CLINICAL AND GENEALOGICAL STUDIES OF FAMILIAL COLORECTAL CANCER TYPE-X IN NEWFOUNDLAND

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CLINICAL AND GENEALOGICAL STUDIES OF FAMILIAL COLORECTAL CANCER TYPE-X IN NEWFOUNDLAND

by

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A thesis submitted to the School of Graduate Studies in partial fulfillment of the requirements for the degree of Masters of Science.

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May 2011

St. John's Newfoundland

ABSTRACT

Familial Colorectal Cancer Type-X (FCCTX) is a syndrome defined by criteria used to identify Lynch Syndrome, but in which the genetic cause is not the result of mismatch repair (MMR) gene mutations with the genetic etiology remaining unknown. In an attempt to facilitate novel gene discovery in FCCTX, families (N = 12) were identified with a strong family history of CRC (≥5 cases of CRC) of unknown genetic etiology, who fulfilled the criteria for FCCTX: meeting the ACI, possessing no known MMR gene mutation, and a probands with MSS CRC. Significant variability was found in terms of age of onset, tumour location, and genetic profile of CRCs amongst the probands of these families. First-degree relatives of the probands of the FCCTX families (N = 126) were compared as a group to first-degree relatives of the probands of fifteen Lynch Syndrome families (N = 153). No difference in lifetime risk of CRC existed between the groups, but the families fulfilling the FCCTX criteria demonstrated a significantly later onset of CRC and fewer cases of extra-colonic cancers. Mapping locations of origin demonstrated that families originated from multiple different geographic isolates. The use of a customized hereditability database failed to demonstrate genealogical linkages between the twelve FCCTX families. In six of the FCCTX families, further archival research also failed to yield a direct link. The heterogeneous clinical and pathological features, geographic distribution of probands in different isolates, and failure to identify genealogical linkages between families suggest that multiple genes underlie the susceptibility to CRC observed in FCCTX.

ACKNOWLEDGEMENTS

I would like to thank the members of my supervisory committee, Dr. Pat Parfrey, Dr. Jane Green, and Dr. Mike Woods, whose guidance and support was central to the completion of this thesis. I would also like to thank Dr. Elizabeth Dicks, Geoff Warden (Ph.D Candidate), Tyler Wish (Ph.D Candidate), Fady Kamel (M.Sc. Candidate), Owen Parfrey, and Astrid Perrot-Daley, who were always willing to assist me despite substantial workloads of their own and whose expertise was invaluable. Additionally, I would like to thank all of the staff of the Patient Research Centre and the Population Therapeutics Research Group for all of their assistance. A sincere thank-you must also be extended to all of the family members who have consented to being involved in research studies such as mine. Furthermore, I gratefully acknowledge the CIHR Team in Interdisciplinary Research on Colorectal Cancer for providing funding for my research.

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LIST OF ABBREVIATIONS

ACI: Amsterdam I Criteria

ACII: Amsterdam II Criteria

APC: Adenomatous polyposis coli

BID: *Bis in die* (twice a day)

CI: Confidence interval

CIMP: CpG island methylator phenotype

CRC: Colorectal cancer

CTC: Computed tomography colonoscopy

ERK: Extracellular signal-regulated kinase

FAP: Familial adenomatous polyposis

FCCTX: Familial colorectal cancer type x

FHS: Family history score

FOBT: Fecal occult blood testing

FW: Family weight

HAI: Hereditability Analysis Infrastructure

HNPCC: Hereditary non-polyposis colorectal cancer

IW: Individual weight

MAPK: Mitogen-activated protein kinase

MMR: Mismatch repair

MSI: Microsatellite instability

MSI-H: MSI-high

MSI-L: MSI-low

MSS: Microsatellite stable

NFCCR: Newfoundland Colorectal Cancer Registry

NGD: Newfoundland Genealogy Database

NSAIDs: Non-steroidal anti-inflammatory drugs

PMGP: Provincial Medical Genetics Program

PTRG: Population Therapeutics Research Group

QD: *Quaque die* (once a day)

RCT: Randomized clinical trial

SEER: Surveillance, Epidemiology, and End Results

SIR: Standardized incidence ratio

TCC: Transitional cell cancer

1. INTRODUCTION

Cancer is a disease with known environmental and hereditary risk factors (Lichtenstein et al., 2000). The lifetime risk of developing colorectal cancer (CRC) as a member of the general population is approximately 6-7% (Green et al., 2007). It is the third most prevalent form of cancer in Canada, following breast and prostate cancer, comprising approximately 13% of all cases of cancer as well as the second leading cause of cancer deaths (Canadian Cancer Society, 2010). Despite having a relatively low incidence of cancers of all types, the Newfoundland population demonstrates the highest incidence rate of CRC in the country (Canadian Cancer Society, 2010). Risk factors for the development of CRC include high consumption of red meat (Sandhu et al., 2001; Norat et al., 2002) and alcohol (Longnecker et al., 1990), cigarette smoking (Giovannucci and Martínez, 1996), high body mass index (Russo et al., 1998), sedentary lifestyle, diabetes (Le Marchand et al., 1997), family history of CRC (Fuchs et al., 1994), a history of inflammatory bowel disease (Bernstein et al., 2001), a history of colorectal polyps (Vogelstein et al., 1988), hereditary syndrome with a predisposition to CRC (Burke et al., 1997), and old age (Turner et al., 1999). The advances in our understanding of human genetics coupled with an increased emphasis on preventive medicine makes the identification of individuals with high predisposition to CRC a high priority and an achievable task.

1.1 Literature Search

A comprehensive literature search of numerous relevant medical databases (Pubmed, EMBASE, The Cochrane Library, Biological Abstracts, Biomedical Reference Collection, CINAHL, Clinical Evidence, PILOTS Database, Proquest, SciFinder Scholar,

and Scopus) was performed using various combinations of the key words: "colorectal cancer", "familial colorectal cancer", "hereditary colorectal cancer", "hereditary non-polyposis colorectal cancer", "microsatellite instability", "genetics", "screening", "surveillance", and "tumourigenesis". Reference lists of relevant papers were also surveyed to identify additional literature on topics of interest.

1.2 Colorectal Tumourigenesis

Neoplasia is the process of abnormal cellular growth and proliferation caused by gene mutations that can result in tumour formation. CRC occurs as a result of the process of neoplasia, which replaces normal colonic epithelium with adenocarcinoma cells (Grady, 2006). The majority of mutations that promote tumour formation are somatic mutations found in the tumour but not within the surrounding cells (Breivik, 2005). Germline mutations in genes associated with cancer susceptibility are responsible only for conferring increased risk for, and not directly causing, tumour formation (Marsh and Zori, 2002). Knudson's two-hit hypothesis proposes that both copies of a particular tumour suppressor gene must be mutated for carcinogenesis to occur (Knudson, 1971). Thus, carcinogenesis is much more likely in the setting of a germline mutation as only one subsequent somatic mutation is required. On the other hand, sporadic neoplasia requires two separate somatic mutations to both copies of the gene (Jackson, 1985). These variants confer cells with survival advantages and are thus able to hyperproliferate within the developing tumour (Nowell, 1976). Somatic mutations in genes involved in the Wingless/Wnt, RAS-RAF-MAPK, phosphatidylinositol 3-kinase, and TGF-β signaling pathways are the most common genetic alterations in the process of colorectal tumour formation (Grady, 2006). CRCs tend to arise from the inactivation of tumour suppressor

genes combined with activation of oncogenes via mutation accumulation (Fearon and Vogelstein, 1990). In their classic review of colorectal tumourigenesis, Vogelstein et al. proposed a roughly sequential model for the mutations involved in tumourigenesis initiating with a mutation in the adenomatous polyposis coli (APC) gene followed by the acquisition of mutations in *KRAS* and *p53* (Vogelstein et al., 1988). However, more recent investigations have emphasized the heterogeneity and complexity of the somatic mutations that are accumulated in the process of tumour development (Wood et al., 2007).

1.3 Colorectal Polyps

Prior to the 1990s, the adenomatous polyp and the hyperplastic polyp were the only two classifications of colonic epithelial polyps, areas of tissue extending into the lumen of the colon (Song et al., 2007; Noffsinger, 2009). The malignant potential of the traditional adenoma has long been recognized. The adenoma-carcinoma sequence, which forms the basis of our understanding of colorectal tumourigenesis, proposed that colorectal tumours develop from precursor adenomas (Morson, 1962; Vogelstein et al., 1988; Bond, 2003; Cappell, 2007). Hyperplastic polyps are typically small in size and occur more frequently in the distal colon (Cappell and Forde, 1989). They are present in approximately 12.5% of asymptomatic adults over the age of 50 (Lieberman et al., 2000). They have been classically viewed as lesions with minimal neoplastic potential and therefore as insignificant findings (Morson, 1962; Arthur, 1968; Williams et al., 1980). This notion has been re-evaluated more recently with support for the view that all hyperplastic polyps have malignant potential (Jass, 1999). The finding of colorectal tumours in mixed hyperplastic adenomatous polyps (Urbanski et al., 1984) and the strong

association of hyperplastic polyposis, a condition of multiple hyperplastic polyps, with CRC served as supporting evidence for this proposal (Jeevaratnam et al., 1996).

In 1990, the term "serrated adenoma" was first used to describe mixed hyperplastic adenomatous polyps that did not fit neatly into either of the two classifications (Longacre and Fenoglio-Preiser, 1990). The epithelium of these polyps had the serrated character typical of hyperplastic polyps, but the atypical finding of an appreciable grade of dysplasia (Hawkins et al., 2002). The malignant potential of these polyps has been well described in the literature with one study reporting 5.8% of randomly selected colorectal tumours being associated with adjacent serrated adenomas (Mäkinen et al., 2001). However, it has been shown that the serrated adenoma has a lower malignant potential than the traditional adenomatous polyp (Song et al., 2007). It has been proposed that this 'serrated neoplasia pathway' represents a different biological pathway to CRC from the traditional adenoma-carcinoma sequence (Hawkins et al., 2002).

1.4 Molecular Pathways to CRC

Research has uncovered four main molecular pathways to CRC emphasizing the heterogeneity of the process. Our understanding of the nature of these pathways is continually evolving as the complex web of carcinogenesis is gradually elucidated. The two main pathways are microsatellite instability (MSI) and chromosomal instability. Both of these processes result in genomic instability, a key underlying mechanism of colorectal tumourigenesis (Stoler et al., 1999). More recently a third novel pathway has been proposed, known as the serrated pathway. Lastly is the familial colorectal cancer type x (FCCTX) classification, which is a blanket term to encompass a heterogeneous group

with molecular changes that do not fit neatly into any of the previous pathways. The FCCTX classification is not so much a pathway as it is a clinical descriptor of families with similar phenotypes who cannot be classified by currently understood hereditary predisposition mechanisms. It is plausible that this classification encompasses several molecular pathways to CRC. It is also thought that right- and left-sided colorectal tumours may evolve from distinct biological pathways. Left-sided CRC is more common in males and middle-aged patients, while right-sided CRC is more frequent in females and tends to be detected at a more advanced stage (Nawa et al., 2008).

1.4.1 Chromosomal Instability

The model of Vogelstein et al. was built around the idea that most colorectal tumours developed from precursor adenomas via the adenoma-carcinoma sequence (Morson, 1962; Vogelstein et al., 1988; Bond, 2003; Cappell, 2007). It also centered on the notion of chromosomal instability as the molecular mechanism leading to CRC as a result of the accumulation of mutations in oncogenes and tumour suppressor genes (Young and Jass, 2006). Chromosomal instability produces genomic instability via changes in chromosomal copy number and structure. This phenomenon is characterized by aneuploidy and loss of heterozygosity (Lengauer et al., 1998). Investigations utilizing comparative genomic hybridization have demonstrated that specific areas of chromosomal gains and losses are associated with the adenoma-carcinoma sequence (Meijer et al, 1998; Hermsen et al., 2002). Tumour development in familial adenomatous polyposis (FAP) occurs via chromosomal instability (Lindor, 2009). This is the result of aneuploid and polyploid chromosomal alterations that occur at the start of the adenoma-carcinoma sequence due to the *APC* mutation (Alberici and Fodde, 2006).

1.4.2 Microsatellite Instability (MSI)

MSI is a change in the number of repeated sequences in the highly repetitive areas of DNA known as microsatellites. The term "instability" refers to the somatic change in length of repeat units from the germline microsatellite allele (De La Chapelle, 2003). The reference panel for the detection of MSI consists of five validated microsatellite markers – *BAT25*, *BAT26*, *D2S123*, *D5S346*, and *D17S250* – collectively known as the Bethesda panel (Boland et al., 1998). A tumour is labeled MSI-high (MSI-H) if two or more of the five markers show instability, MSI-low (MSI-L) if only one of the markers shows instability, and microsatellite stable (MSS) if none of the markers show instability (Boland et al., 1998). However, when none of the markers show instability one cannot exclude the MSI-L classification with the use of only five markers (Halford et al., 2002). CRCs are generally subdivided based on the presence or absence of MSI. However, there is no consensus in the literature on how to deal with the MSI-L category with some investigators grouping this category together with MSS (Laiho et al., 2002), while others contend that MSI-L should be treated as a separate category and may represent a distinct pathway to carcinogenesis (Jass et al., 2002; Rudzki et al., 2003; Bapat et al., 2009).

MSI is a cardinal feature of colorectal tumours in individuals with MMR gene mutations, but also occurs in approximately 15% of sporadic cases of CRC (Halford et al., 2002). Somatic biallelic inactivation of *MLH1* by promoter hypermethylation has been shown to be the cause of the MSI in these sporadic cases (Veigl et al., 1998; Herman et al., 1998; Cunningham et al., 1998). A decline in the expression of *MLH1* has also been associated with increasing age, thereby making the elderly more susceptible to sporadic cases of CRC demonstrating MSI (Kakar et al., 2003). MSI is also thought to

play a role in the development of other cancers, such as sporadic cases of endometrial cancer (Sobczuk et al., 2007) and oral squamous cell carcinoma (Sanguansin et al., 2006).

Prior to 2005, the literature reporting the effect of MSI on CRC prognosis was inconsistent. Two separate papers published in 2005 assessed differences in clinical outcome between individuals with MSI and MSS CRC. The studies both report a significantly reduced risk of death in individuals with MSI-H DNA compared to those with MSS DNA with respective hazard ratios of 0.46 (95% CI: 0.31 – 0.68) and 0.65 (95% CI: 0.59 – 0.71) (Benatti et al., 2005; Popat et al., 2005). The study of Benatti et al. was retrospective in nature, but had a large sample size of CRC patients (N = 1263), used one uniform measure of MSI (the Bethesda panel), and controlled confounding variables via multivariate analysis. On the other hand, Popat et al. conducted a meta-analysis of thirty-two trials (N = 7,642) that suffers from a failure to formally assess the quality of included trials as well as heterogeneity between included studies in terms of general protocol and in the grouping of MSI-L and MSS. The mechanisms underlying the prognostic advantage of MSI have yet to be established, but it is likely that other features that differ between the MSI and MSS pathways underlie the prognostic difference between the two groups.

1.4.3 Serrated Pathway

The term serrated adenocarcinoma was coined to describe CRC with a strong resemblance to hyperplastic polyps (Jass et al., 1992). The precursor lesions for this type of tumour are the hyperplastic polyp, the traditional serrated adenoma, and the sessile serrated adenoma (Torlakovic et al., 2003; Noffsinger, 2009). This serrated pathway has been subdivided into one pathway leading to MSI-H CRC and the other to MSI-L CRC

(Jass et al., 2002). Sessile serrated adenomas appear to be the precursor to sporadic MSI-H CRC (Noffsinger, 2009). They demonstrate a high frequency of somatic activating mutations in the proto-oncogene and serine/threonine kinase *BRAF*, the CpG island methylator phenotype (CIMP), variable levels of MSI, and an increased frequency of right-sided tumours (Young et al., 2005; Wish et al., 2010). On the other hand, the traditional serrated adenoma appears to be a precusor to MSS or MSI-L CRC that is more frequently left-sided, demonstrates a CIMP-low phenotype, and contains predominantly *KRAS* mutations rather than *BRAF* (Noffsinger, 2009; Leggett and Whitehall, 2010). The CIMP is characterized by aberrant DNA methylation resulting in hypermethylation of CpG islands.

KRAS and *BRAF* are oncogenes that are involved in signaling with the *Ras/Raf/MEK/ERK/MAPK* pathway (Figure 1.1). It has been hypothesized that deleterious variants in the *BRAF* and *KRAS* genes have equivalent effects on tumourigenesis (Storm and Rapp, 1993). Somatically acquired *KRAS* variants have been shown to be a key event in the transformation and further growth of small adenomas, which is a vital step in progression in the adenoma-carcinoma sequence (Vogelstein et al., 1988). Thus, it appears that somatic *BRAF* deleterious variants play a similarly important role in colorectal carcinogenesis. The p.Val600Glu *BRAF* variant (c.1799T>A), accounting for 80% of *BRAF* variation, directly causes constitutive activation of the *RAS/RAF/MEK/ERK/MAPK* pathway (Davies et al., 2002). On the other hand, the less common *BRAF* variant require the concurrent presence of a *RAS* variant in order to activate the pathway. It is thought that causal variants in the oncogenes *BRAF* and *KRAS* result in altered regulation of the cell cycle and hyperproliferation of cells

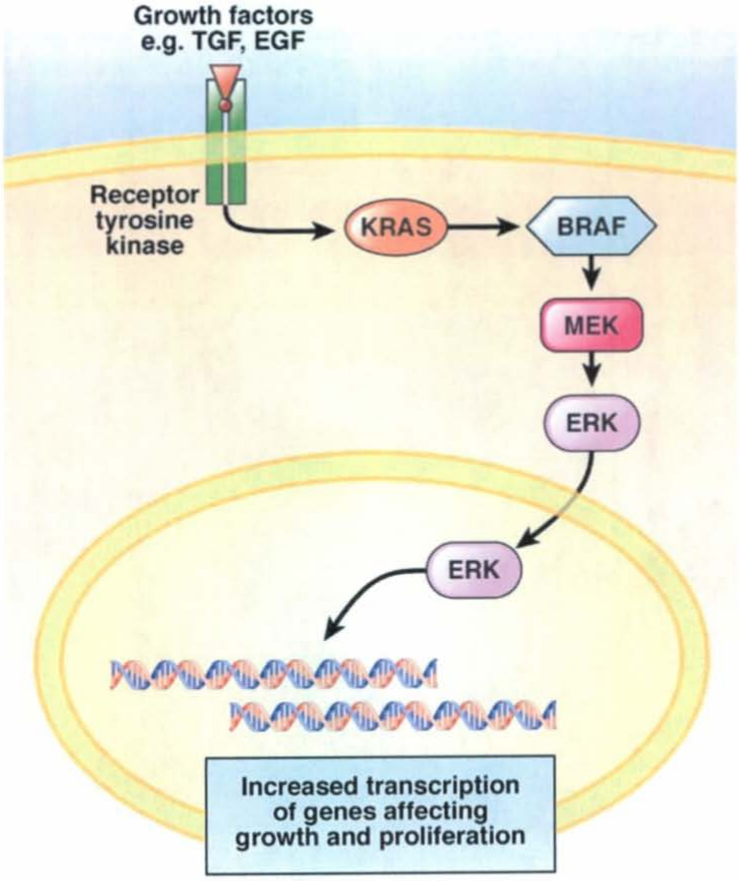

Figure 1.1: Role of *KRAS* and *BRAF* in the *Ras/Raf/MEK/ERK/MAPK* signaling pathway. Adapted from Leggett and Whitehall (2010).

KRAS and *BRAF* activate MEK which phosphorylates and activates extracellular signal-regulated kinase (ERK), which translocates into the nucleus to alter expression of cell signaling molecules.

through constitutive activation of the mitogen-activated protein kinase (MAPK) signaling cascade (Mercer and Pritchard, 2003). Estimations of the prevalence of *BRAF* variants in all cases of CRC vary between 5% and 18%, with the p.Val600Glu variant accounting for between 36.4% and 87.5% of the *BRAF* variants in these studies (Davies et al., 2002; Rajagopalan et al, 2002; Yuen et al., 2002; Samowitz et al., 2005). Reported rates of the p.Val600Glu *BRAF* variant in patients with MSI in the literature are 16%, 45%, and 52% (Domingo et al., 2004; Lubomierski et al., 2005; Samowitz et al., 2005). On the other hand, only 5% of MSS tumours have been shown to have the p.Val600Glu *BRAF* variant and these individuals have been shown to have a poorer prognosis (Lubomierski et al., 2005; Samowitz et al., 2005). *BRAF* variants are associated with the somatic inactivation of *MLH1* by CIMP-associated hypermethylation, the etiology of the 15% of sporadic cases of CRC that are MMR-deficient, and not with germline inheritance of *MLH1* deleterious variants (Wang et al., 2003; Domingo et al., 2004; Weisenberger et al., 2006).

