

**QUALITY-CONTROL STUDY EVALUATING THE IDENTIFICATION, FAMILY  
HISTORY COLLECTION, AND GENETIC COUNSELING REFERRAL OF  
INDIVIDUALS AT-RISK FOR HNPCC (LYNCH SYNDROME) WITHIN THE  
UNIVERSITY OF PITTSBURGH MEDICAL CENTER SYSTEM AND APPLICATIONS  
TO A STATE-WIDE REFERRAL SYSTEM**

by

**Andrew Sardella**

B.S., St. Lawrence University, 2007

Submitted to the Graduate Faculty of  
Graduate School of Public Health in partial fulfillment  
of the requirements for the degree of  
Master of Science

University of Pittsburgh

2011

UNIVERSITY OF PITTSBURGH  
GRADUATE SCHOOL OF PUBLIC HEALTH

This thesis was presented

by

Andrew Sardella

It was defended on

December 17, 2011

and approved by

**Thesis Advisor:**

Candace M. Kammerer, Ph.D.  
Associate Professor  
Department of Human Genetics  
Graduate School of Public Health  
University of Pittsburgh

**Committee Member:**

Randall Brand, M.D.  
Professor  
Division of Gastroenterology, Hepatology and Nutrition  
School of Medicine  
University of Pittsburgh

**Committee Member:**

Robert E. Ferrell, Ph.D.  
Professor  
Department of Human Genetics  
Graduate School of Public Health  
University of Pittsburgh

Copyright © by Andrew Sardella

2011

**QUALITY-CONTROL STUDY EVALUATING THE IDENTIFICATION, FAMILY HISTORY COLLECTION, AND GENETIC COUNSELING REFERRAL OF INDIVIDUALS AT-RISK FOR HNPCC (LYNCH SYNDROME) WITHIN THE UNIVERSITY OF PITTSBURGH MEDICAL CENTER SYSTEM AND APPLICATIONS TO A STATE-WIDE REFERRAL SYSTEM**

Andrew Sardella, M.S.

University of Pittsburgh, 2011

**Background:** Hereditary Non-Polyposis Colorectal Cancer (HNPCC) is a dominantly inherited syndrome predisposing individuals to cancers of the colon and other organs. HNPCC is caused by mutations in one of four mismatch repair proteins responsible for DNA repair. Current guidelines on HNPCC screening have focused on administering molecular testing on tumors of at-risk groups affected with colorectal cancer. Criteria for molecular testing include both tumor pathology and personal and family history of cancer. Abnormal tumor test results warrant referral for genetic counseling and germline testing. **Public Health Significance:** Identifying individuals with HNPCC is crucial for screening and surgical purposes in order to reduce mortality and morbidity. Additionally, at-risk family members can undergo germline testing to determine whether increased surveillance or surgery is warranted. **Results:** The study revealed that 45.3% (total n=44) of patients warranting genetic counseling attended at a genetic counseling appointment within the UPMC system. Patients who had a personal or family history of cancer were more likely to attend a genetic counseling session than individuals who had pathological or age dependant risk factors ( $p = 0.0014$ ; OR = 4.8; 95% CI: 1.78, 12.95). Furthermore, patients with a family history of colorectal cancer were more likely to attend a genetic counseling session than individuals whose families displayed a different type of cancer. The average time interval between molecular tumor testing and genetic counseling was

approximately 63 days. Finally, 24% and 21.5% of individuals with abnormal tumor results were identified independently by family history and pathological criteria, respectively. **Conclusions:** This study indicates that improvements can be made in genetic counseling referral process for at-risk HNPCC individuals within the UPMC system. Several factors were potentially associated with attending a genetic counseling session including: the presence of personal or family cancer history, and type of cancers in the family. Timing may also impact attendance with a genetic counselor. The study reveals that there is an opportunity for more detailed family history collection within the UPMC system, from which health care practitioners can identify and address factors that may influence patient compliance with genetic counseling referrals and clinical management. These results can also inform development of a state-wide screening program.

## TABLE OF CONTENTS

<b>1.0</b>	<b>BACKGROUND .....</b>	<b>1</b>
<b>1.1</b>	<b>GENETICS OF COLON CANCER .....</b>	<b>2</b>
<b>1.2</b>	<b>HEREDITARY CANCERS.....</b>	<b>4</b>
<b>2.0</b>	<b>HEREDITARY NON-POLYPOSIS COLORECTAL CANCER.....</b>	<b>7</b>
<b>2.1</b>	<b>CAUSE.....</b>	<b>8</b>
<b>2.2</b>	<b>CANCER RISKS .....</b>	<b>9</b>
<b>2.2.1</b>	<b>COLORECTAL CANCER.....</b>	<b>9</b>
<b>2.2.2</b>	<b>ENDOMETRIAL CANCER.....</b>	<b>10</b>
<b>2.2.3</b>	<b>OTHER CANCERS.....</b>	<b>10</b>
<b>2.3</b>	<b>DETECTION ON HNPCC INDIVIDUALS .....</b>	<b>12</b>
<b>2.3.1</b>	<b>Family History.....</b>	<b>12</b>
<b>2.3.2</b>	<b>Microsatellite Instability .....</b>	<b>15</b>
<b>2.3.3</b>	<b>MSI in Sporadic Cancer.....</b>	<b>16</b>
<b>2.3.4</b>	<b>Microsatellite Stable and Low Microsatellite Instability .....</b>	<b>20</b>
<b>2.3.5</b>	<b>Other Pathological Characteristics .....</b>	<b>20</b>
<b>2.4</b>	<b>BETHESDA CRITERIA.....</b>	<b>21</b>
<b>2.5</b>	<b>TESTING FOR MICROSATELLITE INSTABILITY.....</b>	<b>23</b>
<b>2.5.1</b>	<b>Limitations of MSI Testing .....</b>	<b>25</b>

2.5.2	Other Applications of MSI Testing .....	26
2.6	IMMUNOHISTOCHEMISTRY .....	27
2.6.1	Limitations of IHC .....	28
2.7	MSI VERSUS IHC .....	29
2.8	BETHESDA CRITERIA AND TUMOR TESTING.....	30
2.9	GENETIC INFORMATION AND TUMOR TESTING .....	32
2.10	GENETIC TESTING FOR HNPCC .....	33
2.10.1	Mutations.....	34
2.10.2	Testing Sensitivity .....	35
2.10.3	Test Results.....	36
2.11	PUBLIC HEALTH RELEVANCE .....	37
2.12	SCREENING AND PREVENTION IN HNPCC INDIVIDUALS.....	38
2.12.1	Screening in Familial Colorectal Cancer Type X .....	42
2.12.2	Future Treatment.....	43
2.13	IMPLICATIONS FOR FAMILY MEMBERS.....	43
3.0	STUDY AIMS.....	46
4.0	METHODS .....	47
5.0	RESULTS .....	49
6.0	DISCUSSION .....	55
7.0	STATE-WIDE REFERRAL SYSTEM.....	71
8.0	STUDY LIMITATIONS.....	76
9.0	CONCLUSIONS .....	80
	APPENDIX : IRB APPROVED CONSENT.....	81

**BIBLIOGRAPHY..... 91**

## LIST OF TABLES

Table 1. Individuals meeting Bethesda Criteria in the study population and their classification.	50
Table 2. Individuals that met additional personal and family history Bethesda Criteria.....	50
Table 3. Attendance of population with abnormal MSI/IHC results with a genetic counselor ....	51
Table 4. Genetic testing status of the population with abnormal MSI/IHC results who met with a counselor .....	51
Table 5. Classification of Bethesda Criteria individuals with abnormal MSI/IHC after medical history review.....	52
Table 6. Individuals with abnormal MSI/IHC results grouped by factor .....	52
Table 7. At-risk family members of those with abnormal MSI/IHC results based on medical history review.....	53
Table 8. Number of cancers reported in family history for individuals with abnormal MSI/IHC results .....	53
Table 9. Identification of Bethesda Criteria individuals with abnormal MSI/IHC results .....	54

## **PREFACE**

I would like to thank Dr. Linda Farkas for providing me with the opportunity to evaluate her research data. I would like to thank my thesis committee, Dr. Randall Brand, Dr. Robert Ferrell, and Dr. Candace Kammerer, for their kind words and helpful recommendations. I would like to thank Elizabeth Gettig, CGC, Dr. Robin Grubs, and Darcy Thull, CGC, for their guidance. Lastly, I would like to thank my friends and family for their ongoing support.

## **1.0 BACKGROUND**

Colon cancer affects approximately 145,000 individuals in the United States per year and is the fourth most commonly diagnosed cancer in the United States, but ranks second amongst cancer deaths (Hampel, Frankel et al. 2008; ACS 2011; Ahnen 2011). The lifetime risk of developing colorectal cancer is approximately 5%, with nearly half of those diagnosed succumbing to the disease (Terdiman, Conrad et al. 1999; Grady 2003). The cause of colorectal cancer can be environmental or genetically influenced, with a proportion generated by a combination of both components.

Several factors contribute to the development of colorectal cancer including biological, physical, environmental and genetic aspects. Biological characteristics that amplify the risk of colorectal cancer are increased age and race; nearly 90% of individuals diagnosed with colon cancer are over the age of 50 and higher rates of cancer are seen in the African American and Caucasian population (NCI 2011). Additionally, a personal history of inflammatory bowel disease and colorectal polyps increases an individual's risk for colorectal cancer. Physical factors such as an inactive lifestyle, diets high in fat, calories, and alcohol consumption and smoking have been associated with an increased risk for colorectal cancer (Rose, Boyar et al. 1986; Martinez, Giovannucci et al. 1997; Slattery, Potter et al. 1997; Cho, Smith-Warner et al. 2004). Those with hereditary colon cancer syndrome and those with family members who have had

colorectal cancer show an increased risk for developing colorectal cancer at higher frequencies and at earlier ages than individuals without genetic risk factors (Burt 2000).

## 1.1 GENETICS OF COLON CANCER

According to Vogelstein and Fearon, colorectal tumorigenesis is a multistep process, beginning with somatic mutations, resulting in cellular abnormalities and corresponding uncontrolled cell growth. Since Vogelstein and Fearon proposed their model, our understanding of colon cancer carcinogenesis has grown exponentially, due to a greater pathological and molecular comprehension of the disease. One way to characterize colon cancer development is based on the initiating pathway, either the tumor suppressor pathway or the mutator pathway. The classification can also be dependent on the precursor lesion from which the cancer derives; in the adenoma-carcinoma pathway, the cancer progresses from conventional adenomas, and in the serrated pathway, cancers originate from lesions such as serrated sessile adenomas and polyps (Snover 2011).

Cancers arising from the tumor suppressor pathway are the result of somatic or germline mutations occurring in genes responsible for cell cycle control. Several genes are frequently mutated in the tumor suppressor pathway, and mutations in these genes are often observed in a sequential pattern dependent on the stage of tumor development. This succession of mutations in relation to the stage of the cancer is supported by studies that document that inactivation of the adenomatous polyposis coli (*APC*) gene and mutations in the *KRAS* oncogene occurred earlier in the sequence, whereas mutations in tumor suppressor genes, such as p53, occur more frequently later in the progression (Vogelstein, Fearon et al. 1989; Snover 2011). Tumors arising from the

tumor suppressor pathway have distinct histological characteristics and typically have a territorial advantage; these tumors are genetically unstable, resulting in losses or gains of chromosome arms, chromosomal translocation or gene amplification, and are appropriately referred to as chromosomal instable tumors (CIN) comprising the majority of observed colorectal cancers (Vogelstein, Fearon et al. 1989).

Mutator pathway tumors are the result of mutations in mutator genes, which lead to an increased rate of somatic mutations in other genes. A number of mutator pathway tumors derive from mutations in mismatch repairs (MMR) genes, which are responsible for DNA base repair and promote development of tumors by increasing the number of mutations in other regulatory cell cycle genes or those involved in carcinogenesis (Halling, Harper et al. 1999). Tumors arising from the mutator pathway do not display the same territorial advantage as CIN tumors, and thus have a better prognosis. Tumors arising as a result of a defect in the mismatch repair system typically display a unique characteristic known as microsatellite instability (MSI). Microsatellites are found throughout the genome and are comprised of repetitive DNA sequences. The DNA repeats are typically mono- and dinucleic repetitions, however, higher order repeats are also observed. As a result of the repetitive nature of their structure, microsatellites are prone to the formation of DNA hairpins causing deletions and duplication errors and base-base mismatches (Vilar and Gruber 2010). The mismatch repair (MMR) genes are primarily responsible for repairing such errors, therefore, an impediment in the functionality of these proteins results in an accumulation of errors. When subsequent mutations occur in cell cycle regulatory genes it can result in the development of tumors. As a result, microsatellite instability, the presence of contraction or expansion in the repeated regions, is commonly observed in MMR deficient tumors (Syngal, Fox et al. 2000). However, microsatellite instable

tumors maintain karyotypic stability and are observed in 5-15% of all colorectal cancers, with the majority being sporadic in nature (Fearon and Vogelstein 1990; Rodriguez-Bigas, Boland et al. 1997; Bedeir and Krasinskas 2011; Snover 2011).

The serrated pathway has been used to describe cancers that arise from mutations that lead to CpG island methylation. Methylation-induced silencing of genes is believed to account for the progression from pre-cursor lesion to carcinogenesis, with the morphology and rate of progression dependant on the genes that are affected (Snover 2011). Alternative mechanisms of the serrated pathway have been described, however, the veracity of these mechanisms have not been molecularly confirmed (Snover 2011).

There is significant overlap between the proposed mechanisms of colorectal carcinogenesis. A large proportion of tumors arising from the adenoma-carcinoma sequence have been observed to derive from the tumor suppressor pathway, with some seen as a result of the mutator pathway. A proportion of sessile serrated adenomas and polyps that develop into cancer have been documented to originate from the mutator pathway and display microsatellite instability, while others do not (Jass 2003; Jass 2005; Snover 2011).

## **1.2 HEREDITARY CANCERS**

Comprehensive models of cancer development have allowed investigators to target genes that might play a role in hereditary predisposition for colon cancer. Studies involving individuals possessing germline mutation in oncogenes or tumor suppressor genes involved in colon carcinogenesis have identified numerous hereditary syndromes associated with an increased risk of colon cancer. Hereditary forms of cancer are associated with approximately 5-10% of all

cancers observed (Nagy, Sweet et al. 2004). Hereditary colorectal cancer syndromes have often been categorized into two groups: polyposis and non-polyposis forms.

Polyposis refers to the presence of multiple polyps that can be localized or distributed throughout the colon. As individuals with a hereditary polyposis syndrome have an increased number of polyps and adenomas compared to the general population, they are at an increased risk for colorectal cancer development through the adenoma-carcinogenesis pathway. Hereditary polyposis syndromes can be inherited in a dominant or recessive manner, and are responsible for less than 1% of all colorectal cancers observed (Bedeir and Krasinskas 2011). Examples of hereditary polyposis syndromes include Familial Adenomatous Polyposis (FAP) and Attenuated Familial Adenomatous Polyposis (AFAP), which both arise from mutations in the *APC* gene, as well as Juvenile Polyposis (JP), *MUTYH*-Associated Polyposis (MAP) and Peutz-Jegher Syndrome (PJS). The polyposis syndromes are often differentiated from one another pathologically, although with the variety of syndromes and range of polyp subtypes, ambiguity still remains. The number and distribution of polyps, as well as molecular and genetic testing, can aid with uncertainties and risk assessment for family members (Roessner, Kuester et al. 2011). However, as a result of the heterogeneity of many hereditary cancer syndromes, cancer risk values can range significantly for each syndrome (Vasen 2000).

Alternatively, the term non-polyposis has been formulated in order to distinguish syndromes different from the polyposis syndromes. Non-polyposis refers to the absence of polyps or the presence of very few polyps. The most common hereditary predisposition to colorectal cancer devoid of multiple polyps or adenomas is known as Hereditary Non-Polyposis Colorectal Cancer (HNPCC), or often referred to as Lynch Syndrome (Senter, Clendenning et al.

2008). HNPCC accounts for approximately 2-5% of all colorectal cancer observed (Hampel, Frankel et al. 2005)

## **2.0 HEREDITARY NON-POLYPOSIS COLORECTAL CANCER**

Hereditary non-polyposis colorectal cancer (HNPCC) syndrome was first reported by Aldred Warthin in 1931, who described a family with a pattern of gastric and endometrial cancer. However, it was Henry Lynch's work that led to the development of a more comprehensive clinical picture of the syndrome (Nagy, Sweet et al. 2004). As a result, the term HNPCC has often been used interchangeably with Lynch syndrome. However, it has been recognized that only those individuals who have undergone genetic testing and have an identified germline mutation have Lynch syndrome, while others are referred to as having HNPCC.

HNPCC is a dominant genetic condition resulting in an increased risk for colorectal and endometrial cancer. Additionally, HNPCC individuals have an increased risk for other cancers including those of the stomach, ovaries, small intestine, biliary tract and pancreas, urinary tract, as well as brain and sebaceous tumors (Vasen, Wijnen et al. 1996; Rodriguez-Bigas, Vasen et al. 1998; Aarnio, Sankila et al. 1999; Park, Shin et al. 2000; Senter, Clendenning et al. 2008; Watson, Vasen et al. 2008). As HNPCC involves multiple organ systems, it can overlap with other hereditary cancer syndromes, which makes identifying individuals with the syndrome difficult. One example of such obscurity is seen in certain HNPCC families presenting with brain tumors, most commonly glioblastomas, who are described as having a subphenotype of HNPCC known as Turcot syndrome (Hamilton, Liu et al. 1995). Further complicating matters, Turcot syndrome is also used to describe a subset of families with Familial Adenomatous Polyposis

(FAP), another hereditary colon cancer syndrome. An additional subclassification of HNPCC is observed in families presenting with skin findings such as keratoacanthomas and sebaceous skin tumors, known as Muir-Torre syndrome (Ollila, Fitzpatrick et al. 2006).

## 2.1 CAUSE

HNPCC is caused by germline mutations in mismatch repair (MMR) genes whose expressed proteins are responsible for DNA single-base pair excision repair. Dysfunction in these genes can lead to increased mutations in cancer inducing genes. The MMR genes implicated in HNPCC are the mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH6*) and postmeiotic segregation increased 2 (*PMS2*) genes; other MMR genes, such as *PMS1* and *MLH3*, may also play a role, but they have not been well established (Syngal, Fox et al. 2000; Umar, Boland et al. 2004; Senter, Clendenning et al. 2008). Additionally, *TGFBR2*, and *EXO1* have been implicated as potential causes of HNPCC, but are still under investigation (Grady 2003). Analysis of the MMR protein function shows that *MLH1* and *PMS2* form heterodimers, as do *MSH2* and *MSH6*. These structures are responsible for base-base mismatch repair or reparation of DNA hairpin loops that can occur as a result of DNA slippage. In HNPCC individuals, the most commonly mutated DNA mismatch repair genes are *MLH1* and *MSH2* (Syngal, Fox et al. 2000), and mutations in these two genes have a greater cancer risk compared to other MMR genes due to increased penetrance. *MLH1* is able to form heterodimers with *MLH3* and *PMS1* in the absence of *PMS2*, while *MSH2* can combine with *MSH3* in absence of *MSH6* (Vilar and Gruber 2010). Consequently, mutations resulting in non-functioning *PMS2* or *MSH6* proteins carry less risk as MMR functionality can be maintained through alternative binding.

Mutations in *MSH2* accounts for approximately 38% of mutations identified in individuals with HNPCC, followed by *MLH1* with 32%, and both *MSH6* and *PMS2* mutations are observed around 15% of the time (Hampel, Frankel et al. 2005; Senter, Clendenning et al. 2008). Alternative mechanisms for hereditary deficiency in MMR functionality have been evaluated. Studies indicate that approximately 20-25% of individuals whose colorectal tumor display a loss of function of *MSH2* or *MSH6*, without an identified germline mutation, have a deletion in the *EPCAM/TACSD1* gene. The *EPCAM/TACSD1* gene is found upstream from *MSH2* and mutations in the gene have been found to result in the hypermethylation of the *MSH2* promoter region, reducing its expression (Ligtenberg, Kuiper et al. 2009; Rumilla, Schowalter et al. 2011). As a result, this mechanism is proposed to account for approximately 5% of all Lynch syndrome tumors with abnormal *MSH2/MSH6* testing.

## **2.2 CANCER RISKS**

### **2.2.1 COLORECTAL CANCER**

HNPCC, like other hereditary cancer syndromes, is associated with an earlier age of cancer diagnosis. Individuals with HNPCC are typically diagnosed with colorectal cancer between 42 and 61 years of age, compared to 71 years of age in the general population (Hampel, Frankel et al. 2005; Horner 2009; Stoffel, Mukherjee et al. 2009).

