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THE GENETIC BASIS OF ENDSTAGE RENAL DISEASE IN THE NEWFOUNDLAND POPULATION

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ABSTRACT

Familial Risk Of Endstage Renal Disease

The objectives of the current research were (1) to determine the contribution of Mendelian inherited disease to the burden of disease caused by Endstage renal disease; (2) to explore the possibility that polygenic disorders could contribute to the development of Endstage renal disease; and (3) to describe the natural history of single-gene disorders associated with Endstage renal disease identified in the Newfoundland population, with particular focus on new data associated with Bardet-Biedl Syndrome.

To determine the risk of renal failure in family members of probands with Endstage Renal Disease (ESRD), all patients who were receiving treatment for ESRD during 1987-1993 in the province of Newfoundland and Labrador, Canada were studied. Detailed family histories were taken from 584 (87%) of the 669 eligible probands. Of the 85 patients with incomplete family histories, 60 (9%) could not be located and 25 (3.6%) refused to participate. The rate of renal failure in relatives of probands was compared to the rate of renal failure in spousal control families. Spousal controls were chosen because they have been shown to be less subject to recall bias and generally are similar to their spouses for environmental influences. Family histories were collected on 499 (85.4%) of the eligible spouses of probands. No spouses or

next of kin could be identified for 65 (11%) of the probands and 20 (3.4%) of potential controls refused to participate.

To determine the original cause of renal disease in the probands the medical records were reviewed. The information gathered was reviewed by a single clinical nephrologist who was blinded to the identity of the patient. Diseases with a Mendelian pattern of inheritance accounted for ESRD in 8.4% of the cases, 4.5% being autosomal dominant polycystic kidney disease, 2.5% Alport's syndrome and the remaining 1.4% to other genetic diseases. This group of cases was excluded from the subsequent familial risk analysis. Glomerulonephritis was the renal diagnosis in 25% of the probands, diabetes mellitus in 20%, unknown in 14%, other in 12%, interstitial in 11%, hypertensive sclerosis in 5% and multiple causes in 4%.

Primary outcomes were defined as a positive family history of renal failure associated with renal replacement therapy in a first, second or third degree relative of a proband or control. In the group without a Mendelian pattern of inheritance, 28% had a first, second or third degree relative with renal failure associated with death or requiring dialysis versus 15% of controls. 1.2% of first degree relatives of probands developed renal failure compared to 0.4% of first degree relatives of controls (OR=3.0, 95% CI: 1.7-5.2). No difference was observed in risk for second degree relatives, but a highly significant increased risk was observed for third degree relatives of probands (OR=2.1, 95% CI: 1.2-3.4). The highest rate of affected first degree

relatives occurred in relatives of probands with hypertensive nephrosclerosis (2.3%), diabetes mellitus (1.6%) and interstitial disease (1.6%).

The second control group utilized was the provincial population. The proportion of relatives of probands registered with the Canadian Organ Replacement Registry (CORR) was compared to the rate of the general population. The provincial incidence of ESRD, registered with CORR, from 1981-1993 was 79/million, excluding 8% of patients with Mendelian inherited disease. The comparable rate of ESRD in first degree relatives of probands without Mendelian inherited renal disease was 297/million almost four times the provincial rate. The comparable rate for first degree relatives of controls was 135/million.

Conclusions: We conclude that not only is the contribution of Mendelian inherited disease to ESRD high, but there is also an increased risk of renal failure in first degree relatives of probands without Mendelian inherited renal disease in a Caucasian population.

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PREAMBLE

The nephrology division in the Medical School at Memorial University has an ongoing interest in the clinical epidemiology of genetic renal diseases. Casual interview of patients at one of the Endstage renal disease (ESRD) treatment centres in Newfoundland indicated a number of renal failure patients without a known Mendelian disorder who had other relatives receiving some form of renal replacement therapy. A descriptive study was therefore undertaken in order to determine the prevalence of ESRD among patient's family members. This study had the overall goal of establishing whether there was a familial tendency for common forms of renal disease that had not previously been considered to have a genetic basis.

I became involved in the study during employment as an undergraduate student in the summer of 1994. The nephrology research unit had already made valuable research gains on such Mendelian renal diseases as Polycystic kidney disease and Bardet-Biedl syndrome. Drs. Patrick Parfrey, John Harnett and John Bear were the coordinators and my supervisors throughout the research. The research was supported by the Kidney Foundation of Canada, Montreal, Canada and funded by Faculty of Medicine, Memorial. Collection of family histories and medical records was done by myself and research nurse Donna Hefferton, without whose valuable help the project would not have been completed. Diagnosis of primary renal disease

in probands was made by nephrologist, Dr. P. Parfrey. The computer and statistical program was set up by our computer consultant Dmitri Gerchikov. Statistical analysis was done by myself.

As part of my Master's work, the natural history of Bardet-Biedl syndrome was described and has recently been published in the *American Journal of Kidney Diseases* (O'Dea et al, 1996; Table E). A consequence of our research has been the collaboration with molecular biochemists Dr. William Davidson, PhD candidate Terry Young and Masters candidate Michael Woods who are undertaking molecular linkage studies of our families with Mendelian renal disease in the hopes of identifying the mutations responsible for the diseases.

Chapter 1

INTRODUCTION

1.1 Genetic Epidemiology

Medical genetic studies have largely concentrated on disorders which follow simple patterns of Mendelian inheritance, including genetic diseases caused by single gene mutations, such as cystic fibrosis. The mechanisms of many of these conditions are relatively well understood. These disorders are rare, however, affecting only a small proportion of the population. Many disorders that are both serious and frequent do not follow a simple mode of inheritance. These include diseases such as diabetes mellitus, heart disease and kidney disease which are responsible for the majority of mortality and morbidity in developed countries such as Canada. In many of these common diseases there is a definite familial tendency, the proportion of affected relatives being greater than in the general population, but less than would be expected on a simple Mendelian basis. One possible explanation is that liability to these disorders is multifactorial, reflecting an interaction of polygenic inherited liability with environmental influences on risk. The assumption with polygenic inheritance is that the number of genes is relatively large and that the contribution of each to liability is small (Mueller et al, 1995).

A search for familial aggregation often serves as an initial step in unravelling the genetic epidemiology of common diseases (Khoury et al, 1986; Wickramartrie, 1995). If a familial

aggregation is found, the next step is an attempt to discriminate among genetic and/or cultural factors that might be causing this clustering. Finally, if evidence of a role for genetic factors is found, analyses designed to test for special genetic mechanisms are performed (Susser et al, 1987; Wickramartrie, 1995) .

The search for genetic factors which influence risk of common diseases is made more efficient by advances in molecular genetics and statistical assessment of polygenic inheritance. This search has practical implications. Improved knowledge of the details of genetic liability to an increasing number of human diseases leads to refined diagnosis, new therapeutic approaches, and disease prevention (Vogel et al, 1979).

1.2 Endstage Renal Disease - prevalence and cost

Kidney failure is a major medical, economic and social problem for patients and their families. Treatment for irreversible chronic renal failure has increased dramatically in the last decade. The Canadian prevalence rate of treated ESRD, of 536 per million Canadians a year, is similar to other developed countries, although it is still lower than the US rate. Differences in prevalence rates between different countries is often difficult to interpret as it is generally unknown to what extent differences in referrals or acceptance for ESRD exist between countries. Thus a risk factor for treated ESRD may reflect an increased probability of developing kidney failure or an increased probability for being treated after kidney failure had occurred. The risk of selection bias is greatest

in populations in which the availability of ESRD therapy is limited. Newfoundland has the second highest rate in the country, with a prevalence rate of 642 persons per million. In 1993, the number of Canadians receiving some form of renal replacement therapy was 24,500, double that in 1983 (CORR, 1994). In the US, the prevalence rate in 1991 was 721 per million persons with an annual increase of 9% between 1980 and 1991 (USRDS, 1993). There are several possible explanations for the increased prevalence rate of ESRD. One likely explanation is that fewer patients are left untreated. There has also been an improvement in the life expectancy of many persons receiving ESRD therapy. The growing fraction of aged and diabetic persons in the population also contributes to an increase in incidence of ESRD. People are living longer and surviving competing risks, such as coronary disease, where they would have died before, making it more likely that they will go onto to renal failure.

In the US it has been estimated that the direct cost of ESRD therapy amounted to 8.6 billion dollars in 1991. Beside the monetary cost there is also the human cost of irreversible renal failure: marked reduction in life expectancy, increased morbidity and substantial loss in the quality of life as a result of the need for continuing therapy and loss of employment (USRDS, 1991).

1.3 Evidence for familial liability to ESRD

In the US disorders with a Mendelian pattern of inheritance account for 3.3% of cases who develop ESRD and in Canada the proportion is 6.3% (USRDS, 1994; CORR, 1993). This probably does not reflect a true difference in the amount of Mendelian cases with ESRD between the two countries as these proportions are dependent on the incidence and acceptance for treatment of patients with other diseases. This value is certainly an underestimate because detailed family histories may not have been obtained from many of the patients enrolled in the national registries. The genetic contribution to ESRD in the remaining majority of patients becomes an important question. Are genetic influences causing a greater susceptibility in developing renal failure. Two questions of practical importance arise: What is the risk of ESRD in first degree relatives? Is this risk higher than the population average?

In addition to ESRD occurring in families as part of a single gene disorder, accumulated evidence from family studies (Roy et al, 1971; Bader et al, 1974; Agar et al, 1980; Walker et al, 1982; Kikuta et al, 1983; Trannin et al, 1983; Julian et al, 1985; O'Connell et al, 1987; Rambausek et al, 1987; Ferguson et al, 1988; Seaquist et al, 1989; Pettitt et al, 1990; Steenland et al, 1990; Borch-Johnson et al, 1992; Noe, 1992; Quinn et al, 1992; Charasse et al, 1993; Freedman et al, 1993B; Nomura et al, 1993; Freedman et al, 1995A; Spray et al, 1995), HLA marker studies (Tolkoff-Rubin et al, 1978; Klouda et al, 1979; Sabatier et al, 1979; Katz et al, 1980; Kashiwabara et al, 1982; Nomoto et al, 1984; Welch et al, 1986; Naito et al, 1987; O'Connell

et al, 1987; Berthoux et al, 1988; Glicklich et al, 1988A; Haskell et al, 1988; Huang, 1989; Hiki et al, 1990; Li et al, 1992; Ogahara et al, 1992; Clark et al, 1993; Freedman et al, 1994A; Freedman et al, 1994B; Muller et al, 1995), racial comparisons (Sisson et al, 1975; Nostrand et al, 1982; Jenette et al, 1985; Berthoux et al, 1988; Hoy et al, 1989; Hughson et al, 1989; Hiki et al, 1990; Van Buynder et al, 1993; Jacobson et al, 1995) and animal studies (Weening, 1986; Brown et al, 1994) indicate a genetic variation in liability to such common renal diseases as diabetic nephropathy, glomerulonephritis, interstitial disease and hypertensive renal damage.

Investigation of familial aggregation using details from family pedigrees is a starting point for evaluating the genetic contribution to a disease. In addition to providing estimates of risks, familial aggregation studies may provide clues about the mode of inheritance. Racial variation in disease rates provide additional supportive data regarding the possible role of genetic factors in disease. Any differences in renal disease rates between different population subgroups may have a genetic origin, but may also reflect social class, culture or interactions of genetic susceptibilities and environmental exposures. It is difficult to disentangle the effects of these factors.

The association of particular Human leukocyte antigen (HLA) alleles with particular diseases offers evidence for genetic liability to several renal diseases (Silver, 1990). While some genetic traits are always associated with a particular phenotype, other traits represent genetic variation present in individuals which may confer increased susceptibility to certain diseases. Different HLA antigens

may exist in linkage disequilibrium with genes causing progressive renal scarring in patients with various renal disease types. There appear to be several disease susceptibility genes of the major histocompatibility complex that have been identified. These studies suggest that the involved HLA allele identifies susceptible individuals who will develop the disease only after appropriate environmental exposure. Some of the HLA associations with renal disease such as idiopathic membranous nephropathy, mesangioproliferative glomerulonephritis, IgA nephropathy and reflux nephropathy are equally as strong or stronger than those found with such well studied diseases as rheumatoid arthritis and diabetes (Clark et al, 1993).

The major aim of the present investigation was the establishment of a more accurate assessment of risk for the development of renal failure of probands arising from a defined Caucasian population. The major strengths of the current study compared to these previous investigations are the size of the population studied, the homogenous composition of the study population (Caucasian of Anglo-Irish descent), and the nature of the control groups utilized. Previous investigators employed age and sex matched controls selected from the general population or a hospital population. This investigation has used spousal control subjects which, as will be discussed, offer several potential advantages and also used the provincial population as a control.

1.3.1 Familial risks for specific renal disorders

1.3.1.1 Glomerulonephritis

A growing body of evidence suggests the possibility of a genetic susceptibility to glomerular damage. African Americans are four times as likely as white Americans to develop ESRD due to glomerulonephritis (USRDS, 1993). ESRD secondary to glomerulonephritis (GN) is increasing in frequency among native American Indians (Hoy et al, 1989; Hughson et al, 1989) and Australian Aborigines (Van Buynder et al, 1993). In an investigation of an island Aboriginal community, proteinuria, glomerular hematuria and ESRD were found to be familial (Van Buynder et al, 1993); the incidence of ESRD in this community was greater than in other communities living in similar environmental conditions. These investigators inferred that glomerular renal disease in this population resulted from environmental factors interacting with a strong genetic predisposition (Van Buynder et al, 1993). Familial risk for glomerulonephritis has also been described in the Zuni (Hoy et al, 1989) and the Navajo American Indians (Hughson et al, 1989). The Zuni have the highest rate of renal disease of all Indian tribes, over half of this renal disease is non-diabetic, presenting as mesangiopathic glomerulonephritis with asymptomatic hematuria in childhood, followed by proteinuria at a later age (Hoy et al, 1989). Genetic drift and high levels of consanguinity may explain the predisposition observed in this group (Hoy et al, 1987).

There are several reports of glomerulonephritis occurring in identical twins (Roy et al, 1971; Bader et al, 1974; Kikuta et al, 1983; Charasse et al, 1993). Bader et al (1974) reported identical twins

who had different clinical courses and renal histological findings of glomerulonephritis, providing support for genetics and the importance of environmental influence in the nephrotic syndrome. Caution should be used when interpreting twin studies. Often such case reports have little more than curiosity value. Structured studies of twin pairs, with known ascertainment, are necessary to begin to accurately infer genetic and environmental influences on liability.

A study on the prevalence of primary glomerulonephritis in relatives of patients with glomerulonephritis in Brittany, France (Charasse et al, 1993), found 7 families, of 480, with at least 2 other family members with biopsy-proven glomerulonephritis, including 4 familial cases of IgA nephropathy, 1 familial case of membrano-proliferative glomerulonephritis, 1 of membranous nephropathy and 1 focal segmental glomerulosclerosis pedigree. These familial cases included a father and child, sibling pairs and a pair of monozygotic twins. The results led the researchers to suspect shared environmental and genetic factors in the occurrence of primary glomerulonephritis. Investigators in Germany were surprised at the proportion of glomerulonephritis patients whose disease was familial; ten percent of their patients had another family member diagnosed with glomerulonephritis (Rambausek et al, 1987). When these families were investigated clinically, 10% of the patient's relatives were diagnosed with glomerulonephritis. While the majority (50%) of these relatives had glomerulonephritis of Alport's type (which is a single-gene condition), a substantial proportion had familial glomerulonephritis of non-Alport type. Twenty-two percent of the relatives had atypical glomerulonephritis forms, 18% IgA nephropathy and 1.9% focal

segmental glomerulosclerosis.

1.3.1.2 IgA nephropathy

There are suggestions of genetic variation in risk of IgA nephropathy. Familial clustering of IgA nephropathy has been described (Julian et al, 1985; Levy et al, 1987; O'Connell et al, 1987; Rambausek et al, 1987; Charasse et al, 1993). Levy identified 22 families with multiple affected instances of IgA nephropathy. A high prevalence is observed in Japan (Hiki et al, 1990), Australia (O'Connell et al, 1987) and France (Berthoux et al, 1988) and certain regions of the US, but not in other countries such as England (Sisson et al, 1975). The incidence of IgA nephropathy is much lower in blacks (Jenette et al, 1985) , Asians and American Indians (Jacobson et al, 1995) than in whites. Abnormalities of IgA immune response in apparently healthy relatives of patients with IgA nephropathy have been described (Egiclo et al, 1987). A Japanese study on relatives of persons with IgA nephritis found the risk of first degree relatives developing proteinuria was 10 times higher than the overall population risk (Nomura et al, 1993).

Data on associations between human leucocyte antigen (HLA) marker alleles and IgA nephropathy are varied and contradictory. Early studies reported HLA identical brothers affected with IgA nephropathy (Tolkoff-Rubin et al, 1978; Sabatier et al, 1979; Katz et al, 1980, Nomoto et al, 1984). An association of HLA-B27 and HLA-DR1 was found in African and white Americans with IgA induced glomerulonephritis (Freedman et al, 1994A). A HLA-B27 association has also

been found in Australian Aboriginals (O'Connell et al, 1987). HLA B35 was found to be relatively frequent in a large population of French patients with IgA nephropathy, and appears to be a risk factor for progression to ESRD (Berthoux et al, 1988). Several studies have reported an HLA-DR4 association in Japanese IgA patients with impaired renal function (Kashiwabara et al, 1982; Naito et al, 1987; Hiki et al, 1990) while a high frequency of HLA-DR12 is found in Chinese IgA patients (Li et al, 1992). Not all studies, however, have found HLA associations (Rashid et al, 1983; Julian et al, 1985).

