

**Family history and molecular features of children, adolescents and young adults with colorectal carcinoma**

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**Background:** Colorectal cancer is extremely rare in childhood. Published case series reporting children and adolescents with colorectal cancer have not focused on the underlying genetic aspects of the tumour or genetic susceptibility of the families.

**Aims:** We examined a cohort of patients with early-onset colorectal cancer to determine whether a specific genetic predisposition could be elucidated. In particular we focused on whether, DNA mismatch repair gene deficiency which causes Hereditary Nonpolyposis Colorectal Cancer (HNPCC), could be elucidated.

**Methods:** Patients with colorectal cancer  $\leq 24$  years of age were identified from a database at the Familial Gastrointestinal Cancer Registry at Mount Sinai Hospital, Toronto. Detailed pedigrees were ascertained from the proband or parents. Tumours were tested for microsatellite instability, a hallmark of HNPCC. Germline mismatch repair gene mutations (*MSH2* and *MLH1*) were sought in some cases. Clinical data were obtained by chart audit.

**Results:** Among 1382 probands in our registry, 16 (1%) colorectal cancer patients were 24 years or younger at the time of diagnosis. Microsatellite instability was identified in tumours from 8 (73%) of 11 evaluated patients. Germline mutations in mismatch-repair genes were identified in 6 of 12 patients including *MSH2* (n=3), *MLH1* (n=2), and *PMS2* (n=1). Ten (63%) of the 16 families met the Amsterdam criteria for HNPCC. Among these, 6 were screened for MMR gene mutations and 3 were found to carry *MSH2* or *MLH1* germline mutations. Location of the colorectal cancers included rectum/sigmoid (n=9), splenic flexure (n=2), hepatic flexure (n=3), and cecum (n=2). Forty-four percent (7 of 16) of these young cases developed additional malignancies [gastrointestinal (n=8) and extra-intestinal (n=4)] during follow-up (mean 12.8 years, SD  $\pm$  12.4 years (range 0.08-30 years)).

**Conclusions:** Patients with early-onset colorectal carcinoma often have an inherited predisposition to the disease. Tumours with high frequency microsatellite instability and germline mutations of mismatch repair genes are sufficiently common in this patient population that they should be considered, even though family histories may not satisfy the stringent Amsterdam criteria for HNPCC. Young colorectal cancer patients are at increased risk of developing second gastrointestinal and extra-intestinal malignancies.

## Introduction

Colorectal cancer is extremely rare in childhood. The published literature consists primarily of case reports with a focus on clinical aspects and less attention to genetic features. A frequency of 1.3 cases among 1 million people under the age of 20 years has been reported (1, 2). However, such series were not population-based studies. Using population-based incidence data, rates for colorectal carcinoma among patients 19 years of age and younger living in the province of Ontario, Canada are between 0.3 and 1.5 cases per million people annually (1980 to 2002) (personal communication C. Herbert, Ontario Cancer Registry May 2004).

Of the cancer predisposition syndromes which cause early-onset colorectal cancer, Hereditary Nonpolyposis Colorectal Cancer (HNPCC) is the most common with penetrance that approaches 60-80% (3,4). HNPCC is an autosomal dominant condition characterized by the development of mostly proximal colon cancer in early adulthood. The mean age at diagnosis of colorectal cancer in patients with HNPCC is 45 years. Subjects with HNPCC are also at higher than average risk for extracolonic tumours involving other sites in the gastrointestinal tract, as well as gynecologic and urinary tract malignancies (5).

Nearly all HNPCC-associated tumours exhibit high frequency microsatellite instability, manifested by expansion or contraction of mono or dinucleotide DNA microsatellite repeats. The genetic basis for HNPCC is due to germline mutations of mismatch repair genes, predominantly *MLH1* and *MSH2* (6,7). Inherited germ-line mutations of mismatch-repair genes are found in up to 50% of HNPCC subjects from families meeting the Amsterdam criteria (8). Approximately 10-15% of sporadic colorectal cancers also exhibit microsatellite instability: however, in these cases the genetic basis is somatic hypermethylation of *MLH1* rather than germline mismatch repair gene mutation (7).

A number of other autosomal dominant cancer predisposition syndromes including Familial Adenomatous Polyposis (FAP), Juvenile Polyposis Syndrome (JPS), and Peutz Jeghers Syndrome (PJS), are characterised by an increased risk of colorectal cancer in children and young adults (9). The hallmark of these conditions is the presence of at least a few polyps in the gastrointestinal tract. However, even subjects with these conditions do not usually present with malignancy until the fourth decade of life.