The overall risks for colorectal cancer in individuals with HNPCC range from 20% to 74% by age 70. Gender differences in colorectal cancer risks have also been reported in the HNPCC population with women having as low as 30-40% less risk compared to men (Hampel,

Frankel et al. 2005; Stoffel, Mukherjee et al. 2009). Cancer risks can be contingent on the MMR gene affected. Lynch syndrome individuals with *MSH6* mutation appear to have lower risks compared to Lynch syndrome individuals with mutation in other MMR genes. Men with *MSH6* mutations have a 22% chance of developing colorectal cancer, while women have a 10% chance of developing the same type of cancer. Similarly, *PMS2* mutation carriers also have reduced colorectal cancer risks including a 20% and 15% risk for males and females, respectively (Senter, Clendenning et al. 2008).

### **2.2.2 ENDOMETRIAL CANCER**

Women with HNPCC have a 26-42% lifetime risk for endometrial cancer with some studies indicating a risk as high as 80% (Hampel, Frankel et al. 2005; Senter, Clendenning et al. 2008; Stoffel, Mukherjee et al. 2009). Phenotype-genotype associations have also been observed in endometrial cancers in women with HNPCC as female *MSH6* mutation carriers having a lower risk (26%) of developing endometrial cancer (Senter, Clendenning et al. 2008). Additionally, female *PMS2* mutation carriers' risk for endometrial cancer is approximately 15% (Senter, Clendenning et al. 2008).

### **2.2.3 OTHER CANCERS**

Increased risks for upper urinary tract, ovarian, gastric, small bowel, biliary tract, pancreatic and brain cancers have been associated with HNPCC. Cancer risks in these other organs vary greatly, but are generally considered to be less than 10-15% (Vasen, Moslein et al. 2007). A large study attempted to quantify risks with greater precision and concluded that the risk for urinary tract

cancer is highest with an approximate 8% lifetime risk, with men at greater risk than women. It was also found that women have a 7% lifetime risk for ovarian cancer. They concluded that the next most prevalent cancer is gastric cancer with a 5.4% lifetime risk, followed by small bowel cancer at 4% and biliary tract/pancreatic cancer at 4%. Individuals with HNPCC have a 2% lifetime risk for brain cancer, however, its association with HNPCC remains cloudy as early age of onset and high mortality rates make mutation testing difficult. The authors also revealed that *MSH2* mutation carriers have a higher risk compared to *MLH1* mutations carriers when assessing incidence of cancers outside of colon and endometrial cancers (Watson, Vasen et al. 2008). HNPCC individuals have a 30% risk of developing a second cancer within 10 years of the first cancer diagnosis and this number increases to 50% at 15 years post diagnosis (Lynch, Harris et al. 1977; Mecklin and Jarvinen 1986).

In rare circumstances, biallelic PMS2 loss has been reported and is a distinct syndrome resulting in hematologic and gastrointestinal malignancies and brain tumors at younger ages than those with Lynch syndrome. Biallelic PMS2 loss results in features similar to Neurofibromatosis-1, including café-au-lait spots, which are also categorically disparate from Lynch syndrome individuals (Senter, Clendenning et al. 2008). Additionally, individuals with Muir Torre, often identified by the presence of specific skin findings, are more frequently observed to have germline mutations in *MSH2* compared to the other MMR genes (South, Hampel et al. 2008).

## **2.3 DETECTION ON HNPCC INDIVIDUALS**

Identifying individuals with HNPCC is an essential public health issue because of its implications regarding surveillance, prognosis, surgical management, treatment and follow-up for patients and their at-risk family members (Rodriguez-Bigas, Boland et al. 1997). Determining the most efficient method of identifying individuals with HNPCC remains a widely debated issue. Population genetic screening for HNPCC is expensive and time-consuming, therefore, efforts have been focused on constructing an algorithm to distinguish high-risk HNPCC individuals from the general population. Multiple factors have been proposed or implemented including family history, tumor pathology, molecular and/or protein staining testing (Rodriguez-Bigas, Boland et al. 1997).

### **2.3.1 Family History**

HNPCC was originally identified through observational studies identifying cancer patterns within families, and was originally described as the diagnosis of endometrial and colorectal cancers presenting at an early age, or as a general familial clustering of colorectal and other types of cancer (Vasen 2000). As a result, several family history models have been proposed for the general screening for Lynch Syndrome (Dinh, Rosner et al. 2011). The model currently implemented most frequently in clinic, the Amsterdam Criteria, is used to identify high-risk individuals and families. As our knowledge of the syndrome has increased, the family history model has been altered in order to improve its efficacy.

Initially, the International Collaborative Group (ICG) proposed a family history based set of guidelines to identify at-risk individuals for HNPCC. The first version of the Amsterdam

Criteria, published in 1990, stated that the presence of three family members with colorectal cancer (with one individual being a first-degree relative to the other two, and one of the cancers diagnosed prior to age of 50), met a clinical diagnosis of HNPCC (Vasen, Mecklin et al. 1991). However, evaluations of the model proved it to be inefficient and the Amsterdam Criteria has been heavily criticized for its stringency. Evaluations of the model showed low levels of specificity and sensitivity for *MLH1* and *MSH2* mutation carriers, at 61% and 67%, respectively, indicating a need to improve the criteria (Rodriguez-Bigas, Boland et al. 1997; Syngal, Fox et al. 2000).

In order to address limitations in the Amsterdam Criteria including those with small families and those with extracolonic cancers, the Modified Amsterdam criteria was drafted. The Modified Amsterdam criteria considered small families with two affected first-degree relatives, with one diagnosed prior to age 55, and a third family member with endometrial cancer or another early neoplasm (Bellacosa, Genuardi et al. 1996). The new criteria improved the sensitivity to 72%, still far from ideal (Syngal, Fox et al. 2000).

In 1999, the International Collaborative Group expanded the Amsterdam Criteria to include those with associated HNPCC cancers. As a result, individuals diagnosed with colorectal, endometrial, small bowel, ureter, or renal pelvis cancer were included within the confines of the original Amsterdam conditions (Vasen, Watson et al. 1999). The revised clinical diagnostic criteria was aptly named Amsterdam Criteria II and increased the sensitivity to 87% and 62% for identifying *MLH1* and *MSH2* mutation carriers respectively. Sensitivities for the less prevalent *MSH6* and *PMS2* were still lacking with documented values of 38% and 48%, respectively (Syngal, Fox et al. 2000; Sjursen, Haukanes et al. 2010).

After extensive evaluation, it became obvious that the Amsterdam Criteria could not be utilized as a paradigm, but more as a framework in an attempt to incorporate characteristics observed across several reports. Nonetheless, the use of family history models as a way to identify HNPCC individuals in a clinical setting has been met with resistance, with many citing that the use of family history as the sole tool for identifying HNPCC individuals is not an effective method. The effectiveness of the method is not only limited by the lack of an unequivocal presentation, but also by other factors including small families, cases of adoption, non-paternity, denial of cancer diagnosis within the family, and families that are geographically or communicatively disconnected from one another, make collecting family histories an unreliable instrument for this purpose. Data suggests that a large proportion of individuals underreport the presence of colorectal cancers in first-degree relatives and an even greater inaccuracy was observed regarding second-degree relatives (Mitchell, Brewster et al. 2004). Therefore, poor patient recall of family cancer history may lead to an underrepresentation of families meeting clinical hereditary cancer syndrome criteria, specifically the Amsterdam criteria. Additionally, surgical removal of tissues at risk to develop cancer can reduce penetrance of the disease. Therefore, as attempts to identify unaffected individuals at risk for carrying a germline MMR mutation using family history alone have been largely unsuccessful, detection of HNPCC individuals has been reduced to molecular testing of affected individuals.

Once the genetic cause of HNPCC had been identified, it became less complicated to identify common attributes associated with the syndrome. Consequently, studies identifying prevalent HNPCC features were initiated to refine the search criteria of affected individuals. Studies suggested that 8.4% of individuals diagnosed with colorectal cancer under the age of 50 were identified as having Lynch syndrome (Hampel, Frankel et al. 2008), characterizing this as a

high-risk group that required increased attention. However, more recent data showed that nearly half of individuals with Lynch Syndrome are diagnosed over the age of 50 (Hampel, Frankel et al. 2008). Thus, testing that was limited to only those with early cancer diagnosis would miss a substantial portion of individuals with the syndrome and it could not be the sole criteria.

### **2.3.2 Microsatellite Instability**

Additional studies have been carried out to identify histopathological markers that correlate with HNPCC individuals so as to improve the identification of these individuals. A significant finding associated with HNPCC is microsatellite instability. Approximately, 8-20% of all colorectal cancers in the United States test positive for microsatellite instability (MSI-H). The phenotype occurs as a result of somatic, germline or epigenetic changes affecting mismatch repair genes (Herman, Umar et al. 1998; Hampel, Frankel et al. 2005; Ligtenberg, Kuiper et al. 2009). The presence of microsatellite instability in HNPCC tumors has led to further investigation of the motif. Data suggests that approximately 95% of microsatellite instable colorectal tumors of HNPCC syndrome patients are a result of loss of expression of one of the four MMR genes, while 5% of these tumors have an unknown reason for their microsatellite instability (Lynch, Shaw et al. 2004). Furthermore, microsatellite instability has been seen in a range of HNPCC associated tumors including gastric, endometrial, ovarian and sebaceous carcinomas, glioblastomas and lymphomas (Vilar and Gruber 2010).

Risk models utilizing multiple components, including family history and molecular test results, have been created in order to predict the likelihood of an individual carrying a germline mutation in an MMR gene as well as cancer risks. Examples of risk models include MMRpro which utilizes components such as family history of colorectal and endometrial cancer, age at

diagnosis, MSI and IHC results, *MLH1*, *MSH2* and *MSH6* mutation prevalence and penetrance, and other characteristics to predict carrier status and cancer risk (Chen, Wang et al. 2006). Another model, PREMM, accounts for colon, endometrial and other HNPCC-related cancers, age at diagnosis, presence of multiple HNPCC cancers in an individual and relationship to the proband to predict the probability of carrying an *MLH1* or *MSH2* mutation (Balmana, Stockwell et al. 2006). The model that appears to provide the greatest sensitivity and specificity, is the two-stage logistical regression model used in MMRpredict (Barnetson, Tenesa et al. 2006). The model incorporates proband sex, cancer locations, endometrial cancer of close relatives, age of diagnosis and presence of multiple tumors in the first stage, and molecular test results in the second stage.

### **2.3.3 MSI in Sporadic Cancer**

Microsatellite instability associated with HNPCC patients is also prevalent in sporadic colorectal cancer (Cunningham, Christensen et al. 1998). Sporadic MSI-H tumors share similar characteristics to HNPCC tumors, but do not follow a hereditary pattern, nor are they observed at early ages, and are seen more often in individuals over the age of 70 (Poynter, Haile et al. 2009). Sporadic MSI-High colorectal cancers arise from sessile serrated adenomas or polyps, as opposed to HNPCC-related colorectal cancer, which are believed to develop from conventional adenomas (Loughrey, Waring et al. 2007).

Sporadic tumors with the MSI-H phenotype are associated with mutations in several oncogenes and tumor suppressor genes including *MRE11A* and *KRAS*. However, it had been determined that the majority of MSI-H sporadic colorectal cancers are the result of hypermethylation of the promoter region of the *MLH1* gene (Cunningham, Christensen et al.

1998). The discovery of the epigenetic pathway associated with the development of sporadic MSI tumors lead to the detection of correlations between the *BRAF* gene and MSI histology (Davies, Bignell et al. 2002; Rajagopalan, Bardelli et al. 2002; Ahnen 2011). The *BRAF* gene is a cytoplasmic protein kinase that plays a role in the raF/meK/erK/maPK kinase signaling pathway and cellular apoptosis (Snover 2011). An activating mutation in the *BRAF* gene causes hypermethylation of the *MLH1* gene, suppressing its expression, which has been corroborated by the absence of MLH1 on protein staining tests (Rajagopalan, Bardelli et al. 2002). *BRAF* mutations have been implicated in sporadic melanoma (66%) and other cancers. However, *BRAF* mutations have not been identified in non-colonic HNPCC related tumors with *MLH1* methylation, therefore, *BRAF* testing for the purposes of excluding HNPCC is limited to colorectal cancer tissue (Deng, Bell et al. 2004). The most common mutation identified in the *BRAF* gene is a single nucleotide polymorphism of thymine to adenine in exon 15 resulting in an amino acid substitution from a valine to glutamic acid (V600E). The mutation is observed in more than 90% of colorectal cancers with a *BRAF* mutation and found in 31-83% of all sporadic MSI colorectal cancers (Rajagopalan, Bardelli et al. 2002; Deng, Bell et al. 2004; Kambara, Simms et al. 2004). Studies have shown a strong correlation (87%) to the presence of a *BRAF* mutation in MSI colorectal tumors with *MLH1* promoter methylation and a marked absence in tumors with germline *MLH1* mutations. Individuals with a *BRAF* mutation are rarely found to have concurrent MMR mutations and studies indicate that positive *BRAF* V600E mutations show a high specificity for predicting negative germline mutations in MMR gene testing (Loughrey, Waring et al. 2007). For these reasons, testing for the V600E mutation in MSI tumors has been adopted as an inexpensive secondary step to discriminate those who do not require more laborious MMR gene testing (McGivern, Wynter et al. 2004). Although rare, concurrent

mutations in *BRAF* and MMR genes are possible, therefore, the presence of a *BRAF* mutation does not completely rule out the possibility of HNPCC (Davies, Bignell et al. 2002; Rajagopalan, Bardelli et al. 2002; Deng, Bell et al. 2004).

MSI colorectal tumors with *MLH1* promoter methylation without an identified *BRAF* V600E mutation comprise another subgroup of cases. Subsequently, some authors suggest the testing of MSI colorectal tumor with absent MLH1 staining for *BRAF* mutation and, if negative, succeeding methylation testing. However, as opposed to the exclusion of HNPCC when a *BRAF* mutation is identified, studies suggest that a small number of individuals within this subset group have concurrent germline mutations and unexplained or somatic *MLH1* promoter methylation (Bouzourene, Hutter et al. 2010). An explanation for this phenomenon has been proposed as the result of MMR germline mutations which cause hypermethylation of the wild-type allele. Therefore, testing tumors for only *MLH1* methylation would result in potential HNPCC individuals being missed (Loughrey, Waring et al. 2007). Although the difficulty in excluding all HNPCC individuals by *BRAF* and methylation testing remains, the rare cases of *MLH1* methylation in HNPCC individuals without a *BRAF* mutation have been described as having less robust methylation when compared to sporadic hypermethylated tumors, and this may be a potential method to discriminate the two groups (Bouzourene, Hutter et al. 2010).

Other possible explanations for suppression of gene expression by hypermethylation have been proposed which could potentially account for a dysfunctional MMR system. Germline epimutations in *MLH1* have been identified, resulting in cancers similar to those seen in Lynch syndrome (Hitchins and Ward 2009). Studies have implicated elevated levels of methyltransferases in hypermethylation of genes, while other literature cites correlations between increasing age and methylation at other gene loci (Issa, Ottaviano et al. 1994). Alternative

methylation pathways have been documented where studies revealed associations between MSI-H colorectal tumors and CpG methylation at other loci including p16, IGF2, TSP-1 and HIC-1 (Ahuja, Mohan et al. 1997).

Mutations in other genes have also been proposed to explain hypermethylation, specifically mutations in the *KRAS* gene. Both *BRAF* and *KRAS* are found in the epidermal growth factor (EGF) pathway, therefore, mutations in either would have a similar biological effect. As a result, it was anticipated that mutations in both genes would not provide any additional biological advantage, and it has been demonstrated that mutations in these two genes are mutually exclusive from one another (Ahnen 2011). These findings resulted in the recognition that classic adenomas with *KRAS* mutations were not precursors to sporadic MSI colorectal cancers, and led to the discovery that MSI colorectal cancers derived from serrated polyps (Ahnen 2011). The concept of the serrated polyp-carcinoma pathway was a direct result of this study, which is distinct from the original adenoma-carcinoma pathway (Ahnen 2011). Other approaches have been explored in order to decipher between sporadic and hereditary microsatellite unstable tumors. Gene expression studies of *MLH1* and *PIWIL1* genes, have been proposed as potential techniques for differentiating to two subgroups of tumors (Kruhoffer, Jensen et al. 2005).

Differentiating between sporadic and hereditary MSI tumors remains problematical and an area of much deliberation. As a result of the phenotypic overlap, current guidelines used to determine candidates for MSI testing for the purpose of identifying germline mutations in MMR genes, intrinsically identifies a larger proportion of individuals with sporadic MSI-H colorectal cancers that do not possess a mutation. It remains imperative that this subset of individuals be distinguished in order to avoid needless and costly genetic testing.

### **2.3.4 Microsatellite Stable and Low Microsatellite Instability**

An additional 3-10% of colorectal cancers display low levels of instability (MSI-L), with the remainder being microsatellite stable (MSS) tumors (Lynch, Smyrk et al. 1993; Aaltonen, Salovaara et al. 1998; Salovaara, Loukola et al. 2000; Hampel, Frankel et al. 2008). Tumors that display lower levels of microsatellite instability have been described as MSI-L. The classification of MSI-L tumors is determined based on the presence of instability in a panel of markers (Aaltonen, Salovaara et al. 1998; Salovaara, Loukola et al. 2000; Hampel, Frankel et al. 2008). The clinical relevance of MSI-L tumors is not well established and several studies suggest that its association with an MMR deficiency is rare. Therefore, the presence of MSI-L tumors in an individual is considered to exclude, or make highly unlikely, the existence HNPCC (Mueller, Gazzoli et al. 2009). *MSH6* mutations have been shown to be associated with MSI-L tumors, presenting a predicament in excluding HNPCC based on MSI results.

### **2.3.5 Other Pathological Characteristics**

Colorectal tumors with microsatellite instability possess other specific profiles, which can be helpful in identifying HNPCC individuals. Data indicates that MSI colorectal tumors are found in greater frequency in the right side of the colon, have a lower stage at diagnosis, and have higher histological grades. As a result, the ICG suggests that clinicians should be aware of characteristics associated with HNPCC colorectal cancer, including an increased proportion of cancers found in the proximal colon and the presence of multiple colon cancers, mucinous phenotypes with marked tumor-infiltrating lymphocytes, a Crohn's-like host response and an absence of necrotic cellular debris (Vasen, Watson et al. 1999; Greenson, Bonner et al. 2003). A

strong association with HNPCC has been observed in tumors displaying mucinous and signet-ring cell component, with less of a consensus on the presence of tumor infiltrating lymphocytes (Wu, Shibata et al. 2001).

Interestingly, despite the poor prognosis of cancers with pathological characteristics commonly seen in Lynch Syndrome colorectal cancer, these cancers typically have a better outcome than CIN cancers (Ahnen 2011). Similarly, gynecological cancers in HNPCC syndrome individuals are largely diagnosed at an earlier stage and are often curable (Watson, Butzow et al. 2001).

## **2.4 BETHESDA CRITERIA**

The Amsterdam Criteria I have been classically focused on high-risk families and was found to be too stringent, therefore a set of criteria using the reported characteristics of HNPCC tumors for population-based screening was needed. The National Cancer Institute (NCI) International Workshop constructed a set of guidelines, known as the Bethesda Criteria, as a tool to capture a greater number of individuals with HNPCC. The guidelines outlined a set of criteria for tumors that should be considered for evaluation of microsatellite instability. Based on the results of the MSI testing, additional testing or corresponding counseling were suggested.

When initially constructed, the Bethesda criteria suggested that MSI testing be carried out on (Boland, Thibodeau et al. 1998; Pinol, Castells et al. 2005):

- individuals with cancer that met the Amsterdam criteria
- those diagnosed with colorectal or endometrial cancer under the age of 45
- diagnosis of an adenoma in an individual less than 40 years of age

- individuals with two HNPCC related cancers (metachronous or synchronous)
- individuals with colorectal cancer and a first degree relative with either an HNPCC related cancer diagnosed under 45 or an adenoma under the age of 40
- those less than 45 years of age with a right-sided colorectal cancer with solid/cribiform histology or a signet ring type colorectal tumor

Based on the age restrictions suggested for each criterion, there was a reduced significance for pathological influence, as any cancer diagnosed under the age of 45 should be captured irrespective of tumor characteristic. Studies measuring the quality of the criteria indicated a superior sensitivity over the Amsterdam criteria with value of ~94%. However, the specificity of the Bethesda criteria was reported to be approximately 25% for identifying individuals with an *MLH1* or *MSH2* germline mutation and 30% for germline mutations in any MMR gene (Syngal, Fox et al. 2000; Pinol, Castells et al. 2005).