1.5 Hereditary Colorectal Cancer

Family history is the most predictive risk factor for the development of CRC with approximately 35% of cases being influenced by genetic susceptibility factors (Lichtenstein et al., 2000). The inherited forms of CRC have been shown to be the result of germline mutations in mismatch repair (MMR) genes, *APC*, *SMAD4*, *STK11/LKB1*, *MUTYH*, and *BMPR1A* (Aaltonen et al., 2007). The two most common forms of inherited CRC, both characterized by autosomal dominant inheritance, are hereditary non-polyposis colorectal cancer (HNPCC), also known as Lynch syndrome, and familial adenomatous polyposis (FAP). Individuals with FAP have an inherited germ-line mutation in *APC* resulting in the formation of numerous adenomatous polyps, which

endows them with a nearly 100% risk of CRC (Steinbach et al., 2000). Lynch Syndrome is caused by inherited or germline variants in the MMR system (Boland, 2005). This system is responsible for repairing errors that occur during DNA replication involving incorrect nucleotide matching (Aquilina and Bignami, 2001). Carriers of mutations in the MMR genes are at approximately an 80% lifetime risk for developing CRC (Mecklin and Järvinen, 2005). Other syndromic forms of CRC include juvenile polyposis (caused by *SMAD4* and *BMPR1A* mutations), Peutz-Jeghers syndrome (caused by *STK11/LKB1* mutations), and *MUTYH*-associated polyposis (Aaltonen et al., 2007). Recent genome-wide association studies have revealed the existence of a multitude of low-risk CRC susceptibility loci, which are contributing factors towards hereditary risk of CRC (Houlston et al., 2008). To account for these newly discovered and currently unknown loci, a new genetic susceptibility syndrome has been described, known as Familial Colorectal Cancer Type-X (FCCTX). Individuals with this syndrome often meet the diagnostic criteria for HNPCC, although the condition has a fundamentally different, and currently unknown, genetic etiology. In terms of nomenclature, it is more appropriate to use the label "Lynch Syndrome" for individuals with a germline MMR deleterious variant and "FCCTX" for individuals who lack MMR defects and meet the clinical diagnostic criteria for HNPCC, the Amsterdam I (ACI) (Abdel-Rahman and Peltomäki, 2008).

1.5.1 Hereditary Non-Polyposis Colorectal Cancer (HNPCC) and Lynch Syndrome

HNPCC was first described by Warthin in 1913 (Warthin, 1913) and then by Lynch more than fifty years later (Lynch et al., 1966). It is characterized by autosomal dominant inheritance, high penetrance, variable expressivity, early age of onset, right-

sided predominance, and increased risk of synchronous and metachronous tumours (Stuckless et al., 2007). Additionally, the incidence of extra-colonic cancers of the stomach, endometrium, ovaries, pancreas, small bowel, brain, and transitional cells of the renal pelvis and ureter, as well as sebaceous tumours is increased (Anaya et al., 2008). In classic cases of Lynch Syndrome, the autosomal dominant inheritance and high penetrance of the disease results in up to 50% of relatives in successive generations being affected by CRC or one of the aforementioned extra-colonic cancers (Vasen, 2007).

Defects in the MMR system have been identified as the cause of Lynch Syndrome (Aquilina and Bignami, 2001). Mutations in *MSH2*, *MLH1*, *MSH6*, and *PMS*2 are responsible for approximately 95% of mutations resulting in HNPCC (Woods et al., 2005). Individuals with Lynch Syndrome experience multiple errors in repetitive DNA sequences called microsatellites within their MMR-deficient tumours. This fundamental change in the structure of their DNA is termed MSI and is characteristic of Lynch Syndrome (Bapat et al., 1999). MSI is the result of the failure of the MMR system to repair mismatched nucleotides following DNA replication resulting in contraction or expansion of short nucleotide sequences between one and four base pairs in length (Ward et al., 2001).

1.5.2 Famlial Colorectal Cancer Type X (FCCTX)

A significant number of individuals who meet the diagnostic criteria for HNPCC actually have microsatellite stable (MSS) DNA and lack a germline MMR gene mutation. According to recent literature, approximately 50% of the individuals who meet the stringent ACI fall into this category (Lindor et al., 2005; Llor et al., 2005). In 2005, Lindor et al. proposed that the label "Familial Colorectal Cancer Type X" (FCCTX) be

applied to subjects who meet the ACI with MSS tumours (Lindor et al., 2005). MSI testing allows families meeting the Amsterdam Criteria to be properly classified as either Lynch Syndrome or FCCTX, with the exception of the 15% of sporadic tumours displaying MMR deficiency as a result of *MLH1* hypermethylation rather than inheritance of a germline MMR deleterious variant (Herman et al., 1998). The term FCCTX encompasses families displaying a pattern of CRC consistent with autosomal dominant inheritance who lack detectable MMR or *APC* deleterious variants (Lipkin and Afrasiabi, 2007). The genetic basis for FCCTX remains to be elucidated and it is hypothesized that novel susceptibility genes exist, mutations to which predispose individuals to the development of CRC (Woods et al., 2005). However, it likely encompasses multiple forms of hereditary CRC of unknown genetic etiology (Woods et al., 2010). As novel susceptibility genes are discovered, additional categories of hereditary CRC can be established and the definition of FCCTX can be adjusted appropriately.

Recent literature suggests that the phenotype of FCCTX is significantly less severe than that of Lynch Syndrome. Jass et al. performed the first direct comparison of the phenotype of individuals meeting the ACI with tumours that were either MSS or MSI (Jass et al., 1995). The main limitation of the study was the small sample size in the MSI (N =62) and MSS (N = 17) groups being compared. The MSS group had a later mean age of onset of cancer (54 years) than the MSI group (48 years), significantly fewer cases of multiple cancers, and more instances of aneuploidy. Another investigation also reports this tendency of MSS tumours to demonstrate aneuploidy, while this rarely occurs in MSI tumours, which demonstrate almost exclusively diploidy (Alberici and Fodde, 2006).

Tumours in the MSI group were also more often right-sided, poorly differentiated, and mucinous compared to the MSS group. This finding agrees with other investigations, which report that colorectal tumours tend to be right-sided in Lynch Syndrome, while those of FCCTX are more frequently left-sided (Renkonen et al., 2003; Llor et al., 2005).

Lindor et al. undertook a study of cancer incidences in 161 ACI pedigrees (N = 3422) divided into two groups: 1.) MSI-H and MMR deleterious variant (Lynch Syndrome group), 2.) MSI-L or MSS with no MMR deleterious variant (FCCTX group) (Lindor et al., 2005). The limitations of this investigation are its retrospective nature and the fact that it groups MSI-L and MSS together rather than considering them independently. The authors used standardized incidence ratios (SIRs) as their measure of risk, which compares the incidence of an event in the study population relative to a normal population. The normal population was derived from the SEER9 database, which is a cancer registry for various regions of the United States between 1973 and 2007. The FCCTX group demonstrated a significant increased incidence of CRC (SIR 2.3, 95% CI: 1.7 – 3.0) relative to this normal population. On the other hand, the Lynch Syndrome group experienced a significantly greater incidence of CRC (SIR 6.1, 95% CI: 5.2 – 7.2) and of extra-colonic cancers of the stomach, urinary tract, small intestine, endometrium and ovary. Thus, the risk of CRC in the FCCTX group was less than half of that of the Lynch Syndrome group without significant risk for any extra-colonic cancers. Additionally, cases of CRC in FCCTX families tended to have a considerably later onset than for individuals with Lynch Syndrome. The mean age of diagnosis of CRC was significantly ($p < 0.05$) earlier in relatives of the Lynch Syndrome group (48.7 years) versus the FCCTX group (60.7 years). This finding is further supported by the

investigation of Renkonen et al., which reported mean age of onset of CRC of 45.2 in the Lynch Syndrome group and 53.7 in the FCCTX group (Renkonen et al., 2003).

In 2005, Llor et al. published the findings of a prospective, population-based cohort study including 1309 individuals recently diagnosed with CRC (Llor et al., 2005). Only 25 individuals (1.9%) met the ACI (N = 17) or ACII (N = 8) with 15 of these individuals (60%) possessing MSS tumours and showing normal MMR gene profiles. This study is significantly limited by its very small sample size of individuals with Lynch Syndrome (N = 10) and FCCTX (N= 15). Thus, any comparisons drawn between the two groups are of questionable reliability and have low generalizability. Another limitation of the study is that mutational analysis for *PMS2* was not performed. A recent study reported that *PMS2* mutations were responsible for approximately 9% of MMR deleterious variants (Hampel et al., 2005). Despite these limitations, this investigation is one of only a few prospective studies that directly compare the phenotype of Lynch Syndrome and FCCTX and as a result its findings possess some value. Its prospective nature is a strength given the higher probability of more complete follow-up and ascertainment of outcome data relative to a retrospective design. The percentage of family members with a diagnosis of CRC in the Lynch Syndrome and FCCTX groups was 31.5% and 18% respectively ($p < 0.05$). However, the authors fail to indicate exactly how family members were defined and how many degrees of relation were included. Despite the fact that the clinical manifestation of FCCTX appears to be less severe than Lynch Syndrome, it does significantly predispose individuals to the development of CRC and thus the elucidation of its genetic basis is a high priority research pursuit.

1.5.3 Diagnostic Criteria

Family history criteria are used to stratify the at-risk population for developing CRC and identify those with a potential genetic susceptibility syndrome. Clinical criteria for the diagnosis of HNPCC were first established by the International Collaborative Group on HNPCC in 1991 during a meeting in Amsterdam (Figure 1.2). The ACI outline the conditions required for a diagnosis of HNPCC: greater than or equal to three relatives, one of whom must be a first-degree relative of the other two, with CRC; the occurrence of CRC in at least two generations; and at least one diagnosis before the age of 50 (Vasen et al., 1991). The ACI were revised in 1999 to include extra-colonic HNPCC-related cancers under the Amsterdam II Criteria (ACII) (Vasen et al., 1999). Individuals fulfilling either the ACI or ACII would be advised to undergo MMR mutation testing. In families who fail to meet the ACI and ACII, the Bethesda Guidelines, developed in 1997 and revised in 2004, identify individuals who should undergo microsatellite instability (MSI) testing (Rodriguez-Bigas et al., 1997; Umar et al., 2004). For patients who fulfill the Bethesda guidelines and are found to have MSI upon investigation, MMR testing is recommended.

These guidelines are designed to predict MSI and MMR gene mutations as accurately as possible. However, according to a 2004 meta-analysis that appraised the suitability of the ACI, ACII, and original Bethesda guidelines, they are not an exact science (Kievit et al., 2004). The results of this investigation, summarized in Table 1 on the next page, demonstrate the inadequacy of the ACI and ACII in predicting the presence of inherited MMR deleterious variants. The results of this study should be

Original (Amsterdam I)	Revised (Amsterdam II)
• At least 3 relatives with CRC, one of whom must be a first degree relative of the other two. • Involvement of 2 or more generations. • At least one case diagnosed before age 50. • FAP has been excluded.	• At least 3 relatives with HNPCC-associated cancer. • One should be 1st degree relative of other two. • At least 2 successive generations affected. • At least 1 diagnosed before age 50. • FAP excluded. • Tumours should be verified by pathological examination.

Original Bethesda	Revised Bethesda
• Individuals with cancer in families that meet the Amsterdam criteria • Patients with 2 HNPCC-related cancers, including synchronous and metachronous CRC or associated extra-colonic cancers (endometrial, ovarian, gastric, hepatobiliary, small bowel, or transitional cell carcinoma of the renal pelvis or ureter). • Patients with CRC and a 1st degree relative with CRC and/or HNPCC-related extra-colonic cancer and/or a colorectal adenoma with one of the cancers diagnosed before age 45 years, and the adenoma diagnosed before age 40 years. • Patients with right-sided CRC having an undifferentiated pattern (solid/cribriform) on histopathologic diagnosis before age 45. • Patients with signet-ring cell type CRC diagnosed before age 45. • Patients with adenomas diagnosed before age 40.	• CRC diagnosed in a patient before age 50. • Presence of synchronous, metachronous colorectal or other HNPCC-associated tumours regardless of age. • CRC with the MSI-H histology diagnosed in a patient before age 60. • CRC diagnosed in a patient with one or more 1st degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed before age 50. • CRC in a patient with two or more 1st or 2nd degree relatives with HNPCC-related tumours, regardless of age.

Figure 1.2: The original and revised Amsterdam Criteria and Bethesda guidelines.
Adapted from Bonis et al. (2007)

interpreted with some caution given the heterogeneity in the inclusion criteria for patients between studies and the fact that the quality of many of the included studies could not be fully assessed given the absence of important information in their protocols.

Table 1.1: Reported sensitivity and specificity values for the ACI, ACII, and original Bethesda Guidelines to predict MMR gene mutations.

	ACI	ACII	Bethesda Guidelines
Sensitivity	54-91%	78%*	89%*
Specificity	62-84%	46-68%	53%*

* When homogeneity of sensitivity and specificity values was significantly proven, sensitivity and specificity values were pooled.

The specificity values of these three sets of criteria are adversely affected by the existence of the FCCTX cohort. Individuals with FCCTX possess features similar to HNPCC, but lack the MSI and MMR gene mutations characteristic of the condition. Thus, FCCTX sufferers would likely be advised to undergo MSI testing or MMR gene sequencing by these guidelines, but would test negative in both regards. These individuals represent false positives which increase type I error rate and lower the specificity of these guidelines. Given that it has been reported that as many as 50% of individuals who meet the stringent ACI have MSS tumours and thus lack an MMR variant, it is easy to understand why these diagnostic guidelines are imprecise in practice. These individuals fall under the realm of FCCTX, which likely represents a collection of different and unknown forms of genetic predisposition and susceptibility to CRC. Identifying the genetic causes of FCCTX has the potential allow more accurate classification of the high-risk individuals identified by these clinical criteria.

1.6 Surveillance of High Risk Individuals

1.6.1 Screening Techniques and Recommendations

CRC is the second most prevalent cancer and the leading cause of cancer death in the Western world, but is actually one of the most preventable forms of cancer (Xiao et al., 2008; Arber et al., 2006). It is thought that the majority of CRCs develop from precursor adenomas that can be identified and removed by colonoscopic polypectomy (Winawer et al., 1993). Screening is recommended for all individuals over the age of 50 (Loffeld, 2009). With the advent of effective screening programs, CRC incidences have been consistently declining in the United States since 1985, the exception being a brief period of slightly increasing incidence between 1995 and 1998 (Edwards et al., 2010). Similarly, CRC mortality rates have been steadily declining since 1984. The majority of the decline in these rates was seen in individuals over the age of 65. Edwards et al. utilized a microsimulation screening analysis (MISCAN) model to demonstrate that the decline in CRC incidence and mortality rates has been primarily the result of screening programs. Risk factor modification and treatment were demonstrated to be less important contributing factors. In 2004, the Canadian Society of Gastroenterology and the Canadian Digestive Foundation released an updated version of their recommendations for colorectal cancer screening (Leddin et al., 2004). Screening has been shown to be a cost-effective strategy to prevent CRC and related mortality (Frazier et al., 2000). The screening options for patients with a negative family history are fecal occult blood testing (FOBT) (every five years), flexible sigmoidoscopy (every five years), the previous two interventions in combination (every five years), double contrast barium enema (every five

years), or colonoscopy (every ten years). Decisions on follow-up intervals are made based on the significance of the colonoscopic findings.

Fecal occult blood testing (FOBT), a test for blood in the stool, has been shown to reduce the incidence of CRC by 20% and decrease CRC-associated mortality by 33% when performed annually (Mandel et al., 1993; Mandel et al., 2000). Flexible sigmoidoscopy has been associated with a similar benefit, reducing mortality between 59% and 79% in case-control studies (Newcomb et al., 1992; Selby et al., 1992). A recently published randomized clinical trial (RCT) has reported reductions of 23% and 31% in CRC incidence and mortality respectively from one-time flexible sigmoidoscopy screening (Atkin et al., 2010). However, the benefits of sigmoidoscopy are reserved for lesions of the rectum and sigmoid colon based on the nature of this endoscopic procedure. The double contrast barium enema, a radiologic imaging of the entire colon following the injection of a barium enema, has been shown to be equally cost-effective as the other strategies (Glick et al., 1998). Its strengths are its ability to image the entire colon and reportedly lower rates of complications (Winawer et al., 1997). However, this supporting data is completely observational with the strengths of the procedure being mainly theoretical and the cost-effectiveness findings being based on mathematical models. The major limitation is that any positive double contrast barium enemas must be followed up with a colonoscopy to confirm findings. A relatively new modality that has been used in CRC screening is the computed tomography colonography (CTC), an imaging technique that produces two- and three-dimensional reconstructions of a patient's colon. The CTC has been shown to be less sensitive and specific than the traditional colonoscopy (Sosna et al., 2003; Mulhall et al., 2005; Rosman and Korsten,

2007). However, it has been advocated as potential alternative to the colonoscopy claims that its less invasive nature has the potential to increase patient compliance with CRC screening (Moawad et al., 2010). The main drawbacks of the CTC are the exposure to radiation during the procedure and that the sensitivity of this investigation decreases with decreasing lesion size, according to the results of two meta-analyses (Sosna et al., 2003; Mulhall et al., 2005). Estimates of per-patient sensitivity relative to the gold standard, the conventional colonoscopy, were 85% and 88% for large lesions (>9mm), 70% and 84% for medium-sized lesions (6-9mm), and 48% and 65% for small lesions (<6mm).

A study of colonoscopy in an asymptomatic patient population has shown it to be an effective measure to detect and remove advanced neoplastic colonic lesions (Lieberman et al., 2000). The cost-effectiveness of such a strategy has been questioned given the availability of less expensive screening tests. Additionally, the effectiveness of the colonoscopy in preventing right-sided cancers, which is the theoretical basis of its benefit versus flexible sigmoidoscopy, has been recently called into question. The case-control study in question reported a 67% reduction in CRC-associated mortality from left-sided tumours, but no mortality reduction (OR = 0.99, 95% CI: 0.86 – 1.14) from right-sided tumours (Baxter et al., 2009). This data supporting the benefit of FOBT and flexible sigmoidoscopy is stronger since it is based on randomized clinical trials (RCTs) as opposed to the observational nature of the evidence supporting colonoscopy. However, despite controversies in the literature, colonoscopy remains the first-choice test for CRC screening and estimates of potential mortality reduction still remain at 60 – 70% (Ransohoff, 2009). An RCT examining the benefit of colonoscopy on CRC incidence and mortality is currently being planned (Bretthauer et al., 2006).

A significant barrier to the effectiveness of colonoscopic screening programs is a lack of patient compliance. Noncompliant patients are obviously unable to avail of the benefits of routine screening and are thus at an increased risk for developing CRC. Reported compliance rates for patients enrolled in such programs range from 52% to 85% in the literature (Yood et al., 2003; Colquhoun et al., 2004). Primary barriers to high compliance rates are discomfort associated with the colonoscopy procedure and required bowel preparation, patient indifference to their risk of disease, and difficulty coordinating follow-up. Suggested measures for improving patient compliance with screening programs include providing patients with information pamphlets upon initial presentation, providing patients with endoscopic pictures of removed polyps and adenomas, and sending patients reminder notifications about upcoming colonoscopy appointments (Rapuri et al., 2008). Improving patient compliance with these surveillance programs is of primary importance to ensure that those at risk seek and receive the necessary preventive medical attention.

1.6.2 Screening for Hereditary CRC

Early screening of individuals with a family history of Lynch Syndrome, FCCTX, or FAP and identification of those carrying MMR or *APC* deleterious variants, thus at high risk for developing CRC, is important as it allows for a proactive, preventive approach to be implemented. Routine colonoscopic screening is also recommended for individuals with a family history of CRC for whom a gene mutation cannot be confirmed. Individuals with a confirmed MMR deleterious variants are recommended to undergo endoscopic investigation via a screening colonoscopy every 1-2 years beginning at the age of 25 or ten years earlier than the age of the youngest onset of CRC in the family

(Burke et al., 1997; Leddin et al., 2004). Follow-up decisions are based on family history and the significance of colonoscopy findings. The effectiveness of screening was proven by a controlled trial comparing individuals at high risk for Lynch Syndrome who underwent screening colonoscopies every 3 years and those who did not, over a 15-year period, which found a 60% reduction in CRC incidence and 65% reduction in mortality in the group that underwent routine screening (Järvinen et al., 2000). Currently, no formal screening recommendations exist for the extra-colonic cancers associated with Lynch Syndrome. Additionally, an annual endometrial biopsy and transvaginal ultrasound has been recommended beginning at age 30 – 35 for females with germline MMR deleterious variants for the prevention of endometrial cancer, the most common of these extra-colonic malignancies (Lindor et al., 2006). Screening recommendations for the other extra-colonic tumours associated with Lynch Syndrome is poorly defined beyond the need for regular general examination (Lindor et al., 2006).

Patients with FAP have an 80-100% lifetime risk of developing CRC, therefore, prophylactic removal of the entire colon plus or minus the rectum is the standard of care for these individuals (Vasen et al., 2008). The two main options are total colectomy with ileorectal anastomosis and proctocolectomy with ileal pouch anal anastomosis, the latter including the removal of the rectum in addition to the colon (McGrath and Spigelman, 2004). Quality of life following these procedures has been shown to be good in this patient population (Church et al., 1996; Erkek et al., 2007), but is significantly higher following ileorectal anastomosis (Günther et al., 2003). Despite this, the ileal pouch anal anastomosis must be performed if there is significant polyposis of the rectum. Given the use of prophylactic colectomy in FAP, it has been proposed that the procedure be

considered for individuals with confirmed MMR gene mutations, the timing of which should be based on the familial pattern of disease (Church, 1996). This is based on the reported 80% lifetime risk of developing CRC in mutation carriers, the high risk of synchronous and metachronous tumours, and the fallibility of screening. A recent study demonstrated a 1 – 2.3 year increase in life expectancy in Lynch Syndrome patients who underwent subtotal colectomy at a young age (Vasen and de Vos Tot Nederveen Cappel, 2005). Decisions regarding prophylactic surgical procedures must be made with careful consideration of the preventive potential of the procedure, the morbidity associated with the operation, post-procedure quality-of-life, and the future risk of cancer and morbidity associated with future endoscopic procedures without the intervention (Syngal et al., 1998). Additionally, prophylactic hysterectomy and bilateral salpingo-oophorectomy has been shown to be an effective option for female carriers of MMR variants in the prevention of endometrial and ovarian cancers (Schmeler et al., 2006). This is based on the 40 – 60% lifetime risk of endometrial cancer and 10 – 12% risk of ovarian cancer in this patient population (Dunlop et al., 1997; Aarnio et al., 1999). Currently, there is no research regarding the utility of prophylactic colectomy in FCCTX. However, it would appear to be less of a suitable option than in Lynch Syndrome given the later onset of cancers and fewer synchronous and metachronous cancers, which favors the use of surveillance in FCCTX.

