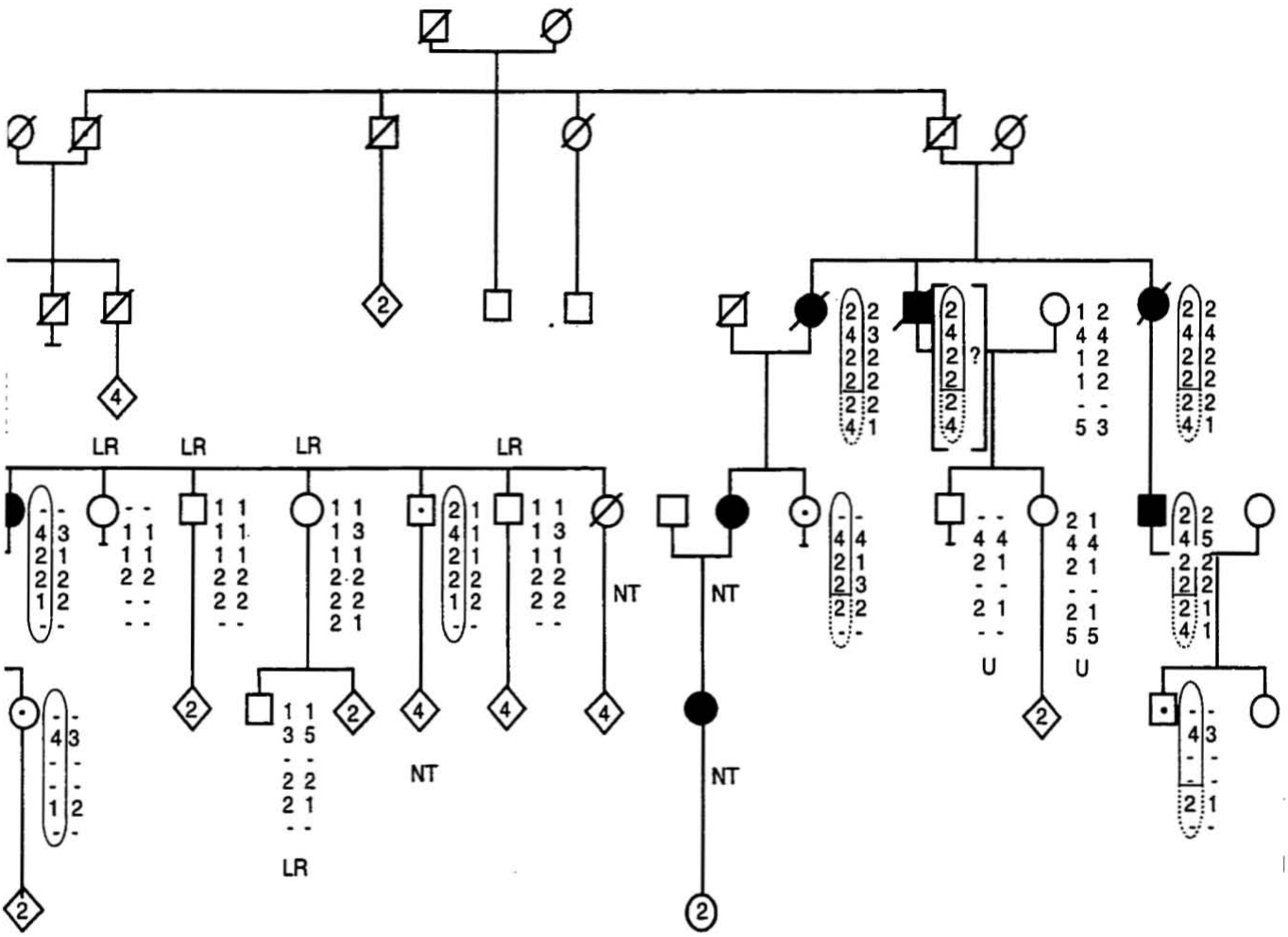


TABLE 2.14. PARTICIPATION IN PREDICTIVE TESTING, AND RESULTS OF LINKAGE ANALYSIS FOR MEN-1(Burin)

PARTICIPATION IN PREDICTIVE TESTING		
At 50% Risk (1990)		169
Not Tested		48
DNA not collected	24	
Testing incomplete	9	
Clinical diagnosis (before predictive testing)	15	
Total Tested		121
RESULTS OF LINKAGE ANALYSIS		
High Risk		50(41%)
With positive clinical screening	23(19%)	
With negative clinical screening	27(22%)	
Low Risk		43(36%)
Uninformative		28(23%)
Total Results		121

time. For those with negative clinical screening, high-risk results (>95%->98% probability of carrying the MEN-1 gene) were given to 27 others (22%), nine of whom were subsequently identified as affected by clinical screening, and low-risk results (<2%-<5% probability) were given to 43(36%) of those tested. Genetic testing was uninformative for 28 of those tested (23%) because of recombination, homozygosity of markers, heterozygosity in both parents, or unavailable DNA samples from key relatives.

Thus, eighteen family members (age 9 to 42) have high-risk results from predictive testing but have no abnormalities as yet on clinical screening. This includes a 42-year-old woman predicted to be homozygous for the MEN-1 (Burin) gene, having inherited the haplotype associated with the disease from each of her affected parents (Figure 2.12). This high-risk group, family members already affected, and those for whom predictive testing was uninformative require continued annual clinical screening to identify and treat any endocrine tumours at an early stage. The low-risk group (n = 43) requires less frequent clinical screening, and will be screened every three years.

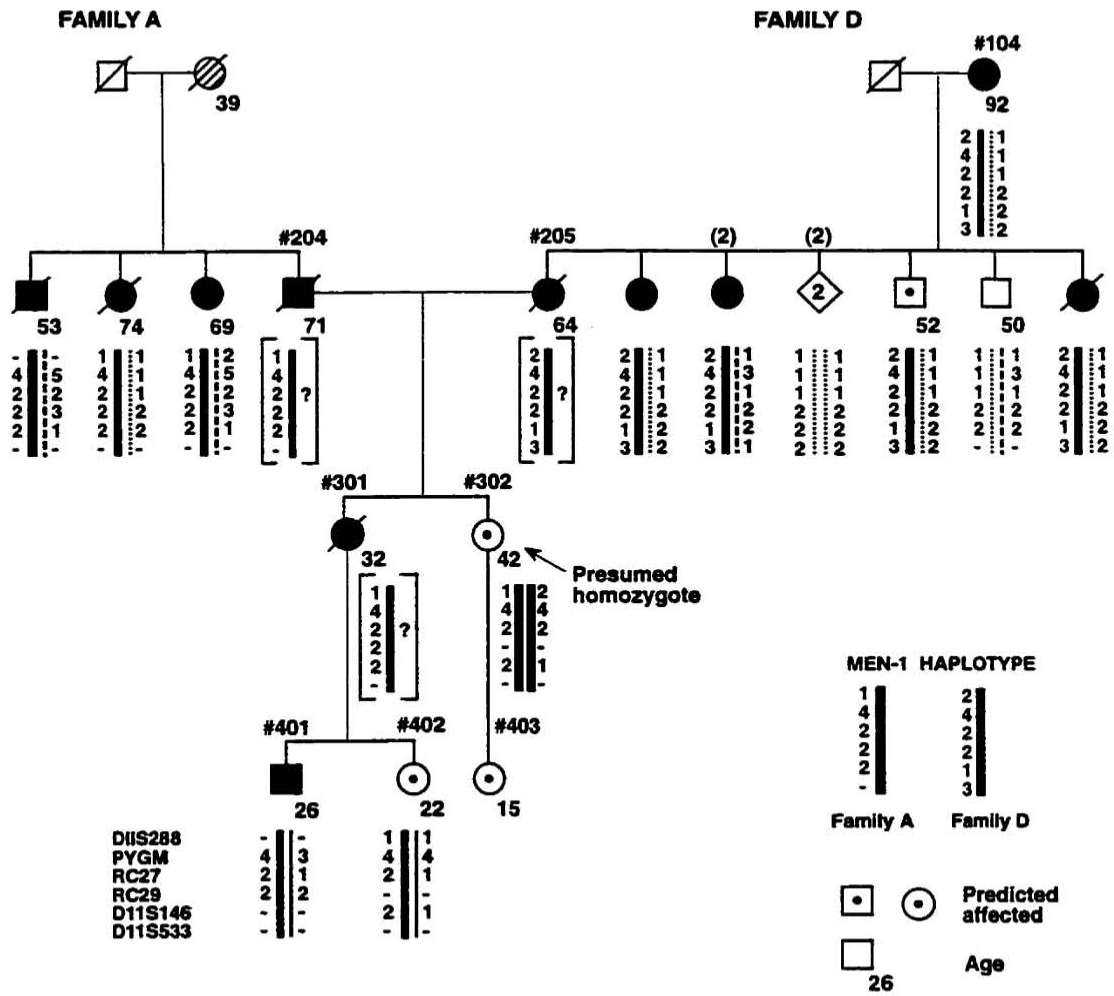
Impact of the Screening Program

Some members of the MEN-1(Burin) families were previously very apprehensive about MEN-1 because of the early deaths or severe disease in close relatives, and because they and their doctors had very little information about MEN-1. They expected a poor outcome for all affected family members,

Figure 2.12. Presumed homozygote for MEN-1 (Burin) identified by linkage analysis.

There was one marriage between two affected persons (#204 from Family A, and #205 from Family D). Their daughter, #302, inherited the haplotype associated with MEN-1 (Burin) from each parent. She is therefore presumed to be homozygous for the MEN-1 (Burin) gene. Although the haplotype of each parent was inferred, in each case it was based on the haplotype of affected and unaffected siblings, the daughter, grandchildren, and multiple affected relatives. At age 42, #302 has no manifestations of MEN-1 (Burin) identified by screening.

Results of predictive testing by linkage analysis indicating presumed homozygote for MEN-1 (Burin)



and now appreciate the information provided on the clinical and genetic aspects of MEN-1(Burin), and the organization of specialized care, as part of the screening program. Many of these feel that family members are much better off medically and psychologically than before. As a result, they comply with clinical screening recommendations, and have been interested in predictive testing to determine whether they and their children should continue with this screening. However none of these family members were interested in prenatal diagnosis, nor would they reduce the size of their families because of the risk of MEN-1(Burin).

Other family members had little previous interest or knowledge about MEN-1(Burin). Typically these had no close relatives who were severely affected, and had no expectation of any adverse effects of MEN-1(Burin). Some of these were not interested in the screening program, and others participated on an irregular basis. Many of this group also requested predictive testing when it was available, to clarify their risk but not to alter reproductive planning.

DISCUSSION

This group of MEN-1(Burin) kindreds was of particular interest because, atypically, each proband presented with a symptomatic prolactinoma (Farid et al 1980), and because of the high frequency of prolactinomas and carcinoid tumours and low frequency of pancreatic islet cell tumours compared to typical

MEN-1 families (Marx et al 1986, Brunt and Wells 1985, Vasen et al 1989b). These kindreds also presented a significant problem for clinical management because of the large number of affected and at-risk members within the original pedigrees, the significant morbidity and mortality in those originally identified, and the independent identification of other distantly related MEN-1 patients from that geographic region which suggested that the full extent of the problem was not yet defined. In addition, because the families reside in a rural area, access to the necessary medical care is difficult. It was felt that a coordinated screening program was necessary to allow early identification and treatment of manifestations of disease (Brunt and Wells 1985, Vasen et al 1989b), and to provide appropriate genetic counselling for affected and at risk family members.

The present study was undertaken to define the clinical phenotype of MEN-1(Burin) so that appropriate clinical screening could be instituted, to map the gene for MEN-1(Burin) so that predictive testing by linkage analysis or direct mutation detection could be offered to those at risk, and to implement and evaluate the clinical and genetic screening program recommended; but first, a few words about the identification of MEN-1 (Burin) families.

Ascertainment of MEN-1(Burin) Families

Ancestors of each of the four independently-identified MEN-1(Burin) families came from a group of very small, isolated, now abandoned communities within a 20-mile radius on the north shore of Fortune Bay. No

single common ancestor was identified. However, three of the pedigrees consist of two separately identified branches joined by pedigree studies, and the small communities in this isolated area of the south coast of Newfoundland were populated by a small number of settlers from southwest England in the middle 1800s. There are no written records to extend the pedigrees beyond the four or five generations documented but there are common surnames in the earliest generations recorded. The fact that all affected in the four families have the same allele of PYGM (a closely-linked marker for which recombination with the MEN-1 locus has not been recorded) supports the inference that a single founder introduced the mutant gene.

Clinical Phenotype of MEN-1(Burin)

Our studies confirmed that MEN-1(Burin) is distinct from typical MEN-1 in the characteristic presentation of disease, and also in the overall frequencies of manifestations detected by comprehensive screening (Table 2.2, p. 88). The high frequency of prolactinomas and carcinoid tumours, and low frequency of pancreatic involvement is not just a bias of ascertainment based on the dramatic presentation of the probands and other early affected individuals. Even though pancreatic islet cell tumours are rare, functional gastrinomas causing Zollinger-Ellison syndrome, and non-functional glucagonomas have occurred. The most difficult manifestations to identify and treat, however, are the

carcinoid tumours of the lung and thymus, and these have caused both morbidity and mortality.

Although the overall pattern of disease in MEN-1(Burin) is different from that in typical MEN-1, there is an extremely variable disease presentation even among close relatives: in age of onset, in number and order of occurrence of manifestations, and in severity of disease. Just over 50% of affected members have hyperparathyroidism only, as in typical MEN-1 families, but 34% have two manifestations, and 11% have three or more. Identification of atypical MEN-1 families depends on having enough patients documented to recognize the atypical statistical distribution of manifestations. Branches of these families seen elsewhere, or small families with a similar genetic defect, might therefore not be immediately recognized as having a different pattern of tumour predisposition. This suggests that a broad screening protocol (Figure 2.2, p. 76) would be desirable for all MEN-1 families, including testing for parathyroid, pituitary, pancreatic and carcinoid tumours. Compliance will be greatest if primary screening is available locally rather than requiring travel to a tertiary care facility. Because of variable availability of medical care for families in different areas of one country or in different countries, the exact set of screening tests used may have to be a compromise.

Mapping of MEN-1(Burin)

The MEN-1(Burin) gene maps to the same chromosome 11q13 region as the gene for typical MEN-1 (Petty et al 1994, Larsson et al 1988). However, until the gene(s) is cloned and specific mutations identified, it will not be possible to determine the basis for the differences in MEN-1 and MEN-1(Burin). Are they different genes (which is unlikely); or different mutations within the same gene, as with the distinct types of mutation in the RET proto-oncogene causing MEN-2A and MEN-2B (van Heyningen 1994); or does the difference reside in factors modifying a common genetic defect as in the different phenotypes of MEN-2 (MEN-2A and familial medullary carcinoma of the thyroid) arising from identical mutations of the RET oncogene (Mulligan et al 1994, Donis Keller et al 1993)?

Results of Clinical Screening for MEN-1(Burin)

The clinical screening program has identified 41 new affected MEN-1(Burin) patients since 1989. Although many have had hyperparathyroidism only, as in typical MEN-1 families (Marx et al 1986, van Heerden 1986, Shepherd et al 1991), others have had prolactinomas or carcinoid tumours only which is not characteristic of typical MEN-1.

It is obviously not possible, as yet, to provide precise data on the long-term medical benefits of the screening program. This and other MEN-1 families must be monitored over a longer time period before appropriate

comparisons can be made. However, as one would expect, the patients who presented symptomatically have had greater morbidity and mortality than those identified by screening, and many had symptoms from at least two involved glands at the time of diagnosis: particularly kidney stones, fatigue and/or muscle pain secondary to hyperparathyroidism; and amenorrhea, galactorrhea, headaches or visual field defects secondary to a prolactinoma. Since screening for most patients is relatively recent, and screening identifies gland involvement at an earlier stage, patients identified by screening have not been affected as long as most symptomatic patients. However, patients with hyperparathyroidism, or hyperprolactinemia identified by screening are being treated, by surgery (Brunt and Wells 1985, Malmaeus et al 1986), or medically with bromocryptine (Dalkin and Marshall 1989), respectively, at an early stage. Since the disease is not progressing, the morbidity or mortality previously seen for these manifestations is less likely to occur.

Surgery is still the treatment of choice for MEN-1-related hyperparathyroidism; however, recognition of MEN-1 is important so that the appropriate type of surgery is done (subtotal parathyroidectomy, or total thyroidectomy with forearm implant) because in MEN-1 (Burin) patients as well as in MEN-1 patients described in the literature, there is typically hyperplasia of all four glands rather than a single adenoma. In the past, removal of only one gland frequently resulted in persistence or early recurrence of symptoms,

and the need for repeat surgery at an early stage (Rizzoli et al 1985, Malmette et al 1987).

No macroprolactinomas have been identified in the MEN-1 (Burin) patients since hyperprolactinemia has been detected by screening and treated medically. Not only have patients not required a difficult surgical procedure, but they also have not developed the severe symptoms caused by a large prolactinoma (galactorrhea, amenorrhea, visual field defects, or severe headaches).

Although two patients had Zollinger-Ellison syndrome with very high gastrin levels and multiple small gastrinomas (as previously described [van Heerden et al 1986, Pipeleers-Marichal et al 1993]), elevated gastrin has not been detected by screening in any other affected or at risk family members. There is evidence from other families with frequent pancreatic islet cell tumours, that treatment of early gastrinomas with H_2-receptor antagonists or ATPase inhibitors (Brunt and Wells 1985, van Heerden et al 1986, Wolfe and Jensen 1987) can prevent Zollinger-Ellison syndrome, but not necessarily the malignant potential of the gastrinoma (Wolfe and Jensen 1987, Pipeleers-Marichal et al 1993). The MEN-1 (Burin) patient who died of sepsis from a perforated ulcer, had liver and lymph node metastases detected at autopsy, and the second Z-E patient has documented slow-growing liver metastases, but the malignant nature of the pancreatic tumours has not been the main concern in the clinical management of these two patients. Both

patients had severe uncontrollable symptoms because of hypergastrinemia. The patients with non-functioning glucagonomas have been identified recently and their long term clinical course remains to be seen.

In many MEN-1 families described in the literature, pancreatic tumours caused the greatest morbidity and mortality, previously because of untractable ulcer disease, and currently because of pancreatic islet cell malignancies (Ballard et al 1964, Vasen et al 1989b, Skogseid et al 1991a). Shepherd et al (1993), however, have described a large Tasmanian family where the majority of patients with radiological or biochemical evidence of pancreatic islet cell disease are asymptomatic. Only 28% of those with pancreatic involvement had symptomatic pancreatic lesions, and deaths from pancreatic disease were rare.

Carcinoid tumours of the lung and thymus cause the greatest problem for clinical management in the MEN-1(Burin) kindreds, and a similar situation is described in the Tasmanian family (Wilkinson et al 1993). Unlike other studies where carcinoid of the lung has been found only in women and carcinoid of the thymus only in men (Shepherd et al 1991, Zeiger et al 1992, Economopoulos et al 1990), we have seen carcinoid tumours in both locations in both sexes, and one male patient has had carcinoid of the lung and carcinoid of the thymus. Carcinoid tumours of the thymus are frequently malignant when identified and are associated with a poor prognosis (Economopoulos et al 1990, Wilkinson et

al 1993). Surgery is difficult because of their location (Teh et al 1994a), and chemotherapy and radiation may not be helpful.

These carcinoid tumours have not secreted 5HIAA as sporadic carcinoid tumours usually do (Duh et al 1987), so have been detectable only by chest x-ray, or the symptoms of advanced inoperable disease. However, there is a concern that annual chest x-ray (or, particularly the diagnostically more useful CT scanning) from an early age may increase the risk of malignancy in a patient with a genetic predisposition to tumours (personal communication, Dr. CE Jackson, Henry Ford Hospital, Detroit, Michigan).

Morbidity and Mortality

Only eight deaths definitely related to MEN-1 have been recorded in the 83 affected members of these kindreds (three other deaths were unrelated, and two were from other malignancies), but some of the MEN-1-related deaths were at an early age (Table 2.4). Four of these eight deceased patients had malignant carcinoid tumours; two deaths were caused by malignant carcinoid of the thymus, and two other patients had metastatic carcinoid of the lung as well as a second metastatic tumour (renal cell carcinoma or breast cancer) as the cause of death. Five of the seven living patients with carcinoid tumours also have malignant tumours, with either local invasion from carcinoid of the thymus, or rapidly enlarging lung and liver metastases from carcinoid tumours of the lung. Various combinations of surgery, radiation and chemotherapy have

been tried with only limited success. Since some of the carcinoid tumours or their metastases have somatostatin receptors, one suggestion has been that somatostatin receptor scanning, and treatment of those with a positive scan with the somatostatin analogue, octreotide, may offer a solution to both the difficulty of detection and the difficulty of treatment of these tumours (Arnold et al 1993). However this is costly (approximately $1000/scan). A limited clinical trial is now being conducted by Dr. Ehud Ur (Endocrinology) and Dr. Peter Hollett (Nuclear Medicine) at St. John's General Hospital to determine the efficacy of this procedure.

Deaths from renal failure (one previously), Zollinger-Ellison syndrome (one previously), and invasive pituitary tumours (two previously) are less likely to occur now because of the early detection and treatment of hyperparathyroidism, hypergastrinemia, and prolactinomas, or other pituitary tumours, because of the screening program.

The relation of other malignancies to MEN-1(Burin), in five patients from one of the kindreds, is not known. Four breast cancers and a renal cell carcinoma were identified in four sisters and their cousin with MEN-1(Burin) (four of whom also had malignant carcinoid tumours). No malignancies have been identified in the non-MEN-1 members of any of the four kindreds, raising the possibility that breast cancer may be a rare manifestation of the MEN-1 (Burin) gene, or that MEN-1(Burin) increases the overall risk of various malignancies including breast cancer and renal cell carcinoma.

The potential for malignancies in MEN-1 other than of pancreatic islet cell or carcinoid tumours has not been described except in the family reported by Johnson and Sawicki (1993) which has a number of atypical features including childhood onset, dysmorphic features and hematological malignancies. In fact, this family may have a contiguous gene syndrome associated with a small deletion rather than variable manifestations of the MEN-1 gene (personal communication, Dr. Carey Johnson, Alberta Children's Hospital, Calgary).

Although some MEN-1 patients in the Newfoundland families have severe disease, there is very mild late-onset disease in other gene carriers, most notably two women (first cousins) identified in their 80s with hypercalcemia, one of whom later developed mild hyperprolactinemia also. Both had severely affected siblings, children or grandchildren who predeceased them. Older patients who are asymptomatic or with very mild disease have also been described by Shepherd (1991) in the large Tasmanian MEN-1 family.

The family member predicted by linkage analysis to be homozygous for the MEN-1 gene has no symptoms of MEN-1 at age 42, and continues to have normal results for all screening investigations. Two other patients predicted to be homozygous for MEN-1 have been reported (Brandi et al 1993). They express manifestations of MEN-1 (age 35 and 40), but are no more severely affected than their heterozygous relatives.

Fifty-two per cent of those who are affected have had hyperparathyroidism only, successfully treated surgically in most cases,

whether identified clinically or by laboratory screening. Because of this mild or easily treatable disease in some family members, some of those at risk have not been interested in the clinical or genetic screening programs, or have been non-compliant following initial screening. Unfortunately, it is not possible to predict the severity of disease in any particular gene carrier, and, as described above, children of mildly affected MEN-1 patients will not necessarily have mild disease also.

Results of Predictive Testing

The mapping of MEN-1(Burin) to chromosome 11q13 allowed predictive testing with a set of markers flanking the MEN-1 region to distinguish those at low or high risk of having inherited the MEN-1(Burin) gene. Discussions during genetic counselling sessions showed that the majority of family members were interested in predictive testing for themselves or for their children, but this was usually to determine whether they required clinical screening, rather than to influence their reproductive decisions.

Nineteen per cent of those tested (23/121) had positive clinical screening results at the same time as high-risk predictive testing results. These will have annual screening, and treatment as necessary. Those who had high-risk predictive testing results (22% [age 10-42 years]) with normal clinical screening results also require annual screening.

Twenty-three per cent of those for whom linkage studies are completed had uninformative results because of unavailable DNA samples from key individuals, homozygosity of markers (a potential problem with inbred populations in genetic isolates), or recombination between flanking markers. This group also requires continued annual clinical screening until the genetic status can be clarified when more informative markers are available (Larsson et al 1992a, Teh et al 1994b), or the MEN-1 gene is cloned and the mutation in this family identified.

For 36% of those at risk who are participating in the clinical screening program and have had negative screening results up to the present time, clinical screening can be reduced because of the low-risk linkage results. Similar predictive testing programs for typical MEN-1 families have been described (Larsson et al 1992a, Teh et al 1994b). When the recommended biochemical testing can be directed to the high-risk group identified by linkage analysis, the screening program is more efficient with reduced costs to individual family members, and to the medical care system.

Costs and Benefits of Screening

The clinical screening has been organized in such a way that the primary testing can be done at the local hospital or clinic, and this is coordinated by the geneticist in St. John's and the various family doctors. For most family members who live in small communities on the Burin Peninsula or Fortune Bay,

this means a 5-60 km drive for screening rather than a drive of at least 400 km to the tertiary care centre in St. John's, and therefore saves both time and money. Follow-up of abnormal screening, however, is done at the Clinical Investigation Unit in St. John's.

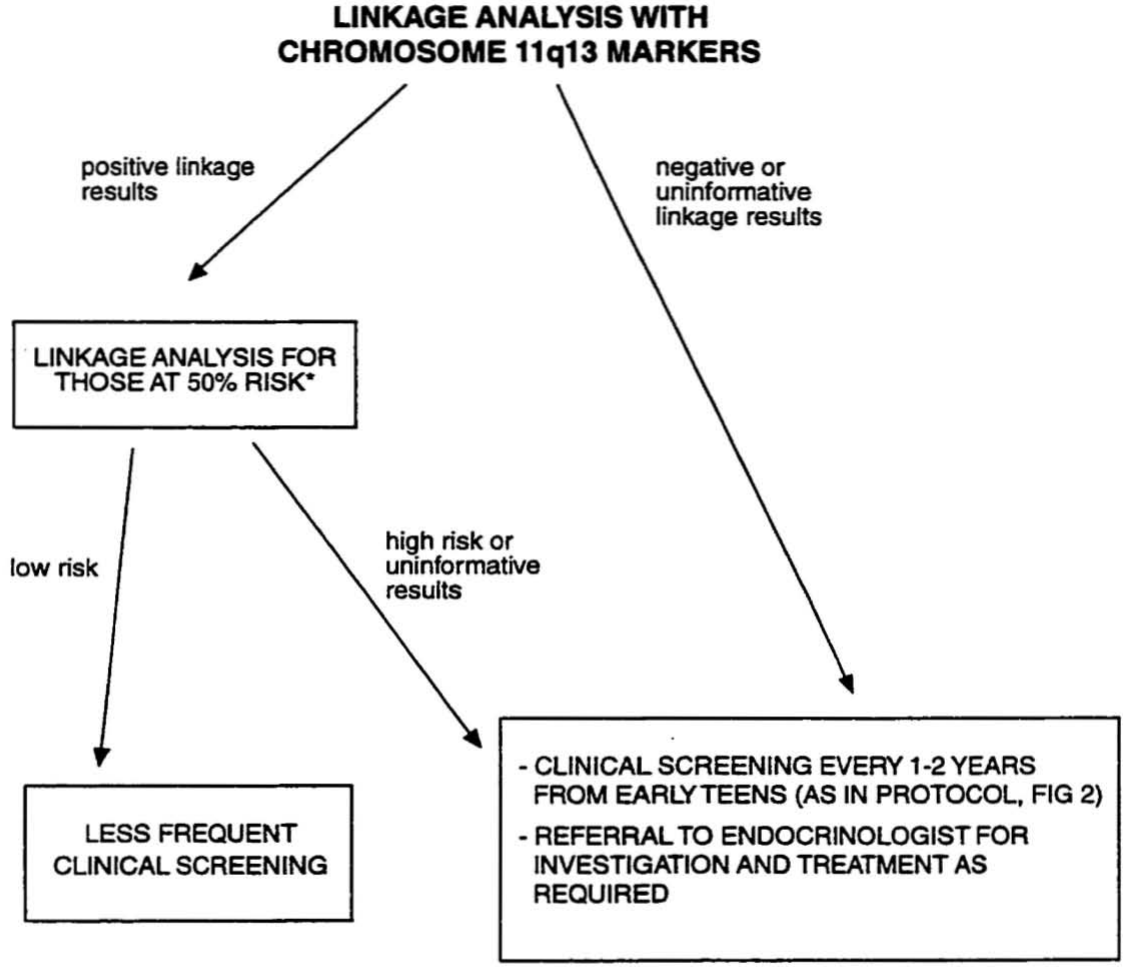
Because of the late diagnosis of very mild disease in some family members, it is not known how long biochemical screening should continue if all biochemical screening results are negative. We have seen that compliance with screening decreases with repeated normal results so some patients may be lost to follow up before diagnosis. The addition of genetic testing by linkage analysis to the screening protocol provides a means to identify those at high risk who should be encouraged to continue with regular clinical screening despite negative testing. The genetic testing also identifies non-carriers who require much less frequent screening (Figure 2.13). Because these are large families, there were over 100 affected and at-risk family members in the screening program in Newfoundland. Clarifying those who require this screening, by genetic testing, is therefore beneficial to the health care system and to the family members. .

Satisfaction with Screening

Family members have expressed their satisfaction with the clinical and genetic screening program because of the early identification and treatment of family members, and the better understanding of the disease, both by themselves and by their family doctors.

Figure 2.13. Management plan for MEN-1 (Burin).

Management plan for MEN-1 families



**for those who decline predictive testing, regular clinical screening is recommended*

They have greater confidence than previously that their special needs are recognized, and that specialists are available, if necessary, to investigate any symptoms or abnormal screening results. Their interest and participation in both the clinical and genetic components of the screening program is correlated with their perception of the burden of MEN-1(Burin), and this, in turn, is correlated with the degree of relationship to those who have had severe disease in the past, or died young of the disease — similar to the situation reported for members of spinocerebellar ataxia (SCA-1) families (Nance et al 1994). The results of predictive testing are used to determine their need to continue with clinical screening or to have their children screened, rather than to alter reproductive planning, unlike in more severe late-onset hereditary diseases where high-risk or affected individuals often choose not to reproduce (Nance et al 1994). Overall, family members feel that they are more healthy and less anxious than before the screening program was offered.

CHAPTER 3 — DEVELOPMENT OF A CLINICAL AND GENETIC SCREENING PROGRAM FOR MULTIPLE ENDOCRINE NEOPLASIA, TYPE 2

INTRODUCTION

Previous Studies

Present Study

PATIENTS AND METHODS

Ascertainment of Family

Clinical Screening

Genetic Screening

Impact of Screening on the Family

RESULTS

Clinical Screening

i) Thyroid disease
   a) Thyroid pathology
   b) Method of diagnosis
   c) Age at diagnosis
ii) Adrenal disease

Genetic Screening

i) Linkage analysis
ii) Predictive testing

Impact of Screening on the Family

DISCUSSION

Clinical Screening

i) Timing of clinical screening
ii) Thyroid disease
iii) Adrenal disease

Genetic Screening

Impact of Screening on the Family

Implications of Screening for Late-Onset Genetic Disease

Recommended Management for MEN-2A/FMTC Families

INTRODUCTION

This chapter will describe the identification of a Newfoundland family with multiple endocrine neoplasia, type 2, the implementation of a clinical screening program, the difficulties in classification of this family based on the results of clinical screening, the development of genetic testing, and the impact of this screening program on family members.

Previous Studies

Multiple endocrine neoplasia, type 2 (MEN-2) is a dominantly inherited cancer predisposition syndrome characterized by medullary carcinoma of the thyroid, pheochromocytoma and hyperparathyroidism (Sipple 1961, Steiner et al 1968, Chong et al 1975). In the classic form of MEN-2, MEN-2A, medullary carcinoma of the thyroid (MTC), or its precursor state C-cell hyperplasia (CCH), occurs in almost 100% of gene carriers but the age of onset is extremely variable (Easton et al 1989), including patients symptomatic in the teens or childhood (Telander et al 1989), and asymptomatic gene carriers in the 6th or 7th decade (Ponder et al 1988). Pheochromocytoma or adrenal medullary hyperplasia occurs in about 50% of gene carriers and may precede thyroid gland involvement (Carney et al 1975, Gagel et al 1988, Howe et al 1993). Parathyroid hyperplasia is present in approximately 20% of those affected, but is rarely associated with significant clinical disease (Gagel et al 1988, Kraimps et al 1992, Decker 1992, Howe et al 1993).

Patients presenting symptomatically typically have advanced disease, with almost half dying of MEN-2 (Calmettes et al 1992, Telenius-Berg et al 1984). In families studied retrospectively, the causes of death have been related equally to metastatic spread from malignant MTC, and to hypertensive crisis due to an undetected pheochromocytoma (Telenius-Berg et al 1984, Vasen et al 1987).

Clinical screening programs (Graze et al 1978, Telenius-Berg et al 1984, Vasen et al 1987, Gagel et al 1988, Calmettes et al 1992) using a pentagastrin and/or calcium-stimulated calcitonin test (Guilloteau et al 1990, Weissel et al 1991, Barbot et al 1994) for identification of thyroid disease, and 24-hour urine measurement of catecholamines and metanephrines for evidence of adrenal disease (Gagel et al 1988, Lairmore et al 1993), allow early detection and treatment of manifestations (Wells et al 1985, Jannson et al 1988, Telander et al 1989, Barry et al 1991), and have resulted in reduced morbidity and mortality (Vasen et al 1987, Gagel et al 1988, Decker 1992, Calmettes et al 1992). Although only 60% of patients have symptomatic evidence of MTC or C-cell hyperplasia by age 70, 93% of those with the MEN-2 gene can be detected by biochemical screening by age 31 (Ponder et al 1988, Easton et al 1989). Those who are detected by prospective screening and have a thyroidectomy as soon as the increased serum calcitonin is confirmed usually have C-cell hyperplasia only and have the best prognosis (Vasen et al 1987). The European Collaborative Group has therefore recommended that screening

should start by age 3 to allow prospective detection of even the earliest onset disease (Calmettes et al 1992), and other groups recommend even earlier screening (Telander et al 1989).

A related but clinically distinct syndrome, MEN-2B (Gorlin et al 1968, Schimke et al 1968, Norton et al 1979), has also been described, with particularly early onset and rapid progression of medullary carcinoma and pheochromocytoma. Hyperparathyroidism is not described in MEN-2B, but associated features including Marfanoid habitus, mucosal neuromas, and intestinal ganglioneuromatosis are often present (Vasen et al 1992). MEN-2B is less common than MEN-2A. Because of the early lethality, often before reproduction, many MEN-2B patients are the result of a new mutation (Montgomery et al 1987, Lairmore et al 1991), but the associated features present in early life may allow early diagnosis (Vasen et al 1992).

It has also been recognized that some MEN-2 families have thyroid disease only, either MTC or C-cell hyperplasia (Noll et al 1984, Farndon et al 1986). In these families with familial MTC (FMTC), the typical age of onset of medullary carcinoma is significantly later than in MEN-2A families and the thyroid disease usually more slowly progressive (Farndon et al 1986). Because of the later age of onset, screening for these families may begin at a later age.

The gene for MEN-2A was mapped to the centromeric region of chromosome 10 in 1987 (Simpson et al 1987, Matthew et al 1987), and subsequently MEN-2B (Norum et al 1990, Lairmore et al 1991) and FMTC

(Narod et al 1989, Lairmore et al 1991) were also mapped to this region. Closely-linked markers have been used for predictive testing in MEN-2A, MEN-2B, and FMTC families (Sobol et al 1989, Telenius et al 1990, Mathew et al 1991, Howe et al 1992, Lichter et al 1992, Shimotake et al 1992, Thakker et al 1993a, Barbot et al 1994), to identify those at high risk of carrying the disease gene and requiring clinical screening.

In 1993, mutations in the RET proto-oncogene were identified in affected members of MEN-2A (Mulligan et al 1993, Donis-Keller et al 1993) and FMTC families (Donis-Keller et al 1993, Mulligan et al 1994). These mutations cluster in five cysteine residues near the transmembrane boundary of the extracellular domain of the RET protein (Eng et al 1994b, Lips et al 1994, Mulligan et al 1994). A missense mutation in this group of cysteine codons has been identified in over 80% of families studied (Mulligan et al 1994, Schuffenecker et al 1994, Xue et al 1994) with the majority of mutations, particularly in MEN2-A families, affecting codon 634 (McMahon et al 1994, Mulligan et al 1994, Schuffenecker et al 1994). Subsequently, missense mutations of codon 918 in the tyrosine kinase domain of the RET proto-oncogene were identified in MEN-2B patients (Carlson et al 1994, Eng et al 1994a, Hofstra et al 1994) and in sporadic MTC (Hofstra et al 1994). Interestingly, inactivating mutations in the intra- and extracellular domains of the RET gene cause a usually unrelated disease, autosomal dominant Hirschsprung disease (Edery 1994, Romeo et al 1994).

This specificity of the site of mutations increases the likelihood of mutation detection for an MEN-2 family, or even for an individual presenting with MEN-2B without family history, and facilitates the identification of gene carriers amongst at-risk relatives by direct mutation analysis (Lips et al 1994, McMahon et al 1994, Tsai et al 1994, Xue et al 1994). Clinical screening to detect thyroid and/or adrenal disease can then be reserved for those identified as gene carriers.

While there is evidence that the clinical screening programs have reduced the morbidity and mortality from MEN-2A, MEN-2B and FMTC (Vasen et al 1987, Gagel et al 1988, Decker 1992), there is some concern about apparent false positive results of calcitonin stimulation testing leading to inappropriate thyroidectomy in unaffected individuals (Simpson et al 1990, Landsvater et al 1993). Use of genetic testing to identify gene carriers, however, will lessen the possibility of this problem.

Present Study

A Newfoundland family with MEN-2, with 4 affected family members and 19 first degree relatives at 50% risk, was referred to genetics in 1989. Clinical screening was organized by Jane Green for all affected or at risk. Genetic screening (initially linkage studies and later a search for the mutation) was undertaken in collaboration with other laboratories in an attempt to

identify gene carriers so that biochemical screening could be restricted to this group.

The results of the clinical and genetic screening are now presented with a preliminary comment on the impact of the screening program on members of the family.

PATIENTS AND METHODS

Ascertainment of Family

The Newfoundland MEN-2 family was first recognized by the Pathology Department of the General Hospital, St. John's, Newfoundland in 1984 when they identified two patients with medullary carcinoma of the thyroid. A 44-year-old patient (Patient 1) who had presented four years earlier with a neck mass, and had biopsy proven MTC, died of metastatic disease. His 67-year-old uncle (Patient 2) died suddenly, and at autopsy a small MTC was identified coincidentally as well as a ruptured aortic aneurysm which was the cause of his death. Screening was instituted for some family members and, in 1985, the 19-year-old grandson of Patient 2 had increased urinary catecholamines, as well as increased blood pressure and tachycardia. After further investigations, including CT scan of the abdomen, MIBG (^{131}I-metaiodobenzylguanidine) scan and venous sampling, he had a bilateral adrenalectomy. The pathology was consistent with bilateral adrenal medullary hyperplasia.

In 1989, screening of a 50-year-old daughter of Patient 2 revealed increased basal and pentagastrin-stimulated calcitonin levels and she underwent a total thyroidectomy. A very small (<2 mm) medullary carcinoma was identified in the pathology specimen. At this time genetics was consulted to assist in the development of a comprehensive screening program for members of the family.

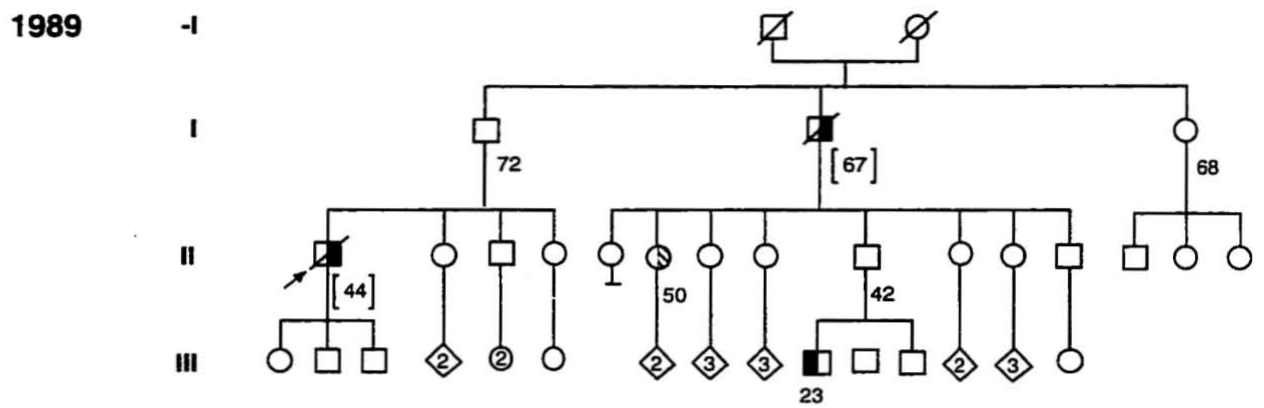
Clinical Screening

Family members were interviewed by Jane Green to complete the pedigree and, after consent was obtained, medical records of living and deceased relatives were reviewed to identify affected family members and their first degree relatives at risk (Figure 3.1 - 1989). Genetic counselling was provided for affected and at-risk family members, including education about the clinical and genetic features of MEN-2, and the recommendation for annual clinical screening from the early teens. Information on MEN-2 was also sent to the family doctors.

Because of the variability in age at diagnosis, and the skipped generation in those already identified as "affected" in this family, a screening program was instituted for first and second degree relatives of affected patients, and for first degree relatives of deceased family members who could have been affected based on family history or medical record review. The screening started at age

Figure 3.1. Initial (1989) and current (1993) pedigrees of a Newfoundland MEN-2 family.

Clinical status of family members, as known in 1989 or in 1993, is indicated on the respective pedigrees.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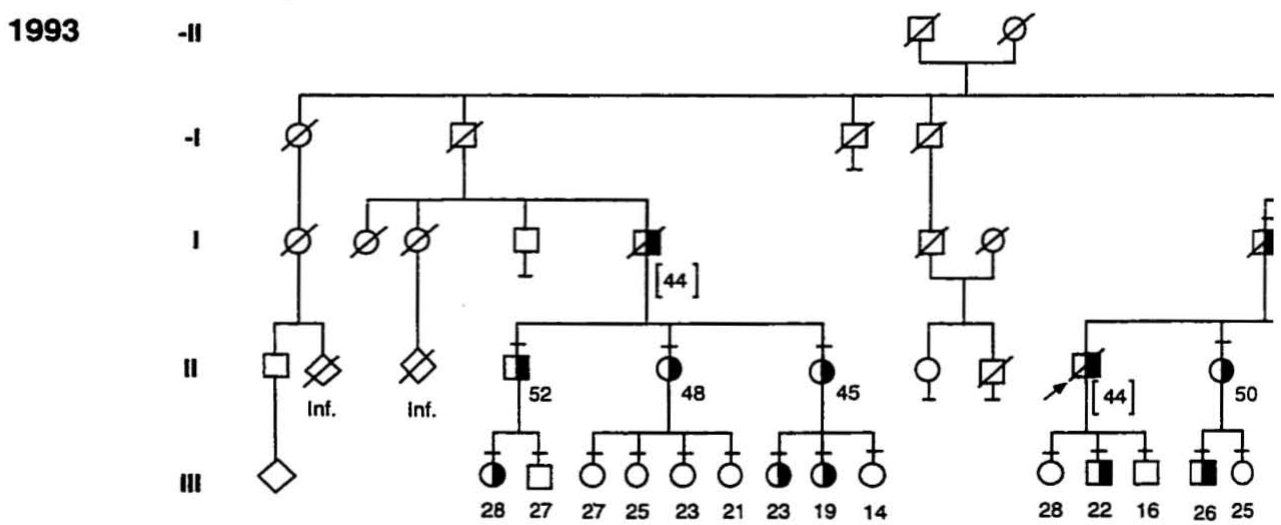
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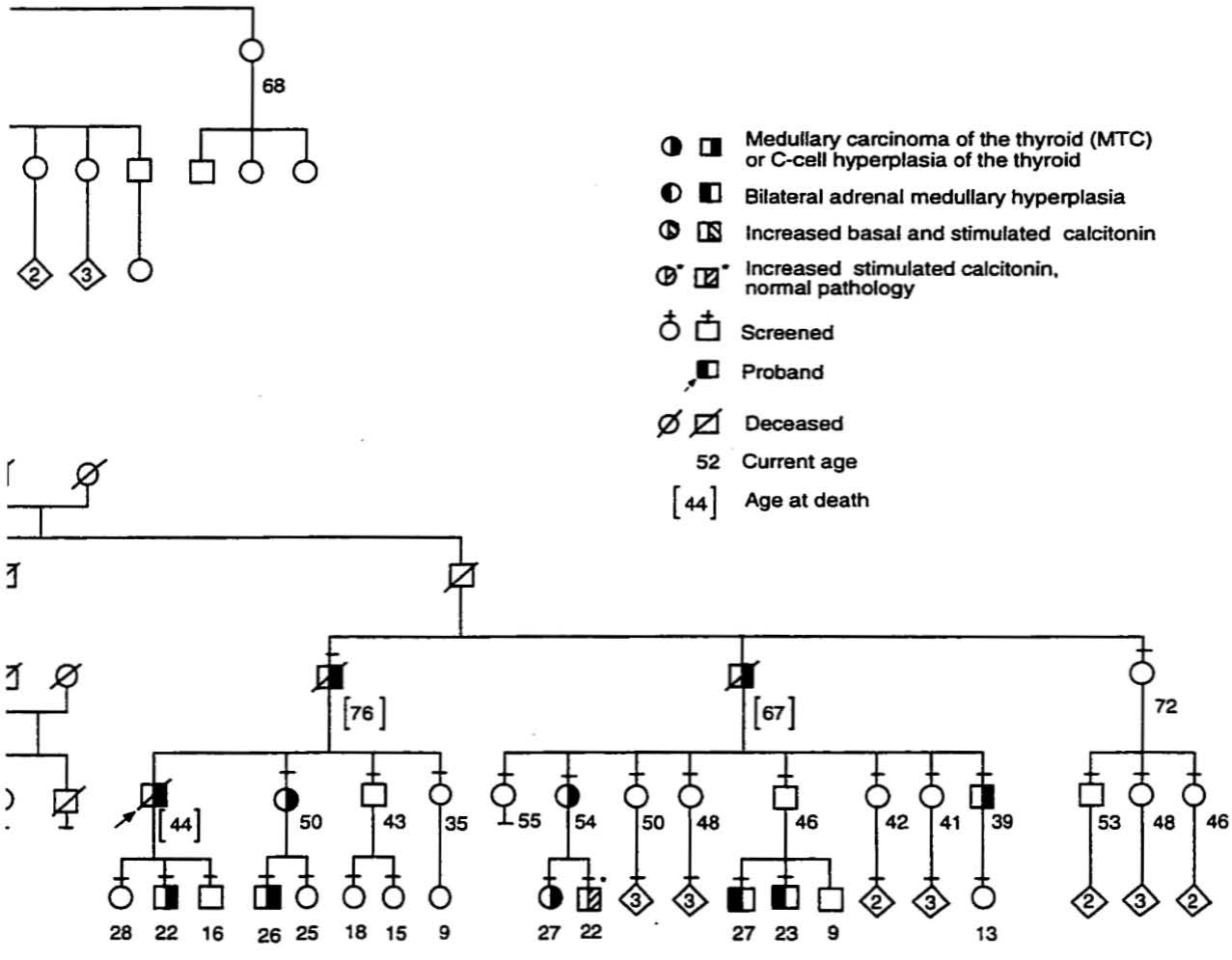
ype 2 in a Newfoundland family



11 and included annual calcium and pentagastrin-stimulated calcitonin testing at the Outpatient Department (OPD) of the General Hospital in St. John's, and annual collection of a 24-hour urine sample for measurement of catecholamines and metanephrines (Figure 3.2).

Appointments were booked with the OPD nurse, and patients contacted about their appointments. When testing was completed, results were reported to patients and their family doctors. Repeat testing and referral to the Endocrine Service was arranged if results were abnormal, and a 1-year recall appointment scheduled if results were normal. Patients with confirmed abnormal stimulated calcitonin or urinary catecholamine levels were referred to the Department of Surgery for pre-operative management and surgery. Annual screening resumed following surgery. Radiological, surgical and pathology reports were reviewed, and age at diagnosis, present age or age at death, and current status, including outcome of surgery, were recorded for all family members.

Later, a computerized system of recall, booking, and reporting of screening investigations was developed by Dr. Marshall Godwin and managed by the Family Practice Unit at the General Hospital. As more family members were identified as affected, the screening was extended to include first degree relatives of these newly identified patients.

Figure 3.2. Clinical screening protocol for multiple endocrine neoplasia type 2 (MEN-2).

CLINICAL SCREENING PROTOCOL FOR MULTIPLE ENDOCRINE NEOPLASIA, TYPE 2 (MEN-2)

1. ANNUAL CALCIUM AND PENTAGASTRIN-STIMULATED CALCITONIN LEVEL*

   Normal values

Basal calcitonin	N < 15pmol/L
Peak calcitonin	N < 30pmol/L

   if confirmed abnormal,

   i) rule out pheochromocytoma
   ii) total thyroidectomy

2. ANNUAL 24-HOUR URINE CATECHOLAMINE AND METANEPHRINE LEVEL*

   Normal values

Urinary catecholamines	N < 700nmol/d
Urinary metanephrines	N < 7μmol/d

   if confirmed abnormal,

   i) abdominal CT scan and MIBG scan
   ii) if pheochromocytoma(s) identified
      — alpha-adrenergic blockade
      — unilateral or bilateral adrenalectomy

* beginning at age 11

Genetic Screening

In 1990-1991, genetic counselling regarding predictive testing by linkage analysis was given to 34 family members known to be at risk and over 12 years of age. Consent was obtained from those requesting predictive testing (and from their parents if less than 18-years-old), and blood samples were obtained for DNA extraction in the laboratory of Dr. Roger Green. Aliquots of DNA from affected and at-risk family members and from unaffected spouses were sent to Dr. Nancy Carson at the Molecular Diagnostic Testing Laboratory at Queen's University, Kingston, Ontario, for linkage studies with RFLP markers in the centromeric region of chromosome 10 (FNRB, D10S34, D10Z1, D10S94, RBP3 [Goodfellow et al 1990, Narod et al 1992]).

In 1992-93, DNA samples from eleven consenting at-risk members of a recently identified branch of the family were sent to Queen's University for PCR-based linkage testing with the marker D10S94 which had been informative in this family. Although an informative flanking marker was not available, no recombination between D10S94 and MEN-2 has been identified in other families (Goodfellow et al 1990, Narod et al 1992). Results of linkage analysis with this marker were tested with the MLINK program of the LINKAGE package.

In 1994, DNA samples from three affected individuals were sent to Dr. Lois Mulligan at Queen's University, to search for the mutation.

Impact of Screening on the Family

There has been extensive contact in person or by telephone with all family members over the five years of the screening program, during counselling sessions and while arranging appointments or reporting results. Although formal interviews were not carried out, family members talked about the significance of the screening program to themselves and their children, and these comments were recorded.

RESULTS

Clinical Screening

i) Thyroid disease

a) Thyroid pathology

Sixteen members of this family have pathological evidence of thyroid disease (Figure 3.1 - 1993, p. 160), ten with medullary carcinoma (MTC) and six with C-cell hyperplasia (CCH). A seventeenth family member underwent thyroidectomy because of elevated stimulated calcitonin levels, but only normal thyroid tissue was identified (Table 3.1). The age range of those with CCH (18-75 years, median 38 years) overlapped that of the MTC patients (18-74 years, median 42.5 years).

b) Method of diagnosis

The proband, age 40, was symptomatic with a neck mass at the time of diagnosis and died at age 44, a 67-year-old was identified coincidentally at

TABLE 3.1. SURGICAL PATHOLOGY IN NINETEEN MEN-2 PATIENTS

PATHOLOGY	NUMBER OF PATIENTS	AGE AT DIAGNOSIS (years) Mean (range)
Thyroid gland		
Medullary carcinoma	10*	42.5 (18-74)
C-cell hyperplasia	6	38 (18-75)
"Normal"	1	19
Adrenal gland		
Adrenal medullary hyperplasia	2	19

* Two patients with metastatic disease
  — 1 symptomatic, age 40; deceased, age 44
  — 1 identified by screening, age 50; asymptomatic, age 53

autopsy, and 15 other asymptomatic family members, age 18-75, were identified by screening with calcium and pentagastrin-stimulated calcitonin testing (Table 3.2). Eight of those identified by initial screening had MTC, but only one, a 50-year-old woman who is still asymptomatic at age 53, has evidence of metastatic disease (markedly elevated basal and stimulated calcitonin post-operatively). Four others identified at initial screening, and two who converted from normal to elevated stimulated calcitonin levels, had CCH only, and in the final patient, identified on subsequent screening, no MTC or CCH was identified at pathology. Screening has identified those with the MEN-2 gene at an early stage when surgery is curative.

c) Age at diagnosis

The three affected members of the earliest generation were 67,74, and 75 years of age at diagnosis, identified either coincidentally, or by screening after their children were affected. Although all three are now deceased, none died of MEN-2. Seven of 15 at risk in the second generation are affected, with diagnosis at age 40-50 years of age; four with MTC (including the proband who died at age 44 of metastatic disease and a 53-year-old with biochemical evidence of metastatic disease), and three with CCH. Seventeen at risk in the youngest generation have been screened, and seven of these were identified as affected (age 18-26). Four had MTC confined to the thyroid, two had CCH only, and one had normal thyroid gland histology. There appears to be an

TABLE 3.2. METHOD OF IDENTIFICATION OF MEN-2 THYROID DISEASE (SYMPTOMATIC, COINCIDENTAL, OR BY SCREENING), AND PATIENT OUTCOME

METHOD OF IDENTIFICATION		NUMBER OF PATIENTS	AGE AT DIAGNOSIS	OUTCOME
Symptomatic		1	40	Deceased, age 44
Coincidental		1	67	Diagnosis at autopsy
Initial screening*		12		
	i)	1	50	Metastatic MTC (increased post-operative calcitonin)
	ii)	7	18-74	MTC confined to thyroid (normal post-operative calcitonin)
	iii)	4	36-47	C-cell hyperplasia (normal post-operative calcitonin)
Subsequent screening*		3		
	i)	2	18-25	C-cell hyperplasia (normal post-operative calcitonin)
	ii)	1	19	Normal thyroid tissue
TOTAL		17		

* elevated basal and/or stimulated calcitonin level

earlier age at onset with successive generations (Figure 3.3), however, this may be a bias of ascertainment.

ii) Adrenal Disease

Two family members, brothers, had a diagnosis of adrenal gland disease (Figure 3.1, p. 160); one presented with increased blood pressure and cardiac arrhythmias, and the second was identified by urinary catecholamine screening. Both underwent bilateral adrenalectomy because of elevated urinary catecholamines, and evidence from venous sampling or MIBG scanning of bilateral adrenal involvement. In each, the pathological diagnosis was adrenal medullary hyperplasia. Both patients have had normal calcitonin testing, and none of the patients with MTC or CCH (or any of those at 50% risk) have had evidence of adrenal involvement. The uncertainty of whether these brothers have MEN-2 creates problems with interpretation of the genetic testing.

Genetic Screening

i) Linkage analysis

The RFLP markers FNRB, D10S34, D10Z1, RBP3 were uninformative for the majority of family members (data not shown), however the marker D10S94 was informative in this family. All family members with MTC or CCH have the same allele with the marker D10S94, but the two patients with adrenal medullary hyperplasia (and their clinically normal father) do not have this allele (Figure 3.4). Are they crossovers, or is their adrenal disease coincidental? If

Figure 3.3. Age at diagnosis by decade, and thyroid pathology in an MEN-2 family.

Age at diagnosis of MEN-2 thyroid disease is extremely variable in this family. The age ranges of those with MTC (18-74 years), and with CCH only (18-75 years) overlap. Patients who are older at the time of diagnosis do not necessarily have more severe disease.