Previous case series reporting children and adolescents with colorectal cancer have not focused on the underlying genetic aspects of the tumour or genetic susceptibility of the families (10-13). Bhatia et al. studied 25 subjects with colorectal cancer diagnosed at age 21 or younger (14) to assess the genetic contribution to disease, characterised by a family history of cancer. A 6-fold excess of colorectal cancer was identified among relatives of probands diagnosed with colorectal cancer before age 15 years. However, tumour microsatellite instability caused by defects in mismatch repair genes was not evaluated.

Young colorectal cancer cases ( $\leq 24$  years of age) are a distinct group of cancer patients from both clinicopathologic and genetic perspectives. This report describes a cohort of 16 subjects with early-onset colorectal cancer in which we evaluated whether a specific genetic predisposition in particular, DNA mismatch repair gene deficiency, could be elucidated.

## Materials and Methods

### Study Population

A retrospective series of subjects  $\leq 24$  years of age at initial diagnosis of colorectal cancer between January, 1960 and December, 2003 was identified using a database at The Familial Gastrointestinal Cancer Registry at Mount Sinai Hospital, Toronto. Clinical data and pathology reports were requested from the treating institutions. Detailed pedigrees were constructed by a genetic counselor after review of clinical material and following interviews with probands and relatives. Paraffin blocks from colorectal resections were requested.

All aspects of this study were reviewed and approved by the Research Ethics Board at Mount Sinai Hospital in Toronto, Canada.

### Analysis of Tumor Microsatellite Instability

For each specimen, regions of invasive cancer with the highest proportion of neoplastic cells (at least 75%) and normal tissue were microdissected separately, and DNA extracted, as described previously (15). Aliquots of extracted genomic DNA were used to amplify sequences by the polymerase chain reaction using 5-10 of the following mononucleotide and dinucleotide microsatellites: BAT-25, BAT-26, D5S346, D2S123, D17S250, BAT-40, TGF- $\beta$  RII, D18S58, D18S69, and D17S787 (Human MapPairs, Research Genetics, Huntsville, Ala.). These specific microsatellites were derived from the National Cancer Institute reference and alternative loci panels (7). Primer sequences and conditions of the PCR assays and gel electrophoresis have been published previously (15).

The presence of additional bands in the PCR product from tumor DNA, not observed in DNA from normal tissue, was scored as instability at that locus. In accordance with the National Cancer Institute consensus on microsatellite instability, any sample that displayed instability in two or more of five loci from the first panel tested, or in greater than 40% of all microsatellite loci was scored as having high-frequency microsatellite instability. A sample with no instability in five loci was scored as microsatellite stable. Any sample with instability in one of the five microsatellite loci tested, underwent screening for a second panel of microsatellite markers. If instability was confirmed, additional loci, up to a maximum of 10, were tested to determine whether the genotype of the sample was low-frequency microsatellite instability; ie. instability at 1 to 3 of 10 loci (7).

### MSH2 and MLH1 Protein Expression in Colon Cancers

Immunostaining was performed using anti-MSH2 (clone FE11, 1/50;Oncogene Research Products, Cambridge, MA) and anti-MLH1 (clone G168-728, 1/250;PharMingen, San Diego, CA), as previously described (16). Tumour cells were judged to be negative for protein expression if they lacked staining when either normal epithelial cells or stromal cells stained positively for MSH2 and MLH1 proteins. If no immunostaining of normal tissue was found, the results were considered ambiguous.

### Germline Mutation Analysis

Blood samples were obtained from affected individuals and DNA and RNA extracted using DNAzol and Trizol (Invitrogen), according to manufacturer's protocols.

Screening for germline mismatch repair gene mutations, (*MSH2* and *MLH1*), was carried out using a combination of complementary techniques, including a protein truncation test assay and genomic DNA sequencing, as described previously (17).

## Results

### Patient Demographics (Table 1)

Of all probands with colorectal cancer referred to our Registry between 1960 and 2003 (n=1, 382), sixteen patients were 24 years or younger at the time of initial diagnosis; eight subjects were younger than eighteen years. Six of the sixteen cases were male (38%).

Eight of the sixteen family histories met the Amsterdam criteria for HNPCC, and two others satisfied the modified Amsterdam criteria (18). Of note, the pedigrees of the four youngest children diagnosed with colorectal cancer (9, 12, 13 and 14 years of age) did not satisfy the Amsterdam criteria.

The six youngest patients had distal tumours while five of the other ten cases had right-sided tumours.