As a result of the poor specificity values, the Bethesda Criteria have been revised subsequent to an NCI workshop in 2002 and outlined in 2004 (Umar, Boland et al. 2004). The updated Bethesda Criteria delineates situations where MSI testing should be carried out which include (I) individuals diagnosed with colorectal cancer under the age of 50, (II) individuals with metachronous or synchronous HNPCC-related cancers, (III) those whose colorectal tumors display histology associated with microsatellite instability and are under the age of 60, (IV) individuals with colorectal cancer and a first degree relative with an HNPCC-associated cancer with the stipulation that one of the cancers were diagnosed prior to the age of 50, and (V) individuals with colorectal cancer that have two or more first or second degree relatives with an HNPCC-associated cancer. The guideline elaborates on tumor histology as the “presence of tumor infiltrating lymphocytes, Crohn’s-like lymphocytic reaction, mucinous/signet-ring

differentiation, or medullary growth pattern” (Umar, Boland et al. 2004). Additionally, the guidelines indicate that a consensus with regards to age limits and tumor histology has not been met. Recent studies suggest that the revised Bethesda Criteria have a sensitivity of 73-91% and an improved specificity of 77-82% (Palomaki, McClain et al. 2009).

In 2009, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) group has recommended that all individuals with colorectal cancer be screened for Lynch syndrome (EGAPP 2009). Additionally, the same group has endorsed testing of women with endometrial cancer diagnosed under the age of 50, with some centers testing all endometrial cancers.

## **2.5 TESTING FOR MICROSATELLITE INSTABILITY**

Microsatellite testing has been praised and scrutinized for its use in identifying HNPCC individuals. The benefits of MSI testing are its reproducibility and that it can be carried out on small amounts of tissues (Zhang 2008). This is an important factor as MSI testing can be easily carried out on biopsies obtained during colonoscopies, where abnormal test results may play a role on future surgical decision.

Additionally, extensive research has been performed on determining the ideal markers for MSI testing. Since the most common microsatellites are mononucleotide and dinucleotide repeats, markers for these sequences have been investigated. Mononucleotides were evaluated for MSI testing and two markers were valued for their microsatellite instability sensitivity. Mononucleotide markers Bat25 and Bat26 were supported for MSI testing as a result of studies indicating that all tested colorectal tumors displayed instability at the two loci (Loukola, Eklin et

al. 2001). An additional benefit of using the monomorphic Bat25 and Bat26 markers, is their ability to detect instability in the absence of normal tissue in non-African populations (Zhou, Hoang et al. 1998; Vilar and Gruber 2010). Therefore, biopsies of abnormal tissue can be collected without associated normal tissue for comparison. However, the increased prevalence of polymorphisms at the Bat26 loci in the African population, and rare frequency in other populations, emphasizes the benefit of DNA comparison to normal tissue in order to validate results (Loukola, Eklin et al. 2001). Nonetheless, additional testing is typically not recommended if there is an absence of instability in the tumor DNA. Similarly, researchers have investigated the use of dinucleotide markers and their efficacy of identifying microsatellite instability. Data indicates that tumors with MSI-L phenotypes typically show instability for dinucleotide markers, therefore it was determined that testing using only dinucleotide markers could lead to misclassification of tumors (Vilar and Gruber 2010).

Originally, a lack of procedural standards resulted in a number of markers being used in order to determine instability. MSI-High had been defined as instability in >30% of loci, while MSI-Low is designated when 10-30% are unstable, and instability in <10% of loci is considered microsatellite stable (MSS). As a result of the variability in MSI protocol, guidelines were constructed to provide uniformity for testing purposes. The Bethesda guidelines recommend the utilization of a 5-marker panel, which includes two mononucleotides, Bat25 and Bat26, and three dinucleotides, D5s346, D2s123 and D17s250, (Loukola, Eklin et al. 2001; Vilar and Gruber 2010). MSI-H histology was defined as the presence of instability in two or more markers, while MSI-L was classified as instability in only one marker (Boland, Thibodeau et al. 1998). Additionally, tumors that display instability at only dinucleotide markers are recommended to undergo testing with additional mononucleotide markers (Bedeir and Krasinskas 2011). The

marker panel continues to be under investigation with some groups suggesting the inclusion of additional mononucleotide markers to increase sensitivity, however, revisions to the Bethesda Guidelines have yet to be made (Umar, Boland et al. 2004).

### **2.5.1 Limitations of MSI Testing**

Tumors tested for the purposes of identifying Lynch syndrome individuals inherently have limitations. As with many tests, sensitivity for MSI testing is not 100%, therefore, the potential for missing microsatellite instability is a plausible shortcoming. As the marker panel continues to be investigated, future revisions may result in an increased sensitivity for MSI testing.

As previously noted, microsatellite instability is not exclusive to HNPCC tumors and is observed in sporadic neoplasms, serrated adenomas and individuals with hyperplastic polyposis syndrome (Hawkins and Ward 2001). As colorectal cancer is common in the general population, there is potential for the cancer to be of sporadic nature, representing a phenocopy of an HNPCC individual, and tumor testing in one individual may not be indicative of the risk within the family (de la Chapelle and Hampel 2010). Additionally, not all HNPCC tumors display MSI histology. It has been well established that tumors with *MSH6* mutations are complicated in part by the variety of tumor phenotypes associated with them including MSI-H, MSI-L and MSS stable phenotypes, which can result in the omission of HNPCC individuals (Umar, Boland et al. 2004).

### **2.5.2 Other Applications of MSI Testing**

There are additional advantages for MSI testing in colon tumors besides identification of HNPCC individuals, including information on prognosis and treatment. MSI tumors typically have better prognoses and implications for response to chemotherapeutic drugs. The enhanced prognosis was based on the decreased likelihood of the cancer spreading to lymph nodes (Watanabe, Wu et al. 2001). With regards to treatment, some literature suggests that individuals with MSI tumors have superior response to 5-FU. However, other reports indicate that individuals with stage II or stage III MSI tumors do not benefit from FU-based adjuvant therapy compared to those with similar staging with MSS or MSI-L tumors (Ribic, Sargent et al. 2003; de la Chapelle and Hampel 2010). Additionally, studies indicate that survival and recurrence-free survival do not significantly differ whether or not treated with FU-based chemotherapy in individuals with MSI-H tumors (Des Guetz, Schischmanoff et al. 2009). This data supports that stage II and stage III MSI positive individuals should not be subjected to hazardous chemotherapy treatment, opposed to the current standard of practice (de la Chapelle and Hampel 2010). Although MSI tumor response to 5-FU has been widely debated, studies on the efficacy of irinotecan on MSI tumors have been encouraging, but are still in their infancy (Vilar and Gruber 2010).

For these reasons, implementing MSI testing as a standard of practice can have an impact on hereditary cancer screening, therapy and as a prognostic indicator for patients (Vilar and Gruber 2010).

## 2.6 IMMUNOHISTOCHEMISTRY

The limitations of MSI testing have prompted investigations for alternative tumor tests. The most successful alternative test for the purposes of identifying HNPCC individuals is immunohistochemistry (IHC), therefore this protocol was proposed as substitute to MSI testing. Immunohistochemistry utilizes monoclonal anti-bodies against the four mismatch repair genes associated with HNPCC, the presence of which is represented by positive staining. Immunohistochemistry detects a mutation resulting in a deficiency MMR protein by the absence of staining in the tumor tissue. Additionally, the lack of expression of a protein corresponds to their dependence as heterodimers, where the degradation of MLH1 or MSH2 protein results in the concurrent loss of its partner protein. Alternatively abnormalities in MSH6 and PMS2 result in a loss of staining for only those proteins, due to the alternative binding capability of MLH1 and MSH2. Some *MLH1* mutations that are antigenically active show only a loss of PMS2 (Shia 2008). Nonetheless, the heterodimerization staining pattern concept has lead to the proposition of transitioning to a two-antibody system, of MSH6 and PMS2, which would reduce the costs of testing. The model is founded on the principle that abnormal IHC testing of MSH6 or PMS2 would prompt reflex testing of the heterodimer partner in order to determine which protein is affected, helping to target gene testing to a single genes. The Bethesda Criteria were constructed for the purposes of outlining situations where MSI testing should occur, and not IHC. However, it has been suggested that the recommendations be expanded to include IHC testing (Lenz 2005).

There are several benefits of IHC testing. Proponents of IHC indicate that testing can be done at the time of surgery, identifying the prospective MMR gene, decreasing the need for postoperative genetic service providers to test archived tumor tissue. Another benefit of IHC is

its wide availability, as it does not need to be performed in a molecular diagnostic laboratory, but in more prevalent pathology laboratories (Hampel, Frankel et al. 2008).

### **2.6.1 Limitations of IHC**

In the interest of uncovering the most efficacious testing for identifying HNPCC, the limitations of IHC testing have been evaluated and its shortcomings identified. Proteins that remain antigenically intact but carry mutations that result in a loss of protein function pose a significant problem when IHC is carried out independently, as they stain positively on testing. The result has been documented to occur in various *MLH1* and *MSH2* mutations including missense, truncating and large in-frame deletions. Similarly, antigenically stable somatic changes in the wild-type allele may produce positive staining within the analyzed tumor tissue and distort interpretation of IHC testing. Another limitation of IHC is the difficulty interpreting the variation in staining patterns. Due to the nature of the procedure, immunohistochemistry is prone to weak, focal, and ambiguous staining, leading to indefinite results or the requirement of additional testing (Shia 2008). IHC is also susceptible to poor staining if the obtained biopsies are small, which becomes problematic in situations where decisions on medical management need to be made prior to surgery. Additionally, as opposed to the Bethesda Criteria for MSI testing, a standard of procedure for IHC testing is lacking. Variations in the laboratory fixatives, tissue handling protocols, and processing methods for IHC testing pose a potential risk of discrepancy in result evaluation (Umar, Boland et al. 2004; Shia 2008).

## 2.7 MSI VERSUS IHC

The shortcomings of both MSI and IHC testing have sparked great debate regarding the ideal test to identify germline mutations in MMR genes. Comparisons of each test type have been explicitly carried out to evaluate efficacy of HNPCC identification. Studies indicate that when MLH1 and MSH2 staining was performed, abnormal results were obtained in 85% of the known mutation carriers, and another study suggests IHC to have specificity of 95% with regards to *MLH1* and *MSH2* mutations (Shia, Klimstra et al. 2005). However, when four MMR antibodies were included in the IHC testing, the sensitivity improved to 92% as the addition of the PMS2 antibody was able to detect *MLH1* mutations that were initially missed by IHC (Shia 2008).

In comparison, approximately 93% of mutation carriers had microsatellite instability detected on their tumors (Shia 2008). Further corroborating the detection ability of MSI testing, one study indicated that all MLH1 or MSH2 negative tumors displayed microsatellite instability (Lindor, Burgart et al. 2002). As previously discussed, due to the heterogeneity in microsatellite instability phenotype observed in MSH6 absent tumors, MSI testing was not as efficient in detecting *MSH6* mutation carriers compared to IHC.

Reviewers indicate that IHC and MSI testing are not equivalent tests as evidence supports that both tests are able to detect cases that the other cannot (Shia, Ellis et al. 2004). Therefore, although carrying out both types of testing is not the most cost-effective method, it is certainly the most sensitive.

## 2.8 BETHESDA CRITERIA AND TUMOR TESTING

The current Bethesda criteria are guidelines for MSI testing for colorectal tumors only, however the use of MSI and IHC in extracolonic tissue has also been assessed.

Microsatellite instability has also been observed in extracolonic tumor and attempts to quantify the prevalence and association with HNPCC have been carried out. Similar to colorectal cancer, approximately 9% of women with endometrial cancer diagnosed younger than 50 years of age have an MMR mutation, however, it has been reported that nearly half of women with Lynch syndrome are diagnosed beyond that threshold (Lu, Schorge et al. 2007; Kwon, Scott et al. 2011). Paralleling increased rate of tumor development from MSI adenomas in colon cancers cases, studies investigating endometrial cancer indicate that MSI has been detected in complex atypical hyperplasia associated with uterine endometrioid carcinoma, while MSI was absent in atypical tissue not associated with endometrial carcinoma (Esteller, Levine et al. 1998). However, it has been documented that complex atypical hyperplasia is only present in small amounts on biopsies, making testing for MSI difficult. Additionally, microsatellite instability due to altered protein expression from MMR germline mutations may develop over time and for that reason instability may not be detectable when endometrial tissue is obtained (Staebler, Lax et al. 2000). Therefore, it has been suggested that IHC testing may be a more appropriate method for analysis of endometrial tissue over MSI testing. Microsatellite instability is also observed in 20-25% of sporadic uterine endometrioid cancer, the most common form of endometrial cancer (Staebler, Lax et al. 2000). Similar to the methylation of *MLH1* observed in sporadic colorectal cancer, studies have revealed that correlations to *MLH1* silencing by epigenetic factors also described in a significant number of sporadic endometrial cancers (Esteller, Levine et al. 1998). When evaluating MSI in gastric tumors, MSI was observed in approximately 13-44% of gastric

carcinomas, with the Bat26 marker being the most sensitive marker in identifying instability (Wirtz, Muller et al. 1998; Halling, Harper et al. 1999).

Literature summarizing MSI testing on extracolonic cancer tissue indicates that although MSI and IHC testing in these tissues have revealed similar characteristics, the sensitivity and specificity of testing extracolonic cancers has not been well established. Furthermore, the markers recommended for MSI are specific to colorectal cancer, therefore, MSI testing of extracolonic cancers using the same marker panel may not display the same pattern, resulting in inaccurate result interpretations (Kuismanen, Moisio et al. 2002; Weissman, Bellcross et al. 2011).

It has been debated whether polyps are a viable option for MSI and IHC testing. Polyps have been shown to be sensitive to the mutator effect, therefore, as precancerous lesions, they are potential candidates for testing. Additionally, studies suggest that the polyps in individuals with Lynch syndrome develop into cancer at a much higher rate (1-3 years from detection) compared to the general population (8-17 years). Therefore, identifying HNPCC individuals prior to tumor development would significantly reduce morbidity and mortality (Jass and Stewart 1992; Vasen, Nagengast et al. 1995).

One study suggested that 88% of large (>5mm) and proximal adenomas in HNPCC individuals display loss of expression of an MMR protein. However, it was concluded that IHC testing could be carried out on adenomas in cases suspicious for HNPCC, with the caveat that intact staining of MMR proteins could not exclude HNPCC due to the poor sensitivity (Halvarsson, Lindblom et al. 2005)

Originally, the Bethesda criteria recommended individuals under the age of 40 that were found to have adenomas should undergo MSI testing. This recommendation was supported by

studies showing the detection of MSI in adenomas resulting in the identification of individuals with Lynch Syndrome (Loukola, Salovaara et al. 1999). However, additional studies revealed that the scarcity of appropriate tissue in polyps lead to a lack of yield for MSI testing, resulting in the removal of the recommendation in the subsequent Bethesda Criteria revision (Jass, Pokos et al. 1996; Umar, Boland et al. 2004; Velayos, Allen et al. 2005). However, the International Collaborative group has defined features of adenomas of which clinicians and pathologists should be cognizant including: early age of onset, relatively low number of adenomas, an increased proportion with a villous growth pattern, a high degree of dysplasia, and their rapid development to carcinoma (Vasen, Watson et al. 1999).

## **2.9 GENETIC INFORMATION AND TUMOR TESTING**

Many investigators consider microsatellite testing to be a genetic test (based on its direct assessment of DNA motifs) and, thus, it requires a patient's informed consent. Others contest that microsatellite testing does not elicit information on the patient's inherited risks or the risks of their family members, and avoids potential for psychosocial or psychological harm that is often associated with genetic tests. Furthermore, MSI testing is performed on cancerous tissue, which may not be indicative of an individual's germline. Some researchers suggested that microsatellite testing on tumors is similar to estrogen receptor testing in breast cancer patients, which does not require informed consent (Chubak, Heald et al. 2011). Similarly, researchers suggest that IHC is a test of tumor phenotype and should not be regarded as a genetic test. However, IHC testing has the ability to identify specific MMR genes that are dysfunctional, potentially providing information about the individual's genetic makeup, as well as the genetics

of his or her family members (Chubak, Heald et al. 2011). This result has been corroborated by reports that loss of MSH2, MSH6 and PMS2 protein is exclusive to HNPCC individuals (Abdel-Rahman, Mecklin et al. 2006; Lagerstedt Robinson, Liu et al. 2007). As a result, immunohistochemistry has a greater potential for psychosocial risk for patients and their families.

Guidelines are available for the performance of genetic tests. The American Society of Clinical Oncology policy indicates that genetic testing should be carried out collectively with pre- and post-test counseling in order to ensure the patient's comprehension of the risks and benefits of the testing (ASCO 2003). Additionally, the Evaluation of Genomic Applications in Practice and Prevention (EGAPP) group states that informed consent should be obtained prior to MSI or IHC testing (EGAPP 2009).

## **2.10 GENETIC TESTING FOR HNPCC**

Genetic testing for at-risk individuals can be initiated in several different ways. Individuals that were identified to be at risk for HNPCC as a result of abnormal MSI and/or IHC testing may undergo genetic testing. Lack of MMR staining on IHC can indicate the specific MMR gene to test first. Reflex testing of the heterodimerizing protein partner can occur in cases where a mutation is not detected in the primary gene candidate. As EPCAM mutations can account for a loss of expression in MSH2, reflex testing of the gene can be considered when germline mutations are not identified in MSH2/MSH6 absent tumors.

Individuals may still be considered for a clinical diagnosis of Lynch Syndrome if (1) they test negative for a germline mutation but their tumor displays microsatellite instability, exhibits a

loss of expression of an MMR protein on IHC testing and, in *MLH1* cases, lack a *BRAF* mutation. As mutations may be undetectable with current technology, these individuals and at-risk family members should undergo screening as recommended for Lynch syndrome individuals.

Unaffected individuals whose family history meets the Amsterdam criteria are often referred for genetic testing of the most commonly mutated MMR genes, *MLH1* and *MSH2*. Tumor testing of family members with an HNPCC cancer is beneficial for targeted genetic evaluation of the proband. If tissue is unattainable, the next most prudent step is germline testing of a family member affected with an HNPCC associated cancer, ideally with the youngest age of onset. If there are no eligible individuals diagnosed with an HNPCC cancer in a family suspicious for the syndrome, genetic testing on another family member can be considered with the caveat that there is a significantly lower chance to obtain an informative test result. However, most individuals are not identified in clinics based on family history alone. Many individuals are referred for genetic counseling based on abnormal tissue tumor test results. When test results indicate a risk of HNPCC, referral to a genetics clinic is warranted. Genetic consults can help educate patients on test implications and address other psychosocial issues.

### **2.10.1 Mutations**

There have been over 200 mutations identified in *MLH1*, over 170 mutations in *MSH2* and over 30 mutations in *MSH6* (Peltomaki and Vasen 2004). Large deletions and rearrangements are responsible for approximately 20% of mutations in the *MSH2* and *PMS2* genes, and deletions account for 5-10% of *MLH1* and *MSH6* mutations (Wijnen, van der Klift et al. 1998; Charbonnier, Raux et al. 2000; Senter, Clendenning et al. 2008). Due to the array of mutations,

testing mutations in MMR genes incorporates sequence analysis and methods for detecting gene deletions, duplications and rearrangements.

The majority of *MSH2* mutations are protein truncating (Salahshor, Koelble et al. 2001; Wahlberg, Schmeits et al. 2002). However, more than 1/3 of *MLH1* mutations and approximately 24% of all mutations identified in Lynch syndrome individuals are missense mutations (Peltomaki and Vasen 2004). Missense mutations can be difficult to assess as benign polymorphisms or deleterious mutations, with the classifications having significantly different medical management recommendations (Weissman, Bellcross et al. 2011). Data suggests that individuals with a personal history of cancer who received uninformative genetic test results had similar distress levels to mutation carriers, supporting the need for follow-up discussion or additional counseling so as to ensure these individuals full comprehend their results (Gritz, Peterson et al. 2005).

### **2.10.2 Testing Sensitivity**

Palomaki and McClain indicate an estimate of greater than 90% for the sensitivity of *MLH1*, *MSH2*, and *MSH6* mutation detection, however, many labs cite a sensitivity of greater than 98% (Palomaki, McClain et al. 2009). Reasons for the inability to detect all mutations include improper specimen handling, unanticipated molecular reactions, as well as data analysis and computational errors. Balanced genomic rearrangements may also attribute to reduced sensitivity of genetic testing. Other observed mutations, such as large deletions and splice site mutations, prove difficult to identify in *MSH2* (Wijnen, van der Klift et al. 1998; Nakagawa, Yan et al. 2002).

Initially, *PMS2* proved to be a difficult for mutation testing due to the presence of numerous pseudogenes. However, with the advent of long range PCR, the testing sensitivity has improved with the exception of exons 13-15, where pseudogenes may still interfere with testing accuracy. Currently, more than 60% of individuals with *PMS2*-absent IHC results have been found to possess germline mutations (Senter, Clendenning et al. 2008).