Age at diagnosis by decade, and thyroid pathology in an MEN-2 family

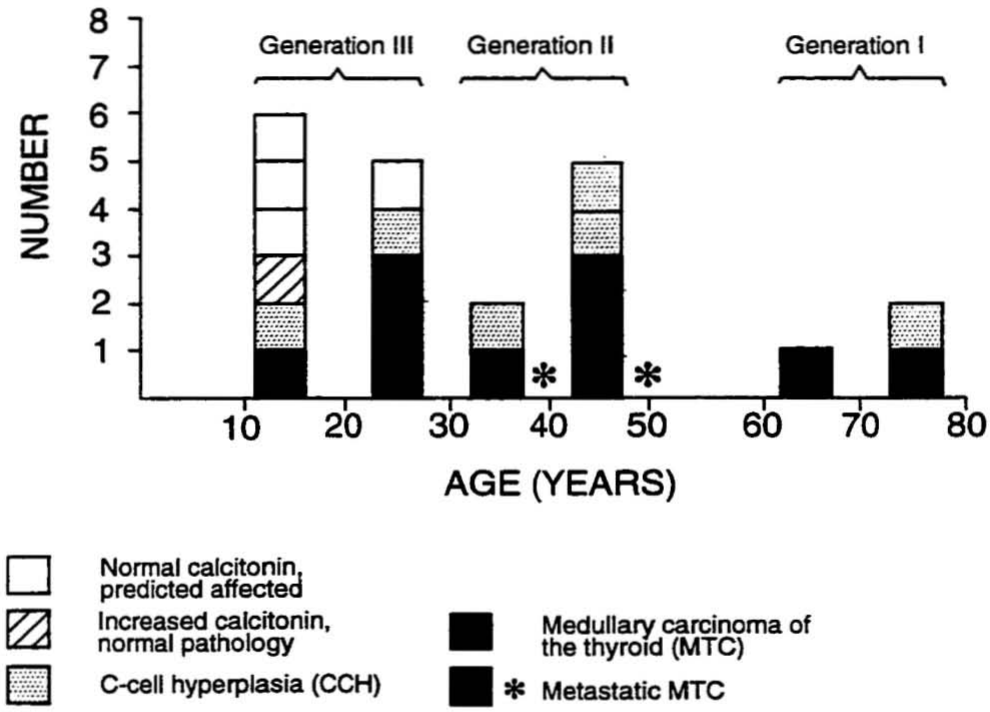
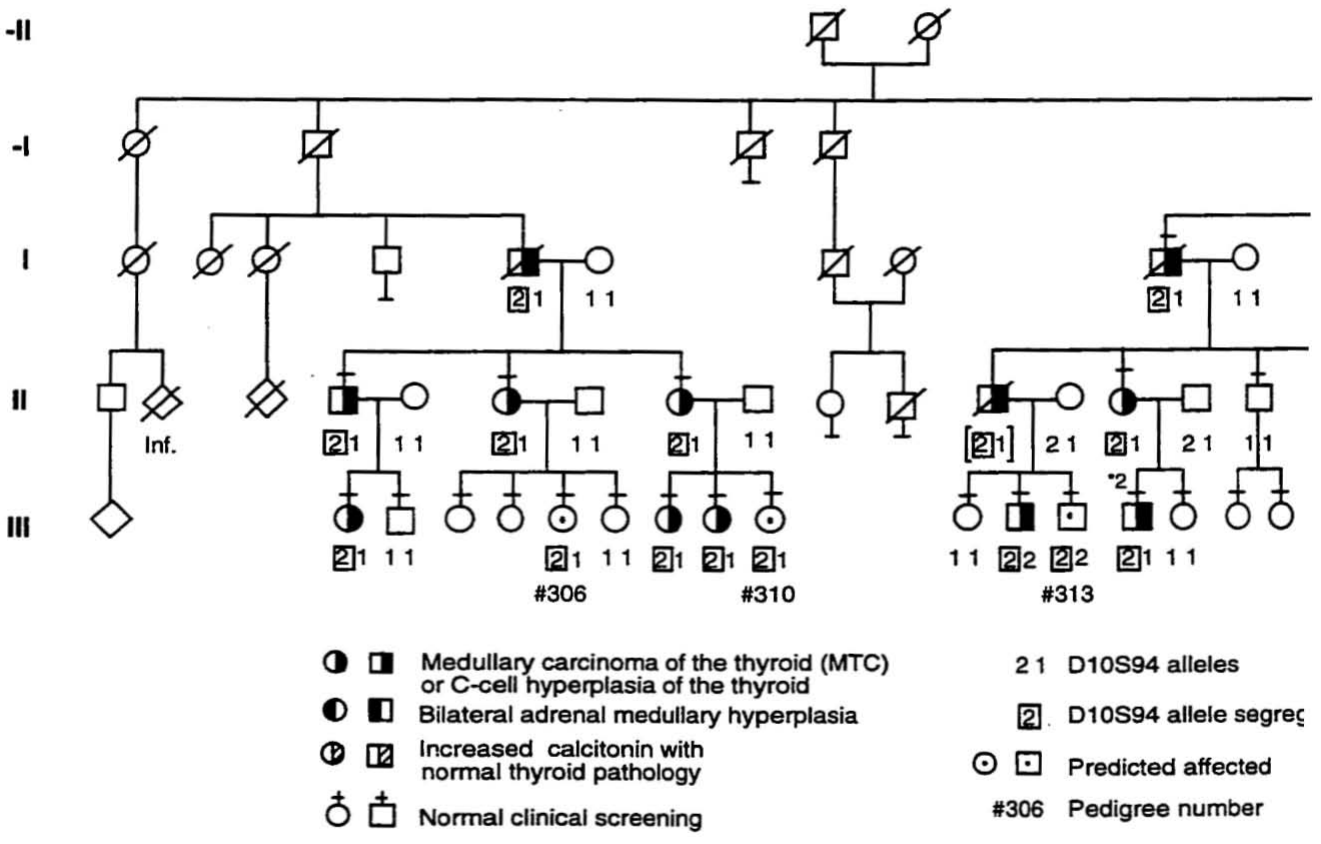


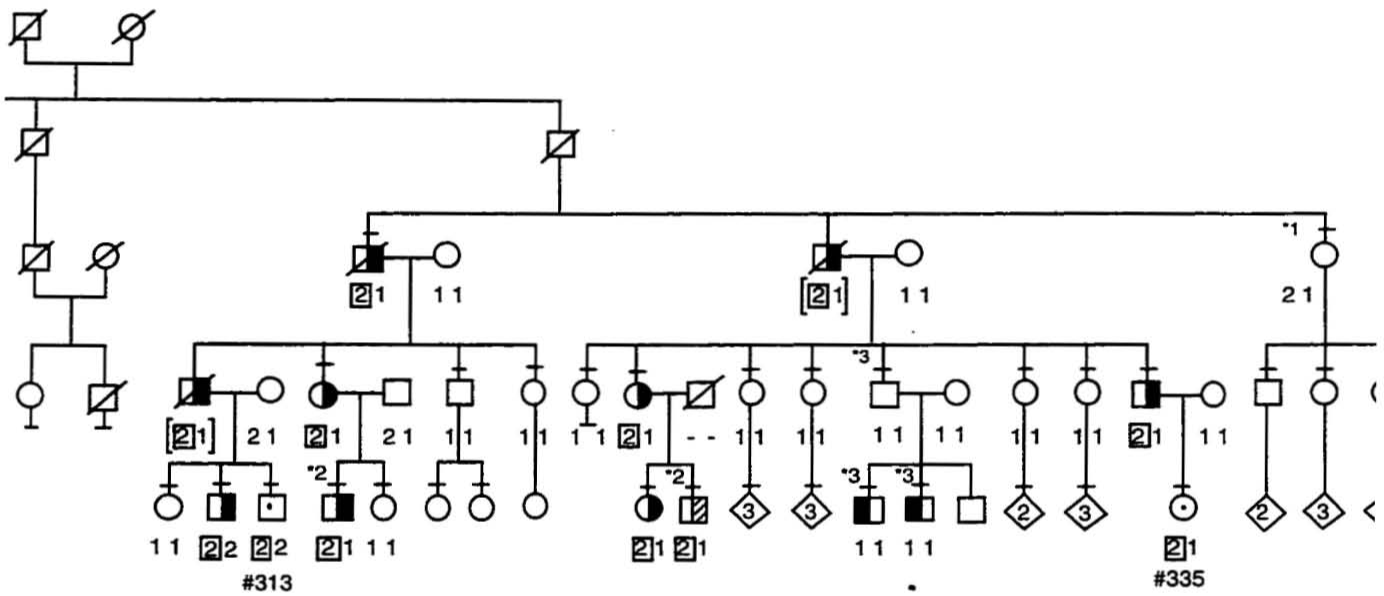
Figure 3.4. Newfoundland MEN-2 pedigree with results of linkage analysis.

The RFLP markers FNRB, D10S34, D10Z1, and RBP3 were uninformative in the majority of family members (data not shown). The marker D10S94 was informative. Allele "2" of D10S94 co-segregated with the presence of MTC or CCH (LOD score of 2.596 or greater, see text). The brothers with adrenal medullary hyperplasia did not inherit allele "2", and are predicted to be unaffected with MEN-2. Four family members, age 13-23, are predicted to be affected; all have had normal clinical screening results so far. The late age at diagnosis, and presence of thyroid pathology only in those with the high-risk allele of D10S94 suggest that this family has FMTC, not MEN-2A.

Newfoundland MEN-2 pedigree with results of linkage analysis



f linkage analysis



d (MTC)
d
olasia

2 1 D10S94 alleles

[2] D10S94 allele segregating with MEN-2

Predicted affected

#306 Pedigree number

*1 - flanking markers suggest a different "2" allele

*2 - consistent with maternal "2" allele based on other markers

*3 - low risk predictive testing results

these two family members are considered to be affected, the LOD score is 2.596 at $\theta = 0.1$; but if their status is classified as unknown, the peak LOD score is 3.608 at $\theta = 0.05$ satisfying the criteria for linkage to chromosome 10q11.2. The brothers would then be predicted to be at low risk of carrying the gene for MEN-2. Despite the evidence for linkage to the chr 10q11.2 region as in other MEN-2A and FMTC families, a mutation in the RET oncogene has not been identified in this family.

ii) Predictive testing

There are 20 clinically unaffected first degree relatives (age 9-64) in this family. Nineteen (all except the nine-year-old) have had regular clinical screening with negative results; 17 of these have had DNA extracted for predictive testing. Thirteen family members (age 18-64) have low-risk predictive testing results with the informative marker D10S94, and four family members (age 13-23) have high-risk results (Table 3.3a and 3.3b). Calcitonin values of those with high-risk results are within normal limits, but each has had detectable or high-normal basal or stimulated calcitonin levels (Table 3.4). This group will continue to have calcium and pentagastrin-stimulated calcitonin testing at 6 month intervals, and will be referred for thyroidectomy if the basal or peak calcitonin levels increase.

Because of the uncertainty of the status of the brothers with adrenal disease (or the cause of their bilateral adrenal medullary hyperplasia if not due to MEN-2), and the implications of this uncertainty to other family members,

TABLE 3.3a. IDENTIFICATION OF AFFECTED AND HIGH-RISK MEMBERS OF A NEWFOUNDLAND MEN-2 FAMILY BY CLINICAL SCREENING AND BY LINKAGE ANALYSIS

CATEGORY		NUMBER OF FAMILY MEMBERS
IDENTIFIED AFFECTED (1989) (thyroid disease — 3, adrenal disease — 1)		4
FIRST AND SECOND DEGREE RELATIVES AT RISK		52
PARTICIPATION IN CLINICAL SCREENING[1]		45
— POSITIVE CLINICAL SCREENING	15	
— high-risk genetic screening	(14)	
— low-risk genetic screening	(1)	
— NEGATIVE CLINICAL SCREENING	30	
PARTICIPATION IN GENETIC SCREENING[2]		46
— HIGH RISK	18	
— positive clinical screening	(14)	
— negative clinical screening	(4)	
— LOW RISK	28	
— (includes 2 brothers with "bilateral adrenal medullary hyperplasia")		
IDENTIFIED AFFECTED (1993) (thyroid disease — 17, adrenal disease [possibly coincidental] — 2)		19

[1] Stimulated calcitonin assay and urinary catecholamine assay
[2] Linkage analysis with chr 10q markers

TABLE 3.3b. SUMMARY OF INFORMATION ON IDENTIFICATION OF AFFECTED AND HIGH-RISK MEMBERS OF A NEWFOUNDLAND MEN-2 FAMILY BY CLINICAL SCREENING AND BY LINKAGE ANALYSIS

CATEGORY	NUMBER OF FAMILY MEMBERS
- High-risk genetic screening with positive clinical screening **(affected)**	14
- High-risk genetic screening with negative clinical screening **(presymptomatic)**	4
- Low-risk genetic screening with positive clinical screening **(false positive)**	2
- Low-risk genetic screening with negative clinical screening **(unaffected)**	26

TABLE 3.4. AGE AND SCREENING RESULTS OF THOSE PREDICTED AFFECTED BY LINKAGE ANALYSIS

PEDIGREE #	AGE	SEX	RECENT SCREENING RESULTS
306	23	F	Detectable basal and stimulated calcitonin*
313	16	M	Detectable basal and stimulated calcitonin*
310	14	F	Borderline stimulated calcitonin
335	13	F	Detectable stimulated calcitonin*

* values within normal limits

those with low-risk predictive testing results are continuing to be screened, at less frequent intervals, until the MEN-2 mutation is identified and their status can be clarified.

The ratio (4:13) of high-risk to low-risk family members identified by linkage analysis is not 50:50 because clinical screening had already identified 15 gene carriers.

Impact of Screening on the Family

Most members of the first two generations of this family lived in one small community at the time of the proband's illness, and were very aware of the diagnosis of "thyroid cancer" and the early death of the proband. They also knew the autopsy results of the proband's uncle (patient 2), and that the identification of a similar thyroid cancer marked this as a "family disease". While they knew that this was called MEN-2, they had found it very difficult to obtain information about the disease before the screening program was implemented.

The two affected members of generation I identified by screening were elderly and did not understand the implications of MEN-2, particularly since neither they nor the third affected member of generation I (patient 2 who was identified coincidentally at autopsy) had had any symptoms or clinical effect from MEN-2. In contrast, the widow of patient 2, now 75 years old, has a very

good understanding of all aspects of MEN-2 and provides a supportive role for children and grandchildren.

The fourteen living affected and at-risk members of generation II all appreciated both the information on MEN-2 provided during genetic counselling, and the organization of the screening program. The six identified as affected by the program were all very grateful for early diagnosis and treatment, since they did not experience symptoms of cancer nor require chemotherapy or radiation treatment, and since (except for one with biochemical evidence, only, of metastatic disease) they have a very good prognosis.

Four of these have children that have also been identified as affected and treated. Three of the four affected parents have stated that they regret having passed this disease on to their children, and one has specifically said that she would not have had children if she had known the risks.

The seventeen members of generation III originally identified as at risk, also appreciated the information they have received, and have been compliant with screening. In general, the seven now affected have accepted diagnosis and surgery as a "fact of life", but because they were young at the time their father/uncle/cousin was sick and died, they do not have such personal experience with the disease as their parents do. One of the seven, who was 26 and had small children at the time of her diagnosis, has more difficulty than the others with accepting the fact that she has a hereditary disease. None of the others have children, but three, who are married or engaged, say that the

MEN-2 gene will not alter their reproductive planning since they expect that the screening program will be available for their future children for early diagnosis and treatment of any thyroid disease.

DISCUSSION

Clinical Screening

MEN-2 is a potentially lethal hereditary disease with families frequently recognized because of symptomatic presentation or early death of one or more family members from medullary carcinoma of the thyroid, or pheochromocytoma (Vasen et al 1987). Once a family has been ascertained, clinical screening of first degree relatives at risk has allowed early identification and curative treatment of the thyroid and adrenal disease in many gene carriers (Vasen et al 1987, Gagel et al 1988, Calmettes et al 1992).

The goal of clinical screening programs in MEN-2 families (MEN-2A, MEN-2B or FMTC) is the presymptomatic identification of thyroid or adrenal involvement so that early treatment by total thyroidectomy (Wells et al 1985, Barry et al 1991, Decker 1992), or unilateral or bilateral adrenalectomy (Jansson et al 1988, Howe et al 1993, Lairmore et al 1993) can prevent the morbidity and mortality often seen with symptomatic disease. The elevation of calcitonin levels after stimulation with pentagastrin and/or calcium has been used to identify early MTC or C-cell hyperplasia, and the elevation of

catecholamines in a 24-hour urine collection has been used to identify presymptomatic pheochromocytoma, or adrenal medullary hyperplasia.

i) Timing of clinical screening

In classical MEN-2A families it is recommended that this screening begin at least by age 3 because of the very early age that metastatic MTC has been identified (Calmettes et al 1992). Clinical screening begins at age 11 in our family, however, since the earliest detection of CCH/MTC, even with prospective screening, has been at 18 years of age. This fits with recommendations for other hereditary cancers (eg, the hereditary colon cancers) where the recommended age to begin screening is five years before the earliest age at diagnosis in the family (Fitzgibbons et al 1987).

ii) Thyroid disease

As in previous studies in which the majority of symptomatic patients had incurable disease (Vasen et al 1987, Calmettes et al 1992), the proband in the Newfoundland family died of metastatic MTC within a few years of diagnosis. Patients identified at their initial screening may also have advanced disease which is difficult to treat, even if asymptomatic at the time of identification (Telander et al 1989). However, many of those with abnormal values at initial screening, and all with normal values converting subsequently to elevated calcitonin levels, have thyroid disease (either early MTC or the precursor stage CCH), that is curable surgically (Wells et al 1985, Vasen et al 1987, Decker 1992). One Newfoundland patient identified at initial screening has significantly

elevated calcitonin levels post-thyroidectomy, and, although asymptomatic, presumably has metastatic disease. Despite extensive investigations, the location of this metastatic disease has not been identified.

Nine patients identified at initial screening and three identified subsequently, all have had reversion to undetectable calcitonin levels post-operatively. The early detection and treatment of thyroid disease facilitated by the screening program has been potentially life-saving for these twelve individuals. Eleven of these had small MTC tumours, or nodules of CCH identified at pathology; in one patient, only normal thyroid tissue was identified.

This 18-year-old patient, with confirmed, but minimally positive, calcitonin stimulation testing and no CCH or MTC identified at pathology illustrates a problem of interpretation sometimes seen in such families — the possibility of false positive tests (Landsvater et al 1993, Barbot et al 1994). This patient did, concurrently, have genetic evidence from linkage analysis that he was a gene carrier and so most likely would have progressed to malignancy, however, members of other families in the literature have had conflicting clinical and genetic screening results (Landsvater et al 1993). Some patients have been identified by abnormal calcitonin test results, and have had CCH identified after total thyroidectomy, but later by linkage studies or direct mutation testing have been shown not to carry the MEN-2 gene (Barbot et al 1994, McMahon et al 1994).

Although presymptomatic thyroid C-cell disease can be identified by a positive pentagastrin stimulation test, it is not possible clinically or biochemically to distinguish early MTC from the precursor stage CCH (Wells et al 1985). For this reason, total thyroidectomy is recommended as soon as a positive pentagastrin stimulation test is obtained (Decker 1992, Telander et al 1989). The recognition that MTC can metastasize when very small emphasizes the need for early thyroidectomy (Telander et al 1989, Calmette et al 1992). However, there is not a clear cut-off between normal and abnormal calcitonin values. The concern is that by setting a low threshold level for abnormal stimulated calcitonin values to increase the sensitivity of the screening test, the specificity is decreased, and some patients may have inappropriate thyroidectomy. At the lower limits of the assay, elevations of calcitonin may result from greater variation in normal values than expected (Simpson et al 1990, Kempter & Ritter et al 1991), or from C-cell hyperplasia unrelated to the MEN-2A or FMTC gene (Libbey et al 1989, Landsvater et al 1993, Lips et al 1994).

The possibility of directly identifying gene carriers in many MEN-2A or FMTC families (Donis-Keller et al 1993, McMahon et al 1994, Mulligan et al 1994, Tsai et al 1994, Xue et al 1994), because of the clustering of mutations in a group of cysteine residues at the transmembrane boundary of the extracellular domain of the RET protein, and in MEN-2B (Carlson et al 1994, Eng et al 1994a, Hofstra et al 1994) where the mutation in the majority of families

studied has been at codon 918 in the tyrosine kinase domain of this protein, will reduce the risk of inappropriate thyroidectomy or adrenalectomy since in informative families only gene carriers will be clinically screened, or proceed directly to surgery (Barbot et al 1994, Lips et al 1994, McMahon et al 1994, Tsai et al 1994).

iii) Adrenal disease

There has been less discussion of false positive values in screening for adrenal medullary disease. However, this was a concern in our family. About 50% of MEN-2A gene carriers are predicted to have unilateral or bilateral adrenal involvement with pheochromocytoma or adrenal medullary hyperplasia, and this may precede thyroid disease. Large families with MTC only (Noll et al 1984, Farndon et al 1986) have been recognized, but the distinction between MEN-2A and FMTC cannot be made in small families. Genetic testing also will not differentiate these two conditions since identical mutations of the RET proto-oncogene have been found to cause either MEN-2A or FMTC in different families (Donis-Keller et al 1993, Mulligan et al 1994, Xue et al 1994). Because of the dangers of surgery with an unrecognized pheochromocytoma, and the variability in frequency of pheochromocytomas (from 5% — 75%) in different MEN-2A families (Narod et al 1989, Narod et al 1992, Xue et al 1994), screening for pheochromocytoma is recommended for all families (except when there is a very large family with thyroid disease only).

The Newfoundland family was originally thought to be a typical MEN-2A family with thyroid and adrenal disease. After symptomatic and coincidental presentation of MTC in the initial two affected members, the first patient identified by screening in this family had increased urinary catecholamines confirmed on several occasions. On further investigation, there was a bilaterally positive MIBG scan with a negative CT scan, these results being compatible with adrenal medullary hyperplasia. Genetic testing to identify or exclude gene carriers was not yet possible and the patient underwent bilateral adrenalectomy. The pathology report indicated bilateral adrenal medullary hyperplasia, a recognized precursor of pheochromocytoma in MEN-2A (Carney et al 1975). Subsequently, this patient's brother, also at age 19, had similar clinical test results and also had a bilateral adrenalectomy.

No other persons with adrenal disease have been detected in this family, either in those at risk, or in the seventeen members with documented MTC or CCH, despite the fact that all affected and at-risk family members have annual 24-hour urine catecholamine testing. However, if the increased catecholamines in the brothers had been due to stress or interfering substances, there should not have been radiological or nuclear medicine evidence of adrenal disease. The genetic linkage studies, when available, also conflicted with the clinical screening evidence since these brothers did not inherit the same chromosome 10q marker allele as affected family members. This raised the question of whether there had been a cross-over between the MEN-2 gene and the marker,

but recombination in males in the centromeric region of chromosome 10 has not been observed (Goodfellow et al 1990, Narod et al 1992).

The possibility of genetic heterogeneity was considered, but there is no strong support in the literature for non-linkage of "MEN-2" to chromosome 10q11.2 markers. Also, there is now evidence of linkage of the thyroid disease in this family to the marker D10S94 near the RET proto-oncogene (LOD score of 3.608 at $\theta = 0.05$), although the mutation within the RET proto-oncogene for this family has not yet been identified. The Newfoundland family, in fact, may have FMTC (with medullary carcinoma of the thyroid or C-cell hyperplasia only), not MEN-2A. The late age at onset of thyroid disease (mean age of diagnosis of 39.4 years in screened family members, with the youngest diagnosis at age 18) also supports the classification of this family as FMTC not MEN-2A. The adrenal medullary hyperplasia may then be unrelated. There is no evidence, however, for another genetic disease such as von Hippel-Lindau disease or neurofibromatosis, type 1 predisposing to pheochromocytoma or adrenal medullary hyperplasia in these brothers or their relatives, yet the bilateral disease in two brothers suggests a hereditary etiology.

Prophylactic bilateral or unilateral adrenalectomy is considered inappropriate by most clinicians even in clearly identified MEN-2A families (Lairmore et al 1993, Utiger 1994), because of the risks associated with long-term adrenal replacement therapy (Jansson et al 1988), because not all MEN-2A patients will develop adrenal disease, and because regular biochemical

screening combined with modern scanning techniques can, in most situations, identify adrenal glands with hyperplasia or pheochromocytoma when adrenalectomy is necessary.

Genetic Screening

The variable age at onset of MEN-2, and the potential false positive or false negative results of biochemical screening tests, emphasize the importance of genetic testing whenever possible to identify gene carriers (Barbot et al 1994, Xue et al 1994, McMahon et al 1994). The specificity of the location of the mutations in the RET proto-oncogene in the majority of MEN-2A, FMTC and MEN-2B families, increases the probability of identifying the mutation. The Newfoundland family, however, is one of the few large MEN-2A or FMTC families, where linkage to chromosome 10q is demonstrated but the specific mutation has not been identified.

Even though gene carriers cannot be directly detected in this family, the results of predictive testing by linkage analysis provide guidance in determining which family members require annual clinical screening, and which require less frequent screening. The four family members with high-risk linkage results have all had detectable or borderline stimulated calcitonin levels so will have pentagastrin stimulation testing at six-month intervals, and will be referred for thyroidectomy if the basal or peak calcitonin levels increase.

Because of the uncertainty of the status of the brothers with adrenal disease (or the cause of their bilateral adrenal medullary hyperplasia if not due to MEN-2), and the implications of this uncertainty to the family members, the 13 family members with low-risk linkage results will have screening every two years until the MEN-2 mutation in this family is identified, and their status clarified.

The ratio of high-risk to low-risk family members identified by linkage analysis is not 50:50 because clinical screening had already identified 15 gene carriers.

Impact of Screening on the Family

In the Newfoundland MEN-2 family there has been reduced anxiety, and very good compliance with the clinical screening program because of the recognized benefits of early treatment, even though it has not yet been possible to definitely identify non-carriers of the MEN-2 gene and exclude them from screening. There will be savings in time, money (for the investigations, and for travel and lost days at work), and from the discomfort of the pentagastrin stimulation test when screening is no longer required for those who are not gene carriers.

Implications of Screening for Late-Onset Genetic Disease

Questions have been raised about the appropriateness of predictive testing for "late-onset" genetic diseases, and, if appropriate, the age at which this testing should be done (Harper and Clarke 1990). For the hereditary tumour syndromes, including MEN-2A/ FMTC, which are potentially lethal but also potentially treatable and possibly curable, clinical screening of those at risk, to identify presymptomatic disease, is desirable (Vasen et al 1987). However, for autosomal dominant diseases, clinical screening of all first degree relatives at 50% risk can be costly in monetary and psychological terms (Eng et al 1994b).

With the identification of mutations in the RET proto-oncogene in the majority of MEN-2A/FMTC families, predictive testing by direct mutation detection or by linkage studies will frequently permit the distinction of gene carriers who would benefit from clinical screening and management, and those who are not gene carriers and therefore require no screening (Utiger 1994). If the group without the MEN-2 mutation no longer require clinical screening, this would result in considerable savings to the health care system, as well as financially and psychologically to the individual family members (Gagel 1993, Eng et al 1994b). Gene carriers and non-carriers would both benefit from knowledge of their status when making reproductive decisions.

It is now clear that MEN-2A and MEN-2B are not "adult-onset" diseases — the mean age at diagnosis in prospectively studied families is 10 years of age (Easton et al 1989). Metastatic MTC has been identified at 6 months of

age in MEN2-B and at 3 years of age in MEN-2A. Large FMTC families with late age at onset (mean 40 years) and slowly progressive disease in the majority of affected members have been described (Farndon et al 1986, and possibly the Newfoundland family), but other large families with FMTC and early aggressive disease have also recently been documented (Xue et al 1994). If clinical screening for MEN2-A and MEN2-B (which is acknowledged to be unpleasant, and is costly to the health care system and to family members) is recommended to begin by 3 years of age by many clinicians, and by 1 year of age by others (and by early teens even for FMTC families) predictive testing should be available before this age (Gagel 1991). Clinical screening or immediate prophylactic surgery can then be reserved for those identified by mutation detection or linkage studies to be at high risk (Gagel et al 1993, Eng et al 1994b). If genetic testing is uninformative in the family or a family member chooses not to participate in genetic testing, then clinical screening should still be available (Gagel et al 1993). Whether prenatal diagnosis should be offered (or is desired for a treatable condition) is a different question, but members of the Newfoundland family were not interested in this option.

Recommended Management for MEN2a/FMTC Families

The most efficient screening for MEN-2A/FMTC families would begin with a search for a mutation within the cysteine codons of the transmembrane domain of the RET proto-oncogene (codons 609, 611, 618, 620, 634) where

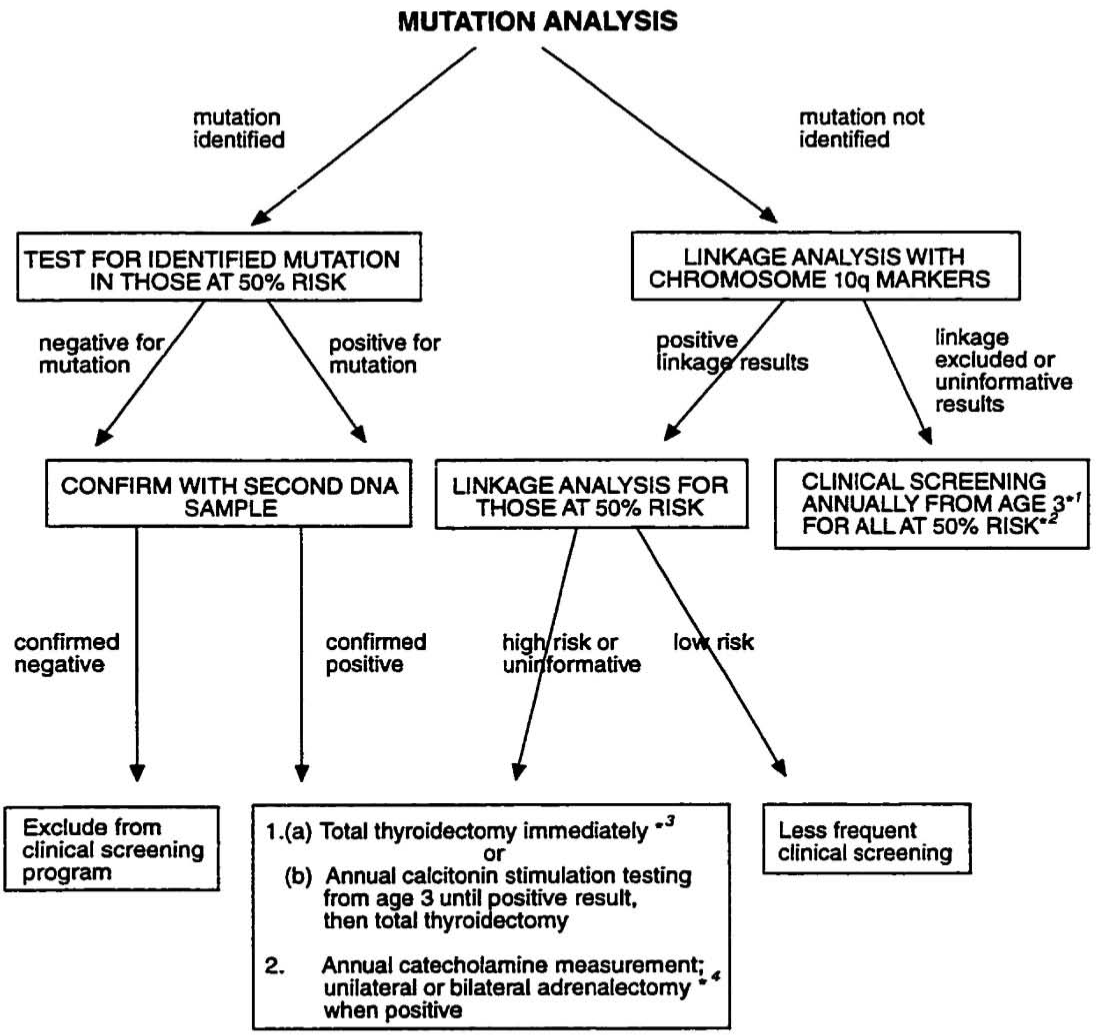
mutations have been identified in approximately 85% of families. If a mutation is found, gene carriers can be identified directly, and clinical screening or immediate surgery arranged for these individuals. Those without the mutation would not require clinical screening. If a mutation is not identified, then linkage analysis using 10q centromeric markers should be utilized, first to clarify linkage to this region, then to predict those who are carrying the MEN-2A/FMTC gene. Those with high-risk results, all first degree relatives of an affected person if linkage analysis is uninformative, and anyone not wishing to participate in genetic screening, would be offered clinical screening annually from childhood. Persons predicted to be at low risk would require less frequent clinical screening (Figure 3.5). With the appropriate combination of genetic and clinical screening, medical care can be maximized, and costs (monetary and psychosocial) can be minimized.

Figure 3.5. Management plan for MEN-2A/FMTC families.

The mutation has been identified in >90% of MEN-2A families,and in >80% of FMTC families. No MEN-2 families have been identified with confirmed linkage to another locus.

Annual clinical screening from age 3 or earlier is recommended for MEN-2A families, and for MEN-2 families not yet classified. For a large MEN-2 family with late age at onset (particularly an FMTC family), clinical screening may begin later, but at least 5 years before the earliest age at diagnosis in the family. For a well-documented FMTC family, annual catecholamine measurement may be excluded.

Management plan for MEN2A/FMTC families



**1 for large family with late age at onset, clinical screening to begin 5 years before earliest age at diagnosis*

**2 total thyroidectomy and/or unilateral or bilateral adrenalectomy as required*

**3 exclude pheochromocytoma before surgery*

**4 alpha-adrenergic blockade prior to surgery*

CHAPTER 4 — CLINICAL AND GENETIC SCREENING IN A FAMILY WITH ATYPICAL FAMILIAL ADENOMATOUS POLYPOSIS

INTRODUCTION

PATIENTS AND METHODS

Ascertainment of Family, and Medical Record Review

Clinical Screening

Genetic Screening

RESULTS

Ascertainment of Family

Clinical Phenotype

i) Number of polyps
ii) Site of polyps
iii) Natural history and course of disease
iv) Upper gastrointestinal neoplasm
v) Associated features

Genetic Screening

i) Identification of family members at risk
ii) Linkage analysis
iii) Predictive testing
iv) Unexpected results
v) Delivery of results

DISCUSSION

Clinical Screening

i) Screening for typical FAP
ii) Screening for atypical FAP
iii) Extracolonic manifestations

Genetic Screening

Implementation and Acceptance of the Screening Program

INTRODUCTION

This chapter describes the ascertainment of a Newfoundland family with familial adenomatous polyposis (FAP), the recognition of an atypical natural history of disease and the revisions of the clinical screening protocol necessitated by this clinical phenotype, the development of genetic screening, and a comparison of the impact and acceptance of the combined screening program by two geographically separated branches of the family.

FAP is an autosomal dominant condition characterized by colon cancer preceded by multiple bowel polyps typically concentrated in the sigmoid colon and rectum (Bulow 1986, Rustgi 1994). Traditionally the diagnosis has required the presence of greater than 100 polyps and often thousands are present (Bussey 1975, Bulow 1991). These polyps usually develop in the teens, with the majority of FAP patients being positive by the end of the third decade. The polyps progress to malignancy, often without symptoms, FAP patients typically developing colon cancer in their 40s or 50s — at a significantly younger age than patients with sporadic colon cancer (Bussey 1987, Rustgi 1994). Before the value of screening was recognized, the majority of FAP patients died of colorectal cancer (Bussey 1975, Arvanitis et al 1990a, Bulow 1991). With appropriate screening and early treatment, the incidence of cancer and the mortality caused by FAP can be markedly reduced (Vasen et al 1990a, Jarvinen 1992, Rhodes and Bradburn 1992).

Because of the characteristic age at onset and location of polyps in FAP, the usual screening protocol recommended for those at risk is a flexible sigmoidoscopy every one to two years from the early teens until the mid thirties, then every 3-5 years until at least 50 years of age (Berk et al 1987, Herrera et al 1990, Jarvinen 1992). The identification of those at risk is initially determined by the pedigree but is modified if possible by genetic testing (Petersen et al 1991, Maher et al 1993, Petersen et al 1993).

In some families originally designated as Gardner's syndrome, extra-colonic features have also been described, including osteomas, epidermoid cysts, dental abnormalities, desmoid tumours (Naylor and Gardner 1977, Jagelman 1987), and retinal pigmentation (congenital hypertrophy of the retinal pigment epithelium or CHRPE [Lewis et al 1984, Baba et al 1988, Berk et al 1988b, Romania et al 1992]) which may provide early evidence of the presence of the FAP gene.

Once multiple polyps have been identified, allowing a clinical diagnosis of FAP to be made, sub-total colectomy is recommended (Berk et al 1988a, Skinner et al 1990, Jarvinen 1985) to prevent the development of colon cancer and thus to improve survival. Because FAP patients are also at risk for adenomatous polyps and cancer of the segment of rectum remaining after the usual surgery, and of the upper gastrointestinal (GI) tract (particularly the duodenum) (Jarvinen et al 1983, Sarre et al 1987, Jagelman et al 1988, Lynch et al 1993b), continued follow-up with sigmoidoscopy of the rectal stump

(Sarre et al 1987), and with upper GI endoscopy (Arvanitis et al 1990, Bulow 1991) is recommended. If only a partial colectomy has been done then surveillance of the remaining colon is also necessary.

In 1987 the gene for FAP was mapped to the long arm of chromosome 5 (Bodmer et al 1987, Leppert et al 1987, Solomon et al 1987) and by 1991 predictive testing by linkage analysis was available for those at risk in some families (Tops et al 1989, Burn et al 1991, Dunlop et al 1991, Petersen et al 1991, Macdonald et al 1992). In 1990, a Utah family was described by Leppert with autosomal dominant colon cancer and a variable number of polyps (from a few polyps to florid polyposis) that was also linked to the 5q21 markers (Leppert et al 1990). This disease presentation was later designated atypical FAP, attenuated adenomatous polyposis coli (Spirio et al 1992), or flat adenoma syndrome (Lynch et al 1992).

In 1991 the adenomatous polyposis coli (APC) gene, responsible for FAP, was identified and cloned (Groden et al 1991, Joslyn et al 1991, Kinzler et al 1991, Nishisho et al 1991). Mutations within the APC gene have been identified for typical FAP, for Gardner's syndrome, and for some families with the atypical FAP presentation (eg, Miyoshi et al 1992, Spirio et al 1992, Olschwang et al 1993a). The variability of disease expression is not entirely explained by allelic heterogeneity, however, since the same base change has been identified in patients originally described as either FAP or Gardner's syndrome (Nishisho et al 1991, Groden et al 1993, Paul et al 1993).

The APC gene is very large with 15 exons spanning greater than 8.5kb, the 15th exon itself being over 6500 nucleotides in length (Groden et al 1991). Although the mutations cluster at the 5' end of exon 15 and some mutation hot spots have been identified (Miyoshi et al 1992, Groden et al 1993, Mandl et al 1994), the majority of families have unique mutations, and up to 30% of FAP patients have a new mutation (Rustin et al 1990, Maher et al 1993). Thus, identification of the specific mutation within a family in order to allow direct identification of gene carriers has only been successful in about 30-60% of families in most studies (Fodde et al 1992, Miyoshi et al 1992, Olschwang et al 1993a).

In this rapidly evolving field, it is necessary to consider carefully the approach to screening and management for a particular familial polyposis family. In some families, particularly those that fit the criteria for typical FAP, either direct mutation analysis for identification of gene carriers, or linkage analysis for predictive testing is possible. Mutation-positive or high-risk individuals are then followed throughout life with a clinical screening protocol and surgical intervention as appropriate, and relatives shown to be mutation-negative or at low risk are freed from the need for follow-up, or require less frequent screening, respectively. In other situations, particularly for small families or where linkage to 5q21 cannot be proven, linkage analysis or direct mutation detection may not be possible, and the clinical screening protocol is the only screening available for identification of gene carriers, as well as for

clinical management. This clinical screening must be appropriate for the disease presentation in the family if it is to identify a premalignant stage of disease when treatment is most likely to be successful.

PATIENTS AND METHODS

Ascertainment of Family, and Medical Record Review

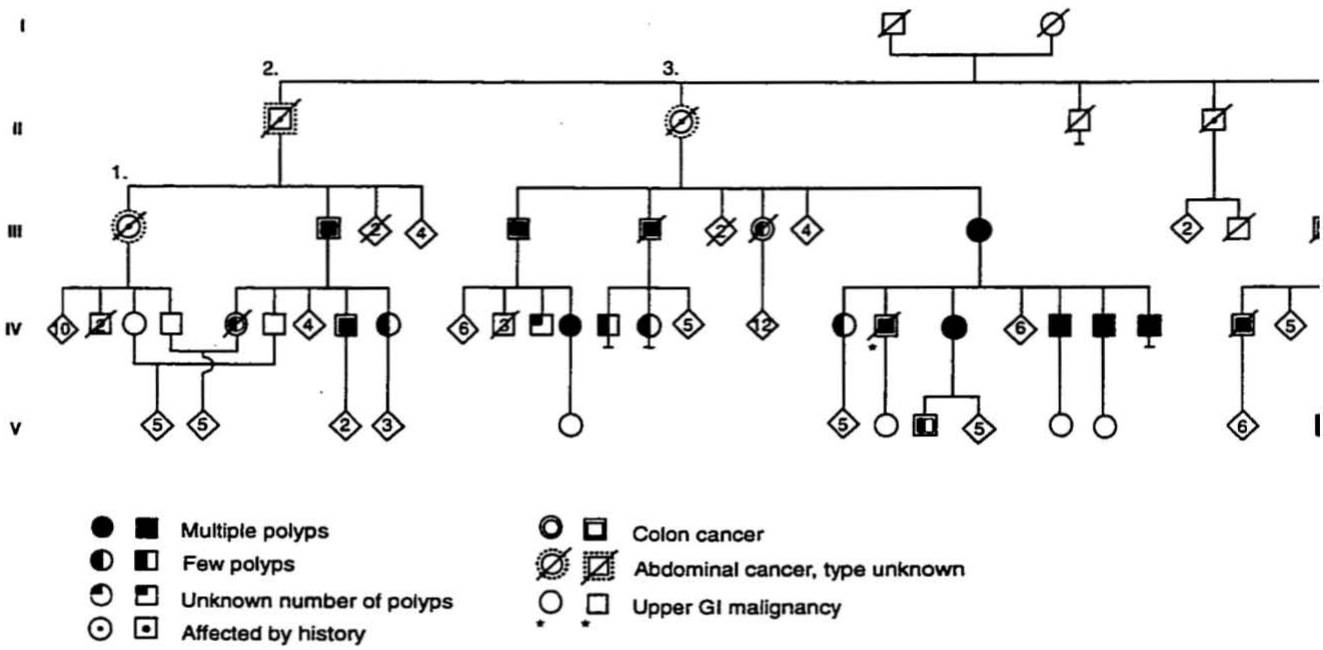
In 1985, a large family from the Notre Dame Bay area of Newfoundland was identified by the family doctor as probably having FAP after several related patients presented with colon cancer and multiple polyps. An initial pedigree was drawn, screening with annual sigmoidoscopy was initiated and a request was made to Medical Genetics in 1989 for assistance in the development of an overall management plan for the extended family.

Family members were interviewed by Jane Green and an extended pedigree was drawn (Figure 4.1). Medical records were reviewed in order to confirm the diagnosis in apparently affected members of previous generations. Several family members were noted to have colon cancer but fewer than the expected number of polyps for a diagnosis of FAP. Consent was obtained from family members, and a more detailed review of all medical records was undertaken in 1991 with particular attention to the surgical and pathological records and the reports of screening investigations. The number and location of polyps, location of cancer, age at onset of bowel-related symptoms, and age at diagnosis of polyps or cancer were recorded. The outcome of surgery, the

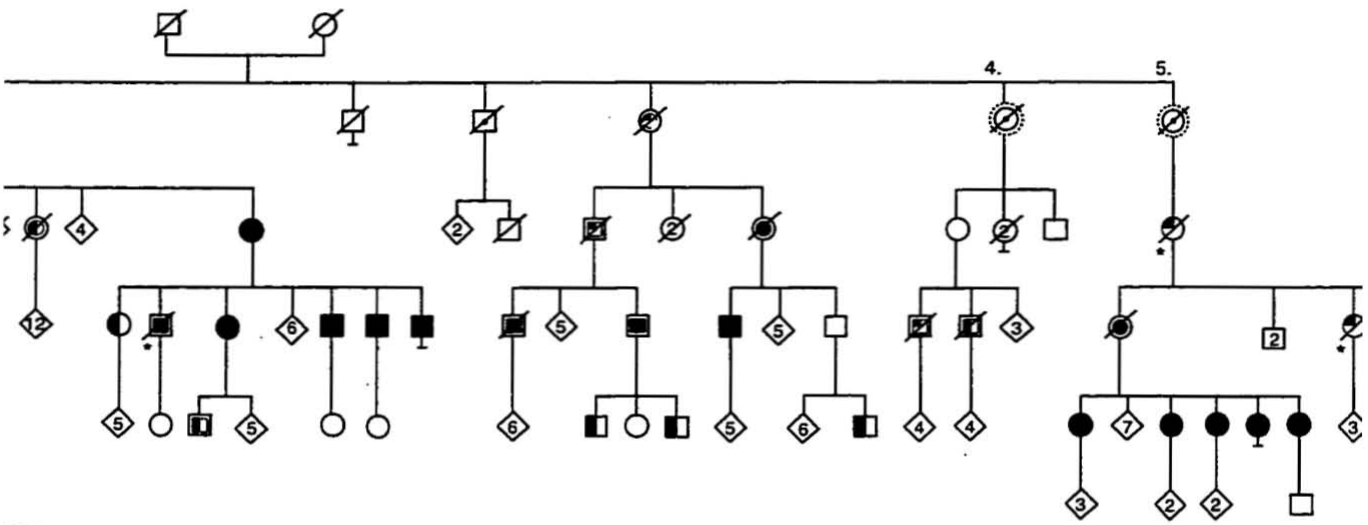
Figure 4.1. Pedigree of a Newfoundland family with atypical familial adenomatous polyposis.

The pedigree has been simplified to emphasize those with FAP (polyps ± colon cancer). The number of polyps, and presence or absence of colon cancer are indicated for each family member.

Newfoundland family with atypical familial adenomatous polyposis



ıl adenomatous polyposis



ıcer

ıl cancer, type unknown

malignancy

development of subsequent manifestations, and the age and cause of death were also documented.

Clinical Screening

Because of the right-sided location of polyps in many family members, recommendations were made for use of colonoscopy instead of sigmoidoscopy for screening. Screening was offered to newly identified at-risk family members including a branch of the family in central Newfoundland. Advice was given on establishing a registry of affected and at-risk family members at the Notre Dame Bay Regional Hospital in Twillingate, and, for the central Newfoundland branch of the family, at the Hugh Twomey Health Care Centre in Botwood.

An Ophthalmology clinic was organized at the Notre Dame Bay Regional Hospital in 1991, and 50 affected and at-risk family members were examined by Dr. Craig Beattie with direct and indirect ophthalmoscopy for evidence of CHRPE. The group examined included nine already affected family members but the status of each individual was not known by the ophthalmologist at the time of examination. At the same clinic an information session was provided by Jane Green on the characteristics of the disease in the family, the inheritance pattern, the recommended clinical screening, and the possibility of genetic screening by linkage analysis if the location of the gene could be confirmed. Blood samples were obtained for DNA extraction from consenting family members including those at risk, affected family members, and spouses.

Genetic Screening

Because of the atypical clinical expression of disease, linkage studies with DNA from known affected and older screening-negative family members were carried out with chromosome 5q markers linked to the APC gene before predictive testing was attempted. The RFLP markers used initially (pi 227 and YN5.48) were uninformative because of homozygosity of alleles in many affected family members. However a CA repeat marker (D5S346) which is 50-70 kb from the APC locus became available (Spirio et al 1991, Spirio et al 1993a), and linkage was demonstrated to this informative marker. One hundred and thirty first degree relatives at 50% risk were then identified from the pedigree.

Predictive testing by linkage analysis was offered to those at 50% risk, and in some circumstances to those at 25% risk, eg, if a parent at 50% risk was deceased or otherwise unavailable for testing, or if the parent had never been screened clinically and for geographic reasons it was easier to collect and test both samples simultaneously. Ninety-six family members were interested in participating in this genetic testing. Blood samples were collected from any remaining at-risk family members, and key affected family members and spouses. DNA extraction and linkage analysis were carried out by Dr. Roger Green's laboratory at the Health Sciences Centre, St. John's, Newfoundland.

A pamphlet was written by Jane Green describing the disease presentation and recommended clinical screening for the family, and also the

method of predictive testing and the meaning of the possible results. This was given to family members when predictive testing results were provided individually in St. John's or at special clinics at the Notre Dame Bay Regional Hospital in Twillingate. The appointments were arranged by the nurse in charge of the registry in consultation with Jane Green, and a team of four geneticists and genetic counsellors travelled from St. John's and Gander to provide individual counselling for each family member. The team also met with the doctors and nurses involved with the family, and Jane Green provided them with information on the methods of genetic testing and the implication of the possible results. The recommendations for clinical screening were then revised for those with low-risk results, but remained unchanged for those with high-risk or uninformative results.

RESULTS

Ascertainment of Family

Medical records were available for documentation of polyps and/or cancer of the gastrointestinal tract in 38 family members, 21 of whom had colorectal cancer. Seven others were deemed affected by verbal history but no records were available for confirmation (Figure 4.1). Nineteen of 38 documented affected (50%) were ascertained because of symptoms, and nineteen (50%) by clinical screening.

Clinical Phenotype

i) Number of polyps

The numbers of polyps in the 32 family members with a documented number of polyps are presented in Table 4.1. Twenty-one (66%) had over 100 polyps and some of these had thousands of polyps, while 11 (34%) had fewer than twenty polyps including four who already had colon cancer. The diagnostic criteria for FAP of the presence of at least 100 polyps is not valid for this family.

ii) Site of polyps

Polyps were clustered in the cecum and ascending colon (right-sided polyps) in nine of 21 patients (42.8%) with multiple polyps, rather than in the sigmoid colon and rectum (left-sided polyps) as in typical FAP (Figure 4.2). In six patients, including three with cancer in the cecum or ascending colon, sigmoidoscopy revealed no polyps in the sigmoid colon or rectum. Therefore, annual screening for those at risk by sigmoidoscopy is not appropriate for this family.

iii) Natural history and course of disease

The mean age at diagnosis of polyps in the Newfoundland family is 39 years (range, 18-68 years) and at diagnosis of cancer is 49 years (range, 19-69 years) (Table 4.2); later than in typical FAP where mean age at diagnosis of polyps is 33 years, and at diagnosis of cancer is 36 years (Bulow 1986). Diagnosis of FAP was 15 years earlier on average in patients identified by

TABLE 4.1. NUMBER OF POLYPS, AND PRESENCE OR ABSENCE OF COLON CANCER IN A NEWFOUNDLAND FAMILY WITH ATYPICAL FAP

COLON CANCER	NUMBER OF POLYPS			
	multiple	less than 20	unknown	TOTAL
present	12	4	3	19
absent	9	7	1	17
unknown	-	-	2[1]	2
TOTAL	21	11	6	38

[1]Obligate carrier; documented cancer of the stomach or duodenum, bowel status unknown.

Figure 4.2. Location of colorectal polyps in typical and atypical familial adenomatous polyposis.

Patients with typical FAP have polyps throughout the colon, increasing in density towards the sigmoid colon and rectum. In the Newfoundland atypical FAP family, 9 of 21 patients with multiple polyps had the greatest density of polyps in the ascending colon or cecum. Six had no polyps visible by sigmoidoscopy, including three with right-sided colon cancer. The location of polyps in typical and atypical FAP determines the type of screening required.

Location of polyps in typical and atypical Familial Adenomatous Polyposis (FAP)

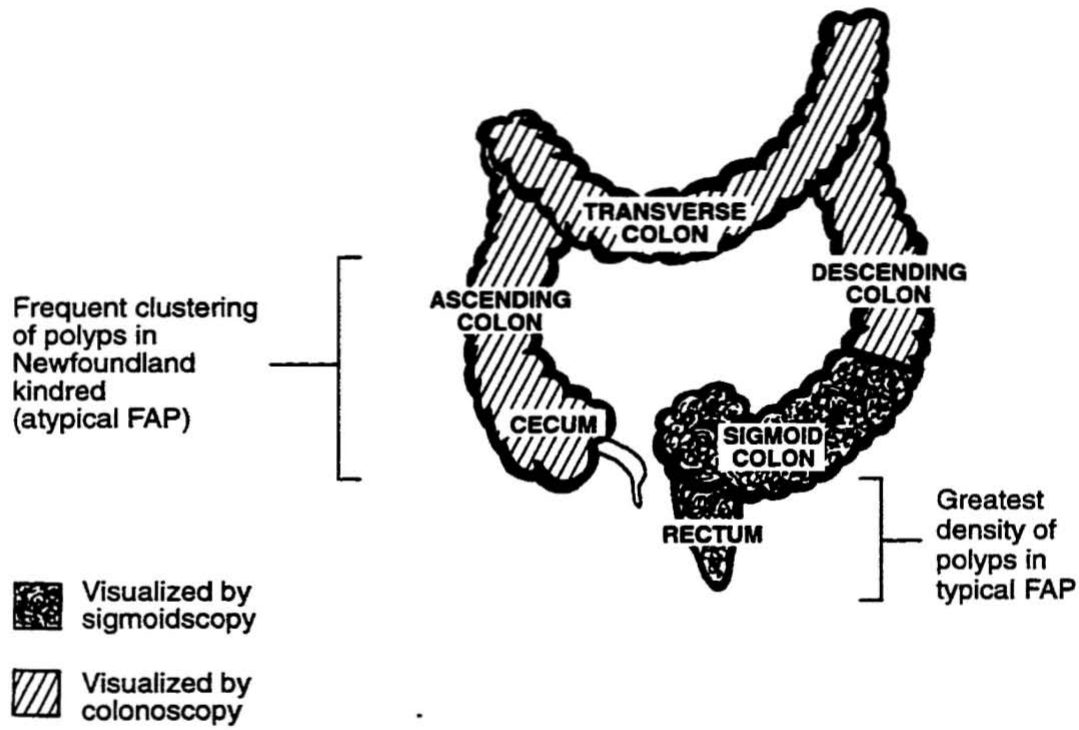


TABLE 4.2. NATURAL HISTORY OF DISEASE IN TYPICAL AND ATYPICAL FAMILIAL ADENOMATOUS POLYPOSIS

CATEGORY	TYPICAL FAP[1]	ATYPICAL FAP (Nfld Kindred)	
	Mean age (yrs)	Mean age (yrs)	Range
Known onset of polyps*	16	25	(18—28)
Onset of symptoms	29	45	(31—65)
Diagnosis of FAP**	33	39	(18—68)
Diagnosis of cancer	36	49	(19—69)
Death due to cancer	40	54	(42—72)

* Patients with previous negative sigmoidoscopy or colonoscopy
** Identification of polyps in those at risk

[1]reference: Bulow 1986

screening than in those who became symptomatic (at a mean age of 32 years for the screened group compared with 47 years for the symptomatic group). All 19 patients diagnosed through screening had disease limited to polyps or carcinoma in situ (CIS). In contrast, 17 of 19 (90%) of the symptomatic patients had invasive cancer at the time of diagnosis (Table 4.3). All twelve cancer deaths (mean age 54 years; range 42-72 years) were in patients who presented with symptoms. Screening resulted in diagnosis at an earlier stage of FAP, and in reduction in mortality from the disease. However, polyps and cancer occur at a later age in this kindred than in typical FAP.

iv) Upper gastrointestinal neoplasms

The presence of frequent upper GI polyps, and of upper GI cancer in about 5% of patients with FAP (Jagelman et al 1988) means that screening of these areas is necessary as well as of the colon. Of the 38 patients with documented colonic polyps or cancer in the Newfoundland family, 5 (13%) had neoplasms of the stomach or duodenum at an average age of 44 years (Table 4.4), however, some of the earlier patients who presented with advanced cancer were not investigated for upper GI polyps or cancer. In three patients the gastric or duodenal neoplasms (multiple gastric adenomatous polyps, gastric polyps with CIS, or duodenal cancer) were identified as a result of screening by upper GI endoscopy after the identification of bowel polyps or cancer. The first two of these patients had successful subtotal colectomy and partial

TABLE 4.3. EFFECT OF SCREENING ON AGE AT DIAGNOSIS AND SEVERITY OF DISEASE IN A FAMILY WITH ATYPICAL FAMILIAL ADENOMATOUS POLYPOSIS

	METHOD OF DIAGNOSIS	
CATEGORY	SYMPTOMATIC #	SCREENING #
Polyps only	1	16
Carcinoma in situ (CIS)	1	3
Invasive cancer	17	0
Total	19	19
Cancer deaths	12	0
Mean age at diagnosis (Range)	47 years (19-68 years)	32 years (18-57 years)

TABLE 4.4. UPPER GASTROINTESTINAL (GI) PATHOLOGY IN AFFECTED AND AT RISK MEMBERS OF AN ATYPICAL FAP FAMILY

Patient	Upper GI Pathology	Age at Diagnosis (yrs)	Bowel Pathology
1	Gastric polyps	16	No polyps or cancer (at high risk)
2	Gastric adenomatous polyps	42	Multiple polyps
3	Gastric polyps with carcinoma-in-situ (CIS)	46	Multiple polyps and cancer of the sigmoid colon
4	Duodenal cancer	40	Multiple polyps and cancer of the cecum
5	Gastric cancer	37	Bowel status unknown (deceased)
6	Cancer of the Ampulla of Vater	54	Bowel status unknown (deceased) (obligate carrier)

gastrectomy, but the third patient died of metastatic colon and duodenal cancer. Two patients in an earlier generation presented with symptomatic end-stage stomach or duodenal carcinoma.