1.3.1.3 Idiopathic focal segmental sclerosis and mesangial sclerosis

For idiopathic focal segmental sclerosis (FS) and mesangial sclerosis (MS) a genetically determined susceptibility is suggested by the occurrence of the disease in siblings, and through successive generations (Agar et al, 1980; Walker et al, 1982; Kikuta et al, 1983; Tejani, 1983; Trannin et al, 1983; McCurdy et al, 1987). Two of 27 patients with FS in Walker's (1982) study were sisters whose mother died of renal failure at a young age. The father of another of these 27 patients died of renal failure at a young age. In a third family, 4 members with ESRD had renal failure due to FS (Walker et al, 1982). A case report of one family found three out of four siblings had nephrotic syndrome with diffuse mesangial sclerosis, two of whom were a pair of identical twins (Kikuta et al, 1983).

There are reports that HLA-DR4 and HLA-A28 are associated with idiopathic FS (Glicklich et

al, 1988A). The HLA BW53 antigen allele was found to be increased in frequency among a group of FS patients whose renal failure was the result of drug abuse (Haskell et al, 1988). The sample sizes in these studies were small and they require confirmation with more extensive data. In a more recent analysis no HLA association was observed in a group of FS ESRD patients (Freedman et al, 1994A). A stronger indication for a genetic predisposition, possibly HLA-linked, comes from transplant studies of individuals with FS. There is a higher recurrence rate of focal sclerosis in transplanted kidneys of FS patients if the donated kidney comes from a relative compared to if the transplant comes from a cadaveric kidney (Zimmerman, 1979). A number of diseases associated with FS or FS-like lesions, such as Alport's syndrome, diabetes and essential hypertension, are known to bear an inherited or familial trait (Weening et al, 1986). Studies in the laboratory rat have shown that FS-like lesions develop spontaneously with aging in most laboratory rat strains, although a considerable variability in the severity of the lesions and the accompanying clinical symptoms is found among different strains (Weening et al, 1986). A gene on chromosome 1 has been suggested as causing glomerulosclerosis in the fawn hooded rat (Brown et al, 1994).

1.3.1.4 Membranous and acute poststreptococcal glomerulonephritis

There have been reports of membranous nephropathy (MN) occurring in a pair of monozygotic twins and five pairs of brothers (Sato et al, 1987). Multiple affected siblings has been reported in nine families with membrano-proliferative glomerulonephritis (Berry et al, 1981; Stutchfield et al, 1986). In one of these families membrano-proliferative glomerulonephritis occurred in four

brothers and a father. Analyses in several different ethnic groups reveal associations between membranous MN and HLA phenotypes, including HLA DR3 (Klouda et al, 1979; Welch et al, 1986; Huang, 1989; Clark et al, 1993; Freedman et al, 1994B), HLA DRB1 (Muller et al, 1995), HLA DR5 (Freedman et al, 1994B) and HLA DR2 in the Japanese (Naito et al, 1987; Ogahara et al, 1992). Sacks et al (1987) have identified unique restriction enzyme cleavage sites in HLA DR3-positive Europeans with mesangial glomerulonephritis, proving the presence of disease-associated DNA polymorphism. There have been suggestions that the epidemic of poststreptococcal glomerulonephritis observed in some families corresponds to recessive inheritance (Rodriguez-Iturbe, 1984).

1.3.1.5 Hypertensive renal disease

There is evidence of a familial risk for hypertensive renal failure. The risk of ESRD from hypertension was found to be 18 times higher in blacks than whites in an early study (Nostrand et al, 1982). Even when black and white patients are matched for prevalence, severity and age of onset of hypertension and diabetes, the relative risks compared to whites are still 5-6 times greater for blacks (Whittle et al, 1991; Powe et al, 1995). The occurrence of renal failure in a close relative was found to increase African Americans' risk of ESRD (Ferguson et al,1988). Freedman et al (1993B) found that hypertensive ESRD patients reported having another family member with ESRD more often than other ESRD etiologies; forty percent of hypertensive patients had a family history of renal failure. The angiotensin-1-converting enzyme has been suggested to increase the

risk of renal artery stenosis (Missouris et al, 1996). In addition , the HLA DR3 allele has been found to be increased among black hypertensive ESRD patients (Freedman et al, 1991).

1.3.1.6 Diabetic nephropathy

Diabetic nephropathy occurs in 35-40% of type I diabetics with disease of 40 years duration or greater. Increasing blood glucose levels, duration of diabetes and presence of hypertension are known risk factors for progression of diabetic nephropathy (Krolewski et al, 1985). This leaves much of an individual's risk unexplained.

Racial differences in the prevalence of diabetic renal disease implicate genetic factors in risk. The African American race appears to be the most important risk factor in predicting an individual's risk for developing subsequent diabetic nephropathy; this condition is 3-7 times more frequent in black patients compared to white patients (Nostrand et al, 1982). Mexican Americans and Native Americans also have a disproportionately higher incidence of renal failure from diabetes when compared to other racial groups (Pugh et al, 1988).

Several studies have sought to determine whether the excess diabetic ESRD could be explained by the disproportionate rates of diabetes and hypertension, and lower economic status of specific ethnic groups. Tierney et al (1985) found, after controlling statistically for the prevalence of diabetes, glucose levels, hypertension, heart failure and male sex, all of which are significantly

related to nephropathy, that the likelihood of increased serum creatinine levels was 92% higher in blacks compared to whites. Black diabetics are at a higher risk for diabetic ESRD than whites, particularly subjects with non-insulin-dependent diabetes (NIDDM), even when access to health care, socio-economic status, prevalence of diabetes and hypertension are controlled for (Brancati et al, 1992). As well, a 2.6 fold higher risk for overt proteinuria has been found in diabetic Mexican Americans compared to diabetic non-Hispanic whites when the effects of glycemic level, blood pressure, smoking and insulin are controlled (Haffner et al, 1989).

Several researchers have reported that the renal status of diabetic siblings best predicts the development of nephropathy in diabetic patients (Seaquist et al, 1989; Quinn et al, 1992; Borch-Johnson et al, 1992). In insulin-dependent diabetes, 83% of diabetic siblings of probands with diabetic nephropathy have evidence of diabetic nephropathy, compared with 17% of diabetic siblings of probands without nephropathy (Seaquist et al, 1989). Familial clustering of diabetic nephropathy has been observed in Pima Indians with NIDDM leading investigators to suspect that a susceptibility to develop diabetic nephropathy exists independently of the susceptibility to develop NIDDM (Pettitt et al, 1990). Pettitt's study (1990) of 499 diabetic family members of a group of NIDDM patients found that proteinuria occurred in 45.9% of the diabetic offspring if both parents had diabetic renal disease. When diabetic renal disease was present in only one parent the prevalence of proteinuria in diabetic offspring was 22.9%, and 14.3% if neither parent had diabetic renal disease. In a study by Freedman et al (1995A), 37% of NIDDM ESRD patients reported

having a first, second or third degree relative with renal failure compared to 7% of diabetic controls without ESRD. It has been hypothesised that susceptibility to diabetic nephropathy may be inherited independently of diabetes, and that those that develop renal complications will do so only in the presence of diabetes (Bennett et al, 1971; Barnett et al, 1986; Seaquist, 1989; Pettitt et al, 1990).

A genetic predisposition to hypertension or cardiovascular damage has been suggested as the risk factor for the development of nephropathy in diabetes. An increased risk of renal disease in diabetic persons was observed if hypertension or cardiovascular disease was present in the parents (Viberti et al, 1987; Krolewski et al, 1988; Earle et al, 1992; Stephenson et al, 1995). Earle et al (1992) studied 61 insulin-dependent patients with nephropathy and 61 insulin-dependent patients without nephropathy and found that 40% of the parents of diabetic nephropathy patients died from cardiovascular disease compared to 22% of parents of diabetic patients without nephropathy, this difference being significant. Further support for the hypothesis that predisposition to hypertension may play an important role in the susceptibility to renal disease comes from studies of red blood cell sodium-lithium countertransport activity (Krolewski et al, 1988; Mangli et al, 1988; Jones et al, 1990; Walker et al, 1990); there is increased activity in diabetic patients with nephropathy compared to diabetic patients without nephropathy.

Studies on ACE gene polymorphism and diabetic nephropathy give contradictory results. Barnas

et al (1995) found that the D-allele for the gene encoding the ACE inhibitors occurred more frequently in patients with diabetic nephropathy compared to controls. Not all studies have confirmed this finding (Bilo et al, 1995).

1.3.1.7 Vesicoureteral reflux

Primary Vesicoureteral reflux occurs infrequently among the black population suggesting that genetic factors play an important role in this condition (Burger, 1972). Since the recording of vesicoureteral reflux in identical twins by Stephens et al (1955) there have been a number of other such observations (Mebust et al, 1972; Hampel et al, 1975; Kier et al, 1983; Sirota et al, 1986) and a report of affected triplets (Hayden et al, 1984). The occurrence of vesicoureteral reflux in members of the same family and in different generations has also been documented in several studies (Breden et al, 1975; Dwoskin, 1976; DeVargas et al, 1978; Jerkins et al, 1982; Baily et al, 1984; Aggarwal et al, 1989; Noe, 1992; Peeden et al, 1992). These reports establish the presence of reflux as a potential familial or hereditary problem. The rate of vesicoureteral reflux in normal healthy children is estimated as being 0.4 and 1.8% (Baily, 1979). A much higher prevalence (11-52%) in siblings of patients has been found (Bredin et al, 1975; Dwoskin, 1976; DeVargas et al, 1978; Jerkins et al, 1982; Baily et al, 1984; Aggarwal et al, 1989; Noe, 1992; Peeden et al, 1992). A prospective study by Sirota et al (1986) on a highly selected group of 16 of these families found a sibling rate of 100%, but sixteen families were selected for investigation because, in each, two family members had previously been identified with reflux and 82% of the

siblings were symptomatic.

While the patterns of aggregation observed in these families suggest an inherited basis for reflux in a proportion of patients, no uniform inheritance pattern is observed. Miller et al (1972) and Lewy et al (1975) proposed an autosomal dominant mode of inheritance with variable expression of the gene and incomplete penetrance. X-linked inheritance has also been suggested (Middleton et al, 1975; Tobekin et al, 1964). Segregation analysis of data from 88 families most strongly supports a single major locus mode of inheritance (Chapman et al, 1985); the locus being dominant, with 45% of gene carriers affected. The occurrence of reflux among siblings has also been explained in terms of multifactorial - polygenic mode of inheritance. Age-related onset and interaction of hereditary factors with environmental factors such as infection provide a reasonable explanation for the variability in severity, type and transmission patterns reported among families (Burger, 1972; DeVargas et al, 1978; Jerkins et al, 1982).

1.3.1.8 Other renal disease types

In patients with urolithiasis, a positive family history is extremely common (McGeown, 1960; Resnick et al, 1968; Churchill et al, 1980; Ljunghall et al, 1985). Risk of developing renal cell carcinoma has been found to be increased in patients with a family history of renal cell cancer (Eng et al, 1993). Molecular studies have implicated a specific gene on chromosome 13 (Stein et al, 1995).

Chapter 2

RESEARCH OBJECTIVES

2.1 Introduction

A prerequisite to measuring familial risk of inherited renal diseases is precise estimates of the prevalence of these diseases. The prevalence of inherited renal disease will be underestimated if epidemiological investigation of families is not conducted.

2.2 Primary Research Objectives

1. To determine the frequency of ESRD due to diseases with a known Mendelian pattern of inheritance in Newfoundland.

2. To determine whether relatives of ESRD patients have a higher risk of ESRD, than relatives of control subjects.

3. To determine whether the rate of renal failure in relatives of ESRD patients, as confirmed by CORR registration, is higher than that in the general population.

4. To identify families with more than one affected member, without a single-gene disease, for further collaborative study.

5. To describe the natural history of diseases with single-gene inheritance identified in the

Newfoundland population, with particular focus on new data associated with Bardet-Biedl Syndrome.

2.3 Improvements in research design compared to earlier studies

This case-control study is the largest to date to examine the influence of familial factors on the risk of developing ESRD. Furthermore, it focuses on a population which arises from a homogenous genetic population. Drawing proband and control subjects from a large population-based case control study with systematic ascertainment of all cases and controls, including deceased patients, represents an improvement over previous family studies, which ascertained their study population from a single hospital setting over a short period of time (Steenland et al, 1990; Freedman et al, 1993B; Spray et al, 1995). This study sought complete ascertainment over a 6 year period (1987-1993). Over 1,000 probands and controls, from three health care facilities which together treat all ESRD patients in Newfoundland and Labrador, were identified. This avoids possible bias arising from whatever selection factors lead individuals to utilize a particular treatment centre. The patients represent different geographic, educational and social backgrounds.

Several features of Newfoundland make it an attractive place to do genetic research. The outport society, with its tendency to large families, geographic remoteness, relative immobility and strength of family ties provide for investigation of relatively large, stable families of several generations. The uniform living conditions in these isolated communities may facilitate studying the relative effects of genetic variation versus environmental differences. Opportunities for founder effect and relatively high levels of consanguinity increase the likelihood of observing rare or previously unrecognised dominant and recessive conditions. Consequently, it has been possible for many genetic disorders

to be investigated in Newfoundland and Labrador.

Previous studies elsewhere did not ascertain prevalence of ESRD among relatives of different generations (Steenland et al, 1990; Freedman et al, 1993B; Spray et al, 1995). Their risk ratios were based on the proportion of index cases that had a positive family history of renal failure without any consideration of family size. The present study calculated risk ratios based on the proportion of relatives having renal failure, as well as on the proportion of index cases with a positive family history. Risk ratios based on proportion of index cases with a positive family history can be influenced by family size. The increase in numbers in larger families will increase the proband's chances of having a family member who has renal failure. Risk ratios based on proportion of index cases who have a positive family history will thus overestimate risk. Calculation of risk ratios based on proportion of relatives positive for a family history of renal failure provides a more accurate assessment of risk. This calculated risk ratio is less influenced by family size.

Collection of family data for both spouses and controls was based on several interviews with several family members; previous investigators appear to have gathered information during a single interview with only one family member.

The use of spouses as a control group represents an additional improvement over previous

investigations by reducing recall bias and allowing fewer differences in environmental exposures (Coughlin, 1990; King, 1992; Drews, 1993; Wickramaratare, 1995). Previous investigators employed age and sex matched controls from the general population or a hospital population. It is believed that use of a random sample from the population provides less statistical power to detect the presence of familial aggregation than those performed with a control group of relatives of probands without the disease of interest (Wickramaratare, 1995). Finally, this study had a low refusal rate for both the proband and control groups.

Chapter 3

STUDY DESIGN

3.1 Design

Two epidemiological approaches used to determine if familial aggregation occurs are: a) to compare disease frequency in relatives of cases versus controls, and b) to compare the frequency in relatives of probands to the rate in the general population (Khoury et al, 1986).

To determine if a familial clustering of renal failure was present in this study population, a case-control study was employed. In this type of observational study persons with a given disease (probands) and persons without the given disease (controls) are selected and compared for a particular risk factor (Sackett, 1991). In the present study, probands were individuals who had ESRD, and the controls were the proband's spouses who were from, in general, the same environment and would be expected to be aware of the nature and significance of ESRD. To determine whether family history played a role in the development of ESRD, the incidence of ESRD in relatives of probands and controls was compared.

The prevalence of renal failure in the proband's relatives was also compared to that in the general population; that rate being the provincial rate reported to the national registry for ESRD therapy

(CORR). Attempts were made to verify reports by family informants of relatives being affected with ESRD after 1981 with CORR.

3.2 Recall bias and other disadvantages of case-control studies

While the case-control studies generally are relatively inexpensive, can be completed in a relatively short period of time and allow a description of incidence of disease, they are susceptible to several sources of bias (Sackett, 1979). A major threat to the validity of case-control studies is recall bias, which occurs when "associations are distorted or created because case informants report differently from controls" (Drews et al, 1993). Differential recall will lead to spurious positive associations or bias away from the null (Copeland et al, 1977; Barron et al, 1977; Raphael, 1987; Drews et al, 1990). The recall of a positive family history could depend markedly on whether the informant is a case or control. Sackett (1979) refers to this type of recall bias as family information bias and the effect upon the relative odds may be profound. For instance, in a study on familial clustering of rheumatoid arthritis the presence of a positive history of rheumatoid arthritis in a parent has been shown to be reported more often by an offspring affected with rheumatoid arthritis than a non-affected offspring (Sackett, 1979). Past exposure to possible risk factors may be more vivid and meaningful to affected persons because of their familiarity with the disease and because of repeated interviewing by nurses and physicians for their family history (Coughlin, 1990). Probands may have previously questioned other relatives for information on the disease when they themselves became ill. Conversely, controls are less sensitised thereby decreasing the chances of recall.

Other factors which may contribute to differential recall include motivation and meaningfulness. The motivation to participate and answer correctly may be greater for patients than for controls (Raphael, 1987). The result will be an inflation of risk estimates. Other potential problems of case-control studies are faulty interpretation of the question by the interviewers (Coughlin, 1990) or extensive probing amongst the probands during the interview for the desired answer. An additional source of error is pedigree error. This may be due to unknown non-paternity, unreported illegitimacy and adoptions. Pedigree errors usually result in an underestimate of familial risk when genes are involved because they substitute random individuals for related ones (Weis, 1982). Case-control studies also make the implicit assumption that as a rule, risk figures are constant in time and space. Considering the environmental changes this assumption is not necessarily true (Vogel, 1979).