1.6.3 Pharmaceutical Prevention

The prevention of progression from adenomatous polyps to CRC, which appears to be a very slow process in sporadic CRC offers a major opportunity for pharmaceutical intervention (Xiao et al., 2008). Currently, no specific pharmaceutical interventions are

recommended for individuals at high risk for developing CRC. However, research in this area has demonstrated significant promise. Chemoprevention of CRC involves the use of pharmaceutical compounds to prevent the formation of adenomatous polyps and, if already formed, inhibit their progression to invasive cancers (Herszényi et al., 2008). An abundance of data from observational studies suggests that non-steroidal anti-inflammatory drugs (NSAIDs) decrease the incidence of colorectal adenomas, CRC, and deaths from CRC (Bertagnolli et al., 2006). The leading hypothesis explaining this effect is based on the upregulation of cyclooxygenase-2 (COX-2), the highly inducible isoform, within adenomas relative to normal intestinal tissue (Bertagnolli et al., 2006; Auman et al., 2008; Glebov et al., 2006).

Previous randomized controlled trials have demonstrated the efficacy of daily low-dose (75mg – 300mg) and high-dose (≥500mg) aspirin in the prevention of colorectal cancer in the general population (Flossmann and Rothwell, 2007; Rothwell et al., 2010). Another randomized controlled trial, The Colorectal Adenoma/carcinoma Prevention Program 2 (CAPP2), examined the effect administration of 600mg QD (*quaque die* = once a day) of aspirin, resistant starch (Novelose), and the combination of the two agents on the incidence of colorectal adenomas or carcinomas over a four year period in adults with Lynch Syndrome (Burn et al., 2008). This study failed to demonstrate a significant reduction in the incidence of adenomas or carcinomas of the colon in any of the intervention groups. A potential explanation for the failure of chemoprevention in this population is that the adenoma-carcinoma sequence appears to be accelerated in Lynch Syndrome (Umar et al., 2004). Additionally, the differences in

the pathogenic mechanism underlying colorectal tumours in Lynch Syndrome relative to sporadic tumours may preclude the efficacy of aspirin in this patient population.

The Adenoma Prevention with Celecoxib (APC) trial demonstrated that celecoxib treatment is associated with significant reductions in colorectal adenoma development over a three year period (Bertagnolli et al., 2006). The authors reported risk ratios for developing adenomas of 0.67 (95% CI, 0.59 – 0.77) and 0.55 (95% CI, 0.48 – 0.64) in the 200mg BID (*bis in die* = twice a day) and 400mg BID celecoxib groups respectively relative to placebo treatment. Similarly, the Prevention of Sporadic Adenomatous Polyps (PreSAP) trial demonstrated a risk ratio for developing adenomas of 0.64 (95% CI, 0.56 – 0.75) in its 400mg QD celecoxib group relative to placebo administration (Arber et al., 2006). Therefore, the evidence supporting celecoxib's ability to impede the development of CRC is quite compelling. Unfortunately, significant cardiovascular toxicities were associated with celecoxib administration in these trials. The Celecoxib Cross Trial Safety Analysis, a meta-analysis of 6 randomized trials (APC, PreSAP, ADAPT, CDME, Celecoxib/Selenium and MA27 Trials) with a total of 16,070 patient-years of follow-up, reported that the overall hazard ratio for an adverse cardiovascular event with celecoxib administration was 1.6 (95% CI, 1.1 - 2.3) regardless of dosage (Solomon et al., 2008). It also reported a roughly dose-dependent cardiovascular risk effect with 400mg BID administration possessing a hazard ratio of 3.1 (95% CI, 1.5 – 6.1), followed by 200mg BID with a hazard ratio of 1.8 (95% CI, 1.1 – 3.1) and 400mg QD with a hazard ratio of 1.1 (95% CI, 0.6 – 2.0). Upon stratifying for baseline cardiovascular risk, the authors found a two-fold increase in risk between the low and moderate risk groups (hazard ratio 2.0; 95% CI, 1.5 – 2.6) and a four-fold increase in risk between the low and high risk

groups (hazard ratio 3.9; 95% CI, 2.3 – 6.7). Additionally, the confidence intervals for the hazard ratios for the low cardiovascular risk subjects overlapped 1.0, indicating no significant increase in risk of an adverse event in these patients. Therefore, celecoxib appears to possess the potential to be safely administered to low cardiovascular risk subjects.

There is a continually expanding body of evidence that suggests that combining cancer chemopreventive agents that possess differing mechanisms of action may result in synergistic interactions that enhance the efficacy of treatment (Reddy, 2007). This facilitates dosage reductions of each agent and thus diminishes the risk for the potentially harmful side effects associated with the higher dosages while improving the cost-effectiveness of the treatment. Recent studies have brought attention to the potential for combination therapy with a coxib and a statin to effectively prevent the development of CRC in high cardiovascular risk individuals (Sacks et al., 1996; Xiao et al., 2008; Herszényi et al., 2008). The combination of an effective pharmaceutical intervention with periodic colonoscopic investigations has the potential to significantly reduce the burden of CRC, however, this potential has yet to be realized.

1.7 Genetic Studies in Newfoundland

1.7.1 The Newfoundland Population

The island of Newfoundland was discovered by Giovanni Caboto in 1497, over 500 years after the establishment of a temporary Viking settlement on the province's Northern Peninsula at L'Anse aux Meadows. The first permanent settlement in the province was established in 1609. However, population growth was very slow because

the majority of the population consisted of seasonal migratory fishermen. As a result, the population of Newfoundland in 1750 was only 6,000 (Froggatt et al., 1999). Rapid population growth occurred between 1780 – 1830 with an influx of Catholics from southern Ireland and Protestants from southwest England (Mannion, 1986). These twenty to thirty thousand migrants essentially represent the founding population of Newfoundland (Rahman et al., 2003). The economy of the province developed around the fishery, resulting in settlements, known as outports, being established almost exclusively in coastal areas (Bear et al., 1987). The general trend was for families to settle and for almost all members to marry and remain within that community. Any migration that occurred tended to be to nearby settlements where resources were more plentiful. Population growth occurred due to large family sizes which were typical of the time. This created a process of internal proliferation that has created genetic isolates within the Newfoundland population. The current population of Newfoundland, according to a 2006 Statistics Canada census, is 505,469 with approximately 60% of the population living in communities with less than 2,500 inhabitants (Statistics Canada, 2006). These characteristics of the Newfoundland population make it ideal for studying particular genetic disorders that are prevalent in the population.

It is possible that the higher incidence of CRC in Newfoundland, relative to the other Canadian provinces, is the result of founder mutations, genetic defects resulting in susceptibility to CRC within the limited number of Irish and English immigrants that established the population of the province (Green et al., 2007). This theory speculates that gene variants conferring susceptibility to CRC were possessed by members of the founding population of Newfoundland and that these variants have been passed through

the generations within the province. Renowned population geneticist Ernst Mayr first described the founder effect in 1942 as a mechanism for producing reduced genetic variability within isolated populations started by relatively few individuals from larger populations, which was the case in the settlement of Newfoundland (Provine, 2004). Mayr described the founder effect as an example of random genetic drift, processes which result in changing population frequencies of specific alleles. In Newfoundland, it has been shown that a founder effect has resulted in higher than normal population frequencies of deleterious variants causing Lynch Syndrome (Green et al., 2002). With the recent categorization of FCCTX, it has been theorized that this founder effect extends to other CRC susceptibility gene variants, resulting in their higher than normal incidence. Additionally, founder effects have also been demonstrated for other hereditary conditions in Newfoundland, such as multiple endocrine neoplasia type I (Olufemi et al., 1998) and hemophilia A (Xie et al., 2002). It is thought that there have been a series of founder effects across the province given the manner in which particular bays were settled by small groups of migrants from whom the population developed. Clearly the founder effect is a well-described phenomenon in Newfoundland, but it is important not to discount the presence of some genetic heterogeneity as communities were not perfect isolates.

1.7.2 Other Populations Similar to Newfoundland

The isolated population and founder effects which make Newfoundland an ideal population for studying hereditary diseases are also attributes of other areas of the world and have facilitated genetic research in these centers. The population of some areas of Quebec demonstrates the founder effect having developed with relative isolation that has

implications in terms of incidence of hereditary diseases and the conducting of genetic research (Laberge et al., 2005). For example, in the geographically isolated region of Saguenay Lac-Saint-Jean, several autosomal recessive conditions have increased frequency but decreased variability consistent with the founder effect and researchers have managed to completely elucidate the transmembrane conductance regulator mutations responsible for increased incidence of cystic fibrosis in the area (De Braekeleer et al., 1998). Longstanding national and regional isolation in Finland has resulted in the overrepresentation of 32 autosomal recessive conditions (Norio, 2003). The Finnish population represents a substantially older founder population than Newfoundland and extensive genetic research has been conducted utilizing its unique population. Similarly high incidences of autosomal recessive diseases have also been reported in Palestinian Arabs in whom a founder effect has been described (Ziotogora, 1997). This is thought to be the result of the unique family relationships that exist in Middle Eastern countries, characterized by cultural preference for marrying relatives, and the high percentage of individuals living in rural areas. Founder effects have been described in various regions in the world and tend to produce high incidences of particular hereditary diseases that are highly suitable for genetic research.

1.8 Study Rationale

The establishment of genealogical linkages would permit the combination of families and thus increase the power of linkage studies and exome sequencing studies aimed at detecting the novel susceptibility genes conferring risk for CRC amongst this cohort of individuals. The primary advantage of having distantly related individuals for these studies is that common regions of DNA sequence are small and sparse. This is the

result of the process of recombination, which occurs at every generation shortening the common regions of DNA sequence between the two lineages. Thus, it becomes easier to identify the deleterious variant. For example, the mapping of the common *MSH2* mutation responsible for a significant number of cases of Lynch Syndrome was made possible only by the genealogical linking of two Newfoundland families inhabiting different communities (Froggatt et al., 1999). The current study was planned to facilitate a sequence of investigations leading to the identification of the gene(s) responsible for CRC susceptibility amongst individuals with FCCTX.

1.9 Study Objectives

The first objective of this study was to identify families meeting the diagnostic criteria for FCCTX with a strong family history, defined as having at least five cases of CRC. The second objective was to describe the clinical and pathological phenotype of the FCCTX probands and families. These features have been directly compared to contribute to the characterization of the clinical phenotype of and to assess the degree of heterogeneity present within these families. The phenotype of the first-degree relatives of the FCCTX probands were compared with first-degree relatives of fifteen population-based Lynch Syndrome probands in a time-to-event analysis. To identify potential genetic isolates, the geographic distribution of the genealogical origin of the FCCTX probands was assessed and compared to the distribution of Lynch Syndrome probands for whom genetic mutations have been confirmed. The next objective was to attempt to genealogically link as many of the FCCTX families as possible using a specialized electronic database and archival research. A summary of the study objectives is listed on the following page:

1. To identify FCCTX families with at least five cases of CRC from the population-based Newfoundland Colorectal Cancer Registry (NFCCR) and from families clinically referred to the Provincial Medical Genetics Program.

2. To compare the clinical and pathological phenotype of the FCCTX probands and family members.

3. To compare the incidence of CRC and other outcomes in first-degree relatives of probands from FCCTX and Lynch Syndrome families.

4. To compare the geographic origin of FCCTX and Lynch Syndrome families.

5. To determine whether any of the FCCTX families have common ancestry.

2. METHODOLOGY

2.1 Study Families

2.1.1 Selection Criteria

Molecular genetics researchers initially selected seven families (families 1-7) of interest for study on the basis of their severe phenotype and high potential for novel gene discovery. These families all had at least five cases of CRC within their extended pedigree, including affected spouses, and fulfilled the criteria for FCCTX: meeting the ACI, possessing no known MMR gene mutation, and having MSS CRC. Family 1 technically does not fulfill the ACI as the earliest diagnosis of CRC was 50 years and 2 months, while the criteria specifically require a diagnosis before age 50. However, for the purposes of this investigation, family 1 will be considered to fulfill the FCCTX criteria, as it may be very useful for novel gene discovery. An additional family (family 3) was initially thought to meet FCCTX classification, but was later proven not to meet the required ACI. This was discovered late in the investigation and so this family underwent detailed analysis. It has been excluded from all analyses in the body of the thesis, but has been included as an appendix (Appendix C). As a result, six families (families 1, 2, 4, 5, 6, and 7) are included in the body of this thesis that underwent extensive genealogical and clinicopathological study.

Subsequently, six additional families were identified that met the initial inclusion criteria: at least five family members with CRC in the extended pedigree with fulfillment of the ACI. These families did not undergo the extensive archival research and additional

clinicopathological profiling that the initial six FCCTX families were subjected to, as they were relatively late additions to the study. However, detailed pedigree construction was possible for these families and so they were involved in the time-to-event comparison with the Lynch Syndrome families and the KINNECT software analysis. The inclusion of these six families makes the total number of FCCTX families included in the investigation to twelve. Six of these families were identified in the Newfoundland Colorectal Cancer Registry (NFCCR), a population-based database compiled of consenting families of patients with pathology-confirmed CRC at age less than 75 between 1999 – 2003 (Green et al., 2007). The other six families were identified from the Provincial Medical Genetics Program (PMGP) having been ascertained clinically due to a strong family history of CRC before the time of the NFCCR. Differentiating between the population- and clinic-based families is important as more uniform follow-up and testing has been performed for the population-based families as they have undergone previous time-to-event study. On the other hand, the purely clinic-based families did not have such uniform recording of ages at last follow-up.

Fifteen families with Lynch Syndrome were used as a comparison group for the FCCTX families. The Lynch Syndrome families were identified from the NFCCR, met the ACI or ACII, and had a proband with MSI CRC and a confirmed MMR gene mutation. These particular Lynch Syndrome families were selected as they had undergone previous investigation with the availability of a database containing follow-up information for time-to-event analyses. Flow charts summarizing the ascertainment of the twelve FCCTX study families and fifteen Lynch Syndrome controls are included (Figure 2.1 and Figure 2.2). The Human Investigations Committee of the Faculty of Medicine at

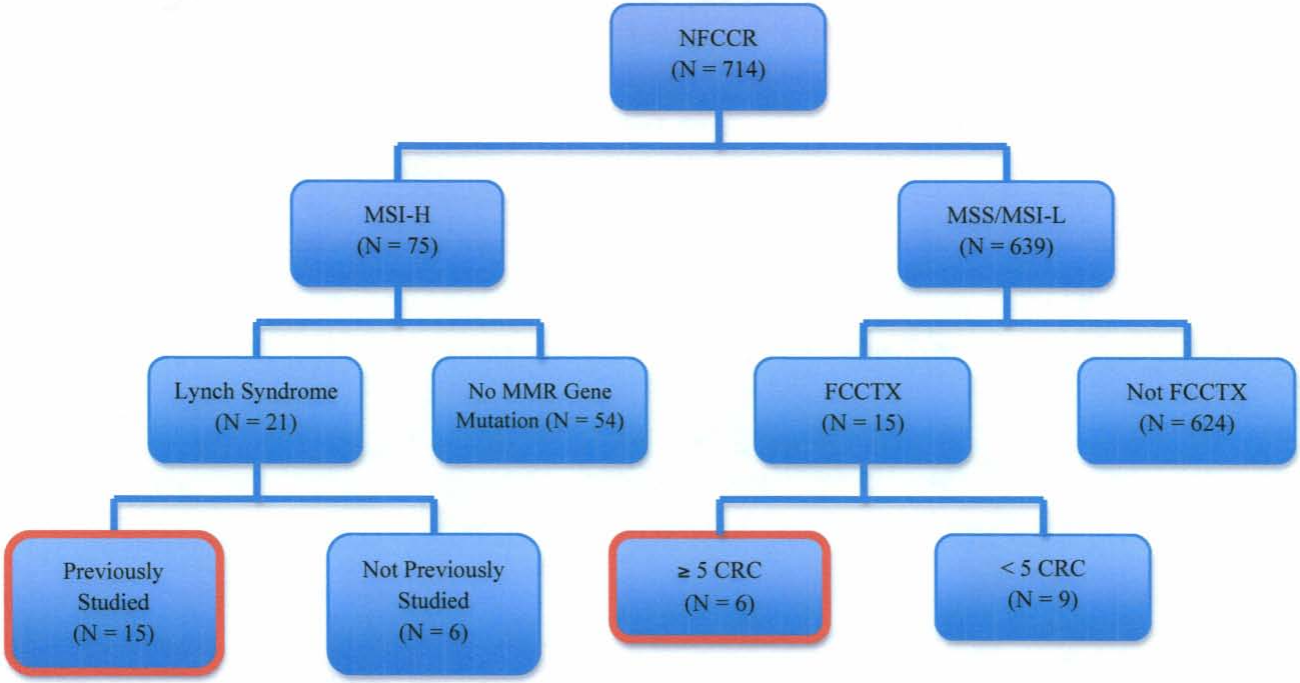

Figure 2.1: A summary of the methodology through which the study families were identified from the NFCCR.

1. N refers to the number of CRC patients from unique families (i.e. those not previously identified).
2. The boxes outlined in red represent the probands of the families selected for investigation in this thesis.

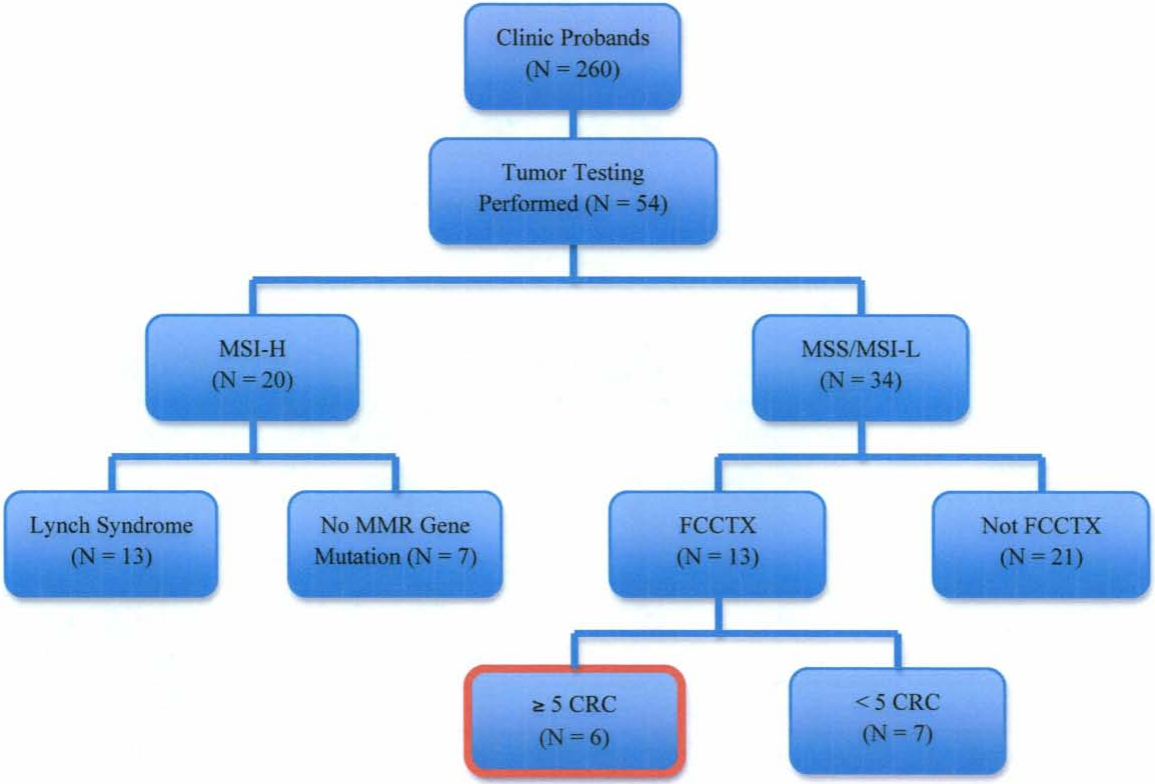

Figure 2.2: A summary of the methodology through which the study families were identified from the PMGP.

Memorial University granted ethical approval for the study. All patients and families involved had previously consented to being included in CRC research and to have their clinical outcomes and family history used for research purposes.

2.1.2 Proband Selection

For the population-based FCCTX and Lynch Syndrome families identified from the NFCCR, the probands was designated as the individual who had the pathology-confirmed case of CRC between 1999 and 2003. For the clinic-based FCCTX families, the probands were designated as the first individual in the family to make contact with the PMGP as a result of referral for a strong family history of CRC. When the proband was not explicitly indicated, the individual with the earliest case of CRC, based on date of diagnosis, was selected as the proband.

2.1.3 Clinical Data Collection

Information of interest in this investigation focused around the occurrence and features of colorectal polyps or cancers in members of the FCCTX families. All incidences of polyps or cancer had to be confirmed by medical records for inclusion in the statistical analyses. Medical records for members of the study families were obtained from the office of geneticist Dr. Jane Green and the PMGP. These records were thoroughly examined in order to ascertain the occurrence of colorectal polyps and/or cancer. All extracted information was entered into an SPSS version 17.0 database. Information on the features of the tumour DNA of probands was obtained through accessing the relevant password protected electronic research databases of the NFCCR.

This permitted access to microsatellite instability testing results as well as *BRAF* and MMR protein status of the tumour DNA. Additional information on tumour location and the occurrence of multiple tumours was also extracted from these databases.