A 16-year-old, examined because of abdominal pain (and later found to be at high genetic risk), was also found to have multiple gastric polyps but no intestinal polyps.

v) Associated features

Congenital hypertrophy of the retinal pigment epithelium (CHRPE) is present as an expression of the FAP gene in 65-80% of kindreds (Baba et al 1988, Heyen et al 1990, Olschwang et al 1993b), and can be a useful presymptomatic marker of the gene in these families. However, CHRPE was not found in the 9 affected or 41 at-risk Newfoundland family members examined by an ophthalmologist. Affected family members were not routinely screened for osteomas, epidermoid cysts or other manifestations of Gardner syndrome. However, large sebaceous cysts were noted by the family doctor on the scalp and neck of several affected members in one branch of the pedigree.

Genetic Screening

i) Identification of family members at risk

As stated previously, thirty-eight members of this family have documented familial adenomatous polyposis, and another seven family

members, including three obligate carriers, were reported to be affected. One hundred and thirty (130) first degree relatives at 50% risk were then identified from the pedigree. After genetic counselling about predictive testing by linkage analysis was given to this family, 96 of those at risk were interested in participation in linkage studies. The majority requesting predictive testing (66/96) were at 50% risk, but those at 25% risk were included in some circumstances such as when the clinical status of the parent at risk was unknown.

ii) Linkage analysis

The original linkage studies with RFLP markers were uninformative because of homozygosity of alleles in many affected family members. However, linkage to a CA repeat marker (D5S346) closely-linked to the APC locus was demonstrated (LOD score of 3.2 at $\theta = 0.0$). Although an informative flanking marker was not available for testing, recombination has not been demonstrated between D5S346 and the APC gene (Spirio et al 1993a).

iii) Predictive testing

Eighty predictive testing results have been obtained and provided to family members. The results delivered include 11 high-risk results (>99.9% probability of carrying the FAP gene) in family members age 10-29, and 64 low-risk results (<0.1% probability of carrying the FAP gene): 36 low-risk results in those at 50% risk, and 28 low-risk results where the parent was determined by linkage analysis to be at low risk (Table 4.5, Figure 4.3). Five

TABLE 4.5. RESULTS OF PREDICTIVE TESTING FOR ATYPICAL FAP IN A NEWFOUNDLAND KINDRED

PREDICTIVE TESTING RESULT		NUMBER
HIGH RISK*		11
LOW RISK**		64
- Parent affected	36	
- Parent at low risk	28	
UNINFORMATIVE		5
TOTAL RESULTS		80

results pending — 16

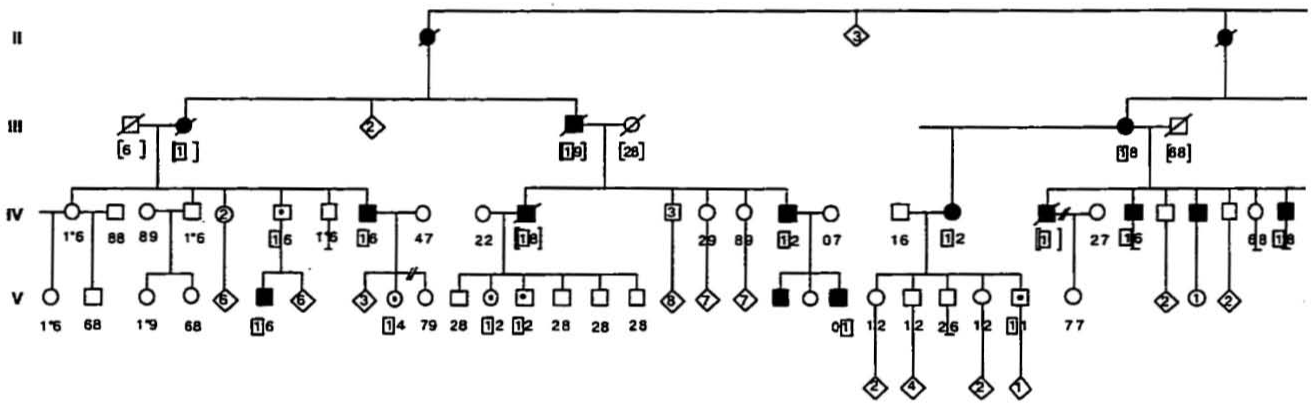
* > 99.9% probability of having inherited the FAP gene
** < 0.1% probability of having inherited the FAP gene

Figure 4.3. Pedigree of a Newfoundland atypical FAP family with results of linkage analysis.

Clinically affected family members (polyps ± colon cancer) are indicated by solid symbols.

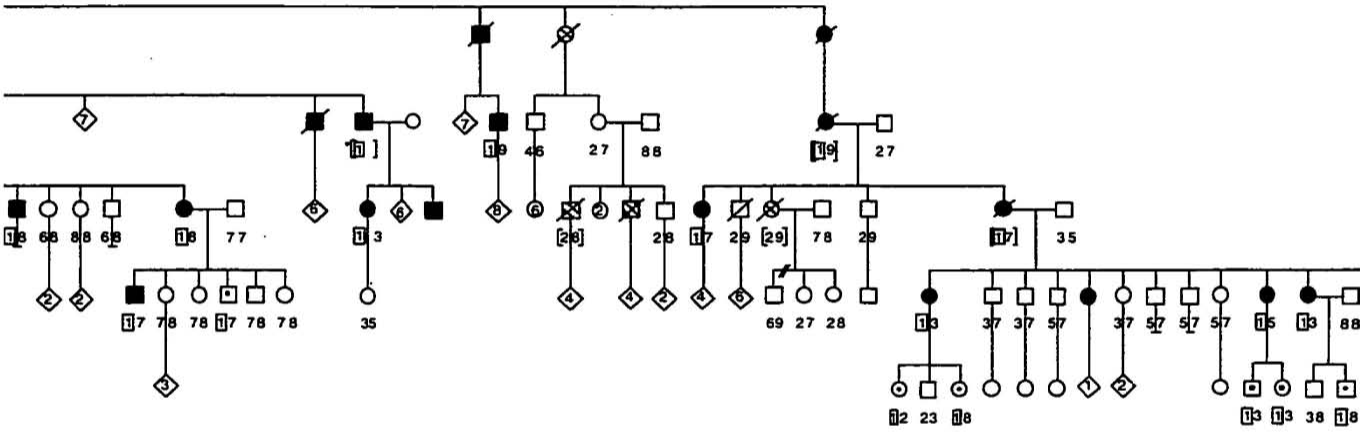
The CA repeat marker D5S346, 50 kb from the APC gene (Spirio et al 1991), was used for linkage analysis. Ten alleles were identified. Allele "1" is segregating with atypical FAP in this family. Genotypes are indicated below each pedigree symbol; inferred genotypes are given in brackets. Predicted affected family members are indicated on the pedigree. Family members with colon or other GI cancer who are predicted to be unaffected with FAP are also indicated (see key).

Newfoundland atypical FAP family with results of linkage analysis



Key

- ● ■ Familial adenomatous polyposis (polyps ± colon cancer)
- ⊙ ⊡ Predicted affected
- 16 Alleles of CA repeat marker
- [1] D5S346 allele segregating with atypical FAP
- 1* Parental origin of allele '1', unknown
- ⊘ ⊠ Colon or stomach cancer, unrelated to atypical FAP


[11]
[19]
27
[28]
[29]
78
29
35
69 27 28
12 23 18
38 18

results were uninformative because both parents were deceased and it was not possible to reconstruct the parental genotypes; the "high-risk" allele could have come from the affected or the unaffected parent. However, there is only one documented instance, of this allele (allele 1) entering the family independently through a spouse.

In those at 50% risk, the ratio of high risk:low risk results (11:36) appears skewed because many gene carriers had already been identified by clinical screening.

iv) Unexpected results

Unexpectedly two brothers with early onset colon cancer who died at age 40 and 56 were at low risk for FAP since their at-risk mother had not inherited the high-risk allele. Therefore their 12 first degree relatives (siblings and children) who were tested were all determined to be at low risk for FAP. Nevertheless, this branch of the family, may be at increased risk of colon cancer for reasons other than the FAP gene. Also, a 37-year-old woman who died of stomach cancer (and whose mother and sisters were affected with FAP), apparently did not carry the FAP gene, based on the alleles present in her husband and children.

v) Delivery of results

The majority of results (n = 56) were delivered at one clinic in Twillingate in November 1992, and the remainder either at a second clinic in Twillingate in October 1993 (n = 16), or at individual counselling sessions (n = 8) in St.

John's or Corner Brook. When the majority of family members live in one community or a group of communities some distance from St. John's, it is more efficient for the geneticist(s) to organize a special clinic at a regional hospital and see the patients there, than for many family members to travel to the tertiary care centre in St. John's.

DISCUSSION

Clinical Screening

Clinical screening and prophylactic surgery for first degree relatives of patients with FAP was introduced as early as 1925 at St. Mark's Hospital, London (Bussey 1975), and has been shown to be an extremely effective means of preventing cancer and prolonging life. This is demonstrated in the Newfoundland family as well as in families described previously in the literature (Berk et al 1987, Vasen et al 1990a, Rhodes and Bradburn 1992). For the family members presenting symptomatically before screening was introduced, 90% (17/19) had invasive cancer, and 12 of 17 died of cancer within 6 years of diagnosis. In contrast, none of those diagnosed as a result of screening had invasive bowel cancer and none have died of the disease. Thus, there has been a dramatic reduction in morbidity and mortality because of the clinical screening program.

i) Screening for typical FAP

Standard clinical screening protocols for FAP are based on the features of FAP described in patients followed by large polyposis registries (Bussey 1975, Bulow 1991). Typically, multiple polyps (often hundreds or thousands) occur throughout the colon, with increasing numbers towards the sigmoid colon and rectum. Diagnosis of FAP is made when over 100 polyps are seen. Polyps usually appear in the teens or early twenties and the majority of gene carriers can be identified as affected by the early thirties (summarized in Bulow 1991). Screening protocols, therefore, usually consist of flexible sigmoidoscopy (to view the region of colon where the maximum number of polyps occur) every 1-3 years from teens to fifties, for those at risk; and review of the rectum and any remaining colon semi-annually, and upper GI endoscopy every 3-5 years, for those already affected.

ii) Screening for atypical FAP

For families with atypical or attenuated FAP such as the Newfoundland kindred, modification of this screening protocol is necessary (Figure 4.4). The number of polyps in the Newfoundland kindred is highly variable. Therefore any number of polyps must be considered significant and followed closely, or prophylactic subtotal colectomy planned, rather than using the typical FAP diagnostic criteria of "greater than 100 polyps". There is frequent clustering of polyps in the cecum and ascending colon (in 43% of those with multiple

Figure 4.4. Recommended screening protocol for atypical familial adenomatous polyposis.

RECOMMENDED SCREENING PROTOCOL FOR ATYPICAL FAMILIAL ADENOMATOUS POLYPOSIS

First degree relatives of those who are affected

a) Predictive testing by linkage analysis in early teens (or direct mutation detection if this becomes available)

b) Clinical screening

i) High risk group
- annual colonoscopy
- upper GI endoscopy every 3-5 years

ii) Low risk group
- colonoscopy in late teens and in mid-thirties

iii) Uninformative group, or those declining predictive testing

Age 15-45 years — annual screening with flexible sigmoidoscopy or colonoscopy (colonoscopy should be used at least every 3rd year); upper GI endoscopy every 3-5 years

After age 45 — colonoscopy every three years

c) Subtotal colectomy with ileorectal anastomosis when polyps identified

Affected patients (following initial surgery)

- sigmoidoscopy of any remaining colon and rectum every 6-12 months
- upper GI endoscopy every 3-5 years

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polyps), and absence of rectal polyps in some affected members in this kindred. A recent patient, for example, had only two polyps in the sigmoid colon but hundreds of polyps in the ascending colon and cecum. Therefore, colonoscopy rather than flexible sigmoidoscopy is recommended for families with this disease presentation (Smith-Ravin et al 1994).

The age at onset is highly variable in the Newfoundland kindred and on average older than for typical FAP. Therefore, if identification of gene carriers by genetic testing is not possible, clinical screening of those at risk must be continued to a later age than for typical FAP.

As in typical FAP families, those who have had prior colectomy require screening of any remaining colon and rectum every 6-12 months for life. Polyps and cancer of the stomach and duodenum have also been identified in members of the Newfoundland kindred, as in other FAP families; therefore regular upper GI endoscopy (every 3-5 years) is recommended for all affected family members. This is of particular concern for those who have had prophylactic bowel surgery and remain at somewhat elevated risk of duodenal or stomach cancer (Jagelman et al 1988, Arvanitis et al 1990). (It was only when early death from colon cancer was prevented by surveillance and treatment that the full extent of the subsequent extracolonic expression of the APC gene was recognized [Jagelman 1987]).

iii) Extracolonic manifestations

The benign but invasive desmoid tumour (Gurbuz et al 1994) found in some FAP patients following colectomy, and hepatoblastoma (Li et al 1987) or brain tumours (Hamilton et al 1995), which occur rarely in FAP gene carriers in childhood, have not yet been seen in this family, nor have CHRPE been identified. These benign pigmented lesions of the retina are a sensitive marker for the development of polyps in about 70% of FAP families (Berk et al 1988b, Heyen et al 1990, Romania et al 1992), but typically occur in FAP families with early rather than late development of polyps (Romania et al 1992) and a more classic presentation of FAP.

A correlation has recently been recognized between the presence of CHRPE and the location of the mutation within the APC gene (CHRPE are only present when the mutation is between exon 9 and codon 1444 in the very large exon 15 [Caspari et al 1995]), and this correlation can be used to facilitate detection of the mutation in specific families (Wallis et al 1994, Olschwang et al 1993b).

A variable number and location of polyps, an older age of onset, and variable age at onset within a kindred have recently been described in several other kindreds (Nagase et al 1992, Evans et al 1993, Spirio et al 1993b, Smith-Ravin et al 1994, Varesco et al 1994); thus, familial polyposis may be a more variable disease than previously recognized. Since the different phenotypes will only be obvious in large kindreds with many affected members, more extensive

screening than previously recommended may also be important for smaller kindreds which lack sufficient numbers to define the characteristics of the disease within their own family.

Even though genetic testing by direct mutation detection or by linkage analysis is possible for many families, clinical screening is still necessary to identify the onset of polyp formation and to plan surgery in identified gene carriers. Furthermore, clinical screening will be the only option for families where the specific mutation has not been found and linked markers are uninformative. Therefore clinical screening protocols should be revised as new clinical or genetic information accumulates, or new technology becomes available.

Genetic Screening

The mapping of the gene for FAP to chromosome 5q21, the cloning of the APC gene, and the identification of mutations within the APC gene for many FAP families allows direct identification of gene carriers in these families (Petersen et al 1993). But the very large size of the APC gene, the many different mutations so far identified, and the high mutation rate increase the difficulty of finding the mutation in each family (Groden et al 1993). There is clustering of mutations at the 5' end of exon 15, particularly in families with "classic" early-onset disease and profuse polyps (Nagase et al 1992, Nagase and Nakamura 1993, Caspari et al 1994, Gayther et al 1994), and several

mutations have occurred more than once, (mutation hotspots have been identified at bp 1061 and 1309 [Miyoshi et al 1992, Nagase and Nakamura 1993, Bhapat et al 1994, Mandl et al 1994]), but the majority of families have a unique mutation (Nagase and Nakamura 1993, Powell et al 1993). Because many of the APC mutations lead to stop codons (large deletions, 4-5bp deletions or nonsense mutations), protein truncation testing by a linked *in vitro* transcription-translation method has recently been developed to increase the efficiency of identifying the APC gene mutation in individual families (Powell et al 1993, Van der Luijt et al 1994). This method is reported to be successful in greater than 80% of families for identification of the specific APC mutation (Liu 1993b), but is not widely available.

Even using these newer methods, and knowing that APC mutations in families with similar phenotype to the Newfoundland family cluster at the 5' end of the APC gene (in exons 1-4) (Spirio et al 1993b), the mutation for the Twillingate family has not yet been identified. Because genetic heterogeneity for FAP has been reported, with linkage to 5q21 being excluded in at least one large family with typical FAP (Tops et al 1993), and also in a family with an atypical expression of FAP (Stella et al 1993), predictive testing by linkage analysis should be used with caution unless linkage to the APC gene region can be demonstrated. The identification of several highly informative closely-linked CA repeat markers, however, has increased the possibility that linkage to the

APC gene region can be demonstrated, and used for predictive testing when the mutation is not identified (Spirio et al 1993a, Eckert et al 1994).

Linkage to one of these CA repeat markers has been established for the Newfoundland family. Predictive testing by linkage analysis has therefore been used for modification of the clinical screening protocol for those at risk (Figure 4.5). Recommendations regarding clinical screening remain unchanged for those with high-risk or uninformative linkage analysis results. For those with low-risk results, a much reduced screening protocol with colonoscopy in the late teens and late thirties is recommended (Petersen et al 1991).

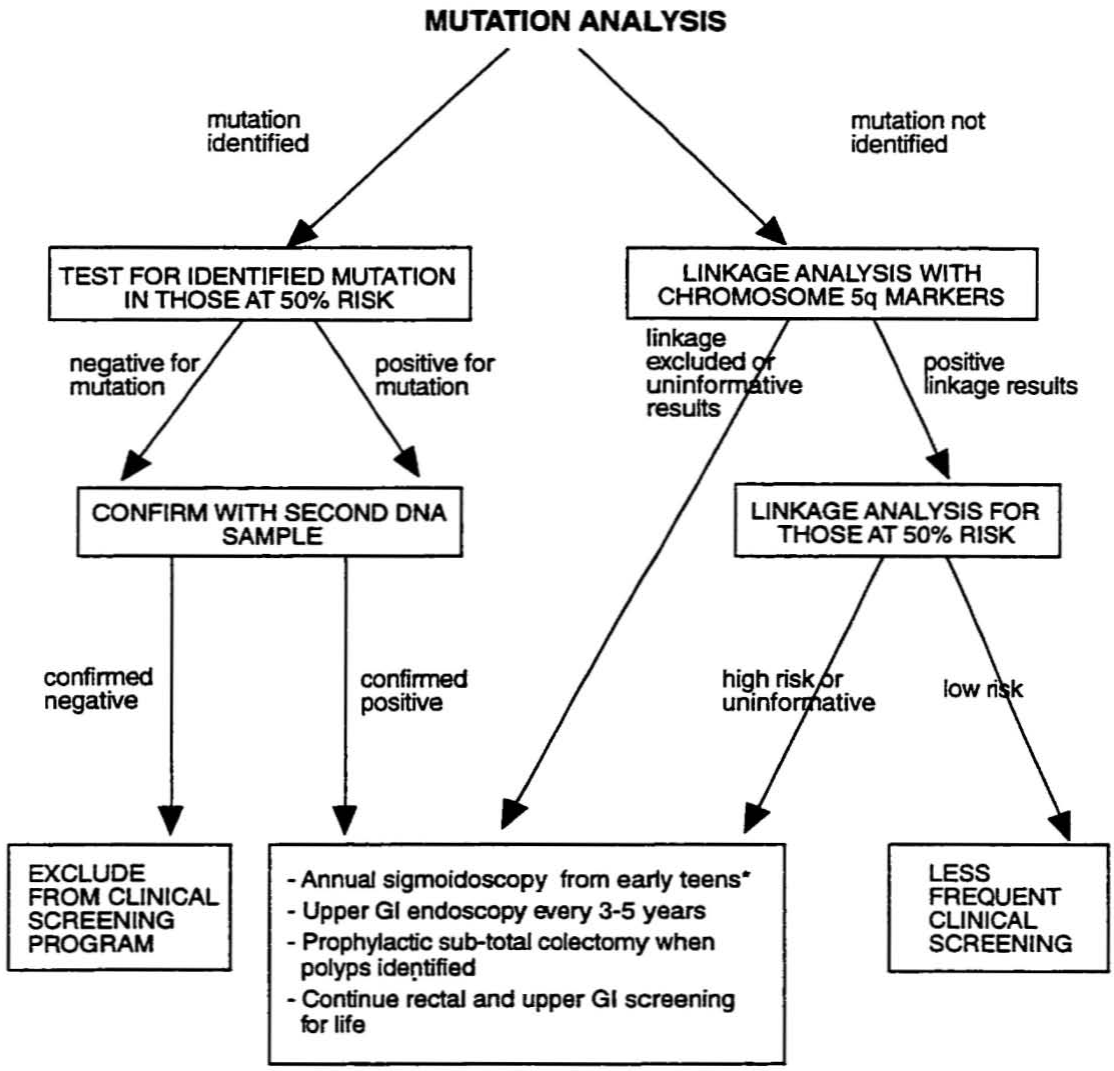
Unexpectedly, the low-risk group includes two brothers with early colon cancer. Colon cancer is frequent enough in the general population that the occurrence of sporadic colon cancer in an older member of a large FAP kindred would not be surprising, but it was unexpected that two brothers who died at a young age of colon cancer (one of whom had several polyps) had not inherited the marker associated with FAP in this family. Because of the early age at onset, they may have had another form of hereditary colon cancer such as HNPCC. Their relatives may have an increased risk of GI cancer, but FAP linkage studies cannot predict the risk of cancer in this part of the pedigree. The presence of individuals within an FAP kindred with GI cancers for other reasons than the APC gene can cause problems with linkage analysis, as well as problems for clinical management.

Figure 4.5. Management plan for FAP families.

Different methods are used for mutation detection by different laboratories (including SSCP, DGGE, RNase protection assay, heteroduplex analysis, and protein truncation testing [PTT]). The mutation is identified in 30-60% of families by most laboratories, and may be identified in over 80% of families if PTT is available (Powell et al 1993, van der Luijt et al 1994). If mutation detection is not available, the management plan begins with linkage analysis.

SSCP - single strand conformation analysis
DGGE - denaturing gradient gel electrophoresis

Management plan for FAP families



** annual colonoscopy is recommended for atypical FAP families with right-sided clustering of polyps*

Although these brothers with colon cancer (and few or an unknown number of polyps) apparently did not have atypical FAP, other family members with colon cancer and only a few polyps did inherit the marker associated with familial polyposis in this family.

Implementation and Acceptance of the Screening Program

Between 1985, when it was first recognized that a type of hereditary colon cancer was responsible for several colon cancer deaths in an extended Notre Dame Bay family, and 1989, a number of family members participated in clinical screening initiated by the family doctor. Because a diagnosis of familial adenomatous polyposis had been made, the FAP guidelines for screening were followed with annual sigmoidoscopy recommended for those at risk. Screening, however, had been offered to "the family" as a whole rather than according to degree of risk, so that some of those participating in this screening program were at 25% risk or less. When Genetics was asked to assist with management of this family in 1989, the degree of risk was established for each individual from pedigree and medical record review. The pedigree was then extended to include another large branch of the family in Central Newfoundland.

Members of the Notre Dame Bay branch live in many small communities on the islands of Twillingate and New World Island. Their health care has always been centred on the regional hospital in Twillingate, and two established

family doctors know the family and the condition very well. In collaboration with the geneticist involved, a registry of affected and at-risk family members was developed at the hospital and is monitored by one of the nurses. A surgeon at the hospital provides the screening by colonoscopy and upper GI endoscopy.

There has been a good understanding by the majority of members of this branch of the family about the disease, the inheritance pattern, the recurrence risk, and the rationale for screening since the information session was held at Twillingate Hospital in 1991, and since educational pamphlets and individual genetic counselling were provided. Compliance with screening recommendations has generally been good, and the majority of those identified as affected by clinical screening have been in this branch of the family. The interest in genetic testing has also been high. Some family members, males more often than females, declined clinical screening but expressed an interest in genetic testing when this became available. Two men at 50% risk refused any screening or genetic testing for themselves or for their children until an 18-year-old son of one of these developed symptomatic disease. All this branch of the family now participate in clinical screening and have recently had blood samples collected for predictive testing.

The central Newfoundland branch of the family live in a number of communities in the Botwood — Phillips Head region, and more members of this part of the family than of the Notre Dame Bay branch have moved to other

parts of Canada. Several different family doctors have clinics in these communities, and over the years there have been frequent changes in the doctors in practice, so that there is no key individual who knows the whole family. The regional hospital in Botwood has become a chronic care facility; as a result, travel to Grand Falls (35 km) or St. John's (300 km) is necessary for any screening procedures or surgery. An attempt to establish a registry similar to that in Twillingate was not successful, partly because of the less centralized care and partly because of the pressures of other work on nurses who originally expressed interest. Relatively few of those at risk in this area have been clinically screened on a regular basis, and it has been more difficult to contact and to provide information for them. However, there has now been a request for information, and for clinical screening and genetic testing from some of these family members. This may provide the impetus for an improved screening program for this section of the family.

As has been demonstrated with other screening programs, such as for carriers of Tay Sachs disease or β-thalassemia, education of the families at risk and of the health care workers is necessary for a successful program (Kaback 1990, Scriver et al 1984, Cao et al 1989, Modell and Kuliev 1991). Genetic registers can also be very beneficial, particularly when on-going screening is required, to facilitate record keeping, regular follow-up, and communication with family members (Bussey 1975, Berk et al 1981, Jarvinen et al 1984, Bulow 1986, Vasen et al 1990a, Rhodes and Bradburn 1992). The registry can

also be used to coordinate the complete management of FAP family members including molecular genetic diagnosis, ophthalmic examination, clinical screening, and treatment (Burn et al 1991, Maher et al 1993).

CHAPTER 5 — DEVELOPMENT OF A SCREENING PROGRAM FOR HEREDITARY NON-POLYPOSIS COLON CANCER

INTRODUCTION

Previous Studies

Present Study

METHODS

Pedigree Studies and Medical Record Review

Genetic Studies

RESULTS

Ascertainment of Families

Pedigree Studies

Natural History of Disease

i) Age at diagnosis
ii) Types of cancer
iii) Initial cancer
iv) Mortality

Clinical Screening

i) Participation in screening
ii) Results of screening

Genetic Studies

i) Mapping of a gene for HNPCC
ii) Cloning of a gene for HNPCC
iii) Request for predictive testing

DISCUSSION

Natural History of HNPCC

Morbidity and Mortality

INTRODUCTION

This chapter describes the ascertainment of two families with hereditary non-polyposis colon cancer (HNPCC), the documentation of the clinical phenotype, the recognition that these were two branches of one large HNPCC family (Family C), the collaborative linkage studies that lead to mapping and subsequent cloning of a gene for HNPCC, the development of a clinical screening program, and a discussion of some of the ethical issues associated with screening for hereditary cancers.

Previous Studies

Familial clusters of non-polyposis colon cancer either alone (Lynch I syndrome) or associated with extra-colonic cancers such as endometrial, other gastrointestinal, or genitourinary cancers (Cancer Family Syndrome or Lynch II syndrome) have been recognized for years, (Warthin 1913, Lynch et al 1966, Lynch and Krush 1971, Mecklin 1987, Orrom et al 1990, Lynch and Lynch 1993a), but until recently these clusters were often explained on the basis of common environmental factors rather than inheritance (Peltomaki et al 1993, Editorial 1993a). In some families, Lynch I or Lynch II syndrome (now referred to as hereditary non-polyposis colon cancer or HNPCC [Fitzgibbons et al 1987, Vasen et al 1990b, Vasen et al 1991b, Lynch et al 1994]) was apparently inherited in an autosomal dominant pattern with variable age of onset, variable

types of cancer and reduced penetrance (Mecklin and Jarvinen 1986a, Vasen et al 1990b, Lynch et al 1993c, Watson and Lynch 1993).

This variability and the lack of a premalignant marker (Lynch and Lynch 1993a, Lynch et al 1993c) made it more difficult to implement a clinical screening program for HNPCC than for familial adenomatous polyposis, discussed in the previous chapter, where the natural history of disease is better known and multiple polyps typically precede the development of cancer. HNPCC is, however, responsible for up to 15% of colon cancer (Vasen et al 1991b, Hamilton 1993, Peltomaki et al 1993), as well as extra-colonic cancers, and, therefore, considerably more common than FAP (responsible for only about 1% of all colon cancer). Clinical screening protocols have therefore been introduced by various groups, recommending colonoscopy at from one to five year intervals, beginning five years before the earliest diagnosis of colon cancer in the family (Mecklin et al 1986b, Fitzgibbons et al 1987, Vasen et al 1989a, Houlston et al 1990, Vasen et al 1993). Women in these families may also be screened for endometrial cancer with endometrial biopsy or dilatation and curettage (D & C), and pelvic ultrasound (Fitzgibbons et al 1987, Watson et al 1994).

Until 1993 efforts to map the gene or genes for HNPCC had been unsuccessful. Several candidate genes known to influence hereditary or sporadic colon cancer progression (APC and MCC on chr 5, DCC on chr 18, and p53 on chr 17) had been excluded (Peltomaki et al 1991, Peltomaki et al

1992), and a search of the whole genome was necessary. DNA was often not available for linkage studies from enough affected individuals to obtain a significant LOD score because HNPCC families are frequently recognized by multiple early cancer deaths (Peltomaki et al 1991). There was concern, however, about combining LOD scores from several families because of the possibility of genetic heterogeneity of HNPCC (Peltomaki et al 1993), or the possibility of lumping families with hereditary and with sporadic cancers.

One attempt to solve this dilemma was the definition of the Amsterdam criteria (Vasen et al 1991a, Vasen et al 1991b) to increase uniformity of families included in linkage studies. Research families were to have at least three first-degree relatives in two generations affected with colon (or endometrial) cancer, and at least one of these persons diagnosed under 50 years of age. Another approach was to study a single HNPCC family, large enough to generate a significant LOD score if there was linkage to a particular map location.

Present Study

In 1990, two Newfoundland patients were referred to Jane Green in Medical Genetics because of their personal and family history of multiple colon and other cancers (Figure 5.1), and their concern about the risk of these cancers to relatives. A family history was taken and medical records reviewed

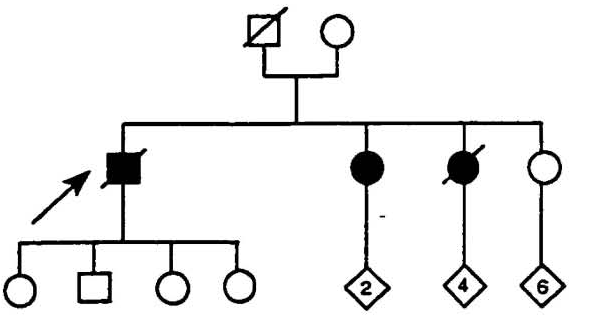
Figure 5.1. Original pedigrees of branch A and branch B of a Newfoundland HNPCC family.

Two probands, each indicated by an arrow, were independently referred to Genetics. Affected family members, as reported by each proband, are indicated by solid symbols.

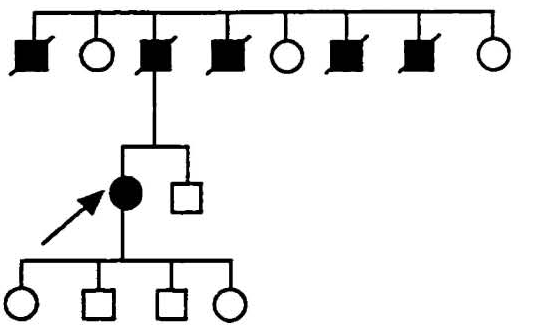
Subsequent pedigree studies and archival review demonstrated that these original families were from separate branches of one large HNPCC family (Figure 5.4).

Original pedigrees of Branch A and B of Newfoundland HNPCC Family

Branch A



Branch B



(by Jane Green) in order to establish the diagnosis, to identify those at risk, and to determine whether screening was appropriate in either family.

Each family documented fit the diagnosis of Lynch II Syndrome (HNPCC with extra-colonic cancers). Extending the pedigrees back six generations identified a common ancestor, and these families were recognized to be two branches of one large HNPCC family.

This family, Family C, was key to mapping the first gene for HNPCC to chromosome 2p (Marshall 1993, Peltomaki et al 1993) and subsequent cloning of a DNA repair gene (hMSH2) by a collaboration between Jane Green and the laboratories of Dr. Bert Vogelstein at Johns Hopkins University and Dr. Albert de la Chapelle at the University of Helsinki (Leach et al 1993), and independently by Fishel and colleagues (Fishel et al 1993, Editorial 1993c). Mutations were identified within hMSH2 which were co-inherited with the disease in each of three large HNPCC families including Family C (see below). Subsequently a second gene for HNPCC was mapped to chromosome 3p (Lindblom et al 1993, Nystrom-Lahti et al 1994b), and mutations identified in another DNA repair gene (hMLH1) mapping to this locus (Bronner et al 1994, Papadopoulos et al 1994, Service 1994).

The documentation of the natural history of HNPCC in the Newfoundland family provides the basis for development of a clinical screening program for this family, and the identification of the mutation predisposing to HNPCC provides the basis for genetic screening. As with screening for other cancer

predisposition syndromes, there are ethical, legal and social concerns about the best method of implementation of this type of screening (National Advisory Council for Human Genome Research 1994). The approach taken in Newfoundland is described below.

METHODS

Pedigree Studies and Medical Record Review

The probands (Appendix, p. 285) were interviewed by Jane Green to obtain details of their family histories. They provided introductions to other family members to extend the pedigrees, and to determine the ancestral geographic location of each family within Newfoundland.

Consent was obtained to review available medical records of those reported to be affected. Details of type(s) of cancer, age at diagnosis, age at onset of any symptoms, type and outcome of treatment, and current age, or age and cause of death were recorded for each individual.

Because of the very similar pattern of cancers in the two families, the English origins of each family, and the geographic location of the ancestors of each family in communities on the northeast coast of Newfoundland, provincial archival information on births, deaths, and marriages was searched for a connection between the families.

Genetic Studies

Family members were approached about participation in a linkage study to try to map the gene predisposing to colon and other cancers in this family. After consent was obtained, blood samples were collected for establishment of cell lines and for DNA extraction from all living affected and older unaffected family members. Tissue blocks were collected, if available, from affected family members who were deceased, to be used as a source of DNA.

Blood samples, tissue blocks and a pedigree indicating disease status were sent to Dr. Vogelstein's laboratory at The Johns Hopkins University for collaborative linkage studies with Dr. de la Chapelle's laboratory in Helsinki, Finland.

Genetic counselling regarding Lynch syndrome (now called HNPCC) was offered to all affected and at-risk family members because of the anxiety expressed by the probands and many other family members about the excess and early age of cancer deaths in the family. This counselling was requested by the majority of family members, and counselling sessions were arranged for 73 individuals at their convenience — in their homes, in local hospitals (in Botwood or Grand Falls), or at the General Hospital in St. John's. The features of HNPCC (including the types of cancers and the age range over which they occur, the pattern of inheritance, and the risk of recurrence to relatives of the affected members), and the clinical screening that is recommended for those at risk (Figure 5.2), were described by Jane Green. When requested, a

Figure 5.2. Recommended screening protocol for hereditary non-polyposis colon cancer (HNPCC).

RECOMMENDED SCREENING PROTOCOL FOR HEREDITARY NON-POLYPOSIS COLON CANCER (HNPCC)

Screening for affected, and at risk in HNPCC families

1. **For all families**

   - annual or biennial (2-yearly) colonoscopy (beginning 5 years before earliest onset of colon cancer in the family)

   - double contrast barium enema and flexible sigmoidoscopy as a second choice, if colonoscopy impossible or unavailable.

2. **For families in which female genital cancers occur**

   - annual pelvic examination, endometrial aspiration biopsy, and pelvic ultrasound

3. **For families in which other extracolonic cancers occur**

   - annual clinical examination and endoscopic or radiological investigation of organs at risk every 2 or 3 years.

4. **For patients with previous colonic surgery**

   - semi-annual or annual endoscopic evaluation of remaining colon or rectum.

NOTE: Subtotal colectomy with ileorectal anastomosis is the treatment of choice for colon cancers in these families because of high risk of synchronous or metachronous cancers.

summary of this information was also sent (by Jane Green) to each family doctor. Surgeons and gastroenterologists, in St. John's and in central Newfoundland, were alerted to the extent of this large family so that screening or treatment offered would be appropriate, and appointments for colonoscopy and/or endometrial biopsy and pelvic ultrasound were arranged when requested.

When the mutation was identified in the Newfoundland family, more detailed counselling was provided to family members about predictive testing by direct mutation analysis. The counselling included information on the variability of disease in this family, and the unpredictable age at which tumours might occur in those identified to be gene carriers. Family members were reminded that regular screening was recommended for those shown to carry the HNPCC mutation, and would remain available for those who declined predictive testing. The possibility of discrimination regarding life insurance, once gene carrier status was known, was also raised by the geneticist. A consent form was signed and DNA banked for family members requesting genetic testing.

RESULTS

Ascertainment of Families

Initial pedigree studies and medical record review delineated two families, each fitting the criteria for Lynch II syndrome. There were multiple affected individuals with non-polyposis colon cancer, and extra-colonic cancers including other gastrointestinal cancers, and endometrial and ovarian cancer in the

affected women. The cancers followed an autosomal dominant inheritance pattern in each family, and many affected members had early onset cancers, bilateral cancers and/or multiple primaries.

The families were both from small communities on the northeast coast of Newfoundland, one from the Wesleyville area on the west side of Bonavista Bay, and the other from Point Leamington at the head of South West Arm, further west in Notre Dame Bay (Figure 5.3). Although these communities are approximately 175 km apart, the Wesleyville area is known to have been settled earlier by immigrants from England, and in a later wave of migration, families moved westward along the coast line towards and past the Point Leamington area (Mannion 1977, Encyclopedia of Newfoundland and Labrador 1981).

By examining archival records including those collected by proband 1 before he died, and old marriage records saved by members of the family of proband 2, a common ancestor of the two families was identified (Figure 5.4), an immigrant from Hampshire, England who settled in 1790 on Greenspond Island, Bonavista Bay, the merchant centre for the Wesleyville area.

Pedigree Studies

Twenty-six affected family members and four obligate carriers were identified by pedigree studies and medical record review. Two of the obligate carriers had died young (age 45 and 53) of other causes, and the others were

Figure 5.3. Geographic location of two branches of a Newfoundland HNPCC family.

Original Newfoundland settlements were along the coastline and transportation between them was by sea. The Greenspond Island area was settled in the 1690s, and the Point Leamington area was settled in the late 1700s.

Geographic location of two branches of Newfoundland HNPCC Family

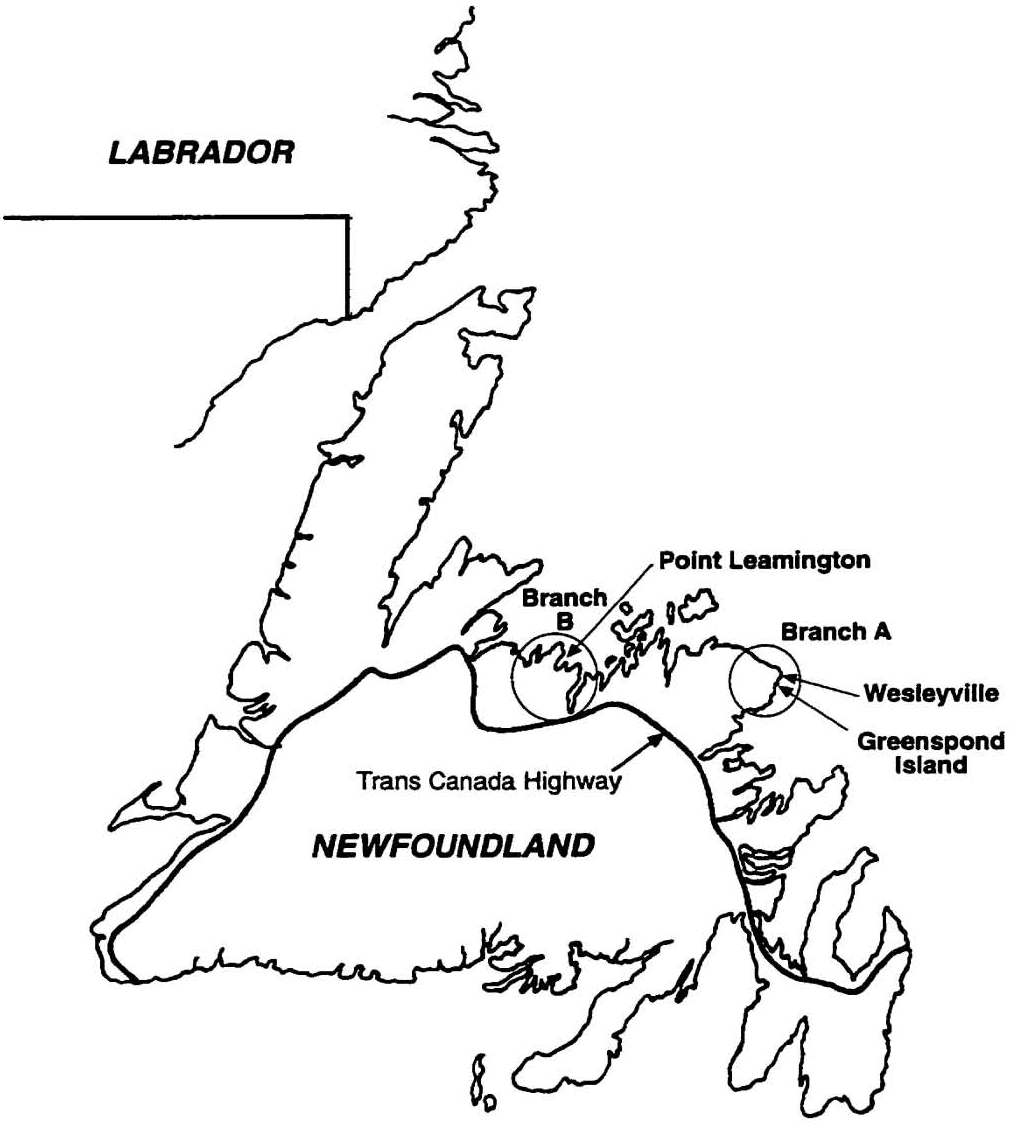
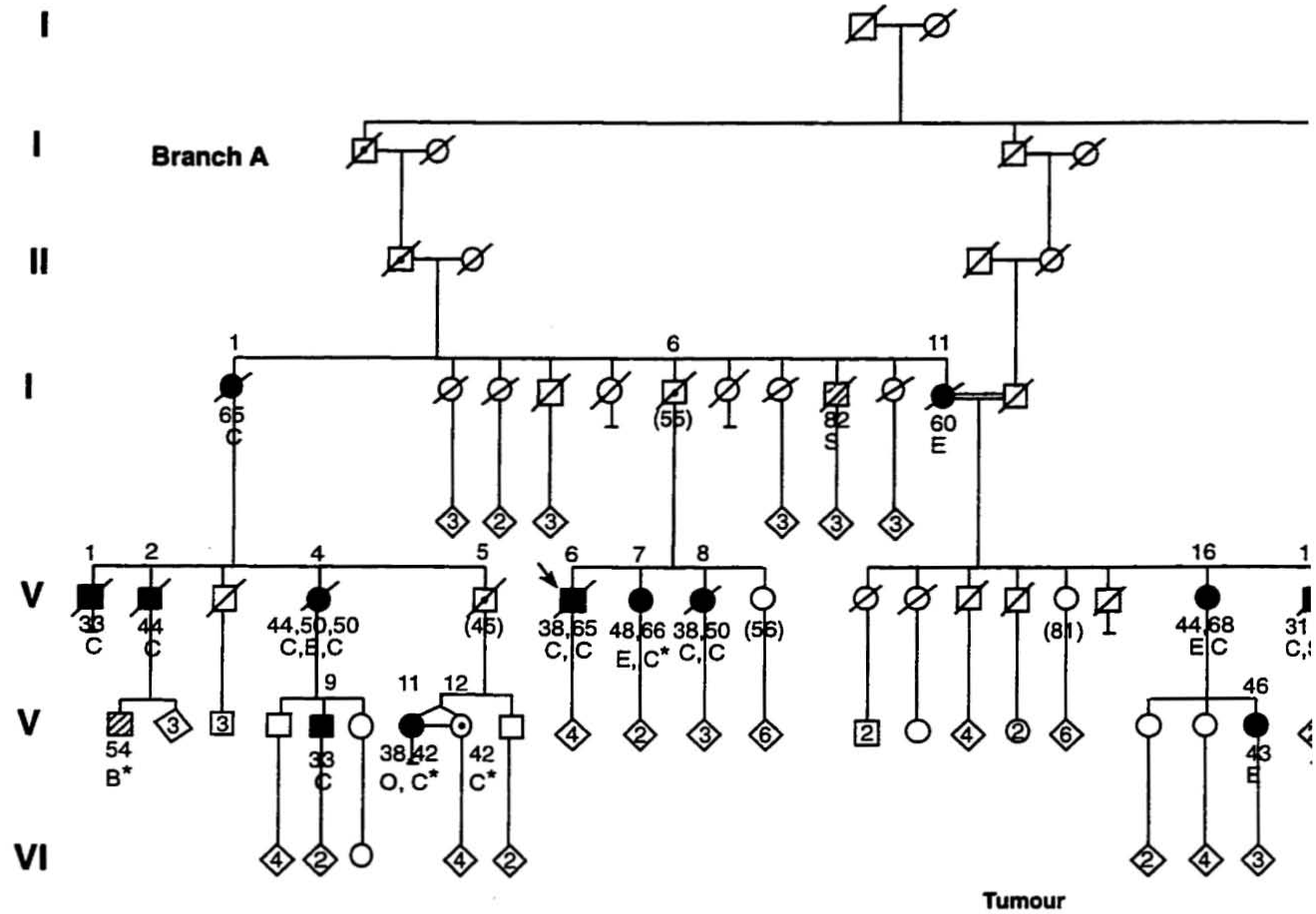


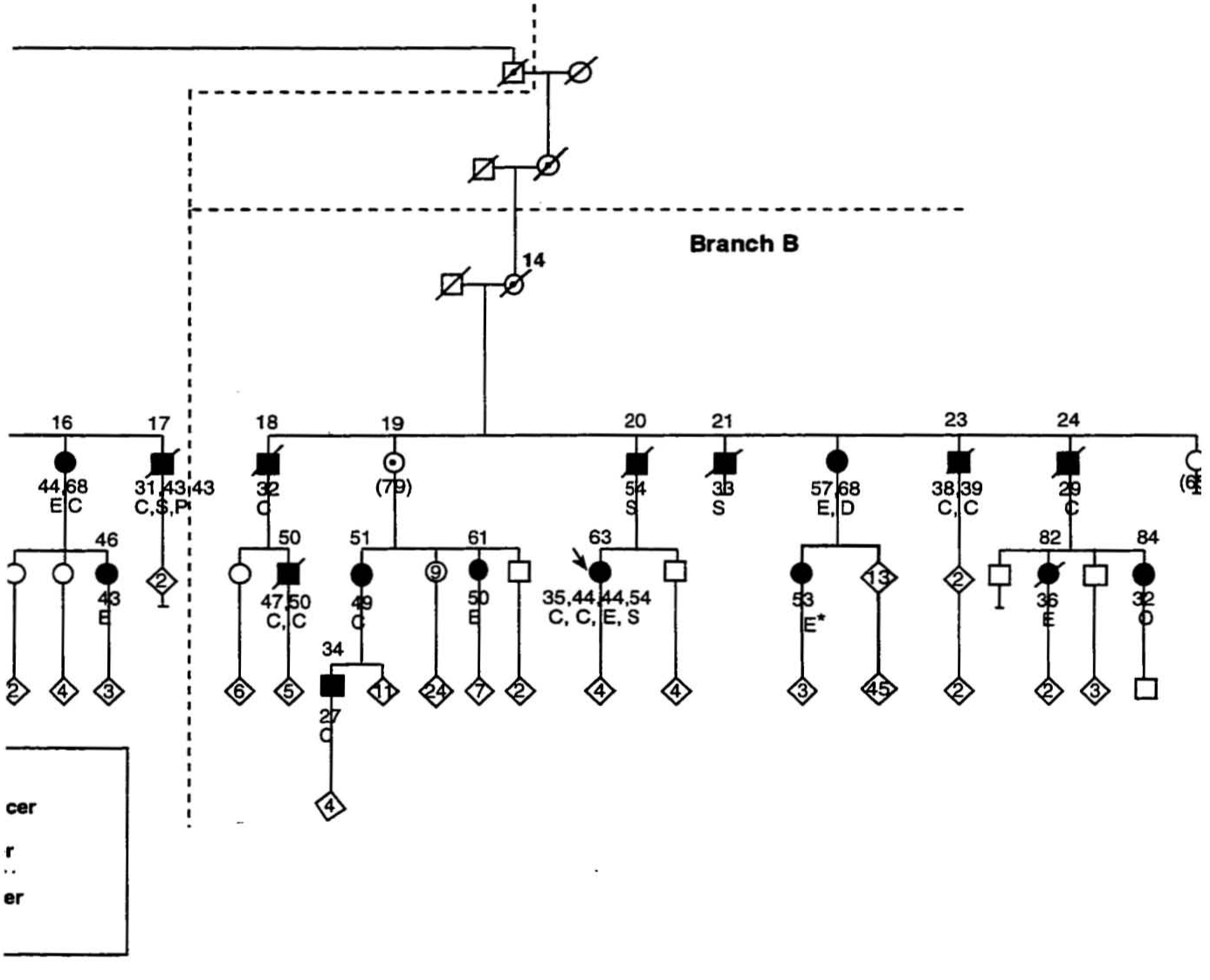
Figure 5.4. Pedigree of a Newfoundland family (Family C) with HNPCC.

The complete pedigree illustrates the connection between the two independently-identified branches of Family C. The types of cancer and age at diagnosis for each affected individual are indicated on the pedigree.

Pedigree of a Newfoundland family (Family C) with HNPCC



IPCC


Branch B
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a 42-year-old identical twin and a 78-year-old woman with affected siblings, children and grandchildren. Ninety-four first degree relatives at 50% risk were identified from the pedigree, 75 of whom were over 20 years of age. Another 153 family members were at 25% risk.

Natural History of Disease

i) Age at diagnosis

In the combined family, twenty-six (12 males and 14 females) were identified as affected (Figure 5.4). The median age at diagnosis of the initial cancer in all affected was 38 years (range, 27-67 years); in affected males, 33 years (range, 27-54 years); and in affected females, 45 years (range, 32-67 years) (Table 5.1, Figure 5.5). There were also four obligate carriers with no evidence of cancer; two men who died of other causes at age 45 and 53, and two women age 42 and 78 still living.

ii) Types of cancer

The twenty-six affected members had a total of 40 cancers typical of the Lynch II classification, with one to four primary cancers in each affected individual. The most frequent cancers were colon cancer (57.5%), endometrial cancer (22.5% of the total cancers, or 39% of the cancers in affected women), and stomach cancer (10%) (Table 5.2, Figure 5.6). Ovarian and duodenal cancer also occurred, and bladder cancer has since been identified in one family member who was at 50% risk. Ten of 20 colorectal cancers (50%), and 2 of

TABLE 5.1. AGE AT DIAGNOSIS, AGE AT DEATH, AND SURVIVAL TIME OF MALE AND FEMALE PATIENTS IN A NEWFOUNDLAND HNPCC FAMILY

	AGE IN YEARS median (range) number	
	Male	Female
Age at diagnosis	33 (27-54) n=12	45 (32-67) n=14
Age at death	41 (29-69) n=10	54 (38-67) n=5
Survival time -deceased only	1 (0-31) n=10	2 (0-16) n=5
-all patients	1 (0-31) n=12	6 (0-34) n=14

Figure 5.5. Time to diagnosis and to disease-specific death for male and female patients in a Newfoundland HNPCC family.

The Kaplan-Meier method was used to estimate the probability of diagnosis or of disease-specific death for male and female patients with HNPCC. Data was censored for those dying of other causes or remaining alive.

The mean age at diagnosis of male ($\bar{x}$, 36.1) and female ($\bar{x}$, 45.4) patients was significantly different ($p < 0.05$). However, the mean age at death of male ($\bar{x}$, 42.8) and female ($\bar{x}$, 53.8) patients was not significantly different ($p > 0.05$).

Time to diagnosis and to disease-specific death for male and female patients in a Newfoundland HNPCC family

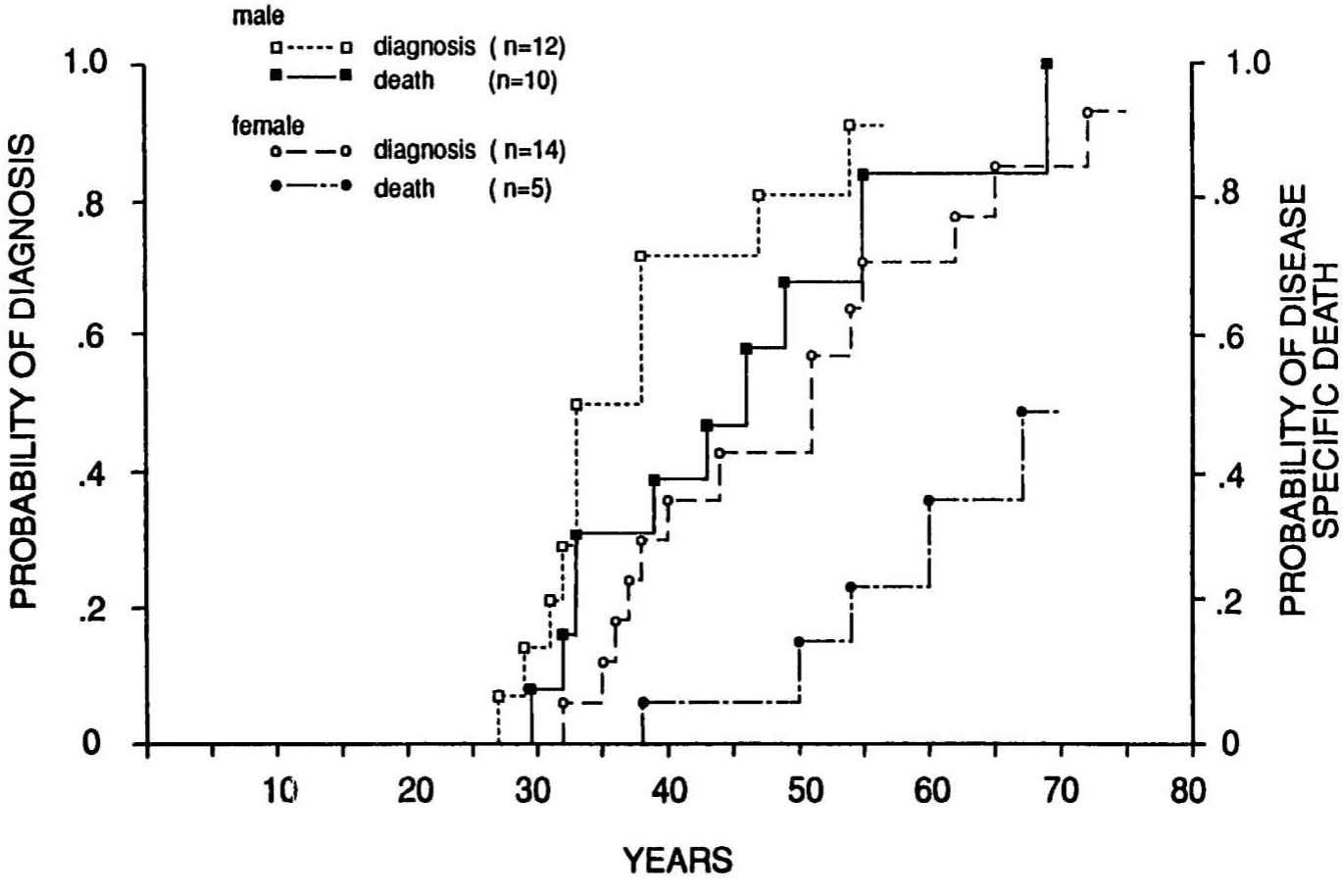


TABLE 5.2. SPECTRUM AND FREQUENCY OF TUMOURS IN A NEWFOUNDLAND FAMILY WITH HNPCC

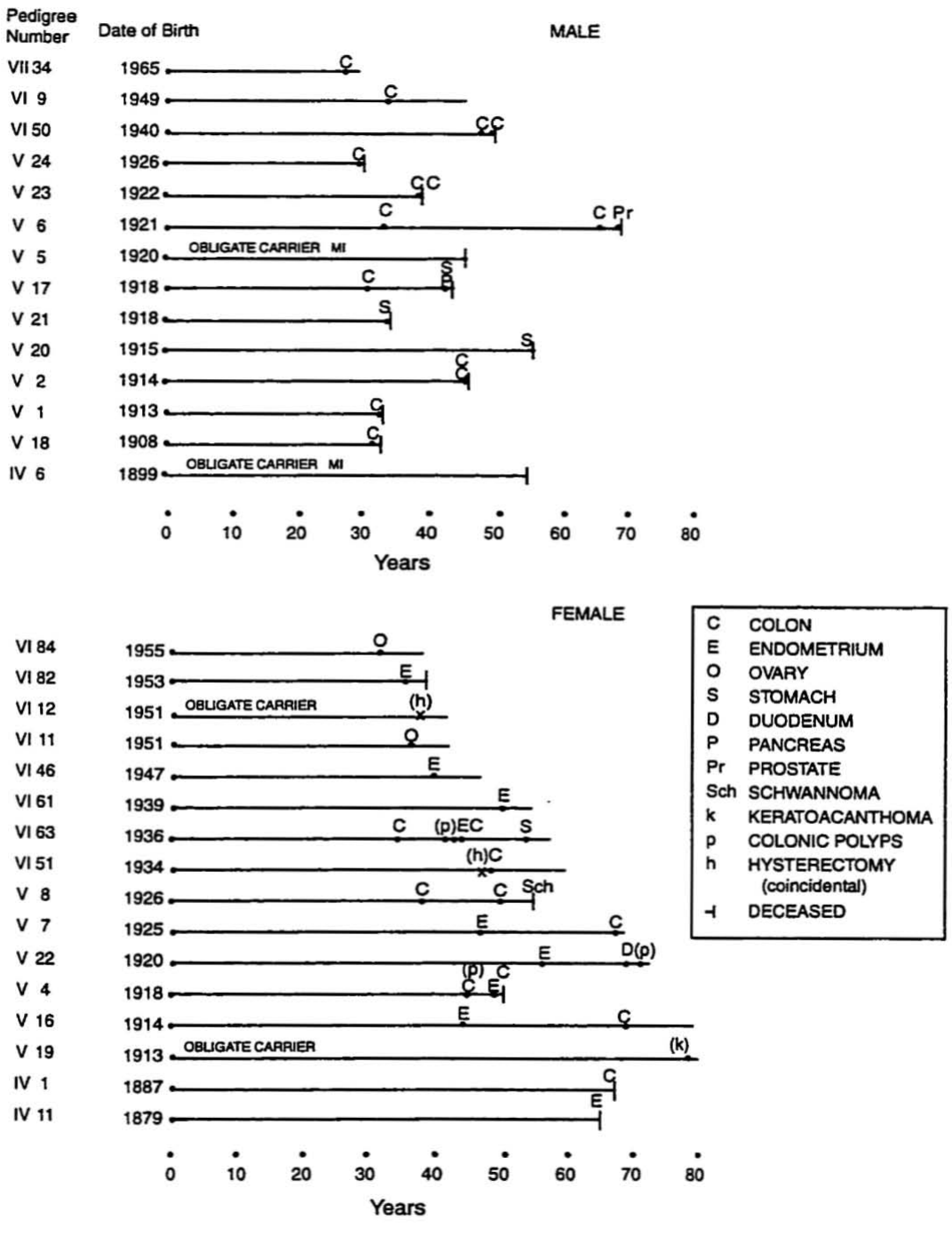
	FREQUENCY				
	INITIAL TUMOUR Number (%)		ALL TUMOURS Number (%)		
TUMOUR	Male	Female	Male	Female	Total
Colorectal	10 (83%)	5 (36%)	13 (76%)	10 (43%)	23 (57.5%)
Endometrial	-	7 (50%)	-	9 (39%)	9 (22.5%)
Ovarian	-	2 (14%)		2 (9%)	2 (5%)
Gastric	2 (17%)	-	3 (18%)	1 (4%)	4 (10%)
Duodenal	-	-	-	1 (4%)	1 (2%)
Pancreatic	-	-	1 (6%)	-	1 (2%)
TOTAL	12	14	17	23	40
Skin			2	4	6
Prostate*			1	-	1
Liposarcoma*			-	1	1
Malignant Schwannoma*			-	1	1

* May be coincidental tumours

Figure 5.6. Combination and order of occurrence of tumours in individual patients with HNPCC.