Twelve patients (75%) had no evidence of synchronous adenomatous polyps at the time of diagnosis of colorectal carcinoma. Four of the sixteen patients had five or fewer adenomatous polyps (2, 3, 3, and 5 polyps). The youngest child (eight years old) had two polyps with moderately differentiated mucin producing adenocarcinoma and one polyp with adenocarcinoma in-situ. None of the subjects in this retrospective review had phenotypic evidence of either JPS or PJS. No patient had evidence of inflammatory bowel disease at the time of diagnosis. However, one patient (patient 10), was diagnosed with Crohn's disease six years following resection of his colorectal carcinoma after presenting with a perianal abscess.

### Molecular Analysis (Table 2)

High frequency microsatellite instability was identified in 8 tumours from 11 evaluated patients (73%). Germline mutations of mismatch-repair genes were identified in six (43%) of 14 patients tested, including *MLH1* (n=2), *MSH2* (n=3) and *PMS2* (n=1) mutations (the *PMS2* mutation was identified at another laboratory (19)). One patient was homozygous for a missense *MLH1* mutation. Her family has been reported in detail previously (20).

Immunohistochemistry for *MLH1* and *MSH2* proteins was performed on tumours from 8 patients. Two of the eight cancers showed protein loss (*MLH1*=1, *MSH2*=1), and both cancers had high frequency microsatellite instability. Six tumours were immunohistochemically intact for both proteins, with three of these showing high frequency microsatellite instability.

Two of two patients tested were negative for FAP or attenuated adenomatous polyposis based on the absence of truncating germline APC mutations (21), and two tested children were found to have a normal karyotype.

Seven of the sixteen (44%) patients developed a second cancer during follow-up (Table 3) (mean 12.8 years, SD +/- 12.4 years) (range, 0.08–30 years). The majority of second tumours (75%) were in the gastrointestinal tract (n=8). Five patients developed two second cancers. Seventy-one percent of subjects (5 of 7 patients) who developed second cancers had high frequency microsatellite instability in their original colorectal tumors. A microsatellite stable tumour was identified in only one of the patients who developed second cancers (22 and 25 years following

the diagnosis of colorectal carcinoma;colorectal and ovarian cancer). One patient's original colorectal tumor was not tested for high frequency microsatellite instability. To date, 6 of 16 patients (38%) are deceased.

**Table 1: Clinical Features of Young Colorectal Cancer Patients**

Patient	Age at colorectal cancer diagnosis	Gender	Site	Presentation	Am I or II	Colorectal polyps	Second cancer, age	
							Gastrointestinal cancer	Non-gastrointestinal cancer
1	9	F	sigmoid	Vague abdominal pain		3 polyps (2 adenocarcinoma 1 adenocarcinoma in situ)		glioblastoma, 24
2	12 (d)	F	rectum	Rectal bleeding Urgency Lethargy		3 adenomatous polyps		
3	13	M	sigmoid	Abdominal pain Bowel obstruction				
4	14	F	rectosigmoid	Bloody diarrhea Abdominal pain Weight loss				
5	16 (d)	M	rectosigmoid	Bowel perforation	I			ovarian, 41
6	16	F	descending	Abdominal pain Obstruction	I		colorectal, 38	
7	17 (d)	F	ascending	Not reported	II	5 adenomatous polyps	colorectal, 20 colorectal, 32	
8*	18 (d)	F	cecum	Not reported	I	3 adenomatous polyps	rectal, 21 duodenal, 28	
9	20	M	sigmoid	Rectal bleeding	I			
10	21	M	hepatic flexure	Anemia	I			
11	22	M	splenic flexure	Rectal bleeding Anemia	II			
12	22 (d)	M	cecum	Anemia Weight loss	I			endometrial, 46
13	22	F	rectum	Not reported			colorectal, 46	
14	22	F	ascending	Not reported	I			endometrial, 40
15*	24 (d)	F	rectum	Abdominal pain Constipation and diarrhea	I		colorectal, 40	
16	24	F	sigmoid	Not reported			colorectal, 26	

\*patient #8 is the daughter of patient #15

(d) = deceased

Am I = Amsterdam I: 3 relatives with colorectal cancer (CRC), one of whom is a first degree relative of the other two, CRC involving at least two generations, one or more CRC cases diagnosed before age 50.

Am II = Amsterdam II: 3 relatives with an HNPCC associated tumour (colorectal, endometrial, small bowel, ureter, renal pelvis), one of whom is a first degree relative of the other two, involving at least two generations, one or more cases diagnosed before age 50.