Recall bias can be minimized by using controls who are likely to have considered the disease to the same extent as probands (Coughlin, 1990; Drews et al, 1993). Wickramatrie et al (1995) found that using randomly selected control will be less efficient, statistically, than using a control group of relatives without the disease. The use of spousal control subjects offers several potential advantages, including a lower tendency to recall bias and fewer differences in environmental exposures between probands and controls. Spouses of patients with renal failure are likely to have considered a given family disease to the same extent as probands and are therefore are less prone to recall bias ("awareness bias") then the general population (Coughlin, 1990; King, 1992; Drews,

1993). Several investigators have also found that differential recall does not always bias estimates of associations away from the null even when probands are more likely to accurately report information than controls (Zierler et al, 1985; Mackenzie et al, 1989; Drews et al, 1990).

Newfoundland's setting and history allow for an ideal location for genetic investigation due to the isolation and immobility of it's communities. These features also may predispose to genetic relatedness between spouses. This might tend to dilute any association between family history and renal disease due to polygenic inheritance.

Despite the limitations of case-control family studies these studies provide an economic practical way to initially examine family risk for common diseases. This study attempts to reduce recall bias through: a) the selection of a control group which is similar in age and environment and has had experience with renal disease and b) by repeatedly interviewing various family members. The primary outcome of interest in this study - ESRD - is a major event and likely to be remembered, particularly if dialysis is a consequence of the disease.

Chapter 4

METHODOLOGY

4.1 Study population

4.1.1 Ascertainment of probands

The study population was ascertained using Endstage Renal Disease (ESRD) registries and the Canadian Organ Replacement Registry (CORR) in the three Newfoundland hospitals where ESRD treatment is available. Both ESRD data bases were used to obtain a complete list as it was observed that there was some degree of underreporting (29.6%) to the CORR registry. The Health Sciences Centre (HSC) and Salvation Army Grace General Hospital (SAGGH) serve the eastern part of the island, including the Avalon Peninsula. Western Memorial Regional Hospital (WMRH) serves the population in the western part of the province. These registries record all new cases of ESRD, demographic data, diabetic and hypertension history, renal diagnosis, and date of first ESRD therapy treatment.

Eligible cases were defined as all adult male and female patients who were receiving ESRD treatment (transplantation, hemodialysis or peritoneal dialysis) in 1987 and any persons who began therapy between 1987 and 1993, inclusive. Verification of a diagnosis of ESRD was made clear based on medical records. Probands had to be at least 17 years of age at the time of the

investigation to be included in the study. Patients who died during this interval were included. The principal exclusion criteria was renal failure that was considered to be potentially reversible (n=150). This judgement was made by a clinical nephrologist who was presented with pertinent clinical data and was blinded to patient identity and outcome. The decision was based on the presence and degree of pre-existing chronic renal impairment and the nature and severity of the process leading to the need for dialysis.

This study population represents the entire adult Newfoundland and Labrador population with ESRD during this period. Because not all ESRD patients were registered with CORR (Table 1) some WMRH patients may have been missed because they were identified mainly through CORR registries. This number should be relatively small, however, as many of the WMRH patients also received treatment at the HSC. Also missed are persons who had progressed to renal failure but refused or were turned down any form of ESRD treatment, because these patients would not have been registered with hospital or CORR registries. It is unknown what this number is.

4.1.2 Classification of Renal Disease in the Proband

The medical records of all eligible patients were reviewed, including records for all hospital admissions up until the diagnosis of ESRD. Pertinent information from clinic records, consultation letters from nephrologists and corresponding letters from physicians were reviewed. If the diagnosis was not specified at the time ESRD therapy was started, subsequent medical admission

records were reviewed. Information obtained on each patient included: discharge summary, history of hypertension (if present, length and any medication), history of diabetes mellitus (if present, duration, type of diabetes, presence of retinopathy or any other diabetic complications), history of tuberculosis, history of renal stones or urinary tract infections, first diagnosis of renal disease, age at first ESRD therapy, discharge reports, renal ultrasound reports, biopsy reports, voiding cystogram, renogram, blood and urine tests, autopsy reports and family history. Any additional medical data relevant to underlying renal disease was also reviewed.

The information gathered was reviewed by a single clinical nephrologist, blinded as to the identity of the patient, and each proband was allocated into one of the following nine diagnostic categories based on defined clinical criteria:

1) Glomerulonephritis - renal biopsy proven or urinary protein excretion of more than 3g/24hr and presence of red blood cell (RBC) casts or 2+ hematuria or greater. Patients with bilateral, smooth, small kidneys and proteinuria are not allocated to this group nor are patients with glomerulonephritis secondary to other causes such as lupus, polyarteritis nodosa, Goodpasture syndrome, Henoch Schonlein purpura, Alport's syndrome. The major forms of glomerulonephritis included are IgA nephritis, post-infective, membranous, membrano-proliferative and diffuse proliferative (including crescentic) glomerulonephritis.

2) Diabetes Mellitus -

Type I - developed diabetes before the age of 35, duration of 10 years or more and remaining on insulin therapy since the diagnosis of diabetes; presence of retinopathy.

Type II - receiving oral agents as therapy, or developed diabetes after the age of 35, with duration of 10 years or more. Patients with a concomitant history of hypertension were retained in this category.

3) Interstitial nephritis - urinary protein excretion of $\leq$ 2g/24hr in the presence of a risk factor for interstitial disease; e.g. renal tuberculosis, nephrolithiasis (bilateral renal stones sufficiently extensive to cause renal failure), vesico-ureteral reflux nephropathy, pyelonephritis in the absence of obstruction or nephrolithiasis .

4) Hypertensive nephrosclerosis - essential hypertension for at least 10 years, urinary protein excretion of $\leq$ 2g/24hr and either hypertensive retinal disease or electrocardiograph evidence of left ventricular hypertrophy. Three probands with extensive vascular disease as primary cause of renal failure were allocated to this group because they had preexisting hypertension. These patients had greater than 60% stenosis in both main renal arteries, or the single renal artery of a patient with a single functioning kidney.

5) Multiple Causes - presence of two or more factors reasonably inferred as leading to ESRD; e.g. uninephrectomy and some other disease.

6) Other - includes patients with diverse etiologies. This category includes: a) obstruction; b) autoimmune disease; c) iatrogenic and; d) other causes. Obstructive uropathy includes congenital malformations, such as posterior urethral valves, pelvic-ureteric obstruction, spina bifida or neurogenic bladder, and acquired obstruction. Scleroderma, lupus, vasculitis, polyarteritis, cyroglobulinemia and pulmonary renal diseases, including Goodpasture syndrome and Wegener syndrome, are allocated as autoimmune diseases. Iatrogenic causes for renal failure include ESRD secondary to radiation damage, analegisic abuse, cholesterol embolism and antibiotic nephrotoxicity. Other less frequent causes of ESRD include hemolytic uremic syndrome, sarcoidosis and amyloidosis.

7) Polycystic Kidney Disease - autosomal dominant polycystic kidney disease as confirmed by imaging and appropriate family history.

8) Other Genetic Diseases- patients with recognised single gene conditions including X-linked dominant and other forms of Alport's syndrome; autosomal recessive Bardet-Biedl syndrome, 2,8 dihydroxyadenine syndrome, hyperoxaluria, Caroli syndrome; and autosomal dominant Charcot-Marie Tooth disease.

9) Unknown - absence of clinical evidence in medical records sufficient to ascribe an etiology. The majority of patients in this category first presented to a physician at the point where renal disease had already progressed to the point of irreversible severe renal failure and elucidation of the original renal pathology was not possible.

The attribution of a primary diagnosis for ESRD is quite difficult and subject to opinion. The broad nature of the classification may lump disorders with different genetic predispositions. Consequently the heterogenous nature of the disorders in each classification group may confound identification of genetic risk. A substantially deeper study would be necessary to overcome this problem.

4.2 Control population

The choice of an appropriate comparison is essential to ensuring valid results in case-control studies (Drews et al, 1993). An association is necessarily more convincing if it is found when different types of control groups are used (Kelsey et al, 1986). The present study utilizes two control groups with which to compare ESRD frequency in patients' families. It compares the prevalence rate of renal failure in the relatives of probands with the prevalence rate of renal failure in relatives of the probands' spouses. It also compares the prevalence of renal failure among probands' relatives with that in the general population, as documented for the province in the Canadian Organ Replacement Registry (CORR).

4.2.1 Primary control group - Ascertainment of spousal families

Potentially, spouses can make up a population similar in age and environment to probands. Spouses share the same geographic and family environment as probands, but are not usually close biological relatives. A finding of increased renal disease frequency in biological relatives of cases compared to biological relatives of spouses argues for genetic factors in liability. Use of spousal controls should reduce recall bias. While the spouse is not directly affected with renal failure and may not be as aware of a family history of renal disease compared to the proband, the spouse's familiarity with ESRD and its severity may make spousal controls more likely to take note and remember relatives who have had a history of renal complications, especially renal failure,

compared to members of the general population, who are much less likely to have had exposure to renal disease. Spouses can also be expected to be motivated and cooperative in their participation in a research study.

For probands without a spouse, a spouse of a sibling who gave information on the proband's family history was used. These individuals were selected in a random fashion to avoid bias. In a few cases the control was a random friend of the proband who was willing to participate.

4.2.2 Secondary control group - CORR registry rate

The Canadian Organ Replacement Register (CORR) is a national information system on organ failure which began in 1981. The data base system was established in an attempt to register all patients who commenced ESRD treatment since it's initiation, irrespective of age. Data collected on patients who began treatment prior to 1981 is incomplete. Presently, CORR estimates that registered patients comprise 94% of all dialysis patients and 75% of all kidney transplant patients (CORR, 1993). A lower registry rate (70.4%) was noted in this ESRD population. This discrepancy is most likely the result of incomplete notification of the CORR registry regarding patients with ESRD in one of the Newfoundland ESRD treatment centres. The CORR registration in this centre was 50%. In addition, enrollment in CORR was incomplete in its initial years.

By comparing the proportions of relatives of probands and controls registered with CORR , it is

possible to determine if the prevalence of renal failure is higher in relatives of probands. The CORR registry also allows determination of whether the rate of renal failure in relatives of probands is higher than that in the general population, as estimated by CORR, compared to the rate of renal failure in relatives of controls compared to the general population. The population prevalence rate is almost certainly an underestimate of the true burden of ESRD due to the poor enrollment with CORR and a failure to register patients who refused, were turned down by physicians for ESRD treatment or have died prior to treatment initiation.

4.3 Collection of data on relatives

Each proband's family was sent a letter detailing the purpose of the study and the information required (Appendix A & B). Informed consent was obtained from all study subjects, and the study protocol was approved by the University's Human Investigation Committee. For families agreeing to participate in the study, pedigrees extending to third degree relatives were obtained using telephone or person-to-person interviews by either a trained research nurse or a trained research assistant. Initial contact was with the proband. If the index case was deceased, another family member (e.g. spouse, offspring, or sibling) was contacted as indicated on the patient's medical record. If the first contacted family member could not provide all information, one or more additional family members were contacted following permission from initial family informant. Interviews generally lasted less than 30 minutes, and the majority of families were contacted 2-3 times for information. Interviews were conducted between March 1, 1993 and February 1, 1996.

Information obtained on each family member included sex, actual or approximate date of birth, history of hypertension (if present , age of onset and use of medication), history of diabetes mellitus (if present, age of onset and treatment regime), history of known renal disease, including renal failure.

If a family member was reported as having ESRD, information on diagnosis, age of onset for ESRD, treatment hospital and CORR registration was sought. For most cases this information was

not available. It was inquired whether the relative was alive or deceased; if deceased, information on cause, year and age at death was sought. If the year of death was unknown, it was ascertained whether the relative had died prior to 1980. When a relative's current age or age at death was unknown to family informants, their ages were estimated approximately from the known ages of siblings in that generation. It was inquired whether any family members were blind and/or deaf because several genetic disorders with these manifestations have renal disease as an additional feature. It was inquired whether any relatives had Bright's disease or dropsy. Bright's disease was a common term used to describe a collection of kidney diseases characterised by edema. Historically, Bright's disease was a unifying term for kidney failure (Perneger, 1995). Dropsy refers to a general accumulation of fluid which may or may not be related to kidney dysfunction and, historically, was more often used to describe individuals with swelling secondary to other medical conditions such as heart failure. Information was sought on infant deaths, stillbirths, half relatives, twins and consanguinity. The community of birth of both proband's and spouse's parents was also acquired.

A checklist was followed to ensure consistency in the response (Appendix C).

Chapter 5

OUTCOME MEASUREMENTS AND ANALYSIS

5.1 Primary Outcome

The primary outcome of interest was defined as a positive family history of renal failure associated with dialysis or death in a first, second or third degree relative of a proband or control. First degree relatives include siblings, offspring and parents. Second degree relatives are aunts, uncles, nieces, nephews and grandparents. Cousins, great uncles and aunts, and great grandparents are third degree relatives.

5.2 Definition of primary outcome in relatives

A family member was considered to be positive for ESRD if he or she had undergone ESRD treatment (transplantation, hemodialysis or peritoneal dialysis), regardless of the underlying cause of ESRD or duration of treatment. In general, reports of renal failure in relatives were not verified from the family member's medical records. Records were, however, examined for family members presently receiving dialysis therapy at WMRH, HSC, SAGGH, but who were not probands because they began ESRD therapy after 1993.12. Because the medical records of affected family members were not reviewed, causes of their renal failure could not be determined. Although no effort was made to determine whether the renal failure was acute or chronic, this should not

influence the risk estimates found for probands and controls because the decision to label a relative positive for renal failure, whether acute or chronic, was made equally between probands and controls by a physician who was blinded as to whether the relative was that of a proband or control. This would also not influence the comparison rate to the general population as only those relatives who were registered with CORR were used for this comparison. Informants were asked whether they knew the cause of renal failure in relatives; in the majority of cases this information was not known.

The decision to label a relative as having died from renal failure was based on accumulated evidence from the interview as to whether renal failure was directly associated with death. While although this method is partial to inaccuracies due to the subjective nature of the decision, the resolution was made by a single nephrologist who was blinded as to whether the affected family member was a proband's or a control's relative thereby equalizing the bias between the two groups. Many of the family members who were reported to have had renal failure died before dialysis became available and easily accessible in Newfoundland, about 25 years ago. If a relative was reported to have had renal failure an attempt was made to confirm the report with another family member. For family members who began ESRD treatment after 1980 confirmation was sought using CORR registries. However, because not all ESRD patients are registered with CORR, any family member who required ESRD therapy after 1981, whether registered with CORR or not, was considered ESRD-positive, if the information was confirmed by another family member. Any

relative who was scheduled to commence ESRD therapy but died before beginning treatment was considered positive for ESRD. Relatives were considered positive for ESRD if their cause of death was indicated as Bright's Disease. Any available information regarding length of renal disease, symptoms (flank pain, hematuria, edema), treatment, hospitalizations, surgeries and autopsy reports were acquired and used in the interpretation. The actual medical records of family members were not reviewed by an investigator; instead, family informants provided such details. Relatives who died with renal cell carcinoma and renal tuberculosis were considered to have ESRD only if they progressed to ESRD treatment.

In some instances, informants recollections of renal failure causing death of a relative were contradictory. In these instances the outcome was classified as possible renal failure and not analyzed for risk estimates. Probands had 41 relatives who were labelled as having possible ESRD. Of these 14 were first degree relatives, 21 were second degree relatives and 6 were third degree relatives. Fifteen relatives of controls were labelled as possibly having renal failure.

5.3 Statistical analysis

5.3.1 Demographic data and measures of risk

Demographic data collected included gender, age of onset for renal failure. Gender differences in age of onset of renal failure were tested using student's unpaired t-test. A p-value less than 0.05 was considered significant.

The statistical significance of differences between relatives of patients and controls in rates of renal failure was tested using 2×2 contingency table and odds ratio (Appendix E) (Jaeschke, 1995). Relative risk estimates the magnitude of an association between exposure and disease and indicates the probability of developing the disease in the exposed group compared to the non-exposed group. A relative risk greater than 1.0 indicates a positive association. Because persons in case-control studies are chosen by virtue of their disease status it is not possible to calculate the risk of development of disease given the presence or absence of exposure. The relative risk was estimated by an odds ratio and a 95% confidence interval. When the lower confidence limit of the odds ratio was >1.0 the odds ratio was considered to be statistically significant. The equations used to calculate relative risk and odds ratio can be found in Appendix D.

5.3.2 Exclusion criteria for statistical analysis

Because this is an investigation of familial risk of non-Mendelian forms of ESRD, probands with a known Mendelian pattern of inheritance for their renal disease are excluded from analyses. These include all patients diagnosed with autosomal dominant polycystic kidney disease (n=30), Alport's syndrome (n=18), Charcot Marie Tooth disease (n=2), hyperoxaluria (n=2), 2,8 dihydroxyadenine disease (n=2), Bardet-Biedl syndrome (n=2) and Caroli Syndrome (n=1). One proband with ESRD due to interstitial disease is excluded from the analysis because this proband belongs to a family in which autosomal dominant polycystic kidney disease occurs. One proband with hypertensive renal failure is excluded because of the occurrence of renal failure due to Alport's syndrome in several first degree relatives. Including the family histories of these two probands would have spuriously elevated the prevalence rate of renal failure among relatives of probands. In four of the control families, a single-gene disorder leading to ESRD was present. Three of these controls belonged to families with a strong history of polycystic kidney disease and the fourth had several family members with Alport's syndrome. These four families are excluded from analysis.

5.3.3 Cases who refused to participate or could not be located

Probands (n=84) and controls (n=85) that could not be located or refused to participate were not analysed. It should be noted that for 6 of the eligible ESRD patients who could not be located or refused to participate, renal failure in another family member has been previously documented in the proband's medical record.