The type(s) of cancer, age at diagnosis of each cancer, and age at death are indicated on a time line for each patient. Colon, endometrial, ovarian, stomach, duodenal and pancreatic cancer are recognized HNPCC tumours; prostrate cancer and schwannoma are not typical of HNPCC and may be coincidental. Keratoacanthoma is a premalignant skin lesion common in the Muir-Torre variant of HNPCC. Colonic polyps are indicated because they may be premalignant manifestations of HNPCC. For women, coincidental hysterectomy is indicated, since this removes the risk of endometrial and possibly ovarian cancer.

Type of Cancer, Age at Diagnosis and Age at Death for Individual Patients



8 colorectal polyps (25%) of known location, were right-sided (Table 5.3, Figure 5.7). Several skin cancers (basal cell carcinoma, sebaceous cell carcinoma, squamous cell carcinoma, and melanoma) were also identified in affected family members as has been seen in some other Lynch II-like families in the literature, designated as Muir-Torre syndrome (Lynch et al 1985a, Hall et al 1994b). Prostate cancer and a malignant schwannoma, identified in affected family members, and a liposarcoma in a 26-year-old at 50% risk, are not typical of Lynch II syndrome; these may be less common cancers of the Lynch II phenotype, or may be coincidental.

iii) Initial cancer

The initial cancer in 10/12 males (83.3%) was colorectal cancer, whereas the initial cancer in 7/14 females (50%) was endometrial cancer, and in two other women was ovarian cancer (Table 5.2). All patients originally documented were identified because of symptoms, most commonly abdominal pain, bleeding or diarrhea in those with colon or stomach cancer, and menstrual abnormalities or post-menopausal bleeding in those with endometrial or ovarian cancer.

iv) Mortality

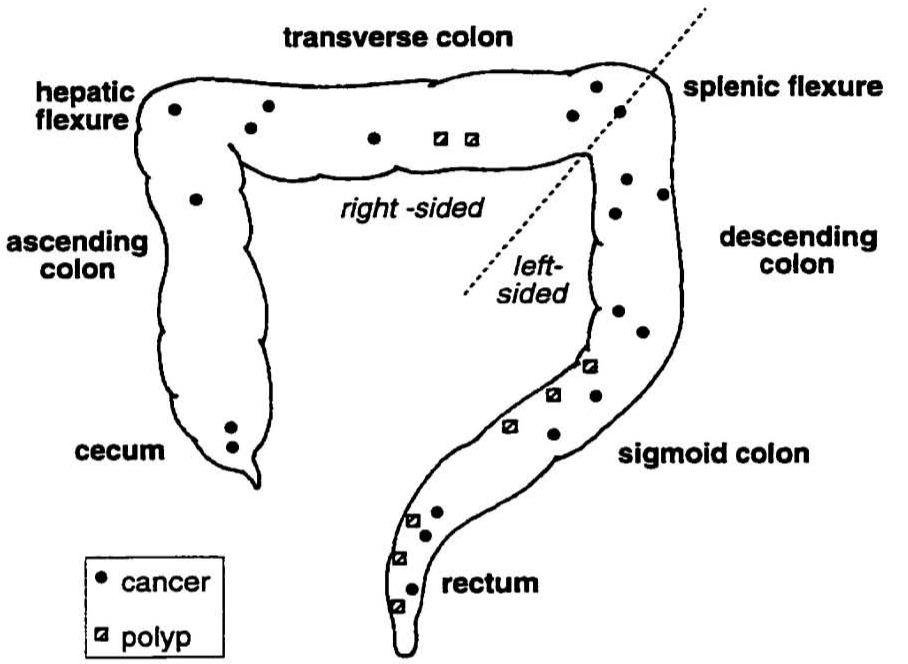
Ten of the 12 affected men have died; the median age at death was 41 years (range 29-69) and the median survival time was 1 year (range 0-31 years). Five of the 14 affected women have died; their median age at death was 54 years (range 38-67 years) and the median survival time was 2 years

TABLE 5.3. LOCATION OF COLORECTAL NEOPLASTIC LESIONS IN AFFECTED MEMBERS OF A NEWFOUNDLAND HNPCC FAMILY

LOCATION	TUMOURS (number)	POLYPS (number)
COLON	17	5
Right-sided	(10)	(2)
Cecum	2	-
Ascending colon	2	-
Transverse colon	6	2
Left-sided	(7)	(3)
Descending colon	5	-
Sigmoid colon	2	3
RECTUM	3	3
UNKNOWN	3	1
TOTAL	23	9

Figure 5.7. Location of colorectal cancer and polyps in patients with HNPCC.

Location of colorectal cancer and polyps in patients with HNPCC



	cancer	polyp
right-sided colon	10	2
left-sided colon	7	3
rectum	3	3
unknown	3	1
total	23	9

(range 0-16 years) (Table 5.1, p. 253; Figure 5.8). The two living affected men have survived 10 and 2 years since diagnosis, but the second of these, a 27-year-old, has terminal colon cancer. The nine living affected women have survived 4-34 years (median, 10 years) since diagnosis, and their current median age is 57 years (38-78 years). All deaths of known affected family members were due to cancer. Two male obligate carriers in a previous generation, however, died at ages 45 and 53 of heart attack without evidence of having cancer, although no investigations had been carried out.

Clinical Screening

i) Participation in screening

Forty-three of those at 50% risk and over 20 years of age (28/42 female, and 15/33 male) have been screened with colonoscopy (35 with normal results, and eight with polyps). Eighteen women have also had pelvic ultrasound, and endometrial biopsy or D & C (fourteen with normal examinations, and four with abnormal results including one with endometrial cancer). Ten women had had a previous unrelated hysterectomy with bilateral oophorectomy (thus, are no longer at risk for endometrial cancer, and have very reduced risk for ovarian cancer). Eight of 11 affected family members have been screened for subsequent cancers, and one of two obligate carriers was also screened (Table 5.4).

Figure 5.8. Kaplan-Meier survival curves for male and female patients with HNPCC in a Newfoundland family.

The Kaplan-Meier method was used to construct survival curves, using age at disease-related death, and censoring data for unrelated deaths and for persons remaining alive. Ten of 12 affected male patients died of HNPCC-related cancers and two are still alive; two obligate carriers died of other causes. Five of 14 affected female patients died of HNPCC-related cancers and nine are still alive; two obligate carriers are living at ages 42 and 79.

Kaplan-Meier survival curves for male and female patients with HNPCC

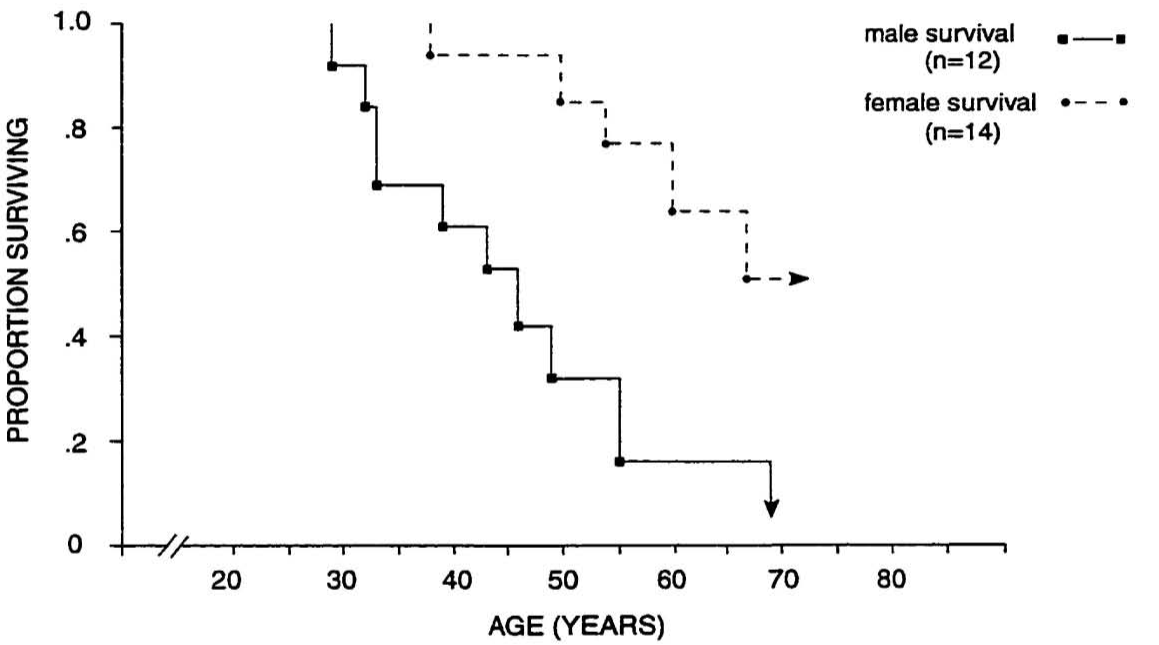


TABLE 5.4. PARTICIPATION, AND RESULTS OF CLINICAL SCREENING FOR AFFECTED AND AT-RISK MEMBERS OF A NEWFOUNDLAND HNPCC FAMILY

	PARTICIPATION	RESULTS OF SCREENING			
		Normal	Abnormal		
Category	No. Screened/ Total		Colon	Endometrium	Other
Affected	8/11	4	4(C-2,P-2)	0	0
Obligate Carrier	1/2	0	1(C)	0	1*1
50% Risk	43/94	30*	8	4	1
Age ≤ 20	0/19	..... Screening Not Offered			
21-30	6/14	5	1(P)	0	-
31-40	15/26	11	3(P)	1*2	-
41-50	14/22	10	3(P)	1(P)	-
51-60	6/9	3	1(P)	1(P,C)	1*3
≥ 60	2/4	1	0	1*4	-

*normal colon (30), normal endometrium (14), previous hysterectomy (10)

*1keratoacanthoma (coincidental detection); *2endometrial thickening; *3transitional cell cancer of the bladder; *4enlarged uterus

abbreviations: C-cancer, P-polyp

ii) Results of screening

Five cancers have been identified since this screening was introduced two years ago: three colon cancers, in two previously affected women, and in the 42-year-old obligate carrier; and endometrial cancer and bladder cancer, each in a family member at 50% risk (Table 5.5). One or more adenomatous colonic polyps were also identified and biopsied in eight at-risk family members, and in two women with previous endometrial or ovarian cancer. These individuals will be screened more frequently than those with a normal colon. Thirty five at-risk and four affected family members had a normal colonoscopy. Fourteen women had normal pelvic ultrasound and endometrial biopsy. Three women with endometrial polyps or thickened endometrium will also be screened more frequently than those with normal examinations.

Genetic Studies

i) Mapping of a gene for HNPCC

The HNPCC gene in Family C was mapped to chromosome 2p15-16 after an extensive search of the genome for linked markers (Peltomaki et al 1993) (Figure 5.9). A LOD score of 6.39 was obtained for linkage to the anonymous marker D2S123 and this linkage was confirmed in a large New Zealand family (Peltomaki et al 1993). Deletions of chromosome 2p or loss of heterozygosity (LOH) were not identified in the tumours from this and other HNPCC families, as would be expected if the gene were a tumour suppressor. However,

TABLE 5.5. DETECTION OF CANCER BY CLINICAL SCREENING IN AFFECTED AND AT-RISK MEMBERS OF A NEWFOUNDLAND HNPCC FAMILY

CATEGORY SCREENED	TYPE OF CANCER DETECTED		
	Colon	Endometrial	Other
Affected*1	2	-	-
Obligate carrier*2	1	-	-
At 50% risk	-	1	1*3

*1 Previous endometrial or ovarian cancer.

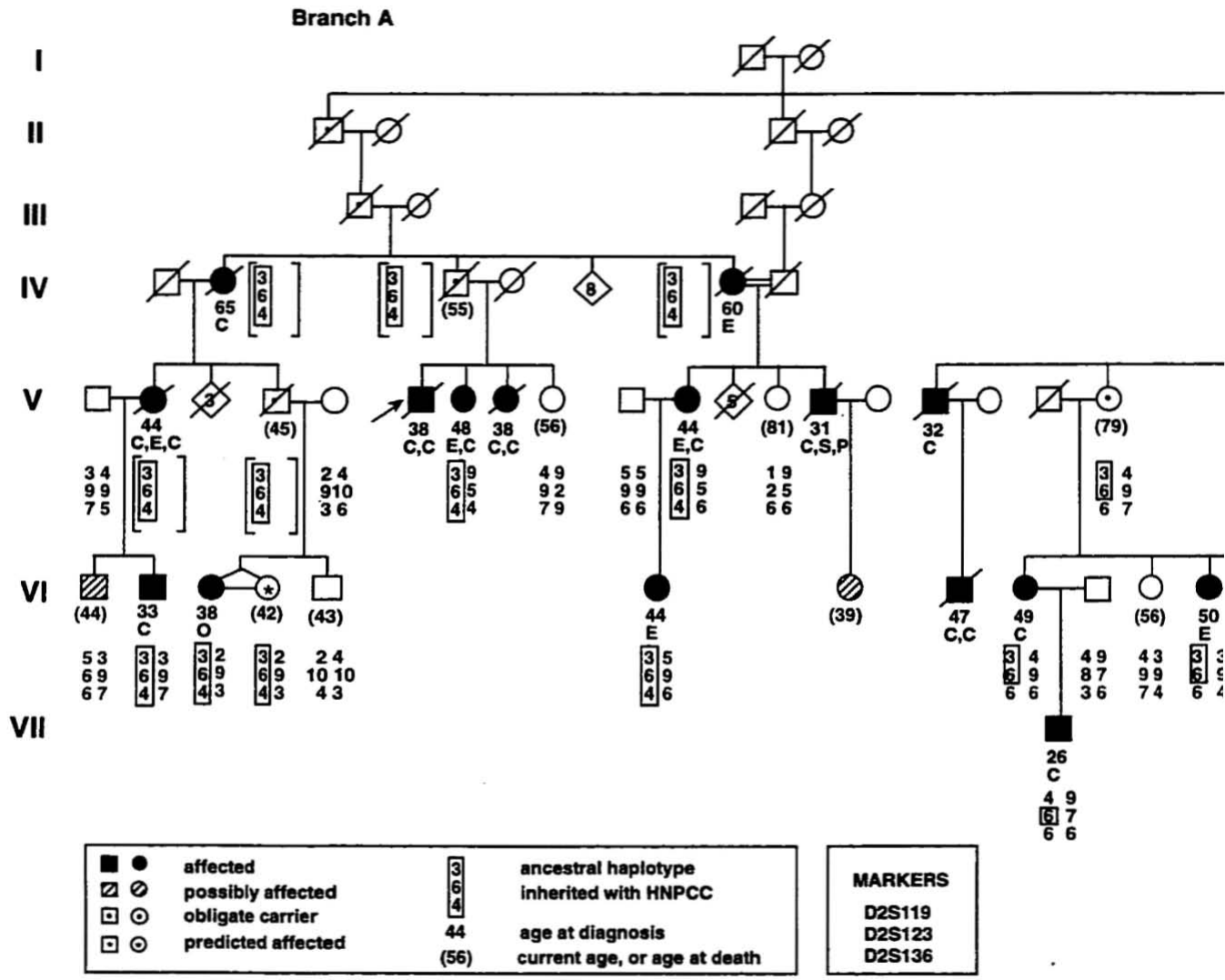
*2 Monozygotic twin affected.

*3 Transitional cell bladder cancer in 54 year old male. (Hematuria was identified and investigated following referral to Internal Medicine for screening for HNPCC.)

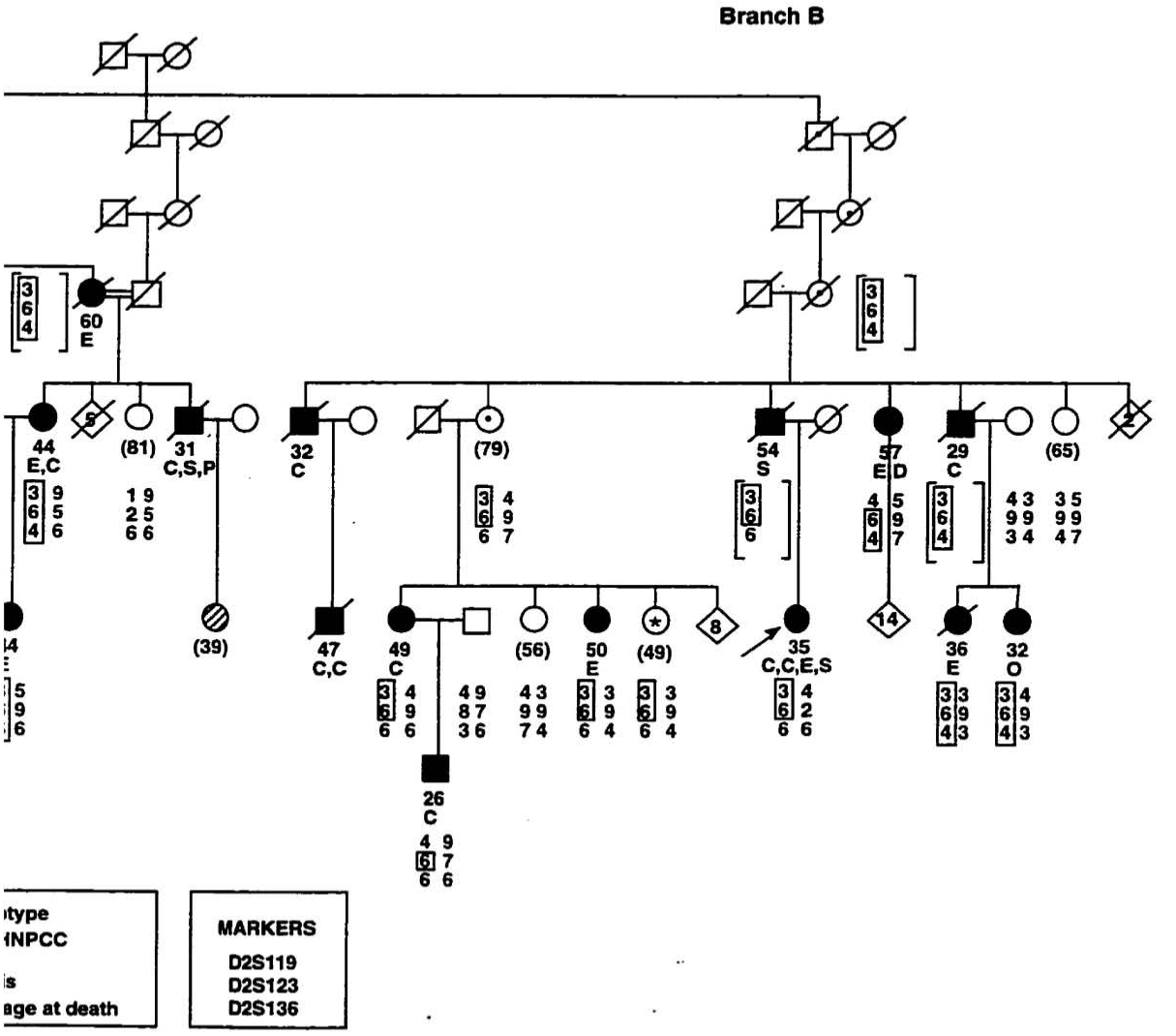
Figure 5.9. Linkage of HNPCC with anonymous markers on chromosome 2p in a Newfoundland family (Family C).

The pedigree of Family C is simplified to highlight those from whom DNA samples were available. Type(s) of cancer and age at diagnosis are given for each affected individual as on the previous pedigree (Figure 5.4). Linkage to three markers on chromosome 2p (D2S119, D2S123, D2S136) was identified by Peltomaki, Aaltonen and colleagues (Peltomaki et al 1993), and data on the alleles present for each marker were provided for each family member tested. Haplotypes were constructed and are indicated on the pedigree. Assumed haplotypes of deceased family members are given in brackets. All affected family members have inherited allele "6" of marker D2S123. Crossovers occurred between D2S123 and flanking markers in some affected individuals as indicated on the pedigree.

Linkage of HNPCC with anonymous markers on chromosome 2p



'kers on chromosome 2p



instability of microsatellite DNA was demonstrated with accumulation of mutations at chromosome 2p and throughout the genome (Aaltonen et al 1993, Thibodeau et al 1993), suggesting a different mechanism of cancer predisposition, possibly a defect in DNA repair (Strand et al 1993, Palombo et al 1994, Parsons et al 1993).

Microsatellite instability was identified serendipitously because of the use of CA repeat (microsatellite) markers in testing for LOH; instead of loss of an allele of markers at chr 2p15-16, multiple new alleles (mutations) of altered CA repeat size were identified for loci on all chromosomes. Slippage during DNA replication can result in this type of mutation, but the mutations are normally repaired. In HNPCC patients the ability to detect and repair these mutations during DNA replication was apparently reduced.

ii) Cloning of a gene for HNPCC

Subsequently, by studying recombinants in Family C and other families, and by using physical mapping methods, the critical region of chromosome 2p16 was defined, and the human homologue, hMSH2, of the bacterial mismatch repair gene, MutS (which corrects errors in microsatellite allele size), was identified in this region (Fishel et al 1993, Leach et al 1993). Mutations of hMSH2 were identified in three HNPCC families including a splice site mutation in Family C which coincided with the disease in this family (Leach et al 1993, Liu et al 1994). Although the laboratory work for mapping and cloning the gene, hMSH2, was done elsewhere, the success of these studies was based on the large, well-defined Newfoundland family.

iii) Request for predictive testing

There are 94 family members identified from the pedigree at 50% risk. Nineteen of these are less than 20 years of age; clinical screening has not been offered to this group, nor was predictive testing offered. Fifty-four of 75 family members over 20 years of age live in Newfoundland, and 36 (66%) of these have requested predictive testing. DNA has been banked and arrangements have now been made for a laboratory to conduct this testing. When results are available, they will be provided in another counselling session in a location convenient for the family member (ie, at the General Hospital in St. John's, in the local hospital, or at home).

DISCUSSION

Natural History of HNPCC

Hereditary non-polyposis colon cancer (HNPCC) has been defined as the autosomal dominant predisposition to colon and extra-colonic cancers, particularly endometrial cancer in women, without the pre-existence of multiple polyps (Lynch et al 1988, Vasen et al 1990b). Age at onset is frequently earlier than for similar sporadic cancers, multiple or bilateral tumours are common, and colon cancers are frequently right-sided (Mecklin and Jarvinen 1986a, Lynch et al 1993c). HNPCC was previously divided into Lynch I and Lynch II syndromes depending on whether or not extra-colonic cancers occurred in the kindred (Lynch et al 1988), but this classification does not seem to have a genetic basis (Nystrom-Lahti et al 1994a).

The most common extra-colonic cancers in HNPCC families (Vasen et al 1990b, Watson and Lynch 1993, Lynch et al 1993c) are other gastro-intestinal cancers (eg, stomach [Cristofaro et al 1987], duodenum [Lynch et al 1989], pancreas [Lynch et al 1985b]); female genital cancers (eg, endometrium [Mecklin et al 1986b, Watson et al 1994] and ovary [Lynch and Lynch 1979]); genito-urinary cancers (eg. transitional cell cancer of the kidney, bladder or ureter [Lynch et al 1990]); and skin cancers (eg, sebaceous cell carcinoma, basal cell carcinoma, and keratoacanthoma [Hall et al 1994a, Hall et al 1994b]). Although heterogeneity among families is observed in the frequency of specific extracolonic cancers (Watson and Lynch 1993), there is also significant intrafamilial variability in the age at onset, site(s) and order of occurrence of HNPCC cancers. Thus the rarer cancers such as stomach, or pancreatic cancer, and transitional cell cancers of the genitourinary tract, may be more common in some families, but cannot be ignored in any HNPCC family.

Morbidity and Mortality

Some authors have referred to colon cancers in HNPCC families as less aggressive and therefore providing a better prognosis than sporadic colon cancer (Mecklin and Jarvinen 1986a, Hall et al 1994b, Jass et al 1994). This is not the case in the Newfoundland family and some other families in the literature, that have very aggressive tumours and early death (Lindblom et al 1993). Both age at diagnosis and age at death were earlier in men than in

women in the Newfoundland family, with 90% of affected men deceased by age 54 (Figure 5.5, p. 255).

HNPCC causes a high burden to families because of the early morbidity and mortality from colon and other cancers (Mecklin and Jarvinen 1986a), and the resulting anxiety to close relatives at risk (National Advisory Council for Human Genome Research 1994). Although it is more common than FAP, it has been more difficult to develop clinical and genetic screening programs because of the poorer understanding of the natural history of disease, the variability of expression including non-penetrance of the gene (Lynch et al 1993c), the lack of a consistent pre-malignant biological marker (Lynch et al 1993c), and until very recently, the lack of information on the gene or genes involved (Lynch et al 1993c, p. 1545).

Clinical Screening

Recent advances in the understanding of HNPCC should improve the management of families. Increased knowledge of the clinical phenotype of HNPCC has come from retrospective and prospective documentation of age of onset and frequency of cancers in large families or groups of families (Mecklin and Jarvinen 1986a, Vasen et al 1990b, Watson and Lynch 1993). For instance, in affected women, in the Newfoundland and other families, endometrial cancer is more frequent and occurs earlier than colon cancer. This must be reflected in the development of appropriate clinical screening (Figure 5.2).

It will not, however, be possible to use routine prophylactic surgery, as in FAP, to prevent cancer, because of the multiple sites in which cancer may occur, the lack of premalignant markers (although one or a few colonic polyps may occur before colon cancer in some patients), and the lack of penetrance in about 10% of gene carriers. Variable age of onset will also continue to be a problem in clinical screening programs. Early onset of cancer requires early screening, but persons at risk with late onset may become non-compliant if early screening investigations are all normal.

The International Collaborative Group on Hereditary Non-Polyposis Colon Cancer (ICG-HNPCC) has provided screening recommendations (Vasen et al 1993), including a recommendation that screening begin five years before the earliest age at diagnosis of cancer in the family. Because some interval cancers (cancers developing between two consecutive investigations) have been identified when screening was every three years, the recommended timing is two-yearly (Vasen et al 1994).

Mapping and Cloning the Genes for HNPCC

Mutations in the DNA repair genes, hMSH2 on chromosome 2p, and hMLH1 on chromosome 3p, have now been shown to be responsible for HNPCC in many families. The large well-characterized Newfoundland family (Family C) played a key role in mapping hMSH2. Family C was large enough to convincingly detect linkage to an anonymous marker on chromosome 2p (Peltomaki et al 1993).

The microsatellite instability (Aaltonen et al 1993, Thibodeau et al 1993, Aaltonen et al 1994, Honchel et al 1994) found in HNPCC tumours from Family C and other families, rather than loss of heterozygosity characteristic of tumour suppressor genes, suggested a defective DNA repair mechanism as the underlying genetic defect. Human homologues of the bacterial mismatch repair genes MutS and MutL, were the obvious candidate genes. In fact, hMSH2, the human homologue of MutS, mapped to chr 2p16 (Leach et al 1993, Fishel et al 1993), and specific mutations in this gene that co-segregated with the disease phenotype have been identified in Family C and other families (Liu et al 1994), a striking example of the productive interplay of research in humans and lower organisms. Subsequently, linkage to chr 3p (Lindblom et al 1993) was demonstrated in HNPCC families not linked to chr 2p, and hMLH1, the human homologue of MutL was mapped to this region and mutations similarly identified (Bronner et al 1994, Papadopoulos et al 1994).

It is estimated that mutations of hMSH2 or hMLH1 are responsible for the predisposition to cancer in 80-90% of HNPCC families (Nystrom-Lahti et al

1994a). Two related DNA repair genes, hPMS1 on chr 2 and hPMS2 on chr 7, have recently been identified, with mutations of each of these genes responsible for predisposition to HNPCC in a few families (Nicolaides et al 1994). Other genes for HNPCC may yet be identified.

Heterogeneity of HNPCC

Both clinical and genetic heterogeneity are seen in the HNPCC families. Families with mutations of different genes (hMSH2, hMLH1, or the less common genes hPMS1 or hPMS2) cannot be distinguished by a specific clinical phenotype. At the same time, the disease expression varies both within and between families with mutations of the same gene (Nystrom-Lahti et al 1994a). Within one family, some family members may have early onset cancers or multiple primaries while others have late onset cancers or non-penetrance of the gene. This is seen in Family C, where a 27-year-old presented symptomatically with metastatic colon cancer, and his 78-year-old grandmother with the same mutation has had no cancer identified. Similarly, there is variability of expression between families with allelic mutations. Nystrom-Lahti and colleagues (1994a) have recently identified hMSH2 mutations in families previously described, because of the spectrum of cancers in each family, as Lynch I syndrome, Lynch II syndrome and Muir-Torre syndrome.

Availability of Predictive Testing for HNPCC

Direct presymptomatic identification of gene carriers is now technically possible for families with an identified mutation of hMSH2, hMLH1, hPMS1, or hPMS2, and has been reported for a large New Zealand family (van de Water et al 1994). Identification of the specific mutation for each HNPCC family, however, is hampered by the number of genes involved and the variability of the mutations so far identified. Protein truncation testing has been useful in identifying mutations of hMSH2 (Liu et al 1994), but missense mutations (mutations in which there is substitution of an amino acid in a normal length protein, rather than a shortened protein) will not be detected by this method.

Although the majority of HNPCC families for whom mutations have been identified have unique mutations (Liu et al 1994), there is an interesting cluster of fourteen HNPCC families from central Finland with the same mutation. Through archival research, nine of these families were shown to have a common ancestor in the early 1500s. The disease phenotype mapped to chromosome 3p in several of the families and a conserved disease-associated haplotype was identified (Nystrom-Lahti et al 1994b). When the second HNPCC gene (hMLH1 on chr 3p21) was cloned, the identical mutation was identified in each of the fourteen families (personal communication, Dr. Albert de la Chapelle, University of Helsinki, Finland). Another cluster of five families in southeastern Finland with a second hMLH1 mutation has also been identified.

Because of this founder effect, genetic screening for one or other of these two mutations is available for the majority of Finnish HNPCC families. It is possible that in Newfoundland a similar situation will exist, with the Family C mutation being identified in other families. Recall that Family C was ascertained through two independent probands, and the extended pedigree of a single family was constructed through archival research. Other Newfoundland HNPCC families with ancestors from the same region of the northeast coast are known, and may also be branches of Family C with this same mutation. If this is the case, predictive testing for HNPCC will be available for a greater number of Newfoundland HNPCC families.

Predictive testing has recently been arranged for members of Family C, and when this is completed, affected members of other Newfoundland HNPCC families will be tested for the Family C mutation.

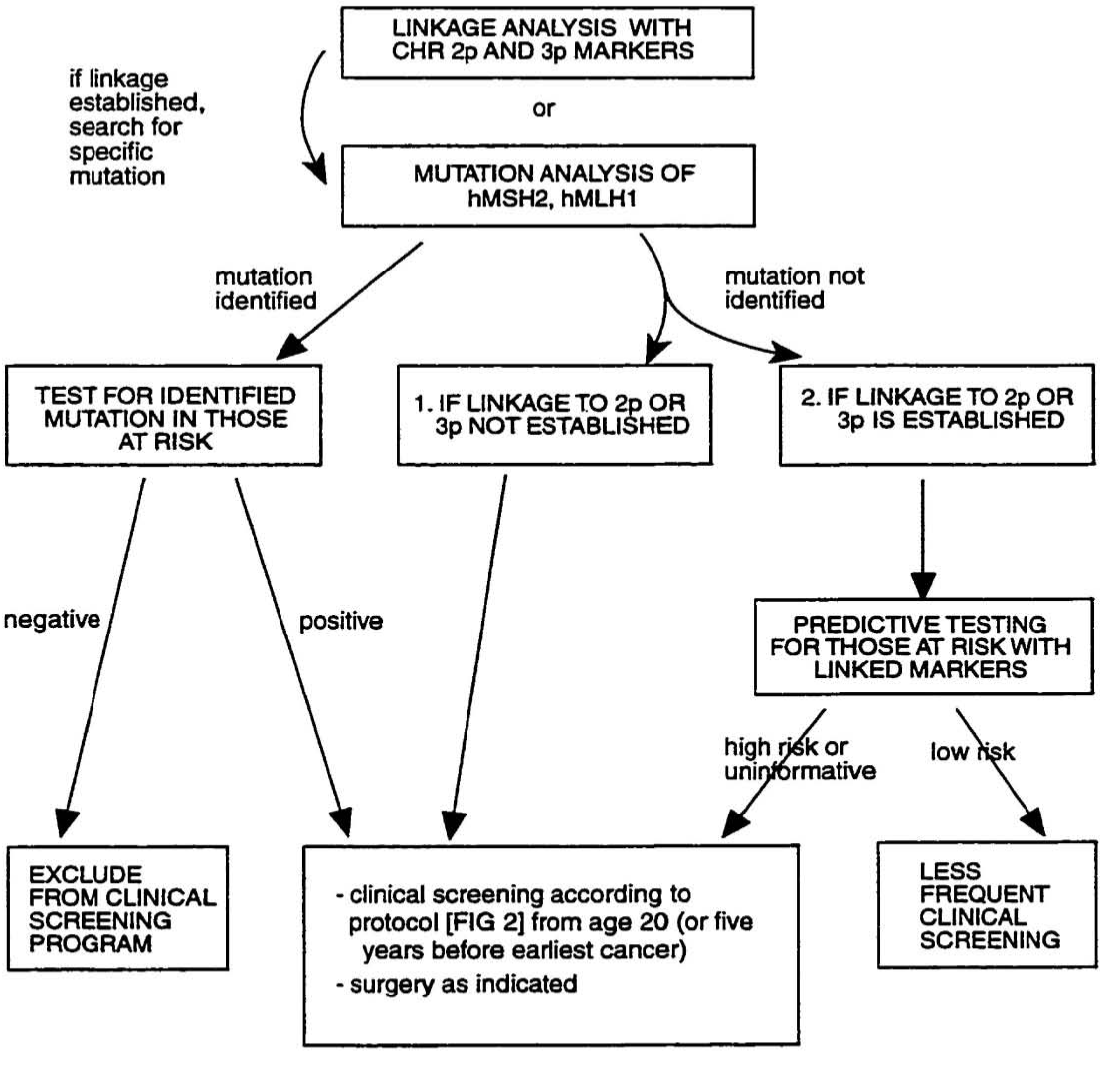
Predictive testing by linkage analysis is possible for some other families for which the individual mutation has not been identified, but linkage is demonstrated to either chr 2p (Green et al 1994) or chr 3p (Lindblom et al 1993). Establishing linkage to one of the identified HNPCC loci, however, is rendered difficult by the lack of DNA from deceased affected family members, and the difficulty in classifying living family members due to the variable expression and reduced penetrance of HNPCC genes. If predictive testing by mutation detection or linkage analysis is possible in a particular family, then the clinical screening protocol can be modified according to the results of genetic testing (Figure 5.10).

Figure 5.10. Management plan for HNPCC families.

Management for HNPCC is complicated by the clinical and genetic heterogeneity demonstrated, the absence of genotype/phenotype correlations which could guide clinical screening, and the lack of sensitive and specific tests for some of the typical cancers.

Depending on family structure, and laboratory facilities available, linkage analysis and/or mutation analysis may be attempted to identify the gene involved and/or the specific mutation. Mutation analysis includes testing for "common" mutations in the specific population and/or a mutation screen (eg, SSCP, DGGE, PTT [Caskey 1993a, Roest et al 1993]).

Management plan for HNPCC families



Demand for Predictive Testing

There is already demand for genetic testing for HNPCC. Originally this was from members of large families, such as Family C, where there had been considerable anxiety because of the frequency of cancer and early cancer deaths. Some members of recognized HNPCC families have had previous screening with colonoscopy and now request genetic testing to determine whether they should continue with clinical screening. Others, who were concerned about their risk of cancer but who had declined unpleasant investigations such as colonoscopy "unless really necessary", also request genetic testing. The demand has increased recently because of the publicity surrounding the recent identification of the HNPCC genes and the suggestion that a blood test for colon cancer was imminent (Globe and Mail 1993). Many members of the general public, appropriately or inappropriately, now expect that a blood test will clarify their risk of colon and other cancers.

Thirty-six of the 54 at-risk members of Family C to whom predictive testing has been offered, have requested this testing. All of these family members were anxious because of the frequency of cancer and cancer deaths in their family, before the question of predictive testing was raised. The majority find colonoscopy (and, if female, the endometrial biopsy) to be unpleasant procedures, and would like to know whether these investigations are necessary. It is encouraging to others in the family that three family

members with early colon cancer, and one with endometrial cancer, identified by clinical screening, have done well.

Even for families where presymptomatic diagnosis or predictive testing is now possible, there are many social, ethical and legal questions to be answered before genetic testing becomes common usage (Assessing Genetic Risk 1994, National Advisory Council for Human Genome Research 1994, Danks 1994, Collins 1994). These concerns have been addressed by the National Institutes of Health, and National Cancer Institute in the United States of America who have recommended extensive research into the psychosocial outcomes of the provision of genetic counselling and testing for heritable breast, ovarian and colon cancer risks, before implementation of genetic testing for these cancers as a general clinical service (NIH Guide 1994).

Implications of Screening for HNPCC

It is important to determine the effect of early identification and treatment of gene carriers by clinical screening on long-term morbidity and mortality, and to establish protocols for genetic testing, so that benefits can be maximized and harms minimized (Collins 1994). These protocols must include methods for education and counselling before genetic testing, for ensuring informed consent, and for provision of follow-up, whether clinical or psychological, to those with increased or decreased risk of cancer. There are fears that there will be discrimination (Billings et al 1992b, Ostrer et al 1993),

particularly in terms of employment or insurance coverage (less of a problem in Canada than in the United States, but still a concern), or stigmatization with "cancer" risk (which engenders fear in many individuals), without significant benefit from early diagnosis and treatment. The benefit from clinical screening is yet to be determined, but it is recognized that HNPCC differs from FAP where there is a more consistent natural history, with a premalignant marker (multiple polyps) and fewer potential tumours, and therefore, a reliable clinical screening protocol for gene carriers, and an effective prophylactic treatment.

APPENDIX

Patient 1 (pedigree # VI-15)

Patient 1, a 68-year-old male, had a history of two primary colon cancers at ages 38 and 65, and, as well, had prostate cancer at age 67. He died at age 69 of metastatic disease from the colon and prostate cancers. One sister died at age 54 having had two primary colon cancers, and another sister had endometrial cancer at age 47 and was well at age 65. Patient 1 was concerned about the risk of cancer to a third sister age 54, and particularly to his four children ages 19-35.

Patient 2 (pedigree # V-8)

Patient 2, a 54-year-old female, had a history of two primary colon cancers at ages 35 and 44, and endometrial carcinoma at age 43. She subsequently developed stomach cancer at age 54. Her father died at age 54 of stomach cancer and four paternal uncles died of bowel or stomach cancer at ages 29, 32, 33 and 39. She had one brother age 49 and four children ages 23 - 28 who were apparently unaffected, and also many more distant relatives, including children of the deceased affected uncles. Many of these relatives were very apprehensive about the risk of cancer to themselves or to their children.

CHAPTER 6 — CLINICAL AND GENETIC SCREENING FOR VON HIPPEL-LINDAU DISEASE

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INTRODUCTION

This chapter will describe the identification of five Newfoundland families with von Hippel-Lindau (VHL) disease; the development, implementation and revision of clinical and genetic screening programs; and the review of medical and psychosocial outcomes of screening.

Previous Studies

VHL is a potentially lethal autosomal dominant disorder with multiple manifestations including benign and malignant tumours of the central nervous system and many internal organs. Although over thirty different manifestations have been described, the major morbidity and mortality is caused by retinal angiomas, cerebellar and spinal cord hemangioblastomas, renal cell carcinomas and pheochromocytomas (Melmon and Rosen 1964, Horton et al, 1976, Lamiell et al 1989).

The age at onset of VHL is variable; the mean age at diagnosis estimated from a literature review was 24 years (Lamiell et al 1989), with age at diagnosis ranging from infancy to the sixth or seventh decade (Jennings et al 1988, Maher et al 1990, Filling-Katz et al 1991b). However, many patients had not been investigated previously, and some asymptomatic patients who were identified at a late age had advanced lesions (Glenn et al 1990). The most common manifestations of VHL are retinal angiomas and cerebellar hemangioblastomas, followed by renal cell carcinoma, pheochromocytomas and

spinal cord hemangioblastomas (Lamiell et al, 1989, Maher et al 1990), but angiomas, adenomas and cysts of many other organs also occur (Horton et al 1976, Hough et al 1994).

Retinal angiomas and cerebellar hemangioblastomas, are also the most common presenting manifestations for individual patients (Glenn et al 1990, Maher et al 1990), often in their teens or twenties. Different large families, however, have different patterns of manifestations, and in some families, including the large Newfoundland family, pheochromocytomas are particularly common (Atuk et al 1979, Green et al 1986, Glenn et al 1991), and, in these families, are frequently the first manifestation (Neumann et al 1993). Renal cell carcinomas were thought to be a late manifestation of VHL occurring after 40 years of age (Horton et al 1976, Lamiell et al 1989, Maher et al 1990), but are now recognized to occur much earlier (Keeler and Klauber 1992). Besides the variability of disease expression between families, the combination and order of occurrence of manifestations within a family is extremely variable (Jennings et al 1988).

The mean age at death from VHL, as estimated from the literature review, was 38 years of age (Lamiell et al 1989). The most common causes of death are cerebellar hemangioblastoma and renal cell carcinoma (Horton et al 1976, Jennings and Gaines 1988, Sato et al 1988, Neumann et al 1992), but in families with frequent pheochromocytomas, hypertensive crisis from an unrecognized pheochromocytoma has been a frequent cause of death (Rho

1969, Neumann et al 1993). Retinal angiomas are a frequent cause of blindness unless detected and treated early (Hardwig and Robertson 1984, Ridley et al 1986, Moore et al 1991).

Because of the variability of VHL, and because of the advanced disease in many symptomatic patients, screening is recommended for early identification and treatment of individual manifestations to improve the prognosis of the disease (Green et al 1986, Jennings et al 1988, Choyke et al 1990, Maher 1990). Early identification of gene carriers also allows genetic counselling to be offered before reproductive decisions are made (Jennings and Gaines 1988, Lamiell et al 1989).

Present Study

A large VHL family from the Bonavista Peninsula of Newfoundland was identified in the early 1980s when several sisters were referred to the ocular genetics clinic at the General Hospital, St. John's. Individual family members had previously seen a number of different physicians when symptomatic but the relationship of the different presenting symptoms was not well understood by the family members or their physicians, and coordinated follow-up had not been arranged for early detection of subsequent manifestations in those already affected, or for review of first degree relatives. Many of the patients had advanced disease at the time of diagnosis, and morbidity, including blindness or neurological deficit, and early deaths were common. Pheochromocytomas

were more common in this family than in most families reported in the literature, and were a frequent cause of death (Rho 1969, Green et al 1986). Cerebellar hemangioblastomas were less common than in most families in the literature, and renal cell carcinoma reported in only one patient. Besides the medical problems, there was a great deal of anxiety, psychological stress, and mistrust of the medical system in affected and unaffected family members because of the deaths and disabilities caused by VHL (Hogan and Sparrow 1972).

Several smaller Newfoundland VHL families were subsequently identified (see Appendix A, p. 361). Screening was recommended to improve the clinical management for these families. Since the location of the VHL gene was unknown at that time, genetic screening was not possible, but a clinical screening protocol was developed based on the frequency and age of onset of manifestations from the literature review and the initial medical record review for the families. The screening protocol, using clinical examination and biochemical or radiological investigations to detect the common manifestations of VHL at a presymptomatic stage, was offered to all affected and at-risk members of the Newfoundland families over three years of age. Patients with positive screening tests were referred for diagnostic investigations and, if necessary, for surgery. Annual screening for subsequent manifestations resumed following the investigations or treatment.

The clinical screening program has been monitored continuously and adapted as necessary as data on the natural history of the disease in the Newfoundland families accumulated, and as new investigative procedures became available.

Although the VHL screening program was originally developed to improve the clinical management of family members, the objectives of the program were extended and formalized to include:

- early identification of those who are affected to allow informed reproductive decisions
- early identification of specific manifestations to facilitate early treatment or intervention and to reduce the morbidity and mortality associated with advanced disease
- genetic counselling for affected and at-risk family members to increase the understanding of all features of VHL disease
- collection of data to allow refinement of the screening protocol.

The large Newfoundland family was included in collaborative studies which localized the gene for VHL to chromosome 3p25-26 in 1988 (Seizinger et al 1988), but closely-linked informative markers have only been available for genetic screening since 1991 (Seizinger et al 1991, Glenn et al 1992, Maher et al 1992). Predictive testing by linkage analysis for the Newfoundland VHL families was developed in collaboration with Dr. Eamonn Maher at Cambridge University, Cambridge, UK, and was underway when the VHL gene was

identified and cloned in May 1993 (Latif et al 1993). The specific mutation responsible for the disease in many families, including three Newfoundland VHL families, has been identified (Crossey et al 1994, Whaley et al 1994, Chen et al 1995). For these families it is now possible to directly identify those with the VHL mutation and provide clinical screening for mutation carriers only, sparing family members without the mutation.

As with other hereditary tumour syndromes there are concerns about the ethical, legal and social issues associated with this type of screening (Motulsky 1994). It is necessary to weigh the benefits against the possible harms which might result from VHL screening programs before recommending their general implementation. As a preliminary approach to review these issues, affected and unaffected members of VHL families who have participated in the clinical and genetic screening program were questioned about their views on the benefits and costs of this program.

An initial description of the natural history of VHL in the large Newfoundland family and recommendations for clinical screening were reported previously (Green et al 1986, Green et al 1991). This chapter will review the clinical screening that has been ongoing for over 10 years, the genetic screening more recently implemented, and the attitudes of family members towards this screening. The following chapter will evaluate the costs and benefits of the overall VHL screening program.

PATIENTS AND METHODS

Ascertainment of Families

Five families with von Hippel-Lindau disease have been identified in Newfoundland. A large family (Family A) from Bonavista Bay, ascertained in 1982, had 24 affected members, some of whom lived in Ontario. Two smaller families, one from Trinity Bay, and the other originally from Nova Scotia had three and four affected members respectively, and two other families from Bay d'Espoir and central Newfoundland had only one definitely affected member each (Appendix A, p. 361-3).

Development and Implementation of Clinical Screening

The pedigree studies and initial medical record review to identify affected and at-risk family members had been completed for Family A in the mid 1980s. A screening protocol was then developed by a group of specialists (internist, pediatrician, ophthalmologist, neurosurgeon, radiologist and geneticist) using the frequency and age at diagnosis of VHL manifestations from a literature review and from the medical record review for this family as the guidelines for the type and timing of investigations (Figure 6.1). This screening protocol, including annual investigations from age three, was implemented for affected and at-risk members of Family A wishing to participate, and records were kept of the results of all screening tests. The frequency and age at diagnosis of

Figure 6.1. Clinical screening protocol for von Hippel-Lindau disease.

The clinical screening protocol has been revised as necessary, as new information on the natural history of VHL, or new technology became available. The protocol shown was used from 1986-1994. Since 1994, MRI (instead of CT scan) has been used to screen for cerebellar hemangioblastomas, and CT scan of the abdomen (instead of renal ultrasound) has been used to screen for renal cell carcinoma.

CLINICAL SCREENING PROTOCOL FOR VON HIPPEL-LINDAU DISEASE

1. Eye examination with indirect ophthalmoscope annually for those at risk, and at least every 6 months for those who are affected.

   (TREATMENT: laser or cryotherapy)

2. Annual examination by internist or pediatrician

   - full physical examination with BP lying and standing
   - 24-hour urine collection for urinary catecholamines and metanephrines
   - plasma catecholamines if increased blood pressure or postural hypotension found but normal urinary catecholamines
   - abdominal CT and MIBG scans if biochemical abnormalities found

   (TREATMENT: surgery after adequate blocking)

3. Annual neurological examination with particular note of any cerebellar signs

   - baseline CT or MRI scan in early teens
   - CT or MRI scan repeated if any suspicious neurological findings

   (TREATMENT: surgery when recommended by the neurosurgeon)

4. Annual ultrasound of kidneys starting in the teens for evidence of cysts or renal cell carcinoma.

   - CT scan of kidneys if any abnormalities seen
   - Annual CT scan of abdomen for those with previous renal cell carcinoma

   (TREATMENT: surgery for renal cell carcinoma)

manifestations in Family A, initially and after 3 years of screening, have already been reported (Green et al 1986).

The screening program was expanded to include the other VHL families as they were identified. When each family was ascertained, family members were interviewed by Jane Green to extend the pedigrees, and consent was obtained for medical record review to identify affected and at-risk family members. Retrospective information was collected from medical records for each affected individual on the type and age at diagnosis of each manifestation, reason for diagnosis (screening or symptomatic), treatment given, and outcome of treatment. Age and cause of death were documented when appropriate.

Genetic counselling was provided for each affected and at-risk family member by Jane Green. This counselling included a discussion of the features of VHL, the age at which these might occur, and typical symptoms which these manifestations might cause; the inheritance pattern and risk of recurrence of disease; and the recommended screening protocol. Similar information was sent to each family doctor, and a pamphlet was written for family members for reference. Copies of the protocol and pamphlet were sent to family members in other provinces when requested.

Appointments were made for the set of investigations for affected and at-risk family members wishing to participate in the screening program. Prospective records were kept of the results of all screening tests, and of the follow-up investigations and any treatment required when screening tests were

abnormal. The age at diagnosis of each manifestation and the order in which manifestations occurred were recorded for each affected family member. The outcome of treatment, and age and cause of death of those who died were also recorded. Record of the date of each appointment was kept so that those with normal test results, and others following treatment, could be recalled annually for repeat screening investigations.

Recently, the appointments have been made as required by one of the secretaries in the Faculty of Medicine, Health Sciences Centre, St. John's, who also notifies the family members of the appointment times.

Development of Genetic Screening

In 1992, after the VHL gene was mapped and closely-linked flanking markers identified (Glenn et al 1992, Maher et al 1992), genetic counselling about predictive testing by linkage analysis was given to each family member involved in the clinical screening program. This counselling included a discussion of the method of linkage analysis, the possible results (high-risk, low-risk or uninformative), and any revisions of the clinical screening protocol which would occur because of each possible result. The importance of having blood samples for DNA extraction from affected family members and spouses was stressed. A pamphlet on predictive testing was written and provided to family members to reinforce this information.

Consent was obtained from all those wishing to participate in predictive testing for themselves or for their children, and a blood sample was obtained for DNA extraction in Dr. Roger Green's laboratory at the Health Sciences Centre. Arrangements were made for predictive testing by linkage analysis to be carried out by Dr. Maher, Cambridge University. At the time, there were only two children at risk under 10 years of age; they were not yet participating in clinical screening, and predictive testing was not offered to them.

Sixty-one DNA samples were collected from members of families A, B, C and E, including 15 samples from affected family members and 33 from those at 50% risk, and these samples sent to Dr. Maher. Initially, RFLP markers flanking the VHL map location were used (including D3S18, D3S601 and D3S1250) (Table 6.1), and later a microsatellite marker, D3S1038, identified by Dr. Maher's laboratory, was included. Marker allele data on all individuals tested were returned to Jane Green.

When the VHL gene was cloned and the specific mutation identified in Family A (a C→T missense mutation at base pair 712 which converts an arginine to a tryptophan and abolishes a restriction enzyme cutting site [Crossey et al 1994]), detection of gene carriers by direct mutation analysis was possible in this family. Family members were notified that a direct test for the VHL gene mutation could be done, and further genetic counselling was given about the method of testing and the implications of the possible results. A consent form was signed by those who wished to proceed with this more

TABLE 6.1. CHARACTERISTICS OF RFLP AND MICROSATELLITE MARKERS USED FOR PREDICTIVE TESTING BY LINKAGE ANALYSIS IN VHL FAMILIES

LOCUS	PROBE	RESTRICTION ENZYME	ALLELE SIZES	FREQUENCY OF HETEROZYGOSITY
D3S18	c-LIB-1	BamHI	8.7, 4.7	0.43
D3S601	c-LIB-7.1	Bgl II	15.4, 13	0.42
D3S601	LIB 19-63	Taq1	4.3, 3.9	0.5
D3S1038	C13-946	*	8 alleles	-
D3S1250	c-LIB-56	EcoRI	2.8, 1.2	0.32

*Microsatellite marker (CA repeat)
Reference: Crossey et al 1993
Richards et al 1993

definitive testing. This included family members who had previously been given results of predictive testing by linkage analysis, and others who had not yet been tested. The direct mutation analysis was also carried out in Dr. Maher's laboratory, and results were sent to Jane Green for delivery to family members.

The mutations in Family B (a 2bp deletion in codon 201), and in Family C (a G→A transition at base pair 713) have now been identified (Crossey et al 1994) and direct mutation detection is underway for five at-risk members of these families.

Results of predictive testing by linkage analysis or direct mutation testing were given individually to family members in a counselling session, either in their own homes or at the General Hospital, St. John's.