### **2.10.3 Test Results**

Identification of a germline mutation in an MMR gene is interpreted as diagnostic for Lynch Syndrome. The result allows at-risk family members to undergo single-site genetic testing for the mutation in order to definitively determine their mutation status. Subsequent, positive results in family members infer an increased risk for HNPCC associated cancers, while a negative result assumes the mutation was not inherited and risk values lower to that of the general population. Probands whose parents test negative for mutations should be counseled for de novo mutations. Literature reveals that de novo mutations in MMR genes are observed in 1-5% of cases, much more rare in HNPCC compared to other CRC cancer syndromes (Win, Jenkins et al. 2011). However, health care providers may also consider other issues, such as non-paternity.

Negative genetic test results are more difficult to assess as reduced testing sensitivity or testing an individual who developed a sporadic cancer within a HNPCC family can lead to inaccurate test result interpretation. Consequently, as genetic testing sensitivity for the MMR genes has developed, a wide spectrum of genetic alterations has been obtained.

There are several benefits for at-risk family members who choose to undergo genetic testing. Firstly, they need only carry out single-site mutation for the identified mutation, and predictive single-site genetic testing is less costly than full sequence analysis. Secondly,

compared to numerous outcomes and implications of full mutation sequencing, result interpretation of single-site testing is relatively unambiguous.

## **2.11 PUBLIC HEALTH RELEVANCE**

Colon cancer is the 4<sup>th</sup> most commonly diagnosed cancer in the United States and HNPCC has been documented to be responsible for 2-5% of all colorectal cancers (Lynch and de la Chapelle 2003; de la Chapelle 2004; Senter, Clendenning et al. 2008). Additionally, endometrial cancer is the fourth most common cancer observed in North American women and HNPCC, the most common hereditary endometrial cancer syndrome, accounts for 1-4% of all endometrial cancers (Hampel, Frankel et al. 2005).

The prevalence of MMR mutations in the general population is approximately 1 in 2700, however, it has been documented to be as high as 1 in 360-440 (Chen, Wang et al. 2006; Hampel and de la Chapelle 2011; Win, Jenkins et al. 2011). Despite its prevalence, impact on individual morbidity and burden on the health care system, Lynch Syndrome remains clinically under diagnosed (Singh, Schiesser et al. 2010).

Individuals with hereditary cancer syndrome develop cancers at a younger age, leading to a greater loss of years of life. Identifying those diagnosed with Lynch Syndrome is important as these individuals are at an increased risk for metachronous and synchronous cancers. Several auxiliary medical management options would be available to these individuals including early detection through cancer screening and risk reduction procedures. Furthermore, identifying these individuals could lead to counseling of unaffected, at-risk family members, who would also be

offered supplemental medical management opportunities. Family members who do not carry an MMR mutation are relieved from increased screening and surgery.

## **2.12 SCREENING AND PREVENTION IN HNPCC INDIVIDUALS**

Increased cancer surveillance and prophylactic surgery have decreased mortality and morbidity in HNPCC individuals (Renkonen-Sinisalo, Aarnio et al. 2000; Schmeler, Lynch et al. 2006). Both unaffected and affected HNPCC individuals can benefit from colon cancer screening through colonoscopies. HNPCC colorectal cancers have been shown to derive from adenomas and develop into cancer earlier and more rapidly when compared to sporadic cancer (Ahnen 2011). Therefore, early and more frequent colonoscopies are beneficial for the removal of precancerous lesions prior to their development to cancer, or for detecting cancers at a less advanced stage. As a result, studies indicate that cancer surveillance in unaffected HNPCC individuals reduce mortality and morbidity by 65% over a period of 15 years (Jarvinen, Aarnio et al. 2000), Studies indicate that colonoscopies reduce the risk of metachronous colon cancers in affected HNPCC individuals as well (Jarvinen, Aarnio et al. 2000). Colonoscopies in HNPCC patients are cost effective, and it is recommended that these individuals undergo earlier and more frequent screening compared to the general population (Vasen, van Ballegooijen et al. 1998). The current guidelines by the National Comprehensive Cancer Network for colon cancer screening in Lynch syndrome individuals recommends that colonoscopies be initiated between the ages of 20-25 at a frequency of every 1-2 years. The guidelines indicate that if there is a

family history of colon cancer diagnosed prior to the age of 25 screening should occur 2-5 years prior to the age of diagnosis (NCCN 2010)<sup>1</sup>.

Hereditary colorectal cancers are managed and treated differently than sporadic tumors. More extensive surgeries may be recommended for HNPCC individuals because of the increased risk for secondary colorectal cancer development (Vasen 2000). However, the decision should be balanced against the treatment's impact on quality of life (Chen, Chiang et al. 2008). The current guidelines by the National Comprehensive Cancer Network indicate that HNPCC individuals that are not amenable to screening or non-compliant to recommendations can consider subtotal colectomy. HNPCC individuals with unresectable adenomas or high-grade dysplasia are recommended to undergo ileorectal anastomosis, during which the surgeons perform a total colectomy and connect the ileum to the rectum (NCCN 2010).

Gynecological screening in Lynch syndrome individuals has the capability of identifying pre-malignant lesions, but debates remain regarding its effect on mortality and the appropriate time intervals between sampling (Auranen and Joutsiniemi 2011). The difficulty in screening for endometrial cancer in women with Lynch syndrome is the lack of understanding surrounding its development. As not all Lynch syndrome endometrial cancers develop from hyperplasia, and sampling does not detect all cases of hyperplasia, endometrial sampling can fail to detect many cancers and hyperplasia (Auranen and Joutsiniemi 2011). Additionally, endometrial sampling is an invasive procedure and can be painful, leading to reduced compliance; current recommendations should be broached during discussion of medical management in order to

---

<sup>1</sup>Cited with permission from the NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines<sup>(tm)</sup>) for [GUIDELINE NAME] V[GUIDELINE VERSION NUMBER](c) 2011 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guidelines<sup>(tm)</sup> and illustrations herein may not be reproduced in any form for any purpose without the express written permission of the NCCN. To view the most recent and complete version of the NCCN Guidelines, go online to [NCCN.org](http://NCCN.org). NATIONAL COMPREHENSIVE CANCER NETWORK<sup>(r)</sup>, NCCN<sup>(r)</sup>, NCCN GUIDELINES<sup>(tm)</sup>, and all other NCCN Content are trademarks owned by the National Comprehensive Cancer Network, Inc.

increase effectiveness. A meta-analysis study by Auranen and Joutsiniemi indicates that there is a lack of evidence regarding the benefit of gynecological cancer screening to serve as the foundation of clinical recommendations for Lynch syndrome patients (Auranen and Joutsiniemi 2011). As a result, the National Comprehensive Cancer Network's current recommendations for gynecological cancer screening indicate that HNPCC women can consider transvaginal ultrasound for endometrial and ovarian cancer. The guidelines indicate that endometrial sampling may be helpful in some cases, however, all women should be educated regarding endometrial cancer, associated symptoms, and management (NCCN 2010).

Another available option for women with HNPCC include prophylactic surgeries to reduce the risk of tumor development. Prophylactic hysterectomy and bilateral salpingo-oophorectomy (BSO) reduces the risk of ovarian and endometrial cancer in women with HNPCC. The decision to have prophylactic surgery is complex and personal, and women should be well informed on several important aspects. Discussions with family members, gynecologists, oncologists, additional health care providers, or other women from HNPCC families may aid a woman with the decision process of prophylactic surgery. Issues to consider that can influence a woman's decision include the risks of the procedure such as loss of fertility and the ensuing onset and management of menopause. Comparisons between gynecological screening and prophylactic surgery have been assessed to determine the effectiveness at an individual and population level. There is evidence that prophylactic TAH-BSO results in longer life expectancy, higher number of quality-adjusted life-years and is the most cost-effective option when compared to those undergoing gynecological examinations and surveillance for Lynch Syndrome (Chen, Yang et al. 2007; Yang, Caughey et al. 2011). The study suggests that the underpinning of the benefit of prophylactic surgery is derived from the increased probability for cancer

diagnosis for those who opt for exams and surveillance, as well as the associated treatment costs of continuous surveillance. Many women choose to pursue surveillance until time of prophylactic surgery, conclusion of childbearing or onset of menopause. However, studies suggest that women should undergo prophylactic surgery as early as possible for greatest risk reduction and cost-effectiveness purposes (Chen, Yang et al. 2007; Yang, Caughey et al. 2011). The National Comprehensive Cancer Network current recommendations for gynecological prophylactic surgery for HNPCC women indicate that women who have completed childbearing can consider prophylactic hysterectomy and bilateral salpingo-oophorectomy (NCCN 2010).

Patients and clinicians should also be aware of the continued cancer risks even after treatment of cancer and 5-year survival period. Lynch syndrome women who survive a gynecological cancer still have the same inherent risk for colorectal cancer as they had previously. Their understanding of this perpetual risk may play a role in their decision to undergo simultaneous prophylactic gynecological and colon surgeries (Yang, Caughey et al. 2011).

Additionally, HNPCC individuals should consider screening for other HNPCC-associated cancers. The National Comprehensive Cancer Network recommendations indicate that HNPCC individuals should undergo Esophagogastroduodenoscopy (EGD) for gastric cancer screening, initiating at the age of 30-35 with follow-up every 2-3 years. Similarly, HNPCC individuals should also consider upper gastrointestinal endoscopy or capsule endoscopy for small bowel cancer at the same age and interval. An annual physical examination and urinalysis may also be considered to detect urothelial and central nervous system cancers (NCCN 2010).

Preventative therapies for colorectal cancer have also been investigated. Research indicates that the consumption of NSAID as a chemoprevention method for colorectal cancer may reduce the risk for individuals to develop polyps. However, chemoprevention medication's

effect on individuals with HNPCC appears dissimilar. Studies on chemoprevention of colorectal cancer in Lynch syndrome individuals revealed an absence of a significant reduction in the incidence of adenomas or cancer in these individuals. However, it does not rule out the possibility of minor reductions in risk (Lindor, Petersen et al. 2006; Burn, Bishop et al. 2008).

### **2.12.1 Screening in Familial Colorectal Cancer Type X**

Failure to identify a germline mutation in individuals at risk of HNPCC can result from reduced testing sensitivity, epigenetic factors or an absence of a mutation. In these cases, medical management is unclear, but the presence or absence of a strong family history should be incorporated into recommendations for the patient.

Approximately 50-60% of individuals meeting the Amsterdam I Criteria have a germline mutation identified in *MLH1* or *MSH2* (Syngal, Fox et al. 2000; Lindor, Petersen et al. 2006). Families that meet the Amsterdam I Criteria without an identified mutation are referred to as familial colorectal cancer type X. Data indicates that familial colorectal cancer type X individuals are at an increased risk for colorectal cancer compared to the general population. The guidelines regarding surveillance in familial colorectal cancer type X individuals are not well defined. Recommendations for increased surveillance, that are less aggressive than those with HNPCC, may be appropriate (Lindor, Rabe et al. 2005; Lindor, Petersen et al. 2006). Familial colorectal cancer type X at-risk family members should also be considered for increased screening. Studies indicate there is no significant evidence to suggest an increased risk for extracolonic cancers, and increased screening for these cancers is not recommended. However, it has been noted that since Amsterdam I Criteria only accounts for families with colorectal

cancers, extracolonic cancer risks of familial colorectal cancer type X family members may not be representative (Lynch and de la Chapelle 2003).

### **2.12.2 Future Treatment**

With the increasing knowledge of genomics and its role in tumor development, continuous progress on treatment methods has unfolded. Similar to genomic-centered therapies seen in BRCA mutation carriers, in whom PARP-1 inhibitors are used to induce cellular lethality in tumors, MSI tumors or MMR mutation carriers may be candidates for alternative treatment (Vilar and Gruber 2010). Additionally, drug development based on molecular subtypes is a burgeoning field in cancer treatment and may soon have implications for HNPCC individuals.

## **2.13 IMPLICATIONS FOR FAMILY MEMBERS**

It is apparent that the implications for individuals with HNPCC are considerable and identifying this high-risk group is a significant public health issue. The importance of identifying affected individuals with HNPCC is not only vital for the patient, but also for at-risk family members. If an MMR mutation has been identified in the family, relatives may choose to undergo genetic testing to determine their personal cancer risks. One study indicated that 75% of individuals meeting a clinical diagnosis of HNPCC or who underwent genetic testing for HNPCC, encouraged family members to undergo medical assessments (Ishii, Arai et al. 2011). The study

illustrates the impact that probands may have on at-risk family members seeking medical consultation, further accentuating the significance of identifying HNPCC individuals.

As HNPCC is an autosomal dominant condition, probands' siblings and children have a 50% risk for also carrying a deleterious MMR mutation. Additionally, identification of a mutation in a proband also infers that a parent is an obligate carrier. Anxiety and guilt are emotions observed in families with genetic cancer conditions and need to be addressed appropriately by health care providers. Identifying MMR mutations in families may also provide an explanation in families struggling to understand the overwhelming number of cancers in the family.

Identifying HNPCC individuals may also have implications for family planning. Prenatal genetic testing is a service typically offered as a screen for childhood conditions that are associated with severe health implications such as Cystic Fibrosis and Tay-Sachs (Strom, Ginsberg et al. 1998). However, prenatal genetic evaluation for highly penetrant, familial cancer syndromes has been investigated (Dewanwala, Chittenden et al. 2011). Preimplantation genetic diagnosis has been carried out on conditions such as Li-Fraumeni Syndrome (LFS), Neurofibromatosis (NF), Hereditary Breast and Ovarian cancer syndrome (HBOC), Familial Adenomatous Polyposis (FAP), Von-Hippel Lindau (VHL) and Lynch Syndrome (Offit, Sagi et al. 2006). However, the American Medical Association indicates that prenatal testing is most appropriate “for women or couples whose medical histories or family backgrounds indicate an elevated risk of fetal genetic disorder” (Association 1994). Nonetheless, a recent study indicated that 42% of Lynch syndrome patients would consider prenatal diagnostic testing in future pregnancies (Dewanwala, Chittenden et al. 2011). While the sample size is not large, this study also revealed the affect of Lynch Syndrome genetic testing on family planning (Dewanwala,

Chittenden et al. 2011). Approximately 20% of women with Lynch syndrome would consider childbearing at younger ages in order to pursue prophylactic surgery at an earlier age (Dewanwala, Chittenden et al. 2011). The study further suggested that health care providers should be aware of the availability of prenatal testing for Lynch Syndrome, be able to discuss testing options prior to or during pregnancy, or make appropriate referrals in order to provide the best care possible to these individuals.

### **3.0 STUDY AIMS**

Aim 1: A quality control analysis of the genetic counseling attendance for at-risk HNPCC patients and potential contributing factors.

Aim 2: Assessment of the quality of patient family history documentation for the purposes of Bethesda Criteria classification compared to family history obtained from medical record review.

Aim 3: Evaluate the impact of family history, pathological and surgical factors in identifying individuals meeting the Bethesda Criteria.

## 4.0 METHODS

Using a research protocol approved by University of Pittsburgh International Review Board (IRB0408094), participants were consented into the research study. Additionally, blinded data on patients undergoing MSI/IHC testing, those with colorectal cancer under the age of 50, and those seeing a genetic counselor in the UPMC system were also provided for study purposes. In total, 589 participants who were seen by UPMC genetic counselors, gastroenterologists or oncologists as a result of cancer diagnosis, polyps or family history, or attended the UPMC colorectal cancer high-risk clinic, or underwent MSI or IHC testing at UPMC between 2004-2009 were selected for the study. This time frame was selected to reflect a consistent protocol period between the revision of the Bethesda Criteria in 2004 and the EGAPP statement regarding testing of all colon cancer in 2009.

Participants meeting either the Bethesda criteria, Amsterdam I or Amsterdam II Criteria were selected for further evaluation. The rationale for MSI/IHC testing was determined by medical documentation review and classified by the researcher. Bethesda Criteria classification was based on medical documentation for MSI or IHC testing of the individual at the time of testing. Researchers completed secondary Bethesda Criteria classification if medical documentation revealed additional criteria met. Concurrent pathological and family history criteria were classified by priority given to the criterion leading to testing if this could be assessed chronologically.

Blinded surgical, pathological and medical records, patient history documentation, and physician or genetic counseling notes were reviewed to ascertain genetic test results, molecular tumor test results, tumor location, surgical history and personal and family cancer history. Individuals that underwent MSI/IHC testing on physicians' request due to strong family history or suspicion of Lynch syndrome, but did not meet Bethesda Criteria, were also evaluated. Families with known polyposis syndromes were excluded from data analysis as per the Amsterdam criteria. The Revised Bethesda criteria were interpreted so that only probands with colorectal cancer tumors were eligible for MSI/IHC testing. The fourth Bethesda Criteria was fulfilled if either the proband with colorectal cancer or their first-degree relative, with an HNPCC-related tumor, were diagnosed under the age of 50. Differentiation between Bethesda II criteria, metachronous and synchronous tumors, was attempted in each case in order to assess the impact of personal cancer history and surgical identification of patients.

At-risk individuals included all individuals with abnormal microsatellite instability (MSI) or immunohistochemical (IHC) analysis of MMR proteins, including both those that met and those that did not meet the Bethesda Criteria. The genetic counseling referral process for at-risk HNPCC individuals was evaluated by comparing the total number of individuals with an abnormal MSI/IHC that attended with a genetic counselor to the entire at-risk population. Genetic counseling notes were used to verify individuals that attended with a counselor. Statistical analyses were performed to compare characteristics of individuals who did or did not meet with a genetic counselor. Contingency tables and Cochran-Armitage test was used to determine differences and trends in the subject cohorts.

## 5.0 RESULTS

The total number of individuals who had IHC/MSI testing between 2004-2009 at UPMC and met one of the Bethesda Criteria was 389 (Table 1). Of the 389 individuals who met a Bethesda Criteria, a total of 79 (20.3%) patients obtained an abnormal MSI/IHC result; an additional 7 individuals displayed absent MLH1 staining on IHC testing and subsequent testing revealed that they had a *BRAF* mutation.

Eighteen individuals not meeting a Bethesda Criteria also had an abnormal MSI/IHC test result. Included in this group were individuals with endometrial cancer, polyps, other HNPCC associated cancer, as well as individuals with colorectal cancer that did not meet one of the Bethesda Criteria. The individuals in this subgroup were referred for MSI and IHC testing at the request of the treating practitioner based on suspicion for HNPCC.

Of those 227 individuals classified as meeting Bethesda Criteria I, 6 (2.65%) did not undergo MSI/IHC testing. No information was provided to explain why these individuals were tested. Of the remaining 221 individuals with Bethesda Criteria I, 14% (31) had abnormal MSI/IHC result.

**Table 1. Individuals meeting Bethesda Criteria in the study population and their classification**

	BI	BII	BIII	BIV	BV	AMS II	Total
Number of individuals	227 (58.5%)	49 (12.6%)	84 (21.6%)	5 (1.2%)	18 (4.6%)	6 (1.6%)	389

\*BI: <50y ; BII: metachronous/synchronous HNPCC tumors ; BIII: tumor histology ; BIV: CRC with 1<sup>st</sup> degree relative with HNPCC tumor, one diagnosed <50y ; BV: CRC with ≥ 2 1<sup>st</sup> or 2<sup>nd</sup> degree relatives with HNPCC tumor

Family history information was further assessed via medical record review (Table 2). In total, 60 individuals met additional personal or family history-based criteria, which were not identified upon initial consultation.

**Table 2. Individuals that met additional personal and family history Bethesda Criteria**

	BII (metachronous)	BIV	BV	AMS II	No additional personal or family history	Total # of individuals meeting another Bethesda Criteria
BI individuals	6 (2.6%)	25 (11%)	3 (1.3%)	11 (4.8%)	182 (80.3%)	45
BII individuals	0	2 (4%)	1 (2%)	4 (8%)	42 (86%)	7
BIII individuals	3 (3.5%)	0	5 (5.9%)	0	76 (90.6%)	8
Total	9	27	8	15	200	60

Data in Table 3 and Table 4 display the number of individuals eligible for genetic counseling (abnormal MSI/IHC) who saw a genetic counselor and those who underwent genetic testing, respectively. Of 97 patients with abnormal MSI/IHC, 44 (45.3%) met with a genetic counselor. Within this subset of 44 patients, 37 (84%) also had genetic testing carried out. In this cohort, there was no significant difference between the proportion of women attending genetic counseling compared to men (OR = 2.33; 95% CI: 0.96, 5.49) .

The most frequently reported reason for patients not undergoing genetic testing was a lack of insurance coverage or other financial limitations. Among the group that underwent genetic testing, testing identified 14 (37.8%) germline mutations, 6 (16.2%) variants of uncertain significance and 2 (5.4%) results were unknown. The 14 germline mutations were contained in the following loci: *MLH1* (5), *MSH2* (7), and *MSH6* (2).