Chapter 6

PROGNOSIS OF MENDELIAN RENAL DISEASES FOUND IN THE NEWFOUNDLAND POPULATION

6.1 Introduction

Inherited renal disease was the cause of ESRD in 6.7% patients who started dialysis in Canada in 1993 (CORR, 1993). In the present study, Mendelian conditions were responsible for 8.2% of all cases receiving ESRD treatment between 1987 and 1993. By far the most frequent cause was autosomal dominant polycystic kidney disease (ADPKD), followed by Alport's syndrome and other rare genetic diseases such as Caroli syndrome, 2,8 di-hydroxyadeneine, Charcot-Marie-Tooth disease, hyperoxaluria, and Bardet-Biedl syndrome. The clinical course of several of these conditions is relatively well described; although that of Bardet-Biedl syndrome is not. This chapter will briefly describe the prognosis of the Mendelian diseases identified in this population.

6.2 Autosomal dominant polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is among the most common autosomal dominant diseases in the world, occurring in 1/1000 of the population. ADPKD was responsible for 4.5% of the Newfoundland ESRD population between 1987 and 1993. It involves cyst formation in the kidneys, liver, pancreas and spleen. Three forms of ADPKD have been identified,

involving mutations at different loci, which differ in onset of symptoms. It is estimated that 85% of ADPKD is due to mutations at the PKD1 locus (Reeders, 1985) and 15% due to PKD2 mutations (Peters, 1993). Subsequently, a few families have been described which are genetically distinct from PKD1 and PKD2 (Daoust et al, 1995; Almeida et al, 1995).

ESRD is the most serious manifestation of ADPKD. There is a variation in the clinical course of the different PKD forms. Comparison of PKD1 and PKD2 have shown PKD2 to be a milder condition (Parfrey et al, 1990). The natural history of PKD1 includes the development of renal cysts before the age of 30, with subsequent development of hypertension, followed by renal failure in the fourth and fifth decades. ESRD occurs about 10 years after the development of renal impairment. Life expectancy of PKD1 is approximately 67 years (Parfrey, 1993). In PKD2, the mean age of onset of ESRD in a Newfoundland population was found to be 68.7 years (Bear et al, 1992). Newfoundland data show no difference between males and females for onset of ESRD (Parfrey et al, 1990); however, larger studies have shown male gender to be associated with worse renal function (Gabow et al, 1992; Choukroun et al, 1995).

Hypertension is another complication of ADPKD, which may hasten renal deterioration (Gabow, 1992). Parfrey et al (1990) found hypertension occurred in 25% of children with cysts and in 62% of adults with cysts; similar to the findings of other investigators (Zeirer et al, 1988).

6.3 Alport's syndrome

Alport's syndrome is an inherited, hematuric, nonimmune glomerulonephritis characterised by progressive renal failure, in which neurosensory hearing loss and ocular abnormalities may also occur (Schrier et al, 1993). The syndrome is genetically heterogenous. The commonest mode of inheritance is X-linked dominant, less frequently it is autosomal dominant and rarely autosomal recessive. Patients with Alport's syndrome constituted 2.3% of ESRD probands in the present study; similar to the proportions found among transplantation patients at the Mayo clinic (Milliner et al, 1982).

The clinical course of Alport's syndrome differs for males and females. The renal prognosis is much more severe in males. Microscopic hematuria is present at birth, or shortly after, in almost all affected males. Renal function inevitably deteriorates and ESRD occurs roughly 6 years from onset of decline in renal function (Grunfeld, 1985). Males typically develop ESRD between the ages of 15 and 40 (Grunfeld, 1985). However, there are reports of cases where onset occurs at an earlier age (Grunfeld et al, 1973). There is a wide clinical spectrum of renal involvement in females. The majority have slight urinary abnormalities with no significant renal impairment. Female carriers can be asymptomatic. A few affected females have progressive nephropathy, developing ESRD between the ages 15 and 30 years (Grunfeld, 1985; Schrier et al, 1993) or earlier (Schrier et al, 1993).

Eighteen Alport's syndrome probands, from 10 families, were identified in the present study. 10 were male and 8 female. Age of onset of ESRD for males ranged from 18-67 years with a mean of 32.7 years. For females, the age of onset ranged from 18-60 with a mean of 35.0 years. The medical records were reviewed to confirm the diagnosis in all Alport's cases. No attempt was made to determine age of onset of symptoms in the affected siblings. Ongoing molecular genetic linkage analysis by Young et al (unpublished data A, Memorial) indicates a X-linked dominant pattern in 6 of these families, and has ruled out X-linked transmission in one family; in one other family the pattern of inheritance could not be determined. The remaining families could not be enrolled for linkage analysis.

6.4 Charcot-Marie-Tooth disease

Charcot-Marie-Tooth disease is a progressive motor and sensory neuropathy usually inherited in an autosomal dominant pattern (Thoene, 1995). Its global prevalence is estimated at 1/2,500 (Theone, 1995). Renal abnormalities have been observed as a complication of the disease in a few individuals (Gheradi et al, 1985; Lemieux et al, 1967). The present study ascertained a mother and daughter presenting with renal failure secondary to Charcot-Marie-Tooth disease at ages 28 and 16, respectively. A clinical description of these two patients has been published (Paul et al, 1990).

6.5 Hyperoxaluria (Oxalosis)

Hyperoxaluria is an autosomal recessive disorder characterised by excessive urinary excretion of oxalic acid leading to oxalate crystals and stones (Theone, 1995). Clinical manifestations usually first occur in childhood, but may occur in infancy or adulthood. Progressive renal damage occurs at a young age (Hockaday et al, 1964). Two male probands, from two separate families, were identified in this population. These individuals progressed to renal failure at 7 and 23 years of age. Their successful treatment with renal transplantation has been described (Paul et al, 1987).

6.6 2,8 Dihydroxyadenine urolithiasis

2,8 Dihydroxyadenine urolithiasis results from an autosomal recessive metabolic enzyme deficiency, for the purine enzyme adenine phosphoribosyltransferase (Simmonds et al, 1995). Approximately 90 families with the deficiency have been identified in North America, Europe, Japan and Australia. The true incidence of the disorder is unknown, because renal stones are not routinely analysed for the presence of dihydroxyadenine. The formation of these stones results in a wide diversity of clinical manifestations - symptoms can range from absence or mild to longstanding renal damage. Kamatani et al (1987) reported a mean age at onset of 24.9 years (ranging 8 months - 72 years) for symptoms such as hematuria, renal colic, dysuria and urinary tract infections. The disorder may lead to renal failure (Kamataui et al, 1987; Manyak et al, 1987; Glicklich et al, 1988B; Fye et al, 1993). Longstanding urolithiasis led to renal failure at ages 72 and 75, respectively, for a brother and sister who were identified as probands in this study (Gault et al, 1981) .

6.7 Bardet-Biedl syndrome

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive disease. Three distinct genes have been identified as causing this syndrome. Ongoing linkage analysis has indicated that a possible fourth gene may be involved (Young et al, unpublished data B).

The syndrome is characterised by dysmorphic extremities, retinal dystrophy, obesity, hypogenitalism in males and renal structural abnormalities (Green et al, 1989). Two probands, from separate families, had BBS as a cause of ESRD in the present population. Because the clinical course of this condition is not well described, 38 BBS patients and 58 unaffected siblings, from 21 families in Newfoundland, were prospectively studied as part of my Master's work has recently been published in the *American Journal of Kidney Diseases* (O'Dea et al,1996). A copy of this paper is appended (Appendix E).

BBS has an adverse prognosis, with early onset of blindness, obesity, hypertension and diabetes. 50% of patients were legally blind by age 18 and 100% by age 30 (O'Dea et al , 1996). Obesity is predominantly truncal and is present from early childhood (Green et al, 1989). Hypertension and diabetes occur early, with 50% of individuals becoming hypertensive by age 34 and 50% becoming non-insulin dependent diabetics by age 55 (O'Dea et al, 1996).

Structural renal abnormalities have been observed in up to 100% of patients reported in clinical series (Harnett et al, 1988). 25% of Bardet-Biedl patients had impaired glomerular filtration rate, and in the group with normal renal function, a further 25% had diffuse cortical loss on renal ultrasound. The earliest age of onset of chronic renal failure in these Newfoundland patients was 2 years. Other case studies have reported even earlier onset (Bluet et al, 1977; Tieder et al, 1982; Linne et al; 1987). Life expectancy was reduced in these individuals, with 25% dying by age 44. In the eight deaths observed in this cohort study, 3 resulted from ESRD and 3 were associated with chronic renal failure (O'Dea et al, 1996).

Chapter 7

RESULTS

7.1 Baseline Results

7.1.1 Proband population

Using CORR and hospital registries, 669 baseline ESRD patients were identified as eligible for the study. This group is representative of the entire Newfoundland population, encompassing all geographic areas of Newfoundland and Labrador. Family histories were collected on 584 (87.3%) of eligible patients, who are referred to as probands. Sixty (9.0%) patients could not be located, and 25 (3.6%) refused to participate.

The mean age of probands beginning ESRD therapy was 50.1 years, lower than the national average of 57 years in 1993 (CORR, 1993). The age range of first ESRD was 2-87 years of age. 32 of the probands began ESRD treatment before the age of 17. Mendelian disease accounted for 60% of these 32 probands. ESRD was more prevalent in males (62.9%) than females (37.1%), similar to other studies (Silliger, 1995). Males began ESRD at an earlier age than females. All except one patient were Caucasian. The majority of probands received ESRD treatment at the HSC (59.5%), followed by the SAGGH (28.4%) and WMRH (12.1%) (Table 1).

7.1.2 Canadian Organ Replacement Registry

70.4% (n=471) of eligible patients had been registered with CORR (Table 1). There was a low registration rate for patients at the SAGGH (46.8%). Patients at WMRH were identified primarily through the CORR registry, which may account for the high registration rate seen for this centre.

7.1.3 Control population

Family histories were collected on 85.4% (499/584) of the eligible spouses of probands. The majority of controls were female (59.1%) (as expected for spousal controls of a disproportionately male group). No spouse or any next of kin could be located for 65 potential controls (11.0%) and 20 (3.4%) declined to participate.

7.2 Primary Renal Disease in probands

7.2.1 Mendelian inherited diseases leading to ESRD

Table 2 classifies the cases of Mendelian diseases leading to renal failure identified in this study. These cases represent 8.5% of baseline patients, similar to the reported Canadian prevalence rate of 6.3% and higher than the reported US prevalence of 3.3%. Autosomal dominant polycystic kidney disease was the most common Mendelian renal disease noted, accounting for 4.5% of baseline patients and 52.6% of the Mendelian patients. This value is lower than that for Canada as a whole (6.1% in 1993), but higher than the US rate of 3.2% (CORR, 1993; USRDS, 1993).

It should be kept in mind that the quoted prevalence is that of treated ESRD. It is unknown to what extent differences in referral or acceptance for ESRD contributes to different prevalence rates of treated ESRD in various countries. This rate may be comparatively low in other countries as a result of inaccurate ESRD diagnosis. This inaccuracy could be the result of less extensive searching for a genetic cause for renal failure. The Newfoundland population has been extensively investigated for many genetic conditions owing to its ideal isolated, close knit population base. More comprehensive exploring of family histories by physicians who are aware of the genetic diseases present in this population may have lead to correct identification of patients with Mendelian disease. Patients with a Mendelian cause for renal failure are less likely therefore to be under reported in the data registries. Mendelian disease may also account for a smaller proportion

of ESRD as a result of increasing prevalence rates of diabetic and hypertensive renal failure. This increase in proportion of other renal conditions may obscure a true high incidence of Mendelian diseases leading to renal failure.

The rate of single-gene forms of renal disease, other than autosomal dominant polycystic disease in the study population was 4.0%; higher than the comparable rate of 1.6% of ESRD cases in Canada and 0.7% in the USA (CORR, 1993; USRDS, 1993). Alport's syndrome is the second most common cause of Mendelian renal disease in the study population, accounting for 2.7% of baseline patients and 31.6% of Mendelian patients. There were 9 probands with rarer forms of inherited disease leading to ESRD, including autosomal dominant Charcot-Marie-Tooth disease (n=2) autosomal recessive Bardet-Biedl syndrome (n=2), 2,8 dihydroxyadeninine disease (n=2), hyperoxaluria (n=2) and Caroli syndrome (n=1). The detailed family histories failed to identify any families with previously unrecognized Mendelian inherited disease.

In that a major goal of this study was to gain a better understanding of genetic liability in non-Mendelian forms of kidney failure, the remainder of this chapter will exclude from analysis the 57 probands with Mendelian disease and discuss those renal disease cases which do not appear to arise from simple Mendelian inheritance. The family histories on 52 of Mendelian patient's spouses

were acquired and used as control family cases.

7.2.2 Probands without Mendelian disease

Simple Mendelian patterns of inheritance cannot be used to explain the majority of cases of ESRD in Newfoundland. 91.5% (n=612) of probands did not have a recognised Mendelian form of ESRD (Table 1). One of the forms of primary glomerulonephritis accounted for ESRD in 25.1% of our eligible patients. The next most prevalent cause of ESRD was diabetic nephropathy, accounting for 19.9% of eligible patients. In a large proportion of patients (14%) an underlying cause of ESRD could not be determined. These three categories accounted for 59.3% of eligible patients, similar to national registries (CORR, 1993; USRDS, 1993). The other causes of ESRD in study population are shown in Table 1. Family histories were collected on 532 of the baseline patients. Table 3 shows the diagnoses for these probands.

7.2.2.1 Glomerulonephritis

168 ESRD patients were identified in which the original renal disease was glomerulonephritis and family histories were completed in 158 of these cases (Table 1 & 3). Similar to the findings of other investigators, males made up a greater proportion of GN cases than females (p=0.0004) (Silbiger et al, 1995). The mean age glomerulonephritis probands entered an ESRD program was 44$\pm$ 18 years, with no difference between sexes. Age at first ESRD treatment ranged from 7 to 77 years.

7.2.2.2 Diabetes mellitus

Diabetics are classified as either type I (insulin-dependent diabetes mellitus, IDDM) or type II (non-insulin-dependent diabetes mellitus, NIDDM). Type II is ten times more frequent in the general population than type I, has a much stronger familial risk and is associated with insulin resistance (McPhee, 1995). Although both type I and type II diabetics suffer the complete spectrum of diabetic complications, the occurrence of these varies with each type. Approximately 30% of type I patients ultimately die of renal disease or require ESRD treatment (Anderson et al, 1983; Krolewski et al, 1985). Type II diabetic patients seem to develop renal failure less frequently than type I patients. Although ESRD occurs more frequently in type I than type II patients, type II patients account for a greater proportion of the ESRD population because of the greater prevalence of type II diabetics. Type II patients accounted for 11.2% (n=58) of the baseline patient population and 56.4% of the diabetic ESRD population. Type I patients constitute 8.7% (n=75) of baseline population (Table 1). We were able to collect family histories on 114 baseline diabetic ESRD patients (Table 3).

ESRD attributed to diabetic nephropathy affects more males than females (males: n=81; females: n=52). Diabetics with type I diabetes progressed to renal failure at an earlier age than persons with type II diabetes. The mean ages at which diabetic type I and II patients commenced ESRD therapy were 39.5 years (range: 21-63) and 65.8 years (range: 50-87), respectively.

7.2.2.3 Interstitial disease

Interstitial disease accounted for ESRD in 10.5% (n=70) of baseline patients; it was possible to complete family histories on 61 of these patients (Table 1 & Table 3). Primary vesicoureteral reflux accounted for 45.7% (n=34) of probands with interstitial disease (5.1% of baseline patients). The majority of reflux patients were female (n=25) and the mean age of onset of ESRD for both males and females was 27.3 years. Nephrolithiasis was the cause for ESRD in 12 probands (1.8% of baseline patients), of whom 6 were male and 6 female. The average age of renal failure in these patients was 54.7 years (range: 32-74). No attempt was made to diagnose specific stone disorders. For 11 patients, tuberculosis was responsible for ESRD. Several patients had a history of renal tuberculosis leading to renal damage with other renal complications also predisposing individuals to ESRD. These patients were classified as having multiple causes of ESRD. In 13 cases, pyelonephritis was the cause.

Sixty-seven percent (n=47) of patients with interstitial disease were females (p=0.04). Patients with interstitial disease had the earliest age of onset of ESRD of study categories, the mean being 36.5±18.4 years (range 6-76).

7.2.2.4 Hypertensive nephrosclerosis

Hypertensive nephrosclerosis accounted for 8.9% of the Canadian population receiving ESRD in 1993 (CORR, 1993) even though we included renovascular disease in this category. This small group subgroup had preexisting hypertension. The rate of renovascular disease as a cause of ESRD is probably rising, as the age of patients starting ESRD therapy increases. Our rate is probably an underestimate because we used a strict predefined criteria, which allowed assignment of patients to this category only if sufficient evidence was documented in the patient's medical record. Of the hypertensive group, 57% were female (p=0.0475). The mean age of onset for renal failure for this group was 69.8± 9.3 (range: 41-81) years of age, higher than for any of the other categories.

7.2.2.5 Other

Other types of renal disease were responsible for 12.4% (n=84) of baseline patients, for which 71 family histories were completed (Table 1 & 3).

Obstructive uropathy was responsible for ESRD in 3.7% (n=25) of the population. Underlying causes of obstructive uropathy in the baseline patients included neurogenic bladder (n=4), posterior urethral valves (n=5), spina bifida (n=3), spinal stenosis (n=2), prostatic hypertrophy (n=2), ileal conduit obstruction (n=1), bilateral megaureter (n=2), other congenital obstruction (n=3) and other acquired obstruction (n=3). Males comprised a significantly greater proportion of obstructive

ESRD patients than females ($p<0.005$). The mean age of renal failure for patients with obstructive ESRD was 41.5 (range: 4-77) years.

Among patients with an autoimmune etiology (n=17; 2.5% of the baseline patient population), systemic lupus erythematosis (n=5), pulmonary renal disease (n=5), scleroderma (n=2), vasculitis (n=2), polyarteritis (n=1) and cyroglobulinemia (n=1) were found.