An SPSS database was constructed to record the occurrence of any form of cancer and the age at diagnosis for each cancer for the purposes of time-to-event analysis. The majority of the variables in the database are nominal or categorical data. Information collected and recorded in this database on the subjects, identified by numerical codes to protect their confidentiality, includes age, gender, family number, whether or not they are dead or alive at the time of last follow-up, and cancer history. The cancer history portion of the database is by far the most extensive and focuses on colorectal as well as extracolonic cancers. It contains dichotomous variables on whether or not each subject has ever had cancer and if so, what specific type of cancer (colorectal, other Lynch Syndrome-related extracolonic cancers, or other non-Lynch Syndrome related cancers), the age at which these cancers were diagnosed, and the age at death or last follow-up. For individuals who failed to experience specific outcomes, age at last follow-up or age at death were recorded. Other Lynch Syndrome-related extracolonic cancers were defined to include stomach, ovarian, endometrial, pancreatic, small bowel, and transitional cell carcinomas (TCC) (Anaya et al., 2008). The follow-up information for the Lynch Syndrome subjects was adapted to conform with the format used for families in the present study in order to ensure direct comparability.

For the purposes of the pathology analyses, surgical reports of colonic resections as well as colonoscopy reports were utilized to obtain the gross features (location,

approximate size) of cases of CRC. Associated pathology reports were reviewed and data was systematically extracted using forms (Appendix A) including all standard cancer pathological variables according to the College of American Pathologists (Washington et al., 2008). The variables recorded include tumour location, size, histologic type and grade, depth of invasion, presence of lymphatic or vascular invasion, status of margins, and occurrence of distant metastases. Colonoscopy operative reports were useful in that they reported the number of polyps identified, their location, and often their approximate size. Pathology reports were then analyzed in order to record the specific features of the polyps. Data from these pathology reports was systematically extracted using forms (Appendix B) including all standard variables according to the College of American Pathologists (Washington et al., 2008). The variables recorded include polyp location, type, size, configuration, and level of dysplasia. The pathological data recorded on the extraction forms for colorectal polyps and cancer was entered into an SPSS database for the purpose of statistical analysis.

2.1.4 Family History Score Calculation

In 1984, a quantitative family history score (FHS) measure for assessing familial risk of retinoblastoma was developed (Chakraborty et al., 1984). The purpose of calculating FHS is to identify families with a higher than expected incidence of a particular disease. The FHS compares the phenotype of each first-degree relative of a proband to age and sex-matched population controls in terms of probability of disease. This involves comparing the observed number of cases for a family over a specific time period to the expected number of cases, calculated based on family member covariates

(age, sex, and race) and overall family structure. The statistic is also powered to account for unusual values of risk variables, such as early onset of disease, and not just the number of cases of disease.

The FHS calculation for estimating relative incidence of CRC was adapted from a 1998 breast cancer study, which was based on the previously described methods proposed by Chakroborty et al. (Yang et al., 1998). The expected CRC incidences were calculated using the Surveillance, Epidemiology, and End Results (SEER) program (National Cancer Institute – NCI). All of the FHS calculations were carried out using a Microsoft Excel spreadsheet specifically configured to solve the equations described below. Risk values were calculated using person-years extracted from data of age at CRC or age at last follow-up for unaffected family members. Only first-degree relatives of the probands were included in the calculations. The expected risk of an individual developing CRC (E_{ij}) was estimated by age, gender, and race (Caucasian, non-Hispanic) using the following equation:

$$E_{ij} = 1 - Exp[-\sum_{kj} ID_k \times \Delta t]$$

k = the age group

ID = the incidence density in the kth age group

Δt = the age interval

Using the calculated expected risk values, the FHS for each family was then calculated using the following equation:

$$T_i = \frac{\sum_j O_{ij} - \sum_j E_{ij}}{\sqrt{\sum_j E_{ij}(1 - E_{ij})}}$$

T_i = the family history score for family i

O_{ij} = the observed CRC status for the jth member in family i

E_{ij} = the expected risk for CRC for the jth member in family i

One limitation of using the FHS statistic to predict CRC risk is that it does not account for the risk of CRC conferred by colorectal polyps. Many of the first-degree relatives included in the analysis have had colorectal polyps identified and removed, but have never had CRC. The exclusion of these events from the analysis, which signify an increased risk of CRC, indicate that the calculated FHS values are likely conservative estimates of the true risk.

2.2 Comparison of Probands From Families Fulfilling the FCCTX Criteria

The comparison of probands from families meeting the criteria for FCCTX was based on available clinical and pathological features of colorectal tumours. Information on tumour location, age of onset, and the occurrence of multiple tumours was obtained through a review of the probands' medical records. The CRC tumour DNA of the probands also underwent testing for the presence of the c.1799T>A *BRAF* variant (p.Val600Glu) given its hypothesized role in the pathogenesis of CRC and association with the sessile serrated adenoma pathway.

2.3 Comparison of FCCTX and Lynch Syndrome Families

SPSS version 17.0 was utilized to perform all statistical analyses described in this section unless otherwise stated. For all tests, the standard significance level (α value) of 0.05 was used to specify the threshold for what would be considered a statistically significant difference.

2.3.1 Pearson Chi Square Test

The Pearson chi-square statistic was used to test for differences in the baseline characteristic gender, cancer, and mortality outcomes. Each comparison is a two-sided test on the null hypothesis that no difference existed for each specific characteristic between the groups. The chi-square distribution is a suitable technique when dealing with frequency or count data. It compares the observed frequencies, the number of subjects that fall within the categories being assessed, and the expected frequencies, the number of subjects that are expected to be in each category assuming the null hypothesis is true. This technique was employed to compare the distribution of gender and cancer outcomes. It is useful in that it ensures the comparability of the groups, by demonstrating that important, potentially confounding variables such as gender are not unevenly distributed. In addition, it provides an initial assessment of differences in outcome risk between the groups. 95% confidence intervals were also calculated to assess the power of the Chi square analysis. The following equations were utilized in to achieve this:

$$CI_{95\%} = ED \pm 1.96 \times SE$$

$$ED = p_1 - p_2$$

$$SE = \sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}}$$

p1 = proportion of first-degree relatives of FCCTX probands with outcome of interest

p2 = proportion of first-degree relatives of Lynch Syndrome probands with outcome of interest

ED = estimated difference between the two proportions p_1 and p_2

SE = standard error of the estimated difference

2.3.2 Time-to-Event Analysis

The Kaplan-Meier method, a simple form of survival analysis, was used to statistically analyze the time-to-cancer and time-to-death data collected and to produce survival curves (Kaplan and Meier, 1958). This method measures the percentage of subjects from a particular group who survive (i.e. do not experience the outcome of interest) over a particular period of time. It is not just restricted to subjects who have experienced an outcome, as the survival time data on subjects who did not experience an outcome contains useful information. Subjects who fail to experience the outcome of interest can be censored by removing them from the analysis at the time that they were last known to be free of that outcome (Bewick et al., 2004). Kaplan-Meier analysis was also used to compare survival curves of study families with population-based Lynch Syndrome subjects using the Log-Rank test. This involves computing a test statistic and an associated p-value that is used to test the null hypothesis that there is no significant difference in the probability of the outcome between the survival curves of the two groups.

This analysis was a comparison of first-degree relatives of the probands of the twelve study families (N = 126) to first-degree relatives from fifteen population-based families (N = 153) with Lynch Syndrome. The outcomes assessed were time to CRC, other Lynch Syndrome-associated extra-colonic cancers, and death. The mean survival time to each outcome and associated 95% confidence interval were obtained for each group as well as a Kaplan-Meier survival curve to directly profile differences between the two groups. Mean survival times were used rather than median values as SPSS was unable to calculate median survival times for some outcomes. Additionally, a Mantel-Cox log rank test was carried out to test the significance of the survival differences between the FCCTX and Lynch Syndrome individuals. To obtain a further evaluation of risk between the two groups, hazard ratios with 95% confidence intervals were computed for each outcome using the Cox Regression analysis. The assumption of proportional hazards was tested for each time-to-event outcome by generating log minus log plots and stratifying by group (i.e. FCCTX and Lynch Syndrome first-degree relatives). Converging, diverging, or crossing log minus log plots indicate violation of the assumption of proportional hazards (Box-Steffensmeier and Zorn, 2001).

2.4 Geographic Distribution of FCCTX Families

The location of origin of the FCCTX probands was mapped using Geographic Information Systems (GIS) software. To determine place of origin, families were retroactively contacted and asked the question: "Where did your family live when you were born?" Place of origin was reported for 12 FCCTX and 13 Lynch families respectively. Reported locations of origin were then matched to corresponding postal

codes from 2006 census records and latitude and longitude coordinates associated with these postal codes were extracted. In the case where places of origin had no postal code, the closest postal code available was used. When multiple postal codes were available for the location, an average of the latitude and longitude of all postal codes for that place of origin was calculated. Latitude and longitude coordinates were imported into the ARCGIS software (ARCMAP version 9.3) where a Transverse Mercator projection with North American 1983 Geographic Coordinate System and Datum were used to display the data. For the purposes of comparison, a similar procedure was carried out for all Lynch Syndrome families from the PMGP and not just the fifteen families used as a comparator for the time-to-event analysis.

2.5 Genealogical Investigations

The establishment of genealogical linkages would permit the combination of families and thus increase the power of genome-wide scans aimed at detecting the novel susceptibility genes conferring risk for CRC amongst this cohort of individuals. The primary advantage of having distantly related individuals for genome-wide scan studies is that common regions of DNA sequence are small and sparse. This is the result of the process of recombination, which occurs at every generation shortening the common regions of DNA sequence between the two lineages. Thus, it becomes easier to identify the deleterious variant, which is the reason for the increase in power of these genome-wide scan investigations. For example, the mapping of the common *MSH2* mutation responsible for a significant number of cases of Lynch Syndrome was made possible only by the genealogical linking of two Newfoundland families inhabiting different

communities (Froggatt et al., 1999). The goal of the genealogical investigation was to explore whether direct connections existed between any of the FCCTX families studied in the form of common ancestors and, in the absence of such a link, increase the certainty of ruling out a connection between the families. The genealogical investigation consisted of all twelve of the FCCTX families being analyzed by the KINNECT software, while six of these FCCTX families underwent further investigation in the archives.

2.5.1 KINNECT Software

Preliminary genealogical research was undertaken using census data and church records (birth, marriage, and burial data) available in the online resources NL GenWeb (http://nl.canadagenweb.org) and Newfoundland Grand Banks Genealogy (http://ngb.chebucto.org). The purpose of the initial genealogical investigations was to extend and expand the pedigrees as much as possible and to add new information on town of origin, dates of birth, as well as ensuring the accuracy of previously ascertained information. The twelve pedigrees of the study families were then converted from electronic records in Progeny® into the proper format to be analyzed by the KINNECT software. This software is powered to match individuals from study pedigrees to individuals in the Newfoundland Genealogy Database (NGD), a collection of family trees based on pre-confederation census records from 1880 - 1945. The NGB contains 522,000 unique individuals and represents a historical form of the population of Newfoundland. This database is a computer-based genealogical collection compiled by the Population Therapeutics Research Group (PTRG) of Memorial University. KINNECT creates an individual match score that is based on the number of fields that correspond between the

pedigrees and the NGD. The individual level match is broken out into a series of 66 parallel match comparisons, including for example: name, married name, date of birth, location, spouse and parents. This parallel design is necessary to determine the best match as records can be matched in a variety of ways. When matching on location, KINNECT considers all individuals within a 50km radius of the specified location, as this was considered a reasonable distance for traveling between the years the NGD covers.

Two variables, individual weight (IW) and family weight (FW), are used to determine a match between pedigree and NGD information, The IW is determined by the amount of pedigree information that matches to the NGD for a particular individual; a higher score represents a greater number of matches. An IW score of 60 is considered a significant match, but each result must be examined individually for two reasons. First, IW is influenced by the amount of pedigree information available and thus when little information is available, a lower score will be reported even when a match corresponds to the person of interest. Second, common names create many matches that had to be individually checked to find the person of interest. To aid in this process, KINNECT uses the second variable, FW, to help find the correct match. The FW score is based on matches to an individual's mother, father and spouses. When an individual matches both parents they receive a score of 85, while matching to only one parent receives a score of 55. Finally when IW and FW are computed, summations of the scores are displayed in descending order, with each score representing an individual that may be the person of interest from the pedigree. The output from the database was used in coordination with the GenWeb and Newfoundland Grand Banks Genealogy online resources to further extend the pedigrees.

This method employed by the PTRG has been was validated by genealogically connecting Attenuated Familial Adenomatous Polyposis patients. Five patient pedigrees were extensively extended by genealogists over several years and were linked to a common founder. 376 individuals within the multiplex family fell within the scope of the software for testing purposes by virtue of being born before 1946. Of these individuals, PTRG manually identified 237 individuals as being present in the NGD. Using KINNECT, PTRG was able to match 229 of the 237 manually identified individuals, a success rate of 97%.

2.5.2 Archival Research

Upon exhausting the resources of the PTRG, archival research was conducted at The Rooms Provincial Archives to further extend the pedigrees of six of the FCCTX families under investigation. The GIS software was used to map towns of origin for the study families in order to identify sites of clustering and to provide direction to archival pursuits. The majority of this research consisted of manually searching through birth, marriage, and burial parish records from the Roman Catholic, Anglican, United, Salvation Army, and Presbyterian churches in the various regions of the province. The records from these approximately two hundred parishes are the centerpiece of all Newfoundland genealogical studies as no central registry for births, marriages, and deaths existed prior to 1891, with all records being recorded and retained independently by the parishes of the province. Pre-Confederation provincial vital statistic microfilm collections (1892 – 1949) of assorted births, marriages, and deaths were also consulted. Birth records provide not only dates of birth for individuals of interest, but also list their place of birth as well as

their parents' names. Marriage records are useful in that they list the maiden name of the bride and the town of origin for both the bride and groom. Burial records often provide dates of birth as well and are especially useful in determining dates of birth for individuals who pre-date the earliest available birth records. In addition to parish burial records, the Stonepics database, a digital collection of headstone pictures from Newfoundland cemeteries, was also utilized. Additionally, the collections of census records (1675-1945), lists of registered voters (1832- 1980), a registry of Crown land purchases (1830-1930), and all available resources were utilized in the process. The website Our Full DataBank of Canada, Newfoundland and England was consulted for genealogical records for the Bonavista Bay area (Roberts, 2002). This collection of records on births, deaths, marriages, and family units was incredibly detailed, providing information as far back as 16th century England. The genealogical research involved exhaustive use of all available resources in an attempt to reconstruct the history of the six study families as completely as possible.

2.6 Additional Data Analysis

2.6.1 Time-to-Event Analysis

The second analysis involved individual profiling of six of the study families with the same outcomes as the previous time-to-event analysis, but with inclusion of subjects beyond the level of the first degree of relation. To be included in this analysis, study family members were required to be at 50% risk for inheriting the CRC susceptibility genes, assuming autosomal dominant inheritance. Additionally, for an individual to be included in the analysis, follow-up information had to be available for that person as well

as at least 50% of his or her siblings. This condition minimized bias by allowing exclusion of individuals from older generations who experienced cases of CRC, but whose siblings lacked follow-up information. Subjects who experienced the outcome of interest, but for whom an age of onset could not be confirmed, were excluded from the analyses.

2.6.2 Pathology Analysis

To be included in the pathology analysis, individuals had to have experienced a case of colon cancer, rectal cancer, or colorectal polyps, for which a pathology report was available. Family members with documented cases of CRC were required to be at 50% risk for inheriting the putative CRC susceptibility allele, assuming autosomal dominant inheritance, to be included in the analysis. Individuals with reported events but for whom pathological reports could not be obtained or were not performed were excluded from the analysis. Multiple cases of primary CRC (either synchronous or metachronous) within the same individual, defined as multiple tumours, were included as separate events in the analysis. Instances of local or regional recurrence of CRC, defined as the detection of cancer in the anastomosis, mesentery, tumour bed, or surgical wound following curative resection, were excluded from the analysis (Funzun et al., 2004). Additionally, extra-colonic cancers for which pathology reports were available were also excluded from the analysis given the focus of the research on CRC. Each polyp was treated as a separate event in the analysis. After exclusion of ineligible subjects, 37 cases of CRC from 33 different individuals and 210 cases of colorectal polyps from 56 different individuals were analyzed. The right colon was defined as the cecum, ascending colon, hepatic

flexure, and transverse colon. The left colon was defined as the splenic flexure, descending colon, sigmoid colon, and rectum (Distler and Holt, 1997). Polyps were analyzed in terms of type (hyperplastic polyps or tubular, villous, and tubulovillous adenomas) and location (right or left side). Tumours were analyzed in terms of location (right or left side) and the occurrence of multiple tumours. All other variables collected were excluded from the final analysis due to an unacceptable number of missing values.

3. RESULTS

Twelve families fulfilling the criteria for FCCTX with at least five cases of CRC were identified. The average FHS for these families was 24.2 with values ranging from -1.3 to 46.5. The number of cases of CRC per family ranged from 5 to 15.

3.1 Comparison of Probands From Families Fulfilling the FCCTX Criteria

The clinical and pathological features of the FCCTX probands have been summarized in the table on the following page (Table 3.1). Mean age of onset of first primary CRC was 56.4 and ranged from 39 to 76 amongst the FCCTX probands. Of the probands, six had a single left-sided CRC, four had one case of right-sided CRC, and two demonstrated multiple primary tumours, including both right- and left-sided CRCs. Three of the probands (2, 4, and 6) had the p.Val600Glu *BRAF* variant in their tumour DNA with 60% of these tumours being right-sided. Interestingly, two of these three probands (probands 4 and 6) experienced two primary tumours. However, only one of the two primary CRCs underwent *BRAF* testing for both of these probands. The other nine probands had *BRAF* wild-type alleles in their tumour DNA and demonstrated a markedly left-sided pattern of tumours, with 80% of the tumours being found distal to the transverse colon.

Table 3.1: A summary of the clinical and pathological features of the probands of the study families.

Proband	Location of Origin	Criteria	CRC (N in family)	FHS	*BRAF*	CRC Location	Multiple Primary CRCs	Age at CRC
1	Greenspond	ACI	10	34.7	Wild-type	Right	No	53
2	N. Peninsula	ACI	7	46.1	p.Val600Glu	Right	No	67
4	Greenspond	ACI	6	41.0	p.Val600Glu	Left & Right	Yes	1st CRC: 60 2nd CRC: 69
5	St. Josephs	ACI	5	37.1	Wild-type	Right	No	39
6	Indian Isl & Hare Bay	ACI	5	18.8	p.Val600Glu	Left & Right	Yes	1st CRC: 62 2nd CRC: 62
7	N. Peninsula & Conc. Bay	ACI	15	-1.3	Wild-type	Left	No	43
8	Bishop's Falls	ACI	5	2.6	Wild-type	Left	No	47
9	St. John's	ACI	7	35.8	Wild-type	Left	No	60
10	N. Pensinsula	ACI	7	-0.7	Wild-type	Left	No	68
11	Long Harbour	ACI	5	5.6	Wild-type	Left	No	45
12	Green's Harbour	ACI	5	24.1	Wild-type	Left	No	57
13	St. John's	ACI	7	46.5	Wild-type	Right	No	76

N. Peninsula = Northern Peninsula; Conc. Bay = Conception Bay, Indian Isl = Indian Islands

3.2 Comparison of FCCTX and Lynch Syndrome Families

3.2.1 Pearson Chi Square Test

The Pearson chi square statistic was used to compare first-degree relatives of the twelve FCCTX probands (N = 126) to first-degree relatives of the Lynch Syndrome population-based probands (N = 153) in terms of outcomes as count data (Table 3.2). The comparison demonstrated no significant difference in gender distribution (p-value = 0.889) or number of events of cancer (p-value = 0.426), CRC (p-value = 0.912), and death (p-value = 0.241). The Lynch Syndrome group experienced significantly more cases of extra-colonic Lynch Syndrome-related cancers (p-value = 0.044) than the study families. The fact that the 95% CI (-0.104, -0.004) comparing the proportion of FCCTX first-degree relatives with other Lynch Syndrome-related cancers did not cross zero further confirms the occurrence of a significantly less of the other Lynch-Syndrome related cancers in the FCCTX group.

Table 3.2: Summary of the Pearson chi square test used to compare the two groups.

Cohort	N	Gender (N males, N females)	Cancer*	CRC	Other Lynch Syndrome-related Cancer¶	Dead
FCCTX Group	126	62 (49.2%) 64 (50.8%)	52 (41.2%)	37 (29.4%)	3 (2.4%)	41 (32.5%)
Lynch Syndrome Population Group	153	74 (48.4%) 79 (51.6%)	56 (36.6%)	44 (28.8%)	12 (7.8%)	40 (26.1%)
p-value (2-sided)		0.889	0.426	0.912	0.044	0.241
95% CI¥ (p_1-p_2)		(-0.110, 0.126)	(-0.069, 0.161)	(-0.101, 0.113)	(-0.104, -0.004)	(-0.043, 0.171)

Note: The "Cancer", "CRC", "Other Lynch Syndrome-related Cancer", and "Dead" columns represent the number of outcomes recorded for each of these events in each group.

* The "Cancer" outcome included all forms of cancer including CRC and Lynch-Syndrome related cancers.

¶ Lynch Syndrome-related cancers included stomach, ovarian, endometrial, pancreatic, small bowel, and transitional cell carcinomas.

¥ The 95% CI refers to the comparison of the proportion of first-degree relatives in the FCCTX and Lynch Syndrome groups that experienced each outcome of interest.

3.2.2 Kaplan Meier Time-to-Event Analysis

Kaplan Meier survival analysis was used to compare the difference in time-to-CRC, other Lynch Syndrome-related cancer, and death between first-degree relatives of the probands from the FCCTX and Lynch Syndrome groups. Cox regression analysis was also employed to obtain hazard ratios for all comparisons. The mean survival time to CRC was 75.2 (95% CI: 70.3 – 80.2) for the FCCTX group and 67.3 (95% CI: 63.1 – 71.6) for the Lynch Syndrome group. Although these confidence intervals overlap, the hazard ratio reveals that the individuals in the FCCTX families are at significantly less risk for developing CRC earlier in life (HR = 0.64, 95% CI: 0.42 – 0.997). This trend is represented in the survival table (Table 3.3), which demonstrates much greater

proportions of individuals being affected in the Lynch Syndrome group at the ages of 30, 40, 50, and 60 respectively, while the percent of individuals affected roughly equalizes later in life. The value of the log rank statistic comparing the survival of the two groups was 4.03 (p-value = 0.045), indicating a significant difference in the survival distributions between the two groups. This is illustrated by the survival curves of the two groups (Figure 3.1), which demonstrate that the Lynch Syndrome subjects experience CRC at a significantly earlier age than the individuals in the FCCTX families. The assumption of proportional hazards was met for the time-to-CRC outcome as the log minus log plots for the first-degree relatives of FCCTX and Lynch Syndrome probands did not converge, diverge, or cross over time.