**Table 3. Attendance of population with abnormal MSI/IHC results with a genetic counselor**

	Saw GC	No GC	Total
Females	33 (52.4%)	30 (47.6%)	63
Males	11 (32.4%)	23 (67.6%)	34
Total	44	53	97

\*GC = genetic counselor

\*\* Population includes both individuals meeting a Bethesda Criteria and those who did not

**Table 4. Genetic testing status of the population with abnormal MSI/IHC results who met with a counselor**

	Underwent Genetic Testing	Genetic Testing not completed	Total
Female	28 (84.84%)	5 (15.15%)	33
Male	9 (81.81%)	2 (18.18%)	11
Total	37	7	44

To investigate whether other factors might influence whether individuals attend a genetic counseling session, all individuals with abnormal MSI/IHC results and their personal and family history of cancer were assessed. In table 5, the Bethesda criteria cohort is further classified by age less than 50 years of age, pathological criteria, family history of cancer, and personal history (metachronous tumors) of cancer. Genetic counseling attendance rates of individuals with abnormal MSI/IHC results were compared and grouped by personal and family history factors versus other factors (Table 6). The classification was based on medical documentation review. The data reveals a significant difference ( $p = 0.0014$ ; OR = 4.8; 95% CI: 1.78, 12.95) between

the proportion of individuals who attended at a genetic counselor with a family or personal history compared to those with other factors.

**Table 5. Classification of Bethesda Criteria individuals with abnormal MSI/IHC after medical history review**

	<i>Females attending GC</i>	<i>Females not attending GC</i>	<b>Total</b>	<i>Males attending GC</i>	<i>Males not attending GC</i>	<b>Total</b>
Age as factor (BI)	3 (37.5%)	5 (62.5%)	<b>8</b>	2 (25%)	6 (75%)	<b>8</b>
Pathological factor (BIII)	3 (30%)	7 (70%)	<b>10</b>	1 (14.28%)	6 (85.72%)	<b>7</b>
Family History of Cancer (BIV, BV, AMS)	13 (68.4%)	6 (31.6%)	<b>19</b>	6 (54.5%)	5 (45.5%)	<b>11</b>
Personal history of HNPCC cancer (metachronous)	6 (85.72%)	1 (14.28%)	<b>7</b>	2 (40%)	3 (60%)	<b>5</b>
<b>Total</b>	<b>25</b>	<b>19</b>	<b>44</b>	<b>11</b>	<b>20</b>	<b>31</b>

\*GC = genetic counselor

**Table 6. Individuals with abnormal MSI/IHC results grouped by factor**

	Saw GC	No GC	Total
FH or PH	27 (64.3%)	15 (35.7%)	42
BI and BIII	9 (27.3%)	24 (72.7%)	33
<b>Total</b>	<b>36</b>	<b>39</b>	<b>75</b>

\* PH = Personal History; FH = Family history

Among individuals who had an abnormal tumor test and saw a genetic counselor, the average difference between tumor testing and seeing a genetic counselor was 63 days, with 83.33% of individuals seen within 90 days (3 months) of tumor testing.

Individuals with abnormal MSI/IHC testing were further evaluated for other factors that might influence genetic counseling compliance including number of at-risk family members

(Table 7) and the type of cancers reported in the family (Table 8) based on medical history review. The Cochran-Armitage test was performed to assess genetic counseling compliance was associated with (1) having no at-risk siblings or children, (2) having children or siblings at risk, or (3) having both children and siblings at risk, having, and having neither children nor siblings at risk. There was no significant difference among these groups, ( $|Z| = 0.4626$ ). However, genetic counseling compliance was associated with type of cancer ( $p = 0.0167$ ); individuals with a family history of colorectal cancers were more likely to seek genetic counseling.

**Table 7. At-risk family members of those with abnormal MSI/IHC results based on medical history review**

	Children only	Siblings only	Both	Neither	Total
Saw GC	11 (29%)	7 (18.4%)	15 (39.5%)	5 (13.1%)	38
Did not see GC	10 (20.4%)	10 (20.4%)	18 (36.75%)	11 (22.45%)	49
Total	11	17	33	16	87

**Table 8. Number of cancers reported in family history for individuals with abnormal MSI/IHC results**

	# of colorectal cancers reported	# of HNPCC associated cancers reported	# of other cancers reported	Total
Saw GC	34 (60.7%)	16 (28.6%)	6 (10.7%)	56
Did not see GC	22 (43.1%)	12 (23.5%)	17 (33.3%)	51
Total	56	28	23	107

The number of abnormal MSI/IHC results in the Bethesda Criteria population and identified by pathologists or surgeons or based on family history are presented in Table 9. Data was based on three criteria including whether patients were identified by only family history criteria (BIV, BV and metachronous HNPCC-associated cancers) or by pathological or surgical criteria (BIII and synchronous tumors). The last classification was whether individuals were identified by standard patient information documentation (BI) or possessed both pathological,

surgical or family history features, either of which would have prompted MSI/IHC testing. The data elucidates the importance of both family history and the role of pathologists and surgeons in identifying at-risk patients, as each independently identified approximately 20-25% of at-risk individuals.

**Table 9. Identification of Bethesda Criteria individuals with abnormal MSI/IHC results**

	Family History	Pathological or Surgical	Identified by both or <50 y	Total
Abnormal MSI/IHC Bethesda Population	19 (24.1%)	17 (21.5%)	43 (54.4%)	79

## 6.0 DISCUSSION

The central aim of the study was to assess the efficiency of the UPMC with referrals of at-risk HNPCC individuals to genetic counselors. Compared to a previous study of high-risk colon cancer patients, in which 30% of eligible patients attended a genetics clinic (Overbeek, Hoogerbrugge et al. 2008), the current study revealed an increased frequency of patients who attended a genetics clinic. Among 97 individuals who were eligible for genetic counseling as a result of abnormal MSI/IHC testing including both Bethesda and non-Bethesda individuals, 45.3% (n=44) met with a genetic counselor at a UPMC facility. This study possesses several inherent limitations that influence interpretation of results. The lack of patient attendance to genetics clinic may be due to various reasons, and the primary assumption is that patients received referrals from clinicians. Additionally, these results reflect a problem in tumor test result interpretation and appropriate, subsequent referrals. The low frequency of eligible individuals attending a genetics clinic in the UPMC system indicates a possible breakdown in communication between pathologists, surgeons, clinicians and patients. Thus, there is an opportunity to improve the referral process in order to identify and counsel a greater number of at-risk individuals. One factor that might influence the low frequency of individuals being counseled is that the University of Pittsburgh Medical Center does not have a defined protocol to channel at-risk colorectal cancer patients to a counseling service or to a single service location. The data collected for the study was from a cohort of individuals, seen by UPMC

gastroenterologists, oncologists, or surgeons and subsequently underwent MSI/IHC testing at a UPMC facility or attended a UPMC high-risk colorectal cancer clinic. The referral to high-risk clinics or genetic counselors was at the physician's discretion. The results of this study indicates a clear need for a more comprehensive approach to patient care in the UPMC system, in which a large scale, high-risk cancer clinic with an institutional referral process should be established.

The development of a comprehensive high-risk colorectal clinic at UPMC would unquestionably be considered a public health service. The clinic would provide the essential functions that define public health and its goals. The high-risk clinic would provide and connect patients with appropriate care. Those attending the clinic would engage in discussions and be educated about colorectal cancer, heritability, genetic testing, result implications, medical management options, and general colorectal cancer information including behavior and lifestyle recommendations. The clinic will provide patients with information on community resources, events and support groups. The clinic would be able schedule or refer patients for medical appointments, supply medical documentation for insurance providers and build relationships with patients for continued care purposes. As a result, the high-risk clinic will be an integral component of patient care within the community and UPMC system.

Another component of public health is maintaining personnel competency in order to provide a high level of quality care. It is imperative that the high-risk clinic organizes education and review sessions for staff. Updated information on colorectal cancer, hereditary polyposis and non-polyposis disease practice guidelines, guidelines, current research, disease facts, new or altered policies and community resources should be communicated regularly with involved parties. The sessions will provide opportunities for deliberation about current protocols and areas requiring further consideration or development.

Instituting a new program that provides care to a sizeable population requires the education and empowerment of the community. Therefore, the high-risk colorectal cancer clinic should consider educational interventions that will provide information to health care providers, community members, and other clinics in order to market its services and potentially identify high-risk families not referred through the UPMC system. By increasing community awareness of hereditary cancer risk and the benefits of genetic counseling, it empowers patients to further investigate their family history and independently contact the high-risk clinic. The clinic will be able to raise general awareness about colorectal cancer and screening in the community. Interventions to consider include participation in local or institutional health fairs, dissemination of informational pamphlets, develop a website and create social media outlets.

Policy development for the purposes of public health will also be fulfilled by the clinic. Institutional policies related to the consent process, tissue testing, result reporting and patient referral would result in standardized process across the system. Collaboration between representatives of the high-risk colorectal cancer clinic and UPMC policy officials should occur in order to discuss policy requirements and potential obstacles as the process would be aided by institutional buy-in. As previously discussed, a standardized UPMC referral process to the high-risk colorectal cancer clinic would permit the utilization of this important institutional resource. The comprehensive care of colorectal cancer patients in the UPMC system and subsequent policies will permit accurate monitoring and data analysis of several components of colorectal cancer and HNPCC in the region.

Other public health essential service criteria that would need to be satisfied are the identification and mobilization of stakeholders. The success of the UPMC high-risk colorectal cancer program will undeniably require partnerships between several groups of health care

practitioners. Relationships between UPMC pathologists, surgeons, clinicians, oncologists and the high-risk clinic needs to be developed for appropriate referrals of patients with family history of colon cancer, abnormal MSI/IHC results, young-age onset disease or increased number of colonic polyps.. A prohibitive upstream factor of attendance with genetic counselors may be access to services. By establishing supplemental satellite clinics or teleconferencing sessions in western Pennsylvania, access to genetic counselors for patients that are farther removed would increase. Formulating affiliations with other high-risk cancer clinics in the UPMC system will be integral to comprehensive care of patients at-risk for HNPCC. Current recommendations for gynecological tumor testing for HNPCC requires excellent communication and involvement of gynecological clinics and practices within the UPMC system. Additionally, formalized interactions with laboratory technicians and directors may need to be considered. UPMC labs are accredited by Clinical Laboratory Improvement Amendments (CLIA), therefore, processing genetic testing for HNPCC on-site could significantly reduce the cost of testing for patients.

Stakeholders not directly involved in patient care will also need to be mobilized to address other factors and improve the efficacy of the high-risk program. Genetic testing costs may preclude individuals from attending clinics or undergoing genetic testing. Collaboration with insurance companies, community and support groups, as well as local or organizational grants may subsidize costs of genetic testing. Additionally, the implementation of the high-risk program would result in greater numbers of individuals being apprised of genetic testing options and potentially electing for testing. It would be prudent to discuss the opportunity for economic growth with genetic testing companies in order to potentially drive down testing costs.

As outlined, many components of the program innately fulfill the public health service requirements, while some still need to be developed. Nonetheless, as a public health service, the high-risk colorectal cancer program will be a tremendous addition to the UPMC system and the surrounding community.

Organizing a restructured comprehensive UPMC protocol for at-risk cancer patients may aid health care practitioners. Placing the responsibility of genetic counseling service referral on a single care provider may be overwhelming and laborious and reduce efficiency of the process. Because numerous health professionals are accountable for the care of a patient, members of this team should share the responsibility in indentifying appropriate candidates for additional testing and referrals. Therefore, one approach would be to bestow on pathologists a greater responsibility in the referral process. As the knowledge and association between molecular diagnostics and genetics continues to unfold, the role of pathologists in the care of patients will undoubtedly increase. The role of pathological markers in hereditary colon cancer is well documented and the importance of pathological markers has also been illustrated in other fields as well. For example, studies have shown that specific morphological features of breast cancer tissue are more likely to be present BRCA1 carriers versus non-carriers. Therefore, the presence of these features in a patient has been proposed as biomarker for increased risk and a recommendation for clinicians and pathologists to refer the patient for genetic testing (Gadzicki, Schubert et al. 2009). Likewise, metabolic storage disorders and hemoglobinopathies identified by pathology studies of placental tissue are examples of diagnoses initiated by pathologists. The involvement of pathologists in identifying at-risk individuals is not only a method of early detection of genetic diseases, but also has implications for genetic counseling and future pregnancies (Oppitz, Klee et al. 2009; Staretz-Chacham, Lang et al. 2009). Therefore, in order to

improve the rate of referrals to genetic counselors, the roles of pathologists in disease recognition should be better utilized.

One way to coordinate a functional, efficient method of communication between current and future health care providers could include a process whereby the notification of abnormal MSI/IHC test results is directly reported to genetic counselors from the pathologists. This strategy would allow counselors to engage in discussions with patients, facilitate appointments, clarify test results, and address other pertinent information if necessary. A previous study assessing patients that were contacted regarding genetic counseling services by mail had a response rate of 26% (Keller, Jost et al. 2004). Although, the low response rate suggests that this method of contact is less than ideal, it may be utilized as a supplement to the current system to increase the number of high-risk patients attending genetic counseling. An automated computer system could be configured to notify genetic counselors of abnormal test result. The process could address some of the obstacles of referral by reducing the workload of pathologists and treating practitioners without a loss of information delivery. The counselor-initiated process also avoids the potential constraint of relying on an intermediary source to recognize the need for and broach the issue of genetic counseling. However, the importance of conversations between patients and treating practitioners should not be overlooked as data indicates that patients whose treating practitioners discussed referrals to a genetics clinic attended more frequently (Overbeek, Hoogerbrugge et al. 2008). Therefore, it is necessary that both genetic counselors and clinicians play an active role in the process of patient referral and attendance at a genetics clinic.

If a counselor-initiated method were implemented, there are several issues to consider. Introducing the issue of genetic referrals or discussing genetic test result implications with individuals during the time surrounding cancer diagnosis may be overwhelming for both the

patient and family. As genetic testing may have implications for surgical decisions, counselors must balance attempting to contact patients prior to surgery and respect for patients' right to privacy during a difficult time. Genetic counselors that make contact with patients need to have a heightened sensitivity for patient emotions and be prepared for a variety of responses. As every patient will cope with the situation differently, genetic counselors' attuned skills for assessing patients' signals will allow them to determine appropriate questions to ask, issues to probe and gauge whether patients are emotionally ready for a detailed discussion. For these and many other reasons, genetic counselors are well equipped to reach out to these at-risk patients during this emotional time.

It is imperative that alterations to the informed consent process occur if counselors are the ones contacting patients. The patients must be informed and consent to the disclosure of information to other appropriate health care practitioners, which would require patient sanctioned communication between pathologists, health care and genetic service providers. The concept has been noted in a previous study, where it was recommended that colorectal cancer patients seen in clinic for treatment or diagnosis should be briefly counseled on the heritability of colorectal cancer and that testing on the tissue will be carried out in order to determine the possibility of this risk (Chubak, Heald et al. 2011). The authors further elaborate that patients be notified that they will be contacted by a genetic counselor or other health care practitioner to discuss their results, define familiar risk or make appropriate referrals (Chubak, Heald et al. 2011). Informed consent and written authorization to share patient information with other health care providers would obviate violations of HIPAA laws or other confidentiality issues. Moreover, informed consent and pre-test counseling may influence genetics clinic attendance. Studies have revealed that educational materials and decision aids, including computer-based resources, provided to at-

risk patients prior to MSI testing improved patients' comprehension of tests implications for themselves and family members. The material also increased patients' preparedness in making informed decisions about MSI testing (Manne, Meropol et al. 2010). Therefore, provision of education materials or appropriate counseling prior to MSI testing may increase the proportion of patients attending a genetic counseling appointment if indicated.

Another benefit to the adjusted consent process would allow for counselors to call to discuss implications of negative test results. Although obtaining a negative MSI/IHC result reduces the likelihood of the presence of a hereditary cancer syndrome, close family members of affected individuals are at an increased risk to develop the disease compared to those in the general population without a family history. Grady et al (2003) report that individuals with a first degree relative with colon cancer are twice as likely to develop colon cancer compared to individuals without a family history of colon cancer. The risk would become more elevated for those with more than one affected close family member. The current study indicates that just over 10% of individuals diagnosed prior to age 50, had a close family member diagnosed with colorectal cancer or an HNPCC associated cancer. The American Cancer Society has outlined screening guidelines for all individuals with a family history of colon cancer, that also would be pertinent for family members of at-risk individuals whose tumor tests results are not abnormal. Although the time required to properly communicate all the pertinent information may not be feasible, providing supplementary information in the counseling session or through educational material, as mentioned previously, is not unreasonable. Thus, discussions with counselors, provision of educational materials to patients and families on sporadic, hereditary, and familial cancers prior to or after testing, may serve to promote family members to seek earlier cancer surveillance because of their increased risk compared to the general population.

A standardized process utilizing automated computer software would benefit system resources and it may have additional clinical applications as well. As the utilization of electronic health information systems evolves and becomes more prevalent, interventions founded on the technology could be implemented to benefit the quality of treatment patients are provided. An electronic notification system would be a practical application of the technology to signal physicians on a variety of facets of patient care including abnormal test results, clinical diagnoses, research eligibility, referrals, general inquiries or topics for discussion. For the purposes of identifying HNPCC individuals, the software program could notify health care providers of individuals meeting a criteria for MSI/IHC testing, a clinical diagnosis of Amsterdam I, and those requiring a referral to a genetics clinic. Previous studies have investigated the utilization of an electronic alert system for notifying physicians of clinical trial availability based on electronic patient health information. The study reported a significant increase in patient referral by physicians that responded to the electronic alert and also found that 85% of those physicians were amenable to the notification process. Additionally, even the majority (66%) of those physicians who did not utilize the alert, appreciated the electronic reminder (Embi, Jain et al. 2008). The study demonstrated that the electronic reminder system is an effective tool for increasing the number of physician-referred patients to clinical trials, and the concept should be readily translatable to genetic services. The study further elaborated that the limitations of the process to include the presence of a functional electronic health records system, shortcomings of the notification, as well as physician acceptance and utilization.

The current study also assessed the time interval between tumor testing and attendance with a genetic counselor. The data revealed that individuals who attended with genetic counselors were seen on average within two months of tumor testing, with over 80% of

individuals seen within three months. The data suggests that it is imperative to begin the process of referring to a genetic counselor soon after testing. In order to accomplish this, a potential plan could include having genetic counselors present or available when treating practitioners disclose abnormal MSI/IHC test results, so that patients could be seen during the same appointment. Patients may be psychologically unprepared for immediate conversations about hereditary factors of their cancer or time restrictions may preclude additional appointments; however, counselors can be available during subsequent follow-up visits. Unfortunately, the busy clinic schedules of genetic counselors and the variety of medical locations where patients are seen for test result disclosure are major obstacles to the “stand-by” genetic counseling concept. However, by centralizing genetic counseling resources to a high-risk clinic and establishing a regulated process of patient referral or recruitment to the clinic could improve the proportion of at-risk individuals seen by genetic counselors within the UPMC system.

Understandably, enhancing the referral process may not necessarily correlate to a significant improvement in attendance to a genetic counseling service as a lack of patient compliance to medical recommendations is an obvious impediment in attaining the goal of complete attendance. Patients have several reservations regarding genetics and genetic testing that may preclude them from seeking genetic counseling. These reservations include a lack of knowledge about genetic testing and counseling services, concerns regarding genetic discrimination, and concerns regarding autonomy and handling of personal and genetic information (Martin, Greenwood et al. 2010). Availability of resources also play a role because limited access or insufficient financial support for genetic services can influence an individual from obtaining applicable and appropriately timed services. As observed in the current study, financial limitation was the most reported factor for not undergoing genetic testing. Distress

regarding familial implications and disclosing information to close family members, including children, has also been frequently documented as reasons for skepticism regarding seeking genetic services (Martin, Greenwood et al. 2010).

Another objective of the study was an analysis of the family history collection of UPMC practitioners. The data elucidates an opportunity for improvement in its attainment as 60 individuals met an additional Bethesda Criteria with a more personal or family history criteria component when medical history documentation was reviewed. Specifically, nearly 5% and 10% of individuals meeting Bethesda I and Bethesda II Criteria, respectively, met Amsterdam II Criteria after review of medical history documentation. Currently, there are no clinical guidelines for individuals who meet the Amsterdam II Criteria, however, with more detailed family history collection, an increase in the identification and follow-up of families meeting the Amsterdam II Criteria may result in more accurate risk estimates and potential clinical screening guidelines for this population. Additionally, improved family histories may result in more compelling genotype-phenotype associations between specific Bethesda Guidelines or family history criteria and germline MMR mutations, which may eventually initiate discussions campaigning for direct DNA analysis or focused gene-specific testing on certain individuals as a way to increase cost-efficiency.