**Table 2: Molecular Features of Young Colorectal Cancer Patients**

Patient	MSI	Immunohistochemistry		MMR germline testing	Other molecular genetic information
		MLH1	MSH2		
1	H	I	I	<b>Homozygous MLH1 mutation</b> (20) Exon 18 codon 687 CGG>TGG Arg287Trp	Normal Karyotype
2	H	NT	NT	<b>PMS2 mutation</b> (19) Exon 5 codon 134,CGA ->TGA Arg ->stop	
3	H	I	D	<b>MSH2 mutation</b> Exon 8 del codons 427-462	
4	S	I	I	No mutation found ( <i>MLH1</i> and <i>MSH2</i> )	Normal Karyotype
5	S	NT	NT	NT	
6	S	I	I	NT	
7	NT	NT	NT	NT	Kin had MSI-H and MSH2 deficient tumour, no germline mutation found
8*	NT	NT	NT	NT	Kin had MSI-H and MLH1/MSH2 immuno intact tumour, no germline mutation found
9	H	I	D	<b>MSH2 mutation</b> Exon 12 codon 636 G -> C (1906G>C A636P)	
10	H	D	I	<b>MLH1 mutation</b> Exon 19 codon 730 (4bp ins AACAA) STOP nucl2234-2236	
11	NT	NT	NT	No mutation found ( <i>MLH1</i> and <i>MSH2</i> )	Kin had MSS tumour, no germline mutation found
12	H	D	I	No mutation found ( <i>MLH1</i> and <i>MSH2</i> )	Tumour was methylated at the MLH1 promotor
13	NT	NT	NT	No mutation found ( <i>MLH1</i> and <i>MSH2</i> )	
14	NT	NT	NT	<b>MSH2 mutation</b> Exon 15 codon 878 2633_2634delAG (E878fsX88)	
15*	H	I	I	No mutation found ( <i>MLH1</i> and <i>MSH2</i> )	
16	H	I	I	No mutation found ( <i>MLH1</i> and <i>MSH2</i> )	

\* patient #8 is the daughter of patient #15

(d) = deceased

NT, not tested

MSI: Microsatellite instability

H – high frequency microsatellite instability S – microsatellite stable

Immunohistochemistry:

I – intact D – deficient

**Table 3. Second cancers in 7 of 16 young colorectal cancer patients**

Site of Second Cancer	No.
Gastrointestinal tract	8
Duodenal	1
Colorectal	7
Extra Gastrointestinal	4
Ovarian	1
Endometrial	2
Glioblastoma	1

## Discussion

The purpose of this report is to describe the genetic features of a cohort of very young colorectal cancer patients, with emphasis on inherited mismatch repair gene deficiency. The three youngest children with colorectal carcinoma had germline mismatch repair gene mutations. The eight year old child with colorectal carcinoma was homozygous for a *MLH1* missense mutation and is part of a unique pedigree (20). Both of her parents, who are first cousins, are confirmed carriers and are currently unaffected. Both siblings are also homozygous for the missense *MLH1* mutation. Her older brother was diagnosed with duodenal adenocarcinoma at eleven years of age. A sister (6 years old) has a plexiform neurofibroma and other characteristics of neurofibromatosis type 1. Homozygous germ-line *MLH1* mutations associated with hematological malignancy and neurofibromatosis have been reported at an early age in occasional families with HNPCC (22). Among the other two youngest children with mismatch repair gene mutations in our cohort, each has one parent confirmed as a carrier.

In adults with HNPCC, the location of colorectal cancer is predominantly right-sided. In contrast, in this series the six youngest children had rectosigmoid tumours. Of these individuals, five of six had either a germ-line mutation identified, confirming HNPCC, or strong support of HNPCC through pedigree evaluation and molecular testing of relatives. This raises the possibility of a different phenotype, with less right-sided dominance in young children and adolescents with HNPCC.