Table 3.3: Time-to-CRC outcomes for the study families and Lynch Syndrome population group.

Cohort	N	N Events	Percent Affected by Age						Mean Survival Time (95% CI)	HR (95% CI)
			30	40	50	60	70	80		
FCCTX Group	126	37	0	1	11	22	47	65	75.2 (70.3 – 80.2)	0.64 (0.42 – 0.997)
Lynch Syndrome Population Group	153	44	2	6	25	39	56	56	67.3 (63.1 – 71.6)	--

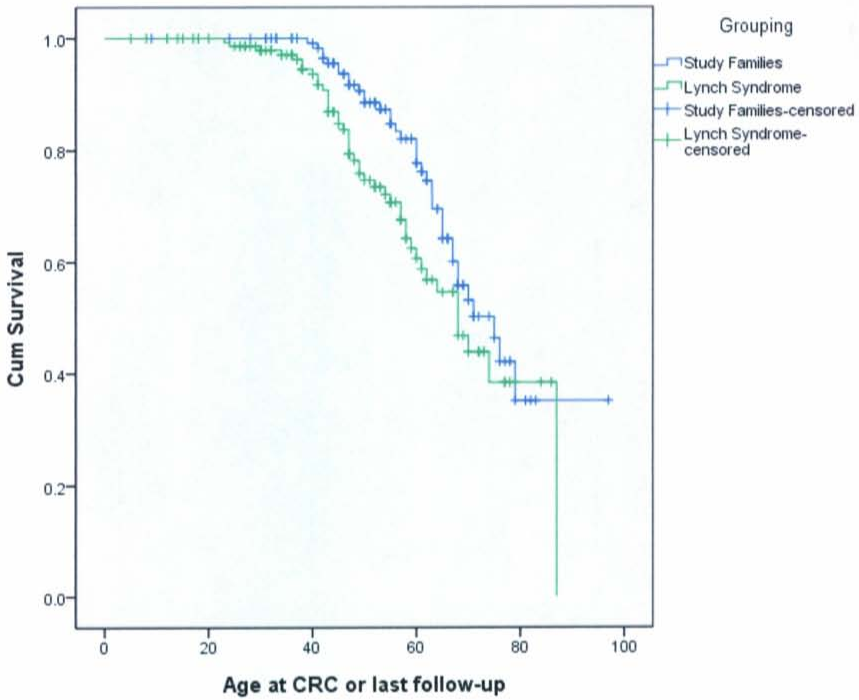

Figure 3.1: Kaplan Meier survival curves for time-to-CRC for study families and the Lynch Syndrome group.

The mean survival time to other Lynch Syndrome-related extra-colonic cancers was 95.2 (95% CI: 93.3 – 97.2) and 94.4 (89.8 – 99.1) respectively for the first-degree relatives of the FCCTX and Lynch Syndrome probands (Table 3.4). Although the mean survival times are similar between the families, this value is non-contributory given the small percentage of individuals experiencing the outcome, which limits its utility. More meaningful is the fact that the hazard ratio reveals that members of the FCCTX families are at significantly lower risk for developing other Lynch Syndrome-related cancers (HR = 0.20, 95% CI: 0.06 – 0.72). The assumption of proportional hazards was satisfied for the time-to-other Lynch Syndrome-related cancer outcome as the log minus log plots for the first-degree relatives of FCCTX and Lynch Syndrome probands did not converge, diverge, or cross over time. Additionally, Kaplan Meier analysis indicates that FCCTX family members experience significantly longer survival for this outcome (log rank statistic = 7.50, p-value = 0.006), which is apparent from a comparison of the survival curves for the two groups (Figure 3.2).

Table 3.4: Time-to-other Lynch Syndrome-related cancer outcomes for the study families and Lynch Syndrome population group.

Cohort	N	N Events	Percent Affected by Age					Mean Survival Time (95% CI)	HR (95% CI)
			30	40	50	60	70		
FCCTX Group	126	3	0	0	0	3	5	95.2 (93.3 – 97.2)	0.20 (0.06 – 0.72)
Lynch Syndome Population Group	153	12	2	2	6	14	17	94.4 (89.8 – 99.1)	--

Note: Lynch Syndrome-related cancers included stomach, ovarian, endometrial, pancreatic, small bowel, and transitional cell carcinomas.

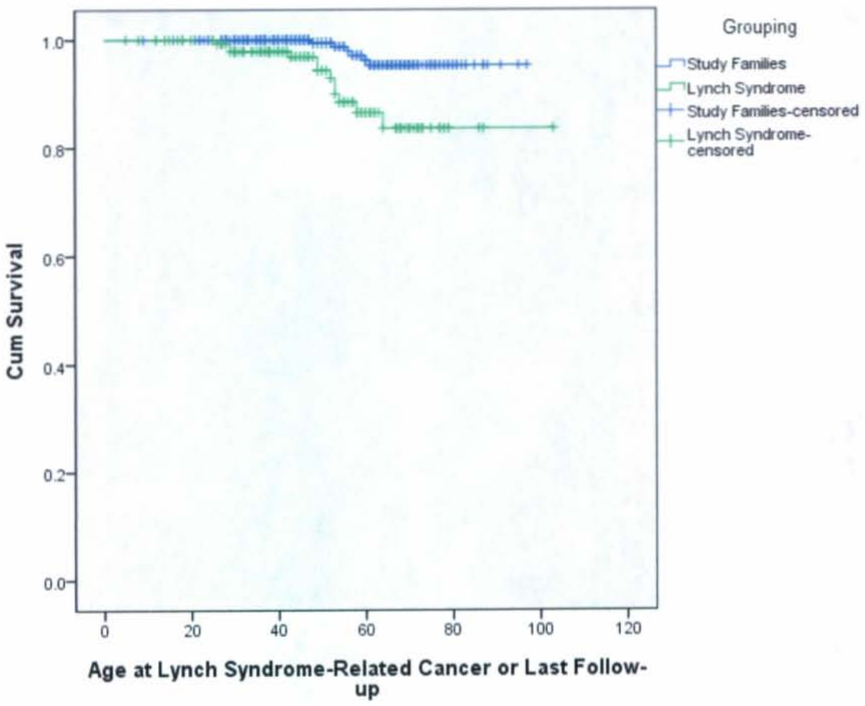

Figure 3.2: Kaplan Meier survival curves for time-to-other Lynch Syndrome-related cancer for study families and the Lynch Syndrome group.

Life expectancy in FCCTX first-degree relatives was 74.3 (95% CI: 69.9 – 78.7) and 70.9 (71.2 – 76.8) for the Lynch Syndrome group (Table 3.5). Again the confidence intervals overlap and the confidence interval for the hazard ratio (HR = 0.80; 95% CI: 0.51 – 1.24) crosses 1, indicating the absence of a significant difference in survival between the two groups. A log rank value of 1.03 (p-value = 0.309) further establishes this similarity, which is illustrated in the survival curves for the two groups (Figure 3.3). The assumption of proportional hazards was not met for the time-to-death outcome as the curves converged and crossed over time. Such converging hazards are biased towards an underestimation of the true hazard ratio (Box-Steffensmeier and Zorn, 2001). Therefore, the hazard ratio of 0.80 (95% CI: 0.51 – 1.24) may be an underestimation of true hazard ratio.

Table 3.5: Time-to-death outcomes for the study families and Lynch Syndrome population group.

Cohort	N	N Events	Percent Affected by Age						Mean Survival Time (95% CI)	HR (95% CI)
			30	40	50	60	70	80		
FCCTX Group	126	41	1	2	10	16	42	60	74.3 (69.9 – 78.7)	0.80 (0.51 – 1.24)
Lynch Syndrome Population Group	153	40	3	5	12	25	49	71	70.9 (66.1 – 75.6)	--

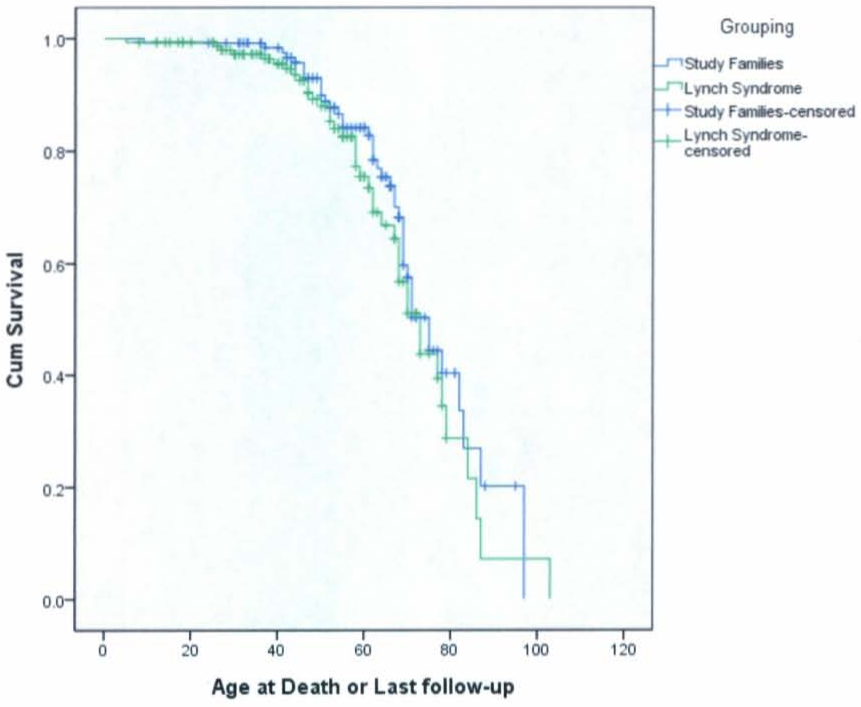

Figure 3.3: Kaplan Meier survival curves for time-to-death for study families and the Lynch Syndrome group.

3.3 Geographic Distribution of FCCTX Families

The geographic distribution of the FCCTX families was mapped using the GIS software (Figure 3.4). The general pattern that is evident from this map is that the FCCTX probands are scattered across the province. However, several instances of clustering have been identified. Two of the probands trace their origin to the town of Greenspond in the Bonavista Bay area. Interestingly, three of the probands originate in the Northern Peninsula, two of whom are in very close proximity. It is hypothesized that, on the basis of a founder effect, families that cluster geographically and have similar phenotype may possess identical inherited mutations. This is demonstrated in the fifteen Lynch Syndrome families with obvious geographical clustering of families with identical types of MMR gene variants (Figure 3.5). There are two obvious areas of clustering: the *MSH2* mutation with a deletion in exon 8 on the Avalon Peninsula and the MSH2 p.Val265_Gln214del variant clustering in the Bonavista Bay and Notre Dame Bay areas. These areas of clustering represent distinct genetic isolates each containing families with identical MMR gene mutations. As such, different types of mutations in different MMR genes tend to cluster across the province in these isolates. A full assessment of the geographical clustering of FCCTX probands with consideration of clinicopathological features is present in the discussion.

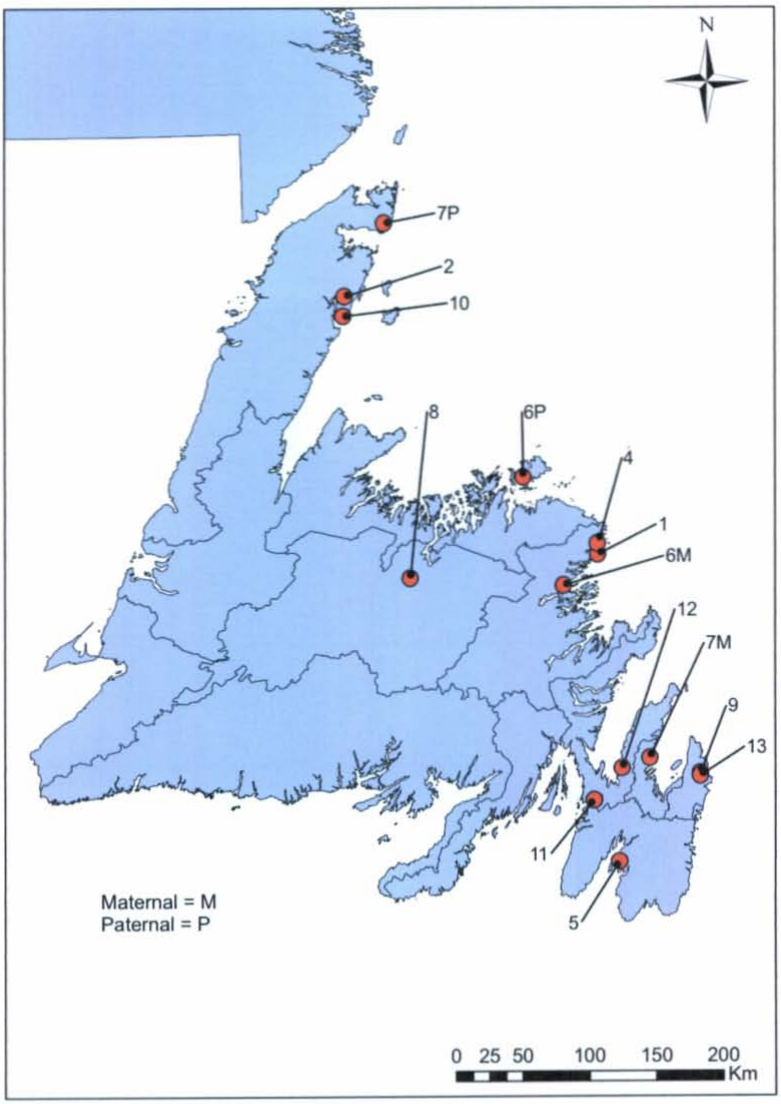

Figure 3.4: A map displaying the geographical distribution of the twelve families studied. Credit for figure to Geoff Warden.

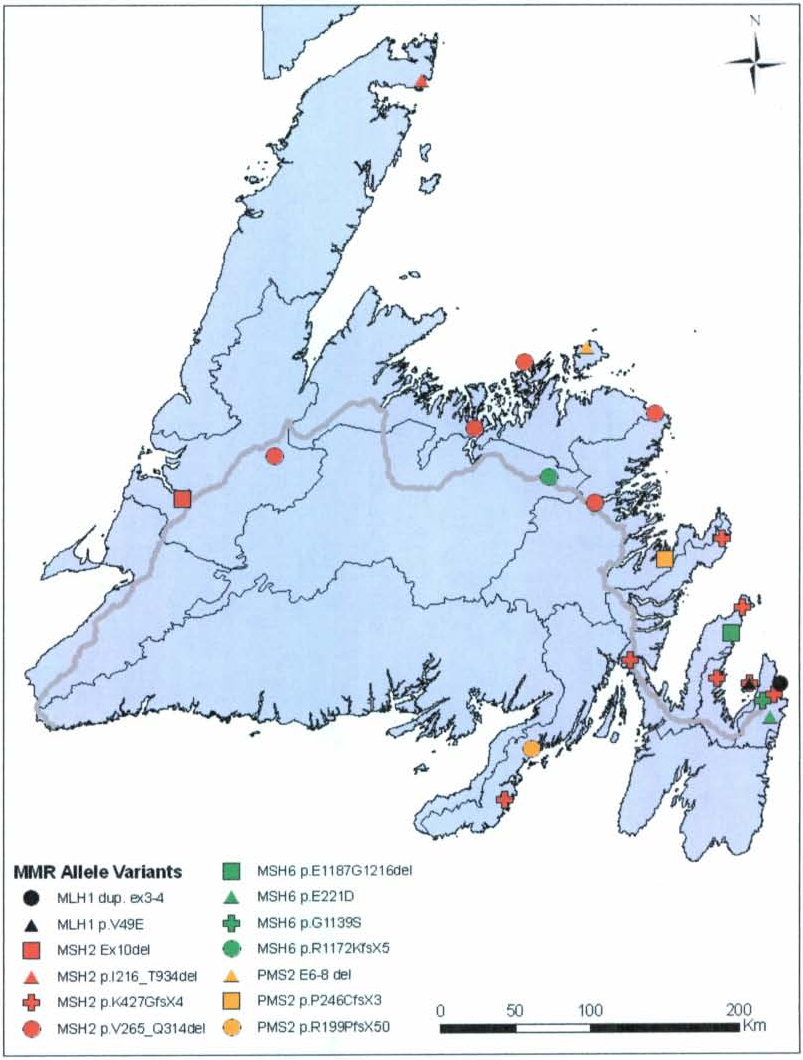

Figure 3.5: A map displaying the distribution of the Lynch Syndrome families selected from the PMGP. Credit for figure to Geoff Warden.

3.4 Genealogical Investigation

3.4.1 KINNECT Software

Analyzing the twelve FCCTX study families through the KINNECT software of the PTRG failed to yield any common ancestry. However, the output from the database did allow for the acquisition of date of birth information for some family members and the identification of previously unknown individuals through the census records.

3.4.2 Archival Research

The pedigrees of six FCCTX study families identified initially were extended as far into the past as the records would allow. A total of 658 new individuals were added to the pedigrees with the number of new generations added ranging between one and ten (see Table 3.6). In all cases, extension was possible as far back as the early 19th century, with one pedigree being extended into the early 16th century. No common ancestry between the families was observed. Interestingly, there is some geographical clustering of families in terms of town of origin. Two of the families originate in Greenspond, two other families originate close to one another in the Northern Peninsula, and two families have roots in the nearby Conception Bay communities of Bradley's Cove and Harbour Grace.

Table 3.6: Summary of the results of genealogical extension of the study families' pedigrees.

Family	Town(s) of Origin	N New Individuals	N New Generations	Earliest Year of Records
1	Damerham, Hampshire, England → Greenspond, NL	195	10	1520
2	Croque and St. Julian's, NL (Northern Peninsula)	44	4	1820
4	Greenspond, NL	305	4	1747
5	St. Joseph's, NL (St. Mary's Bay)	13	1	1820
6	Indian Islands and Hare Bay, NL	35	3	1800
7	St. Anthony and Harbor Grace, NL	38	2	1800

3.5 Detailed Profile of Six of the Study Families

The six FCCTX families examined in detail in this section are the same six whose pedigrees underwent genealogical reconstruction through archival research. Four of the families (families 1, 5, 6, and 7) are clinic-based, having had a family member referred to the PMGP due to a significant family history of CRC. The other two families (family 2 and family 4) are population-based, having been recruited as a result of having a family member with a case of pathology-confirmed CRC before the age of 75 between 1999 and 2003. The average FHS for the families was 29.4. The mean age of onset of CRC was 59.1. A survival curve profiling the time-to-CRC for all six families individually is presented below (Figure 3.6). This figure demonstrates that the survival time to CRC was relatively comparable amongst the six families, but that some degree of heterogeneity exists between them. Specific findings for each of the study families are presented in the following section. The findings that are presented in the next six sections have been summarized and arranged in a geographical manner (Table 3.7).

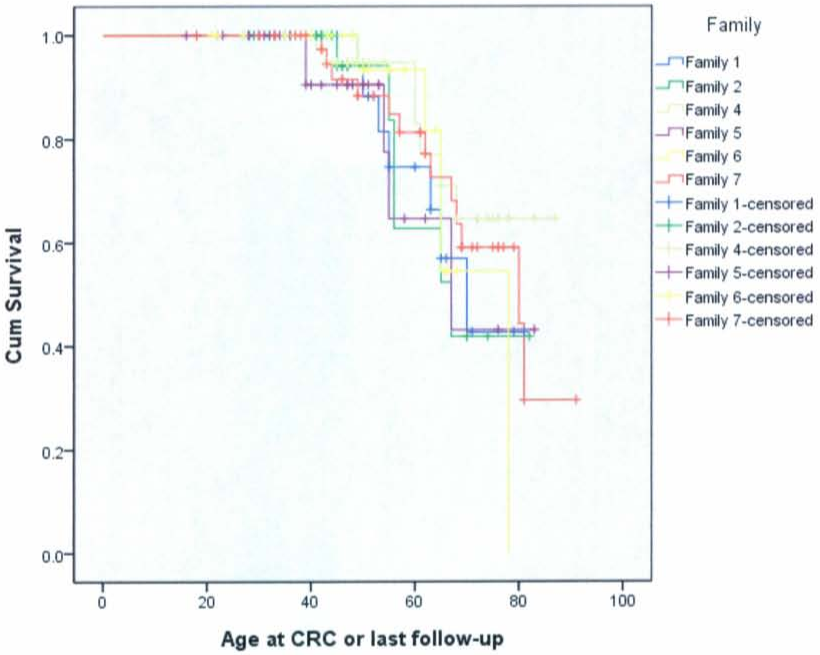

Figure 3.6: Kaplan Meier survival curves for time-to-CRC for each of the six FCCTX families studied in detail.

Table 3.7: Summary of the clinicopathological features of the study families who underwent additional investigation.