The shortcoming of extensive family history attainment has notable clinical impacts on other individuals. Failure to identify Amsterdam I criteria family members is a crucial misstep from a medical management standpoint, as these families, given an abnormal MSI/IHC result, would be provided altered medical management opportunities. Familial colorectal cancer type X families would be considered for increased screening for colon cancer and therefore, the clinical diagnosis of Amsterdam I Criteria families is an important step for clinicians to undertake for

patient care purposes irrespective of genetic test results. Amsterdam I Criteria patients that do not receive genetic counseling referrals, or do not attend genetics appointments or choose not to have genetic testing should still be engaged in conversations about alternative medical management options. As previously discussed, family history has clinical implications for individuals of non-HNPCC family members affected with colorectal cancer therefore, improved cancer history collection may be critical for screening recommendations of unaffected family members.

As individuals with a family history were more likely to attend a genetics clinic, the data also identifies a group that requires additional attention. Individuals with a lack of family history may perceive themselves at a lower risk as seen in the relatively low number of individuals (31%) who met only the Bethesda I Criteria attending at a genetic counseling appointment. However, this study reveals that when medical documentation is further investigated approximately 17% of individuals under the age of 50 met other family history-based Bethesda criteria. Efforts should be made to engage this group more intensely in an attempt to stress features about hereditary cancer syndrome that may be important for the patient and other family members. Characteristics that reduce cancer prominence in a family, including small families, reduced penetrance of the disease, early ages of death, may be important to discuss with this population. Likewise, the issue of reduced cancer penetrance is critical for treating practitioners as well, especially when obtaining a detailed family history. Among the issues previously mentioned, one that can be overlooked in familial cancer syndrome families, specifically HNPCC, is prophylactic surgeries. Risk reducing procedures can lead to the perception of lower risk by both health care providers and patients. It is important for health care providers to inquire about surgeries when obtaining family histories.

Another important reason for obtaining detailed family histories for individuals at risk for HNPCC, is that by obtaining the information it may guide health practitioners towards other differential diagnoses and genetic testing. Alternative colon cancer syndromes are identified through marked presence of polyps, however attenuated polyposis syndromes may be difficult to differentiate from HNPCC. It has been documented that 6.1% and 2.8% of individuals that are diagnosed with colon cancer below the age of 50 and 55, respectively, have been identified to have MYH-Associated Polyposis (Fleischmann, Peto et al. 2004; Balaguer, Castellvi-Bel et al. 2007). The former is important as these individuals meet the Bethesda I Criteria for MSI testing. Therefore, individuals with a lack of family history or those with a recessive inheritance pattern should be considered for MAP. Additionally, for the purposes of identifying differential cancer diagnoses, health care practitioners should be educated on characteristics other than cancer to investigate within families. Inquiries regarding features including skin manifestations, such as those observed in the HNPCC variant Muir-Torre or in Cowden Syndrome, may reveal information that would influence or focus patient care, testing and referrals, resulting in a more cost-effective process. Additionally, more detailed family histories may help to identify other non-cancer syndromes in the family that may require discussion.

Although the current EGAPP recommendations may influence the importance of family history taking for identifying at-risk HNPCC individuals affected with colorectal cancer, the study data reveals that family history can still play a role in various components of patient care. Our data suggests that individuals who have a strong personal or family history of cancer were significantly more likely to attend genetic counseling sessions if they were found to have abnormal tissue testing. The elevated compliance in individuals with a stronger family history is a reasonable finding and has been seen in other cancer syndrome studies (Gadzicki, Schubert et

al. 2009). Unfortunately, as family histories were obtained and pieced together through a review of medical history documentation, the exact influence of obtaining family history is unclear. However, one could surmise that obtaining a more detailed family history with associated discussions may serve to better enlighten patients on the potential hereditary factors of similar and associated cancers, thus increasing patient compliance with referrals to genetic counselors. Therefore, the collection of a more detailed family history is vital for comprehensive patient care and reduced patient and institutional costs.

Other potential influencing factors of individuals who attended genetic counselors were assessed including the presence of non-colorectal cancers in family history and the presence of at-risk family members. The presence of colorectal cancer, HNPCC-associated cancers and non-HNPCC cancers were assessed in order to determine their impact on genetic counseling attendance. The data suggests that the prevalence of colorectal cancer in families compared to those with non-HNPCC associated cancers may influence the likelihood of attending with a genetic counselor. While the data indicates that individuals with a stronger personal or family history were more likely to attend genetic counseling appointments, reduced genetic literacy may play a vital role in the perception of risk in families with non-HNPCC related cancer history. Families may not be apprised of the association of specific cancers in the family due to the prevalence of numerous disparate types of cancer. Genetic counselors are skilled at filtering through extensive family cancer histories with patients and discussing relatedness of certain cancers in the family to explain risk for hereditary syndromes. As such, front-line health practitioners may need to address diverse and substantial family cancer histories by promoting the ability of genetic counselors to dissect it. Although the data does not support the idea, a similar effect could potentially be seen in families with extra-colonic HNPCC-associated

cancers. Families may recognize the relationship between similar cancers in a family and the potential for a hereditary syndrome, but the presence of cancer in various, seemingly unconnected organs may not be as apparent to the population. Therefore, education of individuals with family history of extra-colonic HNPCC associated cancers and the relatedness of those cancers due to a hereditary syndrome may be integral to their perception of risk and, thus, may influence their likelihood to attend at a genetics clinic. Further research should address determining whether the presence of a strong family history of cancer alone is the driving element in attendance with a counselor, or whether the protraction of the family history and discussion of relatedness of cancers is the motivating factor for patient compliance with genetic referral.

Another factor that was considered for influencing patient attendance at a genetic counseling appointment was the presence of at-risk family members. Medical documentation was reviewed to determine which individuals had siblings and children that may be affected by genetic testing results. When grouped for at-risk family members, the data did not reveal any trends in genetic counseling attendance rates between those with no at-risk siblings or children, those with both at-risk siblings and children, and those with either siblings or children at risk. The data appears to undermine the concept that individuals are motivated to undergo testing to help at-risk family members, however the small sample size may have impacted these results. However, the number of at-risk family members was not assessed in this study and this may prove to be significant for influencing attendance with a genetic counselor.

An additional aim of the research was to reveal the methods of identification of at-risk HNPCC individuals. The identification of an individual with HNPCC requires the contribution of numerous health care practitioners at a multitude of checkpoints along the way. Primary care

providers, surgeons, oncologists, pathologists, and geneticists all play a role in the care, management, treatment and identification on an individual with HNPCC. One of the goals of the study was to assess the importance of both pathological and family history factors in identifying at-risk individuals. This was accomplished by categorizing the method of identification into solely pathologically or surgically based, family history based, patient intake information or a combination of multiple factors. The data reveals that pathologists and clinicians independently identified approximately 20-25% of individuals meeting a Bethesda Criteria, who would have otherwise gone unrecognized. The data emphasizes the importance of both pathologists and clinicians, separately and as a health care team. The data suggests that the presence and role of both groups providers are integral, and the absence of either party would result in a reduction in the effectiveness of the process. However, given that the current guidelines indicate that all colorectal cancer tissues undergo MSI or IHC testing, the responsibility of both groups to identify at-risk individuals has consequently been altered. The benefits of the change in protocol will noticeably include the recognition of HNPCC individuals that are missed by the Bethesda Criteria. Conversely, the limitations may not be as conspicuous. An increased number of tumors undergoing molecular testing will put pressure on health professionals carrying out these tests. Additionally, the role of family history in affected individuals has shifted away from identifying at-risk individuals and transformed into having a greater emphasis on other aspects of patient care including potentially influencing compliance with genetic counseling referrals, as discussed above.

## **7.0 STATE-WIDE REFERRAL SYSTEM**

The implementation and success of the high-risk colorectal cancer program in Pittsburgh may lead to greater downstream effects. The cost-effectiveness of colorectal tumor screening in identifying HNPCC individuals on a local level should facilitate recommendations for population screening program funded at a state and, potentially, federal level. Additionally, the program is an ideal opportunity to implement genetic and genomics knowledge to improve health care and health policy.

As discussed previously regarding the high-risk clinic at UPMC, colorectal cancer screening fulfills the essential public health functions for many similar reasons. A large-scale tumor screening and referral program would necessitate assessment of policy development and quality assurance for colorectal cancer and HNPCC screening. Similar to other screening programs, the testing of colorectal cancer tumors will provide comprehensive societal information on disease prevalence of colorectal cancer and HNPCC. The increase in testing, especially in underserved populations, will produce an abundance of information on variants of uncertain significance, cancer risks and prevalence in these communities. Additionally, the information obtained on such a large scale will provide insight on the efficacy of the screening and community education programs and other interventions on reducing the incidence or effect of colorectal cancer in the population.

Genetic testing interventions for the purposes of clinical care are meticulously scrutinized for several key components. With regards to HNPCC testing, the analytic validity, clinical validity, clinical utility, and diagnostic testing has proven to be of high quality (I.O.M. 2010). As such, it seems appropriate to juxtapose the proposed screening program to other genomic screening programs, most notably, the Newborn Screening (NBS) program. With the inception of the NBS, a set of screening criteria drafted by Wilson and Jungner outlined determinants of a disease that should be met to be included in the screening program (Wilson and Jungner 1968). The criteria included: the presence of a pertinent health issue, accepted treatment for the diseased, available diagnostic services, facilities to receive treatment, comprehension of the natural history of the disease, early or pre-symptomatic stages of the disease and testing that is accepted by society. Colorectal tumor testing meets the majority of these criteria for population screening of HNPCC. The significant prevalence of colorectal cancer in the United States, and considerable proportion deriving from HNPCC, as well as the increased risk for other HNPCC associated cancers makes it an important health issue. The reduction in mortality and morbidity from supplemental medical management options such as increased screening and prophylactic surgery for HNPCC can be interpreted as treatment for diseased individuals. Additionally, the aforementioned medical procedures, as well as tumor testing and genetic testing, are available throughout the state and screening can lead to identification of pre-symptomatic mutation carriers who are eligible for similar services. Furthermore, extensive HNPCC research has broadened the knowledge surrounding disease characteristics and progression. Currently, colorectal tumor testing is already carried out on select individuals and is widely accepted as few individuals decline testing after consent.

However, the aforementioned benefits require an implementation protocol for testing as well as referral to genetic counseling. One of the most salient issues for the effectiveness of population colorectal tumor testing is that the program will target unaffected HNPCC family members. Increased screening and surgery can reduce cancer sequelae for affected individuals; however, the greatest impact will be observed in screening family members for disease prevention. A previous study of unselected colorectal cancer patients found that those with HNPCC had an average of greater than three relatives diagnosed with the mutation (Hampel, Frankel et al. 2008). The value of the program is greatly compromised if affected individuals are not referred for counseling and if at-risk family members are not informed about the potential for a hereditary cancer risk. However, there are several ethical dilemmas with communicating risk to third-party family members by health care practitioners, such as patient confidentiality and duty to warn. Discussions with a genetic counselor can mitigate some of these concerns. Furthermore studies have shown that after a genetic counseling session, there is a cascade effect in HNPCC families where five to six relatives per proband subsequently undergo genetic testing (Hampel, Frankel et al. 2005). Therefore, standardized consent approaches and rigorous education programs should be developed to emphasize the implications of tumor testing for family members to improve communication in families. Moreover, a screening program run at a state level may be able to circumvent the fragmentation of the health care system. Irrespective of service provider, affected individuals and their at-risk family members would obtain tumor screening and referrals for genetic counseling services.

The effectiveness of the program requires reaching as many at-risk individuals as possible. MSI testing has the potential to affect a greater proportion of individuals as part of a population-screening program. MSI testing has various, potential applications, while IHC testing

is only useful to identify non-functioning MMR proteins for germline testing. However, for a large-scale program, IHC testing is more cost-effective due to its wide accessibility and resultant targeted genetic testing. Therefore, opt-in and opt-out policies for MSI and IHC testing could be considered as an alternative. Those opting out of testing reduce the effectiveness of the screening program, however, the infringement on patients' rights are potentially avoided.

To determine cost-effectiveness of a screening program, Mvundura et al (2010) compared different screening strategies for HNPCC on a population-wide and age-specific level, and also considered all associated costs and risks. Using relatively conservative values for HNPCC family members that would be contacted as a result of screening program, they calculated the number of life years (LY) saved. The data was used to determine the incremental cost-effectiveness ratios (ICER), total cost per LY. The study revealed that population screening utilizing a preliminary test, MSI or IHC, yielded ICER ranges within accepted values for implementation. The most cost-effective method of IHC testing, BRAF testing and subsequent germline sequencing yielded values closer to that of general population screening for colorectal cancer in those 50 years of age and older. However, the study did not include the cost-effectiveness of screening other HNPCC-associated cancers or prophylactic surgery, which may have further improved the numbers. The study did not evaluate the cost-effectiveness of implementing both MSI and IHC testing. However, one would surmise that testing for both MSI and IHC would decrease the effectiveness of the program due to the increased costs associated with performing both tests (Mvundura, Grosse et al. 2010).

The cost-effectiveness of the population-screening program has been further corroborated from a provider's perspective. The study also supported the IHC preliminary testing in order to maximize efficiency (2010). Additionally, the study indicated that although subsidized genetic

testing of the proband is costly, there is potential for financial benefit. As the overlying goal is to reduce morbidity and mortality in unaffected HNPCC individuals, genetic testing of affected individuals is essential to identifying familial mutations. Subsequent single-site testing for family members is much more affordable and would cause an influx of patients into the health system. Increases in screening frequencies of unaffected individuals would also be profitable. Additionally, while screening is currently limited to colon tumor, there is potential for a similar screening program expanding into gynecological tumors.

A screening and referral program on a state level has the opportunity to benefit a variety of involved parties from both a financial and health perspective. Imminent developments in genetic technology make it even more essential to develop and establish a screening and referral process on a large scale. This intervention can serve as a foundation or model for similar genetic programs.

## 8.0 STUDY LIMITATIONS

There are several marked shortcomings of the current study that will be elaborated upon, however, the general composition of the study population appears comparable to other populations. Approximately 20% of individuals meeting Bethesda criteria in our study had an abnormal MSI/IHC, which is similar to a larger study conducted on 1,721 individuals meeting the Bethesda Criteria where 23.6% of tumors displayed microsatellite instability (Mangold, Pagenstecher et al. 2005). The obvious shortcoming of the study is that the analysis of the data was based on review of provided medical documentation. The completeness of the data obtained affects the accuracy of several aspects of study including clinician referrals, molecular and genetic testing completed, personal and family surgical history and personal and family cancer history. Consequently, the comprehensiveness of documents was a considerable limiting factor. Moreover, medical information on the number of children in the family was easier to ascertain for women within the study compared to men. Additionally, many women are known to be the gatekeeper with regards to family medical information, which may have resulted in stronger and more detailed family histories compared to men. Moreover, medical reports obtained and reviewed were restricted to those found in the UPMC system. As a result, individuals who reported more detailed family histories, sought follow-up genetic counseling services or genetic testing outside the UPMC system would result in an underrepresentation of the data. Limited patient information restricted the geographical origin of patients, precluding inferences about the

number of out of the area patients that may have been assessed elsewhere. Similarly, reduced patient adherence to physician recommendations may have played a role in the study and attributed to the reduced number of individuals seeking genetic counseling services. Data could not differentiate between those referred for genetic counseling but did not attend for unknown reasons, and those that did not receive a referral at all. Reasons for not seeking genetic counseling services could not be ascertained from the data, however, explanations may include apprehension for genetic counseling services, reservations about costs, feelings of guilt regarding familial risks, and limited access to genetic counseling services.

As previously discussed, in the absence of corroborating medical information on family members, patient recall of family cancer history is a major obstacle in accurate risk assessment. Underreporting of cancer diagnosis within a family may result from communication breakdowns due to family dynamics or because of geographical separation. Other factors may also influence the type of cancer reported in families including a lack of literacy on the disease. The sinister feature of cancer is its ability to spread, and therefore, it can affect a profound number of tissue sites other than the site of origin. The affected tissue that ultimately leads to the demise of an individual may be the type of cancer reported by family members resulting in misguided family history documentation. Social networking sites have allowed families to connect with one another from across the globe. With the availability of information sharing and family history applications on these networking sites, an improvement of the accuracy and specificity of family medical history is inevitable, although health practitioners will still need to confirm many of reported diagnoses. An evolution in family history is taking place and we as health practitioners need to embrace and utilize it for improved patient care.

Another potential limitation of the evaluation was that the study cohort represents a selection bias as a proportion of the individuals were assessed at the High-Risk colorectal cancer clinic, whose focus on the recognition of hereditary colon cancer syndromes may have led to more detailed family history documentation and identification of at-risk individuals. Physician prompted questioning and clinical environment may have influenced the accuracy of family history reporting. Individuals in attending a high-risk genetics clinic discussing the potential hereditary factors of cancer may be more inclined to report similar cancers within the family. Additionally, health practitioners' probing for genetically associated cancers may ask about specific cancers which in turn could lead to individuals over reporting these syndrome associated cancers. Furthermore, patients assessed at a high-risk genetics clinic or by UPMC clinicians with special interest in hereditary cancer syndromes would be more cognizant of strong family history, abnormal test results and their possible implications. As a result, patients of these informed health care practitioners may be more likely to obtain referrals to genetics counseling services.

Technological shortcomings are also observed in the study as the majority of patients that had immunohistochemistry testing, had MLH1, MSH2 and MSH6 proteins assessed. Therefore, as staining for PMS2 was not applied to these patients, pathogenic mutations in *PMS2* gene may have been neglected. Studies indicate that approximately less than 4.3% of microsatellite unstable tumors display absent PMS2 proteins, without simultaneous absence of other MMR proteins (Senter, Clendenning et al. 2008). Additionally, a smaller number of individuals that had IHC evaluation were only tested for MLH1 and MSH2, thus overlooking the potential to identify MSH6 dysfunctional proteins. As previously discussed, a proposed initiative to move towards a

two-antibody system of MSH6 and PMS2, not only for cost effectiveness purposes, but also to capture mutations in these less prevalent MMR genes, may remedy this issue in future studies.

## **9.0 CONCLUSIONS**

The current study reveals there is an opportunity for the UPMC system to improve the genetic counseling process of individuals at risk for HNPCC. Suggestions have been outlined that could potentially address some of the current deficiency in the UPMC system. Alterations in referral protocols, clinical policies, and use of technologies may prove to have an impact on the issue. However, study suggests that the overall fragmentation of the health care system needs to be addressed by developing a single clinical pathway for at-risk patients to be managed.

Several factors have been identified as potentially influencing the attendance of at-risk HNPCC individuals with a genetic counselor. The information uncovered in the study can be utilized by health practitioners to effectively discuss genetic counseling referrals with patients and address possible barriers to compliance.