Malignant diseases caused ESRD in 2.1% (n=14) of baseline patients.

Iatrogenic causes were responsible for a small proportion of cases (1.8%; n=12).

Miscellaneous causes of ESRD accounted for 2.4% (n=16) of baseline patients, and included such etiologies as amyloidosis (n=2) and hemolytic uremic syndrome (n=1).

The mean age for ESRD observed for patients within the Other category was 53±17; 66% were male.

7.2.2.5 Multiple Causes

Twenty eight baseline patients (4.2%) were identified as having more than one underlying determinant of ESRD (Table 1). Family histories were taken for 25 of these 28 patients (Table 3). The mean age of onset of ESRD in this group was 67.6±8.2 (range:52-81) years; with equal

numbers of males and females.

7.2.2.7 Unknown

The primary origin of renal disease leading to ESRD could not be determined for 14% (n=94) of baseline patients, similar to national registries (CORR, 1993) (Table 1). Family histories were collected on 77 of these patients (Table 3). Of these patients, 63.6% are male. The mean age of renal failure was 58.6±18.7 years, with a wide range (8 to 85 years).

7.3 Prevalence and familial risk of ESRD among relatives

Familial clustering of renal disease can be assessed in two ways. One is to compare the proportion of probands and controls reporting a family history of ESRD, regardless of the number of relatives affected. A better approach is to compare the proportions of first, second and third degree relatives positive for ESRD in proband control families.

7.3.1 Prevalence of ESRD in families

In this study, 11.9% (63/530) of probands had a first degree relative with ESRD, 9.1% (48/530) had a second degree relative with ESRD and 8.3% (44/530) had a third degree relative with ESRD (Table 4A). The percentage of probands having at least one relative with ESRD was 27.7% (147/530). Of controls, 2.8% (14/495) had a first degree relative with ESRD, 9.1% (45/495) had a second degree relative and 2.8% (14/494) had a third degree relative with ESRD;

14.7%(73/495) had at least one relative with ESRD. ESRD was significantly associated with the presence of renal failure in a first degree relative (OR: 4.63, p<0.0001; Table 4B) and third degree relative (OR:3.11, p=0.0003; Table 4B), but not for a second degree relative. The proportion of controls with a second degree relative with ESRD was surprisingly high.

7.3.2 Prevalence of ESRD among first, second and third degree relatives

1.20% of first degree relatives of probands had ESRD compared to 0.39% of first degree relatives of controls (Table 5A). A person's risk of developing renal failure leading to death or renal replacement therapy, if a first degree relative had renal failure, was nearly three times that of the controls (OR=2.96; 95% CI: 1.7-5.2) (Table 5B). No difference was observed when risks were compared for second degree relatives, but a significant increased risk was observed for third degree relatives (0.37% vs 0.18%; OR=2.06, 95% CI: 1.2-3.4; Tables 5A & 5B).

7.3.3 Familial risk of ESRD by cause of ESRD in proband

The occurrence of renal failure in relatives of probands according to the etiology of ESRD is shown in Table 6. The risk of ESRD in first degree relatives is highest in families of probands with hypertensive ESRD, interstitial renal disease and diabetic nephropathy.

7.4 CORR Results

The numbers of affected relatives of probands and controls registered with CORR is small, but the

trends are consistent with the family history data (Table 7). First degree relatives of probands were twice as likely to be registered with CORR as first degree relatives of controls. Second degree relatives were three times as likely and third degree relatives 2.4 times as likely to be registered. Overall, the risk of a proband having another relative registered with CORR was 3.2 times that for relatives of controls ($p=0.002$).

7.5 ESRD in families of probands and controls versus the general population

The Newfoundland provincial incidence of ESRD, as registered with CORR, from 1981-1993, was 79/million/year, excluding patients with a Mendelian disease (CORR, 1993). The rate for first degree relatives of probands was 297/million/year, almost four times the general population rate. The comparable rate for first degree relatives in controls was 135/million/year.

Chapter 8

IS THERE A FAMILIAL RISK FOR ENDSTAGE RENAL DISEASE?

8.1 Introduction

As a clinical entity, ESRD, is characterised by progressive renal dysfunction leading ultimately to kidney failure requiring persons to be maintained on dialysis or undergo transplantation for survival. The prevalence of treated ESRD has increased substantially over the past decade, making it an important chronic disease in terms of morbidity, mortality and monetary costs. Despite intense research, the etiology of ESRD remains to a large extent unknown. Disease following a classic Mendelian pattern of inheritance appear to be responsible for only a small percentage of cases (CORR, 1993; USRDS, 1993).

Evidence is accumulating to demonstrate the role of genetic factors and genetic-environmental interactions in the etiology and pathogenesis of common human diseases, including renal disease (Khoury et al, 1993). This is attributable in large part to advances in molecular biology, which have expanded our understanding of diseases at the molecular level and have facilitated investigation of the genetic nature of many common diseases.

Genetic susceptibility in common forms of renal disease is suggested by the appearance of clusters

of renal disease in certain families (Julian et al, 1985; Ferguson et al, 1988; Seaquist, 1989; Steenland et al, 1990; Borch-Johnson et al, 1992; Quinn et al, 1992; Freedman et al, 1993B; Spray et al, 1995). Because they do not appear to segregate in a classic Mendelian fashion, some forms of these diseases have been considered to have a multifactorial liability, reflecting the interaction of multiple genes with environmental influences.

8.2 Glomerulonephritis

Glomerulonephritis was the most common cause of ESRD identified in this population. Although a strong genetic liability to glomerulonephritis was not indicated in the present data - 0.76% of first degree relatives had ESRD - other epidemiological studies suggest a genetic predisposition to glomerular disease. 26% of glomerular ESRD probands in this study had a first, second or third degree relative with renal failure. This supports the results found by Spray et al (1995). In their study, 24% of Caucasian patients with glomerular ESRD had either a first, second or third degree relative with ESRD. Freedman et al (1993B) observed a smaller proportion of African-American glomerular ESRD patients having other family members also with renal failure; 14% had either a first, second or third degree relative with renal failure.

There is evidence of familial and genetic liability to specific glomerular diseases. Julian et al (1985) found that at least 18% of Kentucky-born IgA patients belonged to potentially related pedigrees.

The clustering of IgA nephropathy in these cases was not restricted to individuals sharing a common environmental factor. Familial IgA nephropathy has also been described in German (Rambusek et al, 1987), Japanese (Nomura et al, 1993), French (Charasse et al, 1993) and Australian Aboriginal (O'Connell et al, 1987) families. Certain HLA alleles are more frequent among patients affected with IgA nephropathy than controls (Tolkoff-Rubin et al, 1978; Sabetier et al, 1979; Katz et al, 1980; Kashiwabara et al, 1982; Nomoto et al, 1984; Naito et al, 1987; Julian et al, 1989; Hiki et al, 1990; Li et al, 1992; Freedman et al, 1994A; Pei et al, 1995); these antigen markers for IgA nephropathy varying with racial group. Recently, an increase in the AGT/TT polymorphism of the renin-angiotenin system was found to possibly increase IgA nephropathy in certain patients with IgA nephropathy suggesting a possible genetic determinant (Pei et al, 1995).

Family studies, animal investigations and HLA associations suggest a genetic role in focal segmental glomerulosclerosis (Zimmerman et al, 1979; Agar et al, 1980; Walker et al, 1982; Kikuta et al, 1983; Trannin et al, 1983; Weening et al, 1986; Glicklich et al, 1988A; Freedman et al, 1994A), mesangial proliferative glomerulonephritis (Kikuta et al, 1983) and membranous glomerulonephritis (Klouda et al, 1979; Welch et al, 1986; Naito et al, 1987; Huang et al, 1989; Ogahara et al, 1992; Clark et al, 1993; Sacks et al, 1987; Freedman et al, 1994B; Muller et al, 1995;).

Few of the patients of the present study were biopsied or fell neatly into one of the

glomerulonephritis classifications. The family data was analysed in aggregate to assess whether a predisposition to progressive glomerulonephritis occurred within the broad category of chronic glomerulonephritis disease. This heterogeneity may have obscured increased familial risks of specific types of glomerular diseases.

Several investigators have postulated that it is not a genetic susceptibility to develop glomerulonephritis as such, but rather a genetic predisposition to develop hypertension which in turn produces glomerular damage, with different genetic mechanisms predisposing individuals to become hypertensive on the one hand and develop glomerular damage as a result of hypertension on the other hand (Dworkin et al, 1986; Schmidt et al, 1990; Cusi et al, 1993). The interaction of these distinct genetic mechanisms with the rest of the genotype and the environment could manifest as distinguishable etiologies of ESRD. Schmidt et al (1990) proposed increased genetic risk of hypertension in glomerulonephritis patients based on the observation that the prevalence of hypertension in parents of glomerulonephritis patients was twice that for controls. Dworkin et al (1986) observed differential development of glomerular damage in different strains of hypertensive rats.

8.3 Hypertensive nephrosclerosis

In the present study, the group of probands who were classified as having ESRD secondary to hypertensive nephrosclerosis or renal vascular disease were found to have the highest familial risk of renal failure. 44% of the probands in this group had an affected relative with renal failure and 2.5% of their first degree relatives had renal failure. It is unclear whether the familial risk associated with hypertension and ESRD results from true hypertensive nephrosclerosis or from vascular causes of renal failure. It is well documented that there are significant racial differences in the incidence of ESRD from hypertensive nephropathy, with up to a 20- fold increased risk in the black population compared to the whole population (Qualheim, 1991; McLellan, 1989). This difference does not appear to be explained by race related differences in the prevalence, age of onset or severity of hypertension (Whittle, 1991). Within a black population evidence of familial risk for ESRD has been reported. Ferguson et al (1988) found that ESRD was associated with the presence of hypertension and chronic renal failure in first or second degree relatives of hypertensive ESRD patients in a subset of African American families. They suggested a heritable liability in this subset of families. In a study comparing prevalence rates of renal failure among relatives of 131 African Americans grouped by ESRD etiology and 115 age-sex and race-matched controls without ESRD, the greatest familial risk was found for patients with hypertensive nephropathy - 40% had a relative with ESRD (Freedman et al, 1993B). These findings were supported by Bergman and colleagues who determined that 24% of African American dialysis patients with clinically suspected hypertensive nephropathy as the cause of their ESRD had a first

degree relative with evidence of nephropathy, i.e. elevated serum creatinine or proteinuria (Bergman, 1996). Freedman et al (1991) found an increased frequency of HLA DR3 among black hypertensive renal failure patients.

It has been postulated that the kidney in blacks, compared to other races, may be more susceptible to hypertensive injury by virtue of renal vasculature (Levy et al, 1978), renal hemodynamics (Frochlich et al, 1983), renin levels (Dunne et al, 1973) and salt sensitivity (Dustan et al, 1987). Further, black hypertensives are more likely to be volume expanded (Lilly, 1976). Any of these traits may have an inherited basis, and hence a familial influence on the likelihood of ESRD.

The inclusion of renal vascular disease in this group of probands complicates the interpretation somewhat although only 3 probands had this diagnosis and they had preexisting hypertension. Atheromatous renal artery stenosis is associated with numerous factors such as smoking and hyperlipidemia. The role of genetic predisposition to renal artery disease has received relatively little attention to date. Missouris (1996) found a higher incidence of the ACE-D allele in patients with renal artery stenosis than in age, sex and race matched controls (OR:1.7, 95% CI:1.0-2.8). The presence of this allele has been previously reported to be associated with coronary artery re-stenosis in patients who have undergone coronary angioplasty (Ohishi, 1993; Kaski, 1994).

8.4 Diabetes mellitus

In the present study, 22% of the 114 diabetic ESRD patients reported having a family member with renal failure, and ESRD occurred in 1.34% of diabetic probands' relatives. If only probands with Type I diabetes were considered, 1.6% of first degree relatives were affected. A large proportion of diabetics do not experience the renal complications of diabetes - proteinuria, hypertension and progressive renal failure - despite long term diabetes (Krolewski et al, 1985). Risk factors for the progression of diabetic nephropathy include duration of diabetes for both IDDM and NIDDM (Nelson et al, 1988), blood glucose level (Krolewski et al, 1988; Kunzelman et al, 1989) and hypertension history (Parving, 1981); however, these risk factors leave much of the individual risk variation unexplained.

Genetic factors are known to play an important role in the etiology of diabetes mellitus of all types (Brosius et al, 1992; Leahy et al, 1993). Numerous studies have shown associations of IDDM liability with specific HLA and complement phenotypes (Brosius et al, 1992). Davies and colleagues (1994) have recently undertaken a genome-wide search for human type I diabetes susceptibility genes and have identified several chromosomal regions where such genes probaly occur. Separate inherited risks may exist for diabetes on the one hand and the development of diabetic nephropathy on the other hand.

A genetic basis for diabetic nephropathy may be inferred from associations with family history of

hypertension or cardiovascular disease (Viberti et al, 1987; Krolewski et al, 1988; Earle et al, 1992; Stephenson et al, 1995), with increased sodium-lithium counter transport activity (Krolewski et al, 1988, Mangili et al, 1988), with race (Tierney et al, 1985; Haffner et al, 1989; Brancati et al, 1992), and from familial clustering of diabetic nephropathy (Seaquist et al, 1989; Pettitt et al, 1990; Borch-Johnson, 1992; Brancati et al, 1992; Quinn et al, 1992; Freedman et al, 1995A). The most convincing evidence of this distinction comes from a study by Seaquist et al (1989) which found that 83% of IDDM siblings of IDDM patients with nephropathy also had diabetic nephropathy, as compared to 17% of IDDM siblings of IDDM patients without nephropathy. Nephropathy in the proband was the only factor predictive of the renal status of the diabetic sibling. Quinn et al (1992) found that renal impairment occurred in 53% of IDDM siblings of probands with nephropathy compared to 18% of IDDM siblings of diabetics without nephropathy. These results have been confirmed by Borch-Johnson et al (1992) who point out that the clustering may be due to genetic inheritance or sib's-similarities due to sharing the same environment. More recently Quinn et al (1996) examined concordance for diabetic nephropathy in families with multiple IDDM siblings. A siblings risk of diabetic nephropathy after 25 years of IDDM was significantly higher if the proband had persistent proteinuria then if they did not (71.5% versus 25.4%), which the authors suggest is consistent with an autosomal dominant effect.

Genetic susceptibility to NIDDM nephropathy has been investigated extensively in the Pima Indians, who experience both a high prevalence of NIDDM and early age of onset of NIDDM

(Pettitt et al, 1990). Proteinuria was observed in 45% of diabetic offspring if both parents had NIDDM and proteinuria, compared to 14% if neither diabetic parent had proteinuria. The risk remained after adjustment for blood pressure. The aggregation of renal disease in these diabetic families led Pettitt et al (1990) to suggest that susceptibility to renal disease was independent of the susceptibility to develop diabetes. In African Americans, Freedman et al (1995A) found that 37% of NIDDM ESRD patients had either a first, second or third degree relative with ESRD, compared to 7% of diabetic controls who did not have nephropathy.

It has been hypothesised that in diabetic nephropathy one or more genes, in combination with environmental factors, is required to produce clinically significant insulin resistance, while different gene(s) produce the renal manifestations of diabetes (Seaquist et al, 1986; Freedman et al, 1995A). Susceptibility to diabetic nephropathy is recognised only after prolonged diabetes. Seaquist et al (1986) proposed that the biochemical abnormality encoded by the "susceptibility gene" may involve the metabolism of the glomerular basement membrane; diabetics who remain free of nephropathy after years of disease must have a metabolism that can tolerate the stress of the diabetic state.

Another possibility is that susceptibility to diabetic nephropathy is due to genetic polymorphism of enzymes that determine the composition of the glomerular basement membrane and extracelluar matrix (Deckert et al, 1989). According to this theory, diabetic patients with genetic defects in the

regulation of the glomerular basement membrane and extracellular matrix glycoprotein, namely heparan sulphate proteoglycan, are at a greater risk of developing functional and structural abnormalities that lead to glomerulosclerosis and eventually clinical nephropathy (Deckert et al, 1989).

Several other genetic mechanisms for diabetic nephropathy have been suggested, including familial predisposition to hypertension as a risk factor. Several studies have found a greater prevalence of hypertension and cardiovascular disease in the parents of persons who later develop diabetic nephropathy (Viberti et al, 1987; Krolewski et al, 1988; Earle et al, 1992; Stephenson et al, 1995). Cusi et al (1993) propose additive action of genes causing high blood pressure and genes causing diabetes, together causing hypertension and glomerular damage. Other studies have not supported these findings (Mangili et al, 1988; Walker et al, 1990; Norgaard et al, 1991).

Because sodium-lithium counter transport activity is higher in patients with diabetic nephropathy compared to those without (Krolewski et al, 1988; Mangili et al, 1988; Walker et al, 1990), higher in a large subgroup of essential hypertensive patients (Canessa et al, 1980), and has a genetic basis, a genetic predisposition to hypertension or sodium-lithium counter transport activity could increase the susceptibility to renal disease in diabetic patients and could be considered as a possible marker for diabetic nephropathy. Microalbuminuric patients, a group at high risk of developing overt renal disease, have also been found to have increased rates of sodium-lithium activity (Jones et al, 1990).

Not all investigations support these findings (Jensen et al, 1990; Elving et al, 1992). Jensen's (1990) study found increased sodium-lithium counter transport activity for both diabetic patients with nephropathy and diabetic patients free of nephropathy, leading these authors to speculate that the increased activity may be a consequence of diabetes per se and not a risk marker for the development of nephropathy.

The D-allele of the ACE gene (Barnas et al, 1995) and HLA associations (Freedman et al, 1993A) have also been suggested as possible risk markers for diabetic nephropathy.