	Family								Proband			
Family	**Town of Origin**	**Criteria**	**FHS**	**CRC (N)***	**%CRC by 70**	**% Left Sided**	**Multiple Primary CRCs (N)¶**	**Villous Polyps**	***BRAF***	**CRC Loc.**	**Multiple Tumours**	**Villous Polyps**
1	Greenspond	ACI	34.7	7	57	71	0	Yes	Wild-type	Right	No	No
4	Greenspond	ACI	41.0	8	40	86	1	Yes	p.Val600 Glu	Left & Right	Yes	No
2	N. Peninsula	ACI	46.1	6	58	50	0	Yes	p.Val600 Glu	Right	No	No
7	N. Peninsula & Conc. Bay	ACI	-1.3	14	41	100	0	No	Wild-type	Left	No	No
5	St. Josephs	ACI	37.1	6	57	20	1	Yes	Wild-type	Right	No	Yes
6	Indian Island & Hare Bay	ACI	18.8	6	46	40	1	Yes	p.Val600 Glu	Left & Right	Yes	No

Note: N. Peninsula = Northern Peninsula, Conc. Bay = Conception Bay, Loc. = Location

*The numbers reported in the "CRC" column reflect the total number of primary CRCs in each family, including instances of multiple primary tumours.

¶The numbers reported in the "Multiple Primary CRCs" column reflect the number of individuals in each family with more than one primary CRC.

3.5.1 Family 1

For the purposes of this investigation, family 1 (N = 21) are said to meet the ACI, despite the earliest diagnosis of CRC being at age 50 rather than before it. The family had a FHS of 34.7 and displayed a pattern of CRC consistent with autosomal dominant inheritance (Figure 3.7). 195 new individuals were added to the pedigree, which was traced back ten new generations to the early 16th century in Damerham, Hampshire, England (Appendix D). The records indicate that founding members of this family migrated to Greenspond, Newfoundland in the early 19th century. The phenotype of family 1 is presented below with a profile of the polyps and tumours (Table 3.8) and a summary of the Kaplan Meier time-to-event analysis (Table 3.9). The time-to-CRC data was consistent with the FCCTX phenotype, with the majority of cases occurring after the age of 50. There were seven colorectal tumours and twenty colorectal polyps reported within the family. Polyps (71.4%) and tumours (60.0%) tended to be left-sided, while tubular adenomas (60.0%) were the most common type of polyp. The left-sided predominance of polyps and tumours as well as the mean age of onset of 57.9 (95% CI: 50.3 – 65.4) are consistent with the FCCTX phenotype. However, the three cases of extra-colonic Lynch Syndrome-related cancers are uncharacteristic of FCCTX. The proband had a tumour with wild-type *BRAF* alleles, which in combination with the left-sided predominance of colorectal tumours is may be suggestive of a molecular pathway to CRC other than the sessile serrated adenoma pathway.

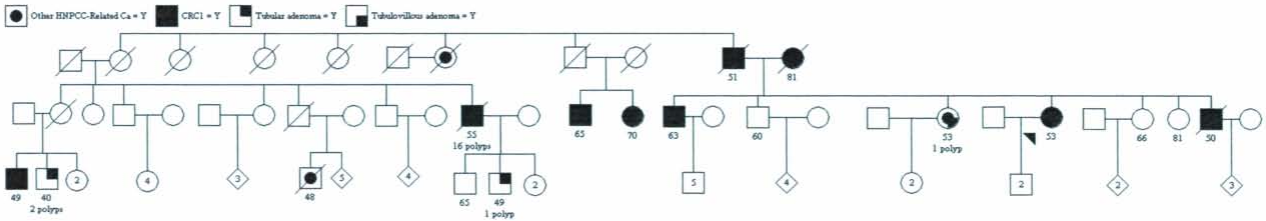

Figure 3.7: A pedigree displaying the three most recent generations of family 1.

Table 3.8: Summary of the phenotype of the proband and family 1 collectively.

	Proband	Family
Number of Primary CRCs	1	7
Number of Primary CRCs With Pathology Report	1	7
Mean Age at Onset of CRC (95% CI)	53	57.9 (50.3 – 65.4)
CRC Location	Right (Hepatic flexure)	Left: 5 (71.4%) Right: 2 (28.6%)
Multiple Tumours	No	0
Polyps	No	20*
Polyp Type	No polyps reported	Hyperplastic: 2 (10.0%) Tubular: 12 (60.0%) Villous: 2 (10.0%) Tubulovillous: 4 (20.0%)
Polyp Location	n/a	Left: 12 (60.0%) Right: 6 (30.0%) Missing: 2 (10.0%)
BRAF Status	Wild-type	--
FHS	34.7	

*The number of polyps per affected individual ranged from 1 to 16.

Table 3.9: Kaplan Meier time-to-event analysis results for family 1.

Event	N Events	Percent Affected by Age 30	40	50	60	70	80	Mean Survival Time (95% CI)
CRC	7	0	0	12	25	57	57	69.1 (62.6 – 75.5)
Death	3	0	0	6	13	13	30	75.6 (70.1 – 81.0)

3.5.2 Family 2

Family 2 (N = 43) fulfilled the ACI, had a FHS of 46.1, and displayed a pattern of CRC consistent with autosomal dominant inheritance (Figure 3.8). The family was traced back four new generations to the Northern Peninsula communities St. Julian's and Croque with the pedigree being extended back to the early 19th century with the addition

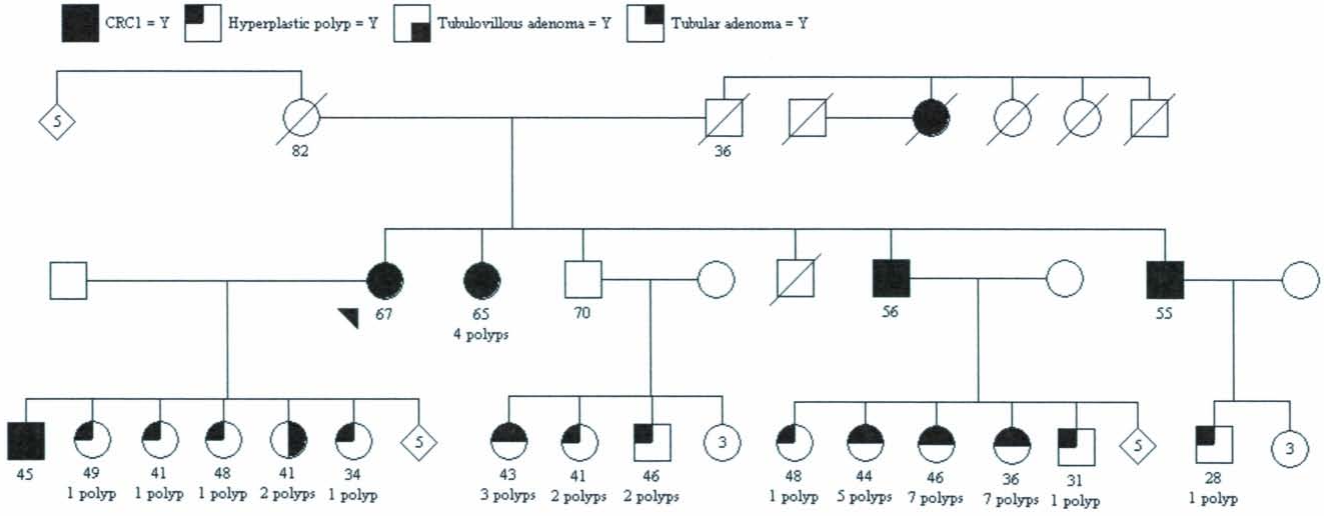

Figure 3.8: A pedigree displaying the three most recent generations of family 2.

of 44 new individuals (Appendix E). The phenotype of family 2 is presented below with a profile of the polyps and tumours (Table 3.10) and a summary of the Kaplan Meier time-to-event analysis (Table 3.11). The time-to-CRC data was consistent with the FCCTX phenotype with the majority of cases occurring after the age of 50. There were six colorectal tumours and thirty-eight colorectal polyps reported for the family. Polyps were most commonly hyperplastic (71.0%) in nature, but location was not available for the majority of lesions as direct access to the pathology reports was not possible. Tumours were evenly distributed between left- and right-sided. The p.Val600Glu *BRAF* variant was found in the tumour DNA of the proband of family 2. The presence of the *BRAF* variant and the absence of predominantly left-sided tumours suggest that the sessile serrated pathway may be involved.

Table 3.10: Summary of the phenotype of the proband and family 2 collectively.

	Proband	**Family**
Number of Primary CRCs	1	6
Number of Primary CRCs With Pathology Report	1	4
Mean Age at Onset of CRC (95% CI)	67	57.3 (49.0 – 65.6)
CRC Location	Right (Transverse colon)	Left: 2 (50.0%) Right: 2 (50.0%)
Multiple Tumours	No	No
Polyps	No	38*
Polyp Type	No polyps reported	Hyperplastic: 27 (71.0%) Tubular: 8 (21.0%) Villous: 2 (5.3%) Tubulovillous: 1 (2.6%)
Polyp Location	n/a	Left: 2 Unknown: 36
BRAF Status	p.Val600Glu	--
FHS	46.1	

*The number of polyps per affected individual ranged from 1 to 7.

Table 3.11: Kaplan Meier time-to-event analysis results for family 2.

Event	N Events	Percent Affected by Age 30	40	50	60	70	80	Mean Survival Time (95% CI)
CRC	6	0	0	6	37	58	58	68.2 (60.5 – 75.9)
Death	5	0	3	7	7	26	50	74.8 (68.4 – 81.1)

3.5.3 Family 4

Family 4 (N = 40) met the ACI, had an MSS tumour, a FHS of 41.0, and demonstrated a pattern of CRC that may be consistent with autosomal dominant inheritance or could be a chance aggregation of CRC (Figure 3.9). In total, 305 new individuals from four new generations were added to its pedigree, which has been traced back to the mid-18th century in Greenspond (Appendix F). The phenotype of family 4 is presented below with a profile of the polyps and tumours (Table 3.12) and a summary of the Kaplan Meier time-to-event analysis (Table 3.13). The time-to-CRC data demonstrated that the majority of cases occurred after the age of 50. There were eight colorectal tumours, including one instance of multiple primary CRCs, and fifty-eight colorectal polyps. Polyps (67.2%) and tumours (85.7%) were more commonly left-sided, while hyperplastic polyps (51.7%) were the most common type of polyp identified. The left-sided predominance of polyps and tumours, the absence of extra-colonic Lynch Syndrome-related cancers, and the mean age of onset of CRC of 61.3 (95% CI: 54.3 – 68.2) appear to fit the FCCTX phenotype. The proband had multiple primary CRCs with *BRAF* mutations. These findings provide mixed evidence for the underlying molecular pathway with the p.Val600Glu *BRAF* variant suggesting possible involvement of the

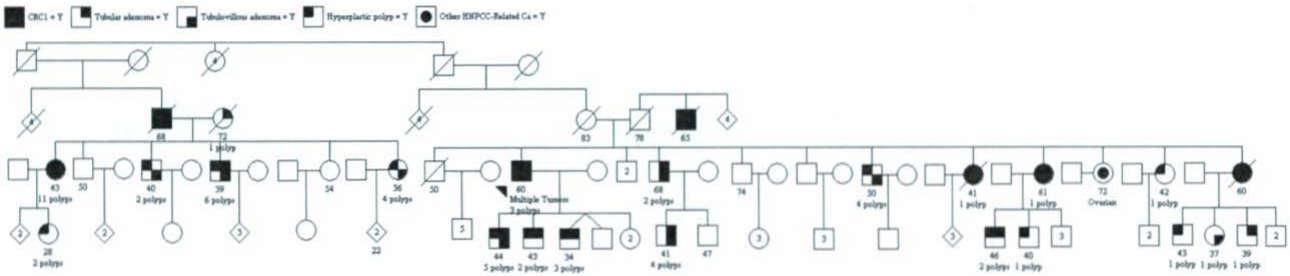

Figure 3.9: A pedigree displaying the four most recent generations of family 4.

sessile serrated pathway, while the left-sided predominance of tumours and the MSS tumour DNA is more typical of another pathway.

Table 3.12: Summary of the phenotype of the proband and family 4 collectively.

	Proband	Family
Number of Primary CRCs	2	8
Number of Primary CRCs With Pathology Report	2	7
Mean Age at Onset of CRC (95% CI)	1st: 60 2nd: 69	61.3 (54.3 – 68.2)
CRC Location	1st: Left (Descending colon) 2nd: Right (Ascending colon)	Left: 6 (85.7%) Right: 1 (14.3%)
Multiple Tumours	Yes	1
Polyps	3	58*
Polyp Type	Tubular: 3 (100%)	Hyperplastic: 30 (51.7%) Tubular: 16 (27.6%) Tubulovillous: 12 (20.7%)
Polyp Location	Left: 1 (33.3%) Right: 2 (66.7%)	Left: 39 (67.2%) Right: 19 (32.8%)
BRAF Status	p.Val600Glu	--
FHS	41.0	

*The number of polyps per affected individual ranged from 1 to 11.

Table 3.13: Kaplan Meier time-to-event analysis results for family 4.

Event	N Events	Percent Affected by Age 30	40	50	60	70	80	Mean Survival Time (95% CI)
CRC	7	0	0	5	17	35	35	77.4 (71.4 – 83.3)
Death	10	0	0	8	8	33	58	75.7 (70.1 – 81.3)

3.5.4 Family 5

Family 5 (N = 35) met the ACI, had MSS tumour DNA, a FHS of 37.1, and demonstrated a pattern of CRC consistent with autosomal dominant inheritance with variable expression (Figure 3.10). The pedigree was traced back one new generation with

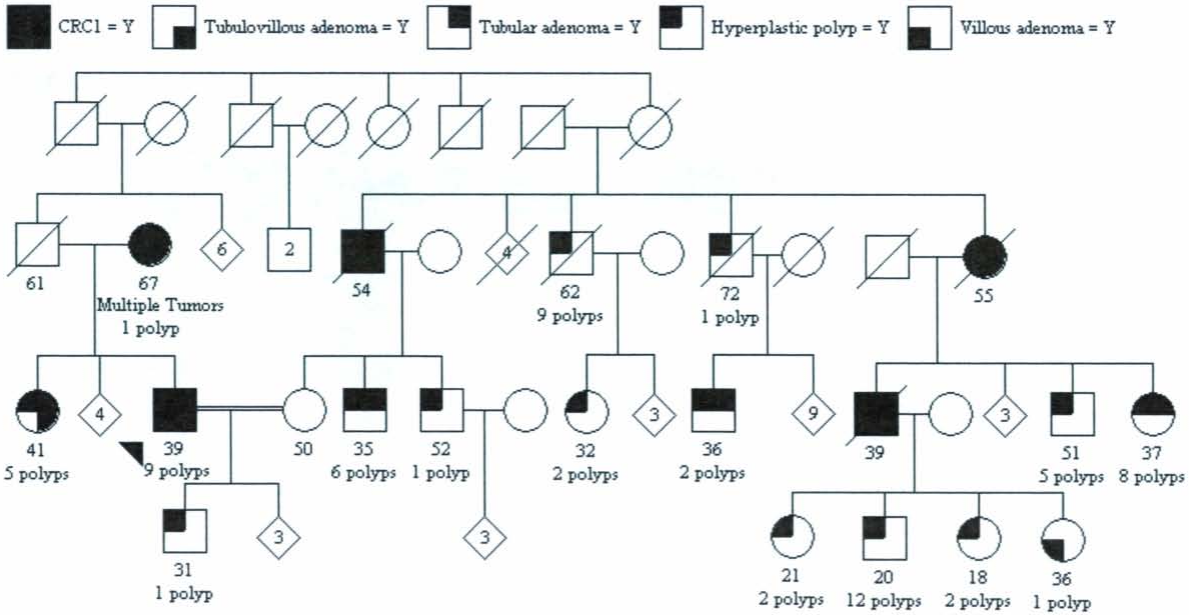

Figure 3.10: A pedigree displaying the four most recent generations of family 5.

the addition of 14 new individuals to St. Joseph's in the St. Mary's Bay region (Appendix G). The archival records for this area are quite poor and reconstruction to the early 19th century was only made possible by the high pre-investigation quality of the pedigree. The phenotype of family 5 is presented below with a profile of the polyps and tumours (Table 3.14) and a summary of the Kaplan Meier time-to-event analysis (Table 3.15). This survival analysis reported that only 10% of the family was affected with CRC before the age of 50, which is consistent with the general FCCTX phenotype. There were six cases of CRC, one instance of multiple primary CRCs, and sixty-seven polyps reported within the family. Tumours (60.0%) were more commonly right-sided, while polyps (67.2%) tended to be left-sided. The most common type of polyps identified was hyperplastic (85.1%). Mutation testing indicated that the proband had a CRC with wild-type *BRAF* alleles. These findings provide mixed evidence for the underlying molecular pathway with the wild-type *BRAF* being atypical of the sessile serrated pathway, while the right-sided predominance of tumours is more typical of this pathway.

Table 3.14: Summary of the phenotype of the proband and family 5 collectively.

	Proband	Family
Number of Primary CRCs	1	6
Number of Primary CRCs With Pathology Reports	1	5
Mean Age at Onset of CRC (95% CI)	39	50.8 (36.0 – 65.6)
CRC Location	Right (Transverse colon)	Left: 1 (20.0%) Right: 3 (60.0%) Missing: 1 (20.0%)
Multiple Tumours	No	1
Polyps	9	67*
Polyp Type	Hyperplastic: 8 (88.9%) Tubulovillous: 1 (11.1%)	Hyperplastic: 57 (85.1%) Tubular: 7 (10.4%) Villous: 1 (1.5%) Tubulovillous: 2 (3.0%)
Polyp Location	Left: 6 (66.7%) Right: 3 (33.3%)	Left: 45 (67.2%) Right: 22 (32.8%)
BRAF Status	Wild-type	--
FHS	37.1	

*The number of polyps per affected individual ranged from 1 to 12.

Table 3.15: Kaplan Meier time-to-event analysis results for family 5.

Event	N Events	Percent Affected by Age 30	40	50	60	70	80	Mean Survival Time (95% CI)
CRC	5	0	10	10	35	57	57	68.0 (58.2 – 77.8)
Death	4	0	5	5	18	18	18	77.8 (68.1 – 87.4)

3.5.5 Family 6

Family 6 (N = 30) met the ACI, had MSS DNA, a FHS of 18.8, and demonstrated a pattern of CRC consistent with autosomal dominant inheritance (Figure 3.11). The pedigree was extended back three new generations with the addition of thirty-five new individuals (Appendix H). The family was traced back to the early 19th century in the

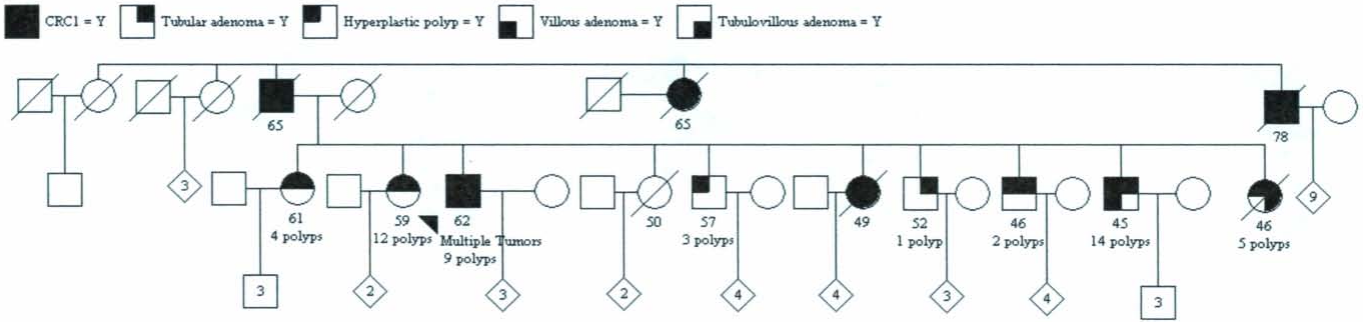

Figure 3.11: A pedigree displaying the three most recent generations of family 6.

Indian Islands, a now resettled island located just south of Fogo Island, and Hare Bay, a community in the Bonavista Bay region. The phenotype of family 6 is presented below with a profile of the polyps and tumours (Table 3.16) and a summary of the Kaplan Meier time-to-event analysis (Table 3.17). This family demonstrated the late age of onset of CRC that is typical of FCCTX, with only 7% of individuals affected prior to the age of 60. There were six cases of CRC, one of which represents a second primary CRC within the same individual, and fifty cases of polyps on record. Polyps (60.0%) were more commonly left-sided, while tumours (60.0%) tended to be right-sided. Hyperplastic polyps (64.0%) were the most commonly identified pre-cancerous lesion. The proband experienced synchronous right-sided CRCs within the same year and represents the only instance of multiple tumours within the family. In addition, the proband's CRC expressed the *BRAF* mutation. This finding combined with the right-sided predominance of tumours is suggestive that the sessile serrated pathway may possibly be the underlying mechanism.

Table 3.16: Summary of the phenotype of the proband and family 6 collectively.

	Proband	Family
Number of Primary CRCs	2	6
Number of Primary CRCs With Pathology Report	2	5
Mean Age at Onset of CRC (95% CI)	1st: 62 2nd: 62	63.8 (51.0 – 76.6)
CRC Location	1st: Right (Hepatic flexure) 2nd: Right (Cecum)	Left: 2 (40.0%) Right: 3 (60.0%)
Multiple Tumours	Yes	1
Polyps	9	50
Polyp Type	Hyperplastic: 7 (77.8%) Tubular: 2 (22.2%)	Hyperplastic: 32 (64.0%) Tubular: 16 (32.0%) Villous: 1 (1.0%) Tubulovillous: 1 (1.0%)
Polyp Location	Left: 9 (100%)	Left: 30 (60.0%) Right: 20 (40.0%)
BRAF Status	p.Val600Glu	--
FHS	18.8	

*The number of polyps per affected individual ranged from 1 to 14.

Table 3.17: Kaplan Meier time-to-event analysis results for family 6.