Colorectal cancer in children and adolescents has been reported in subjects with underlying predisposing conditions such as inflammatory bowel disease and hereditary polyposis syndromes. None of the patients in our study had evidence of inflammatory bowel disease at the time of diagnosis of colorectal carcinoma. Three quarters of the patients in this series had no polyps identified at the time of diagnosis of colorectal carcinoma. Therefore, we conclude that these patients likely did not have FAP. The youngest child (patient 1, nine years old) was homozygous for a missense *MLH1* mutation. She had two adenomatous polyps with adenocarcinoma and one polyp with adenocarcinoma in situ. Two patients with three and five adenomatous polyps (patients 7 and 8) have not undergone genetic testing. However, in both cases, other family members with colorectal cancer had high frequency microsatellite instability tumours, but no specific germline mismatch repair gene mutations has been identified to date. The tumour from the relative of patient 7 had normal expression of *MSH2* and *MLH1* by immunohistochemistry. The relative of patient 8 had a tumour which was identified as *MSH2* immunodeficient. These two families meet the Amsterdam and modified Amsterdam criteria. Therefore, HNPCC is likely even though germline mismatch repair gene mutations have not been found. The fourth patient (patient 2) who had three adenomatous polyps developed a glioblastoma twelve years after the initial diagnosis of colorectal carcinoma. She had a *PMS2* mutation, but no *APC* mutation (19).

High frequency microsatellite instability was identified in tumours from 70% of patients evaluated in this study. Half (n=8) of the families did not meet the Amsterdam criteria. Therefore, much of this cohort does not represent HNPCC, at least from a family history perspective. Within those families not meeting the Amsterdam criteria, four of five probands

tested had tumours with high frequency microsatellite instability and germline mutations were identified in three. HNPCC is suspected in two additional probands; one with a relative who had a colorectal cancer identified as *MSH2* immunodeficient with high frequency microsatellite instability, and a second proband with a tumour showing intact *MLH1* and *MSH2* protein by immunohistochemistry, but exhibiting high frequency microsatellite instability. For the latter case, *MSH6* testing may be considered in ruling out HNPCC. Of the remaining three probands from non-Amsterdam criteria families, one child had a microsatellite stable tumor and a normal karyotype. A second patient had an *MLH1* and *MSH2* intact tumour by immunohistochemistry and his affected mother (endometrial cancer) had a microsatellite stable and immunohistochemical intact tumour. The last patient had no tumour available for testing, but germline DNA sequenced for *MLH1* and *MSH2* was wild type.

Of the 8 patients meeting the Amsterdam criteria, three had germline HNPCC mutations. Two of 5 tumours tested were microsatellite stable with or without intact *MLH1* and *MSH2* protein expression. One of the patients with a high frequency microsatellite instability and *MLH1* deficient tumour was negative for mutations in the *MLH1* gene. This tumour was methylated at the *MLH1* promotor (data not shown), possibly accounting for the high frequency microsatellite instability, *MLH1* deficient status.

Terdiman et al compared rates of genetically defined HNPCC among individuals with colorectal cancer diagnosed before 36 years of age who were identified at a high-risk clinic in California (median age 30 years, range 16-35 years) and at a population-based cancer registry (median age 31 years, range 14-35 years). Seventy percent of tumours in 40 subjects had high frequency microsatellite instability and 30% had germline *MLH1* and *MSH2* mutations. In contrast, among the population-based registry, only 33% of the tumours had high frequency microsatellite instability and no germline *MLH1* and *MSH2* mutations were identified (23).

In our series, colorectal cancer was seen in a parent of the affected child in 7 of 16 families, with the age of first diagnosis in the parent ranging from 20 to 63 years. Current colorectal screening recommendations include surveillance colonoscopy starting 10 years prior to the age of the youngest first-degree relative diagnosed with colorectal carcinoma (24). It should be noted that use of such guidelines would have captured only three (19%) of 16 probands identified in this series. Six parents of 5 affected children with known or obligate HNPCC carrier status remain unaffected to date. One of the youngest probands (age 14) had no family history of colorectal cancer, and was found to have a microsatellite stable, immuno-intact tumour. This raises the possibility that the subject has a de-novo mutation, or an autosomal recessive syndrome causing such a young case of colorectal cancer. This patient (patient #4) and three others (patient #11,12, and 16) in this series whom did not have *MLH1* and *MSH2* mutations identified had *MYH* analyzed by dHPLC and no mutations were identified (25) (data not shown).

Gafanovich et al evaluated pediatric patients with a variety of primary malignancies including colorectal adenocarcinoma who developed second tumours (26). In agreement with our observations high frequency microsatellite instability was identified in all nine second tumours from children evaluated. These findings suggest the presence of a mutator phenotype that predisposes to the development of second malignancies.

In summary, patients with early-onset colorectal carcinoma often have an inherited predisposition to the disease. They are at increased risk of developing second gastrointestinal and extra-intestinal malignancies. Tumours with high frequency microsatellite instability and germline mutations of mismatch repair genes are sufficiently common in this patient population that they should be sought for, even though family histories may not satisfy the stringent Amsterdam criteria for HNPCC.

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