Review of Screening Program

To determine whether a screening program should be continued, revised, or discontinued, the outcomes of the program should be reviewed. Long-term follow-up of many families being screened is required in order to demonstrate statistically significant differences in morbidity and mortality because of the screening program. It is valuable, nevertheless, to review the program on an ongoing basis to provide preliminary data: on the screening process, on the medical and psychosocial outcomes, and on the costs associated with the program. Revisions to the screening protocol can then be made as new information and new technology become available, and hypotheses can be

generated, particularly regarding psychosocial outcomes, for more rigorous testing in a larger number of families and over a longer period of time.

i) Screening protocol

The specialists involved with the VHL patients met in October 1994 to review and revise the clinical and genetic screening protocols. Jane Green provided a summary of the results of clinical screening (see medical outcomes), and also an overview of genetic screening methods including the possible results of this screening and the availability of the different methods for the Newfoundland VHL families. The projected direct costs of clinical screening with or without the implementation of genetic screening were estimated and presented (see direct costs). The radiologists reported on the acquisition of new imaging equipment, including a Magnetic Resonance Imager (MRI) and a new CT scanner.

ii) Medical outcomes

To determine the effect of the clinical screening program on medical outcomes, the types of tumours detected, the age at diagnosis, the severity of disease, the type and outcome of treatment, and the age and cause of death were compared for two groups: those who had been identified because of symptoms, and those who had been identified by screening.

iii) Psychosocial outcomes

To determine the effect of the clinical and genetic screening programs on the well-being of family members, 15 affected and unaffected family

members were interviewed individually about the psychological and social implications of VHL disease and of the screening program, for themselves and for other members of their families. The individuals interviewed included males and females, in both reproductive and post-reproductive age groups. Although the interviews were only semi-structured, the topics covered (Appendix B, p. 364-5) included:

- knowledge and understanding of the disease and of screening methods;
- compliance with screening recommendations;
- anxiety and stress because of the disease or because of screening;
- support systems available;
- financial implications of the disease and of screening;
- discrimination because of the disease;
- effect of the disease, or of the availability of genetic screening on reproductive decisions;
- quality of life before and after the screening program began.

These interviews were taped and reviewed to identify common themes brought up by different family members. The interviews were supplemented by relevant information documented during counselling sessions or telephone calls with affected and at-risk family members over the duration of the screening program. This method of assessment of psychosocial outcomes was chosen

(after discussions with Dr. Michael Murray, Division of Community Medicine, Memorial University of Newfoundland), because of the relatively small number of family members available, and the preliminary nature of this review.

iv) Direct costs

To determine the effect of genetic screening on the direct costs of the screening program, the annual costs and projected lifetime costs of clinical screening for at-risk family members before and after genetic testing was introduced, were compared.

RESULTS

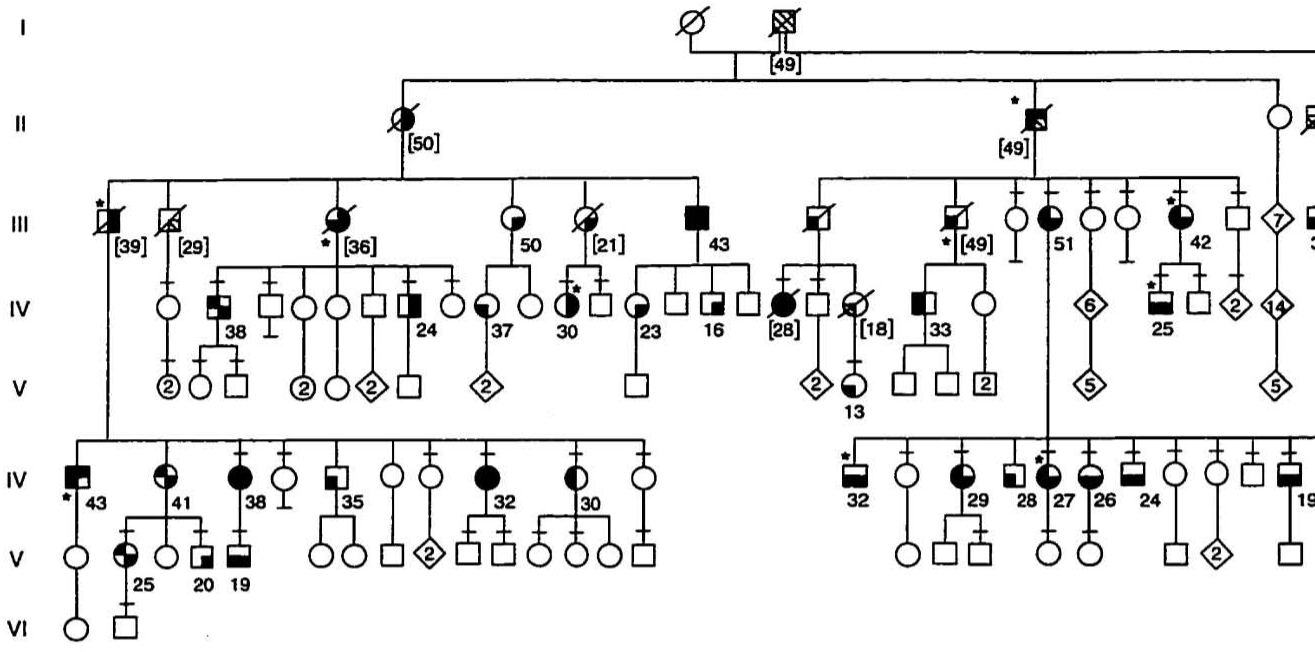
Ascertainment of Families

One large and 4 smaller VHL families (Figure 6.2) have been identified in Newfoundland, from different geographic regions of the province, or originally from another province (Figure 6.3). At the time of ascertainment of the families, there were 33 affected individuals identified through medical record review (24 of whom were in Family A), and 76 first degree relatives at 50% risk. The mean age at diagnosis was 28 years of age, and all but three patients were symptomatic at the time of diagnosis. Sixteen of the 33 affected members had unilateral or bilateral blindness. Sixteen of the 33 affected members were deceased at a mean age of 37 years (all, except one accidental death, as a result of VHL disease).

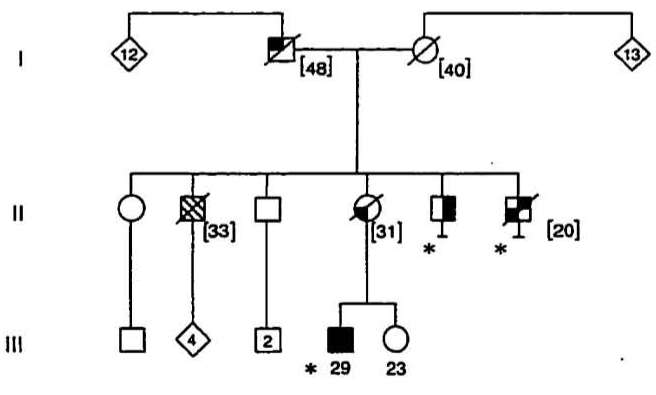
Figure 6.2. Pedigrees of Newfoundland VHL families with manifestations for each family member.

The common VHL manifestations (retinal angioma, pheochromocytoma, renal cell carcinoma, cerebellar and spinal cord hemangioblastoma, and pancreatic islet cell tumour) are present as indicated by the key. Less common manifestations (indicated as "other manifestations") include AV malformation of the colon, liver angioma, stomach angioma, carotid body tumour, cerebral hemangioblastoma, and chordoma.

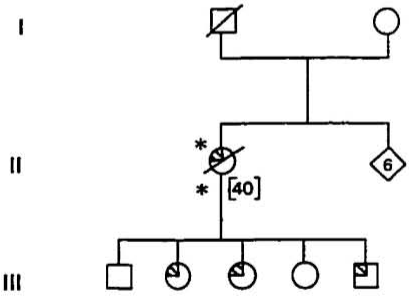
FAMILY A



FAMILY C



FAMILY D



Cerebellar hemangioblastoma
Retinal angioma
Pheochromocytoma
Renal cell carcinoma
Spinal cord hemangioblastoma
Pancreatic islet cell tumour

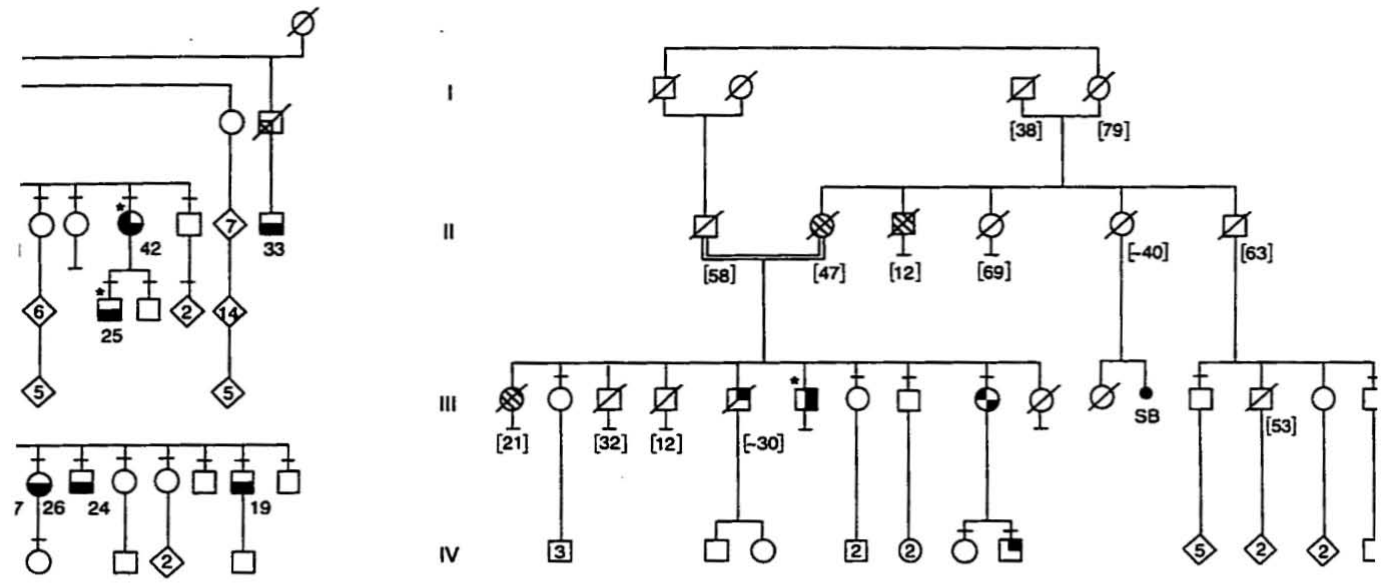
Probable cerebellar hemangioblastoma
Probable retinal angioma
Probable pheochromocytoma
Renal cysts
Affected, details unknown
Examined in St. John's

Other r
(include
cerebra
stomac
angiom

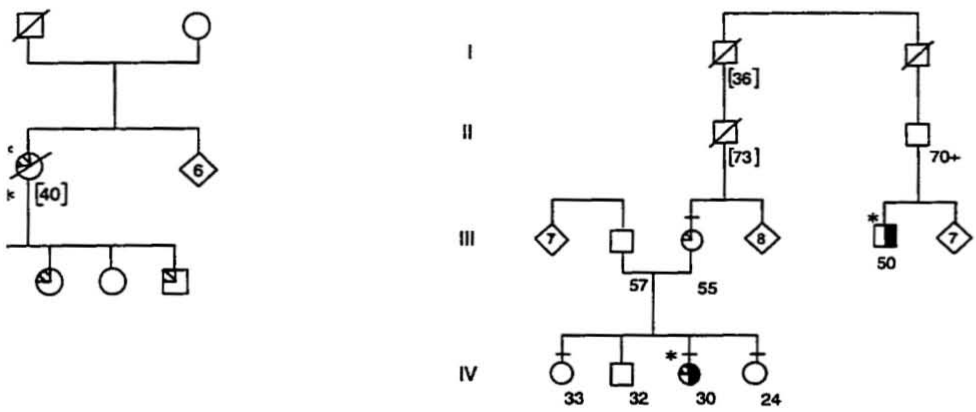
Deceas

32 [29] Age [ag

FAMILY B



FAMILY E



astoma

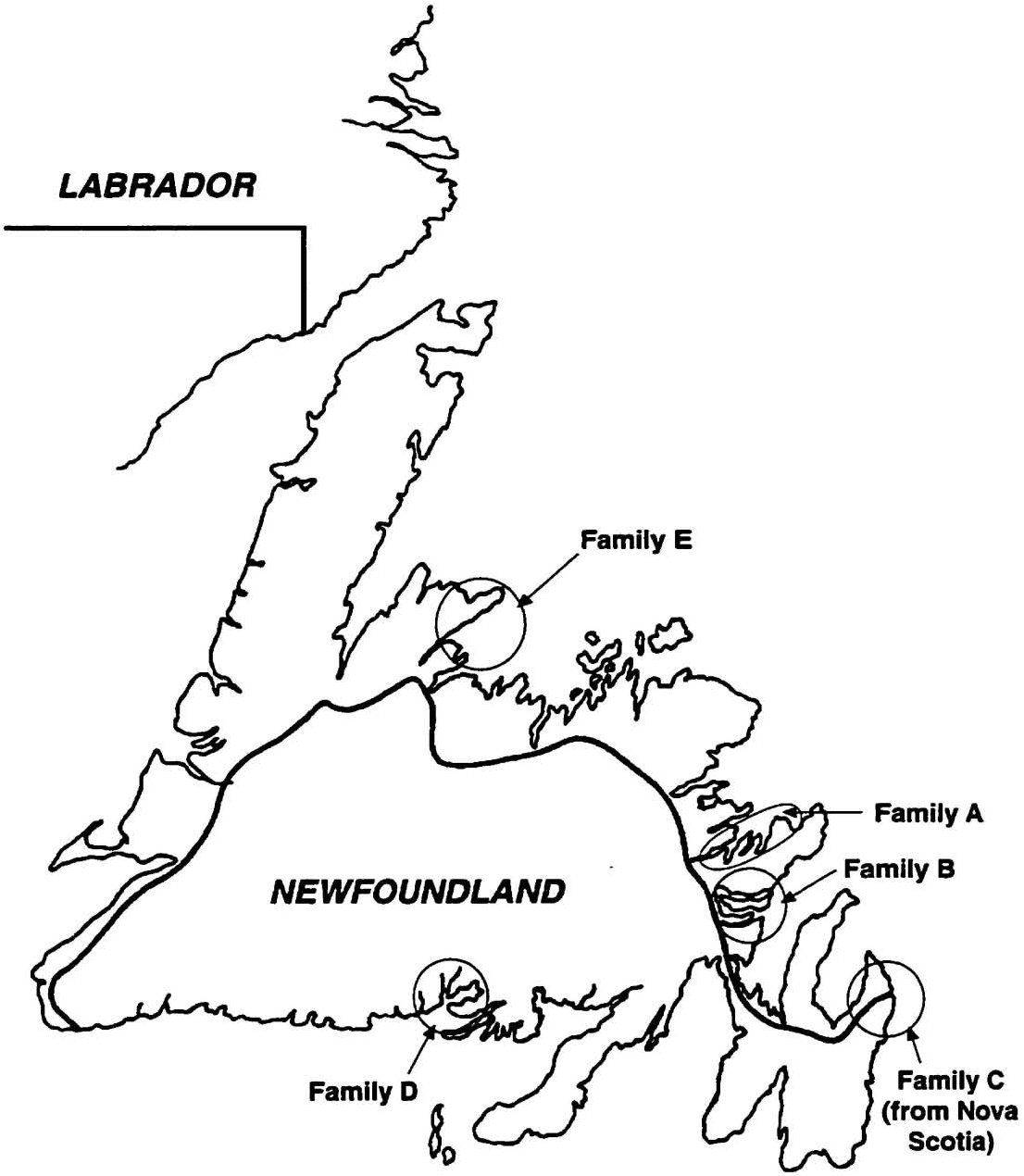
Other manifestation (includes paraganglioma, cerebral hemangioblastoma, stomach angioma, liver angioma, craniopharyngioma)

Deceased

32 [29] Age [age at death]

Figure 6.3. Geographic distribution of Newfoundland VHL families.

Geographic distribution of Newfoundland VHL families



Clinical Screening

i) Age at diagnosis and frequency of manifestations

Since 1982, 21 others (18 from Family A) have been identified as affected; 19 by the clinical screening program, at a mean age of 16 years, and 2 symptomatic patients age 26 and 49 years. Thus the clinical screening program resulted in a shift to an earlier age at diagnosis; the mean age at diagnosis in the screened group was 16 years, compared to 28 years of age in the previous symptomatic patients (Figure 6.4). This shift to a younger age at diagnosis is expected to increase, since many of the screened group were identified at their first investigation, with manifestations which would have been present for some time.

The initial tumour detected varies both within and between families (Table 6.2). In families A and C where pheochromocytomas are frequent, 22/45 family members (49%) presented with pheochromocytoma at a mean age of 20.2 years. Twenty-one of 45 (47%) presented with retinal angioma (mean age 21.5 yrs), one presented with cerebellar hemangioblastoma and one with renal cell carcinoma. In families B, D and E where pheochromocytomas have not been identified, 4 of 6 patients (where records are available) presented with cerebellar or spinal cord hemangioblastomas, and the other patients with retinal angioma, and with renal cysts.

Since 1982, a total of 96 tumours have been identified as initial or subsequent manifestations of VHL, 88 of these by screening, and 8 because

Figure 6.4. Cumulative age at diagnosis of VHL in symptomatic and screened patients.

Before 1982, 30 VHL patients were identified because of symptoms, at a mean age of 28 (range, 5-55 yrs; SD, 12.05) 90% of these patients were identified by age 41. Since 1982, 19 VHL patients were identified by screening, at a mean age of 16 (range, 9-28 yrs; SD, 3.72). Ninety percent of these patients were identified by age 26. The age at diagnosis by screening is expected to decrease, since 9 of 19 were identified as affected at their first investigation. For comparison, the cumulative age at diagnosis of 538 VHL patients from the literature is also shown.

Cumulative age at diagnosis of VHL in symptomatic and screened patients

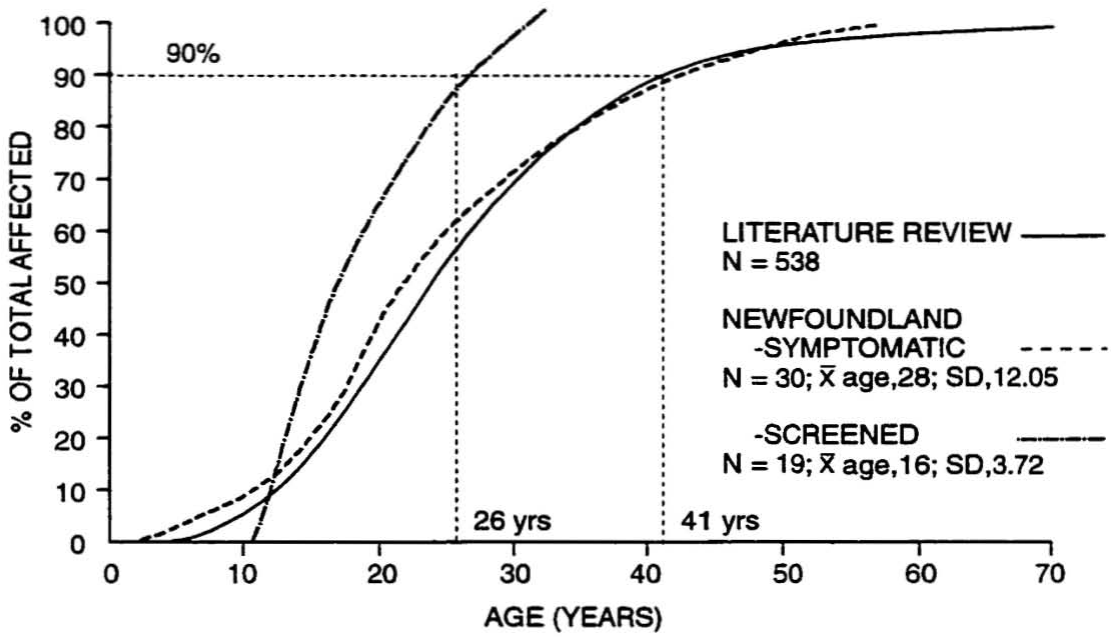


TABLE 6.2. INITIAL MANIFESTATION OF VHL IN NEWFOUNDLAND FAMILIES

	NUMBER, AND AGE AT DIAGNOSIS				
	Number (age)				
MANIFESTATION	Family A	Family B	Family C	Family D	Family E
Retinal angioma	20($\bar{X}$,21.3)*1	1 (32)	1 (27)	-	-
Pheochromocytoma/ Paraganglioma	19($\bar{X}$,21.1)*2	-	3 (11,17,17)	-	-
Cerebellar Hemangioblastoma	1 (22)	2 (15,30)	-	-	1 (18)
Spinal Cord Hemangioblastoma	-	1 (27)	-	-	-
Renal Cysts	-	-	-	1 (35)	-
Renal Cell Carcinoma	-	-	1 (48)	-	-
Total Documented	40	4	5	1	1
Total Affected in Family	42	5	5	1	1

*1 Range: 6-28 yrs.
*2 Range: 9-47 yrs.

of symptomatic presentation (Table 6.3). Although this is an increase in the number of tumours detected, the diagnosis for the majority of these was at an earlier, presymptomatic stage than for the tumours identified before 1982.

In 1992, family A had 42 affected members identified, of whom 30 were living (mean age of 32 years), and 50 first degree relatives at *a priori* 50% risk. (The numbers of affected and at-risk family members are constantly changing as new diagnoses are made and a new group at risk identified, or as genetic screening excludes family members from at-risk status).

Retinal angiomas (70%) and pheochromocytomas (67%) are the most common manifestations, and frequently the first manifestations diagnosed in this family (Table 6.4) (and also in Family C, originally from Nova Scotia). Renal cell carcinoma, which had been identified in only one member of Family A prior to 1986, is now present in 36% of affected individuals, and also in one member each of Family B and Family C. Cerebellar hemangioblastomas (24%) and spinal cord tumours (14%) are less common manifestations in Family A, but are frequent manifestations in Families B and C.

ii) Morbidity and mortality

At the time of ascertainment of these families, 16 of 33 affected members were deceased at a mean age of 37 years (range 18-55 years). Only two patients have died since 1982; one, age 49, who had a malignant pheochromocytoma at the time of diagnosis, and one, age 28, who presented symptomatically in 1981 with a cerebellar hemangioblastoma, and died in 1986

TABLE 6.3. DETECTION OF VHL MANIFESTATIONS IN FAMILY A SINCE 1982, BY CLINICAL SCREENING AND BY SYMPTOMATIC PRESENTATION

MANIFESTATION	METHOD OF IDENTIFICATION			
	BY SCREENING			SYMPTOMATIC
	Initial Diagnosis	Subsequent Manifestation	Recurrence	
Retinal Angioma	9	8	30	1
Pheochromocytoma	8	5	5	1
Hemangioblastoma Cerebellar	1	4	-	2
Spinal Cord	-	-	-	4
Renal Cell Carcinoma	-	15	2	-
Pancreatic Islet Cell Tumour	-	1	-	-
SUBTOTAL	18	33	37	8
TOTAL (96)		**88**		**8**

TABLE 6.4. FREQUENCY OF MAJOR CLINICAL MANIFESTATIONS OF VHL IN AFFECTED MEMBERS OF A NEWFOUNDLAND FAMILY, AND IN A LITERATURE REVIEW

MANIFESTATION	FREQUENCY			
	LITERATURE REVIEW*	NEWFOUNDLAND FAMILY A		
			Age at diagnosis (yrs)	
	%	% No.	Median	(Range)
Retinal angioma	57	70 (30)	19	(5-42)
Cerebellar hemangioblastoma	54	25 (11)	30	(15-55)
Renal cell carcinoma	23	36 (15)	31	(19-55)
Pheochromocytoma	19	67 (28)	22	(9-55)
Spinal cord hemangioblastoma	14	14 (6)	33	(16-55)
TOTAL NUMBER OF PATIENTS	**538**	**42**		

*Reference: Lamiell et al 1989.

of complications of VHL and a concurrent illness (Table 6.5). The majority of patients, however, have multiple manifestations of VHL, with development of subsequent tumours following successful treatment or surgery for the initial tumour (Table 6.6, Figure 6.5). The effect of the screening program on the medical outcomes related to the different manifestations is described in the following sections.

a) Retinal angioma

Before 1982, eleven patients in family A developed unilateral or bilateral blindness because of untreated retinal angiomas. Since 1982, small retinal angiomas detected by annual screening have been successfully treated with laser or cryotherapy in 18 patients, some of these patients having multiple recurrences. However, three patients, who were asymptomatic but had large or multiple angiomas when first examined, developed inoperable retinal detachments several years after treatment, and have progressed to unilateral blindness.

b) Pheochromocytoma

Prior to 1982, five of 10 deaths in Family A were a result of a hypertensive crisis from an unrecognized pheochromocytoma and one death in Family C was the result of a malignant pheochromocytoma. Since 1982, twenty patients with unilateral or bilateral pheochromocytomas or paragangliomas detected by screening have had the tumours successfully

TABLE 6.5. AGE AND CAUSE OF DEATH OF AFFECTED MEMBERS OF NEWFOUNDLAND VHL FAMILIES

CAUSE OF DEATH	NUMBER, AND AGE AT DEATH		
	Number (age)		
	Family A	Family B	Family C
Cerebellar/spinal cord hemangioblastoma	2 [39,50]	3 [21,30,48]	-
Pheochromocytoma -hypertensive crisis	5 [18-55]	-	-
-malignancy	1 [49]	-	1 [31]
Renal cell carcinoma	-	-	1 [48]
Unknown	2 [21,49]	-	-
Other	2 [28,29][*1,*2]	-	1 [20][*3]
Total Dead	12	3	3

*1 sepsis and addisonian crisis
*2 accidental
*3 chordoma

TABLE 6.6. NUMBER AND COMBINATION OF MANIFESTATIONS IN VHL PATIENTS (FAMILY A)

MANIFESTATIONS		PATIENTS
Number/Combination		Number (%)
One*		12 (29%)
RA	5	
PHEO	7	
Two		9 (21%)
RA, PHEO	4	
RA, RCC	3	
RA, CEREB	1	
PHEO, RCC	1	
Three		10 (24%)
RA, PHEO, RCC	4	
RA, PHEO, SPC	2	
RA, PHEO, CEREB	1	
RA, CEREB, SPC	1	
RA, CEREB, PANC	1	
PHEO, PANC, other*1	1	
Four		8 (19%)
RA, PHEO, RCC, CEREB	4	
RA, PHEO, RCC, SPC	2	
RA, PHEO, CEREB, other*2	1	
PHEO, RCC, CEREB, other*3	1	
Five		1 (2%)
RA, PHEO, RCC, CEREB, SPC	1	
Unknown		2 (5%)
	TOTAL	42

RA — retinal angioma; PHEO — pheochromocytoma;
RCC — renal cell carcinoma; CEREB — cerebellar hemangioblastoma;
SPC — spinal cord hemangioblastoma; PANC — pancreatic islet cell tumour

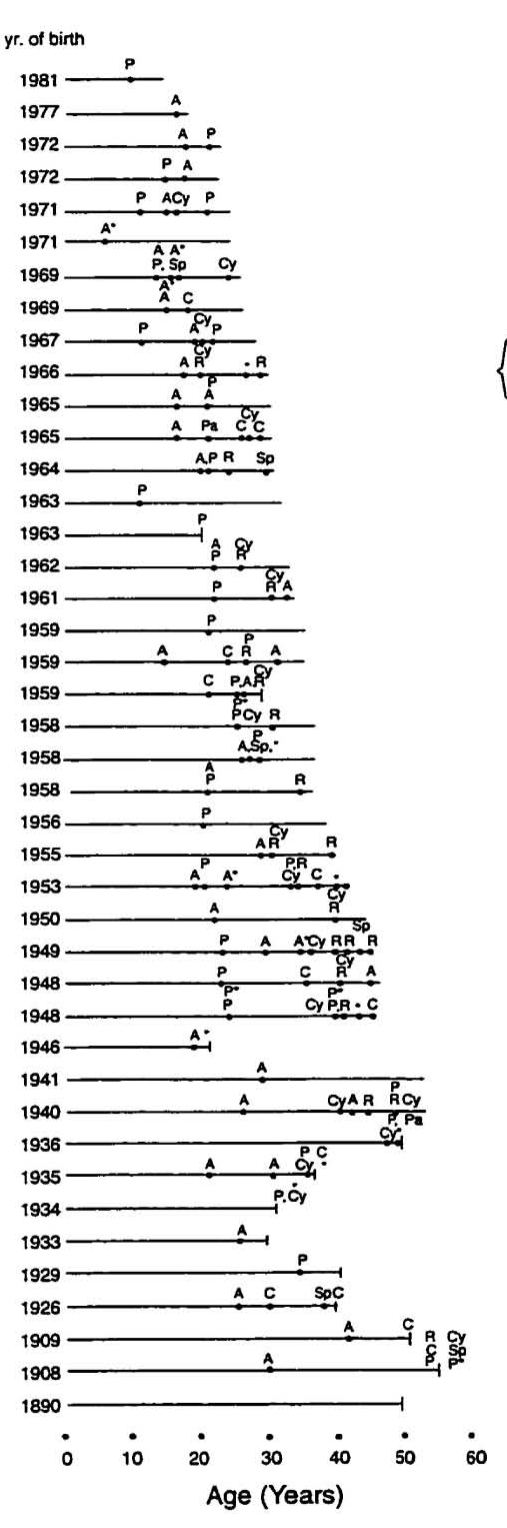
*1stomach angioma; *2arteriovenous malformation of the colon
*3carotid body tumour

*NB: five of twelve with only one manifestation are deceased.

Figure 6.5. Age at diagnosis, order of manifestations, and age at death of affected members of a Newfoundland VHL family.

Age at diagnosis of each manifestation, and age at death are indicated on a time-line for each affected family member. Except for retinal angiomas which have been multiple in all persons with this manifestation, each primary tumour that occurred is indicated (eg, a second pheochromocytoma, or second cerebellar hemangioblastoma).

Age at Diagnosis, Order of Manifestations, and Age at Death of Affected Members of a VHL Family



Other manifestations

A	retinal angioma
A*	angioma of optic disk
P	pheochromocytoma
P*	paraganglioma
R	renal cell carcinoma
C	cerebellar hemangioblastoma
Sp	spinal cord hemangioblastoma
Pa	pancreatic islet cell tumour
Cy	cysts (renal cysts unless specified)
*	other

pancreatic, renal cysts; Crohn's disease (probably coincidental)

renal & pancreatic cysts

renal & pancreatic cysts

epididymal cysts

parathyroid adenoma (probably coincidental)

carotid body tumour

infarct of pituitary

renal, pancreatic & liver cysts

hemangioma of stomach

AV malformation of colon

renal, adrenal, liver & pancreatic cysts

renal, liver, spleen & pancreatic cysts

details unknown

removed after pre-operative alpha-adrenergic blockade. The youngest of these was nine years old at the time. One patient (in another province) with long-standing intractable hypertension had a malignant pheochromocytoma at the time of diagnosis, and died of metastatic disease.

c) Renal cell carcinoma

Renal cell carcinoma had only been detected in one patient prior to 1982, as a coincidental observation at autopsy in a 55-year-old man with multiple manifestations of VHL. Since 1986, renal cell carcinomas have been identified by ultrasound screening in 14 previously affected individuals, 19-45 years of age. All were treated surgically with partial or total nephrectomy. None had metastatic disease at the time of surgery, but 6 have had a subsequent tumour in the second kidney and 4 of these have only part of one kidney remaining. Because onset of renal cell carcinoma was found to be earlier than expected, the screening protocol was revised, with ultrasound of the kidneys being introduced in the early rather than the late teens.

d) Cerebellar and spinal cord hemangioblastoma

In the past, two of 10 deaths in Family A, and all three deaths in Family B for which medical records are available, were due to inoperable cerebellar or spinal cord hemangioblastomas. Since 1982 five patients had cerebellar hemangioblastomas detected by screening. Three of these patients have had successful surgical removal of the tumour, and the other two are being monitored. Three other patients in families A and B (two in Newfoundland and

one in Ontario) had cerebellar hemangioblastomas identified after presentation with symptoms of headache or ataxia. All have had surgery. The patient in Ontario is on long-term disability because of his post-operative neurological deficit.

Four patients in Family A presented with symptoms of a spinal cord hemangioblastoma, subsequently confirmed by CT scan or MRI. Two are being followed by the neurosurgeon, and two have had surgical removal of the tumour. One of these, living outside of Newfoundland, had late investigation of symptoms. He required lengthy rehabilitation following surgery and has some neurological deficit remaining.

In two families with no previous family history of VHL, spinal cord or cerebellar hemangioblastomas were identified in each proband only after advanced disease had developed. The proband of Family E, an 18-year-old girl, presented with decreased vision. Five cerebellar hemangioblastomas were identified and removed, however, she has bilateral blindness because of optic atrophy secondary to the papilloedema which had developed prior to removal of the tumors. The proband of Family D, a 40-year-old, had a stroke-like event when terminally ill with "polycystic kidney disease". A spinal cord hemangioblastoma was identified at autopsy.

Before the availability of MRI, screening for spinal cord hemangioblastomas was not possible. CT scanning was appropriate for investigation of symptoms, but not for screening, because of the amount of

radiation necessary for visualizing the whole spinal cord. For those within the VHL screening program, however, symptoms were recognized and investigated at an earlier stage than for those at risk who were not closely monitored, or for members of new families where the risk was not known.

Genetic Screening

i) Linkage analysis

Twenty-four at-risk members of Families A, B and C (age 12-53) with negative clinical screening results participated in predictive testing by linkage analysis. Twenty of these received low-risk results (<2% risk of inheriting the VHL gene from the affected parent); three (ages 12, 13, and 25) received high-risk results (>98% risk of inheriting the VHL gene); and one, age 15, had an uninformative result (Table 6.7, Figure 6.6a and 6.6b).

The frequency of clinical screening was reduced to 3-5 year intervals for the twenty family members who received low-risk results. Continued annual screening was recommended for the four family members with high-risk or uninformative results.

ii) Direct mutation analysis

Twenty-eight members of Family A, including all those with previous predictive testing by linkage analysis, participated in direct mutation analysis after the VHL mutation was identified in this family (Table 6.8, Figure 6.7a and

TABLE 6.7. RESULTS OF PREDICTIVE TESTING FOR VHL BY LINKAGE ANALYSIS

AGE (YRS)	NUMBER TESTED	RESULTS		
		HIGH RISK*	LOW RISK**	UNINFORMATIVE
<10	0	-	-	-
10-20	7	2	4	1
21-30	7	1	6	0
>30	10	0	10	0
TOTAL	24	3	20	1

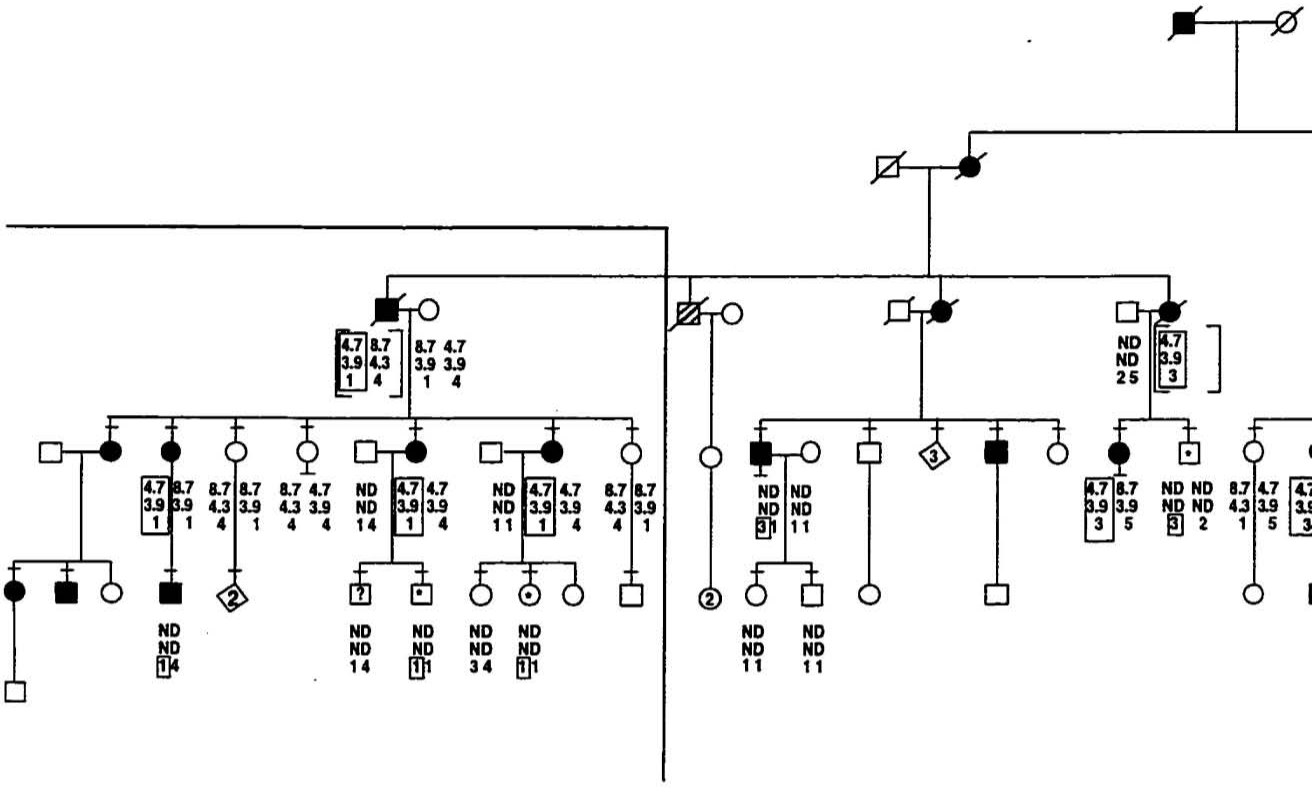
* >98% risk
** <2% risk

Figure 6.6a. Pedigree of Family A with results of predictive testing for VHL by linkage analysis.

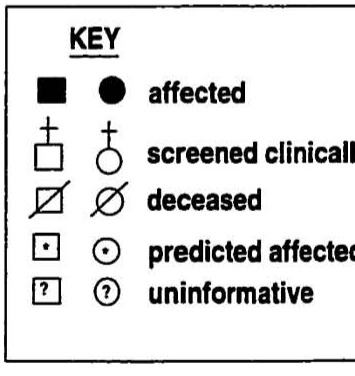
The pedigree is simplified compared with Figure 6.2, to emphasize family members who participated in genetic testing. The alleles for the three chromosome 3p markers used (D3S18, D3S601, D3S1038) are arranged in haplotypes and indicated for each family member tested. Inferred genotypes are given in brackets. The haplotype inherited with VHL is indicated by a box.

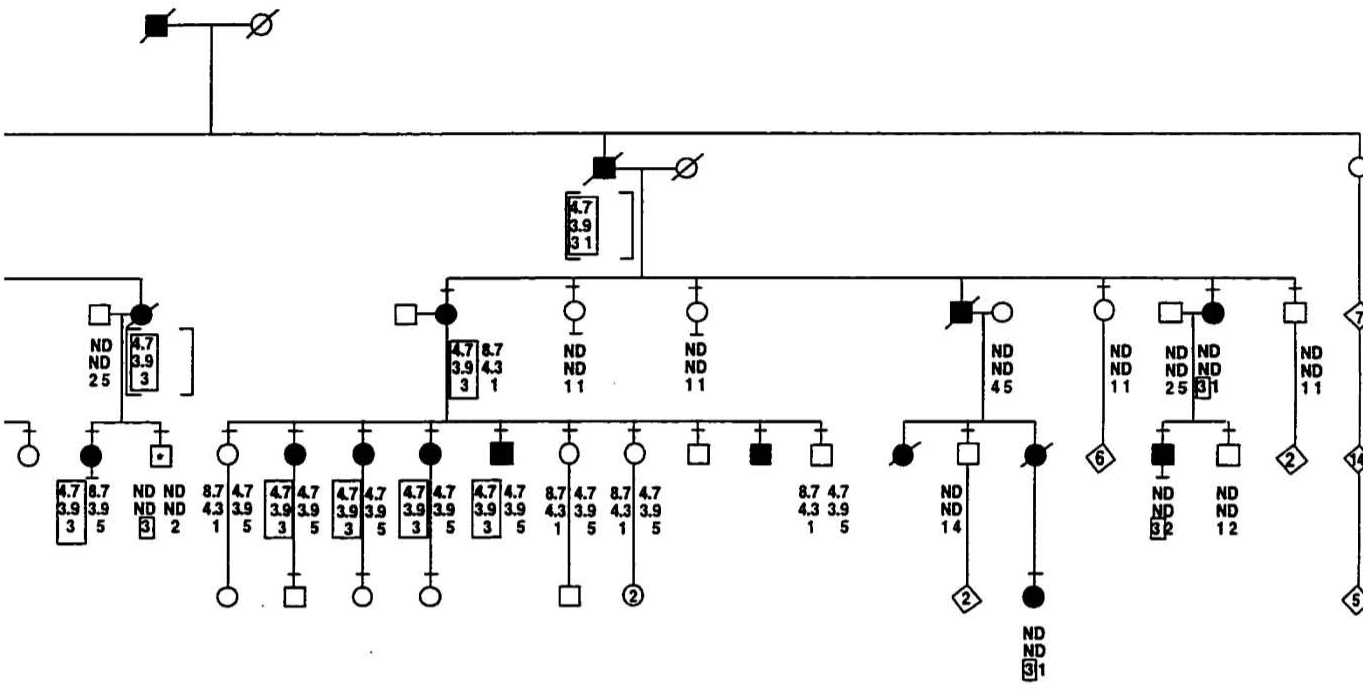
There has been a cross-over between D3S601 and D3S1038 in one branch of the family. Allele "1" co-segregates with VHL in the branch of the family at the left; allele "3" co-segregates with VHL in the rest of the pedigree. Six of 11 affected family members tested for all three chromosome 3p markers were homozygous for alleles at D3S18 and D3S601. Only D3S1038 was informative in these persons.

Family members with high-risk (n = 3), low-risk (n = 16), and uninformative (n = 1) results are indicated.



MARKERS	ALLELES
D3S18(C-LIB-1) (VHL)	4.7, 8.7
D3S601(19-63)	3.9, 4.3
D3S1038(946)	1-8





KEY

- affected
- screened clinically
- deceased
- predicted affected
- uninformative

haplotype inherited with VHL disease

4.7 3.9 3 or 4.7 3.9 1

Figure 6.6b. Pedigree of Family A (enlarged) with results of predictive testing for VHL by linkage analysis.

One branch of the pedigree is enlarged to demonstrate the high-risk, low-risk, and uninformative results more clearly.

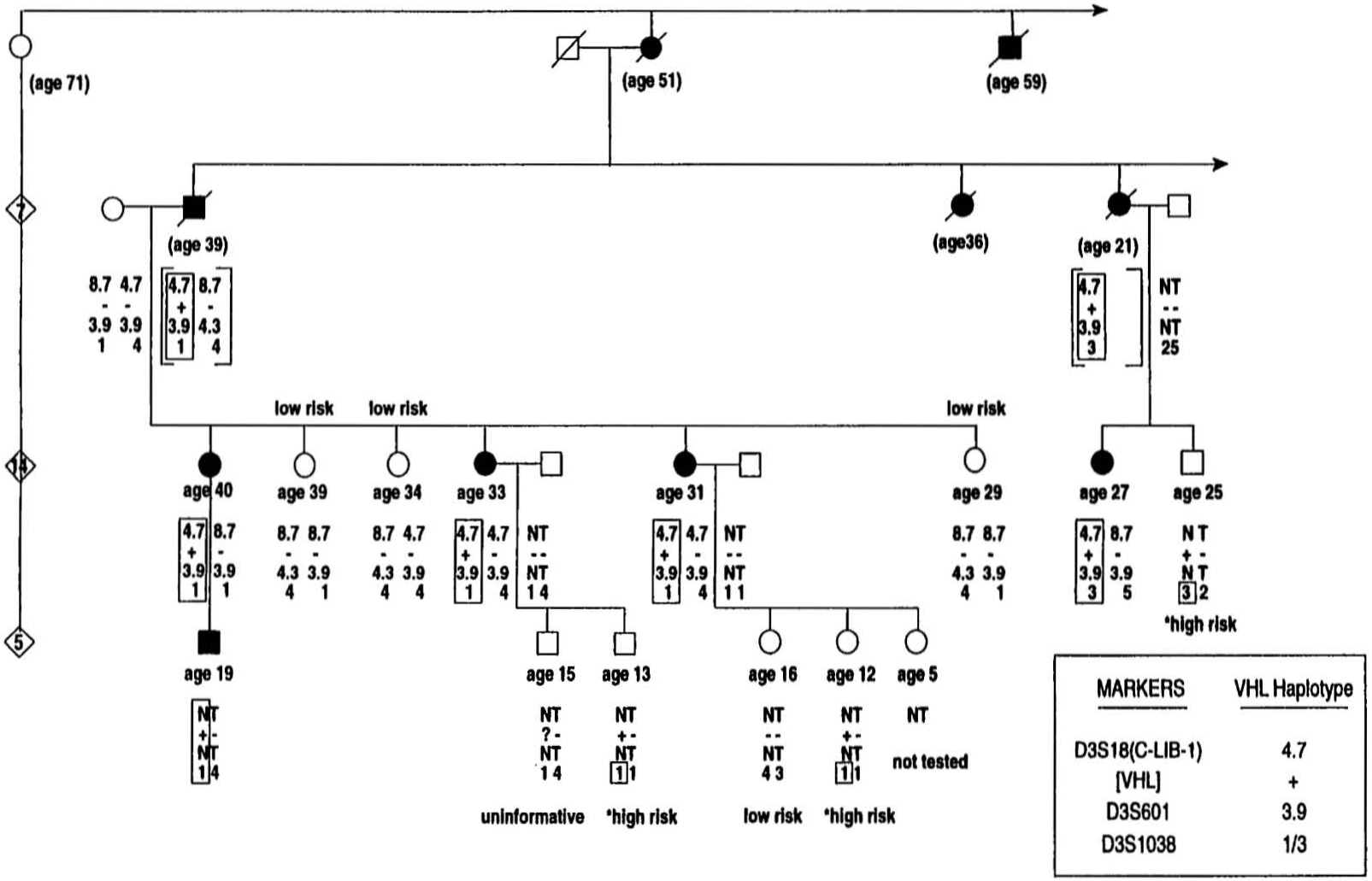

(age 71)
(age 51)
(age 59)
(age 39)
(age36)
(age 21)
low risk
low risk
low risk
age 40
age 39
age 34
age 33
age 31
age 29
age 27
age 25
age 19
age 15
age 13
age 16
age 12
age 5
not tested
uninformative
*high risk
low risk
*high risk
*high risk
MARKERS
VHL Haplotype
D3S18(C-LIB-1)
4.7
[VHL]
+
D3S601
3.9
D3S1038
1/3

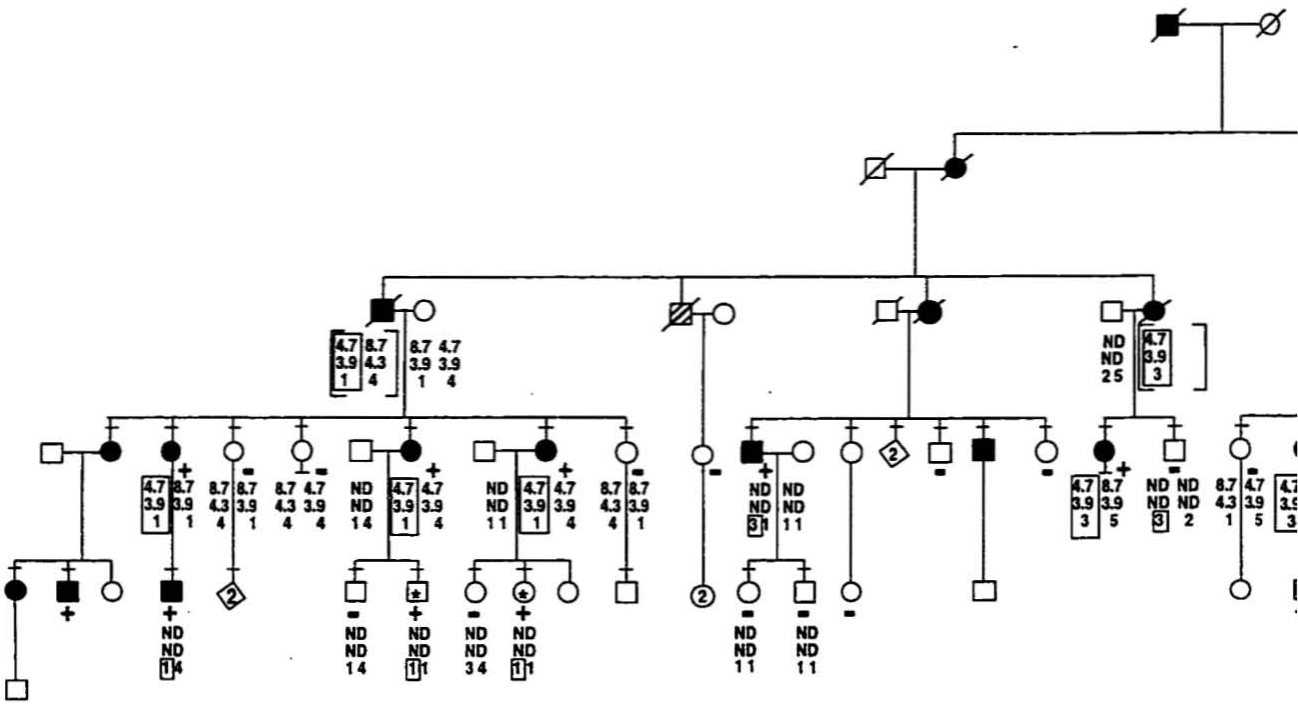
TABLE 6.8. RESULTS OF PRESYMPTOMATIC DIAGNOSIS FOR VHL BY MUTATION ANALYSIS

AGE (YRS)	NUMBER TESTED	RESULTS	
		MUTATION POSITIVE	MUTATION NEGATIVE
<10	0	-	-
10-20	10	4	6
21-30	7	0	7
>30	11	0	11
TOTAL	28	4	24

Figure 6.7a. Pedigree of Family A with results of presymptomatic diagnosis of VHL by mutation detection.

A direct mutation test was possible for Family A, when a C→T transition at bp 712 of the VHL gene was identified. This mutation abolishes an Msp1 restriction site.

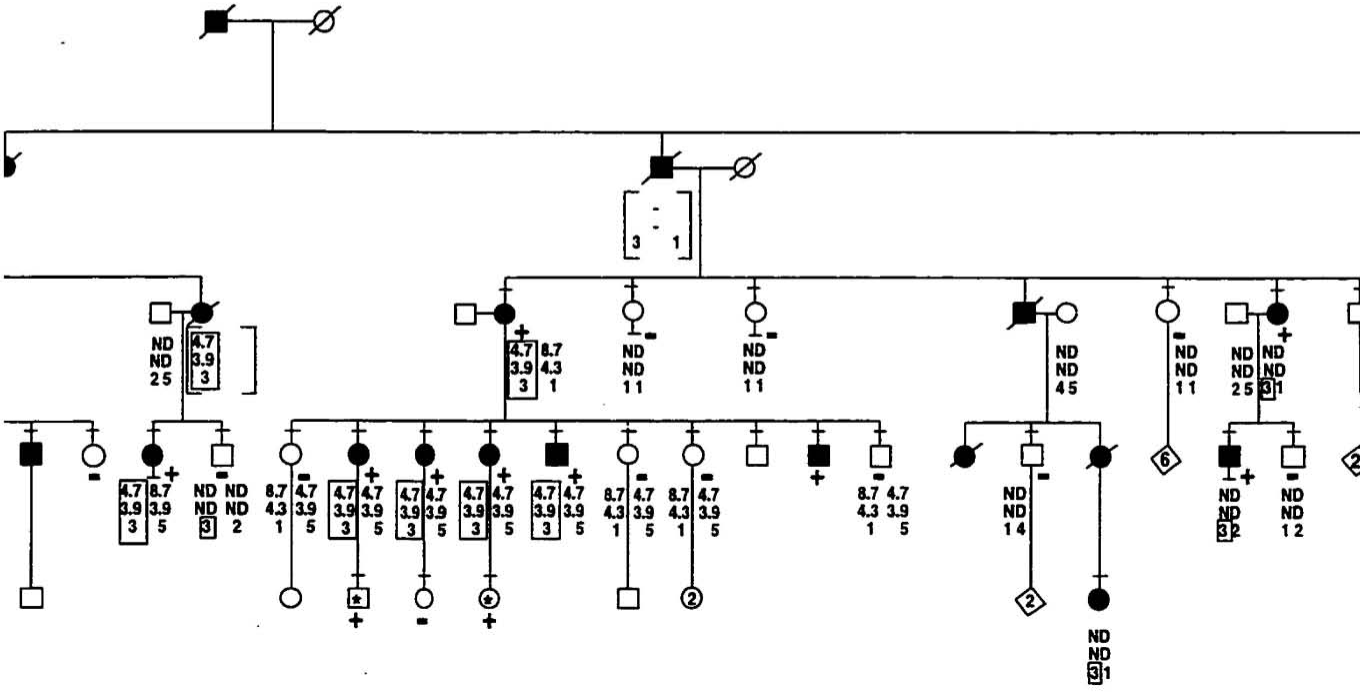
The same pedigree as in Figure 6.6a is used to demonstrate the results of direct mutation analysis. Presence of the mutation is indicated by "+" and absence of the mutation is indicated by "-".



MARKERS	ALLELES
D3S18(C-LIB-1) (VHL)	4.7, 8.7
D3S601(19-63)	3.9, 4.3
D3S1038(946)	1-8

KEY

- affected
- screened clinically
- deceased



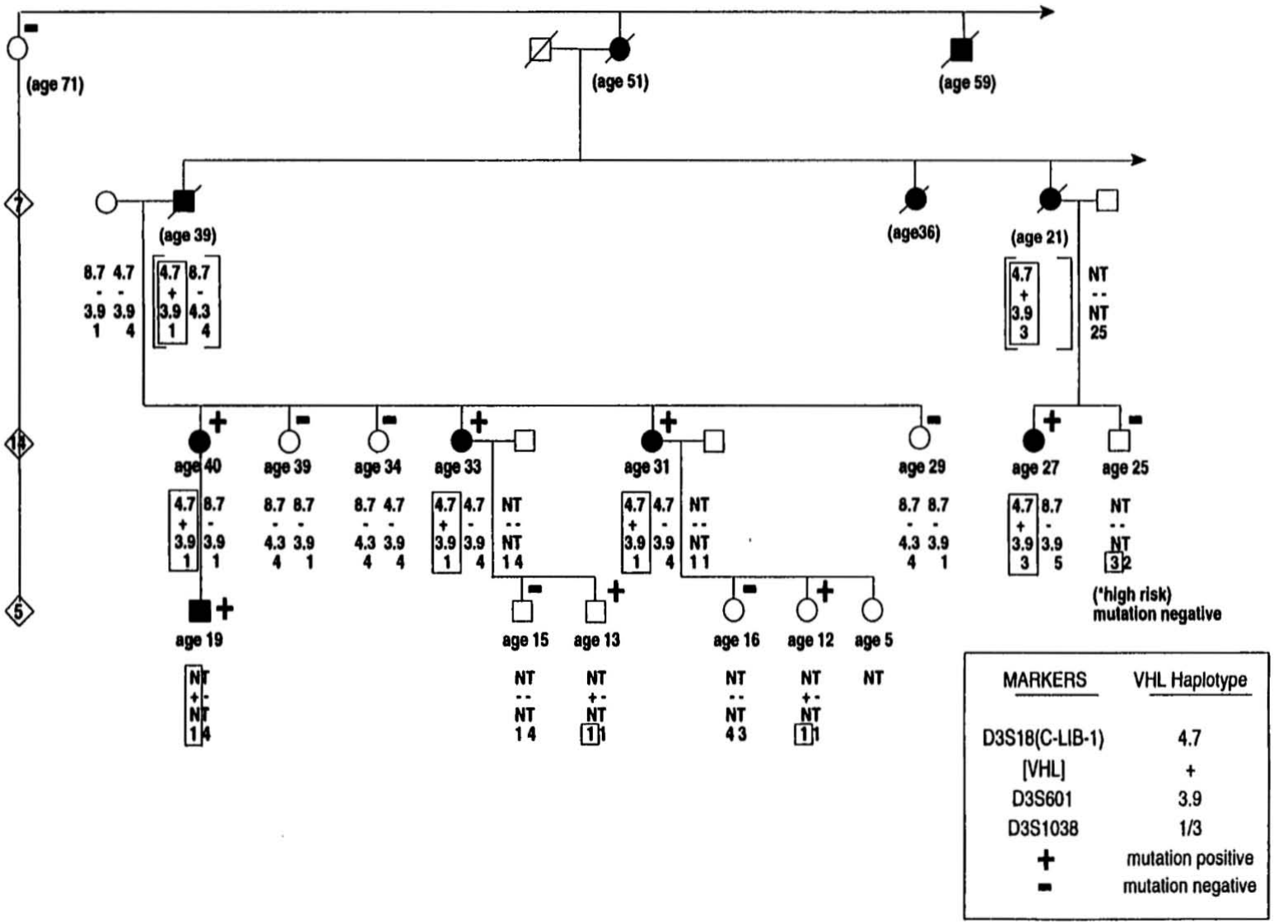
affected
screened clinically
deceased

+ mutation positive
- mutation negative
* predicted affected

Figure 6.7b. Pedigree of Family A (enlarged) with results of presymptomatic diagnosis of VHL by mutation analysis.