Event	N Events	Percent Affected by Age 30	40	50	60	70	80	Mean Survival Time (95% CI)
CRC	5	0	0	7	7	46	100	70.7 (64.5 – 76.9)
Death	5	0	0	14	23	61	100	69.3 (60.6 – 78.1)

3.5.6 Family 7

Family 7 (N = 43) met the ACI, had MSS DNA, a FHS of -1.3, and demonstrated a pattern of CRC consistent with autosomal dominant inheritance with reduced penetrance (Figure 3.12). The family was traced back to the beginning of the 19th century in the Northern Peninsula community St. Anthony and Harbour Grace, located in Conception Bay. Thirty-eight new individuals from two new generations were added to

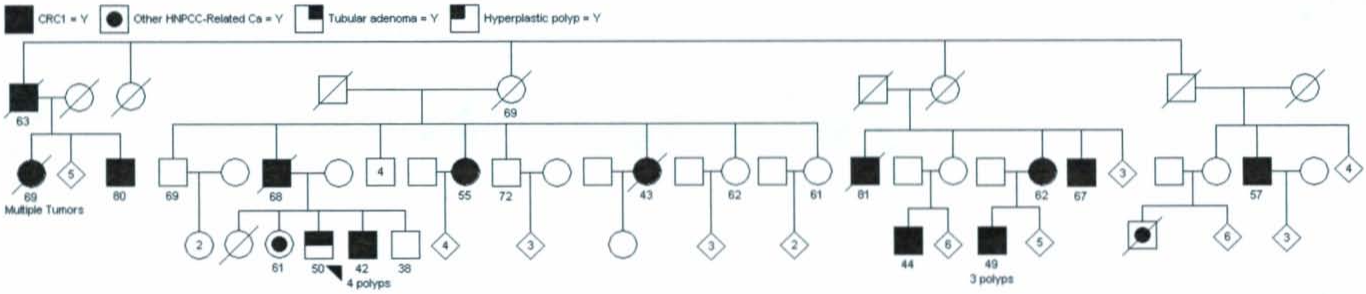

Figure 3.12: A pedigree displaying the three most recent generations of family 7.

the pedigree (Appendix I). The phenotype of family 7 is presented below with a profile of the polyps and tumours (Table 3.18) and a summary of the Kaplan Meier time-to-event analysis (Table 3.19). This family also demonstrated the relatively late age of onset of CRC typical of FCCTX, with only 12% of individuals affected by age 50. There were fourteen cases of CRC, but only four with available pathology reports, one case of multiple primary CRCs, and seven reported polyps. Polyps (85.7%) were most commonly left-sided and hyperplastic, while all of the tumours with pathology reports were left-sided. The left-sided predominance of tumours and polyps and the mean age of onset of CRC of 60.0 (95% CI: 52.1 – 67.9) are all consistent with the FCCTX phenotype. The two cases of extra-colonic Lynch Syndrome-related cancers are atypical of the FCCTX phenotype. The proband was found to have CRC containing wild-type *BRAF* alleles and all of the cases of CRC within the family were left-sided. These findings support the possibility of the involvement of molecular pathway other than the sessile serrated adenoma pathway.

Table 3.18: Summary of the phenotype of the proband and family 7 collectively.

	Proband	Family
Number of Primary CRCs	1	14
Number of Primary CRCs With Pathology Report	1	4
Mean Age at Onset of CRC (95% CI)	42	60.0 (52.1 – 67.9)
CRC Location	Left (Sigmoid colon)	Left: 4 (100%)
Multiple Tumours	No	0
Polyps	No	7*
Polyp Type	No polyps reported	Hyperplastic: 6 (85.7%) Tubular: 1 (14.3%)
Polyp Location	n/a	Left: 6 (85.7%) Right: 1 (14.3%)
BRAF Status	Wild-type	--
FHS	-1.3	

*The number of polyps per affected individual ranged from 3 to 4.

Table 3.19: Kaplan Meier time-to-event analysis results for family 7.

Event	N Events	Percent Affected by Age 30	40	50	60	70	80	Mean Survival Time (95% CI)
CRC	13	0	0	12	19	41	56	74.4 (68.2 – 80.7)
Death	15	0	0	11	11	34	62	75.2 (69.3 – 81.0)

4. DISCUSSION

4.1 Comparison of Probands From Families Fulfilling the FCCTX Criteria

There was significant variability in the clinical experience of disease amongst the probands of the families studied meeting the FCCTX criteria with at least five cases of CRC. The age of onset of CRC ranged from 39 to 76 with half of the probands being affected before the age of 60. The FHS values calculated for the FCCTX probands ranged from -1.3 to 46.5. The variability is likely partially attributable to the variable degrees of screening to which these families have been subjected. Naturally, increased surveillance will tend to decrease the FHS through the prevention of cases of CRC via colonoscopy (Lieberman et al., 2000). Additionally, differences in lifestyle are also a likely contributor to the degree of variation (Longnecker et al., 1990; Giovannucci and Martínez, 1996; Sandhu et al., 2001; Norat et al., 2002). The main limitation of the FHS usage in this context is the fact that it is so greatly affected by which individual is designated as the proband. For example, family 7 has the fifteen cases of CRC, the most of all twelve families, but has the lowest FHS due to the fact that they were ascertained clinically and the first individual in the family referred to the PMGP had no first-degree relatives with CRC.

In terms of CRC location, four probands had a right-sided tumour, six probands had a left-sided tumour, and two probands had both a right- and left-sided tumour. This finding further supports the heterogeneity of the FCCTX phenotype, while also confirming the observation that tumours are most commonly left-sided in individuals under the FCCTX classification. Previous studies have reported between 76.8% and

82.0% of colorectal tumours in individuals meeting the criteria for FCCTX are left-sided (Jass et al., 1995; Llor et al., 2005).

Three of the twelve FCCTX probands had the p.Val600Glu *BRAF* variant in their colorectal tumour. Two of these three probands had two primary CRCs. The observation that all three of these probands with the *BRAF* variant in their tumour had cases of right-sided CRC suggests possible involvement of the sessile serrated adenoma pathway. This pathway is thought to underlie CRCs that are more typically right-sided, demonstrate a variable degree of MSI, and frequently contain the p.Val600Glu *BRAF* variant (Young et al., 2005; Wish et al., 2010). The tumours in these probands all had MSS DNA, which does not support the involvement of the sessile serrated adenoma pathway. All six of the left-sided cases of CRC occurred in probands with tumours containing wild-type *BRAF* alleles. These tumours appear more likely to have arisen from other molecular pathways to CRC. The definitive association of the tumours of these probands with specific molecular pathways and epithelial architectures requires a standardized pathological review of tumours and genetic testing for *KRAS* variants. Such a standardized pathological review is the planned subject of future research and until such an investigation is complete, these observations are merely speculation.

4.2 Comparison of Study Families to Population Lynch Syndrome Group

First-degree relatives in the FCCTX families (N = 126) demonstrated significantly longer survival to CRC in comparison with the Lynch Syndrome group (HR = 0.64, 95% CI: 0.42 – 0.997). However, the lifetime risk of CRC did not differ appreciably between the groups as 47% of the study family members and 56% of the Lynch Syndrome subjects

had developed CRC by age 70. Additionally, the Pearson chi-square coefficient reported no significant difference in number of cases of CRC between the two groups ($p = 0.912$). This finding is consistent with previous investigations, which similarly report a later age of onset for individuals with FCCTX compared with Lynch Syndrome (Renkonen et al., 2003; Lindor et al., 2005). As expected, study families were also found to be at significantly lower risk for developing Lynch Syndrome-related extra-colonic cancer (HR = 0.20, 95% CI: 0.06 – 0.72). This data supports the notion that the FCCTX phenotype is less severe, with significantly later onset of CRC and less risk of extra-colonic cancers, than that of Lynch Syndrome. However, it does reinforce that the lifetime risk of CRC in the FCCTX classification is substantial.

The comparison with Lynch Syndrome families is limited by the inherent differences between the families in the two groups. Six of the FCCTX families were population-based, having been identified in the NFCCR, while the remaining six FCCTX families were ascertained clinically and identified through the PMGP. On the other hand, all fifteen of the Lynch Syndrome families utilized for comparison were population-based, having been identified in the NFCCR. Additionally, to be included in this investigation, FCCTX families were required to have at least five cases of CRC in their extended pedigree, while the same criterion was not applied to the Lynch Syndrome families. This inclusion criteria was applied in order to select high risk families for novel gene discovery and genealogical reconstruction, but was not an ideal method through which to select families for phenotypic comparison with Lynch Syndrome families. It biases towards inflating the number of cases of CRC in the FCCTX cohort, given the pre-

selection of families with greater than five individuals affected by CRC. The fact that the first-degree relatives in the FCCTX families tended to experience colorectal tumours at a later age remains a significant result, but the equivalence of the lifetime risk of CRC between the groups must be called into question. The exact degree of screening that each group was subjected to also could not be quantified for comparison in this study. The absence of this data is a significant limitation in the comparison as differences in the degree of screening between the groups could potentially bias the results of the analysis. Given the absence of this data, the potential of screening to affect the number of colorectal tumours detected and the age at diagnosis of these tumours cannot be discounted as a confounding factor in the time-to-event analysis. Another limitation of this study is that one of the six families investigated (family 1) does not actually meet the ACI required for the FCCTX classification given that the earliest case of CRC in the family is at age 50 rather than before it. The differences in ascertainment and potential differences in screening between the two groups certainly must be viewed as limitations of the time-to-event analyses.

4.3 Geographic Distribution of FCCTX Families

The locations of origin of the Lynch Syndrome probands were widely distributed across the recognized geographic isolates of Newfoundland, defined by the great bays of the province. Two areas of geographical clustering have been observed that correspond with unique MMR mutations. Individuals possessing the *MSH2* deletion of exon 8 obviously cluster in the Avalon Peninsula, while families possessing the MSH2 p.Val265_Gln214del variant cluster in the Bonavista Bay and Notre Dame Bay areas

(Figure 3.5). This clustering is hypothesized to be the result of founder effects whereby members of the founding population passed these genetic variants through the generations within these communities. The hypothesis of a founder effect in FCCTX would suggest that groups of FCCTX families would be found to originate in close geographic proximity.

The twelve FCCTX probands have origins that are widely distributed across the province with instances of clustering in geographic isolates. Of particular importance in this investigation is the geographical clustering of probands with similar clinicopathological features. Such clustering could result from a common genetic variant that is predisposing these individuals to CRC. Three of the FCCTX probands originated in close proximity in the Northern Peninsula. Two of these probands (7 and 10) from the Northern Peninsula had a very similar clinicopathological phenotype, each with a left-sided CRC which did not contain the p.Val600Glu *BRAF* variant and the absence of multiple tumours. On the other hand, the other Northern Peninsula proband had a very different phenotype having experienced a right-sided tumour containing the p.Val600Glu *BRAF* variant. Proband 7 also has maternal lineage in the Conception Bay area placing it in close proximity to the origin of proband 12, who demonstrates a similar left-sided tumour with wild-type *BRAF* alleles. Two probands (1 and 4) also originated in Greenspond, but their clinicopathological features were drastically different. Proband 1 had a right-sided tumour with wild-type *BRAF* alleles, while proband 4 had multiple CRCs containing mutant *BRAF* genes. Proband 6 has roots approximately 50km away in the Hare Bay area and demonstrates an identical phenotype to proband 4 with multiple tumours, both right- and left-sided, containing the *BRAF* variant with a similar age of

onset. Outside of these areas of clustering, the FCCTX families appear to be relatively diffusely located around the province. These areas of clustering could conceivably represent distinct genetic etiologies of FCCTX, but based on the small number of tumours analyzed and the failure of the genealogical investigation to yield any common ancestry, this seems unlikely.

4.4 Genealogical Investigation

4.4.1 KINNECT Software

The potential of genealogical research to connect families creating the link that facilitates novel gene discovery is a very exciting area of research. In the past, these studies were based entirely on tedious work by the individual. Recent technological developments, such as the KINNECT software, have automated the process to a degree. The automation of the process, which is being undertaken by the PTRG, is in its infancy with this search representing the second ever use of the KINNECT software. Although, the software failed to yield any direct links between the twelve families investigated, the output did provide valuable information in the forms of dates of birth, towns of origin, and in clarifying familial relationships. Currently, the software serves as a tool to help verify information contained within pedigrees and guide future archival inquiries. As more census records and other forms of reference are added, the KINNECT software has the potential to quickly make genealogical links that were previously possible only by many hours of archival work.

4.4.2 Archival Research

The genealogical investigation failed to yield any common ancestry in the study families. The reconstruction was carried out to the full degree made possible by the archival and on-line genealogical records. One family was traced back to the 16th century, two others to the mid-18th century, and four to the early 19th century. The most complete reconstructions were achieved with the two families traced back to Greenspond, one of which was traced back to Damerham, England. A connection was found between these two families, but it represented an indirect connection via a first cousin who did not directly contribute to one of the pedigrees. Two other families were traced back to the Northern Peninsula, while two families had roots in the Conception Bay district. These families were investigated thoroughly for potential connections given the fact that migration between Conception Bay and the Northern Peninsula was common for fishermen. After following up on numerous exciting leads and reconstructing the pedigrees as much as possible, it must be concluded that there are no direct genealogical connections between the study families based on the available records. Despite the absence of a direct genealogical connection between the six families, several positive findings must be noted. The extended pedigrees represent comparators when other FCCTX families in the PMGP have their pedigrees reconstructed. The extensive mapping of the two study families originating in Greenspond represents a valuable resource to future genealogical studies in the area.

The absence of common ancestry in these six FCCTX families significantly reduces the probability that one or two highly-penetrant mutations underlie the FCCTX

classification. If a highly-penetrant mutation was the etiological agent of FCCTX, one would expect these families to share common ancestry given the extensive genealogical reconstruction and the hypothesis of a founder effect. Common ancestry was readily documentable in Newfoundland for families with Lynch Syndrome, which is caused by highly-penetrant mutations in MMR genes (Froggatt et al., 1999). The absence of common ancestry in these FCCTX families increases the likelihood that multifactorial inheritance with moderately-penetrant mutations in multiple genes provides the etiological explanation for the classification. This would account for the failure to find common ancestry in the six families investigated given that they could potentially represent differing combinations of the moderately-penetrant genes conferring the increased susceptibility to CRC observed within the FCCTX classification. Another possibility is that these families represent chance aggregations of sporadic CRC in higher than normal frequency. This explanation cannot be discounted as a possibility given the heterogeneity observed within the FCCTX classification and the reduced probability of highly-penetrant genes being involved as a result of the absence of common ancestry.

The potential of genealogical research to drive genetic discoveries in Newfoundland must be considered in context of the fundamental limitations of the process. The primary limitation of genealogical research is the incomplete nature of the records. The amount of information available varies dramatically by region of the province, with records before the 20th century being almost entirely unavailable in some regions. The incomplete nature of these records can be attributed to a variety of factors: the loss of records in fires, resettlement, or for other reasons; the fact that agencies

responsible for census data and population statistics did not exist in the 19th century with the responsibility being entrusted to the churches; 19th century censuses did not include women or children, only men old enough to work. Regardless of the reason for the incompleteness of the records, it is important to note that it is a primary obstacle to the success of genealogical research. Another fundamental issue is that dates of birth are often unavailable with only baptism dates being recorded. This can make estimating an individual's age difficult given the fact that the age at which an individual was baptized was variable. It is also very difficult to follow maternal lineages due to the frequent absence of information on maiden name. As a result, the majority of the reconstruction in this investigation has been through paternal lineages. In autosomal dominant inheritance, the mutation could have been inherited from the maternal or paternal lineage at each generation. There is no historical clinical information to determine which is the relevant lineage so the pedigree extension may not have included the correct ancestors. This represents a major limitation that is an inevitable consequence of the nature of the records. There was also a significant difference in the pre-investigation quality of the pedigrees, based on the variability in family members ability to trace their family tree. Another limitation of the process worth noting is the unavoidable involvement of the subjectivity of the investigator in the process. The investigator is required to use logic, intellect, and instinct in exploring potential genealogical connections, while simultaneously investigating multiple kinships. The process allows a significant amount of decisions to be made by the individual rather than adhering to a strict protocol. In this way, genealogical research is as much an art as it is a science. One must consider these

fundamental limitations of the genealogical reconstruction process when interpreting the results of such investigations.

4.5 Detailed Profile of Six of the Study Families

All six of the study families appear to fit the FCCTX grouping displaying a pattern of CRC consistent with autosomal dominant inheritance, MSS tumour DNA, a general left-sided predominance of CRCs, and a relative absence of Lynch Syndrome-related extra-colonic cancers. The mean age of onset of CRC ranged from 50.8 to 63.8 between the study families and was 59.1 overall, which conforms with the reported values for the mean age of onset of 53.7 and 60.7 reported in the literature (Renkonen et al., 2003; Lindor et al., 2005).

4.5.1 Time-to-Event Analysis

The Kaplan Meier time-to-event analysis further reinforced that the study families fulfilling the criteria conform to expected phenotype as described in the literature. There was only one case of CRC before the age of 40, less than or equal to 11% of family members affected by the age of 50, and an average of 23% affected by age 60 across all study families. The mean time of survival to CRC ranged from 68.0 to 76.1 amongst the study families. Similarly, the mean of survival to the outcome death ranged from 69.3 to 77.8. These patterns are consistent with the late-onset pattern of CRC in the FCCTX classification. It must be noted that the families included in the study represent a biased sample when compared to the general population due to their enrollment in genetic databases and close follow-up. Thus, one would expect some inflation in mean time to survival figures due to the early detection of colorectal tumours that is associated with

regular screening. However, this a common feature of families who are the subject of genetic research so making comparisons between these groups is fairly valid. The generalizability of these findings to unknown mutation carriers in the general population is more questionable. Thus, it will be important to also study this condition in the general population once its genetic basis has been elucidated.

A primary limitation of the time-to-event analysis is the mixed ascertainment of the included families, two of whom were population-based and four of whom were clinic-based. In fact, all six of these families were originally discovered clinically, but two of them were investigated in population-based research projects having had a family member with a case of CRC between 1999 and 2003. This is an important factor as more uniform follow-up and testing has been performed for the population-based families as they have undergone previous time-to-event study. On the other hand, the purely clinic-based families did not have such uniform recording of ages at last follow-up. Despite having been extensively followed, more thorough searching through records was necessary to find dates of onset of cancer events and dates of last follow-up for this group. The retrospective nature of the investigation means that ages of onset were unavailable in certain cases and some reported cases of CRC in pedigrees could not be confirmed by pathology reports.

4.5.2 Pathology Analysis

The pathology analysis of colorectal tumours and polyps from the six FCCTX families studied in detail demonstrated a left-sided predominance of colorectal tumours (66.7%) and polyps (66.2%). This is in agreement with previous studies that report

between 76.8% and 82.0% of CRCs in individuals meeting the criteria for FCCTX are left-sided (Jass et al., 1995; Llor et al., 2005). The most common type of polyp found during screening was the hyperplastic polyp (62.6%). Similar to the findings of the comparison of FCCTX probands, the molecular mechanism underlying the development of CRC could not be explained by one pathway amongst these families. There was a mixture of left- and right-sided predominance of tumours and the wild-type *BRAF* gene and the p.Val600Glu *BRAF* variant.

A more detailed analysis of the pathological features of tumours and polyps was planned (Appendix A and Appendix B), but it was not feasible given the amount of missing information. As a result of this finding, a more formal evaluation of these features is planned for the future involving the reassessment of CRC and polyp biopsies by a pathologist, with specific focus on the occurrence of serrated polyps. Given that the pathology reports were analyzed in a retrospective manner, significant discrepancies exist in terms of the amount of information and the level of detail recorded in the reports. Another major limitation was that many of the pathology reports were not available. The files of the PMGP and those of geneticist Dr. Jane Green were the records that were consulted. These collections represent substantial, but incomplete records of the families under study. The pathological features of significant numbers of tumours and polyps were unavailable as a result. Additionally, pathological investigations were not always performed as many polyps were simply cauterized and others were snared but not retrieved. The fact that pathology reports dated back as far as 1978 also added an additional degree of heterogeneity to the process. Significant advancements have been

made in the field of pathology and guidelines have been altered over the last thirty years, which is reflected in the level of detail and the dated terminology used in some of the older reports. Another issue was the existence of discrepancies between colonoscopy report and pathology report on variables such as polyp location, depth of invasion, and the nature of the specimen (biopsy or intact). Despite its limitations, the pathology analysis provides meaningful insight into the location and features of polyps and cases of CRC providing valuable phenotypic information for the attempt to identify the novel susceptibility genes underlying FCCTX.

4.6 Screening Recommendations

The risk of CRC in individuals from families meeting the FCCTX criteria has been well established by several studies (Jass et al., 1995; Renkonen et al., 2003; Lindor et al., 2005; Llor et al., 2005). It would appear appropriate to initiate screening later than the recommendations for individuals with Lynch Syndrome. The family history of disease, specifically the earliest age of onset of CRC in the family, should be used to guide the timing of initiating screening. The recommendations in Lynch Syndrome are to begin screening at age 25 or ten years before the earliest age of onset of CRC in the family (Burke et al., 1997; Leddin et al., 2004). There are no clinical guidelines currently available with screening recommendations for FCCTX. It seems reasonable to initiate screening later than age 25 given the later onset of CRC in individuals fulfilling the FCCTX criteria, but formal recommendations require further investigation and are outside the scope of this thesis. Advances in the area of pharmaceutical prevention are also likely in this area and will warrant attention in the surveillance and prevention of

CRC in this patient population. The observed left-sided predominance of CRCs is also a useful piece of information for physicians responsible for colonoscopic surveillance of these patients. Extra attention should be given to the surveillance of the left colon without compromising the assessment of the right colon as tumours in this location occur, but less frequently. However, in family members of probands with *BRAF* deleterious variants in their tumour, the right colon should be given particularly close surveillance given the predominance of right-sided tumours in these individuals.