**APPENDIX : IRB APPROVED CONSENT**



**University Pittsburgh  
Physicians**

**CONSENT TO ACT AS A PARTICIPANT IN A RESEARCH REGISTRY**

**TITLE:** Hereditary Colorectal Tumor Registry (UPCI 04-112) Consent Version Date 08-18-11

**PRINCIPAL INVESTIGATOR:** Randall E. Brand, MD  
Hillman Cancer Center  
5117 Centre Avenue, Suite 2.32/Research Side  
Pittsburgh, PA 15213  
Phone: 412-623-0021  
[brander@upmc.edu](mailto:brander@upmc.edu)

**CO-INVESTIGATORS:**

<p>Andrew Sardella, BS UPMC Shadyside Shadyside Medical Bldg. 5320 Centre Ave. Pittsburgh, PA 15232 <a href="mailto:sardellaa@upmc.edu">sardellaa@upmc.edu</a></p>	<p>Lin Zhang, PhD. Assistant Professor of Pharmacology and Medicine Hillman Cancer Center 5117 Centre Avenue, Room L211.03 Pittsburgh, PA 15232 Phone: 412-623-1009 Fax: 412-623-7778 <a href="mailto:zhanglx@upmc.edu">zhanglx@upmc.edu</a></p>	<p>Darcy Thull MS, CGC, Genetic Counselors Magee Womens Hospital 300 Halket Street Pittsburgh, PA 15213 Phone: 412-641-1466 <a href="mailto:dthull@mail.magee.edu">dthull@mail.magee.edu</a></p>
<p>Christina M. Chimera, MS, CGC Ruth E. Dudley, MS Elena Infante, MS Kelly Knickelbein, MS Genetic Counselors UPMC Cancer Genetics Program Magee-Womens Hospital 300 Halket Street, Rm 3522 Pittsburgh, PA 15213</p>	<p>Andrew Baum, PhD Professor, University of Pittsburgh School of Medicine Iroquois Building, Room 405 Pittsburgh, PA 15213 Phone: 412-624-4890 <a href="mailto:baum@pitt.edu">baum@pitt.edu</a></p>	<p>Jian Yu, PhD University of Pittsburgh Cancer Institute, UPCI Research Pavilion 5117 Centre Avenue, Room 2.26h Pittsburgh, PA 15232 Phone 412-623-7786 Fax 412-623-7778 <a href="mailto:Yuj2@upmc.edu">Yuj2@upmc.edu</a></p>

<p>Joseph Kelley, MD  OB/GYN Oncology  Magee Womens Hospital  300 Halket Street  Pittsburgh, PA 15213  Phone: 412-641-5411  <a href="mailto:jkelly@upmc.edu">jkelly@upmc.edu</a></p>	<p>James F. Pingpank, MD  University of Pittsburgh Cancer Institute  UPMC Cancer Pavilion  5150 Centre Avenue, Rm 406  Pittsburgh, PA 15232  (412) 692-2852  <a href="mailto:pingpankjf@upmc.edu">pingpankjf@upmc.edu</a></p>	<p>James Celebrezze, Jr, MD  University of Pittsburgh Physicians Division of Colon &amp; Rectal Surgery  UPMC Cancer Center at UPMC Passavant  9100 Babcock Blvd.  Pittsburgh, PA 15237  Phone: 877-684-7189  <a href="mailto:celebrezzejp@upmc.edu">celebrezzejp@upmc.edu</a></p>
<p>Alyssa Krasinskas  UPMC Cancer Center  UPMC Presbyterian  200 Lothrop Street  Pittsburgh, PA 15213  (412) 647-3600  <a href="mailto:krasam@upmc.edu">krasam@upmc.edu</a></p>	<p>David Bartlett, MD  University of Pittsburgh Cancer Institute  Division of Surgical Oncology  UPMC Cancer Pavilion  5150 Centre Avenue  Pittsburgh, PA 15232  (412) 692-2852  <a href="mailto:bartdl@upmc.edu">bartdl@upmc.edu</a></p>	<p>Sheila Solomon, MS  Genetic Counselor  UPCI Research Pavilion  Suite 2.3  5117 Centre Ave.  Pittsburgh, PA 15232  Phone: 412-623-4124  Fax: 412-623-7828  <a href="mailto:solomons@upmc.edu">solomons@upmc.edu</a></p>
<p>Matthew Holtzman, MD  University of Pittsburgh Cancer Institute  Division of Surgical Oncology  UPMC Cancer Pavilion  5150 Centre Avenue  Pittsburgh, PA 15232  (412) 692-2852  <a href="mailto:holtzmanmp@upmc.edu">holtzmanmp@upmc.edu</a></p>	<p>UPMC Cancer Centers Physicians and staff</p> <p>Complete listing available on request</p>	

**SOURCE OF SUPPORT:** UPMC University of Pittsburgh Physicians, Department of Surgery

**What is the purpose of this Research Registry?**

Many advancements in medicine have resulted from research involving the collection and analysis of the medical record information of patients with a certain disease or condition. Because you are being seen by the University of Pittsburgh Medical Center Hereditary Colorectal Tumor Program Center (UPMC-HCRTP), we are asking for your permission to allow

	<p>University Of Pittsburgh  Institutional Review Board</p>	<p>Approval Date: 8/22/2011  Renewal Date: 3/2/2012</p>	<p>IRB #: IRB0408094</p>
---	---	---	--------------------------

us to place your past, current and future medical record information into the Hereditary Colorectal Tumor Registry (HCRTR)

By placing the medical record information of many patients such as you into the HCRTR, researchers will be able to conduct research studies directed at increasing our knowledge about Genetic diseases related to cancer.

It is anticipated that the HCRTR will assist our investigators in two important ways.

First, it will allow researchers to review and study the medical records of many individuals to answer questions about your disease and its treatment.

Second, it will help researchers identify and recruit patients who are eligible for participation in future research studies. For example, physicians and other researchers associated with the UPMC Center for Genetic Disease are also frequently involved in research studies directed at evaluating the safety and effectiveness of drugs, devices or procedures for the treatment of HNPCC (Hereditary Nonpolyposis Colorectal Cancer) related to genetic disease. If you agree to participate in the HCRTR, physicians and researchers will be able to review your medical record information to determine if you might qualify for various future research studies. Please note a separate consent will be required from you to participate in any other studies.

**Who is being asked to participate in this Research Registry?**

You are being asked to participate because you are seeking treatment or are being treated at the Hereditary Colorectal Tumor Program because of your early onset colorectal cancer diagnosis. This is voluntary and you are not obligated to participate in future studies. Your first degree relatives are also being asked to participate in the Hereditary Colorectal Tumor Registry. Adults who are  $\geq 18$  years diagnosed with colorectal cancer before the age of 50 are being asked to participate. This registry will accrue an indefinite number of participants.

**What will my participation in this Research Registry involve?**

If you agree to participate in the Hereditary Colorectal Tumor Registry, your past, current and future medical record information will be placed into the Research Registry. This will permit research studies to be conducted on the medical record information contained within the registry. You are being asked to allow us to contact you if one of our researchers determines, through review of your medical record information contained in the Hereditary Colorectal Tumor Registry that you are eligible for participation in a future research study directed at the study of Genetic disease. Please note that if you qualify for any future research studies, you will be asked to sign a separate consent form that outlines in detail the nature of that research study, including its potential risks and benefits. Participation is voluntary and you are not obligated to participate in any research study here at the University of Pittsburgh Medical Center. Your future care under Dr. Brand or any investigator in this research registry will not be affected should you decide not to participate.



All information gathered in the registry will be the same information found in your clinical medical records.

If you have first-degree relatives (parents, brothers and sisters and children), and if you desire, we can help send letters from you to them to explain any change they may need to consider in their future health care, or future assessment they may desire to explore.

Procedures:

Your voluntary consent to participate in this registry will be obtained before any screening takes place during the initial visit to the Hereditary Colorectal Tumor Program. Attending clinicians and genetic counselors will assess you for eligibility and you will be asked to participate by the Hereditary Colorectal Tumor Registry staff. The staff will conduct the informed consent process.

If you decline participation in the registry, you will still be able to receive the standard counseling and care. You may still decide to have tumors analyzed for MSI or IHC or to undergo genetic testing after counseling as standard of care if you fit the Bethesda criteria accordingly.

A complete medical and family history will be obtained from all who participate in the HCTR. You will be asked complete a questionnaire about your health and colon polyp history in the clinic or take it home to complete and return in a self addressed stamped envelope provided by the research staff. The questionnaire takes approximately 15 minutes to complete

First Degree Relatives

You will be asked to contact any first degree relative that has a history of early onset colorectal cancer. You will be given a Letter of Introduction to give to your first-degree relatives. If your first degree relative is interested in participation in the Hereditary Colorectal Tumor Registry, he/she will be provided with the Letter of Introduction. Your first degree relative must sign the Letter of Introduction and send it back via self-addressed stamped envelope supplied by you to show interest in participation. The Letter of Introduction will inform your first degree relative that he/she was referred to the Hereditary Colorectal Tumor Registry because of a shared family history of colorectal, ovarian, endometrial, gastric, biliary, renal pelvis, bladder, and or brain tumor.

If your first degree relative is interested in learning more about the Hereditary Colorectal Tumor Registry, he/she will be asked to include a phone number where he/she can be reached and the Registry staff will call them to describe the Hereditary Colorectal Tumor Registry. If your first degree relative agrees to participate, he/she will be asked to sign a consent form. Your first degree relative will also be asked to sign a release of medical information form and to complete the questionnaire. None of your medical or personal history information provided by you will not be discussed with any other family members and their information will not be discussed with you.



If you or your first degree relative consents to enter the study, the investigator who is a physician will review the results of the tests/procedures that are part of you or your first degree relative's clinical care and the results will become part of the research record.

All identifiers will be removed such as name, social security number will be removed from registry participants. They will be assigned a code number, which is in compliance with HIPAA guidelines.

**What are the possible risks of my participation in the Research Registry?**

Breach of confidentiality:

- Participation in the Hereditary Colorectal Tumor Registry does involve the possible risk that individuals outside of the UPMC treating centers might know information about subject's health. In unusual cases, the researchers may be required to release subject's identifiable medical record information from the Hereditary Colorectal Tumor Registry in response to an order from a court of law.
- Authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may also review information contained within the Hereditary Colorectal Tumor Registry to ensure that the research registry adequately protects subject's privacy.
- We will attempt to preserve your medical record confidentiality by assigning a special research code number to your medical record information stored in the research registry, and by removing personal identifiers (for example, your name, social security number, medical record number) from information stored about you in the Hereditary Colorectal Tumor Registry. Information linking the research code number to your name and other personal identifiers will be stored in a separate secure location. Access to any identifiable information about you that is contained within the Hereditary Colorectal Tumor Registry will be limited to investigators associated with the Hereditary Colorectal Tumor Program and their research staffs, as well as the University of Pittsburgh Cancer Center Research Staff.
- Should a Breach of Confidentiality occur, you will be notified of information breached, it will be documented and reported to the University of Pittsburgh IRB.

Genetic Research Risks

Genetic research data related to inheritable characteristics could potentially impact your future insurability, employability, or reproduction plans; or have a negative impact on family relationships, and / or result in stigmatization.



### Physical Risks

The possible risk with the Hereditary Colorectal Tumor Registry is the potential of a Breach of Confidentiality. A breach of the confidentiality of genetic research data related to inheritable characteristics could potentially impact future insurability, employability or reproduction plans: or have a negative impact on family relationships, and/or result in stigmatization. In stating all this there has never been a case brought before US Courts in regards to Employment Discrimination. HIPAA also protects those with group insurance plans from Insurance Discrimination. Prior to any genetic testing being performed on you, a full consent process will be performed with you and a genetic counselor.

We will attempt to preserve your medical record confidentiality by assigning a special research code number to your medical record information stored in the Hereditary Colorectal Tumor Registry, and by removing personal identifiers (for example, your name, social security number, medical record number) from information stored about you in the Research Registry. Information linking the research code number to your name and other personal identifiers will be stored in a separate secure location. Access to any identifiable information about you that is contained within the Hereditary Colorectal Tumor Registry will be limited to investigators associated with the Hereditary Colorectal Tumor Program and their research staffs, as well as the University of Pittsburgh Cancer Center Research Staff.

#### **What are the possible benefits of my participation in the Research Registry?**

It is unlikely that you will receive any direct benefit as a result of your participation in the Hereditary Colorectal Tumor Registry. However, medical record information contained within the Hereditary Colorectal Tumor Registry will be used for research studies directed at improving our knowledge and treatment of cancer related to genetic disease and this knowledge might benefit patients with HNPCC in the future.

#### **Will my insurance provider or I be charged for my participation in the Research Registry?**

There will be no costs to you or your insurance provider to participate in the Hereditary Colorectal Tumor Registry.

#### **Will I be paid for my participation in the Research Registry?**

No, you will not receive any payment for participating in the Hereditary Colorectal Tumor Registry.

A description of this clinical trial will be available on [www.clinicaltrials.gov](http://www.clinicaltrials.gov), as required by US Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.



**Who will know about my participation in this Research Registry?**

Any information from your medical records that is placed into the Hereditary Colorectal Tumor Registry will be kept as confidential (private) as possible. In addition, you will not be identified by name in any publication of the results of research studies involving the use of your medical record information unless you sign a separate consent form (release) giving your permission.

**What is the nature of my medical record information that will be placed into the Research Registry?**

All of your past, current and future medical record information related to your HNPCC will be recorded into the Hereditary Colorectal Tumor Registry. Since medical conditions and treatments not related directly to your HNPCC may affect genetic disease and/or its treatment, it is likely that all of your existing and future medical record information will be placed in the Hereditary Colorectal Tumor Registry. This information will be collected from your Hereditary Colorectal Tumor Program Clinic records, hospital records and, if applicable, private physician records.

**Who will have access to my identifiable medical record information contained in the Research Registry?**

Access to your identifiable medical record information contained within the Hereditary Colorectal Tumor Registry Research Registry will be limited to investigators associated with the Hereditary Colorectal Tumor Program and their research staffs.

In addition, the following individuals may have access to your identifiable medical record information contained within the Hereditary Colorectal Tumor Registry: authorized representatives of the University of Pittsburgh Research Conduct and Compliance Office may review information contained within the Hereditary Colorectal Tumor Program to ensure that the Hereditary Colorectal Tumor Registry adequately protects your privacy.

In unusual cases, the researchers may be required to release your identifiable medical record information from the Hereditary Colorectal Tumor Registry in response to an order from a court of law.

**For how long will my medical record information continue to be placed in the Research Registry and for how long will this information be used for research purposes?**

We will continue to place your medical record information into the Hereditary Colorectal Tumor Registry until 1) you are no longer living; or 2) you withdraw your permission for participation in the Hereditary Colorectal Tumor Registry. Your medical record information contained within the Hereditary Colorectal Tumor Registry will be used for research purposes for an indefinite period of time.



**Is my participation in the Research Registry voluntary?**

Your participation in the Hereditary Colorectal Tumor Registry, to include the use of your medical record information for the research purposes described above, is completely voluntary. Whether or not you provide your permission for participation in the Hereditary Colorectal Tumor Registry will have no affect on your current or future medical care at the University of Pittsburgh Medical Center, affiliated health care provider, or your current or future relationship with a health care insurance provider.

**If your physician is also an investigator in this study, you are not obligated to participate and your care will not change.**

**May I withdraw, at a future date, my consent for participation in this Research Registry?**

Your doctor may be an investigator in this research study, and as investigator, is interested in both in your medical care and in the conduct of this research. Before entering this study or at any time during the research, you may discuss your care with another doctor who is in no way associated with this research project. You are not under any obligation to participate in any research study offered by your doctor.

You may withdraw, at any time, your consent for participation in the Hereditary Colorectal Tumor Registry, to include the additional collection of your medical record information and its further use for the research purposes described above. However, any research use of your medical record information prior to the date that you formally withdraw your permission will not be destroyed.

To formally withdraw your permission for participation in the Hereditary Colorectal Tumor Registry, you should provide a written and dated notice of this decision to Dr. Randall Brand of the Hereditary Colorectal Tumor Registry at the address listed on the first page of this consent form.



\*\*\*\*\*

**VOLUNTARY CONSENT**

All of the above has been explained to me and all of my current questions have been answered. I understand that I am encouraged to ask questions about any aspect of my participation in the Research Registry at any time, and that such future questions will be answered by Dr. Brand at 412-692-2520 and other researchers associated with the Center for Genetic Disease or their research staffs. I understand that a copy of this consent form will be given to me.

I understand that any questions which I have about my rights as a participant in the Hereditary Colorectal Tumor Registry will be answered by the Human Subject Protections Advocate of the IRB Office, University of Pittsburgh (1-866-212-2668).

**By signing below, I agree to participate in the Hereditary Colorectal Tumor Registry**

\_\_\_\_\_  
Participant's Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Printed name of Participant

**CERTIFICATION OF INFORMED CONSENT**

I certify that I have explained the nature and purpose of the Hereditary Colorectal Tumor Registry to the above-named individual, and I have discussed the possible risks and potential benefits of participation in this Research Registry. Any questions the individual has about the Hereditary Colorectal Tumor Registry have been answered, and the physicians and research staff associated with the Hereditary Colorectal Tumor Registry will be available to address future questions as they arise.

\_\_\_\_\_  
Printed Name of Person Obtaining Consent

\_\_\_\_\_  
Role in Research Registry

\_\_\_\_\_  
Signature of Person Obtaining Consent

\_\_\_\_\_  
Date



## BIBLIOGRAPHY

- Aaltonen, L. A., R. Salovaara, et al. (1998). "Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease." The New England journal of medicine **338**(21): 1481-1487.
- Aarnio, M., R. Sankila, et al. (1999). "Cancer risk in mutation carriers of DNA-mismatch-repair genes." International journal of cancer. Journal international du cancer **81**(2): 214-218.
- Abdel-Rahman, W. M., J. P. Mecklin, et al. (2006). "The genetics of HNPCC: application to diagnosis and screening." Critical reviews in oncology/hematology **58**(3): 208-220.
- ACS (2011). Cancer Facts & Figures 2011. Atlanta, American Cancer Society.
- Ahnen, D. J. (2011). "The American College of Gastroenterology Emily Couric Lecture--the adenoma-carcinoma sequence revisited: has the era of genetic tailoring finally arrived?" The American journal of gastroenterology **106**(2): 190-198.
- Ahuja, N., A. L. Mohan, et al. (1997). "Association between CpG island methylation and microsatellite instability in colorectal cancer." Cancer research **57**(16): 3370-3374.
- ASCO, A. S. o. C. O. (2003). "American Society of Clinical Oncology policy statement update: genetic testing for cancer susceptibility." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **21**(12): 2397-2406.
- Association, A. M. (1994). "Code of medical ethics." Arch Fam Med **3**: 633-642.
- Auranen, A. and T. Joutsiniemi (2011). "A systematic review of gynecological cancer surveillance in women belonging to hereditary nonpolyposis colorectal cancer (Lynch syndrome) families." Acta obstetricia et gynecologica Scandinavica **90**(5): 437-444.
- Balaguer, F., S. Castellvi-Bel, et al. (2007). "Identification of MYH mutation carriers in colorectal cancer: a multicenter, case-control, population-based study." Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association **5**(3): 379-387.
- Balmana, J., D. H. Stockwell, et al. (2006). "Prediction of MLH1 and MSH2 mutations in Lynch syndrome." JAMA : the journal of the American Medical Association **296**(12): 1469-1478.
- Barnetson, R. A., A. Tenesa, et al. (2006). "Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer." The New England journal of medicine **354**(26): 2751-2763.

- Bedeir, A. and A. M. Krasinskas (2011). "Molecular diagnostics of colorectal cancer." Archives of pathology & laboratory medicine **135**(5): 578-587.
- Bellacosa, A., M. Genuardi, et al. (1996). "Hereditary nonpolyposis colorectal cancer: review of clinical, molecular genetics, and counseling aspects." American journal of medical genetics **62**(4): 353-364.
- Boland, C. R., S. N. Thibodeau, et al. (1998). "A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer." Cancer research **58**(22): 5248-5257.
- Bouzourene, H., P. Hutter, et al. (2010). "Selection of patients with germline MLH1 mutated Lynch syndrome by determination of MLH1 methylation and BRAF mutation." Familial cancer **9**(2): 167-172.
- Burn, J., D. T. Bishop, et al. (2008). "Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome." The New England journal of medicine **359**(24): 2567-2578.
- Burt, R. W. (2000). "Colon cancer screening." Gastroenterology **119**(3): 837-853.
- Charbonnier, F., G. Raux, et al. (2000). "Detection of exon deletions and duplications of the mismatch repair genes in hereditary nonpolyposis colorectal cancer families using multiplex polymerase chain reaction of short fluorescent fragments." Cancer research **60**(11): 2760-2763.
- Chen, J. R., J. M. Chiang, et al. (2008). "Mismatch repair protein expression in Amsterdam II criteria-positive patients in Taiwan." The British journal of surgery **95**(1): 102-110.
- Chen, L. M., K. Y. Yang, et al. (2007). "Gynecologic cancer prevention in Lynch syndrome/hereditary nonpolyposis colorectal cancer families." Obstetrics and gynecology **110**(1): 18-25.
- Chen, S., W. Wang, et al. (2006). "Prediction of germline mutations and cancer risk in the Lynch syndrome." JAMA : the journal of the American Medical Association **296**(12): 1479-1487.
- Cho, E., S. A. Smith-Warner, et al. (2004). "Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies." Annals of internal medicine **140**(8): 603-613.
- Chubak, B., B. Heald, et al. (2011). "Informed consent to microsatellite instability and immunohistochemistry screening for Lynch syndrome." Genetics in medicine : official journal of the American College of Medical Genetics **13**(4): 356-360.
- Cunningham, J. M., E. R. Christensen, et al. (1998). "Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability." Cancer research **58**(15): 3455-3460.

- Davies, H., G. R. Bignell, et al. (2002). "Mutations of the BRAF gene in human cancer." Nature **417**(6892): 949-954.
- de la Chapelle, A. (2004). "Genetic predisposition to colorectal cancer." Nature reviews. Cancer **4**(10): 769-780.
- de la Chapelle, A. and H. Hampel (2010). "Clinical relevance of microsatellite instability in colorectal cancer." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **28**(20): 3380-3387.
- Deng, G., I. Bell, et al. (2004). "BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer." Clinical cancer research : an official journal of the American Association for Cancer Research **10**(1 Pt 1): 191-195.
- Des Guetz, G., O. Schischmanoff, et al. (2009). "Does microsatellite instability predict the efficacy of adjuvant chemotherapy in colorectal cancer? A systematic review with meta-analysis." European journal of cancer **45**(10): 1890-1896.
- Dewanwala, A., A. Chittenden, et al. (2011). "Attitudes toward childbearing and prenatal testing in individuals undergoing genetic testing for Lynch Syndrome." Familial cancer.
- Dinh, T. A., B. I. Rosner, et al. (2011). "Health benefits and cost-effectiveness of primary genetic screening for Lynch syndrome in the general population." Cancer prevention research **4**(1): 9-22.
- EGAPP (2009). "Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives." Genetics in medicine : official journal of the American College of Medical Genetics **11**(1): 35-41.
- Embi, P. J., A. Jain, et al. (2008). "Physicians' perceptions of an electronic health record-based clinical trial alert approach to subject recruitment: a survey." BMC medical informatics and decision making **8**: 13.
- Esteller, M., R. Levine, et al. (1998). "MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas." Oncogene **17**(18): 2413-2417.
- Fearon, E. R. and B. Vogelstein (1990). "A genetic model for colorectal tumorigenesis." Cell **61**(5): 759-767.
- Fleischmann, C., J. Peto, et al. (2004). "Comprehensive analysis of the contribution of germline MYH variation to early-onset colorectal cancer." International journal of cancer. Journal international du cancer **109**(4): 554-558.
- Gadzicki, D., A. Schubert, et al. (2009). "Histopathological criteria and selection algorithms for BRCA1 genetic testing." Cancer genetics and cytogenetics **189**(2): 105-111.