As in Figure 6.6b, one branch of the pedigree is enlarged to demonstrate mutation-positive and mutation-negative results more clearly.

The family member with uninformative results by linkage analysis did not inherit the VHL mutation. The 25-year-old man with high-risk linkage analysis results also did not inherit the VHL mutation (confirmed on two separate DNA samples). Because DNA was not available from his mother who died many years previously (see text), his high-risk linkage results were based on inheritance of the same allele from his mother as his affected sister. His mother could have been homozygous for this allele, or a cross-over could have occurred.


(age 71)
(age 51)
(age 59)
(age 39)
(age36)
(age 21)
8.7 4.7
- -
3.9 3.9
1 4
4.7 8.7
+ -
3.9 4.3
1 4
4.7
+
3.9
3
NT
--
NT
25
age 40
age 39
age 34
age 33
age 31
age 29
age 27
age 25
4.7 8.7
+ -
3.9 3.9
1 1
8.7 8.7
- -
4.3 3.9
4 1
8.7 4.7
- -
4.3 3.9
4 4
4.7 4.7 NT
+ - --
3.9 3.9 NT
1 4 1 4
4.7 4.7 NT
+ - --
3.9 3.9 NT
1 4 1 1
8.7 8.7
- -
4.3 3.9
4 1
4.7 8.7 NT
+ - --
3.9 3.9 NT
3 5 3 2
(*high risk)
mutation negative
age 19
NT
+ -
NT
1 4
age 15
age 13
age 16
age 12
age 5
NT
--
NT
1 4
NT
+ -
NT
1 1
NT
--
NT
4 3
NT
+ -
NT
1 1
NT
MARKERS
VHL Haplotype
D3S18(C-LIB-1)
4.7
[VHL]
+
D3S601
3.9
D3S1038
1/3
+
mutation positive
-
mutation negative

6.7b). Twenty-four of those tested, age 11-53, did not have the VHL mutation. This included the 25-year-old with high-risk linkage analysis results. Four, ages 13-15, did have the mutation. These four have all been screened clinically for 8-10 years with negative screening results so far. The proportion of gene carriers identified by genetic testing (4/28) is much less than 50% because of the prior identification of many gene carriers by symptomatic disease or by clinical screening.

Because of the significance of these predictive testing results for medical management and reproductive decision making, the mutation analysis is now being established by Dr. Peter Bridge in St. John's, and all testing is being repeated with a second DNA sample. Clinical screening will no longer be required for those confirmed negative for the mutation. Those who are confirmed to have the mutation will require life-long clinical screening, as will all of those previously identified as affected by screening or by symptomatic disease, and any family members who decline predictive testing.

Cost of Clinical Screening

Although two of the goals of the screening program (early diagnosis of VHL, and early identification and treatment of individual tumours) were being fulfilled by the clinical screening protocol, the costs to both the health care system and to individual family members were high because of the number of investigations required and the number of family members at risk. When

closely-linked flanking markers became available, and later the gene was cloned and the mutation identified in three of the Newfoundland families, predictive testing to identify gene carriers by linkage analysis or mutation detection became possible. One result of this predictive testing was the reduction in the number of family members requiring clinical screening.

The cost of clinical screening before and after the availability of genetic screening can best be evaluated in Family A because the majority participating in clinical screening are from this family, and only members of this family have so far received results of mutation analysis.

Forty-two affected members and 50 first degree relatives at 50% risk had been identified in Family A by 1992 prior to the availability of predictive testing for VHL. Thirty-one of those at 50% risk lived in Newfoundland, and participated regularly in the clinical screening program. Twenty-eight of these have now had predictive testing for VHL.

The average cost per person, in 1994, of the annual set of investigations recommended by the VHL clinical screening protocol was $288.00. Before genetic testing was available, annual clinical screening was recommended for everyone at risk from age 3 until at least age 50. The lifetime costs of basic screening for each person at risk were therefore estimated to be at least $13,800 (using 1994 values). Based on their present ages, the projected cost for the remaining annual screening for these 28 family members was $186,010. Genetic screening has identified that 24 of the 28 are not gene

carriers. Therefore, $145,690 of this cost would have been spent unnecessarily on those without the VHL mutation (Table 6.9). Costs of the investigations, however, are only a portion of the total costs of screening and management of VHL families. A more extensive discussion of direct and indirect costs of VHL care and management, before and after screening was introduced, is presented in the following chapter.

Psychosocial Impact of VHL Disease

From structured interviews with 15 family members, and conversations during counselling sessions or phone calls with 15 others, it is clear that there is stress and anxiety associated with the VHL screening program because of the even greater stress and anxiety caused by VHL itself (Langsley et al 1964, Yuen et al 1984). Even before family members knew the name of the disease within the family, they were aware of and very apprehensive because of the early and sudden deaths, the progressive and disabling neurological disease, and the severe visual loss in many relatives. Those who were already affected were afraid of developing subsequent manifestations, or of their children also being affected, and unaffected family members worried that they would be next to develop some expression of "the disease".

In the past, family members were distrustful of the medical care system because of the poor outcome of treatment for many affected individuals (although this was frequently because of severe disease at the time of

TABLE 6.9. ESTIMATED COST OF CLINICAL SCREENING FOR AT-RISK FAMILY MEMBERS BEFORE AND AFTER GENETIC TESTING

Cost of annual set of investigations*1	$288
Lifetime cost of annual screening from age 3 to age 50 (at 1994 rates)	$13,824
Cost of remaining screening for 28 family members who participated in genetic testing*2	$186,010
Cost of remaining screening for 4 gene carriers identified*2	$40,320
Savings if 24 non-carriers no longer require clinical screening	$145,690

*1 Average cost of ophthalmology appointment, pediatric/internal medicine appointment, catecholamine assay, and renal ultrasound (screening costs will be higher when CT scan of the abdomen replaces renal ultrasound to screen for renal cell carcinoma).

*2 Based on present ages.

presentation and treatment). One of the benefits of the screening program is that family members now feel that the medical care system is working with and for them. Although family members are still very apprehensive before their own or their close relatives' screening appointments and until they receive results, they have greater confidence now that problems will be identified and treated. For the rest of the year, the majority (14/15) are able to push the disease and the screening out of mind. (Over and over the comment was made, "If I didn't put it on the back burner, I would go crazy".) One family member is unable to do this and feels that her life is "under a cloud", or "on hold" because of VHL disease.

The majority of family members (45/54) comply with screening recommendations because they have seen the improvement in medical outcomes since the screening program began. Some family members originally resisted the recommendations for screening, either because they did not trust the medical care system which they thought had failed them in the past, or because they were afraid of what might be found. Non-compliance is less common now, but when it occurs is attributable to: a) fear of VHL (n=1); b) lack of understanding or nonchalance about the disease (n=4); or c) confidence, after years of negative test results, of not being affected (n=4).

Family members feel that they understand the medical and genetic features of VHL better than before, and the reasons for screening. Most (13/15) appreciate having the combination of personal genetic counselling and

pamphlets for reference, but a few prefer one or the other method of information delivery.

For support, participants in the screening program primarily turn to other family members (particularly those who are affected) who have directly or indirectly experienced the disease. They also depend on the geneticist, and their key specialist for support regarding medical matters, rather than their family doctors, who they think do not have sufficient knowledge of VHL. Persons being screened rarely turn to friends or colleagues for support because the medical and psychological implications of VHL are unknown to the average person.

Most of the members of the VHL families live outside St. John's and therefore have travel costs associated with screening and treatment. Few have insurance to cover these costs but consider them to be necessary costs. ("Screening is my life, and this money comes first.") The costs of all screening tests (and follow-up investigations and treatment if necessary) are covered by the provincial medical insurance plan. In a medical care system where these costs are the responsibility of the individual (or the individual's health insurance), there would be an additional direct financial burden to the patient.

When participants in the screening program were questioned about discrimination, the immediate answer was that there was no discrimination because of VHL or the screening program. (One patient looked surprised and said that there was more discrimination because of her diabetes, than because

of "the disease".) However, when directly questioned about insurance, family members acknowledged that this was a problem — they cannot obtain personal life insurance or extended health care plans once they have VHL disease. Few members of the Newfoundland families had group insurance through employment because they were frequently self-employed, seasonal or part-time employees, or worked for small fish plants without these benefits. When applying independently for insurance, *it is the disease, not the screening program*, which causes the discrimination. Because of the early age at diagnosis of VHL (diagnosis of disease requiring treatment, not just presymptomatic disease to be monitored), few family members would be applying for insurance before the clinical diagnosis is made.

Although there has been no direct discrimination in terms of education or employment, one husband lost his job when he would not accept a transfer to Labrador. He felt that this distance from the tertiary care centre in St. John's would be detrimental to his wife's medical care for known VHL disease.

VHL disease has had a very significant impact on reproductive decisions for all family members, whether affected or unaffected. Almost all family members (18/20) thought that the disease should be stopped ("stamped out") and therefore that those who were affected should not have children. Because of uncertainty about who was affected (before the screening program was introduced), some non-carriers (n = 6) had chosen to have no children. Some family members have thus had no children, or fewer children than desired,

while others (n = 6), in earlier generations when the diagnosis was made after reproduction, regretted the number of children that they did have, because of the occurrence of VHL in their children, or the risk that children would be affected. The clinical and genetic screening programs now identify those who did, or did not, inherit the VHL gene, and permit reproductive choices that were not previously available.

None of the affected family members interviewed (0/15) said that they would choose prenatal diagnosis and therapeutic abortion of an affected fetus. This may be a choice in other cultures, but Newfoundlanders have been less likely to accept this option even in situations where there is a risk of severe congenital disease. Even though affected and at-risk family members consider VHL to be a severe burden, the "late" age of onset made the choice of therapeutic abortion unacceptable, and, if they had the VHL gene, they preferred not to have any children.

In the past, the morbidity and mortality associated with VHL affected the quality of life of all family members. Besides the direct medical problems, psychological and financial stresses sometimes disrupted marriages or family units. Other family units, however, felt closer because of "the common enemy". Overall, the screening program has made family members more confident and hopeful about the future.

Affected family members think that the genetic testing has provided major benefits to non-carriers because the non-carriers are freed from the need

for clinical screening and the worry about developing VHL, and can now have children without fear of the children being affected. Those demonstrated to be non-carriers, however, have some equivocal feelings because they are concerned that "enjoying" their mutation-negative status would "hurt" family members who are affected and, therefore, not so fortunate.

DISCUSSION

Family Characteristics

The Newfoundland VHL families were recognized because of the morbidity and early mortality in multiple affected members of previous generations, or in individual probands of the smaller families. The one large and four small VHL families were from different geographic areas, four from Newfoundland and one originally from Nova Scotia, with no apparent connection between them (Figure 6.3). The two families with greatest geographic proximity (from Bonavista Bay and Trinity Bay) had different clinical features (Family A with, and Family B without, pheochromocytomas). On the other hand, the two families with similar clinical presentation (ie, frequent pheochromocytomas) were from different geographic regions (Family A from Bonavista Bay, Newfoundland, and Family C from Nova Scotia). Subsequent molecular analysis has shown that these three families each have a different mutation, confirming their independent origin.

In family A (with 42 family members now identified as affected), pheochromocytomas and retinal angiomas are particularly common and frequently the first manifestations detected, while cerebellar and spinal cord hemangioblastomas are less common than in many VHL families (Table 6.4). The suggestion that a particular VHL family will have either pheochromocytomas or renal cell carcinomas (RCC) (Neumann and Wiestler 1991), and therefore that screening is necessary for only one or other of these tumours, appears to be wrong (Filling-Katz and Choyke 1991a, Maher 1991). RCC has now been identified in 36% of the affected members of Family A, including thirteen with both RCC and pheochromocytoma.

RCC has also been identified in this family at an earlier age (mean age of 34 years; range, 19-45 years) than had been predicted by the literature (mean age of 39-44 years in different studies [Hardwig and Robertson 1984, Lamiell et al 1989, Maher et al 1990]). This necessitated earlier screening for RCC. A pancreatic islet cell tumour, a less common manifestation but previously reported clustered in a few VHL families (Fishman and Bartholomew 1979), was identified in one 23-year-old family member. Pheochromocytomas have not been identified in family B, but cerebellar and spinal cord hemangioblastomas are more frequent than in family A. Family C is similar to family A in the high frequency of pheochromocytomas, but also has frequent hemangioblastomas of the cerebellum or spinal cord, as in family B.

Although different VHL families have different frequencies of the major manifestations, the combination, age and order of occurrence of manifestations varies even between individuals within a family (Figure 6.5). In all families VHL is a complex disease; as patients live longer they develop more tumours, either bilateral or multifocal tumours of a single type, or other types of tumour of the VHL spectrum.

Clinical Screening

Clinical screening has been recommended for affected and at-risk members of VHL families to allow earlier identification and treatment of tumours because of the advanced disease present in many symptomatic patients (Huson et al 1986). The type and timing of investigations of early clinical screening protocols were based on the age at diagnosis and frequency of manifestations in individual families. Screening for pheochromocytomas was excluded for some families in the literature because they were thought not to occur (Neumann and Weistler 1991). However, pheochromocytomas may not have been identified because of small family size rather than because of absence of risk. Because of the variability of disease expression even within families, a comprehensive screening protocol is recommended for all families (Maher et al 1990). Particularly, the danger of an unrecognized pheochromocytoma in a patient requiring investigations with administration of contrast material, or possible surgery for other VHL manifestations (either of

which could precipitate a hypertensive crisis if a pheochromocytoma is present), outweighs any savings from omitting this testing. Conversely, not screening for RCC in a family with known pheochromocytomas may delay diagnosis of RCC until too late for successful surgical treatment, since these tumours rarely cause symptoms before there is metastatic disease.

The mutation has now been identified in many VHL families. Families with pheochromocytomas are more likely to have missense mutations, and families without pheochromocytomas to have inactivating mutations (Crossey et al 1994, Whaley et al 1994, Chen et al 1995). If the genotype-phenotype correlation can be more clearly defined, it may become possible to tailor the clinical screening protocol to the specific mutation in an individual family.

i) Results of clinical screening

Since the clinical screening program was introduced in 1982 for the Newfoundland families, there has been an increase in the number of tumours identified, either as the initial or subsequent manifestation of VHL. The majority of patients were asymptomatic at the time of diagnosis and treatment has been successful. There has been a decrease in mortality and a decrease in adverse sequelae such as blindness or neurological deficit. Renal cell carcinoma (RCC) has been detected at an earlier age than expected. None of the patients with RCC have had metastatic disease, and therefore none have required chemotherapy or radiotherapy.

The clinical screening program has also resulted in a shift to an earlier age at initial diagnosis of VHL (the mean age at diagnosis in the screened group was 16 years, compared to 28 years in the previous symptomatic group). This allows reproductive decisions to be made based on accurate genetic counselling about the recurrence risk of the disease. In previous generations, affected members (n = 6) frequently had many children before being identified as affected. Other family members (n = 4) chose not to have children rather than risk passing on the VHL gene, and subsequently have been shown to be non-carriers.

The mean age at diagnosis of individual tumours has also decreased because of screening, and the timing of investigations has been revised because of this. Initial scanning of the head and abdomen, for evidence of cerebellar hemangioblastoma or renal cell carcinoma respectively, has been advanced to the early rather than the late teens. Recently an MRI, which provides better resolution for cerebellar screening and the possibility of spinal cord screening without excessive radiation (Filling-Katz et al 1989, Nelson et al 1991, Hoff et al 1993), has been available. The screening protocol has therefore been revised; MR imaging replaces CT scanning for screening for central nervous system tumours. A decision has also been made to replace ultrasound screening of the kidneys by CT scanning now that a higher speed CT scanner is available, giving better resolution while keeping radiation to acceptable levels.

Thus, the goals of the screening program (early diagnosis of VHL, and early identification and treatment of individual tumours) were being fulfilled, by clinical screening. However, there are costs to both the health care system and to individual family members, in order to achieve these goals. These will be outlined here, and discussed in detail in the following chapter.

ii) Cost of clinical screening

Clinical screening for a disease such as VHL with multiple manifestations, variable age of onset, and variable combination and order of occurrence of tumours is expensive because of the number of different investigations required, the early age at which screening must start, and the many years over which repeat testing is necessary. This is acceptable clinical management when screening improves the prognosis, as has been demonstrated with the Newfoundland and other VHL families. However, if identification of those at high risk is from pedigree analysis, screening necessarily includes some family members who did not inherit the gene and therefore do not require screening, as well as those with the gene who could benefit from clinical screening. If identification of those at high risk is by genetic testing, the clinical screening can be provided just for gene carriers, thus reducing the number requiring screening, and saving money.

Monetary costs are not the only costs of screening (see Chapter 7), however the potential savings in direct costs can be considerable if genetic screening is combined with clinical screening in an overall VHL screening

program. In Newfoundland, the lifetime screening costs, for a first degree relative who is subsequently found not to have the VHL gene, have been estimated at $13,800/person (in 1994 dollars). This is based on the cost of the recommended annual screening from age 3 (because of the early age of onset in some patients) until at least age 50 (because of the uncertain upper limit of age of onset) using our current screening protocol ($288/per person per year). This means that a total of $145,690 could be saved in screening for Family A by first identifying gene carriers and then applying clinical screening to this group only (Table 6.9). With implementation of the recommendation to use CT scan of the abdomen instead of renal ultrasound for screening for renal cell carcinoma, the annual cost per person of the scanning investigations would be $402, and the total cost of screening, and of the sum saved, proportionately higher.

Screening investigations and their costs will change as technology changes, and the nature of these changes cannot be foreseen, so the figure is an estimate only. Allowance for inflation and detailed discounting of the lifetime costs were therefore not done (Robinson 1993b): some costs will increase because of inflation, others will decrease because diagnostic equipment such as CT scanners and MR imagers is becoming less expensive, and costs related to potential new investigations cannot be anticipated. However, the figure presented does give an indication of the direct savings if

family members who did not inherit the VHL gene can be identified and excluded from clinical screening.

Genetic Screening

As noted previously, it became possible to provide genetic screening by linkage analysis or direct mutation detection for individuals at risk in many VHL families. This permits the identification of gene carriers (or high-risk individuals) who require clinical screening, and of those without the VHL mutation (or low-risk individuals) who no longer required screening.

i) Results of genetic screening

Genetic screening was offered to at-risk members of the Newfoundland VHL families who were participating in the clinical screening program. All of these were over 10 years of age. Results of direct mutation detection and/or linkage analysis have been given to 33 members of Families A, B, and C. This genetic screening has identified VHL gene carriers (or those at high-risk), and others who do not carry the gene (or are at low-risk). The clinical screening recommendations have been modified on the basis of these results; continued annual screening is recommended for the gene carriers (n = 4) along with family members previously identified clinically as affected. Clinical screening has been discontinued for those who did not inherit the VHL gene (n = 24), and has been reduced in frequency for those with low-risk linkage analysis results (n = 5). (The mutations have now been identified in Families B and C, and direct mutation testing is underway for the 5 low-risk members of these families).

If genetic testing was not yet available, continued clinical screening would be recommended for all 33 family members. Thus, the number requiring clinical screening has been markedly reduced by the genetic testing so far completed (only 4 of 33 at risk who were previously being screened need to continue with annual clinical screening, and 5 others require screening every 3 years). First degree relatives who do not have the VHL mutation are freed from regular screening, with reduction in health care costs, both for the medical care system and, financially and psychologically, for themselves. The information provided also allows all family members tested to make informed reproductive decisions.

ii) Comparison of methods of identification of gene carriers

The genetic testing results for the large Newfoundland family have also allowed a comparison between the methods of identification of VHL gene carriers: by symptomatic presentation, by clinical screening, by linkage analysis, and by direct mutation detection, with direct mutation detection being considered the gold standard.

Twenty-six members of Family A (age 5-55) presented with symptoms, all prior to 1985. Since 1982, another 16 members (age 9-28) were identified as affected by clinical screening. Twenty-eight first degree relatives at 50% genetic risk but with negative clinical screening participated in genetic testing. Four of these, all 13-15 years of age, were identified as gene carriers by mutation analysis, while 24 others age 11-53 do not carry the VHL gene. In

this group that participated in genetic testing, everyone with the VHL gene over 15 years of age had already been identified because of symptomatic disease or positive screening tests.

There is linkage information for 20 of those for whom direct mutation analysis is complete. For 18 of these the linkage analysis and mutation detection results agreed: sixteen with low-risk results were later confirmed to be mutation negative, and two with high-risk results were identified as gene carriers (two others identified as gene carriers had not been included in linkage analysis). For 2 of 20 tested (10%), the linkage information was uninformative or misleading. One 16-year-old who was uninformative for linkage analysis because of the combination of alleles in his parents, was later shown not to carry the mutation. A 25-year-old received high-risk linkage results, but was later found not to carry the mutation — confirmed on two separate DNA samples. Because DNA was not available from his affected mother who died many years ago, his linkage results were based on inheritance of the same high-risk allele as his affected sister. In this situation, a recombinant event between the marker and the VHL gene could not be detected, nor could homozygosity of the high-risk allele in his affected mother.

This comparison of linkage analysis and mutation detection shows the advantage of direct detection of the mutation where possible. Linkage analysis is least reliable when not all relevant DNA samples are available, for example, because of previous deaths from the disease in question. A false positive result

(high-risk predictive testing result in a mutation-negative individual), as in the 25-year-old patient mentioned above, would have meant continuing the clinical screening program unnecessarily. With a potentially lethal disease, a false positive result is much less serious than a false negative result (low-risk predictive testing result in a mutation-positive individual). Even if clinical screening at reduced frequency were recommended to such a person with false negative results, it might not have been frequent enough to detect a tumour early, or it might have been discontinued if the subtle message received was that low-risk implied being free of the disease.

Although most VHL families have a unique mutation, in three large studies the VHL mutation has been identified in greater than 60% of families (Crossey et al 1994, Whaley et al 1994, Chen et al 1995) — a much higher success rate than for diseases such as Marfan syndrome or neurofibromatosis, type 1 (Heim et al 1994, Sutherland and Richards 1994). If members of a VHL family request predictive testing, it is worth searching for the specific mutation, because of the likelihood of finding the mutation, and because of the greater precision in distinguishing carriers and non-carriers if the specific mutation is known.

The comparison of genetic testing (either linkage analysis or mutation detection) and clinical testing demonstrates that manifestations of VHL frequently occur early, and that regular clinical screening is reliable in detecting presymptomatic disease (all gene carriers over age 15 in this family were

already identified by screening or by symptoms). This is important for those families where linkage analysis or mutation detection is not possible because the mutation has not been detected, the family is uninformative for linkage, or key samples are not available.

The fact that, in this family, there were no gene carriers over age 15 that had not already been identified by clinical screening or symptomatic disease, does not imply that every VHL gene carrier will be detected clinically by that age. Where genetic screening is not possible, regular clinical screening must continue much longer than this.

Variable Age at Onset of VHL

The age at onset of VHL is variable and the upper age limit at which VHL might initially present is unknown (Jennings et al 1988, Maher et al 1990). Age at diagnosis in the literature ranges to the sixth or seventh decade (clinical screening has therefore been recommended until at least 50 years of age), but many of those with late diagnosis had not been investigated previously and had advanced disease at the time of diagnosis (Huson et al 1986, Lamiell et al 1989). However, a 65-year-old obligate carrier of the VHL gene with negative clinical screening, has recently been described (Davies et al 1994). From our own experience, the latest age at diagnosis of VHL with previous negative screening was in a member of Family B who developed a retinal angioma at age 33 and, later, a renal cell carcinoma. The variability of age at onset even within

a family is emphasized by the fact that this patient's son had a large cerebellar hemangioblastoma identified by screening at age 15, just 4 years after his mother's diagnosis.

In family E, there was no known family history of VHL when the 18-year-old proband presented. However, a maternal second cousin had had "arteriovenous (AV) malformations of the cerebellum and spinal cord." When reviewed by the radiologist, this was considered compatible with the diagnosis of VHL, but intervening family members were not known to be affected. Although most were not investigated, the proband's mother, now age 54, has been extensively investigated and has a complicated renal cyst but no other evidence of VHL. If this is in fact an expression of VHL, she has very mild, late onset disease. Identification of the mutation in this family will be necessary to clarify whether anyone other than the proband is a gene carrier.

In Family A, there is no evidence of reduced penetrance or late onset of disease. Studies in other VHL families, comparing clinical and genetic screening results and following gene carriers prospectively, are necessary to determine the upper limit of age at onset of VHL, and the frequency with which non-penetrance occurs.

Genotype-Phenotype Correlation

In three recent studies comprising 291 VHL families (Crossey et al 1994, Whaley et al 1994, Chen et al 1995), specific mutations have been identified

in over 60% of families, including missense, nonsense, and deletion mutations. Over 90% of families with pheochromocytomas have missense mutations, whereas nonsense and frame-shift mutations, and rearrangements (all truncating mutations) are associated with "non-pheochromocytoma" families. There are two clusters of missense mutations at bp 505, and bp 712/713, occurring frequently but not exclusively in families with pheochromocytomas. However, the frequency of pheochromocytomas in families with missense mutations varies from 0% to 70%, and pheochromocytomas are also found in a few families with truncating mutations.

Eventually genotype-phenotype correlations may allow tailoring of clinical screening protocols for carriers of specific VHL mutations. At present, a comprehensive clinical screening protocol is still recommended for members of all VHL families.

Psychosocial Evaluation

Increasingly, concerns are being voiced about the possible detrimental aspects of screening, particularly the discrimination and psychological costs associated with presymptomatic diagnosis for a late-onset condition (Assessing Genetic Risk 1994). Even if predictive testing is considered appropriate, there is a question as to the age at which this should be done (Harper and Clarke 1990, Working Party of the Clinical Genetics Society (UK) 1994), and there are objections from some geneticists to any genetic testing of children. However,

others argue that the decision should be based on the age at which clinical disease occurs. In Newfoundland, VHL, for most patients, is not an adult-onset disease. Over 50% of patients in our families had clinical disease requiring treatment before age 18. The demonstration that early diagnosis and treatment improves prognosis emphasizes the value of early implementation of a comprehensive clinical and genetic screening program.

Psychosocial evaluation of the clinical and genetic screening program in the Newfoundland VHL families may answer some of the questions about the relative benefits and harms of screening for VHL disease and other AD hereditary predispositions to tumours. Many members of the families (particularly the large family, Family A) have undergone annual clinical screening for over 10 years, and more recently have participated in genetic screening. Detailed prospective records have been kept of the results of screening, of follow-up investigations, and of treatment when necessary. Records of previously affected family members were available for retrospective review. Some older unaffected family members are still alive who remember the medical and psychological problems of earlier generations, and members of the younger generation were interested and available for interviewing regarding the psychosocial aspects of screening.

Both affected and unaffected members of VHL families consider VHL to be a severe disease and one that should not be passed on to children. Family members experience considerable anxiety now (and even more in the past)

because of the early deaths, disabilities, and recurring medical problems caused by the disease. Typically they experience increased stress at the time of the screening appointments for themselves or close relatives because of the fear that there will be positive results. However, they unanimously agreed that the clinical screening program has improved their health, both medically and psychologically, and that the genetic screening program will allow more appropriate reproductive decisions and will free those without the VHL mutation from financial and psychological costs associated with clinical screening.

Recommended Management for VHL Families

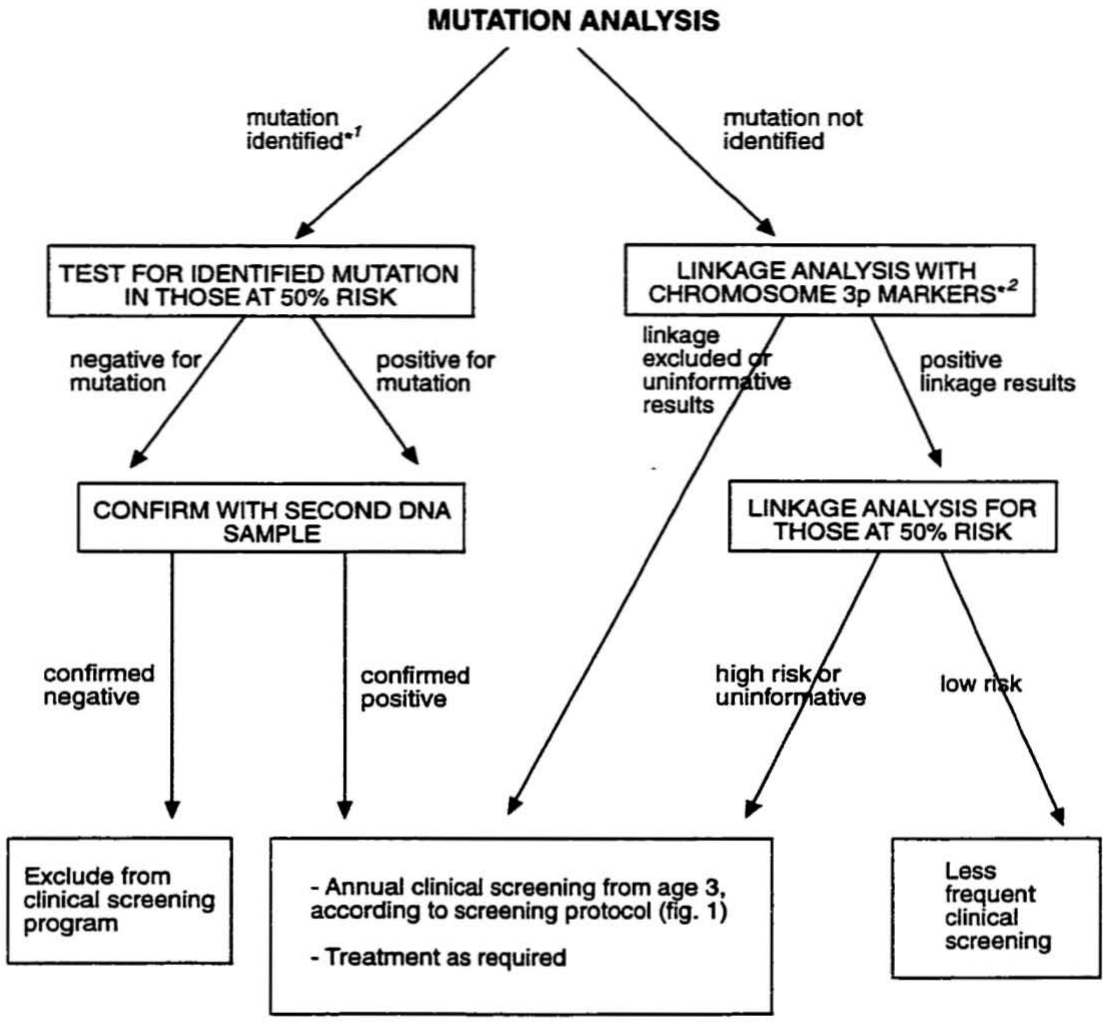
An efficient screening program for VHL (Figure 6.8) includes:

- genetic testing (ideally by direct mutation detection, otherwise by linkage analysis) to identify gene carriers, and provide reproductive counselling to both those who have and those who do not have a VHL mutation, and
- clinical screening of gene carriers throughout life, using a clinical screening protocol based on the natural history of the disease (revised as necessary) to identify and treat individual tumours.

Distinguishing those with the VHL gene will provide accurate reproductive information, will concentrate the clinical screening on those who require it, and will reduce psychological and financial costs to family members while reducing

Figure 6.8. Management plan for VHL families.

Management plan for VHL families



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*1 *mutations have been identified in approximately 60% of VHL families studied*

*2 *no families have been identified with confirmed linkage to another location*

costs to the medical care system. Since genetic testing must be voluntary, some family members at risk may choose not to participate.

The excess of low-risk results in the group at *a priori* 50% risk but with negative clinical screening, indicates that in the families described here, there is a high penetrance of the gene with respect to disease, and that clinical screening has been very efficient in identifying those who are affected. This is important for families where genetic testing is not possible (eg, small families, or those in which key individuals are deceased or unavailable so that linkage studies or mutation detection cannot be done). In such families, clinical screening can be reliably used for early diagnosis of VHL as well as for identification and treatment of individual tumours.

Predictive testing for VHL disease in childhood is justified, because of the early age of onset of VHL and therefore the early age that clinical screening is required, and the availability of treatment particularly when tumours are identified early. This approach is supported by the views of members of the Newfoundland families. This is unlike the situation with Huntington disease (Ball and Harper 1992), a late-onset and untreatable condition, but may apply to other hereditary tumour predispositions such as the multiple endocrine neoplasias (MEN-1 and MEN-2) and FAP, where disease onset is early and treatment available (Working Party of the Clinical Genetics Society (UK) 1994, Petersen et al 1993, Eng et al 1994b).

APPENDIX

A: VHL families

Family A:

Several sisters from the Bonavista Peninsula were referred to the ocular genetics clinic in 1982 because of a family history of eye, brain and "kidney" tumours. On examination, two of these sisters had retinal angiomas, and one had previously had an adrenal tumour (pheochromocytoma). Pedigree studies and a review of medical records indicated that 24 family members (10 of whom were deceased) had one or more manifestations of VHL. There were 50 first degree relatives at 50% risk, many of whom had never been investigated, and thirty-two relatives at 25% risk. Since the implementation of the clinical screening program in 1982, VHL disease has been identified in another 18 family members (a total of 42 members are now known to be affected). As family members continued to be screened, subsequent tumours were identified in recently and previously affected family members.

Family B:

A second Newfoundland VHL family originally from Trinity Bay was identified when the proband, a 32-year-old man with previously treated retinal angioma, and cerebellar and spinal cord hemangioblastomas, returned to the province from Ontario. Pedigree studies and medical record review identified one definitely and two probably affected first degree relatives, all now

deceased: a brother who died following surgery for cerebellar hemangioblastoma, and his mother and sister who died of "brain tumours". Four siblings at 50% risk and their nine children at 25% risk live in Newfoundland. One of the four siblings and her son have now been identified as affected.

Family C:

A teenage brother and sister who had recently moved to Newfoundland were referred for VHL screening because their mother had died at age 31 of a malignant pheochromocytoma, having had a previous pheochromocytoma removed at age 17. Two of her brothers in Nova Scotia had VHL: one brother was blind from bilateral retinal angiomas and has cerebellar and cerebral hemangioblastomas, and another brother died at age 22 with multiple manifestations including pheochromocytomas, and spinal cord and cerebellar hemangioblastomas. The teenage brother, originally referred, has now been identified as affected.

Family D:

A 40-year-old female patient with "polycystic kidneys" presented to a central Newfoundland hospital with renal failure. She suffered a stroke-like event, her condition deteriorated, and she subsequently died of sepsis. At autopsy, multiple bilateral kidney cysts, spinal cord hemangioblastoma and liver angioma were identified and the diagnosis of VHL was made by the pathologist.

She had no family history compatible with VHL disease, but had 5 children (age 10-20) each at 50% risk, and seven siblings, also possibly at risk.

Family E:

An 18-year-old girl from central Newfoundland presented to an ophthalmologist because of decreased vision. She was referred to the Health Sciences Centre in St. John's because of bilateral papilloedema; further investigations revealed five cerebellar hemangioblastomas and bilateral retinal angiomas. The diagnosis of VHL was made by the neurosurgeon. In retrospect, this patient had had periods of nausea and vomiting and progressive uncoordination over the previous 9 months, but the significance of these symptoms had not been recognized.

Her parents and three siblings were well, with no apparent VHL manifestations. However, pedigree studies and medical record review identified a maternal second cousin who had been disabled for a number of years because of "AV malformations of the cerebellum and spinal cord." When previous scans were reviewed by the radiologists, these lesions were considered to be compatible with cerebellar and spinal cord hemangioblastomas, and a diagnosis of "probable VHL" was made. This raises the possibility that the proband's mother is a carrier and that the proband's siblings are at 50% risk for VHL disease — genetic and clinical screening are, therefore, indicated.

B: Interview topics for psychosocial evaluation of the impact of VHL, and of the screening program on VHL family members

1. Knowledge/understanding of VHL and of screening methods

   - how was it obtained
   - is it sufficient; would you like to know more (or less)
   - how should it be provided
   - do family doctors, public health nurses, etc, know enough?

2. Compliance with clinical screening; satisfaction with the program

   - reasons for non-compliance (financial, fear, disinterest, lack of concern)
   - are there any things that should be done differently?

3. Anxiety/stress because of the disease, or because of screening

   - death and disability in the past
   - concern for yourself or your children about medical aspects of VHL
   - worry at time of screening appointments
   - worry because of false positive/false negative results
   - negative side effects of screening or treatment

4. Support systems

   - family, spouse, friends
   - family doctor, public health nurse
   - specialists, geneticists, psychologist/psychiatrist

5. Financial implications of the disease, or of screening

   - for appointments/treatment (travel, accommodation, etc): are these expenses covered by insurance?
   - are there other expenses due to the disease or screening?

6. Discrimination or stigmatization because of VHL or because of screening

   - has the disease or screening affected
     - education
     - employment
     - insurance
     - social/marriage relationships
     - family life?

   (has it affected your choices; or other peoples' choices or decisions with respect to you?)

7. Effect of the disease, or of availability of genetic screening on reproductive decisions

   - no children, fewer children, worry about children
   - interest in predictive testing/mutation testing (for self or for children)
   - interest in prenatal diagnosis
   - does possibility of predictive testing/prenatal diagnosis change approach to reproduction?

8. Effect on quality of life

   - of the disease
   - of screening

CHAPTER 7 — HEALTH CARE EVALUATION OF THE VON HIPPEL-LINDAU DISEASE SCREENING PROGRAM

INTRODUCTION

NEEDS ASSESSMENT

SUMMATIVE EVALUATION

Introduction

i) Evaluation design
ii) Collection and analysis of data
iii) Ongoing analysis of program

Methods and Results

Objective/Question 1. Does clinical screening for VHL result in earlier identification of affected family members?
i) Method
ii) Results

Objective/Question 2. Does the screening program cause a decrease in morbidity and mortality from VHL by facilitating earlier treatment or intervention for specific manifestations of disease?
i) Method
ii) Results
   a) Quantitative results
   b) Qualitative results (In-depth case studies)
      - Retinal angioma
      - Pheochromocytoma
      - Cerebellar hemangioblastoma
      - Renal cell carcinoma

Objective/Question 3. Has the provision of genetic counselling for affected and at-risk family members increased their understanding of VHL, and decreased their anxiety concerning the adverse aspects of the disease?
i) Method
ii) Results

Objective/Question 4. Has the screening program been revised when appropriate?

i) Method
ii) Results

Discussion

i) Selection of control groups
ii) Threats to internal validity
    a) Threat of history
    b) Threat of maturation
    c) Threat of instrumentation
    d) Threat of selection
iii) Threats to external validity

FORMATIVE EVALUATION

Introduction

Method

Results

i) Personnel
ii) Participants
iii) Record keeping
iv) Sensitivity and specificity of investigations
v) Genetic testing
vi) Side effects of screening

Discussion

COST-BENEFIT ANALYSIS

Introduction

Method

Results

Discussion

OVERALL DISCUSSION OF HEALTH CARE EVALUATION

INTRODUCTION

This chapter provides a complete health care evaluation of the von Hippel-Lindau disease screening program, including Needs Assessment, Summative Evaluation, Formative Evaluation and Cost-Benefit Analysis.

As previously noted, the impetus to develop a screening program for VHL was provided when a large Newfoundland family with the disease (Figure 6.2a) was identified in 1982. Individual members of the family had previously been seen by a variety of specialists for urgent medical treatment of symptomatic disease. Many had suffered blindness or early death, and yet there had been no coordinated approach to the management of the multiple manifestations of the disease. The relationship of the different presenting symptoms was not well understood by the family members or their physicians, and the frequent morbidity and mortality in this family, and four smaller VHL families subsequently identified, caused a great deal of anxiety and mistrust of the medical system.

It was recognized that a multi-disciplinary approach, with the development of a screening program, was necessary for improved medical management of family members identified through pedigree studies and review of medical records as affected or at risk (Jennings et al 1988).

The objectives of the VHL screening program were:

- early identification of individual manifestations to facilitate early treatment or intervention, and to reduce the morbidity and mortality associated with advanced disease,
- early identification of those who carry the VHL gene to allow informed reproductive decisions,
- genetic counselling for affected and at-risk family members to increase the understanding of all features of VHL disease, and to reduce the anxiety caused by the disease,
- collection of data to allow refinement of the screening protocol,

Since the location of the VHL gene was not known in 1982, the identification of gene carriers and the early identification of individual manifestations were both dependent on a clinical screening protocol which included a set of investigations at least annually to detect presymptomatic disease (as described previously, Figure 6.1). After the VHL gene was mapped (Seizinger et al 1988), and later cloned (Latif et al 1993), methods for direct identification of gene carriers by linkage analysis and by direct mutation detection were developed. Comprehensive genetic counselling was provided for all family members when first identified as affected or at risk and, when necessary, has been reinforced subsequently. Results of the clinical screening program and of the genetic testing, and relevant information from recent literature, have been used to review and revise the program.

From the review of past medical records and documentation of the results of investigations and treatment since screening was implemented, several advantageous results of the program have been demonstrated. These include an earlier age at diagnosis (Figure 6.4), detection of manifestations at an earlier and more treatable stage (although the number of tumours detected has increased), and a decrease in the morbidity and mortality associated with these manifestations (see Summative Evaluation). It is also our impression, from interviews and frequent contact with the family, that family members now have a better understanding of the implications of VHL for themselves and for their children, and are less anxious than previously about the threats of the disease to their well-being (see Chapter 6, Psychosocial evaluation).

The purpose of this section is the evaluation of the program:

- to review the objectives of the VHL screening program, including whether or not the need for the program still exists (The Needs Assessment);
- to determine whether the objectives have been achieved and, if so, whether it is appropriate to attribute these results, or outcomes, to the screening protocol (Summative Evaluation);
- to determine whether there are better or cheaper ways to meet the objectives (Formative Evaluation); and
- to identify the costs and benefits, both financial and psychosocial, of this health care program (Cost-Benefit Analysis).

NEEDS ASSESSMENT (Does the problem still exist?)

In von Hippel-Lindau disease, as in a number of other hereditary tumour predisposition syndromes, patients who are symptomatic usually have advanced disease which is difficult to treat successfully and often leads to death or severe deficits (Vasen et al 1987, Lamiell et al 1989). To prevent this, individual manifestations in those who are affected must be identified and treated earlier. A clinical screening protocol based on the natural history of the disease was designed for this purpose (see Chapter 6).

The autosomal dominant inheritance pattern means that children of an affected individual have a high (50%) risk of also being affected. Because of the severity of VHL, many family members prefer not to risk passing the gene on to their children. To make appropriate reproductive decisions they must know whether or not they carry the VHL gene before considering having children. When the Newfoundland VHL screening program was initiated, the location of the VHL gene was unknown so that gene carriers could only be recognized by diagnosis of the initial manifestation of VHL in each individual, and this was also facilitated by the clinical screening protocol.

The recent advances in molecular genetics, including the mapping and cloning of the VHL gene, permit the identification of gene carriers by genetic testing in some families; by linkage analysis with closely-linked flanking markers or by direct detection of the VHL mutation (Glenn et al 1992, Maher et al 1992, Crossey et al 1994, Chen et al 1995). Thus, genetic testing may now

be added to the screening program to allow reproductive planning for affected and unaffected family members. Genetic testing will also focus clinical screening on those with the VHL gene, reducing by half the number requiring clinical screening, and therefore reducing the costs associated with clinical screening. A clinical screening program, however, continues to be necessary for early identification and treatment of specific manifestations in all those with the VHL gene, since the age and order in which manifestations occur in any particular individual is unknown (Maher et al 1990).

Genetic counselling in its broadest sense is education about the natural history, management, and risk of inheriting a genetic disease, and about the options available for dealing with the risk. For VHL, this includes knowledge of the rationale for screening, of the recommended clinical screening protocol, of the types of symptoms which should be reported to the family doctor between screening appointments, and of the availability and implications of genetic screening. Genetic counselling should also include psychological support for the patient facing the negative aspects of genetic disease. All of these components of genetic counselling continue to be necessary for VHL family members.

Continuous review and refinement of the screening program allow incorporation of new technology, and new clinical and genetic knowledge about VHL.

Assuming that benefits of the program can be demonstrated, and considering that there are 78 known affected or at-risk VHL family members in Newfoundland, a screening program including clinical testing and genetic counselling, and with the recommended inclusion of genetic testing, is needed.

SUMMATIVE EVALUATION (Have the objectives been met?)

Introduction

If the need for a VHL screening program is accepted, then the Newfoundland program should be evaluated in terms of its effectiveness. This is the summative evaluation (Shortell and Richardson 1978, FitzGibbon and Morris 1987) answering the questions of whether or not the objectives (as previously stated) have been achieved; if so, whether they can be attributed to the screening program; and whether there are any unexpected outcomes.

i) Evaluation design

An appropriate evaluation design should gather comparative information on how things might have been if there had been no program, or a different program (FitzGibbon and Morris 1987). A good design will rule out the effect of variables other than the treatment (the screening program) on the outcome (FitzGibbon and Morris 1987, Shortell and Richardson 1978), to reduce the possibility of alternative interpretations of the results. There must be an operational indicator (something to measure) that will tell whether or not each objective has been achieved (Shortell and Richardson 1978).

Ideally, individuals from the "population" of interest (in this case, affected and at-risk members of VHL families) should be assigned randomly to experimental and control groups (Shortell and Richardson 1978, FitzGibbon and Morris 1987), those in the experimental group to have regular clinical screening according to the designated protocol, and those in the control group to have no screening or a different screening program. However, the medical literature now recommends some form of clinical screening for members of VHL families (Jennings et al 1988, Maher et al 1990), and families with other tumour predisposition syndromes (Ponder et al 1988, Vasen et al 1990a, Skogseid 1991a), because of the morbidity and mortality in unscreened families. It would therefore have been unethical to assign those at risk randomly to screening and non-screening groups and, thereby, to withhold medical care from the unscreened group (FitzGibbon and Morris 1987, Sackett 1980). Furthermore any randomization process would be confounded by the fact that family members communicate. A quasi-experimental rather than a true experimental design was therefore used (Shortell and Richardson 1978, Cook and Campbell 1979). Non-equivalent control, or comparison, groups were chosen that were as similar as possible (members of the family living in other provinces where regular screening program has not been implemented, and members of the family in Newfoundland in previous decades), and the differences documented (FitzGibbon and Morris 1987). Patient outcomes have been compared in light of these differences.

ii) Collection and analysis of data

Because VHL is an extremely variable disease even for members of the same family with the same genetic defect, the screening process must be individualized according to the results of the primary investigations. Screening proceeds according to an algorithm rather than a step-by-step protocol. It is difficult, therefore, particularly in a small sample, to do any statistical analyses, except the most basic frequency comparisons. Qualitative data collection is appropriate, however, for evaluation of this type of program, using observations, interviews or in-depth chart reviews of "information-rich" cases, for comparison of the effects of the disease at a very personal level (Patton 1987). The key to this part of the evaluation is the appropriate selection of cases, or "purposeful sampling" (Patton 1987).

For the VHL program evaluation, typical case sampling, maximum variation sampling, and deviant case sampling are appropriate. The typical and extreme cases are the most important ones in the comparison of the medical outcomes between experimental and comparison groups. The extreme or deviant cases are particularly significant, because the program cannot facilitate treatment or intervention for manifestations of VHL which are unknown or for which the type or timing of screening investigations is inappropriate. The deviant cases may be difficult to provide for, but it is the prevention of severe "typical" cases, that is the intent of the screening program.

Naturalistic inquiry (Patton 1987) based on observations over the duration of the screening program and recent interviews using open-ended questions, is appropriate for evaluating the psychosocial outcomes and the effect of genetic counselling.

The use of different data types (quantitative and qualitative), different data sources (medical records, direct observations and interviews), and different methods of inquiry (quasi-experimental and naturalistic) is an example of triangulation (Patton 1987), and is used to combine the strengths and offset the weaknesses of the various approaches.

iii) Ongoing analysis of program

Any screening program will require adaptation over time. To determine when revisions are appropriate, the results of screening and treatment should be monitored, and reviewed with the specialists responsible for the medical management of patients. The relevant literature should also be reviewed to identify new investigations or treatments which may be appropriate, current information on the natural history of VHL which may necessitate revision of the type or timing of investigations, and changes in the status and availability of molecular genetic testing.

Methods and Results

Clinical data have been collected retrospectively or prospectively for each affected family member, including age at diagnosis of initial and subsequent

manifestations of VHL, method of diagnosis (by symptomatic presentation or by clinical screening), outcome of treatment, and age and cause of death when appropriate. Specific methods related to individual objectives are discussed in each section.

Objective 1: Early identification of those who are affected to allow reproductive planning (Does clinical screening for VHL result in earlier identification of affected family members?)

i) Method

Age at diagnosis was compared in screened and unscreened VHL family members using a quasi-experimental design with similar but non-equivalent control groups. The experimental group was the cohort of members of three VHL families residing in Newfoundland and participating in the screening program from 1982-1991. The control groups included untreated cohorts (ie, cohorts of 1962-71 and 1972-81 that were not screened), and a differently treated group (VHL family members in Ontario and New Brunswick, 1982-1991) with minimal screening.

ii) Results

The mean age at diagnosis of 16 members of the large VHL family identified by the clinical screening program (1982-91) was 16 years, compared with 29 years in five family members presenting with VHL in other provinces in the same time period, and 27 years in the 24 family members identified

previously because of symptomatic disease presentation (a mean age at diagnosis of 36 years in those identified in 1962-71, and 19 years in those identified in 1972-81) (Table 7.1).

Nine of 16 of those identified by screening already had presymptomatic manifestations of VHL when first investigated. As the clinical screening program continues and manifestations are detected prospectively in young family members, it is expected that the mean age at diagnosis will decrease further.

Objective 2: Early identification of individual manifestations to facilitate early treatment or intervention and to improve the prognosis (Does the screening program cause a decrease in morbidity and mortality from VHL by facilitating earlier treatment or intervention for specific manifestations of disease?)

i) Method

The status at diagnosis (presymptomatic or symptomatic disease expression); the frequency and age of death from VHL; and the frequency of unilateral or bilateral blindness, or of disability preventing normal employment, were compared in the experimental and comparison groups. In-depth case studies using observations and descriptive information from medical records were prepared for patients identified presymptomatically by screening, and patients who presented symptomatically, to compare medical outcomes and post-treatment quality of life of screened and unscreened family members.

TABLE 7.1. IDENTIFICATION OF NEW PATIENTS, AND OF NEW TUMOURS IN AFFECTED MEMBERS OF A VHL FAMILY, BY SCREENING OR BY SYMPTOMATIC PRESENTATION

CATEGORY	Regular Screening Nfld 1982-91	Minimal Screening Ont./N.B. 1982-91	Minimal Screening Nfld./Ont. 1972-81	No Screening Nfld. 1962-71
Number of New Patients	16	5	13	11
Mean Age at Diagnosis (yrs.)	16 SD, 3.72	28.8 SD, 12.15	19 SD, 6.21	35.9 SD, 11.14
Number of Tumours Identified	74	14	16	19
— Asymptomatic	70	8	4	0
— Symptomatic	4	6	12	19

N.B. The mean age at diagnosis for screened patients was 16.1 (SD, 3.72) and the mean age at diagnosis of all symptomatic patients was 28.9 (SD, 13.16). Because the variances are significantly different, parametric statistics are not appropriate. However with a non-parametric procedure, the means are significantly different ($p<0.001$).

ii) Results

a) Quantitative results

There was a marked increase in the number of VHL tumours detected in affected family members when the clinical screening program was introduced (74 tumours identified by screening in Newfoundland in 1982-91 in 18 affected individuals, compared with 14 tumours in 14 patients identified in other provinces during the same time period, and 16 tumours in 16 patients [1972-81] and 19 tumours in 11 patients [1962-71] in unscreened cohorts previously). However, the majority of tumours (70/74) in the screened group were identified at a presymptomatic stage, compared to 8/14 tumours detected presymptomatically in other provinces in the same time period. In the earlier cohorts only 4 tumours were identified presymptomatically, 31/35 tumours being identified because of symptoms of advanced disease (Table 7.1).

One of 18 patients in the screened group died, and 3 developed unilateral blindness during 1982-91. However, each of these patients had presented symptomatically prior to the institution of screening, or with advanced disease at initial screening. Fourteen of the 18 patients in the screened group were free of debilitating disease. In contrast, during 1962-81, of 24 family members presenting with symptomatic disease, 10 died at a mean age of 37 years, 10 developed unilateral or bilateral blindness, and 7 had a severe neurological or other disability preventing employment for several years prior to death (Table 7.2).

TABLE 7.2. MORBIDITY AND MORTALITY IN COHORTS OF VHL PATIENTS MANAGED WITH OR WITHOUT A CLINICAL SCREENING PROGRAM

CATEGORY	NUMBER, AND AGE AT DEATH OR DISABILITY			
	Nfld. 1982-91	Ont./N.B. 1982-91	Nfld./Ont. 1972-81	Nfld. 1962-71
Deceased (Age)	1* (28 yrs)	1 (49 yrs)	1 (18 yrs)	9 (21-55 yrs) $\bar{x}$, 38.9; SD, 11.0
Blind - Unilateral (Age)	3** (23-26 yrs)	1 (5 yrs)	-	8 (18-45 yrs) $\bar{x}$, 27.9; SD, 8.8
- Bilateral (Age)	-	-	1 (21 yrs)	1 (45 yrs)
Neurological Disability (Age)	-	2 (27,34 yrs)	1 (18 yrs)	6 (19-54 yrs) $\bar{x}$, 38.2; SD, 13.6
Total living affected	18	12	14	2

* symptomatic presentation in 1980

** i) identified in 1973 and not followed
ii) symptomatic presentation before screening started
iii) large angioma on first examination

b) Qualitative results (In-depth case studies)

The in-depth case studies comparing the medical outcomes and quality of life in those identified with symptomatic or presymptomatic manifestations of VHL, provide insight into the impact of this disease on individual patients.

- Retinal Angioma. In 1981, a 16-year-old girl was seen by an ophthalmologist because of unilateral loss of vision. She had a retinal detachment because of multiple retinal angiomas (>20). Two attempts at surgical repair of the detachment were unsuccessful and she is now blind in one eye. She also had multiple angiomas in the second eye on initial examination. Laser and cryotherapy have so far kept these under control, although she has extensive scarring and is at risk of a traction retinal detachment which would cause loss of vision of the second eye. This patient's mother died at age 21 of VHL, but the only screening the patient had had was an annual "water test" (ie, VMA [vanillylmandelic acid] measurement, which was the previous screening test for pheochromocytoma).

A 19-year-old at-risk family member in Ontario, who had never been screened, presented similarly with decreased vision and was found to have multiple bilateral retinal angiomas. Despite extensive laser treatment and cryotherapy, within two years he had retinal detachments in both eyes and was registered blind at age 21.

Since 1982, retinal angiomas have been detected by screening in twelve at-risk family members in Newfoundland. All but one, who had a large angioma

when first seen, have had an excellent outcome with small circumscribed scars where angiomas have been treated. Except in this one patient, no retinal detachments and no loss of vision have occurred. Angiomas have recurred in all patients, but because of the regular screening they were small when identified, and have been relatively easy to treat.

- Pheochromocytoma. In 1981, before the screening program began, a 17-year-old girl (whose father had died previously of hypertensive crisis from bilateral pheochromocytomas) had increased blood pressure during pregnancy, attributed to toxemia of pregnancy. The day after delivery of a healthy daughter she had a hypertensive crisis with subarachnoid hemorrhage and was comatose until she died 5 months later. No autopsy was done.

When her medical records were reviewed, we suspected that she had had a pheochromocytoma and was, therefore, affected (an undetected pheochromocytoma is associated with at least 27% mortality during pregnancy or delivery [Griffin et al 1984, Freier et al 1993]). Her daughter was assumed to be at risk and was screened completely from age three. At age nine the daughter's urinary catecholamines were increased, and a CT scan demonstrated a unilateral pheochromocytoma. She had a successful adrenalectomy and is well at the age of 13 years.

Six of the twelve deaths from VHL in the family have been secondary to pheochromocytoma; five because of hypertensive crisis during surgery or

pregnancy (all before the screening program), and one because of malignancy in 1983 in another province.