5. CONCLUSION

The variability in the clinicopathological features and severity of disease experienced by the FCCTX probands and family members reinforces the heterogeneity of the FCCTX classification. The colorectal tumours of the twelve probands displayed mixed features in terms of right- and left-sided location and tumour DNA with wild-type and p.Val600Glu *BRAF* alleles. These mixed features provide evidence to suggest potential involvement of multiple molecular pathways to CRC amongst these individuals. The absence of common ancestry in the genealogical investigation reduces the likelihood of the involvement of a highly-penetrant mutation in one or two undiscovered genes. Genetic heterogeneity is also supported by the widespread distribution of place of origin of probands in geographic isolates across the province. This genealogical study represents one of the first usages of new technologies developed by the PTRG, lessons from which will be used to improve the planning and execution of future studies. Furthermore, the families studied represent the beginning of a collection of fully extended pedigrees in the FCCTX families of Newfoundland. Future additions to this collection may very well yield the direct link necessary to facilitate novel gene discovery.

The clinical experience of disease amongst first-degree relatives in the FCCTX study families conformed to the few previously published reports in the literature. The age of onset and lifetime risk of CRC, the left-sided predominance of tumours, and the relative absence of Lynch Syndrome-related extra-colonic cancer agreed with the previous reports. This study provides further evidence to the growing body of data supporting the notion that the FCCTX classification represents a less severe phenotype

than Lynch Syndrome. This understanding of the clinical manifestations of the condition can be used to guide the development of appropriate screening programs for these individuals.

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5. APPENDICES

Appendix A: Form used to extract variables from polyp pathology reports

Patient Name: _____

MCP Number: _____

Study Number: _____

Family Number: _____

Date of Polypectomy: _____

Presence of Colorectal Cancer (If yes, date): _____

Record of Other Cancer (If yes, date): _____

1.) Tumour Site

__ Cecum
__ Right (ascending) colon
__ Hepatic flexure
__ Transverse colon
__ Splenic flexure
__ Left (descending) colon
__ Sigmoid colon
__ Rectum
__ Not specified

2.) Specimen Integrity

__ Intact
__ Fragmented
__ Not specified

3.) Polyp Type

__ Hyperplastic
__ Tubular adenoma
__ Villous adenoma
__ Tubulovillous adenoma
__ (Traditional) serrated adenoma

__ Sessile serrated adenoma
__ Hamartomatous polyp
__ Indeterminate
__ Not specified

4.) Level of Dysplasia
__ None
__ Mild
__ Moderate
__ Severe
__ Not specified

5.) Polyp Size
Greatest dimension: __ cm
Additional dimensions: __ × __ cm
__ Cannot be determined
__ Not specified

6.) Polyp Configuration
__ Pedunculated with stalk
Stalk length: __ cm
__ Sessile

7.) Additional Pathologic Findings (check all that apply)
__ None identified
__ Inflammatory bowel disease
__ Active
__ Quiescent
__ Other (specify): _____

8.) Ancillary Studies
Specify: _____
__ Not performed

9.) Comments:

Appendix B: Form used to extract variables from CRC pathology reports.

Patient Name: _____
MCP Number: _____
Study Number: _____
Family Number: _____
Primary CRC, Second Primary CRC, or Recurrence (Date): _____
Record of Previous Cancer (Date): _____

1.) Tumour Site

__ Cecum
__ Right (ascending) colon
__ Hepatic flexure
__ Transverse colon
__ Splenic flexure
__ Left (descending) colon
__ Sigmoid colon
__ Rectum
__ Not specified

2.) Specimen Integrity

__ Intact
__ Fragmented
__ Not specified

3.) Size of Invasive Carcinoma

Greatest dimension: __ cm
Additional dimensions: __× __ cm
__ Cannot be determined

4.) Histologic Type

__ Adenocarcinoma
__ Mucinous adenocarcinoma
__ Signet-ring cell carcinoma
__ Small cell carcinoma
__ Squamous cell carcinoma
__ Adenosquamous carcinoma
__ Medullary carcinoma
__ Undifferentiated carcinoma
__ Other (specify): _____
__ Carcinoma, type cannot be determined

5.) Histologic Grade

__ Not applicable
__ Cannot be determined
__ Low-grade (well differentiated to moderately differentiated)
__ High-grade (poorly differentiated to undifferentiated)

6.) Tumour Extension

__ Cannot be determined
Invasion (Deepest)

__ Lamina propria
__ Muscularis mucosae
__ Submucosa
__ Muscularis propria

7.) Margins (check all that apply)
Deep Margin (Stalk Margin)
__ Cannot be assessed
__ Uninvolved by invasive carcinoma
Distance of invasive carcinoma from margin: __ mm
__ Involved by invasive carcinoma
Mucosal/Lateral Margin
__ Not applicable
__ Cannot be assessed
__ Uninvolved by invasive carcinoma
__ Involved by invasive carcinoma
__ Involved by adenoma

8.) Venous (Large Vessel) Invasion (V)
__ Not identified
__ Present
__ Indeterminate

9.) Lymphatic (Small Vessel) Invasion (L)
__ Not identified
__ Present
__ Indeterminate

10.) Type of Polyp in Which Invasive Carcinoma Arose (note **G**)
__ Tubular adenoma
__ Villous adenoma
__ Tubulovillous adenoma
__ Traditional serrated adenoma
__ Sessile serrated adenoma
__ Hamartomatous polyp
__ Indeterminate

11.) Additional Pathologic Findings (check all that apply)
__ None identified
__ Inflammatory bowel disease
__ Active
__ Quiescent
__ Other (specify): _____

12.) Ancillary Studies
Specify: _____
__ Not performed

Appendix C: Detailed Profile of Family 3

Family 3 (N = 21) did not meet the ACI, rather meeting the Bethesda guidelines, and therefore does not fit the FCCTX classification (Figure 5.1). The proband had MSS CRC and the family had a FHS of 3.8 and demonstrated a pattern of CRC consistent with autosomal dominant inheritance with reduced penetrance. The pedigree was traced back four new generations with the addition of 28 new individuals to Bradley's Cove in Conception Bay as far back as the mid-18th century (Figure 5.2). The phenotype of family 3 is presented below with a profile of the polyps and tumours (Table 5.1) and a summary of the Kaplan Meier time-to-event analysis (Table 5.2). The time-to-CRC data was consistent with the FCCTX phenotype with the earliest case occurring at age 57 and a mean age of onset of 61.3 (95% CI: 54.3 – 68.2). Members of the family experienced six cases of CRC, including one instance of multiple primary tumours, and five colorectal polyps. Polyps (60.0%) and tumours (80.0%) were more commonly left-sided, while hyperplastic polyps (60.0%) were the most common pre-cancerous lesions identified on colonoscopy. The proband for this family had a tumour lacking the p.Val600Glu *BRAF* variant and represents the only case of multiple primary CRCs (synchronous) in the family, both of which were diagnosed at age 57. This family appears to represent a pathway to CRC other than the sessile serrated adenoma pathway, given the left-sided predominance of tumours, and the absence of the *BRAF* variant in the proband's tumours.

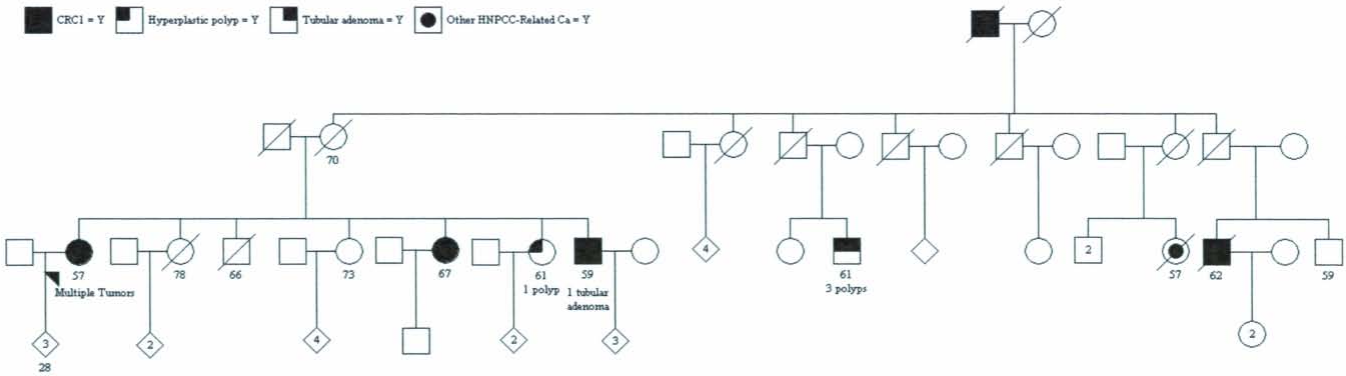


Figure 5.1: A pedigree displaying the four most recent generations of family 3.

Table 5.1: Summary of the phenotype of the proband and family 3 collectively.

Events	Proband	Family
CRC	Yes	6
CRC Pathology Report	Yes	5
Mean Age at Onset of CRC (95% CI)	1st: 57 2nd: 57	61.3 (54.3 – 68.2)
CRC Location	1st: Left (Descending colon) 2nd: Right (Ascending colon)	Left: 4 (80.0%) Right: 1 (20.0%)
Multiple Tumours	Yes	1
Polyps	No	5*
Polyp Type	No polyps reported	Hyperplastic: 3 (60.0%) Tubular: 2 (40.0%)
Polyp Location	n/a	Left: 3 (60.0%%) Right: 2 (40.0%)
BRAF Status	Wild-type	--
FHS	3.8	

*The number of polyps per affected individual ranged from 1 to 3.

Table 5.2: Kaplan Meier time-to-event analysis results for family 3.

Event	N Events	Percent Affected by Age 30	40	50	60	70	80	Mean Survival Time (95% CI)
CRC	4	0	0	0	15	40	40	71.7 (66.7 – 76.7)
Death	7	0	7	13	13	24	100	70.4 (63.9 – 77.1)

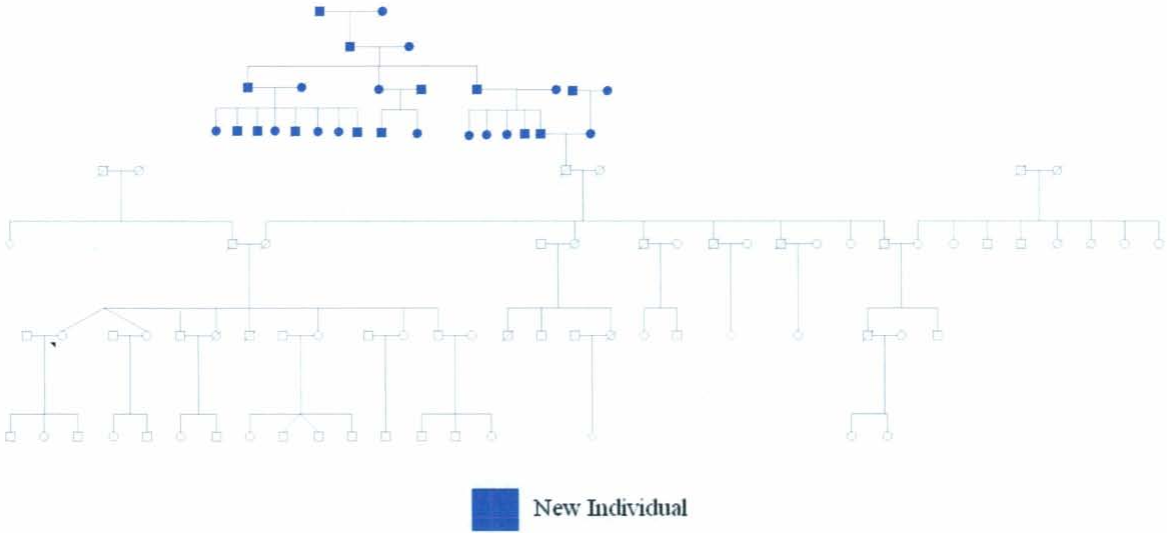
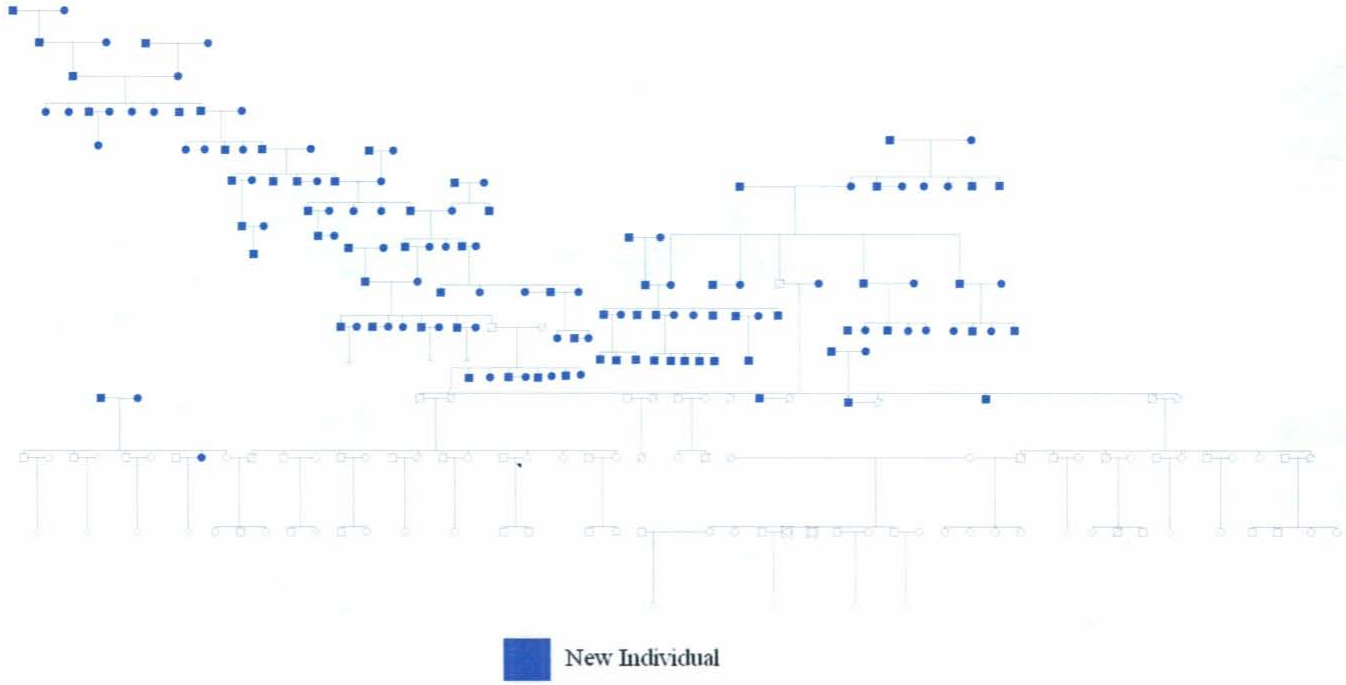
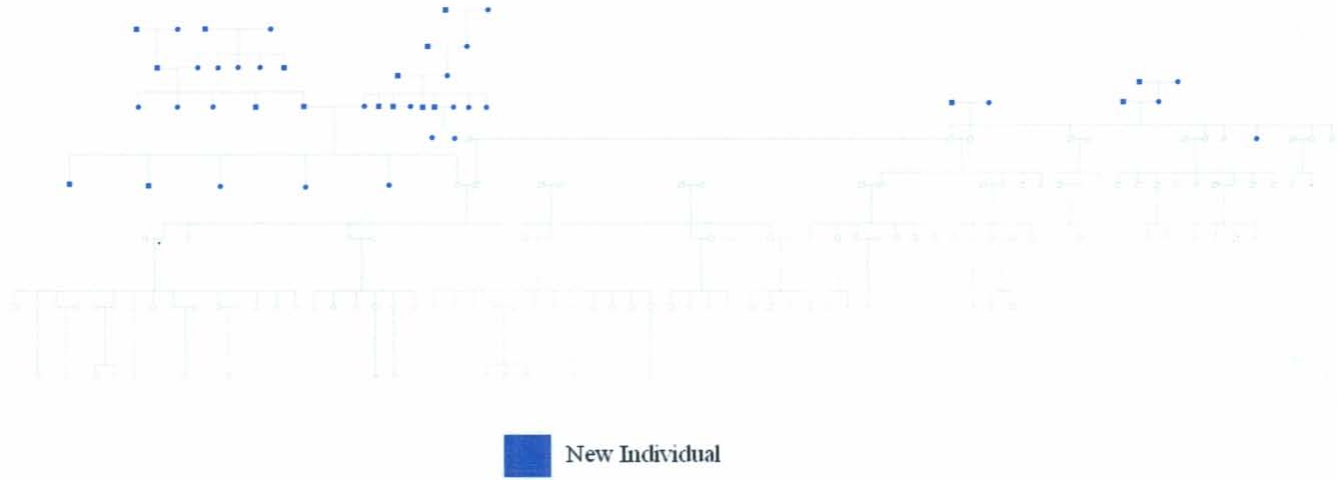


Figure 5.2: Extended pedigree of family 3 following genealogical reconstruction.

Appendix D: Extended pedigree of family 1 following genealogical reconstruction.

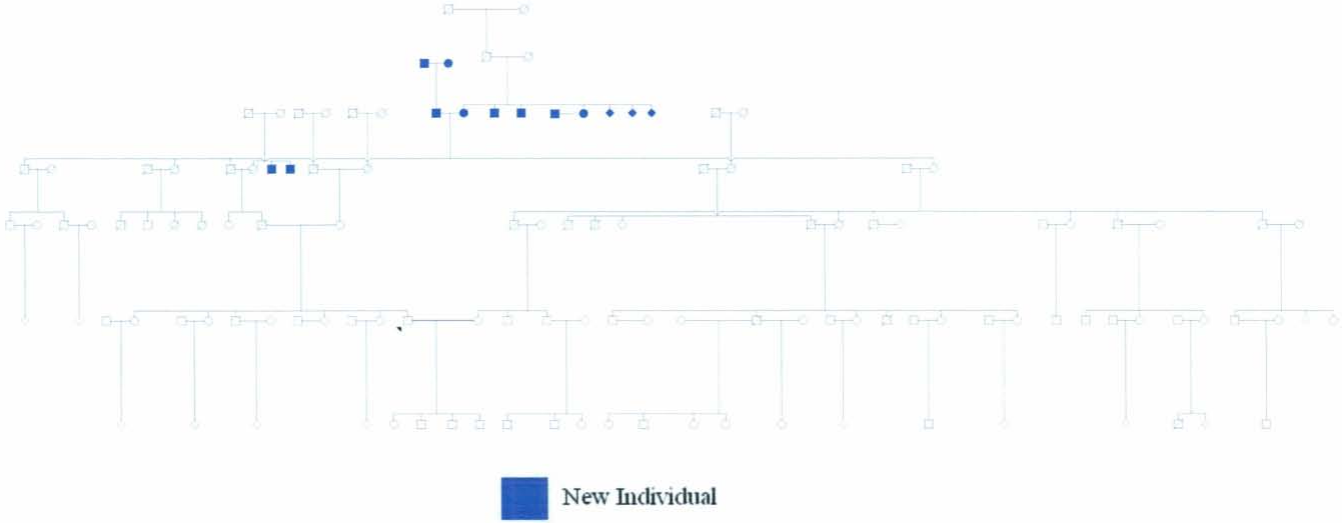


Appendix E: Extended pedigree of family 2 following genealogical reconstruction.

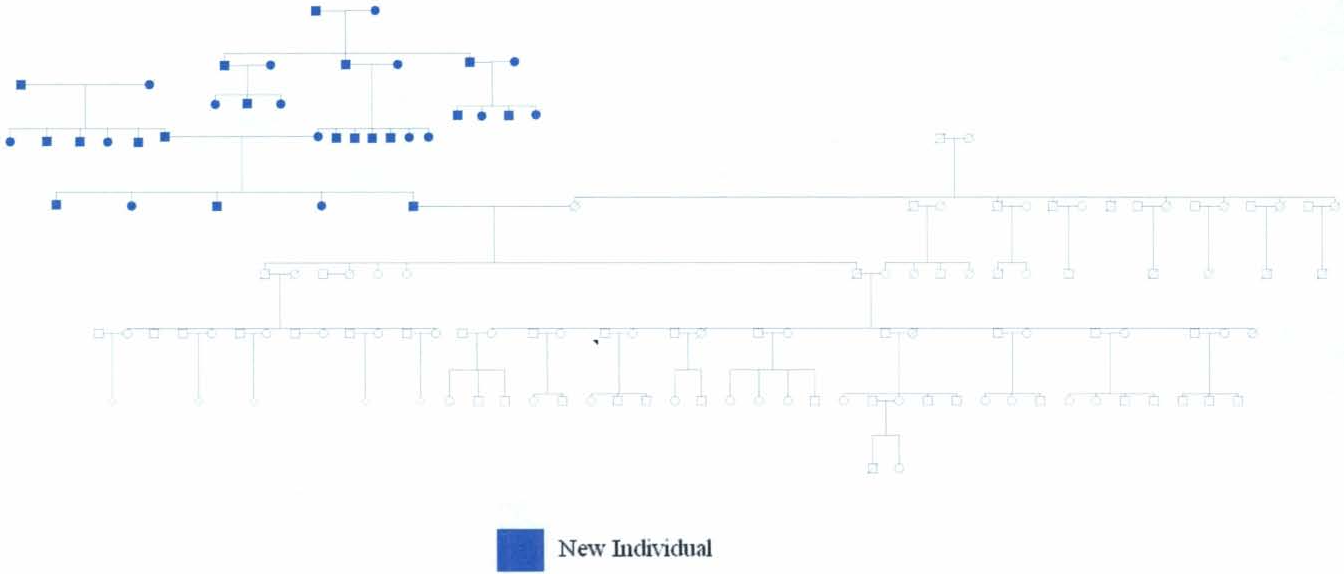


Appendix F: Extended pedigree of family 4 following genealogical reconstruction.

Appendix G: Extended pedigree of family 5 following genealogical reconstruction.



Appendix H: Extended pedigree of family 6 following genealogical reconstruction.



Appendix I: Extended pedigree of family 7 following genealogical reconstruction.