8.5 Interstitial disease

Genetic susceptibility appears to be relevant in the development of ESRD in probands with interstitial disease in the present study. 34% of probands with interstitial disease reported renal failure as occurring in a first, second or third degree relative and 1.4% of first degree relatives were affected. Other investigations have excluded interstitial patients from analysis, therefore comparisons are not possible. The strong familial clustering is probably driven by the diagnosis of reflux nephropathy. Vesicoureteral reflux patients accounted for over half of the interstitial group and evidence points to a genetic causation in the development of renal failure for this etiology (Bredin et al, 1975; Dwoskin et al, 1976; DeVargas et al, 1978; Jerkins et al, 1982; Baily et al, 1984; Hayden et al, 1984; Sirota et al, 1986; Aggarwal et al, 1989; Noe et al, 1992; Peeden et al, 1992).

Of interest was the observance of a proband having ESRD secondary to reflux, who had an identical twin. Although both the proband and the twin had the same type of reflux, only the proband had progressed to renal failure; showing the influence of genetic and environmental interactions in the progression of renal deterioration. There have been other reports of vesicoureteral reflux occurring in twins (Stephens et al, 1955; Mebust et al, 1972; Hampel et al, 1975; Kier et al, 1983; Hayden et al, 1984; Sirota et al, 1986).

8.6 Discussion

Like the etiology of ESRD itself, the problem of determining a genetic causation for renal disease is complex. Observations of familial concentrations in the present study may be due to genetic resemblance between relatives, to common environment or, as is probably the case, a mixture of both. Argument for multifactorial inheritance is plausible, as it is becoming more apparent that most diseases are not purely genetic or environmental in etiology, but depend on a complex interaction of the two. According to the multifactorial theory, ESRD is a complex final phenotype determined by many intermediate phenotypes and environmental influences. There is a multi-involvement of genes at independent loci which predispose the individual to kidney disease with environmental factors influencing expression of the gene. No mechanism of disease causation can be discerned by this approach, and the number and nature of the genes involved remain unknown. Evidence shows that even for Mendelian disorders there is considerable environmental influence on disease occurrence (Khoury et al , 1993). Many of these single gene conditions do not manifest clinically at all, unless triggered by specific environmental exposures (Grandjean et al, 1991). Even with diseases caused by environmental factors, genetic susceptibility may be involved in determining the ultimate clinical manifestations.

The major strengths of the current study compared to the previous investigations on the familial

basis of renal disease are the size of the population group studied, the homogenous composition of the study population and the fact that spouses were used as controls. This study demonstrates that first degree relatives of probands without Mendelian disease are at a three fold risk of renal failure compared to those of controls. The reported incidence of ESRD, registered with the national registries, is two times higher in the relatives of probands with ESRD of non-Mendelian etiology than in the general population. The renal failure rate of 297/million/year found in first degree relatives of probands is approximately four times higher than the ESRD rate for the entire Newfoundland population; higher than one would expect by chance. One must be cautious when interpreting these rates due to the incomplete registration rates with CORR on which the population rates are estimated. The incomplete enrollment however should be equal between relatives of probands and controls therefore allowing for a comparison between the two groups.

Second degree relatives of probands had the same rate of renal failure as second degree relatives of control subjects (0.4%). Third degree relatives of probands had a higher rate than third degree relatives of controls, but not higher than the first and second degree relatives of controls. The basis for this latter observation is not clear. It is possible that the rate in third degree relatives of controls is not actually as low as 0.2% given that a rate of 0.4% was observed in first and second degree relatives of controls. We could not identify any methodological errors that could have influenced

the rates other than unreliable or invalid informants. This error is certainly possible for information given on second and third degree relatives since confirmation of renal failure by medical records was not done. When analysis was done based on those registered with CORR a difference was observed in the proportion of second degree relatives of probands and controls who have ESRD. One can speculate that the failure to find a concentration of renal disease in second degree relations, while observing it in first and third degree relatives, results from recessive genetic liability in a proportion of families increasing risk to, in particular, cousins of probands. Long term secular trends in diagnosis and treatment of ESRD, and environmental influences could result in a higher proportion of cousins having ESRD compared to second degree relatives (Susser, 1987). Second degree relatives included aunts, uncles and grandparents. Most of these relatives belong to a generation in which the availability to medical services in many parts of Newfoundland was inadequate. Even if renal disease was present in these relatives it is highly possible that a diagnosis of renal disease was never made. Third degree relatives of index cases are largely cousins. These individuals belong to the same generation as the index case making the likelihood of diagnosis and treatment for renal disease much more probable. In essence, the effects of environmental change are observed with the genetic components held constant.

Evidence for genetic liability has been addressed for a number of common renal diseases. The

present study's finding of a risk ratio of 3.0 for the presence of renal failure among first degree relatives of probands can be compared with a value of 2.63 reported by Spray et al (1995) in their study of 103 Caucasian families. These risks are lower than the odds ratio of 9.1 observed fro African American families found by Freedman et al in 131 African American families (1993B). Steenland and associates (1990) studied 325 men with ESRD and found that they had a family history of renal failure more frequently than controls (OR:9.3; 95% CI:8-10), although female and diabetic patients were excluded in this study. 50% of this population was Caucasian. An obvious explanation for the lower odds ratio found in the current study as compared to prior reports (Freedman, 1993B; Steenland, 1990) is the differences in the etiology of ESRD in the African American population studied by Freedman et al (1993B) versus the Caucasian population used in the study and the study by Spray et al (1995). For example, 21% of the African American study group had ESRD attributed to hypertensive nephropathy, which appears to be among those diseases with a higher familial aggregation, while only 5% of probands in the current study and none of the probands in the Spray study (1995) were classified as such.

The familial risk of ESRD observed in the present study and others may not be due to an inherited predisposition to a single disease entity but instead could be the result of an inherited susceptibility to renal damage. It is likely that susceptibility will vary by disease process and by individual. In

Freedman's study (1993B) on familial clustering of ESRD among African American families, affected family members of multiplex ESRD families typically had renal failure of different etiologies. Members within the same family had ESRD diagnosed as the result of hypertension, diabetes or glomerulonephritis. Freedman et al (1993C) proposes that the inherited susceptibility to ESRD observed in African Americans was a genetic predisposition to microvascular injury. Bergman et al (1994) reported that ESRD relatives of patients with hypertensive ESRD were as likely to have diabetic nephropathy as hypertensive nephropathy. It seems reasonable to suspect a genetic susceptibility to progressive renal disease that is unrelated to the primary illness. The final cause of ESRD would then depend on environmental exposures and other genes. This hypothesis is in line with the suspicions of several investigators of diabetic nephropathy (Seaquist et al, 1986; Freedman et al, 1995A) and glomerular disease (Schmidt et al, 1990; Cusi et al, 1993), who propose that the contribution of genetic factors in the development of renal damage in diabetic nephropathy, hypertensive sclerosis and glomerulonephritis occurs independently of the determinants of insulin resistance and glomerular dysfunction. Indeed, the HLA DR3 antigen is increased among patients with diabetic nephropathy (Freedman et al, 1993A), membranous (Klouda et al, 1979; Welch et al, 1986; Huang et al, 1989; Clark et al, 1993; Sacks et al, 1987) and hypertensive ESRD (Freedman et al, 1991). It may be a genetic propensity towards hypertension, cardiovascular disease, and glomerular damage which in combination with

environmental exposures such as infection that determines the final phenotype.

There are a number of limitations inherent in a study of this type. It is recognized that not all individuals with ESRD will be treated. Unfortunately, these people will not be accounted for in any ESRD data bases. The apparent familial aggregation of a disease will be reduced if there are a high proportion of "sporadic" cases in the study population. This is almost certainly the case in the ESRD population, given the heterogeneity of diseases in this group. In general caution must be exercised when interpreting the significance of familial aggregation of disease. A high degree of familial aggregation of any trait by no means proves an inherited cause. Conversely, a low familial risk does not exclude an inherited mechanism. Familial clustering of renal disease could be due to sharing of similar environment experiences during childhood and adolescence, for example diet, exercise habits and exposure to infection, all of which can influence progression to renal disease. If these similarities included factors of importance in the etiology of nephropathy their effects may mimic genetic inheritance.

A relatively low degree of association with family history was observed. The implications of the odds ratio of 3.0 for renal failure in first degree relatives of probands compared to controls, reported in this study, may be judged by comparing it to the reported familial risk of other chronic

diseases which may or may not be due to inherited factors (table 8). The mechanisms of the diseases listed are not known, but inherited predisposition with some type of environmental trigger is hypothesised in many cases. This study investigates a very heterogenous group of disorders which may account for the low association observed. Considering the etiologic heterogeneity and pathologic diversity of renal diseases it is not surprising that a lower odds ratio is observed in this investigation compared to diseases such as Alzheimer and Parkinson's which are clearly more "narrow" in their etiology. A lower odds ratio may also be accounted for by the fact that the phenotypic end point of renal disease - renal failure - was measured as the outcome variable. Utilization of an outcome which occurs earlier in the course of the disease such as raised serum creatinine, may have produced higher odds ratios.

Several factors complicate the genetic study of renal disease. An important problem in interpreting the current data is that of incomplete penetrance in which individuals may possess a liability genotype without expressing it (Weiss et al, 1982). Phenotypic manifestations of genotype may be reduced by variable expressivity and late age of onset of disease symptoms, and gene-environment interaction (Khoury et al, 1993). The extent to which genetic and environmental factors interact is hard to establish.

A limitation of the present investigation is the accuracy of the methods used to classify the cause of ESRD. Overall, some of the classification is going to be erroneous, but it is difficult to estimate how much. The implicit underlying assumption for the multifactorial model for common diseases is that any given disease has the same etiology (King, 1992). This is not the case in this investigation. The calculated odds ratio was based on a group of renal diseases which are obviously independent from one another in their etiology, manifestations and inheritance. This etiologic heterogeneity of renal diseases complicates its genetic investigation. The diagnostic criteria are not mutually exclusive and the limits between various disease entities are not clearly established. Diseases of discrete entities that may have different genetic and/or non-genetic contributions have been lumped together under the same disease classification such as glomerulonephritis and interstitial disease. A single disorder, such as ESRD or even glomerulonephritis, is in reality not one disease, but a group of diseases. Such a broadly defined disorder can have a number of different subtypes that are genetic or non-genetic in origin. A specific subtype may be caused by two or three interacting major genes, by a set of genes acting in a polygenic pattern or by an environmental agent. When the subtypes of a disease are indiscriminately combined, the overall familial aggregation may not resemble a genetic predisposition or a pattern expected in multifactorial inheritance. Renal artery stenosis and essential hypertension are two such renal diseases that were allocated to the same diagnostic category

despite the two diseases having separate etiologies, physiological effects and clinical consequences. Similarly, many investigators consider ESRD attributed to hypertension to be a nonspecific label that is used on patients with a variety of underlying kidney diseases (Weisstuch, 1992). The accuracy with which other diseases were excluded as contributing to ESRD was often not clear in the patient's medical records. This was true for patients with diabetes type II. Because the diagnosis was very subjective there is the problem of misdiagnosis. Misclassification may obscure true associations. Given the potential for error in classification of the cause of ESRD in the proband it is difficult to estimate the errors that would result in determining the specific familial risk associated with any given disease.

It could be argued that the clustering of ESRD in probands results from differential recall, which would bias the results in favour of probands. It is certainly possible that patients on dialysis would be more likely to know if a relative had kidney disease and thus introduce a differential reporting bias. However, this is unlikely for the present study, given that both probands and controls should be equally aware of the presence of renal failure among their siblings and parents due to the severe nature of the disease. Evidence shows that the uses of spousal controls offers several potential advantages in the study of familial disease (Wickramatrie, 1995; Coughlin, 1990; Drews, 1993; King, 1992). Souses of patients with renal failure are likely to have considered a given family

disease to the same extent as probands and are therefore less prone to recall bias. A positive family history was considered based on the data given by the informants. Because there was no verification of the accuracy of ESRD in the reported family member this subjectivity could introduce an additional source of reporting bias. However, this reporting bias should have minimal affect on the risks calculated as the bias should occur equally between proband and control families. The criteria used to label a relative positive for renal failure was similar for both groups and the decision was made by a single clinical nephrologist who was blinded as to whether the relative in question was that of a proband or control. Overall, reporting bias seems unlikely to be responsible for the results reported here, especially for the results observed for first degree relatives.

In the present study, the outcome of interest - renal failure - often occurs at the end of life or as the final stage of renal disease. Many persons with renal disease such as glomerulonephritis and diabetic nephropathy do not progress to ESRD. This is true even of Mendelian inherited diseases. Individuals with the genotype for ADPKD, for example, will all develop cysts by the age of 30, but only a 50% chance of dying or going on to dialysis by the age of 55 (Parfrey et al, 1990). Identical twins can also be used to demonstrate how ESRD may be a poor genetic marker for renal disease. The appearance of a disease in one twin is probable, but far from certain indicator of its eventual appearance in the other. Indeed, in this study a pair of identical twins was observed, both of whom

had vesicoureteral reflux, but only one of whom progressed to ESRD. Furthermore, although individuals in our study may have the genetic predisposition to develop renal disease they may succumb to other co-morbid conditions such as heart failure, stroke or other unrelated diseases before ESRD occurs. These persons are not accounted for in the ESRD data base. Similarly, not all persons who suffer ESRD will be treated. As a result, ESRD may be a poor marker for a genetic defect, in that renal failure may not occur in all people who inherit the genetic propensity.

Ascertaining only family members who had ESRD and not earlier signs of renal impairment may seriously underestimate the total prevalence of renal disease within families. In one study of patients with glomerular renal disease, 10% of their relatives had glomerular dysfunction. 80% of index cases with affected family relatives, were not aware that this was the case until the investigators discovered it with clinical screening (Rambausek et al, 1987). Bergman and colleges studied the families of 40 black ESRD patients with a diagnosis of hypertensive nephropathy; evidence of nephropathy was found in 39 first degree relatives of 26 of the index patients through clinical screening. 11 of the first degree relatives had ESRD. This suggests that the familial risk for renal disease, at least glomerular and hypertensive disease, may be higher than suspected, based on case-control studies employing reported family histories. Perhaps if some other measure of renal dysfunction were used as a primary outcome, higher risks of familial renal impairment would have

been demonstrated in the present study.

The appropriate next step in the investigation of potential polygenic inheritance of renal disease, therefore is to clinically screen family members of index cases for evidence of nephropathy at a stage prior to the development of ESRD. The focus of the research should centre first on those group of renal diseases appearing to behave in a familial pattern. In the present investigation, the risk of ESRD in first degree relatives was highest in families of probands with hypertensive ESRD, interstitial renal disease and diabetic nephropathy. Clinical investigation of families with diabetic nephropathy has already demonstrated a familial predisposition to diabetic renal disease (Seaquist, 1989). In this investigation, patients with reflux nephropathy comprised the greater majority of our patients with interstitial disease and there is strong existing evidence of genetic liability for reflux nephropathy. To date, little clinical investigation of families with hypertensive renal disease has been done. This investigation did show a familial tendency for renal failure in patients with hypertensive renal disease. Clinical screening of family members from this group and an appropriate control may demonstrate a higher risk of renal disease. Observation of higher odds ratios would support a molecular genetics approach in affected siblings to identify which genes were associated with the trait.

Given the present study's findings and those of other investigators, the risk of ESRD not attributable to Mendelian conditions is well worth further investigation. Evaluation of relatives of patients from multiplex families may be an effective means of identifying individuals at increased risk for ESRD. The identification of a high risk subset of individuals can be important to an understanding of the course of the disease and to assigning risk to individuals based on symptoms which may be identified through studying them. Although the genetically predisposed may comprise only a small proportion of all cases of a disease in the population they may be a numerically important group. In the long run, screening family members may be an appropriate intervention strategy for prevention and control. This is especially true for the group of probands who were classified as having ESRD secondary to hypertensive nephrosclerosis or renovascular disease. A substantial proportion (44%) of the probands reported having another affected relative with ESRD. This familial aggregation was observed to be greater than that observed for diabetic nephropathy, which has been found to have a strong familial component. By studying specific renal disease more closely for detection of possible asymptomatic earlier signs of kidney disease one can reduce the problem of disease heterogeneity with a more accurate estimate of risk.

The aim of the present investigation was the establishment of a more accurate assessment of risk for renal failure in susceptible families. We conclude that there is an increased risk of renal failure

in first degree relatives of probands without a Mendelian inherited renal disease in a Caucasian population. The argument that polygenic inheritance contributed to this predisposition is certainly plausible, with individuals inheriting a particular combination of alleles and thus becoming more genetically susceptible to developing a clinical condition, perhaps in response to additional environmental triggers. The specific mechanisms responsible for genetic susceptibility for the majority of these diseases are not fully understood or in any cases not known. This ignorance represents the real challenge for the study of the genetic basis of diseases. It is generally hoped that molecular understanding of those aspects of these diseases that have genetic components will improve their therapy and allow for their prevention. As more genetic markers become mapped through the Human Genome Project, the testing of certain families with multiple affected members may reveal the genomic regions most closely linked to the development of kidney diseases such as diabetic nephropathy, glomerular and interstitial disease (Brosius et al, 1992).