- Cerebellar Hemangioblastoma. In 1983 an 18-year-old girl in central Newfoundland saw an ophthalmologist because of decreased vision and was eventually referred to St. John's. She had bilateral papilloedema and was referred for a CT scan of the head and neck. This showed five cerebellar and one spinal cord hemangioblastomas for which she had successful surgery. In the postoperative period, retinal angiomas developed and were successfully treated. However, she is totally blind because of optic atrophy from the long-standing preoperative papilloedema caused by the multiple hemangioblastomas. In retrospect, she had had nausea and vomiting and decreased coordination over the previous 9-12 months. Because there was no significant family history (she was the proband of VHL family E) these symptoms were not thought to be significant and were not investigated.

A 44-year-old affected member of the minimally-screened Ontario branch of VHL family A has a significant neurological deficit, and has not worked since neurosurgery in 1984 for a cerebellar hemangioblastoma. He had bilateral pheochromocytomas removed in 1972 but did not have any subsequent screening. His cerebellar tumour was identified in 1984 after he became symptomatic with incoordination and ataxia. Surgery was only partially successful because of the large size of the tumour and the extensions around the brain stem.

In contrast, a 15-year-old boy at risk for VHL was in St. John's in 1990 for his annual screening (his mother had retinal angiomas and renal cell carcinoma). His parents were concerned about the recent occurrence of morning headaches. Even though he had had a normal CT scan of the head two years previously and had a normal neurological examination otherwise, he was referred for a repeat CT scan because of the headaches and the positive family history. The CT scan showed a very large cerebellar hemangioblastoma which was removed successfully. He has no visual or neurological deficit post-operatively.

- Renal Cell Carcinoma. None of the patients in this family have had symptomatic renal cell carcinoma (RCC). Prior to 1982 there had been only one diagnosis of RCC — in a severely affected 55-year-old man at autopsy. Since 1986, RCC has been identified by routine ultrasound in thirteen asymptomatic affected family members. They all had unilateral or bilateral partial or total nephrectomies. None of these tumours extended through the kidney capsule or into the blood vessels, and no metastatic disease has been detected, so these patients have not required chemotherapy or radiotherapy. However, three patients have had subsequent primary tumours in the second kidney. In other VHL families documented in the literature, metastatic RCC is the second most common cause of death.

Objective 3: Genetic counselling to increase the understanding of VHL and reduce anxiety (Has the provision of genetic counselling for affected and at-risk family members increased their understanding of VHL, and decreased their anxiety concerning the adverse aspects of the disease?)

i) Method

Knowledge of VHL and anxiety concerning the disease were not formally evaluated before the screening program was implemented. The clinical needs of family members were so pressing when they presented to the Ocular Genetics clinic in 1982 that the original clinical screening protocol was designed and implemented, and genetic counselling including education about VHL and the rationale for screening given immediately. However, observations were made by Jane Green (who was always in close contact with the family), at the outset, and throughout this program (Patton 1987). Relevant information or opinions stated by family members during counselling sessions or telephone conversations were also recorded. A medical student report (Hogan and Sparrow 1972), written when several family members died from what was later recognized to be VHL disease, was also reviewed. The feelings of family members documented at that time were compared with those of family members interviewed recently. This allowed some subjective conclusions about the changing views of the family members towards the disease.

Six affected and nine unaffected family members were interviewed, about their knowledge of VHL and the clinical and genetic screening methods; about the benefits and costs of the screening program, and their satisfaction and compliance with it; and about the overall effect of screening on their quality of life. The group interviewed included male (n = 5) and female (n = 10) family members, and those within the reproductive (n = 8) and post-reproductive (n = 7) age groups.

The interviews were conducted by Jane Green at the homes of the family members or at the General Hospital in St. John's, depending on the preference of the individual. The same open-ended questions were asked of each person (Appendix B, p. 364-5). Those interviewed were free to discuss these points in as great depth as they wished.

ii) Results

The first group of six affected and at-risk family members seen (and their family doctor) did not know the name of the "family disease", nor did they know the names of individual manifestations, and incorrectly referred to pheochromocytomas (adrenal gland tumours) as "kidney tumours". They did not understand the connection between the eye, brain, and adrenal gland tumours occurring in different family members, and also thought that all family members would develop the disease. Family members now know the main clinical and genetic features of VHL, and they and their family doctors have pamphlets to refer to, or geneticists to contact, if further information is

required. They regularly tell medical practitioners that they have, or are at risk for, VHL when investigated for any medical problem.

They are now making reproductive decisions based on their knowledge of gene carrier status rather than because of fear that everyone in the family will be affected. Five family members identified as affected by clinical screening in their teens have chosen to have sterilization rather than risk passing on the VHL gene, and three others have had one or two children only, rather than larger families. All family members at risk in the reproductive age group (n = 14) chose to have genetic testing (direct mutation analysis) when this became available, for reproductive planning as well as for clinical management. Four family members who had chosen voluntary sterilization to ensure that they did not have affected children, are now known to be non-carriers. Two of these plan to have the sterilization reversed. One other family member had only one child, and plans to have more children now that she knows that she does not carry the VHL gene. Conversely, two affected family members in one of the earlier generations had 10 and 12 children before the VHL diagnosis was known, and now regret that the gene was passed on to so many (13/22) of their offspring.

The medical student report from 1972 (Hogan and Sparrow 1972) documents the extreme anxiety in affected and unaffected family members caused by the frequent sudden deaths and severe disabilities within the family. Family members still feel anxious about the devastating potential of VHL.

However, for 14 of 15 family members interviewed, this is controlled except during the period immediately before and after annual screening investigations, or if symptoms develop, because they now trust the medical care system and the screening program. One patient feels that VHL is a permanent cloud over her life and prevents her enjoyment of other things. The family members also state that their compliance with the screening program is based on an understanding of the rationale for screening, and concern about the effect of VHL if not detected early.

Although there was no opportunity for a formal pre- and post-intervention evaluation of knowledge about VHL disease and of the stress caused by this disease, it appears that the current understanding of VHL is being used appropriately, and that the quality of life of family members has improved because of the screening program.

Objective 4: Collection of data to refine the screening protocol (Has the screening program been revised when appropriate?)

i) Method

Literature on the natural history, screening recommendations, and molecular genetics of VHL was monitored continuously. Clinical data were collected retrospectively or prospectively for each family member. A summary of the natural history of VHL in the Newfoundland families (including frequency and order of occurrence of manifestations, and age at diagnosis), and of the

medical outcomes before and after the screening program was implemented, was prepared and presented to the medical specialists. Each specialist reported on new technology that could facilitate screening or treatment.

The availability and acceptability of molecular genetic testing for the Newfoundland families, and the results of linkage analysis and mutation detection conducted under a research protocol were also presented and discussed.

ii) Results

In 1986, renal ultrasound from the mid or late teens was included as one of the annual investigations for affected and at-risk family members, because of new data in the literature on earlier age at onset of renal cell carcinoma than previously described. In 1994, it was recommended by the radiologists that staged CT scanning of the kidney/adrenal area replace ultrasound screening. A new, faster CT scanner had been obtained which allowed the better sensitivity of CT scanning while minimizing the radiation exposure. Similarly, when an MRI was obtained in 1993, MRI of the head, and spinal cord if necessary, replaced CT scanning because MRI provides better resolution of central nervous system tissue (Filling-Katz et al 1989, Hoff et al 1993), without exposure to radiation.

In 1994 it was recommended that genetic testing be offered to all at-risk members of VHL families that were informative for linkage analysis or mutation detection, because of the precision with which gene carriers and non-carriers

can now be identified, and because of the acceptability of this testing to family members. Thus the screening program has been revised according to advances in diagnostic and genetic technology.

Discussion

A health care program should be evaluated to determine whether the goals of the program are being achieved and, if so, whether the results can be attributed to the program, and whether they can be generalized to other situations (Shortell and Richardson 1978). It would take many years and the follow-up of many families to determine definitively the degree to which a screening program for a hereditary cancer syndrome actually changes the long-term medical and psychosocial outcomes for affected individuals and members of their families, and whether this is a cost-beneficial method of clinical management. A preliminary evaluation, can, however, provide some guidelines as to the effectiveness of such a program, and will allow necessary revisions to be made (Objective 4). For this reason the Newfoundland VHL screening program, which has been underway for 12 years (for 78 affected and at-risk members of 5 families) has been reviewed and evaluated.

i) Selection of control groups

Patients were not randomized to "treated" and "untreated" groups, since it was considered unethical to withhold screening from a control group (FitzGibbon and Morris 1987). Instead, a quasi-experimental design was used

with two similar comparison groups: a historical unscreened group (Cook and Campbell 1979), and a less extensively screened group living outside Newfoundland. There are some differences in the numbers, age profile and disease profile of members of the experimental and comparison groups because of the variability of the disease, and because the groups were defined by the family structure and geographic location. In fact, without extensive investigations, the actual number of affected, and number of manifestations per patient are not known.

The goals of the screening program [i) earlier age at diagnosis of VHL to allow reproductive decisions, ii) earlier diagnosis and treatment of individual manifestations to decrease morbidity and mortality from the disease, iii) increased knowledge and reduced anxiety regarding VHL to improve the quality of life, and iv) fine-tuning of the screening protocol as new information, or new technology becomes available] have all been achieved since the screening program was implemented. But, can these results be attributed to the program rather than to other factors?

ii) Threats to internal validity

Various threats to internal validity have been considered (Shortell and Richardson 1978, Cook and Campbell 1979):

a) Threat of history (the effect seen could result from changes in medical care during the past decade rather than from the intervention)

Overall, medical care has improved and new diagnostic procedures have become available since 1972 or even since 1982, which could result in earlier diagnosis now than previously. However, without the screening protocol, these diagnostic procedures and examinations would not be used on asymptomatic patients. Surgical procedures have also improved so that patients with the same degree of disease should have better outcome now than previously, however, it is the screening program which allows early access to these procedures.

b) Threat of maturation (the effect seen could be related to the natural history of the disease rather than to the intervention)

VHL is an extremely variable disease, in its manifestations and age of onset. There is no way of knowing, for any particular patient, what the age or order of manifestations will be. Late age at diagnosis may result from late age of onset, rather than late detection of disease because screening was not utilized; and good outcome of treatment may result from mild disease, rather than from early diagnosis and treatment because of screening.

There is no reason, however, to think that the variability of the disease is different now than it was in previous generations, or in those living in different provinces; in each group, patients with early onset severe disease, and others with later onset milder disease were identified.

c) Threat of instrumentation (the apparent effect could be biased by the greater availability of current, local data)

The data for control groups (either archival data or data from another province) may not be as complete or as accurate as data for the experimental group. However, for this evaluation, records were available and were reviewed for all members of the control groups.

d) Threat of selection (the effect seen could be the result of less severe disease in the experimental group rather than the result of the intervention)

The groups were not randomly assigned and are relatively small so may have different representations of those with mild or severe disease, or of different ages. Even if the numbers were large enough, patients could not be stratified according to severity, because the number and type of manifestations any individual will have cannot be predetermined. However, in the control groups the two most recent deaths, and 4 of 6 disabilities during the 1980s were because of the initial VHL manifestation. These patients had unrecognized rather than particularly severe disease.

iii) Threats to external validity

Threats to external validity were also considered (Shortell and Richardson 1978), to determine whether these results have significance to other VHL families, or other similar diseases.

Because VHL is so variable, both within and between families, a comprehensive clinical screening protocol should be recommended for other VHL families as well as for the Newfoundland families. This clinical screening protocol is based on the natural history of the disease described in the literature and in large well-studied families, but possibly modified for an individual family. Earlier diagnosis and treatment of initial disease and of subsequent manifestations should be achieved by screening members of VHL families elsewhere with the protocol developed for the Newfoundland family.

The genetic counselling process as carried out in Newfoundland, including counselling and educational components, is similar to that done elsewhere. Therefore, the review of psychosocial costs and benefits, and of the response to predictive testing in this family should provide guidelines for other centres developing screening programs for small or recently identified VHL families.

As described in earlier chapters of this thesis, screening for other hereditary tumour syndromes has been developed in a similar way, using a clinical screening protocol determined by the natural history of the specific disease. Those at risk (defined by genetic principles) are screened for the manifestations causing significant morbidity or mortality, beginning prior to the age that these manifestations can be recognized symptomatically, and using a presymptomatic diagnostic procedure. Genetic screening methods to identify

gene carriers are included in the screening program when linked markers are available, or specific mutations are known.

In conclusion, there is evidence that the outcomes identified in this study can be attributed to the VHL screening program. It is also felt that the results and recommendations of the Newfoundland screening program can be generalized to similar situations elsewhere. However, one of the reasons that the Newfoundland program works particularly well may be the special interest of personnel involved here.

FORMATIVE EVALUATION (Is there a better way to provide the VHL screening program for those at risk, either in terms of the type and timing of screening investigations, or in terms of the administration of the program?)

Introduction

In addition to evaluation of outcomes, a program should be evaluated in terms of its implementation, looking at the day-to-day activities of program personnel, the set of investigations recommended for each family member, and the compliance of the patients with these investigations. This is the formative evaluation (Shortell and Richardson 1978), answering the question of "who is doing what to whom, with what resources and within what period of time" (Shortell and Richardson 1978, Sackett 1980). Formative evaluation should take place continuously but particularly in the early stages of implementation in order to arrive at the most efficient and effective process (FitzGibbon and

Morris 1987, Patton 1987). Although program operation is less important than the achievement of the medical and genetic outcomes, these outcomes cannot be attained unless the program implementation is successful (Patton 1987).

Formative evaluation is primarily a descriptive evaluation documented through observation of the daily activities which must take place in order for all aspects of the screening program to proceed. Tables or flow charts should be used to follow the personnel involved in the program, their activities related to each major objective (Shortell and Richardson 1978), and time spent per week or per month at these tasks. The personnel should also be interviewed to elucidate any deficiencies or problems in the procedures and to identify areas for improvement.

The clinical investigations of the screening protocol should be reviewed to clarify the sensitivity and specificity (Sackett et al 1991) of these examinations and procedures, and to determine whether recently available investigations should replace current procedures (such as MR imaging replacing CT scanning).

In population screening for a low-risk event, specificity is more important than sensitivity, in order to reduce the number of false positives. However, in very focused screening, as for those affected or at 50% risk for VHL, high sensitivity is most important, particularly when an undetected manifestation is associated with potential lethality. Since the "cost" of a false negative is so

high, the investigation of choice may be different from that used in other situations.

The results of genetic testing done on a research basis should be reviewed to determine whether this should be included in the overall screening program.

Method

Information was collected from appointment books and medical records on the number of participants in the screening program and their compliance with appointments. The duties and time-commitment of personnel involved with the program were documented and monitored, and the method of obtaining, recording and storing test results was reviewed.

Individual medical records were reviewed to identify false positive or false negative results of screening tests, or any adverse side effects of investigations. The results of screening (frequency of manifestations and age at diagnosis), and the literature regarding screening recommendations from other centres were monitored and reviewed with physicians involved so that the type and timing of clinical investigations could be revised as necessary.

Genetic screening was introduced on a research basis in 1992 as discussed in Chapter 6. The results of predictive testing by linkage analysis and subsequently by direct mutation detection, and the monetary costs and

psychosocial implications of including genetic testing in the screening program, were also reviewed with the group of specialists.

Participants in the screening program were interviewed regarding their satisfaction with the screening program, and reasons for compliance or non-compliance with scheduled investigations.

Results

i) Personnel

The main personnel of the von Hippel-Lindau disease screening program are the geneticist (Jane Green) who also acts as program coordinator, a secretary, medical specialists, and other health professionals (Table 7.3). The geneticist oversees the whole program and provides genetic counselling for each affected and at-risk family member in the program, when first identified and as necessary throughout screening. These subsequent counselling sessions have taken place when any change in the protocol was introduced, particularly when genetic testing became available, and to provide the results of genetic testing for those requesting it.

The geneticist also reviews all clinical test results with the appropriate specialists, records the data, maintains individual files, and identifies when recall appointments are due. Relevant literature has been reviewed, so that appropriate revisions to the protocol can be made. Educational material was also prepared for family members, family doctors and specialists.

TABLE 7.3. DUTIES OF PROGRAM PERSONNEL RELATED TO EACH OBJECTIVE OF THE VHL SCREENING PROGRAM

PERSONNEL	OBJECTIVES OF VHL SCREENING PROGRAM			
	1) Early identification of gene carriers	2. Early identification and treatment of individual tumours	3. Genetic counselling	4. Revision of program
GENETICIST/ PROGRAM COORDINATOR	i) identify families at risk and define problem ii) participate in multi-disciplinary group to develop screening protocol iii) monitor screening program and supervise secretary iv) record and file results	i) as for Objective 1 ii) as for Objective 1 iii) as for Objective 1 iv) as for Objective 1	i) provide genetic counselling initially ii) follow-up counselling as new information available iii) report results of genetic testing iv) prepare pamphlet for family members, and information for other physicians	i) review literature ii) review medical and psychosocial outcomes iii) present overview to multidisciplinary group
SECRETARY	i) make appointments as required (coordinate those for out-of-town families) ii) notify patients of appointments	i) as for Objective 1 ii) as for Objective 1	i) make appointments as required ii) notify patients	i) ———
MEDICAL SPECIALISTS	i) participate in multi-disciplinary group to develop screening protocol ii) patient examinations/ investigations according to screening protocol	i) as for Objective 1 ii) as for Objective 1 iii) Provide treatment	i) reinforce counselling in clinic appointments	i) send reports to coordinator ii) review literature iii) recommend new technology for investigations
OTHER HEALTH PROFESSIONALS				
a) Laboratory and x-ray personnel	i) carry out required investigations	i) as for Objective 1	i) ———	i) ———
b) Molecular genetics personnel	i) carry out genetic testing	i) ———	i) report results to geneticist	i) develop new testing procedures

The secretary books appointments that are due, in groups for those living out of town, and notifies the participants of appointment times. The medical specialists (including pediatricians, internists, ophthalmologists, and surgeons) see patients in clinic, provide treatment, make referrals to other specialists as necessary, and send reports to the coordinator. They also review relevant areas of the literature to recommend revisions to the protocol. Other health professionals such as laboratory and x-ray personnel conduct the investigations that are part of the screening protocol, as booked for family members.

ii) Participants

Affected and at-risk members of five VHL families in Newfoundland participate in the screening program. The exact number in each category varies as new clinical diagnoses are made, as genetic testing identifies gene carriers or non-carriers, or as family members have moved to or from the province. In 1982, 12 affected and 34 at-risk family members were identified and entered the program. The maximum number involved was 27 affected and 51 at risk, and currently (since six family members have moved, and genetic testing has identified 24 non-carriers), 25 affected and 23 at risk participate.

The overall compliance with clinic appointments has been 78% compared with 85% compliance for other patients booked in similar clinics. Certain patients (5/42) have been less reliable than the majority participating in the program. When questioned about non-compliance, they say that they are afraid of the disease, and the only way they can handle their fear is by denying it, and

avoiding investigations which might demonstrate an actual manifestation of VHL. Four others, now in their 40s or 50s, became less compliant after many years of normal screening results — they became confident that they did not have the VHL gene.

iii) Record keeping

A file is maintained for each participant with data on all investigations, follow-up appointments, and treatment. An overall summary is also maintained, with date and result of each appointment for each participant, to facilitate recall. A computerized database with automatic flagging of those due for appointments, or for whom results have not been received, would improve the management of files and data.

iv) Sensitivity and specificity of investigations

False positive or false negative results are rare. Four patients had renal, cerebellar or pancreatic lesions identified which on retrospect were evident on previous ultrasound or CT scan examinations, but which had not been reported at that time. These patients were still asymptomatic and had successful surgery when the diagnosis of renal cell carcinoma (n=2), cerebellar hemangioblastoma, or pancreatic islet cell tumour was made. Two patients had symptoms from spinal cord hemangioblastomas which were initially attributed to "mechanical" back problems, but were later correctly identified. These spinal cord tumours both occurred before MRI was available in Newfoundland for appropriate evaluation of such symptoms.

Seven patients had false positive results. Two of these patients had surgery for "query renal cell carcinoma" identified on ultrasound screening and follow-up CT scanning of the abdomen, but the pathology demonstrated renal cyst only. In VHL disease, many renal cysts have renal cell carcinoma cells lining the wall, and solid tumours have appeared cystic on scanning. As a result there is a high suspicion of any lesion identified. Two patients with increased catecholamines and three with increased blood pressure suggesting pheochromocytoma had normal CT and MIBG scanning, however, one of these later had a definite pheochromocytoma. Because of the previous morbidity and mortality from unidentified pheochromocytomas in this family, a false positive result is preferable to late investigation.

v) Genetic testing

In three Newfoundland families, predictive testing by linkage analysis was introduced on a research basis in 1992. Linkage results were received for 24 family members at risk (20 low-risk results, 3 high-risk results and 1 uninformative result). Regular clinical screening continued for those at high risk (or those for whom testing was uninformative) but was reduced in frequency for those at low risk.

The specific mutation has now been identified in each of these families. Mutation analysis of 28 members of Family A, at *a priori* 50% risk but with negative clinical screening results, has been completed and has demonstrated that the majority (24/28 or 85.7%) do not have the VHL mutation. This

includes all those over 15 years of age, and confirms the early age at onset of VHL and the efficiency with which the clinical screening has identified VHL gene carriers.

The implications of the genetic testing to overall cost of the screening program are discussed in the cost-benefit section of this evaluation.

vi) Side effects of screening

Four patients developed allergies to the regular ionic CT scan dye, one requiring admission to the Intensive Care Unit. Because of the severity of the reaction this patient no longer has scanning with contrast — therefore only less sensitive testing is possible. The other patients, with a milder reaction, now require non-ionic dye for any investigations. One patient developed a peripheral visual field defect after laser treatment for an enlarging angioma of the optic disc. The treatment was considered necessary because of the danger of loss of central vision if the disc angioma was not controlled.

Discussion

As is recommended in evaluation literature, the program process has been monitored since the beginning, and revisions have been made both in the activities of the program personnel and in the investigations of the screening protocol. The geneticist has always acted as program coordinator, and originally was responsible for all scheduling, notifying, recording and filing, but recently a secretary has become involved in these activities.

Appointment dates and results are recorded on a summary table which has to be reviewed manually to determine which participants are due for re-testing. A computer program has been recommended to facilitate recall of patients but has not yet been developed. With the number of patients, the number of investigations and the patient individualization necessary, this lack of computerized monitoring is a major handicap and results in excessive time spent in keeping track of the program. Another problem with the process concerns regular receipt of copies of test results by the program coordinator. Some come automatically but some do not. Missing reports must be searched for (which can be a time consuming process) in order to determine whether the appointment was missed or rescheduled, or the result not sent. A reminder to the many specialists involved in clinical management about the benefits of centralized coordination and monitoring of the screening program, should improve this situation.

The clinical components of the screening program have also been revised. Originally screening was directed towards identification of retinal angiomas, pheochromocytomas and cerebellar hemangioblastomas only, because in the Newfoundland families only one renal cell carcinoma (RCC) had been previously identified (at autopsy of a 55-year-old man); and because, in the literature, RCC was considered a late manifestation of VHL and all living affected patients in Newfoundland were under 40 years of age. The recommendations in the literature for screening for RCC were then revised in

the mid 1980s to include earlier screening. Baseline screening by ultrasound of the kidneys was introduced for affected individuals in Newfoundland and several patients as young as 19 years of age were found to have renal cell carcinoma. A renal ultrasound was then introduced annually from early teens for all affected and at-risk persons.

More recently, the radiologists (Dr. Peter Collingwood and Dr. Tom Cummings) have recommended replacing renal ultrasound by staged CT scanning of the kidney/adrenal region. This gives better resolution than ultrasound, and a new, more rapid CT scanner reduces radiation exposure required for the procedure. MR imaging of the head has replaced CT scanning for screening for cerebellar hemangioblastomas. MRI is the investigation of choice for central nervous system lesions because of better sensitivity, and also because radiation is not involved (Filling-Katz et al 1987, Sato et al 1988, Hoff et al 1993). (When dealing with an inherited precancerous condition, the carcinogenic potential of the procedure [eg, the cumulative radiation exposure from repeat CT scanning] should be considered as well as the sensitivity and specificity.)

Early in the screening program, there were some delays for CT scanning as a follow-up for primary investigations because there was only one CT scanner in Newfoundland, at the General Hospital in St. John's. Now that other hospitals have obtained CT scanners, significant delays rarely occur. For screening programs such as this, it is important to ensure that the follow-up

investigations and treatment are available in a timely fashion, otherwise early diagnosis will only decrease the quality of life by labelling the patient with a diagnosis with many negative consequences.

Even on a research basis, genetic testing has so far only been offered to those already participating in the clinical screening program, and these were all over 10 years of age when genetic testing became available. (Because of voluntary infertility, there are very few young children at risk.) Informed consent has been required. Because genetic testing was offered to those already involved in the screening program, the uptake of genetic testing was high in the three families where this testing was possible. All adults (n = 22) informed of the possibility of testing requested it; in their opinion, the potential for beneficial outcomes associated with low-risk or mutation-negative results (no longer requiring annual screening, the freedom to have children without the risk of passing on the VHL gene, and reduced anxiety) far outweighed the negative implications of high-risk or mutation-positive results. For them, continuing clinical screening, worrying about the onset of VHL disease, and forgoing children was the status quo. They recognized from the family's experience that even if they did inherit the VHL gene, clinical screening provided an opportunity for early and successful treatment for gene carriers.

Two of 12 sets of parents declined genetic testing for their children when it became available, although they had requested it beforehand. One mother did not feel she could cope emotionally with mutation-positive results,

and one couple did not want to treat their two children differently if the results were not the same. The children (n = 3) in both families continue with annual clinical screening.

This high uptake of predictive testing for VHL is very different from the situation with genetic testing for Huntington disease (HD) for which uptake is much lower (Wiggins et al 1992), and many at-risk persons feel that the "costs" (of increased anxiety, and potential discrimination) outweigh the benefits (Codori and Brandt 1994a), particularly because HD is untreatable.

COST-BENEFIT ANALYSIS (How does the cost of management of VHL patients using a screening program, with or without genetic testing, compare with the cost of management of VHL patients without screening?)

Introduction

Once the efficacy and effectiveness of a program have been established (Sackett 1980, Robinson 1993a), it is necessary to conduct an economic evaluation to determine whether the program is worth doing, (Stoddart and Drummond 1984a). Previously, health care budgets expanded to cover the costs of new health programs, but these budgets can no longer cover the costs of a multitude of new procedures, treatments, and programs each of which may have some value to some patients (Stoddart and Drummond 1984a). Expenditures must therefore be rationalized (Williams 1974, Robinson 1993a), ideally by using a cost-benefit (Robinson 1993e) or cost-utility analysis

(Robinson 1993d) — comparing the cost of the program in monetary terms with the benefits in non-monetary terms (since it is very difficult to put a dollar value on a human life [Thompson 1987], or by a cost-effectiveness analysis (Robinson 1993c) — comparing the cost to society of caring for VHL patients by treating symptomatic disease, with the cost of implementing a screening program and treating presymptomatic disease.

Health care evaluation is a complex process in which there is considerable disagreement among the experts. The following section therefore presents a brief review of the various approaches, a demonstration of how one of them can be applied to the present program, some of the data needed for such an analysis, and some tentative conclusions.

As discussed in the thesis introduction, cost-minimization analysis is used when the outcomes of programs to be compared are the same, and the goal is to choose the least expensive option. Cost-effectiveness analysis is used when the outcomes of the programs are similar, but differ at least in degree. Neither of these methods is suitable for economic evaluation of a program as complex as the VHL screening program described, with individualized screening protocols, and variable outcomes.

In cost-utility analysis, the outcomes vary and are converted into quality of life units for comparison, the most common unit being the QALY. However QALYs have proved difficult to measure. In cost-benefit analysis, by definition, all costs and benefits are converted into monetary units. However, many cost-

benefit studies have been published in which non-monetary units are used for some costs or benefits, particularly relating to length and quality of life.

Several papers (Hagard and Carter 1976, Chapple et al 1987, Henderson 1991) describe cost-benefit analyses of programs for prenatal diagnosis of severe genetic disorders (eg, Down syndrome, neural tube defect, or Duchenne muscular dystrophy). The authors estimate the costs of these programs as the costs of genetic counselling, and of the diagnostic procedures to allow prenatal diagnosis and therapeutic abortion of an affected fetus. They estimate the benefits of screening as the costs not incurred for treatment, specialized education, and extra family expenses for the relevant disorders because the births are prevented. The assumption is that each affected fetus will be aborted, so that either there are costs for treatment and management (status-quo situation), or there are costs for screening (screening program).

Modell and Kuliev (1991 and 1993) disagree with this approach since not all screening programs for hereditary diseases result in such a clear-cut "either/or" situation, particularly if the disease is of "late-onset", or if treatment is at least partially successful.

The model for this VHL economic evaluation is Modell and Kuliev's evaluation of programs for thalassemia management when different levels of education and screening have been introduced (Modell and Kuliev 1993). These authors stress that prevention and treatment are not necessarily alternatives, but can be complementary objectives of the same program in

which the goal is to obtain a healthy family. For the VHL evaluation, the comparison is between a) the status quo situation with no screening and patients treated when symptomatic (Program 1), and b) management and treatment after screening is introduced (Program 2 including clinical screening only, and Program 3 including genetic and clinical screening).

VHL patients will require medical care, whether or not there is a screening program, because the VHL gene predisposes to tumour development in multiple parts of the body. Without screening (Program 1), patients will present for treatment when symptoms develop, as has been documented for members of the large Newfoundland family prior to 1982. Some of these will have treatable disease, and others will have advanced disease which results in death or disability. The monetary cost of the no-screening situation, therefore, is the cost of treatment of symptomatic patients, and the costs incurred by families because of death or disability from untreatable complications of VHL.

When a screening program is implemented, the cost of management and treatment of VHL family members includes the direct and indirect costs (Stoddart and Drummond 1984b, Robinson 1993b) of the program, and the cost of treating patients identified by screening or any who present symptomatically. From the review of medical outcomes of screened and unscreened cohorts (Chapter 6), it is expected that screened patients will have treatment that is more frequent, but less complex and more successful. These patients have been able to return to work following the postoperative recovery

period, in contrast to the many patients presenting symptomatically who could not work subsequent to treatment.

In the first stage of the screening program (Program 2), clinical screening was offered to all affected and at-risk family members (determined by the position in the pedigree). Annual screening was recommended to continue until at least 50 years of age, even for those with negative results, because of the range of ages of onset of VHL in the literature. The cost of screening therefore is the cost of annual screening for all who are affected and all first degree relatives, including those who in fact do not carry the VHL gene. The direct hospital costs and the cost of administration of the screening program, as well as the out-of-pocket expenses for family members, must be summed for this group.

In the second stage of the screening program (Program 3), genetic testing is offered to first degree relatives to distinguish gene carriers and non-carriers. Then clinical screening is only required by those already affected or shown to carry the VHL gene. The cost of genetic testing is added to other costs, but the cost of clinical screening is much reduced because non-carriers are no longer screened.

There are also indirect psychosocial costs for each of these three programs: in the status quo situation, this is the extreme anxiety of affected and unaffected family members because of the severe disease, the frequent early deaths, and the poor understanding of the cause or means of control of

VHL. As has been documented through interviews, anxiety about VHL disease is reduced for most family members by the educational and counselling component of the screening program. However, repeated examinations for a potentially lethal condition may induce stress, and for some individuals, "labelling" (with the high-risk or presymptomatic diagnosis of VHL disease) may create a high anxiety state (Sackett et al 1991).

Balancing these costs are the medical and social benefits of screening, including life-years gained (Robinson 1993c) and days of disability averted (particularly blindness, neurological deficit, and symptomatic cancer). There is also decreased anxiety for many family members when they have confidence that the medical system recognizes their risks, and actively searches out and treats problems which may exist.

The financial benefits of screening are that treatment of early disease is frequently less costly than treatment of late disease (Shortell and Richardson 1978), and welfare or social services costs to dependents will be decreased if early deaths or disability are prevented.

With multisystem diseases like VHL, however, it is important to recognize that identifying and successfully treating one manifestation does not result in a cure. VHL is a lifetime disease and consequently has a continued risk for subsequent manifestations which will require appropriate treatment.

Method

The components of the alternative programs for management and treatment of VHL family members were identified, as were the costs and benefits of each of these programs, whether monetary or non-monetary. The societal viewpoint (Robinson 1993a, Phatak et al 1994) was used so that costs and benefits to the patients, to other family members, and to the medical care system were all included (Table 7.4).

The direct costs of Program 1 are the costs of treatment of symptomatic disease, including investigations, hospitalization and surgical procedures. For comparison, the present-day (1994) cost was calculated for the medical care documented in patient charts. For investigations and treatment prior to 1981, there is no record of family members' out-of-pocket expenses. The indirect costs of Program 1 are the costs of disability, and premature death from VHL, and the extreme anxiety in affected and at-risk family members.

The direct costs of Program 2 are the costs of clinical screening, including the cost of recommended investigations for affected and at-risk family members, the cost of administration of the screening program (the proportion of salary of the geneticist and secretary attributable to the time commitment to the program), the cost of follow-up investigations and treatment of affected individuals, and the out-of-pocket expenses to family members participating in screening and treatment. The cost of screening for at-risk family members was calculated from the number of participants and the cost of the set of annual

TABLE 7.4. IDENTIFICATION OF COSTS AND BENEFITS OF PROGRAMS FOR MANAGEMENT OF VHL DISEASE WITH OR WITHOUT CLINICAL AND GENETIC SCREENING

	PROGRAM 1	PROGRAM 2	PROGRAM 3
	"Pre-screening"	"Implementation of clinical screening"	"Addition of molecular genetic testing"
PROGRAM	- no screening or counselling - treatment of late (symptomatic) disease	- education and counselling - clinical screening for all affected and at risk - treatment of early (presymptomatic) disease	- education and counselling - genetic testing - clinical screening for gene carriers - treatment of early disease

COSTS	- delayed treatment
	- early deaths
	- disabilities
	- social service/welfare costs
	- severe anxiety for whole family
		- geneticist's salary (re: education, counselling and coordination of program)
		- secretary's salary (re: administration of program)
		- clinical screening (for affected and all at 50% risk)
		  - health care costs (set of appointments)
		  - out-of-pocket expenses for family (travel and accommodation)
		  - anxiety at time of screening
		- early treatment
		- reduced family size (for affected and at risk family members)
			- geneticist's salary (same as Program 2)
			- secretary's salary (less than Program 2)
			- clinical screening for gene carriers only (less than Program 2)
			- early treatment (same as Program 2)

BENEFITS	- some successful treatment	- information - early diagnosis and treatment - reduced morbidity and mortality - much reduced anxiety	- information - reassurance for non-carriers - knowledge for reproductive planning - early diagnosis and treatment - reduced morbidity and mortality - even greater reduction in anxiety

investigations recommended in the screening protocol. The cost of screening and treatment for affected family members was determined directly by calculating the cost of all appointments, investigations and treatments recorded in the medical records of these patients. This approach was taken because of the variability of the disease; the treatment required is different for each patient and even the screening protocol varies depending on the previous manifestations of the disease. Therefore an average cost of screening and medical care for members of this group was not known. Out-of-pocket expenses for all family members (particularly travel expenses) were estimated from the number of trips to the tertiary care centre required by those living out of town.

The direct costs of Program 3 differ from that for Program 2 by a) the addition of costs of predictive testing (by mutation detection or linkage analysis as appropriate) for at-risk family members requesting this, and b) the subtraction of annual screening costs and out-of-pocket expenses for those found not to carry the VHL gene.

The costs of medical appointments, investigations and treatments (including hospitalization and surgery) were obtained from the fee schedules of the radiology department, the medical laboratories, and the provincial medical care plan. If the fees quoted did not already include factors for overhead, and for maintenance and depreciation of major equipment (such as for CT scan, MRI

and ultrasound), appropriate amounts were added to the base fees (Stoddart and Drummond 1984b).

The indirect costs of the programs were assessed by interviewing key participants about the psychosocial costs and benefits of the clinical and genetic components of screening, as described in the previous chapter.

Results

The costs of the three programs are summarized in Table 7.5, and the costs estimated for the three comparison groups in Program 1 (the 1962-71 cohort from Newfoundland, the 1972-81 cohort from Newfoundland and Ontario, and the 1982-91 cohort from other provinces) are presented in more detail in Tables 7.6a,7.6b, and 7.6c, respectively.

For Program 2, monetary costs for screening and treatment of affected and at-risk family members were calculated directly, adding costs of all investigations and procedures required during 1982-1991, a proportion of the geneticist's and secretary's salaries for administration of the program, and out-of-pocket expenses of family members participating. There is no cost included for disability or death; none of the affected members of this group are disabled by the disease (all have been able to return to work following usual postoperative recuperation whenever surgery was required). Although there was one death and three instances of unilateral blindness in the group, these

TABLE 7.5. COMPARISON OF COSTS AND BENEFITS OF PROGRAMS FOR MANAGEMENT OF VHL DISEASE

IDENTIFIED COSTS	PROGRAM 1 (PRE-SCREENING)			PROGRAM 2 (CLINICAL SCREENING)		PROGRAM 3 (GENETIC AND CLINICAL SCREENING)	
DIRECT							
1. For counselling and administration of screening program							
a) % of geneticist's salary		0		(20%)	90,000	(20%)	90,000
b) % of secretary's salary		0		(25%)	53,000	(15%)	33,000
2. For genetic testing		0			0	($350/pers.)	10,850
3. For clinical screening							
a) For affected							
i) set of appointments		0			123,500		123,500
ii) out-of-pocket expenses		0			17,000		17,000
b) For at risk							
i) set of appointments		0		($288/pers.)	89,280	($288/pers.)	20,160
ii) out-of-pocket expenses		0			4,000		1,000
4. For treatment	a) 393,060*	b) 588,860*	c) 334,830*		483,000		483,000
5. For disabilities	a) 192,500**	b) 150,000**	c) 300,000**	(All patients working)		(All patients working)	
TOTAL	a) 585,560	b) 738,860	c) 634,830		859,780		778,510
INDIRECT							
1. Deaths							
Number of deaths	a) 9	b) 1	c) 1	-		-	
Age at death [range (mean)] (yrs)	21-55 (R, 37)	18	49				
Dependent children [# (mean age)]	29 (R, 9.5)	1 (5 mos.)	-				
2. Disabilities							
Disability preventing normal employment							
- Number	a) 8	b) 1	c) 2	(All working)		(All working)	
- Age at disability [range (mean)] (yrs)	19-54 (R, 33)	21	27, 34				
- Dependent children [# (age)]	28 (R, 9.5)	-	4 (R, 10)				
Unilateral blindness [# (mean age)]	8 (R, 28)	-	1 (5)				
				-		-	
3. Anxiety		+ + + +		+ +		+	
4. Reduced family size		+		+ +		+	
NUMBER OF PARTICIPANTS							
- Affected	a) 11	b) 15	c) 13	19		19	
- At risk	a) NA	b) NA	c) NA	31		7	
- Mean age of gene-carriers	a) R, 23.7; SD, 11.49	b) R, 23.4; SD, 9.43	c) R, 29; SD, 9.42	R, 31.5, SD, 9.87		R, 31.5; SD, 9.87	

* An underestimate of medical costs since records are not complete.

** Based on legal or insurance awards for lost wages due to disability of similar-age person (prorated for 10 years).

a) Newfoundland 1962-71 (see Table 7.6a)
b) Nfld./Ont. 1972-81 (see Table 7.6b)
c) Ont./N.B. 1982-91 (see Table 7.6c)

(NA - not applicable)
(pers. - person)

References: Goldsmith's damages for personal injury and death in Canada

TABLE 7.6a. COSTS OF MANAGEMENT OF VHL, 1962-71

PATIENT	MEDICAL COSTS		"COST" OF DISABILITY			"COST" OF EARLY DEATH	
	Hospitalization/Treatment ($)		Blindness (age)	Neurological (age)	Other (age)	Age	No. Dependent Children (age [x̄; range])
1	unknown		bil. (unk.)	+ (unk.)	-	49	-
2	69,160	3,700	unil. (30)	+ (54)	-	55	2 (x̄, 16; 15, 17)
3	9,880	500	unil. (47)	+ (48)	-	50	3 (x̄, 14; 11-18)
4	66,120	2,400	-	-	+ (34)***	40	1 (9)
5	45,600	3,000	unil. (26)	+ (36)	-	39	10 (x̄, 9; 1-17)
6	-	-	unil. (26)	-	-	29	1 (10)
7	19,320	1,200	unil. (17)	+ (19)		21	2 (x̄, 1.5; 1, 2)
8	29,640	2,200	unil. (22)	+ (34)		36	7 (x̄, 9; 1-17)
9	129,200	500	-	-	+ (29)***	31	3 (x̄, 5; 3-7)
10	10,640	500	unil. (28)	-	-	-	-
11	-	-	unil. (26)	-	-	-	-
SUBTOTAL	379,360	13,700	9 (x̄, 27.8; SD, 8.73)	6 (x̄, 38.2; SD 13.57)	2 (x̄, 31.5)	9 (x̄, 38.9; SD, 11.04)	29 (x̄, 9.5; 1-18)
TOTAL	393,060*			192,500**			

N.B. Costs for 11 affected family members (present day cost of treatment and hospitalization documented).
* Underestimate since records incomplete.
** Based on legal awards for loss of future wages for similar disability (prorated for 10 years).
*** Illness related to unrecognized pheochromocytoma.

TABLE 7.6b. COSTS OF MANAGEMENT OF VHL, 1972-81

PATIENT	MEDICAL COSTS		"COST" OF DISABILITY		"COST" OF EARLY DEATH	
	Hospitalization/Treatment ($)		Blindness (age)	Neurological (age)	Age	No. Dependent Children (age)
1	-	-	(unil, prev.)	-	-	-
2	-	-	(unil, prev.)	-	-	-
3	72,200	1,300	-	-	-	-
4	22,800	1,000	-	-	-	-
5	15,960	2,000	-	-	-	-
6	13,500	1,000	-	-	-	-
7	15,960	1,500	-	-	-	-
8	11,400	1,000	-	-	-	-
9	1,000	500	-	-	-	-
10	23,860	1,500	bil (21)	-	-	-
11	53,200	1,200	-	-	-	-
12	15,960	1,200	-	-	-	-
13	290,000	10,000	-	+(18)	18	1 (5 mos.)
14	23,800	1,200	-	-	-	-
15	5,320	500	-	-	-	-
SUBTOTAL	564,960	23,900	1 (+2 prev.)	1	1	1 (5 mos.)
TOTAL	588,860*		150,000**			

N.B. Costs for 15 affected family members (present day cost of treatment and hospitalization documented).
* Underestimate since records incomplete.
** Based on legal awards for loss of future wages for similar disability (prorated for 10 years).

unil - unilateral, bil - bilateral, prev - previously

TABLE 7.6c. COSTS OF MANAGEMENT OF VHL IN OTHER PROVINCES, 1982-91

PATIENT	MEDICAL COSTS		"COST" OF DISABILITY			"COST" OF EARLY DEATH	
	Hospitalization/Treatment ($)		Blindness (age)	Neurological (age)	No. Dependent Children	Age	No. Dependent Children (age)
1	54,720	5,000	-	-	-	49	-
2	25,840	4,500	-	-	-	-	-
3	41,040	7,000	-	+ (34)	4 (x̄, 10)	-	-
4	15,960	2,000	unil. (5)	-	-	-	-
5	-	1,000	-	-	-	-	-
6	15,960	4,500	-	-	-	-	-
7	-	3,000	-	-	-	-	-
8	6,840	1,000	-	-	-	-	-
9	5,000	500	-	-	-	-	-
10	115,410	6,000	-	+ (27)	-	-	-
11	4,560	1,000	-	-	-	-	-
12	-	3,000	(unil, prev)	-	-	-	-
13	10,000	1,000	-	-	-	-	-
SUBTOTAL	295,330	39,500	1 (+1 prev.)	2	4	1	
TOTAL	334,830*		300,000**				

N.B. Costs for 13 affected family members (present day cost of treatment and hospitalization documented).
* Underestimate since records incomplete.
** Based on legal awards for loss of future wages for similar disability (prorated for 10 years).

unil - unilateral, prev - previously

were related to delayed investigation and treatment prior to 1982 so were not included in the cost of Program 2.

The costs of Program 3 are the predicted costs of management of the same group of affected and at-risk family members as in Program 2, assuming that genetic testing had been possible in 1982 as the first step of the screening program. All of the investigations and treatment of affected members, the four gene carriers identified by predictive testing, and the three children for whom genetic testing was declined would have been necessary for Program 3, as they were for Program 2. The cost of molecular genetic testing for all at risk is added to the cost of Program 2, and the cost of clinical screening for those who were later found to be mutation-negative is subtracted. Thus the monetary costs of Program 3 are less than for Program 2, and, from the interviews done previously, the psychosocial costs (particularly from anxiety and lack of reproductive choice) are also less for Program 3 than for Program 2.

The cost of management of VHL with a screening program was then compared with the cost of management of VHL after symptomatic presentation (Program 1). Costs of investigation and treatment, and costs relating to the disabilities and early deaths caused by late treatment, were identified for 3 comparison groups: a) the cohort in Newfoundland when VHL disease was first recognized (1962-71) (summarized in Table 7.6a), b) the cohort of 1972-81 in Newfoundland and Ontario (summarized in Table 7.6b), and c) the cohort of

1982-91 in Ontario and New Brunswick (summarized in Table 7.6c). There was no screening during 1962-71, and only minimal screening during 1972-81, or during 1982-91 for family members in other provinces.

For these groups, medical records were reviewed to identify the type and number of hospitalizations, investigations and procedures that were carried out. For comparison of costs, the value used was the present day (1994) cost of similar medical care in Newfoundland. Some medical records were incomplete, and out-of-pocket expenses are unknown; therefore monetary costs for these comparison groups are underestimated.

Early deaths and disabilities related to delayed diagnosis and treatment of VHL manifestations (particularly bilateral blindness, and neurological or other medical disabilities preventing normal employment) were then identified for each group. Because disability pensions are typically related to previous wages (which were unknown), disability pensions could not be calculated. In order to provide some estimate of the financial implications of these deaths and disabilities, values were obtained for legal awards or insurance payments for "loss of future wages" because of death, or similar disabilities of individuals of comparable age but from the general public. The number and ages of children left by death of a parent were also documented. Although the identified monetary costs within the health care system are greater for screening and management of VHL (Programs 2 and 3), the costs in loss of life and reduced

quality of life when patients present symptomatically, far outweigh any apparent financial savings from not screening.

Finally, the mean age of gene carriers was calculated for the experimental group and the two control groups in Newfoundland. For the experimental group, this includes all those identified by symptomatic presentation, by screening, or by direct mutation detection. In order to have complete identification of gene carriers in the earlier groups, all those with a diagnosis of VHL at the time, and those who subsequently developed VHL were included. The mean age of gene carriers has increased from 23.7 and 23.4 years in the earlier decades to 31.5 years in 1992.

Discussion

This cost-benefit study considers the overall costs and benefits of screening and treating members of families with von Hippel-Lindau disease, and compares this with the costs and benefits of treating these same individuals if no screening program is in place (Program 1). Additionally, the costs and benefits of the screening and treatment program are considered either as originally implemented with clinical screening only (Program 2), or as recently possible, with a combination of genetic and clinical screening (Program 3).

This approach is taken because those who have inherited the VHL gene will require medical care whether or not any form of screening takes place. The goal of the health care system is to maximize the health of the population

(Williams 1974, Robinson 1993a); more specifically, to prevent disease when possible, and to improve the health of individuals who are ill (including patients with VHL). Because resources are not limitless, it is desirable to control health care expenses while pursuing this goal (Sackett 1980).

All costs and benefits of the three VHL programs were identified (Table 7.5). There has been no attempt, however, to convert all costs and benefits into monetary units because of the recognized difficulty in valuing human life or human disability (Modell and Kuliev 1991, Robinson 1993d, Robinson 1993e). Monetary costs, including direct and indirect costs to the patients, their immediate families and the medical care system, for screening tests, follow-up investigations and treatment of manifestations of VHL, were calculated and compared. The ascertainment of costs for treatment of members of Program 1 is less complete than for the screened group, because of historical or geographic reasons for difficulties in obtaining complete data on days in hospital, number of outpatient appointments, and out-of-pocket expenses. Nevertheless, if costs of death and disability are included, monetary costs are greater in Program 1 than for Programs 2 or 3, in which screening has been implemented.

Improved medical and psychosocial outcomes were demonstrated for participants of the screening programs and members of their families, before the cost-benefit analysis was undertaken. There was no attempt to convert these costs and benefits into dollar values, so the cost to society for the VHL

screening and treatment program cannot be compared to the cost to society for some other "program" within or outside the health care sector. However, if these individuals are to be treated, then this is the most cost-beneficial way to do it.

Most economic analyses of genetic screening programs compare the cost of screening the population at risk with the cost of treatment of affected individuals avoided, as an either/or situation (Hagard and Carter 1976, Henderson 1991), rather than comparing the cost of treatment of affected individuals with or without previous screening. Cost-benefit analyses of screening for thalassemia (Modell and Kuliev 1991), and for hemochromatosis (Phatak et al 1994) are the exceptions.

The main reason for the different approaches are the characteristics of the genetic diseases being screened for. If affected individuals will have a lethal disease, or major mental or physical handicaps despite the best treatment, and particularly if the condition is congenital or has its onset in early childhood, the reproductive choice taken is often the termination of pregnancy involving an affected fetus (Chapple et al 1987). Therefore the goal for many individuals being screened is to prevent the birth of affected children, and the economic analysis of the screening program reflects this point of view.

Screening for "late-onset" genetic disease (often called adult-onset genetic disease, although this term is not always appropriate), and particularly screening for treatable late-onset genetic disease has different objectives, and

therefore the evaluation must be different. Gene carriers are unaffected at birth and for varying periods of time before one or more medical problems develop. Frequently these medical problems (whether cardiovascular disease as in Marfan syndrome, or a tumour as in one of the hereditary cancers) can be treated successfully, particularly if identified early, and the "affected individual" can lead a productive life following treatment.

For some late-onset diseases such as the hereditary tumour syndromes, including VHL, disease mutations predispose to multiple tumours, and after successful treatment of one manifestation of disease, other tumours may occur. Still, between the occurrence and treatment of different tumours, the affected individuals may have no symptoms and function normally, particularly if the tumours are identified and treated early. Therefore the main goal of the screening program is to prevent death or disability from the disease, not to prevent birth of affected individuals.

For late-onset diseases, it is rare for those at risk of having affected children to consider prenatal diagnosis and therapeutic abortion as an option. This is documented for autosomal dominant polycystic kidney disease (Hodgkinson et al 1990, Gabow 1993) and for spinocerebellar ataxia (Nance et al 1994), as well as for the VHL families, and families with other hereditary tumours described in this thesis. For the parents involved, it is difficult to accept elective termination of a pregnancy that would result in a normal birth, despite the potential for severe disease in the future. Parents in the VHL

families studied, however, consider VHL to be so severe that many have chosen to have no children rather than risk transmitting the VHL gene.

OVERALL DISCUSSION OF HEALTH CARE EVALUATION

Health care evaluation provides a formal review of the need for, and objectives, outcomes and costs of a health care program, to determine whether it is appropriate to continue the program in its present or another form.

Needs assessment considers the reason for a program. A health care program may have been appropriate previously, but no longer be necessary if the risk factors, or the disease in question, have been significantly reduced or eradicated. A continued need for a VHL screening program in Newfoundland is indicated by the number of affected or at-risk individuals in the province, the severity and complexity of VHL disease, and the medical and reproductive problems resulting from late diagnosis and treatment.

Summative evaluation relates the objectives of a program to its outcomes. Affected individuals identified by the clinical screening protocol utilized in Newfoundland have an earlier age at onset (mean age of 16 years) than those identified by symptomatic disease (mean age of 28 years). Clinical screening allows the majority of affected family members to know that they have inherited the VHL gene in time to make informed reproductive decisions. Identification of those who <u>do not</u> carry the VHL gene, by clinical screening, is not as exact, however, since those with no disease manifestation may be non-

carriers, or carriers with later onset disease. This is an important distinction for a disease so severe that in the past family members at 50% risk chose to have no children rather than risk passing on the VHL gene. The recent identification of the mutation in three of the Newfoundland VHL families means that all gene carriers and non-carriers can be identified prior to the reproductive period, if each family member wants this information. This is of particular benefit to those identified as non-carriers; they no longer require clinical screening, and can have children without fear that they will transmit the VHL gene.

There have been fewer deaths, and less disability (such as blindness or neurological handicap) in family members who have been clinically screened than in those presenting symptomatically. Thus, early diagnosis and treatment appears to improve the prognosis of VHL, but longer follow-up is necessary to confirm this. VHL is, however, an unrelenting disease, and those who have been successfully treated face the prospect of other tumours developing in the future. Individual patients in the screened group therefore have more tumours than those who presented symptomatically and often died at a young age. An increase in the mean age of affected individuals in the screened versus unscreened groups provides evidence that patients who have been managed by screening are living longer.

Based on past and present observations and recent interviews, family members participating in the screening program have a better understanding of the clinical and genetic features of VHL, and have less anxiety about the

disease than family members before the screening program began. Relatives in other provinces do not have the same regular screening as those in Newfoundland, but many have benefited indirectly, particularly in their understanding of VHL and their awareness of possible symptoms, by obtaining copies of pamphlets and screening protocols from family members in Newfoundland. From the point of view of the analysis, this is "contamination" of the control group (Sackett 1980), but this dissemination of knowledge meets the broader objectives of the VHL program, and is both impossible and unethical to prevent.

Formative evaluation assesses the components of the screening program; the personnel, the participants, and the procedures. The investigations making up the VHL screening program have been updated regularly. Participants can therefore benefit from new technology, and from new information about the natural history and the molecular genetics of VHL. Although program objectives are being met, improvements are possible in the process by which the screening protocol is managed. Specifically, it would be more efficient to have a computerized system of storage and recall of patient data, particularly of the date and results of recent screening or interventions, so that subsequent appointments can be scheduled more efficiently. There are similar screening programs for other hereditary cancers in Newfoundland, and a computer-based registry (Littler and Harper 1989, Read 1990) for management of this group of genetic diseases would be invaluable.

Finally, cost-benefit analysis compares the relative costs and benefits of managing VHL disease with or without screening. VHL is expensive to treat and control, and is associated with high psychosocial costs for affected and unaffected family members. Screening reduces monetary and non-monetary costs to the health care system and to family members, through identification of non-carriers who do not require screening, and through earlier identification and treatment of tumours in those with VHL mutations. Family members are using the information provided in the educational and counselling components of the program to voluntarily reduce the number of affected individuals in future generations.

CHAPTER 8 — SUMMARY AND CONCLUSIONS

INTRODUCTION

DEVELOPMENT OF A SCREENING PROGRAM

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ii) Type of genetic testing
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RELEVANCE TO OTHER CENTRES AND TO OTHER DISEASES

i) Relevance to other centres
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THE VALUE OF REGISTERS

CONCERNS ABOUT GENETIC TESTING IN CHILDHOOD FOR ADULT-ONSET DISEASES

THE VALUE OF CLINICAL AND GENETIC SCREENING FOR HEREDITARY TUMOUR SYNDROMES

i) Familial adenomatous polyposis
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INTRODUCTION

Screening for genetic disease has advanced since the first programs for early detection and treatment of phenylketonuria were introduced in the 1960s to prevent severe mental retardation. Although the burden of many genetic diseases was well recognized, screening for these disorders was originally limited because few could be identified presymptomatically (by biochemical or physiological testing), and even fewer could be treated successfully. When no treatment was available, other objectives of screening programs were substituted, including a) provision of counselling regarding reproductive options, to prevent, or prepare for, the possible birth of an affected child, and, later, b) reduction of uncertainty when faced with the possibility of future development of a severe untreatable disease.

An ever-increasing number of genetic diseases can now be identified presymptomatically, using molecular genetic technology to detect specific mutations, or markers linked to disease loci. As mapping and cloning of disease genes accelerates, the number of potential genetic tests has increased rapidly. Availability of tests is no longer a limiting factor for development of screening programs; rather, the danger is that screening may be offered "because a test is available" without prior evaluation to establish that screening is in the best interests of those affected or at risk.

The rationale for screening, as set out by Wilson and Jungner (1968) (but adapted to suit the broader objectives of genetic screening), still provides

useful guidelines for the development of a screening program. According to this rationale, screening is appropriate when:

- there is a recognized burden of disease
- those at risk can be identified
- the natural history of disease is known (or the gene, or map location has been identified)
- a clinical test is available to detect presymptomatic disease (or a genetic test is available to identify gene carriers)
- treatment is available (or reproductive options and counselling are available, or uncertainty can be reduced)
- early treatment (or counselling) improves the prognosis and/or the quality of life

The successes and failures of previous screening programs provide valuable lessons for those embarking on a new program. A screening test must be part of a complete screening program, ideally, planned with the population to be screened, and including education and counselling, informed consent, and confidentiality of testing and reporting for all participants; and availability of follow-up investigations and treatment, or provision of reproductive options, for those testing positive.

As for other health care programs, screening programs for hereditary disease must be evaluated, to assess the medical and psychosocial outcomes,

and to determine the costs and benefits so that best use can be made of health care dollars.