- Grady, W. M. (2003). "Genetic testing for high-risk colon cancer patients." Gastroenterology **124**(6): 1574-1594.
- Greenon, J. K., J. D. Bonner, et al. (2003). "Phenotype of microsatellite unstable colorectal carcinomas: Well-differentiated and focally mucinous tumors and the absence of dirty necrosis correlate with microsatellite instability." The American journal of surgical pathology **27**(5): 563-570.
- Gritz, E. R., S. K. Peterson, et al. (2005). "Psychological impact of genetic testing for hereditary nonpolyposis colorectal cancer." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **23**(9): 1902-1910.
- Halling, K. C., J. Harper, et al. (1999). "Origin of microsatellite instability in gastric cancer." The American journal of pathology **155**(1): 205-211.
- Halvarsson, B., A. Lindblom, et al. (2005). "Loss of mismatch repair protein immunostaining in colorectal adenomas from patients with hereditary nonpolyposis colorectal cancer." Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc **18**(8): 1095-1101.
- Hamilton, S. R., B. Liu, et al. (1995). "The molecular basis of Turcot's syndrome." The New England journal of medicine **332**(13): 839-847.
- Hampel, H. and A. de la Chapelle (2011). "The search for unaffected individuals with Lynch syndrome: do the ends justify the means?" Cancer prevention research **4**(1): 1-5.
- Hampel, H., W. L. Frankel, et al. (2008). "Feasibility of screening for Lynch syndrome among patients with colorectal cancer." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **26**(35): 5783-5788.
- Hampel, H., W. L. Frankel, et al. (2005). "Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer)." The New England journal of medicine **352**(18): 1851-1860.
- Hawkins, N. J. and R. L. Ward (2001). "Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas." Journal of the National Cancer Institute **93**(17): 1307-1313.
- Herman, J. G., A. Umar, et al. (1998). "Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma." Proceedings of the National Academy of Sciences of the United States of America **95**(12): 6870-6875.
- Hitchins, M. P. and R. L. Ward (2009). "Constitutional (germline) MLH1 epimutation as an aetiological mechanism for hereditary non-polyposis colorectal cancer." Journal of medical genetics **46**(12): 793-802.

- Horner, M. J., Ries, L.A.G., Krapcho, M., Neyman, N., Aminou, R., Howlander, N. (2009). "SEER Cancer Statistics Review, 1975–2006, National Cancer Institute." from [http://seer.cancer.gov/csr/1975\\_2006/](http://seer.cancer.gov/csr/1975_2006/).
- I.O.M. (2010). The Value of Genetic and Genomic Technologies: Workshop Summary. Washington (DC).
- Ishii, N., M. Arai, et al. (2011). "Factors affecting encouragement of relatives among families with Lynch syndrome to seek medical assessment." Familial cancer.
- Issa, J. P., Y. L. Ottaviano, et al. (1994). "Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon." Nature genetics **7**(4): 536-540.
- Jarvinen, H. J., M. Aarnio, et al. (2000). "Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer." Gastroenterology **118**(5): 829-834.
- Jass, J. R. (2003). "Hyperplastic-like polyps as precursors of microsatellite-unstable colorectal cancer." American journal of clinical pathology **119**(6): 773-775.
- Jass, J. R. (2005). "What's new in hereditary colorectal cancer?" Archives of pathology & laboratory medicine **129**(11): 1380-1384.
- Jass, J. R., V. Pokos, et al. (1996). "Colorectal neoplasms detected colonoscopically in at-risk members of colorectal cancer families stratified by the demonstration of DNA microsatellite instability." Journal of molecular medicine **74**(9): 547-551.
- Jass, J. R. and S. M. Stewart (1992). "Evolution of hereditary non-polyposis colorectal cancer." Gut **33**(6): 783-786.
- Kambara, T., L. A. Simms, et al. (2004). "BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum." Gut **53**(8): 1137-1144.
- Keller, M., R. Jost, et al. (2004). "Acceptance of and attitude toward genetic testing for hereditary nonpolyposis colorectal cancer: a comparison of participants and nonparticipants in genetic counseling." Diseases of the colon and rectum **47**(2): 153-162.
- Kruhoffer, M., J. L. Jensen, et al. (2005). "Gene expression signatures for colorectal cancer microsatellite status and HNPCC." British journal of cancer **92**(12): 2240-2248.
- Kuismanen, S. A., A. L. Moisio, et al. (2002). "Endometrial and colorectal tumors from patients with hereditary nonpolyposis colon cancer display different patterns of microsatellite instability." The American journal of pathology **160**(6): 1953-1958.
- Kwon, J. S., J. L. Scott, et al. (2011). "Testing women with endometrial cancer to detect lynch syndrome." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **29**(16): 2247-2252.

- Lagerstedt Robinson, K., T. Liu, et al. (2007). "Lynch syndrome (hereditary nonpolyposis colorectal cancer) diagnostics." Journal of the National Cancer Institute **99**(4): 291-299.
- Lenz, H. J. (2005). "First Amsterdam, then Bethesda, now Melbourne?" Journal of clinical oncology : official journal of the American Society of Clinical Oncology **23**(27): 6445-6449.
- Ligtenberg, M. J., R. P. Kuiper, et al. (2009). "Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1." Nature genetics **41**(1): 112-117.
- Lindor, N. M., L. J. Burgart, et al. (2002). "Immunohistochemistry versus microsatellite instability testing in phenotyping colorectal tumors." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **20**(4): 1043-1048.
- Lindor, N. M., G. M. Petersen, et al. (2006). "Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review." JAMA : the journal of the American Medical Association **296**(12): 1507-1517.
- Lindor, N. M., K. Rabe, et al. (2005). "Lower cancer incidence in Amsterdam-I criteria families without mismatch repair deficiency: familial colorectal cancer type X." JAMA : the journal of the American Medical Association **293**(16): 1979-1985.
- Loughrey, M. B., P. M. Waring, et al. (2007). "Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer." Familial cancer **6**(3): 301-310.
- Loukola, A., K. Eklin, et al. (2001). "Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC)." Cancer research **61**(11): 4545-4549.
- Loukola, A., R. Salovaara, et al. (1999). "Microsatellite instability in adenomas as a marker for hereditary nonpolyposis colorectal cancer." The American journal of pathology **155**(6): 1849-1853.
- Lu, K. H., J. O. Schorge, et al. (2007). "Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **25**(33): 5158-5164.
- Lynch, H. T. and A. de la Chapelle (2003). "Hereditary colorectal cancer." The New England journal of medicine **348**(10): 919-932.
- Lynch, H. T., R. E. Harris, et al. (1977). "Role of heredity in multiple primary cancer." Cancer **40**(4 Suppl): 1849-1854.
- Lynch, H. T., T. G. Shaw, et al. (2004). "Inherited predisposition to cancer: a historical overview." American journal of medical genetics. Part C, Seminars in medical genetics **129C**(1): 5-22.

- Lynch, H. T., T. C. Smyrk, et al. (1993). "Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review." Gastroenterology **104**(5): 1535-1549.
- Mangold, E., C. Pagenstecher, et al. (2005). "Spectrum and frequencies of mutations in MSH2 and MLH1 identified in 1,721 German families suspected of hereditary nonpolyposis colorectal cancer." International journal of cancer. Journal international du cancer **116**(5): 692-702.
- Manne, S. L., N. J. Meropol, et al. (2010). "Facilitating informed decisions regarding microsatellite instability testing among high-risk individuals diagnosed with colorectal cancer." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **28**(8): 1366-1372.
- Martin, D. K., H. L. Greenwood, et al. (2010). "Public perceptions of ethical issues regarding adult predictive genetic testing." Health care analysis : HCA : journal of health philosophy and policy **18**(2): 103-112.
- Martinez, M. E., E. Giovannucci, et al. (1997). "Leisure-time physical activity, body size, and colon cancer in women. Nurses' Health Study Research Group." Journal of the National Cancer Institute **89**(13): 948-955.
- McGivern, A., C. V. Wynter, et al. (2004). "Promoter hypermethylation frequency and BRAF mutations distinguish hereditary non-polyposis colon cancer from sporadic MSI-H colon cancer." Familial cancer **3**(2): 101-107.
- Mecklin, J. P. and H. J. Jarvinen (1986). "Clinical features of colorectal carcinoma in cancer family syndrome." Diseases of the colon and rectum **29**(3): 160-164.
- Mitchell, R. J., D. Brewster, et al. (2004). "Accuracy of reporting of family history of colorectal cancer." Gut **53**(2): 291-295.
- Mueller, J., I. Gazzoli, et al. (2009). "Comprehensive molecular analysis of mismatch repair gene defects in suspected Lynch syndrome (hereditary nonpolyposis colorectal cancer) cases." Cancer research **69**(17): 7053-7061.
- Mvundura, M., S. D. Grosse, et al. (2010). "The cost-effectiveness of genetic testing strategies for Lynch syndrome among newly diagnosed patients with colorectal cancer." Genetics in medicine : official journal of the American College of Medical Genetics **12**(2): 93-104.
- Nagy, R., K. Sweet, et al. (2004). "Highly penetrant hereditary cancer syndromes." Oncogene **23**(38): 6445-6470.
- Nakagawa, H., H. Yan, et al. (2002). "Allele separation facilitates interpretation of potential splicing alterations and genomic rearrangements." Cancer research **62**(16): 4579-4582.
- NCCN (2010). NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines). Colorectal Cancer Screening.

- NCI. (2011, 07/15/2011). "National Cancer Institute - Colorectal Cancer Prevention." from <http://www.cancer.gov/cancertopics/pdq/prevention/colorectal/HealthProfessional/page1>.
- Offit, K., M. Sagi, et al. (2006). "Preimplantation genetic diagnosis for cancer syndromes: a new challenge for preventive medicine." JAMA : the journal of the American Medical Association **296**(22): 2727-2730.
- Ollila, S., R. Fitzpatrick, et al. (2006). "The importance of functional testing in the genetic assessment of Muir-Torre syndrome, a clinical subphenotype of HNPCC." International journal of oncology **28**(1): 149-153.
- Oppitz, M., A. Klee, et al. (2009). "[Sickle cell anemia in perinatal placental diagnostics]." Der Pathologe **30**(4): 326-328, 330-321.
- Overbeek, L. I., N. Hoogerbrugge, et al. (2008). "Most patients with colorectal tumors at young age do not visit a cancer genetics clinic." Diseases of the colon and rectum **51**(8): 1249-1254.
- Palomaki, G. E., M. R. McClain, et al. (2009). "EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome." Genetics in medicine : official journal of the American College of Medical Genetics **11**(1): 42-65.
- Park, Y. J., K. H. Shin, et al. (2000). "Risk of gastric cancer in hereditary nonpolyposis colorectal cancer in Korea." Clinical cancer research : an official journal of the American Association for Cancer Research **6**(8): 2994-2998.
- Peltomaki, P. and H. Vasen (2004). "Mutations associated with HNPCC predisposition -- Update of ICG-HNPCC/INSiGHT mutation database." Disease markers **20**(4-5): 269-276.
- Pinol, V., A. Castells, et al. (2005). "Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer." JAMA : the journal of the American Medical Association **293**(16): 1986-1994.
- Poynter, J. N., R. W. Haile, et al. (2009). "Associations between smoking, alcohol consumption, and colorectal cancer, overall and by tumor microsatellite instability status." Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology **18**(10): 2745-2750.
- Rajagopalan, H., A. Bardelli, et al. (2002). "Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status." Nature **418**(6901): 934.
- Renkonen-Sinisalo, L., M. Aarnio, et al. (2000). "Surveillance improves survival of colorectal cancer in patients with hereditary nonpolyposis colorectal cancer." Cancer detection and prevention **24**(2): 137-142.

- Ribic, C. M., D. J. Sargent, et al. (2003). "Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer." The New England journal of medicine **349**(3): 247-257.
- Rodriguez-Bigas, M. A., C. R. Boland, et al. (1997). "A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines." Journal of the National Cancer Institute **89**(23): 1758-1762.
- Rodriguez-Bigas, M. A., H. F. Vasen, et al. (1998). "Characteristics of small bowel carcinoma in hereditary nonpolyposis colorectal carcinoma. International Collaborative Group on HNPCC." Cancer **83**(2): 240-244.
- Roessner, A., D. Kuester, et al. (2011). "[Colorectal polyposis syndrome : A guide to diagnosis.]." Der Pathologe.
- Rose, D. P., A. P. Boyar, et al. (1986). "International comparisons of mortality rates for cancer of the breast, ovary, prostate, and colon, and per capita food consumption." Cancer **58**(11): 2363-2371.
- Rumilla, K., K. V. Schowalter, et al. (2011). "Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated lynch syndrome cases." The Journal of molecular diagnostics : JMD **13**(1): 93-99.
- Salahshor, S., K. Koelble, et al. (2001). "Microsatellite Instability and hMLH1 and hMSH2 expression analysis in familial and sporadic colorectal cancer." Laboratory investigation; a journal of technical methods and pathology **81**(4): 535-541.
- Salovaara, R., A. Loukola, et al. (2000). "Population-based molecular detection of hereditary nonpolyposis colorectal cancer." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **18**(11): 2193-2200.
- Schmeler, K. M., H. T. Lynch, et al. (2006). "Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome." The New England journal of medicine **354**(3): 261-269.
- Senter, L., M. Clendenning, et al. (2008). "The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations." Gastroenterology **135**(2): 419-428.
- Shia, J. (2008). "Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry." The Journal of molecular diagnostics : JMD **10**(4): 293-300.
- Shia, J., N. A. Ellis, et al. (2004). "The utility of immunohistochemical detection of DNA mismatch repair gene proteins." Virchows Archiv : an international journal of pathology **445**(5): 431-441.

- Shia, J., D. S. Klimstra, et al. (2005). "Value of immunohistochemical detection of DNA mismatch repair proteins in predicting germline mutation in hereditary colorectal neoplasms." The American journal of surgical pathology **29**(1): 96-104.
- Singh, H., R. Schiesser, et al. (2010). "Underdiagnosis of Lynch syndrome involves more than family history criteria." Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association **8**(6): 523-529.
- Sjursen, W., B. I. Haukanes, et al. (2010). "Current clinical criteria for Lynch syndrome are not sensitive enough to identify MSH6 mutation carriers." Journal of medical genetics **47**(9): 579-585.
- Slattery, M. L., J. D. Potter, et al. (1997). "Tobacco use and colon cancer." International journal of cancer. Journal international du cancer **70**(3): 259-264.
- Snover, D. C. (2011). "Update on the serrated pathway to colorectal carcinoma." Human pathology **42**(1): 1-10.
- South, C. D., H. Hampel, et al. (2008). "The frequency of Muir-Torre syndrome among Lynch syndrome families." Journal of the National Cancer Institute **100**(4): 277-281.
- Staebler, A., S. F. Lax, et al. (2000). "Altered expression of hMLH1 and hMSH2 protein in endometrial carcinomas with microsatellite instability." Human pathology **31**(3): 354-358.
- Staretz-Chacham, O., T. C. Lang, et al. (2009). "Lysosomal storage disorders in the newborn." Pediatrics **123**(4): 1191-1207.
- Stoffel, E., B. Mukherjee, et al. (2009). "Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome." Gastroenterology **137**(5): 1621-1627.
- Strom, C. M., N. Ginsberg, et al. (1998). "Three births after preimplantation genetic diagnosis for cystic fibrosis with sequential first and second polar body analysis." American journal of obstetrics and gynecology **178**(6): 1298-1306.
- Syngal, S., E. A. Fox, et al. (2000). "Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1." Journal of medical genetics **37**(9): 641-645.
- Terdiman, J. P., P. G. Conrad, et al. (1999). "Genetic testing in hereditary colorectal cancer: indications and procedures." The American journal of gastroenterology **94**(9): 2344-2356.
- Umar, A., C. R. Boland, et al. (2004). "Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability." Journal of the National Cancer Institute **96**(4): 261-268.

- Vasen, H. F. (2000). "Clinical diagnosis and management of hereditary colorectal cancer syndromes." Journal of clinical oncology : official journal of the American Society of Clinical Oncology **18**(21 Suppl): 81S-92S.
- Vasen, H. F., J. P. Mecklin, et al. (1991). "The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC)." Diseases of the colon and rectum **34**(5): 424-425.
- Vasen, H. F., G. Moslein, et al. (2007). "Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer)." Journal of medical genetics **44**(6): 353-362.
- Vasen, H. F., F. M. Nagengast, et al. (1995). "Interval cancers in hereditary non-polyposis colorectal cancer (Lynch syndrome)." Lancet **345**(8958): 1183-1184.
- Vasen, H. F., M. van Ballegooijen, et al. (1998). "A cost-effectiveness analysis of colorectal screening of hereditary nonpolyposis colorectal carcinoma gene carriers." Cancer **82**(9): 1632-1637.
- Vasen, H. F., P. Watson, et al. (1999). "New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC." Gastroenterology **116**(6): 1453-1456.
- Vasen, H. F., J. T. Wijnen, et al. (1996). "Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis." Gastroenterology **110**(4): 1020-1027.
- Velayos, F. S., B. A. Allen, et al. (2005). "Low rate of microsatellite instability in young patients with adenomas: reassessing the Bethesda guidelines." The American journal of gastroenterology **100**(5): 1143-1149.
- Vilar, E. and S. B. Gruber (2010). "Microsatellite instability in colorectal cancer-the stable evidence." Nature reviews. Clinical oncology **7**(3): 153-162.
- Vogelstein, B., E. R. Fearon, et al. (1989). "Allelotype of colorectal carcinomas." Science **244**(4901): 207-211.
- Wahlberg, S. S., J. Schmeits, et al. (2002). "Evaluation of microsatellite instability and immunohistochemistry for the prediction of germ-line MSH2 and MLH1 mutations in hereditary nonpolyposis colon cancer families." Cancer research **62**(12): 3485-3492.
- Watanabe, T., T. T. Wu, et al. (2001). "Molecular predictors of survival after adjuvant chemotherapy for colon cancer." The New England journal of medicine **344**(16): 1196-1206.
- Watson, P., R. Butzow, et al. (2001). "The clinical features of ovarian cancer in hereditary nonpolyposis colorectal cancer." Gynecologic oncology **82**(2): 223-228.

- Watson, P., H. F. Vasen, et al. (2008). "The risk of extra-colonic, extra-endometrial cancer in the Lynch syndrome." International journal of cancer. Journal international du cancer **123**(2): 444-449.
- Weissman, S. M., C. Bellcross, et al. (2011). "Genetic counseling considerations in the evaluation of families for Lynch syndrome--a review." Journal of genetic counseling **20**(1): 5-19.
- Wijnen, J., H. van der Klift, et al. (1998). "MSH2 genomic deletions are a frequent cause of HNPCC." Nature genetics **20**(4): 326-328.
- Wilson, J. M. and Y. G. Jungner (1968). "[Principles and practice of mass screening for disease]." Boletin de la Oficina Sanitaria Panamericana. Pan American Sanitary Bureau **65**(4): 281-393.
- Win, A. K., M. A. Jenkins, et al. (2011). "Determining the frequency of de novo germline mutations in DNA mismatch repair genes." Journal of medical genetics.
- Wirtz, H. C., W. Muller, et al. (1998). "Prognostic value and clinicopathological profile of microsatellite instability in gastric cancer." Clinical cancer research : an official journal of the American Association for Cancer Research **4**(7): 1749-1754.
- Wu, A. H., D. Shibata, et al. (2001). "Dietary heterocyclic amines and microsatellite instability in colon adenocarcinomas." Carcinogenesis **22**(10): 1681-1684.
- Yang, K. Y., A. B. Caughey, et al. (2011). "A cost-effectiveness analysis of prophylactic surgery versus gynecologic surveillance for women from hereditary non-polyposis colorectal cancer (HNPCC) Families." Familial cancer.
- Zhang, L. (2008). "Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing." The Journal of molecular diagnostics : JMD **10**(4): 301-307.
- Zhou, X. P., J. M. Hoang, et al. (1998). "Determination of the replication error phenotype in human tumors without the requirement for matching normal DNA by analysis of mononucleotide repeat microsatellites." Genes, chromosomes & cancer **21**(2): 101-107.