TABLES

Table 1. Characteristics of Baseline Patient Population

Mean Age (yrs) starting ESRD treatment	50.1 ± 19.2	
Median Age (yrs)	53	
Range	(2 - 87)	
Male (%)	62.9	
ESRD Classification	**N**	**%**
Glomerulonephritis	168	25.1
Diabetes Mellitus	133	19.9
Interstitial Disease	70	10.5
Hypertensive Nephrosclerosis	34	5.1
Polycystic Kidney Disease	30	4.5
Mendelian Disease	27	4.0
Other	85	12.7
Multiple Causes	28	4.2
Unknown	94	14.1
TOTAL	**669**	
Treatment Facility		
Health Science Centre	398	59.5
Salvation Army Grace General Hospital	190	28.4
Western Memorial Regional Hospital	81	12.1
CORR Registration		
Yes	471	70.4
No	198	29.6

Table 2. Mendelian Diseases Leading to ESRD in Baselines Population

	N	% Genetic	% Total
Autosomal Dominant Diseases			
Polycystic Kidney Disease	30	52.6	4.5
Charcot-Marie-Tooth Disease	2	3.8	0.3
Autosomal Recessive Diseases			
Bardet-Biedl Syndrome	2	3.5	0.3
2, 8 Dihydroxyadenine Syndrome	2	3.5	0.3
Hyperoxaluria	2	3.5	0.3
Caroli Syndrome	1	1.8	0.1
Alport's Syndrome			
TOTAL	18 57	31.6	2.7

Table 3. Cause ESRD in Probands without a Single-Gene Inheritance for their ESRD

	N	%
Glomerulonephritis	**158**	**29.7**
Diabetes Mellitus		
Diabetes mellitus type I	50	9.4
Diabetes mellitus type II	64	12.0
	114	21.4
Interstitial		
Reflux	32	6.0
Nephrolithiasis	12	2.3
Tuberculosis	7	1.3
Unspecified	10	1.9
	61	11.5
Other		
Obstructive uropathy	22	4.1
Autoimmune diseases	16	3.0
Malignant diseases	12	2.4
Latrogenic	8	1.5
Miscellaneous causes	13	1.7
	71	11.9
Hypertensive Nephrosclerosis	**26**	**4.9**
Multiple Causes	**25**	**4.7**
Unknown	77	**14.5**
TOTAL	**532**	

Table 4. Number and Proportion of Probands (n=530) and Controls (n = 494) who have a Family Member with ESRD, with Odds Ratios and 95% Confidence Intervals

Degree of Relative	Probands		Controls		Odds Ratio	95% CI
	# of Probands	Probands with Family Hx of ESRD	# of Controls	% Controls & Family Hx of ESRD		
First	63	11.9	14	2.8	4.63	2.52-9.07
Second	48	9.1	45	9.1	1.00	0.64-1.56
Third	44	8.3	14	2.8	3.11	1.65-6.22

Table 5. Number and Percentage of First, Second and Third Degree Relatives wit ESRD Among Probands and Controls, with Odds Ratios and 95% Confidence Intervals

Degree of Relative	Probands		Controls		Odds Ratio	95% CI
	Number of Relatives	ESRD in Relatives	Number of Relatives	ESRD in Relatives		
First	5780	67 (1.2)	3807	15 (0.4)	3.0	1.7-5.2
Second	15475	58 (0.4)	12093	53 (0.4)	0.9	0.6-1.2
Third	13194	49 (0.4)	11617	21 (0.2)	2.1	1.2-3.4

Table 6. Percentage of Affected First, Second, and Third Degree Relatives with ESRD According to Etiology of ESRD in Proband

Etiology of ESRD in Proband	% First Degree Relatives	% Second Degree Relatives	%Third Degree Relatives
Glomerulonephritis	0.76	0.46	0.22
Diabetic Nephropathy	1.35	0.26	0.31
Interstitial Disease	1.42	0.23	0.94
Hypertensive Nephrosclerosis	2.47	0.69	0.44
Multiple causes	0.71	0.12	0.68
Other	0.81	0.27	0.65
Unknown	1.25	0.41	0.41

Table 7. Comparison of Probands and Controls for Number of First, Second, and Third Degree Relatives with ESRD who are Registered with CORR

Relation	Probands (%)	Controls (%)
First Degree Relatives		
alive >1981	4407	2846
ESRD & CORR registered	17 (0.4)	5 (0.18)
Second Degree Relatives		
alive >1981	11910	9239
ESRD & CORR registered	14 (0.12)	4 (0.04)
Third Degree Relatives		
alive >1981	11379	10378
ESRD & CORR registered	14 (0.12)	5 (0.05)
First - Third Degree Relatives		
alive >1981	27696	22463
ESRD & CORR registered	45 (0.16)	14 (0.06)

Table 8. Odds Ratios and 95% Confidence Intervals of Having Affected First Degree Relatives in Subjects Diagnosed with Different Diseases, Probands vs. Controls, in Published Case-Control Studies. When the lower Confidence Limit is >1.0 the Odds Ratio is Significantly Increased. 95% Confidence Interval Not Calculated

Disease	Author and Year	Number of Subjects	Control Type and Number	Odds Ratio (95% C.I.)
IDDM	Dalquist et al., 1989[49]	339	population (528)	7.8 (3.6-16.8)
Alzheimer's Disease	Amaducci et al., 1986[50]	116	hospital (116) population (97)	5.0[a] 2.6[a]
	Chandra et al., 1987[51]	64	hospital (64)	1.0[a]
	Graves et al., 1990[52]	130	population (130)	2.2 (1.17-4.18)
Multiple Sclerosis	Midgard et al., 1996[53]	155	hospital (200)	12.9 (1.73-552)
Migraine Headaches	Stewart et al., 1997[54]	73	population (72)	1.5 (0.94-2.40)
Atopic Eczema	Diepgen et al., 1996[55]	426	population (628)	2.2 (1.58-2.96)
Parkinson's Disease	Payami et al., 1994[56]	114	population (114)	3.5 (1.3 - 9.4)
	Marder et al., 1996[57]	233	population (1172)	2.3 (1.3 - 4.0)
Rheumatoid Arthritis	Jones et al., 1996[58]	207	population (180)	1.6 (0.3 - 8.7)

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APPENDICES

A. Initial letter to index patient

Dear

We are interested in learning more about how kidney disease tends to run in families. Since you have been affected by diabetes, I would ask for your participation in a research study to identify other family members with diabetes, kidney disease or high blood pressure.

A research assistant will be contacting you by telephone during the next few weeks to ask if you would be willing to participate. Your participation in this study is entirely voluntary. You may not decide to participate or may withdraw from the study at any time.

With your permission, she will ask you questions about your immediate family's medical history. These questions will focus on whether any other family members have been diagnosed with a diabetes, kidney disease or high blood pressure. The interview will last up to 20 minutes. A mutually convenient time for this interview can be arranged. If any family members are identified as having diabetes, she may ask you to contact them for permission to allow her to contact them for information and to arrange a time where clinally investigation of renal function can assessed through urine tests.

Your participation would be greatly appreciated.

Yours truly,

Dr. Harnett, MD

B. Initial letter of contact to index patient's family

Dear

We are interested in learning more about how kidney disease tends to run in families. Since you have had a family member affected by kidney disease, I would ask for your participation in a research study to identify other family members with kidney disease or high blood pressure.

A research assistant will be contacting you by telephone during the next few weeks to ask if you would be willing to participate. Your participation in this study is entirely voluntary. You may not decide to participate or may withdraw from the study at any time.

With your permission, she will ask you questions about your immediate family's medical history. These questions will focus on whether any other family members have been diagnosed with a diabetes, kidney disease or high blood pressure. The interview will last up to 20 minutes. A mutually convenient time for this interview can be arranged. If any family members are identified as having diabetes, she may ask you to contact them for permission to allow her to contact them for information and to arrange a time where clinical investigation of renal function can assessed through urine tests.

Your participation would be greatly appreciated.

Yours truly,

Dr. Harnett, MD

C. Questionnaire Checklist

From Interviewee

1. Consent

2. Father's name

    Date of birth
    Living
        cause of death
        age of death
        year of death (die before 1980)
    History of renal disease, hypertension, diabetes
    Community of birth

3. Father's parent's names

    Date of birth
    Living
        cause of death
        age of death
        year of death (die before 1980)
    History of renal disease, hypertension, diabetes

3. Father's siblings (including half siblings and infant deaths)

    names
    Date of birth
    Living
        cause of death
        age of death
        year of death (die before 1980)
    History of renal disease, hypertension, diabetes
    Number of children and their gender
        Living
        cause of death
        age of death
        year of death (die before 1980)

6. Mother's name
    - Date of birth
    - Living
        - cause of death
        - age of death
        - year of death (die before 1980)
    - History of renal disease, hypertension, diabetes
    - Community of birth
7. Mother's parent's names
    - Date of birth
    - Living
        - cause of death
        - age of death
        - year of death (die before 1980)
    - History of renal disease, hypertension, diabetes
8. Mother's siblings (including half siblings and infant deaths)
    - names
    - Date of birth
    - Living
        - cause of death
        - age of death
        - year of death (die before 1980)
    - History of renal disease, hypertension, diabetes
    - Number of children and their gender
        - Living
        - cause of death
        - age of death
        - year of death (die before 1980)

9. Propositus' siblings (including half siblings and infant deaths)

- Names
- Date of birth
- Living
  - cause of death
  - age of death
  - year of death (die before 1980)
- History of renal disease, hypertension, diabetes
- Number of children
  - Living
  - cause of death
  - age of death
  - year of death (die before 1980)
  - History of renal disease, hypertension, diabetes

10. Propositus' children in order of birth

- Names
- Date of birth
- Living
  - cause of death
  - age of death
  - year of death (die before 1980)
- History of renal disease, hypertension, diabetes

11. Obtain information on renal failure for all first, second and third degree relatives.

12. Identify who would be willing to give further information and obtain their telephone numbers as needed.

D. 2×2 Contingency Table, Relative Risk Equation and Odds Ratio Equation

2×2 Contingency Table - Unmatched cases and controls*

Table A1. Observed frequencies of individuals relating their disease status to factor exposure

		Disease		
		Present, D+(Cases)	Absent, D-(Controls)	Total
Factor	Present, F+ (exposed)	a	b	$m_1=a+b$
	Absent, F- (unexposed)	c	d	$m_0=c+d$
Total		$n_1=a+c$	$n_0=b+d$	$n=a+b+c+d$
Proportion		$p_1=a/n_1$	$p_0 b/n_0$	

Odds ratio equation: $\frac{a \cdot d}{c \cdot b}$

Relative risk equation: $\frac{a \cdot (c+d)}{c \cdot (a+b)}$

*From Sackett, 1991

F. The Importance of Renal Impairment in the Natural History of Bardet-Biedl Syndrome

INTRODUCTION

Bardet (Bardet, 1920), in 1920, and Biedl (Biedl, 1922), in 1922, described a syndrome characterised by congenital obesity, polydactyly, retinitis pigmentosa, mental retardation and genital hypoplasia. In 1925, Solis-Cohen and Weiss (Solis-Cohen, 1925) mistakenly combined this syndrome with a disorder, described by Laurence and Moon (Laurence, 1866) in 1866, into one syndrome known as the Laurence-Moon-Biedl Syndrome (Solis-Cohen, 1925). Today, Bardet-Biedl Syndrome and Laurence-Moon Syndrome are recognized as two distinct disorders (McKusick, 1992; Editorial, 1988). While polydactyly is frequent in Bardet-Biedl syndrome it is almost absent in the rarer Laurence-Moon Syndrome. As well, neurologic complications observed in Laurence-Moon syndrome are not observed in Bardet- Biedl patients (McKusick, 1992; Editoral, 1988; Schachat et al, 1982). Recent studies suggest that the cardinal manifestations of Bardet-Biedl include not only retinal dystrophy, obesity, dysmorphic extremities, hypogenitalism in males, but also renal abnormalities (Green et al, 1989). Other anomalies observed frequently in this rare autosomal recessive condition include mental retardation, hypertension and diabetes mellitus (Laurence, 1866; Green et al, 1989; Bell et al, 1958; Escallon et al, 1989).

In the past the care of patients with Bardet-Biedl Syndrome has not been optimal, particularly because of their abnormal appearance, incapacitating blindness, and assumed mental retardation. These patients may survive to adulthood and then present to nephrologists with hypertension, abnormal renal imaging tests, or renal failure. However little information is available concerning the natural history of affected individuals and the importance of renal disease in their clinical outcome. Therefore we have re-evaluated Bardet-Biedl patients studied in 1987 (Green et al, 1989), and have investigated additional Bardet-Biedl patients identified since the original study. This paper reports on the age of diagnosis of important clinical manifestations including blindness, hypertension, diabetes mellitus, renal failure and death, and compares the frequency in patients and their unaffected siblings.

METHODS

In our original study, 32 patients with Bardet-Biedl Syndrome were identified through the registry of the Canadian National Institute for the Blind (CNIB), records in the Ophthalmology Department at the Health Sciences Centre, St. John's, Newfoundland, and through subsequent family studies (Green et al, 1989). Medical records were also searched to identify any persons receiving treatment for Bardet-Biedl or Laurence-Moon-Biedl Syndrome. A further 6 patients were identified since 1987. Thus 38 patients from 21 families with Bardet-Biedl Syndrome were studied. Consanguinity was known or assumed in 7 families. 19 were female and 19 male, with ages ranging from 1-63 years at last follow up, with a mean age of 35 $\pm$ 15 years.

There were 58 unaffected siblings in the 21 families and a further 6 infant deaths in whom Bardet-Biedl diagnosis could not be ruled out. 28 of the siblings were female and 30 were male. The ages of the siblings ranged from 7-59, with the mean age being 38 $\pm$ 10 years.

The geographic distribution of families with Bardet-Biedl Syndrome is shown in Figure 1. 260,000 people live around the St.John's area and the remaining 300,000 are distributed in coastal communities around the island. Until recently contacts between the communities were by the sea, as there were few roads. The result of this isolation has been a clustering of many recessive conditions in certain parts of the island, where consanguinity is likely to be high. The families with Bardet Biedl Syndrome, however, were found to be scattered all over the island.

A protocol for investigation was approved by the Human Investigations committee of St. John's General Hospital. For those patients undergoing evaluation an appropriate informed consent was obtained.

17 patients with Bardet-Biedl Syndrome who were fully evaluated in 1986/7 were re-studied in 1993. A further 15 patients had complete evaluation in either 1987 or 1993, and the remaining 6 patients had partial testing. 45 of the 58 unaffected siblings were available for complete testing in 1993. In addition, medical records were reviewed for all 38 cases and 45 of the unaffected siblings to obtain confirmation of information concerning age of onset of legal blindness, hypertension, diabetes mellitus, renal impairment and endstage renal disease.

At each visit blood pressure was recorded. Blood was drawn for measurement of serum urea, creatinine, electrolytes, glucose, glycosylated hemoglobin, calcium, alkaline phosphatase, albumin, total protein, hemoglobin, complete blood count. Blood was also obtained for measurement of follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone and estradiol. Urine samples were obtained and analyzed for blood and protein. 29 Bardet-Biedl patients underwent ultrasound of both kidneys, with 21 having serial studies available, all of these being interpreted by one radiologist (BCC). Unaffected siblings did not undergo ultrasound investigation.

The height and weight of 27 (15 female, 12 male) adult Bardet-Biedl patients and 42 (21 female , 21 male) adult siblings were converted to Body Mass Index scores (BMI) based on a formula which integrates height (m) and weight (kg) (Healthy Weight, 1987).

DEFINITIONS

Bardet-Biedl Syndrome: the presence of dystrophic extremities ± polydactyly, retinal dystrophy and obesity, in the absence of neurological complications. Other manifestations include genital hypoplasia in males, mental retardation, and renal structural abnormalities, but these were not incorporated into defining a case.

Hypertension: sitting systolic pressure >150 and/or diastolic >90mmHg or taking anti-hypertensive agents. Age of onset of hypertension was considered to be the age at which the first record of hypertension was observed in the medical charts.

Renal impairment: estimated creatinine clearance of less than 1.2 ml/sec (70ml/min), using the formula of Cockroft and Gault (Cockroft et al, 1976), or a serum creatinine > 120 μmol per litre (1.35 mg/dl) when creatinine clearance values were not available (2 not available). Age of diagnosis of chronic renal failure was taken to be the earliest recorded date of renal impairment.

Diabetes mellitus: glycosylated hemoglobin levels above the upper normal limit of 0.07 or presently on a therapy (diet, drug or insulin) to control blood sugar levels. Age of onset of diabetes was determined from the earliest clinical recording in the medical records.

Obesity: BMI greater than 27 (+, 1987).

Legal blindness: visual acuity of 20/200 or less, or a visual field of 20 degrees or less.

Primary testicular failure: serum testosterone levels below lower limit of normal with FSH and LH levels above the upper limit of normal.

Primary ovarian failure: serum estrogen levels below lower limit of normal with FSH and LH levels above the upper limit of normal, in females of reproductive age, corrected for time in the menstrual cycle at which blood was taken.

STATISTICAL ANALYSIS

The Kaplan-Meier method was used to compare age of diagnosis of various clinical manifestations in Bardet-Biedl patients and their unaffected siblings (Dixon, 1988). Terminal events included death, chronic renal failure, blindness, hypertension, and diabetes mellitus.

Two tail chi square tests were used to compare proportions. Fisher's exact test was used for evaluating statistical differences for of cells having observed or expected frequency of less than 5. Differences in the quantitative variables were evaluated by student's unpaired t-test . P values less than 0.05 were considered significant.

The total number of patients studied varied for the different investigations.

RESULTS

The manifestations of disease present in Bardet-Biedl Syndrome are presented in Table 1. The earliest manifestations are those associated with dysmorphic extremities including polydactyly, syndactyly and brachydactyly.