This thesis considered the appropriateness of screening for the hereditary tumour syndromes (variable AD diseases predisposing to benign or malignant tumours), using a combined genetic and clinical screening protocol to identify gene carriers and provide reproductive counselling, and to detect and treat tumours early in order to improve the prognosis. This group of disorders was first distinguished from other genetic diseases for which screening programs had already been developed. Then the need for screening, the objectives, the choice of investigations or tests, and the medical and psychosocial outcomes of screening for each screening program were discussed, with a more formal evaluation, including a cost-benefit analysis, of the longest running screening program (that for von Hippel-Lindau disease).

Capitalizing on the unique opportunity offered by the structure and distribution of the Newfoundland population, the author had identified large Newfoundland families with von Hippel-Lindau disease (VHL), multiple endocrine neoplasia type 1 (MEN-1) and type 2 (MEN-2), familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC). Extended pedigrees were constructed, previous morbidity and mortality documented, and relevant literature reviewed. A burden of disease was established for each hereditary tumour syndrome (ie, previous early deaths and disabilities of affected individuals, reduced family size, and anxiety in many affected and

unaffected members), and the "population" at risk determined by the autosomal dominant inheritance pattern.

Information on the natural history of each disease (the type and frequency of tumours, and their age and order of occurrence) was gathered from the family medical records and the relevant literature. For three of the "hereditary cancers" studied (VHL, MEN-1, FAP), the initial data from the family records suggested an atypical disease presentation within the Newfoundland kindreds. For each disease, specialists met to determine the appropriate clinical investigations for presymptomatic detection of tumours. At the outset, less genetic information was available; none of the relevant genes was cloned, although genetic mapping strategies were in progress elsewhere, in several cases involving Newfoundland families. Detection of gene carriers was therefore originally provided by diagnosis of clinical disease. Linked markers or specific mutations have now been identified for predictive testing or presymptomatic diagnosis of gene carriers for each hereditary tumour syndrome.

Educational materials were prepared, counselling was provided, and screening was offered to family members, first for the presymptomatic detection of individual tumours, and later for the identification of gene carriers. Follow-up investigations and treatment were provided for those with positive clinical screening, and counselling regarding reproductive options was given to those identified as gene carriers by clinical or genetic testing.

Records have been maintained throughout the development and implementation of the screening programs, to document the identification and treatment of tumours, and the reproductive decisions made. A full health care evaluation has now been completed for the VHL screening program (the first screening program initiated, and therefore the one with the most data available). An improved prognosis (reduced morbidity and mortality), and better quality of life (reduced anxiety, and informed reproductive decisions) are demonstrated. The other programs have been less formally reviewed, with similar results. For VHL, the overall cost to society of screening and management of presymptomatic disease is less than the combined costs of treatment of symptomatic disease, and the costs related to early deaths and disabilities.

Screening programs combining clinical and genetic testing methods to identify gene carriers and provide reproductive counselling, and to identify and treat presymptomatic tumours are therefore considered appropriate for hereditary tumour syndromes.

As a group, these hereditary cancers differ from untreatable adult-onset disorders (eg, Huntington disease and other neurodegenerative diseases) because the harmful effects of the disease genes can be mitigated. As a result, there is greater participation in these screening programs than in predictive testing programs for HD. Many of the psychosocial issues related to prediction of future rather than imminent disease, however, apply, so that the need for

counselling and support are similar for the hereditary cancers as for HD. Despite many similarities, there are important differences within this group of hereditary cancers (the age at onset, the severity of disease, the spectrum of disease expression, the potential for treatment, and the state of genetic knowledge) that have influenced the development of the screening programs.

Because the information may be helpful in the future development of similar programs, I will summarize the ways in which these factors affected the structure of specific programs, and influenced the attitudes of family members towards participation in the clinical and genetic components of screening. I will then consider the relevance of the Newfoundland experience to other clinical settings, and review the value of registers in managing screening programs. I will also address the issue of genetic testing in childhood, and finally will restate the value of clinical and genetic screening programs for management of hereditary tumour syndromes.

DEVELOPMENT OF A SCREENING PROGRAM

i) Type and timing of clinical investigations

For each screening program, the type and frequency of clinical investigations recommended depends on the tumours that occur, and the age range over which they have been identified. This is initially defined by the literature, but revised as necessary according to family experience. For hereditary tumour syndromes in general, screening is recommended to start five

years before the earliest symptomatic presentation in order to identify presymptomatic disease (Fitzgibbons et al 1987). In the following section, I will describe how the screening programs have been modified as the unique characteristics of the Newfoundland families were defined.

For FAP, the investigation recommended in the literature (annual sigmoidoscopy starting in late childhood or early teens) is based on the characteristic location of the premalignant stage (the multiple polyps) in the sigmoid colon and rectum, and the typical age at onset in the teens or twenties. In the Newfoundland FAP family, by contrast, the number and location of polyps is variable, with one third of patients (including some with cancer) having few if any polyps within view of the sigmoidoscope. The age at onset of polyps is also variable, from the teens until at least the forties. The recommended screening test is therefore a full colonoscopy, with screening continuing until a later age than for typical FAP for those whose genetic risk is not defined. Similar families have been documented elsewhere, and a relationship between this clinical phenotype and the location of the mutations within the APC gene has been established for most of these families. The APC mutation for the Newfoundland family, however, has not yet been identified. Thus there is evidence of clinical heterogeneity, and as a result different screening protocols are appropriate for different families — with a large family this should be obvious, but with a small family this distinction may not be

apparent. Identifying the mutation will then be important to define the expected phenotype, and plan clinical management.

The early data on tumour frequencies in the Newfoundland MEN-1 families were also atypical, and the families originally called the prolactinoma variant of MEN-1. But was this because of genetic or clinical heterogeneity, or because of greater variability of typical MEN-1 than previously recognized? The evidence supports the latter. Large families elsewhere have since been described with an intermediate clinical phenotype (Shepherd et al 1991), and some branches of the Newfoundland families are indistinguishable from typical MEN-1. A more comprehensive screening protocol is, therefore, recommended for all families.

The large Newfoundland VHL family has a higher frequency of pheochromocytomas than many other VHL families, and since these are frequently the initial manifestation (and were a frequent cause of death in the past), there has been greater emphasis on screening for pheochromocytomas in Newfoundland than at many other centres. Since the VHL gene has been cloned and many mutations identified, a genotype/phenotype correlation has recently been recognized between the type of mutation and "pheo" vs "non-pheo" families (predominantly missense mutations in "pheo" families, and predominantly deletion or truncation mutations in "non-pheo" families). Because the genotype/phenotype correlation is not 100% and because an undetected pheochromocytoma is acutely life-threatening, this information

should be used to emphasize screening for "pheos" in families with missense mutations and to reduce but not exclude screening for "pheos" in families with truncation mutations. Documentation of tumour frequencies with different mutations should continue, to confirm and refine the genotype/phenotype classification so that other modifications of screening protocols may be possible. Conversely, knowing the clinical phenotype in a given family will guide a search for the specific mutation. All investigations in the VHL screening protocol now begin earlier than originally recommended (based on the literature review) because of the identification of advanced disease at initial screening in a number of family members.

The Newfoundland MEN-2 family may be an FMTC (familial medullary carcinoma) family, at risk for thyroid disease only, rather than an MEN-2A family, at risk for thyroid and adrenal disease, as originally thought. Screening for both thyroid and adrenal disease continues, however, because of the uncertainty about the cause of bilateral adrenal hyperplasia in two brothers who are at low risk to carry the MEN-2 mutation. Screening is initiated later in this family than recommended for MEN-2A families (11 years of age vs 3 years of age) because of the late age at onset of thyroid disease. Screening does, however, begin more than five years before the earliest documented age at diagnosis (18 years).

For HNPCC families screening is concentrated on detection of colon and endometrial cancers. More comprehensive guidelines for screening are not yet

established, since present methods are not sensitive or specific enough for screening for such tumours as ovarian, pancreatic, or urogenital cancers. There is also the problem of variability in the type of tumour and age at occurrence within and between families. When there is extreme variability of disease expression, as in HNPCC, it is difficult to plan a protocol that will allow early detection of the majority of tumours without excessive false positive results or harmful side effects of investigations, and without loss of interest in screening after several negative tests in those who may be at high genetic risk.

Four different genes have been mapped and cloned but no genotype/phenotype correlation has been identified which could guide the development of different screening protocols, or the recognition of the relevant gene in a specific family. Recent literature does recommend colonoscopy every second year rather than less frequently (previous recommendations from some centres were for screening every 3-5 years) since interval colon cancers have been identified in patients screened every third year. The frequency of screening is thus determined by the rate of growth of typical tumours.

For each hereditary tumour syndrome, the type of investigations will be revised as new diagnostic options are available (whether new scanning techniques such as MRI and MIBG scanning, or new laboratory assays), and the timing of investigations may need to be altered as new information on age at onset of tumours, in general or for a specific family, becomes available. This may be earlier screening than recommended in the literature as for VHL, or later

screening than recommended in the literature as for the Newfoundland MEN-2 family.

ii) Type of genetic testing

The type of genetic screening available depends on the state of molecular genetic knowledge, a rapidly changing field. Before the relevant gene is mapped, only pedigree analysis is available. Predictive testing by linkage analysis is possible if a gene is mapped and informative markers have been identified, or if a gene is cloned but the specific mutation not identified for the family in question. Presymptomatic diagnosis by mutation analysis is available when the specific mutation is known. When the work described in this thesis began, no genetic testing (except pedigree analysis) was available for the Newfoundland families. Either the relevant genes were not mapped, or informative markers had not been identified. It is interesting, therefore how rapidly the results of research have been adapted and applied to clinical practice.

Currently genetic testing by mutation detection or linkage analysis can be offered to almost all families studied. Presymptomatic diagnosis by direct mutation detection is possible for the large HNPCC family, and for three of five VHL families, since the specific mutations in these families have been identified subsequent to the cloning of the relevant genes in 1993. Predictive testing by linkage analysis is available for the FAP family, and was used for three VHL families before each mutation was identified. Linkage analysis has also been

used for the MEN-1 families but the available markers are not informative for all family members requesting genetic testing.

For over 80% of MEN-2A or FMTC families in the literature, the mutation has been identified in one of five cysteine codons in the extracellular domain of the RET proto-oncogene. Mutations in the remaining one sixth of MEN-2A and FMTC families, including the Newfoundland family, have not yet been identified. Linkage analysis provides preliminary predictive testing information for the MEN-2 family.

For small families with VHL, MEN-1, MEN-2, or FAP that are identified in the future, even linkage analysis may not be possible, despite the mapping or cloning of the relevant gene, if the mutation is not known, and if too few affected individuals are living for the marker alleles linked to the disease gene in the particular family to be identified.

The situation for HNPCC is even more complex because of the genetic heterogeneity recognized — four genes have been identified (hMSH2, hMLH1, hPMS1, and hPMS2) and at least one other gene is anticipated. No genotype/phenotype correlation has been recognized which could predict the gene involved in a particular family. Most families are too small to obtain the necessary data to substantiate linkage to one of the four known map locations, and thereby identify the relevant gene. In relatively isolated populations like Newfoundland, however, the number of independent mutations may be limited. The use of detailed pedigree studies, and mutation or haplotype analysis, as in

Finland, may identify a common founder of several families, and facilitate genetic testing.

Another limitation to the availability of genetic testing is the small number of molecular genetics laboratories capable of doing the testing (and of research laboratories with time and funding to search for individual mutations).

iii) Recommended age for genetic testing

Genetic testing should be available before the typical age that clinical screening is recommended since one rationale for genetic screening is the identification of gene carriers requiring clinical screening. The timing of genetic testing therefore depends on the age at onset of the disease. For VHL, genetic testing is offered in early childhood; for MEN-1, the Newfoundland MEN-2 family, and atypical FAP, genetic testing is offered in the early teens; and for HNPCC, genetic testing is offered in the early twenties. Except for HNPCC, these are not adult-onset diseases. Many of the objections to genetic testing in childhood, are inappropriate when applied to VHL, MEN-1, MEN-2 and FAP since many gene carriers will have disease requiring treatment before age 18.

iv) Expected uptake of genetic testing

Although genetic screening should be offered before clinical screening begins, some family members may not wish to participate; for these family members, clinical screening must remain available. The reasons for interest or disinterest in genetic screening vary according to the disease, and will be discussed in the next section (Patient attitudes and participation). However,

extensive education and counselling regarding the advantages and disadvantages of genetic testing are necessary for all family members, even though some, in the end, do not wish to participate.

v) Costs and benefits of screening

A formal cost-benefit analysis was completed only for the VHL screening program, and the other programs were reviewed informally for comparison. Of the conditions studied, VHL is the most expensive to treat, with or without a screening program, because of the many different tumours occurring throughout life. Those who are treated successfully for one tumour are unfortunately at high risk of subsequent disease. The evidence from the cost-benefit analysis, however, is that overall costs to society (including costs within and outside the health care system) are reduced when the management includes a screening program, because screening decreases early death and disability. When genetic screening is included, the costs are reduced because only gene carriers (or those at high risk) participate in clinical screening. Many years of annual testing for those who are found to be mutation-negative are therefore avoided.

The likelihood of multiple tumours is much lower for each of the other hereditary tumour syndromes so that the overall costs of screening and treatment are less. Nevertheless, costs of disease management are reduced by screening. For FAP and MEN-2, the majority of those with symptomatic disease had advanced cancer, and, despite intensive treatment, early death

frequently occurred. The majority of those identified by screening, on the other hand, do not have cancer (or, for MEN-2, have very small cancers confined to the thyroid gland). These patients avoid the symptoms of cancer, and rarely require chemotherapy or radiation treatment following surgery. Therefore the costs of treatment and discomforts of the disease are less in those identified by screening. Although the follow-up of the Newfoundland FAP and MEN-2 families after screening was implemented, has been short, the literature supports our evidence that subsequent cancers are uncommon in FAP and FMTC patients following screening and surgery (Farndon et al 1986, Jagelman et al 1988). Therefore, for many affected family members, the early identification and treatment is curative. For these diseases also, genetic screening, when available, focuses clinical screening on high-risk or mutation-positive family members, thus reducing costs.

For MEN-1, the mortality was lower than for the other hereditary tumour syndromes studied, but many symptomatic patients had serious morbidity. Early identification by screening markedly reduces this morbidity and early mortality, and often decreases medical costs by allowing medical rather than surgical treatment. The cost per person for annual screening is lower than for the other disorders because biochemical screening is utilized, and the blood samples can be taken locally — saving travel costs for family members. However, because of the extremely variable age at onset of MEN-1, a long follow-up time is required for those with negative clinical screening if genetic status is unknown.

Genetic testing will therefore reduce the costs of the screening program despite the lower cost per person of annual clinical screening. This is important because of the large number of family members at risk in Newfoundland.

Screening for HNPCC in Newfoundland is very recent, and therefore difficult to evaluate. Because there is less experience with clinical screening for HNPCC, worldwide, than for the other hereditary tumour syndromes, it is not yet known how much the morbidity and early mortality can be reduced by screening. This reflects the extreme variability of clinical expression of HNPCC, and the lack of sensitive and specific tests, and of successful treatment for some HNPCC tumours (eg, of the ovary or pancreas). However, for those screened regularly with colonoscopy, a reduction in morbidity and mortality from advanced colon cancer is expected, resulting in reduced costs, as well as increased benefits in length and quality of life.

In summary, it is necessary to know the disease, including both the medical and genetic characteristics, in order to provide an effective and cost-beneficial screening program.

PATIENT ATTITUDES AND PARTICIPATION

i) Severity of disease

VHL is the most devastating disease of those studied, from the patients' as well as the professionals' point of view, because of the early age at onset, the many different tumours, the multiple recurrences in any one individual, and

the difficulty in treating some components of VHL. Extreme anxiety occurred in many affected and unaffected family members because of the previous early deaths and disabilities in their families.

FAP and HNPCC also caused many early deaths in the past, and engendered an overwhelming fear of "cancer" in many families. For both disorders, the recommended screening (colonoscopy) adds to the stress, and sub-total colectomy, if required, reduces the quality of life. However, because of the variability of HNPCC, some family members (particularly those whose close relatives had later onset, or treatable disease), are less concerned about their risk of colon or other cancers, than members of FAP families.

In the Newfoundland family reported here, MEN-2 represents a serious burden to the older generation, but is considered a treatable disease by the younger generation. The screening (a stimulation test), and treatment (total thyroidectomy) are more acceptable than screening and treatment for FAP and HNPCC. For MEN-1 family members the perception of the disease is much more variable, and is correlated with the relationship to severely or mildly affected individuals.

ii) <u>Interest in clinical screening</u>

Interest in clinical screening is determined primarily by the family's perception of the severity of the disease, and of the potential benefits of screening (ie, the likelihood of reduction of deaths and disabilities), but may be modified by any unpleasantness or inconvenience of screening.

VHL is considered a very severe disease, but family members recognize that screening may improve the prognosis because of early diagnosis and treatment. Most are very compliant with the many tests of the screening protocol because they see this as their only hope for a "healthy" life. They worry that tumours will be untreatable if not detected early because they know of serious disease and severe anxiety within the family before screening began. A few family members, however, are non-compliant because they deal with the stress of the disease by putting it, and the recommended screening, out of mind.

FAP is now considered by most family members to be a serious but preventable disease, however, the discomfort and embarrassment of the necessary investigation (a colonoscopy) reduces compliance, particularly in males. Even in this group, interest in clinical screening is increased if high-risk predictive testing results are received. MEN-2 family members accept the pentagastrin stimulation testing, despite some unpleasant side effects, because they recognize the sensitivity of this procedure in identifying early treatable thyroid disease (in contrast to the untreatable metastatic thyroid cancer leading to early death present in the previous symptomatic family member).

In the MEN-1 families, where there is extremely variable disease expression, there is also a marked difference in interest in clinical screening depending upon the relationship to those with severe disease. Some family members are clearly uninterested because, in their experience, MEN-1 is so

mild. However, the screening is convenient because bloodwork can be done locally, so some family members, who would not travel for testing, do participate.

The availability of clinical screening for the HNPCC family is more recent. Family members are divided between those eager to participate in a screening program, hoping to prevent advanced cancer or early death, and those not wanting to acknowledge the cancer risk, and therefore declining any screening or discussion of HNPCC. The uptake of clinical screening has been greatest in close relatives of those with early death or multiple tumours, because of the hope of a better outcome. Those with cancers recently detected by screening and successfully treated, encourage other family members to participate. The prospect of colonoscopy, however, is a deterrent. Despite their concern about the cancer risk, some family members cannot bring themselves to have this testing.

In general, compliance with clinical screening increases when the investigations are available locally (saving time and money for travel to a tertiary care centre), and when the tests themselves are not unpleasant. Family support also improves compliance and reduces anxiety. Persons from small families, or without known relatives with the same disease (for instance, an affected individual who had been adopted outside the family) have a more stressful time participating in a screening program.

iii) Interest in genetic screening

The extent to which individuals were interested in genetic screening also varied. In general, the uptake of genetic screening was correlated with the overall severity of the disease, the stress or unpleasantness of the clinical screening tests, and the likely informativeness of the results.

For the most severe disease, VHL, the majority of family members at risk have requested genetic testing. However, those studied in Newfoundland have all participated in clinical screening for a number of years. The consequence of genetic screening, therefore, is continuation of clinical screening for those with a VHL mutation, or freedom from this screening protocol for those without. A few family members declined genetic testing for their children because they preferred the uncertainty of 50% risk status rather than facing the possibility of mutation-positive results. For a newly identified family, the decision regarding genetic testing may be more difficult to make because mutation-positive results (for a disease as severe as VHL) could be more devastating for those who are unprepared.

All adults at risk for VHL chose genetic testing for themselves because of the importance for reproductive planning as well as for determining the need for clinical screening. Several of those at 50% risk had previously put off or forgone having children to ensure that others were not born with the disease. This included family members who were actually non-carriers of the VHL gene.

Knowing that they were mutation-negative would therefore permit non-carriers to have a family.

The numbers requesting or declining genetic testing, and the reasons, differed for the other diseases. The results of genetic testing, however, were used primarily to determine whether clinical screening was needed.

The uptake of genetic testing was high in the FAP family, related to the family perception of the severity of the disease, and the discomfort of screening. Colonoscopy is considered unpleasant and embarrassing, but because of the lethality of FAP if not detected early, many family members agree that colonoscopy is necessary for those at high risk. The outcome of genetic testing is, therefore, whether regular colonoscopy is, or is not, required.

For HNPCC there are similar concerns about colonoscopy. Many of those participating in clinical screening desire genetic testing to define their risk more accurately, and clarify whether they should continue with these investigations. Others at risk want genetic testing to decide whether to begin clinical screening, but the possibility of insurance discrimination creates a dilemma. The extremely variable age at onset and reduced penetrance of HNPCC means that some gene carriers will be unaffected by HNPCC for many years yet might become uninsurable because of the genetic testing results. In VHL families, this situation does not arise. Because of the consistently earlier onset of disease, any discrimination regarding insurance is due to actual disease, not predictive testing results.

Clinical screening for MEN-1 is more acceptable than for the hereditary colon cancers since it is primarily a blood test and can be done in the local community. Some family members were quite willing to continue clinical screening but lacked interest in genetic testing because it was not seen to offer a significant difference in their lifestyle. Others, who had close relatives with severe MEN-1 manifestations, were more interested in knowing whether they were at high or low risk of being affected, and requested predictive testing when offered. The fact that MEN-1 predictive testing was not always informative, also decreased the uptake.

All members of the MEN-2 family desired genetic screening to determine the need for continued clinical screening but definitive genetic testing is not possible in this family until the mutation is identified.

iv) Use of genetic screening

For VHL, the results of genetic screening determine who requires clinical screening, and also provide information for reproductive decisions. Many family members felt so strongly that this disease should be stopped that, even when at 50% risk, they chose not to have children to ensure that another generation was not born with the disease. Genetic screening allows informed reproductive decisions, and particularly allows those who are not gene carriers to have children without worry.

The majority of VHL gene carriers choose to have no children because of the risk of recurrence. None would consider prenatal diagnosis and the

possibility of a therapeutic abortion. Despite the severity of VHL, they could not accept termination of an "affected" pregnancy when the disease was not actually present at birth.

Some of the older affected members of the MEN-2 family said that they would not have had children if they had known of their genetic status and the risk of recurrence. However, younger affected family members accept MEN-2 as a treatable disease and do not consider the recurrence risk a reason to alter reproductive planning.

For the other diseases in this group (FAP, HNPCC, MEN-1) the results of genetic screening have been used to determine whether or not clinical screening is recommended (for those screened, or for their children), but have not been used for reproductive planning.

In summary, it is necessary to understand the psychosocial implications of a disease, as well as the medical and genetic characteristics, to provide an acceptable screening program. For some hereditary diseases, or for some individuals, screening may not be the best approach, although this may change as more is learned about the genetic basis of specific diseases, and new treatments are developed. For others, such as the hereditary tumour syndromes considered in this thesis, there is evidence that genetic and clinical screening will reduce or prevent some of the serious medical and psychosocial consequences of disease, and that a screening-based approach to disease management is cost-beneficial.

RELEVANCE TO OTHER CENTRES AND TO OTHER DISEASES

One conclusion of this thesis is that families with hereditary tumour syndromes in Newfoundland have benefitted medically and psychologically from management with a clinical and genetic screening program. But does the experience in Newfoundland have relevance to other centres; and does the recommendation for screening apply to diseases other than those described?

i) Relevance to other centres

The basic recommendation when screening for hereditary tumour syndromes elsewhere is to use the same approach: ie, to develop a clinical screening protocol based on the natural history of disease, and to add genetic screening as current molecular genetic knowledge permits, but not necessarily to use the same screening protocols as developed in Newfoundland. The investigations of the clinical screening protocol should be determined by the tumours that occur, should start before the earliest age at diagnosis, and should be repeated at an interval determined by the rate of growth of tumours in previous studies — usually annually or every two years. The objective is to identify the majority of tumours at a premalignant or treatable stage, but not to overburden those at risk with too frequent testing because of the financial and psychosocial costs of screening. Genetic screening by linkage analysis or mutation detection, when available, should be offered before the age that clinical screening begins to reduce the monetary and psychosocial costs of screening.

Ideally, information on the clinical phenotype, and the mutation, or map location, of the relevant gene, will be available for the family or families to be screened. However, if the families are recently identified, or too small, the spectrum of disease will be unknown, and the mutation or relevant gene may not have been identified. Then one must rely on the literature; data on intrafamilial and interfamilial clinical variability, and the spectrum and relative frequency of mutations documented for families elsewhere, are valuable.

If genotype-phenotype correlations are recognized, as an increasing number of mutations are identified, these correlations may provide guidance for clinical screening protocols. However, these correlations are more useful for emphasizing the need for a particular type of screening in susceptible families, than for excluding screening investigations.

There are differences in the screening process related to the location of a screening centre; whether in an "isolated", primarily rural area, or an urban centre. In Newfoundland (or Tasmania [Shepherd 1985]), the initial impetus to develop screening was the large number of affected members of individual families, and the impact of the morbidity and mortality previously experienced. The impetus to continue screening is the encouragement of early results, and the interest and expertise developed by the specialists at the single provincial tertiary care centre, and by the family practitioners in the geographic regions where the families are concentrated.

In urban centres the impetus for screening is the number of unrelated families requesting this service. The number of members of each family seen, or accessible, is likely to be much lower than in Newfoundland, both because of smaller family size, and because families may be scattered, with relatives or their records unavailable. Coordination of screening for such families may be more difficult because of the number of different physicians and health care centres involved, and because less is known about the clinical phenotype of each family. A comprehensive screening protocol should then be used until the family can be classified as a typical or a variant form of the particular hereditary tumour syndrome, and a more appropriate screening protocol applied.

Although there are these disadvantages, advantages of screening programs based at a large urban centre include earlier access to new technology, and the availability of greater molecular genetic expertise, facilitating mutation detection and predictive testing.

Support for family members participating in screening programs may be provided in different ways in rural areas like Newfoundland, where there are a few large families, and in urban centres with many small families. In Newfoundland, patient support is provided by members of the same family who have experienced the disease and the screening process; in an urban area, support is more likely to be provided by a support group established by members of a number of families experiencing a particular need.

ii) Relevance to other diseases

The features of the screening programs described are determined by the characteristics of the hereditary tumour syndromes, ie, severe but treatable autosomal dominant disease with variable age at onset and variable manifestations. As stated in the introduction, similar screening programs have been successfully implemented for other diseases with similar features, eg, for NF2 (MacCollin et al 1993) and for Marfan disease (Bridges et al 1992), and a screening program for dysplastic nevus syndrome is administered by the Foundation for Hereditary Tumours in the Netherlands (Vasen 1989). NF-1 and tuberous sclerosis, although AD multisystem diseases, are less suited to this type of screening because many patients are mildly affected and do not require screening (Smalley et al 1994), while others have severe disease which is best managed individually as recommended in recognized standards of care. Although clinical screening for polycystic kidney disease has been considered, there is no definite evidence that the prognosis is improved by presymptomatic detection of disease, and there is little demand for predictive testing for ADPKD (Gabow 1993).

Many of the neurodegenerative diseases such as the cerebellar ataxias and Alzheimer disease are severe AD diseases with variable age of onset, but are not yet treatable (Nance et al 1994). HD predictive testing or presymptomatic diagnosis protocols serve as the prototype for offering molecular genetic testing for these conditions when the gene or specific

mutation is known. If treatment becomes available, a clinical screening protocol may also become appropriate.

The greatest unfilled societal demand for a clinical and genetic screening program for identification of gene carriers, and for early diagnosis and treatment, is for hereditary breast cancer (gene carriers being at risk for ovarian as well as breast cancer) (Statement of the American Society of Human Genetics 1994). Hereditary breast cancer is more common than the hereditary cancers studied, except for HNPCC, even though the majority of breast cancer is sporadic rather than hereditary.

Although there is a need for early diagnosis and treatment, hereditary breast cancer does not entirely fit the guidelines for clinical screening. Those at risk for hereditary breast cancer may not always be identified (because of late onset, low penetrance, or transmission through males, a family with hereditary breast cancer may be missed; or a family with a cluster of sporadic breast cancers may be incorrectly assumed to have hereditary disease); a test to reliably detect presymptomatic disease is not yet available (a mammogram, particularly in young women, may miss early breast cancer, and screening for ovarian cancer is even less reliable); and early treatment does not always improve prognosis (King et al 1993). If clinical screening is recommended for early identification and treatment of tumours in order to improve prognosis (and hopes of those who are affected and at risk, therefore, raised), yet tumours are missed because of insensitivity of the tests, or the medical outcome is not

improved, serious psychological harm can be done to the participants, and future compliance with screening will be reduced. Improvements in the ability to identify, investigate and treat those at risk for hereditary breast cancer are needed before a clinical screening protocol is routinely offered to members of these families.

Molecular genetic testing is, however, available for some families, by linkage analysis or direct mutation detection (Editorial 1994c). As with HNPCC, there is more than one gene causing clinically similar disease which adds to the difficulty of identifying families in which genetic testing is informative. One of the genes (BRCA1 on chromosome 17) has been cloned (Miki et al 1994), and a range of mutations identified, and a second gene (BRCA2) mapped to chromosome 13 (Wooster et al 1994). Predictive testing following a HD protocol has been offered to families where the mutation or chromosomal location is known (Lynch et al 1993d). Participants have used this information, to reduce uncertainty, to guide clinical management, and, less often, to make reproductive decisions (Biesecker et al 1993). Research should continue on the clinical, molecular, and psychosocial aspects of screening for breast cancer to determine whether a full screening program can be offered in the future (NIH Guide 1994).

THE VALUE OF REGISTERS

One of the difficulties of managing an on-going screening program is keeping track of all participants. Unlike screening programs for prenatal or neonatal hereditary disease, or for carrier status of certain autosomal recessive disorders, which are conducted once, screening for those at risk for hereditary tumour syndromes (or for other variable multisystem diseases) must continue on a regular basis. For those who are affected or identified to be gene carriers, life-long testing is required. There may be problems, however, if the responsibility for screening is left to the individual family practitioner or specialist. Each of the hereditary tumour syndromes is relatively rare so primary care physicians may not be familiar with the screening requirements; family members may be scattered and have a number of different family doctors; and patients or their doctors may move; any of which could disrupt the continuity of care. Screening for affected and at-risk family members may, therefore, be best organized through a centralized registry.

A registry can efficiently manage large numbers of participants with a special need, by maintaining pedigrees, and personal and medical records of consenting affected and at-risk members of relevant families (Read 1990). Since date and results of last screening are recorded, the registry can notify family members when screening is due. The actual screening may also be centralized (as at the St. Mark's Polyposis Registry in London, England), or arranged through a local specialist, as from The Foundation for the Detection

of Hereditary Tumours in the Netherlands (Vasen 1989). The registry thus provides a means of timely recall, and also facilitates revision of risk status for other family members when a new diagnosis is made.

Registries have been used successfully for clinical management of FAP families since the St. Mark's Hospital Polyposis Registry was established in London, England in 1925 (Bussey 1975, Berk et al 1981, Jarvinen et al 1984, Bulow 1986, Vasen et al 1990a), and more recently have expanded to include other hereditary tumour syndromes, such as VHL, MEN-2 and retinoblastoma in Wales (Littler and Harper 1989); and MEN-1, MEN-2, HNPCC, and dysplastic nevus syndrome in the Netherlands (Vasen 1989). Besides the main objective of promoting and administering screening for early detection and treatment of tumours, registries can play an important educational role, for the primary care physicians as well as for the participants. Basic information on the disease and on screening recommendations can be provided initially, with updates as new clinical, molecular genetic, or technological advances occur which necessitate modifications to screening protocols.

Because results of screening tests are stored, registries also facilitate epidemiological research, particularly prospective studies of the clinical course of disease, and of medical and psychosocial outcomes following screening and treatment (Vasen et al 1989, Rhodes and Bradburn 1992). Evaluation of screening programs is also facilitated. These studies may be from individual registries, or may be collaborative when large numbers of subjects are required.

Results of the research are then used to revise screening protocols and improve clinical care.

Genetic counselling may be organized through the registry, and DNA banking and predictive testing have been offered when the relevant genes are mapped or cloned (Read 1990, Bulow 1991, Burn et al 1991, Rhodes and Bradburn 1992). It has also been recommended that registries maintain a database of the spectrum of mutations identified (Petersen et al 1993). This information would then be available for genotype-phenotype correlations.

In the Netherlands because of small geographic size, despite a large population, a national registry for several hereditary tumour syndromes has proven to be very successful (Vasen 1989). In larger countries, such as Great Britain, and particularly Canada and the United States, regional registries may be more efficient in maintaining close contact with physicians and participants whom the registry is serving (Rhodes and Bradburn 1992). Regional registries frequently have contact through national or international organizations (eg, Leeds Castle Polyposis Group [Thomson 1988], or International Collaborative Group on Hereditary Non-Polyposis Colon Cancer [ICG-HNPCC] [Vasen et al 1991b]) to maintain expertise and up-to-date knowledge.

Despite the many positive features of registries, there are some concerns, particularly regarding ethical issues. There are concerns about the confidentiality of records. There are also fears from some family members that they will be "labelled" with the disease in question even if only at risk, and

suffer discrimination regarding employment or insurance. To reduce these risks consent must be given before personal or medical records are entered in the registry, and information is only released to third parties with further consent. There may also be a conflict of interest between family members if some do not wish to be included; family or medical records that are withheld may be necessary for recognition of relatives that are at risk.

Finally, a registry requires funding for a dedicated coordinator, and preferably a dedicated computer-based system for storage of data and automatic flagging of those requiring follow-up, if it is to function efficiently.

CONCERNS ABOUT GENETIC TESTING IN CHILDHOOD FOR ADULT-ONSET DISEASES

A group of hereditary diseases, including neurodegenerative disorders, AD polycystic kidney disease, and hereditary tumour syndromes, are classified as late-onset disorders to differentiate them from genetic diseases presenting at birth or in early childhood (neonatal or early-onset disorders). Because the typical age at diagnosis of patients presenting symptomatically was in adulthood, these disorders have also been referred to as "adult-onset" disorders (Harper and Clarke 1990). With a better understanding of early signs and symptoms of many late-onset diseases, and the use of screening to detect presymptomatic disease, diagnosis is now typically much earlier for some of these conditions, for example in the teens or childhood for VHL, MEN-2, or Marfan disease. For these diseases, the designation "adult-onset" is no longer

correct. Because geneticists and ethicists, very appropriately, have established guidelines for genetic testing of children (ie, testing before the age of majority) (Harper and Clarke 1990, Marteau 1994, Report of a Working Party of the Clinical Genetics Society [UK] 1994), this is not just a semantic argument. Because of the autosomal dominant inheritance pattern of many of the late-onset disorders, the risk of recurrence within families is high, and the question of predictive testing frequently arises.

The two most important points to consider regarding whether or not genetic testing in childhood is appropriate, are age at onset, and availability of an effective therapeutic intervention. If onset is typically in adult years, and/or if no treatment is available (eg, for HD or Alzheimer disease), genetic testing of children is not appropriate (Working Party of the Clinical Genetic Society [UK] 1994). The child's future autonomy will have been breached, and there are dangers of discrimination (eg, for education, employment or insurance, and even by parents or other family members), without significant benefits. If onset is usually in childhood and early intervention will improve the outcome, then it is good clinical practice to offer genetic testing in childhood. This is particularly the case if clinical screening in childhood is recommended for those at risk, to allow early identification and treatment of disease manifestations, as with VHL, FAP, MEN-1 OR MEN-2 (Gagel 1993), Petersen et al 1993, Eng et al 1994b). Similarly, genetic testing in childhood is appropriate for Marfan disease, if predictive testing is available for the particular family. On the other hand,

because of the "late" and extremely variable age at onset of HNPCC, clinical screening is not recommended before 20-25 years of age. Genetic testing of children is therefore not appropriate. For NF-2 and hereditary breast cancer, similarly, because onset is rarely before 20 years of age, genetic testing should only be offered to adults. For hereditary breast cancer, additionally, an improved outcome because of screening is not certain (Evans 1995).

It is important to revise the classification of late-onset disease using current knowledge of age at onset. It is also necessary to evaluate clinical screening programs for multisystem late-onset disorders to determine whether medical and psychosocial outcomes are improved by early diagnosis and treatment. Both these forms of evaluation should be completed before the ethical concerns regarding age for genetic testing are applied to a particular disease. Some of the criticisms of genetic testing in childhood may then be recognized as misdirected.

THE VALUE OF SCREENING FOR HEREDITARY TUMOUR SYNDROMES

The Newfoundland families with hereditary tumour syndromes were identified because of a concentration of benign or malignant tumours within each family which had caused serious medical problems and early deaths. It was also apparent from the time of first contact with the families, that these diseases had an impact beyond the family members already medically affected. There was evidence of severe anxiety and stress within families because of the

past illnesses and early deaths, fears were expressed by both affected and unaffected family members that they or their children would develop subsequent tumours, and reproductive plans, particularly for VHL, had been affected.

A similar situation is documented in the literature with regard to other families with VHL, MEN-1, MEN-2, FAP and HNPCC. Symptomatic patients frequently have advanced disease which is difficult to treat, resulting in early deaths and disabilities (eg, Vasen et al 1987, Lamiell et al 1989, Bulow 1991, Shepherd 1991, Lynch et al 1993c), and many family members experience stress when there is a potentially lethal hereditary disorder within the family (Langsley et al 1964, Yuen et al 1984, Gagel 1993).

Although the numbers in each Newfoundland family are too small, and the ages at diagnosis or death, too variable, for rigorous statistical analysis, there is evidence that the clinical and genetic screening programs for hereditary tumour syndromes that have been introduced in Newfoundland and at other centres, have resulted in reduced morbidity, mortality and anxiety; and increased understanding of the relevant disease, and of the reproductive options available. Overall, an improved prognosis and quality of life have been demonstrated. From the health care evaluation of the VHL screening program, there is also evidence that the cost of managing patients with a clinical and genetic screening program to achieve these outcomes, is less than the cost of

treating symptomatic disease if the costs related to early deaths and disabilities are included.

To summarize the conclusions and recommendations emerging from the work described in this thesis, I will briefly recapitulate the main points, beginning with the least complex program, that for FAP.

i) **Familial adenomatous polyposis**

In the Newfoundland FAP family, 17 of 19 symptomatic patients had invasive cancer, and 12 of these died within 6 years of diagnosis: none of the 19 patients identified by clinical screening had invasive cancer, and none died of the disease. Clinical screening also resulted in a shift to an earlier age at diagnosis ($\bar{x}$ of 47 years in those presenting symptomatically, compared with $\bar{x}$ of 32 years in those detected by screening).

Successful clinical screening programs for FAP have been developed at other centres since 1925 (Bussey 1975), because of the easily identifiable premalignant stage of FAP (multiple colorectal polyps). Results of screening similar to those of the Newfoundland program have been obtained. The frequency of cancer, and therefore of cancer deaths, is greatly reduced for screened vs symptomatic patients (for Denmark, 2% vs 67% [Bulow 1991]), for Holland 4% vs 47% [Vasen et al 1990a], for central Canada 5.5% vs 57% [Berk et al 1987] and for England 5% vs 65% [Bussey 1975]); and there is a shift to a younger age at diagnosis in screened patients ($\bar{x}$, 19 yr) compared with symptomatic patients ($\bar{x}$, 33 yr) (Bulow 1986).

It is noted that both symptomatic and screened members of the Newfoundland family were older at the time of diagnosis than the comparable symptomatic and screened groups reported in the literature; this is related to the atypical disease expression in the Newfoundland family (and in a small proportion of other FAP families [Leppert et al 1990, Spirio et al 1993b]. Although there is a different clinical phenotype, regarding the age at onset, and the number and distribution of polyps, the same successful screening results can be obtained if the atypical disease is recognized, and the clinical screening protocol revised accordingly. With this evidence of decreased morbidity and mortality because of the Newfoundland and other screening programs, there can be little doubt that clinical screening for FAP is beneficial.

The recommended investigation for clinical screening (sigmoidoscopy for typical FAP, or colonoscopy for atypical FAP), however, is not acceptable to all family members; there is, therefore, some non-compliance. Predictive testing by linkage analysis (Maher et al 1993, Rhodes and Bradburn 1992), or by direct mutation analysis (Petersen et al 1993), however, is acceptable to many at-risk family members, and focuses clinical screening on those at highest risk. High risk genetic testing results may thus increase compliance with endoscopy.

Because of the large size of the APC gene, and unique mutations in the majority of families, mutations were only identified in 30-60% of families prior to the development of protein truncation testing (PTT) (Powell et al 1993). With the use of PTT, and the evidence from recently described genotype-

phenotype correlations, the likelihood of finding the APC mutation is now improved, and informative CA repeat markers in the region of the APC gene are also available for linkage analysis if the mutation is not identified. Accurate predictive testing should therefore be increasingly available for FAP family members who desire it.

Although colorectal cancer is the most frequent tumour in FAP, upper GI cancers have been documented in approximately 5% of affected persons (Jagelman 1987). Longer follow-up is required for the Newfoundland and other FAP families, to determine the long-term prognosis of typical and atypical FAP, particularly in those who have had prophylactic colectomy. Is there an increased frequency of upper GI cancers and other tumours in these gene carriers, now that longer survival is possible? It will also be important to study attitudes towards clinical and genetic screening in at-risk members of younger generations who do not have personal experience with the early deaths and morbidity from symptomatic cancer.

ii) <u>Multiple endocrine neoplasia, type 2</u>

Only one patient in the Newfoundland MEN-2 family had symptomatic disease, a patient with metastatic medullary carcinoma of the thyroid who died at age 44 within four years of diagnosis. One other patient, now age 53, who was identified at the first screening investigation, has biochemical evidence of metastatic MTC but is clinically well. Sixteen other family members were identified by initial or subsequent screening, had MTC confined to the thyroid

or CCH only, and postoperatively have no evidence of residual disease. Vasen et al (1987) in a more extensive series from the Netherlands, reported similar results of clinical screening for MEN-2, ie, a marked decrease in frequency of metastatic MTC, and improved survival following thyroidectomy; and also earlier diagnosis and more successful treatment of pheochromocytoma.

Although the age at symptomatic diagnosis of MEN-2 is extremely variable, the age at diagnosis by screening, for classic MEN-2A families, is typically in childhood. This is documented in extensive series in Great Britain (Easton et al 1989), Europe (Calmettes et al 1982), and the USA (Telander et al 1989). Clinical screening is therefore recommended to begin at least by age 3, and genetic testing should be offered earlier. Later age at onset is documented for families with FMTC (thyroid disease only), therefore clinical screening can begin at a later age (Farndon et al 1986).

Genetic testing is available for the majority of MEN-2A and FMTC families because, in greater than 85% of families, the mutation is found in one of five cysteine codons near the transmembrane domain of the RET proto-oncogene (Mulligan et al 1994). Every MEN-2A or FMTC family identified should therefore be tested for a mutation in one of these codons. Genetic testing can then be integrated with clinical screening to provide a complete management plan for MEN-2A and FMTC families (Gagel 1993, Eng et al 1994b, Ledger et al 1995), with benefits of improved medical outcome and reduced anxiety.

Although there is a difference in the ability to identify specific mutations and provide accurate genetic testing for MEN-2 and FAP, these two hereditary cancers are very similar in the high disease-related mortality before screening programs were introduced, and the marked reduction in mortality, morbidity, and anxiety after screening programs are implemented. As with FAP, longer follow-up of members of MEN-2 families who have had prophylactic surgery is necessary, to determine the long-term prognosis, and to assess the interest in, and compliance with, clinical and genetic screening in children of these patients.

iii) Multiple endocrine neoplasia, type 1

The Newfoundland MEN-1 families have an atypical clinical phenotype (MEN-1 [Burin]), determined by the relative frequency, and order of occurrence of parathyroid, pituitary, and pancreatic involvement (ie, more frequent prolactinomas which may be the first manifestation, and rare pancreatic tumours),and an excess of carcinoid tumours, compared with typical MEN-1. There is marked intrafamilial variability in disease expression, however, with about 50% of affected family members having hyperparathyroidism only, as in typical MEN-1. Thus the atypical phenotype cannot be recognized in individual affected family members. Because small families with this phenotype may not be identifiable, comprehensive screening is recommended for all MEN-1 families, rather than initial screening for parathyroid disease only as previously recommended (Marx et al 1986).

The disease-related mortality in symptomatic MEN-1 (Burin) patients was low compared with the other hereditary tumour syndromes studied. It was also low compared to typical MEN-1 where mortality is primarily due to pancreatic disease (which is rare in MEN-1 [Burin]). Disease-related morbidity, however, was high in symptomatic patients, and was reduced by screening. Unlike for FAP and MEN-2, immediate prophylactic surgery is not recommended for MEN-1 patients with positive screening results. Timing of parathyroidectomy is determined by the physicians, but the recognition of MEN-1-related hyperparathyroidism determines the appropriate procedure, ie, a subtotal parathyroidectomy, or total parathyroidectomy with forearm implant, instead of removal of one parathyroid gland only. Presymptomatic prolactinomas and gastrinomas (the most common pituitary and pancreatic islet cell tumours, respectively) are treated medically rather than surgically. Besides the benefit of early treatment, possible detrimental side-effects of surgery are avoided by this approach. Carcinoid tumours of the lung or thymus, which are rare in typical MEN-1, remain the most difficult tumours to detect or treat in MEN-1 (Burin).

Clinical screening programs in Sweden and Holland are primarily directed at reducing morbidity and mortality from pancreatic disease (Vasen et al 1989b, Skogseid et al 1991a), but screening for the large Tasmanian family has more similarities to screening for MEN-1 (Burin) because of the similarities in disease phenotype (Shepherd 1991, Shepherd et al 1993).

MEN-1 and MEN-1 (Burin) both map to chr 11q13 but the gene(s) has not yet been identified. Predictive testing is available by linkage analysis (Larsson et al 1992, Thakker et al 1993b, Teh et al 1994), however closely-linked CA repeat markers have not been identified, so predictive testing is not informative for all MEN-1 family members requesting this testing. The demand for genetic testing however was lower in the MEN-1 (Burin) family than for families with the other hereditary tumour syndromes, because clinical screening is relatively convenient, and MEN-1 (Burin) is seen as a mild disease by some family members, ie, those whose close relatives had hyperparathyroidism only, and were successfully treated.

Family members, and their doctors, however, appreciated the education and counselling provided with the screening program, and the availability of appropriate clinical care when the underlying etiology of disease was recognized. Thus, the screening program improves the quality of life of family members as well as improving the medical outcomes.

As with FAP and MEN-2, it is important to continue to document the clinical course in patients with successfully treated tumours to determine the long-term prognosis. When the MEN-1 gene is cloned and mutations identified, it will be possible to search for genotype/phenotype correlations which on the one hand would refine the development of appropriate screening protocols, and on the other hand would guide the search for mutations in new MEN-1 families.

iv) Hereditary non-polyposis colon cancer

Screening for HNPCC is more recent than for the other hereditary tumour syndromes, in Newfoundland and in other centres. Although Lynch I and Lynch II syndromes had been described by Henry Lynch and others (eg, Mecklin and Jarvinen 1986a, Lynch et al 1988, Vasen et al 1990b), families with the Lynch syndromes (now both called HNPCC) were difficult to distinguish from clusters of sporadic colon cancer; the age at onset and type of tumours was variable; and there was no premalignant marker, as in FAP, to reliably detect presymptomatic disease. Clinical screening was therefore less commonly recommended than for FAP, and until 1993 the location of the gene (or genes) involved was unknown so that predictive testing was not an option. Because of very rapid developments involving a Newfoundland family, this situation has now changed.

Two independent probands were referred in 1990 for genetic counselling because of an overwhelming medical and psychological burden of disease, for these individuals and members of their extended families, due to multiple symptomatic cancers and early cancer deaths in the families previously. A correct diagnosis had not been made in either family, and the presence of "hereditary cancer" had even been questioned. Through the initial pedigree studies and medical record review, a diagnosis of Lynch II syndrome (HNPCC) was made for each family, and a very similar pattern of cancers (type and age at onset of tumours) was noted. The geographic origins of the two families

were each in small communities along the north-east coastline, although about 175 km apart. (Were these really two branches of one family as the similar clinical phenotype, and geographic origin hinted?)

Because of the success of screening programs in Newfoundland for other hereditary tumour syndromes, this same approach was taken for the HNPCC families. Education and counselling were provided for family members; clinical screening, particularly for colorectal and endometrial cancer, was recommended; and this information was then summarized for the family doctors. Five cancers (three colorectal cancers, one endometrial cancer, and one bladder cancer), and colorectal polyps in ten other affected and at-risk family members, have since been identified and removed because of this screening. Family members were extremely grateful for the clinical screening program, and for the information leading to a better understanding of HNPCC, but those at risk continued to ask for a "test" to indicate whether or not they would also be affected. In 1991, such a test was not possible because the location of the gene (or genes) for HNPCC was unknown. However the search was on: the APC gene for familial adenomatous polyposis had just been cloned, and several candidate genes for HNPCC had been excluded, but few informative HNPCC families were available for study.

A fortuitous discussion at the short course in Medical Genetics at the Jackson Laboratory, Bar Harbour, Maine in July 1991 with members of Bert Vogelstein's group initiated a collaboration between the laboratories at Johns

Hopkins University and the University of Helsinki (with expertise and manpower for mapping and cloning genes, but lacking informative families), and myself (with two well-documented HNPCC families — soon to be established as two branches of one large family — but without the resources locally to carry out intensive linkage studies).

The goal of medical genetic research is ultimately to provide better genetic services for families with hereditary disease; by developing better methods of prevention, treatment, and provision of reproductive options, and by increasing knowledge. At the same time, genetic research is dependent on individuals or families with well-characterized hereditary conditions identified and documented by those providing genetic service. The study of hereditary tumour syndromes in Newfoundland (particularly of HNPCC) presents a striking example of the beneficial outcomes when genetic services, and genetic research go hand-in-hand.

Thus, the recognition of an informative HNPCC family and the willingness of family members to participate in linkage studies, even though there was no guarantee of any personal benefit, set the stage for mapping the first HNPCC gene. Over the next several months, field trips were conducted to small communities in central Newfoundland for further genetic counselling about the proposed research, to extend the pedigrees (an 1882 marriage certificate seen in one home demonstrated the connection between the families), and to collect blood samples for DNA extraction.

It was then September 1992 — the preliminary work had taken two years, but a clinical screening program and genetic counselling were in place for the original and subsequent families with HNPCC, the connection between the two original HNPCC families was established, and the intensive laboratory studies with Newfoundland Family C and a large New Zealand family were just beginning at Johns Hopkins University and the University of Helsinki. Three hundred and forty-five markers later, in late March of 1993, a gene for HNPCC was mapped to chr 2p16 (to be reported in the May 7, 1993 issue of Science) (Peltomaki et al 1993).

As stated in Chapter 5, the first HNPCC gene (hMSH2) was cloned barely six months later, and mutations detected in three families including Family C; a new mechanism for predisposition to hereditary tumours was identified (a defect in mismatch repair genes); and three other DNA repair genes responsible for clinically indistinguishable disease in other HNPCC families soon identified (Fishel et al 1993, Leach et al 1993, Bronner et al 1994, Papadopolous et al 1994, Liu et al 1994).

In the excitement of the announcements of these breakthroughs, there were predictions of a "blood test for colon cancer" within 6-12 months, for relevant families. Public expectations were raised, but frequently the requirements that defined a "relevant colon cancer family" were misunderstood; first, an HNPCC family, and more specifically, one in which the particular mutation, or at least the specific gene (hMSH2, hMLH1, hPMS1 or hPMS2)

was known, so that predictive testing by mutation detection or linkage analysis was possible. Even for families where the mutation was known (such as family C), services were not necessarily immediately available to provide the genetic testing that was requested. Further genetic counselling was necessary regarding the implications of prediction of future disease (for Family C alone there were 77 family members at risk over 20 years of age), and proficient laboratory personnel were required to do the genetic testing on a service basis. Because of the rapidity with which molecular genetic progress has taken place, the medical care system has yet to "catch up", and provide the funding for the necessary manpower and facilities so that complete programs for clinical screening and predictive testing for late-onset multisystem diseases such as HNPCC can be implemented, and the medical and psychosocial benefits documented in this thesis can be more generally obtained.

For 36 members of Family C, predictive testing is now underway (some family members chose not to accept this option because they prefer that the risk of future serious disease remains indefinite, and others have not yet decided). The results will be provided individually as soon as possible, and counselling, support, and clinical screening will continue as necessary. Affected members of other HNPCC families in Newfoundland will then be tested for the same mutation — if two independent probands were part of one large family, might not other families, particularly those from the north-east

coast, also be related. If so, genetic testing can immediately be offered to other families, as has been requested.

Preliminary review of the HNPCC clinical screening program indicates both medical and psychosocial benefits of screening. Presymptomatic cancers have been identified and successfully treated, and polyps have been removed which may have prevented other cancers. Family members are better informed about HNPCC and the recurrence risks, are less anxious because of the disease in the family, and are more confident that their physicians will provide appropriate care. Long-term follow-up of these families is required to formally evaluate the medical and psychosocial outcomes before and after clinical and genetic components of the screening program were offered.

From a societal point of view, the real value of the HNPCC screening program, however, is the research that was facilitated. Members of Family C presented to Genetics in 1990 requesting a service (predictive testing) which was not yet available. Counselling and clinical screening however were offered. The documentation of the pedigree and medical records to confirm the diagnosis and plan the clinical screening protocol set in train a cascade of events leading to the mapping and cloning of four HNPCC genes, and therefore the availability of predictive testing, not only for Family C but also for hundreds of other HNPCC families worldwide.

v) Von Hippel-Lindau disease

As discussed in Chapters 6 and 7, VHL family members presented to the Ocular Genetics Clinic in 1982 requesting help to prevent or reduce the disabilities and early deaths that had plagued the family. The extreme anxiety in affected and unaffected family members was clearly evident. A multi-disciplinary VHL interest group was formed, and a clinical screening program was immediately established based on recommendations from the literature, and the initial medical review of affected family members. As has been summarized for HNPCC, provision of medical genetic services, and research on VHL were inter-connected from the start. The large Newfoundland VHL family was part of studies to map the VHL gene in 1988 which in turn led to development of genetic testing for this and other VHL families (Seizinger et al 1988, Glenn et al 1992, Maher et al 1992). The clinical description of this family, from medical records and documentation of the results of clinical screening has contributed to the recent recognition of genotype-phenotype correlations (Crossey et al 1994, Whaley et al 1994, Chen et al 1995), but also demonstrates the extensive variability possible within a single family. This documentation of inter- and intrafamilial variability for VHL families is resulting in more appropriate clinical screening protocols for individual families.

In the Newfoundland families, early deaths, neurological disabilities, and blindness have been reduced by early recognition and treatment of tumours, facilitated by the clinical screening protocol. Anxiety in affected and unaffected family members has been reduced, because of the education and counselling

provided, and because of trust in the clinical screening program. Informed reproductive choices are possible for most family members, because of early identification of gene carriers by genetic testing, or by clinical screening if mutation detection or linkage analysis are not available. As detailed in the health care evaluation, all this is accomplished with decreased overall costs, within and outside the health care system, for management of members of VHL families because those affected with VHL (or other hereditary cancers) will require medical care and other support, whether or not screening is provided.

FINAL CONCLUSION

The development and implementation of appropriate clinical and genetic screening programs for management of hereditary cancers and other treatable, late-onset multisystem diseases is strongly recommended because of the results of screening for hereditary tumour syndromes in Newfoundland and at other centres. This is a multidisciplinary and preventive approach to health care in keeping with the trends in other areas of medicine.

Medically, improved outcomes are demonstrated for each disease studied. This is particularly dramatic for FAP and MEN-2 where in Newfoundland or as documented in the literature, there is a very high incidence of cancer and cancer mortality in gene carriers when patients present symptomatically, and a curable disease for the majority of gene carriers after a screening program is implemented, and for VHL where morbidity and

mortality are markedly reduced, although the multiple potential tumours make "cure" impossible.

Psychologically, there are also dramatic reductions in human anxiety and suffering, documented in most detail for the Newfoundland VHL family, because of the education and counselling provided with screening programs, and the confidence in the clinical screening protocols, as well as the direct medical benefits from this type of clinical management.

Because of individual personalities and family dynamics, and because of past experiences, some persons at risk may require more support and counselling before being willing or able to trust the screening program that is offered. Patients may be so devastated by consequences of late treatment of previous symptomatic disease that they have no faith in the medical care system, or may be so depressed by identification of new presymptomatic disease that they have difficulty complying with screening recommendations. A dedicated screening coordinator must be aware of these potential problems, and must be available to counsel and support participants through difficult times so that they can obtain maximum benefit from a screening program.

Finally, continued success of screening programs for hereditary tumour syndromes requires financial investment at the outset, for a geneticist/ coordinator and for maintenance of a registry, for personnel and facilities to develop and provide molecular genetic testing, and for flexibility to respond to future research developments, so that savings in money and human suffering can be realized.

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