Blindness: Of 36 patients with Bardet-Biedl Syndrome 86% were legally blind, as compared to none of the 45 siblings evaluated. Since the BBS patients were identified primarily through the CNIB a possible bias may exist. The blindness was usually incapacitating. At last examination one patient had no light perception, 28% (N=7) could perceive only light, 16% (N=4) could see only had movements, 28% (N=7) could do no better than count fingers. The remaining 24% (N=6) had visual acuity $\leq$ 20/200. The age range of recorded legal blindness was 5-29 years of age. Figure 2 shows that 25% of Bardet- Biedl patients were legally blind by the age of 13, 50% by the age of 18 and 100% by the age of 30. All five Bardet-Biedl patients not yet legally blind were under the age of 12 years. Retinal dystrophy was observed in 100% of our patients. For other ocular abnormalities see Green et al (Green et al, 1989).

Hypertension: 66% (25/38) of Bardet-Biedl patients were hypertensive compared with only 11% (5/45) of the unaffected siblings. 60% of the hypertensive Bardet-Biedl patients were treated with antihypertensive agents. 25% of Bardet-Biedl patients had elevated blood pressure by the age of 26, 50% by the age of 34 and 75% by the age of 53 (figure 3). In

contrast, 25% of unaffected siblings were hypertensive by the age of 49 ($p = 0.001$).

Diabetes Mellitus: 12 out of 38 (32%) Bardet-Biedl patients investigated had diabetes mellitus compared to none of the 45 unaffected siblings. Two patients were insulin dependent, four were prescribed oral hypoglycemic agents and 6 were maintained with dietary management. Figure 2 shows that 25% of Bardet-Biedl patients were diabetic by the age of 35 and 50% by the age of 55. The age of diagnosis ranged from 24-55 years.

Renal Failure: Renal impairment occurred in 9 of 36 (25%) patients, 4 of whom progressed to end stage renal disease. The earliest age of onset observed was 2 years old. By age 48, 25% of Bardet-Biedl cases had chronic renal insufficiency (Figure 2). Only 1 unaffected sibling had mild renal impairment.

Renal Structure: Fetal lobulation was present in 96% of 28 Bardet-Biedl patients investigated with ultrasound, abnormal calyces in 96%, calyceal diverticula or cysts in 58% of patients, diffuse cortical loss in 25% and focal cortical loss in 7%. In three patients with renal impairment at the time of last ultrasound, 2 had diffuse cortical loss and 1 (4%) had focal loss. Of 25 patients with normal renal function at time of ultrasound 1 had focal loss and 5 (20%) had diffuse cortical loss, two of whom showed progressive loss on serial ultrasound.

Survival Analysis: 8 of 38 (21%) Bardet-Biedl patients died by last follow up (3 males, 5 females). The ages of death ranged from 1-63. Three (38%) of these deaths were the result of End Stage Renal Disease (ESRD), 2 from congestive heart failure both of whom had chronic renal failure, 1 from metastatic renal cancer who also had chronic renal failure, 1 from pulmonary embolism and morbid obesity, and the 8th from respiratory failure and sepsis following surgery for Hirschsprung's disease at age 1 year. Thus renal failure was present in 75% of patients at the time of death.

Of the 58 unaffected siblings only one (1.7%) had died (as a result of a myocardial infarct at the age 36).

Figure 4 shows the cumulative survival in 38 Bardet-Biedl patients and their 58 unaffected siblings. Life expectancy was significantly worse in patients with Bardet Biedl Syndrome than in their unaffected siblings with 25% of Bardet-Biedl patients dead by the age of 44 years of age ($p<0.0001$).

Obesity: Figure 5 shows the distribution of Body Mass Index in affected and unaffected siblings. The range of BMI for female Bardet-Biedl patients was 30-55 (mean 40.1 ± 8.3) compared to 20-40 (mean 26.5 ± 5.14) for unaffected female siblings. Female Bardet-Biedl patients had significantly greater BMI compared to their affected brothers ($p<0.01$). For male

Bardet-Biedl patients, the BMI ranged from scores of 21-55 (mean 33±8.7) compared with 20-42 (mean 29.1±5.2) for their unaffected male siblings. The obesity in Bardet-Biedl patients was responsive to calorie restriction, as 5 patients lost substantial amounts of weight on calorie restriction diets.

Gonadal Dysfunction: During the study 10 males had tests of gonadal function 4 of whom had evidence of primary testicular failure. One of the 9 females of reproductive age (excluding 3 being treated with contraceptives) had primary ovarian dysfunction.

Mental Retardation: Forty-one per cent of the patients were considered mentally retarded. Further details of objective IQ testing can be obtained from Green et al (Green, 1989).

DISCUSSION

Bardet Biedl Syndrome is a rare disorder. The incidence rate in Switzerland is 1 in 160,000 live births (Klein et al, 1969). Among the mixed Arab population of Kuwait the prevalence rate is estimated at 1:36,000. However, among the Bedouin in Kuwait, where consanguinity is frequent, the rate is estimated at 1:13,500 (Faraj et al, 1989). A similar prevalence rate has been observed in the Newfoundland population (1:17,500) (Green et al, 1989). The scattered geographic distribution of the families we studied is striking, unlike the clustering often seen in autosomal recessive conditions.

Prognostic data for Bardet Biedl Syndrome is of particular interest to families with affected individuals or at risk of having children with the syndrome, as well as to physicians who must advise and treat them because of the serious clinical manifestations and the lack of demonstrated preventive therapy.

Polydactyly and/or syndactyly is the earliest manifestation of the syndrome and is recognized at birth. A delay in achieving developmental milestones may be expected in many affected individuals due to mental retardation or decreased vision (Green et al, 1989). Obesity is frequently present in childhood. Blindness usually develops in the teen years. In early adulthood hypertension, diabetes mellitus and renal failure occur. Longevity is substantially reduced.

Obesity: This manifestation usually begins in childhood and increases in severity with age (Klein et al, 1969; Dekaban et al, 1972; Bauman et al, 1973). Bauman et al (1973) reviewed 73 cases where birth weight and subsequent weight gain were recorded in order to better define the age of onset of obesity. 71% of Bardet Biedl patients were >50th percentile at birth with 38% being >90th percentile. For those that were above the 50th percentile at birth, 33% of these cases were obese by one year of age. This adiposity noted during early life has usually been described as diffuse and nonspecific in distribution. By adulthood, however, this adiposity becomes the most prominent in the trunk and proximal section of the limbs.

Blindness: Severe retinal dystrophy is another feature presenting early in affected individuals. The earliest manifestation of retinopathy has been observed to be either loss of central visual acuity and or decreased night or peripheral vision (Schachat et al, 1982; Jacobson et al, 1990; Leys et al, 1988; Fulton et al, 1993; Riise et al, 1987; Lyle et al, 1946; Krill et al, 1961). Retinal degeneration leading to blindness has been reported to appear between the ages of 4 and 10 (Dekaban et al, 1972; Krill et al, 1961; Campo et al, 1982) with 73% of cases being blind by the age of 20 (Klein et al, 1969). Other studies have found that visual acuity was moderately reduced at the beginning of their teens, after which it rapidly decreased by the age of 30 (Jacobson et al, 1990; Leys et al, 1988; Riise et al, 1987; Campo et al, 1982). Deterioration of eyesight occurred rapidly in our patients with 25% of Bardet-Biedl patients being legally blind by the age of 13, 50% by the age of 18 and 100% by the age of 30. The

wide range in ages of diagnosis of legal blindness (ages 5-29) observed in our patients may be the result of late or inaccurate recording of precise age at which individuals were legally blind. This may be particularly true for older patients. The severity of blindness is often incapacitating, as demonstrated by the fact that in 76% of patients examined visual acuity was at the level of counting fingers only or worse.

Hypertension: Hypertension was observed frequently and at young age in Bardet- Biedl patients - 50% affected by age 34 years. It is difficult to know whether hypertension is a direct result of the mutant gene, or an indirect result of renal involvement, obesity or diabetes mellitus.

Diabetes mellitus: A higher rate of diabetes was observed in our group of patients (36%) than in those studies by Amman & Klien (14%) (Klein et al, 1969). This abnormality in glucose metabolism occurred early, with 50% of our Bardet-Biedl patients developing diabetes mellitus by the age of 55. The cause of diabetes in Bardet-Biedl patients remains unclear. Pancreatic histological abnormalities have not been observed in autopsy reports to account for the presence of abnormal glucose levels (Fraccaro et al, 1953; Franke, 1938 et al; Churchill et al, 1981). Obesity could lead to the development of diabetes by a reduction of the cellular insulin receptors, which in turn leads to a decrease in insulin sensitivity and an increase in insulin levels (Rizza et al, 1981; Olefsky et al, 1981). Diabetes in Bardet-Biedl syndrome is probably type II

diabetes because increased insulin levels have been reported following a glucose load in Bardet-Biedl syndrome (Green et al, 1989) and only 2 of the 12 Bardet-Biedl patients with diabetes were insulin dependent. Therefore it is likely that dietary management would be helpful, at least in the initial phases.

Renal Impairment: Renal disease is a characteristic feature of the syndrome (Green et al, 1989; Harnett et al, 1988; Churchill et al, 1981; Allon et al, 1973; Hurley et al, 1975; Tieder et al, 1982, Linne et al, 1986). Structural renal abnormalities have been observed in up to 100% of patients reported in recent clinical series (Harnett et al, 1988). The radiographic observations of fetal lobulation, calyceal clubbing, blunting, cysts or diverticula, in our Bardet-Biedl patients are characteristic of the syndrome. Twenty five percent of patients had impaired glomerular filtration rate and in the group with normal renal function a further 20% had diffuse cortical loss on renal ultrasound. One suspects that the latter group will go on to develop renal impairment.

Impairment of glomerular filtration rate often occurred at an early age. By the age 48 years 25% of Bardet-Biedl patients had renal impairment. The earliest age of onset of chronic renal impairment was two years of age. Case studies (Tieder et al, 1982; Bluett et al, 1977; Linne et al, 1986) have shown even earlier onset of chronic renal failure, with a 3 month and 6 month old infant developing chronic renal failure.

Longevity: There are no reported data on the life expectancy of Bardet-Biedl patients. Longevity is certainly reduced, with 25% of individuals affected with Bardet-Biedl Syndrome dying by the age of 44 compared to only 2% of unaffected siblings. Renal failure has been observed to be a frequent cause of death. (Klein et al, 1969; Churchill et al, 1981; Nadjimi et al, 1969). A review of 16 autopsies by Churchill et al showed that 56% of deaths resulted from renal failure (Churchill et al, 1981). 38% of the deaths observed in our study were the result of chronic uremia and a further 38% of patients had renal impairment at time of death.

The early onset and severity of the various manifestations of this syndrome make early diagnosis of the syndrome essential if the patient's function is to be maximized. Only the feature of dysmorphic extremities may be recognized at birth. However, the presence of retinal dystrophy, severe obesity and renal structural changes should lead to an early diagnosis. The fact that many patients are not mentally retarded (Green et al, 1989) and do not become blind until their teen years suggests that special education could maximize patients ability to function independently in adulthood. Screening for hypertension, abnormal glucose levels and renal function is indicated, as early treatment of these manifestations could be beneficial.

Heterozygotes: Swift and Croft (Croft et al, 1990) have postulated that heterozygote siblings of Bardet-Biedl patients are at an increased risk of also developing obesity, hypertension, diabetes and renal disease. This is based on the frequent observation of these complications in a review

of 75 relatives of 2 Bardet-Biedl patients. Other studies also report on unaffected family members with obesity (Ehrenfeld et al, 1970) and renal impairment (Runge et al, 1986). Although 57% of unaffected siblings in our study had a body mass index greater than 27 and 25% of unaffected siblings were hypertensive by age 49 years, only one had mild renal impairment, none had diabetes mellitus and there was only 2% mortality by the age of 50.

The rate of hypertension may be artificially elevated in the unaffected siblings because they were assessed only once, as part of the study. In a group of autosomal dominant polycystic kidney disease families studied in a similar manner we observed hypertension (as defined in the current study) in 23% of unaffected adults aged 40-59 years (Parfrey et al, 1990). It is possible that on repeat testing blood pressure may not remain elevated.

At present the evidence to support an increased risk of clinically important disease in heterozygote siblings is not strong.

Conclusions: Bardet-Biedl syndrome has an adverse prognosis with early onset of obesity, blindness, hypertension and diabetes mellitus. Renal impairment is frequent and an important cause of death. Survival is substantially reduced.

List of Figures

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Fig 1. The geographic distribution of patients in Newfoundland with Bardet-Biedl syndrome.

Fig 2. The age of diagnosis of blindness, diabetes mellitus, and renal impairment in Bardet-Biedl syndrome.

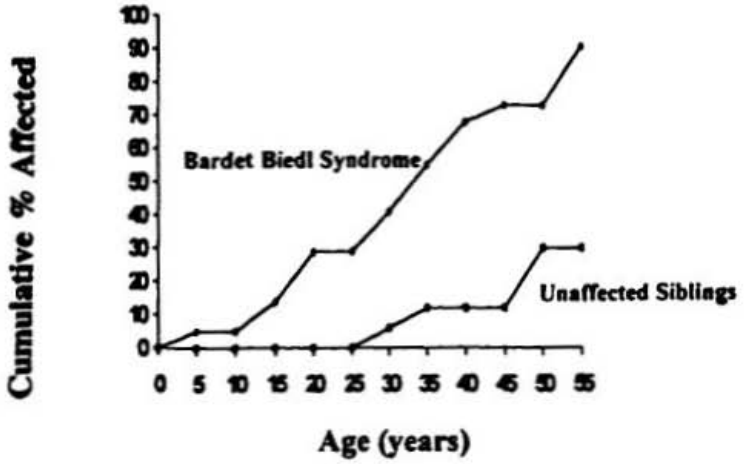

Fig 3. The age of diagnosis of hypertension in Bardet-Biedl syndrome and in their unaffected siblings.

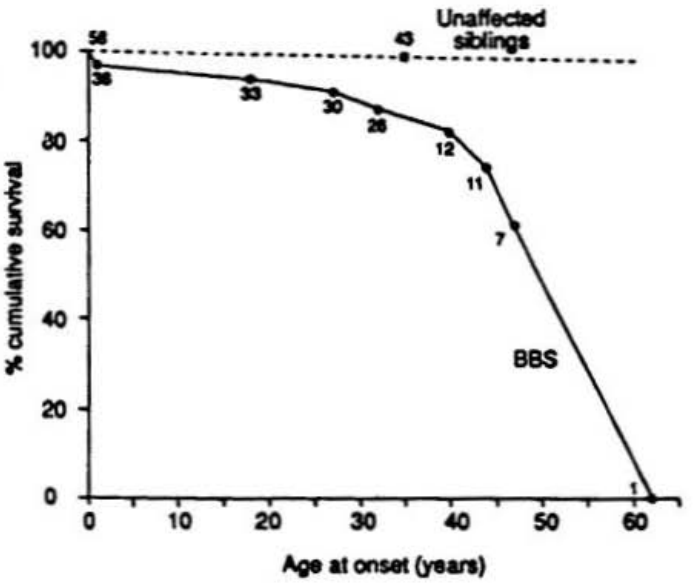

Fig 4. The cumulative survival of patients with Bardet-Biedl syndrome and of their unaffected siblings. Numbers on survival curves refer to subjects alive to that point.

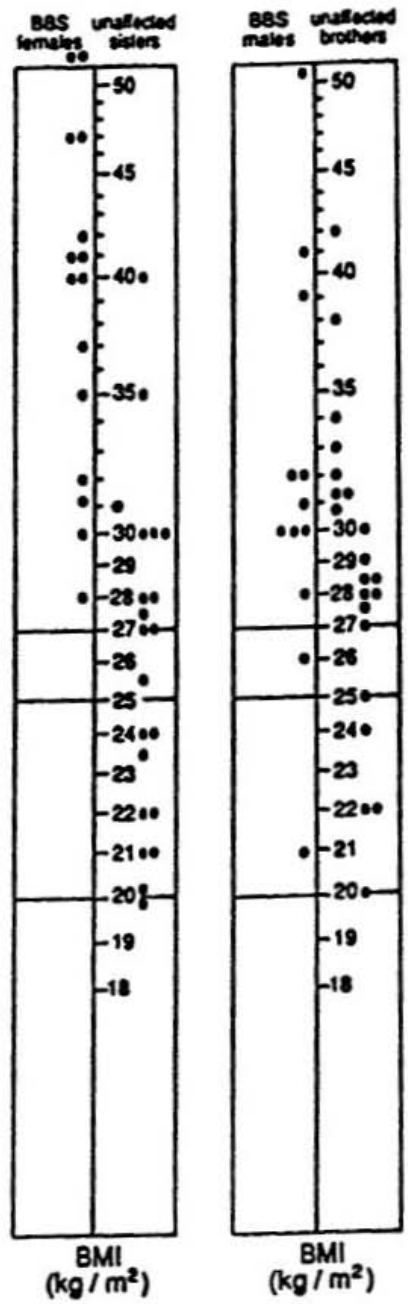

Figure 5. Obesity in siblings of families with Bardet-Biedl syndrome. The diagram on the left show body mass index for females with BBS, compared with their unaffected sisters. The diagram on the right shows body mass index for males with BBS, compared to their unaffected brothers. BMI > 27 denotes obesity.

Table 1: Clinical Manifestations of Bardet-Biedl Syndrome.

	N With Abnormality	N Examined	%
Syndactyly or polydactyly[a]	29	31	93
Polydactyly[a]	18	31	58
Brachydactyly of feet[a]	22	22	100
Legally blind	31	36	86
Severe blindness[b]	19	25	76
Retinal dystrophy[a]	28	28	100
Mental retardation[a]	13	32	41
Obesity	25	27	93
Hypogenitalism in men	7	8	88
Primary testicular failure	4	10	40
Primary ovarian failure	1	9	11
Diabetes mellitus	12	38	32
Hypertension	25	38	66
Reduced glomerular filtration rate	9	36	25
Renal fetal lobulation	27	28	96
Abnormal renal calyces	27	28	96

[a]Data obtained from reference (8).