

## COLON CANCER

## 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 non-polyposis 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 eight (73%) of 11 evaluated patients. Germline mutations in mismatch repair genes were identified in six of 12 patients, including *MSH2* (n=3), *MLH1* (n=2), and *PMS2* (n=1). Ten (63%) of 16 families met the Amsterdam criteria for HNPCC. Among these, six were screened for mismatch repair gene mutations and three 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 caecum (n=2). Forty four per cent (7/16) of these young cases developed additional malignancies (gastrointestinal (n=8) and extraintestinal (n=4)) during follow up (mean 12.8 (SD 12.4) years (range 0.08–30)).

**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 extraintestinal malignancies.

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 one million people under the age of 20 years has been reported.<sup>1,2</sup> 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 (1980–2002) (personal communication C Herbert, Ontario Cancer Registry, May 2004).

Of the cancer predisposition syndromes which cause early onset colorectal cancer, hereditary non-polyposis colorectal cancer (HNPCC) is the most common, with penetrance that approaches 60–80%.<sup>3,4</sup> HNPCC is an autosomal dominant condition characterised by the development of mostly proximal colon cancer in early adulthood. 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 gynaecological and urinary tract malignancies.<sup>5</sup>

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*.<sup>6,7</sup> Inherited germline mutations of mismatch repair genes are found in up to 50% of HNPCC subjects from families meeting the Amsterdam criteria.<sup>8</sup> 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.<sup>7</sup>

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.<sup>9</sup> 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

**Abbreviations:** HNPCC, hereditary non-polyposis colorectal cancer; FAP, familial adenomatous polyposis; JPS, juvenile polyposis syndrome; PJS, Peutz-Jeghers syndrome

genetic aspects of the tumour or genetic susceptibility of the families.<sup>10-13</sup> Bhatia *et al* studied 25 subjects with colorectal cancer diagnosed at age 21 years or younger<sup>14</sup> to assess the genetic contribution to disease, characterised by a family history of cancer. A sixfold excess of colorectal cancer was identified among relatives of probands diagnosed with colorectal cancer before the age of 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 clinicopathological 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 were 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 counsellor 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, Toronto, Canada.

### Analysis of tumour 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.<sup>15</sup> 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, Alabama, USA). These specific microsatellites were derived from the National Cancer Institute reference and alternative loci panels.<sup>7</sup> Primer sequences and conditions of the polymerase chain reaction assays and gel electrophoresis have been published previously.<sup>15</sup>

The presence of additional bands in the polymerase chain reaction product from tumour 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

**Table 1** Clinical features of the young colorectal cancer patients

Patient No	Age at colorectal cancer diagnosis (y)	Sex	Site	Presentation	Am I or II	Colorectal polyps	Second cancer, age (y)	
							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 diarrhoea, 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	Caecum	Not reported	I	3 adenomatous polyps	Rectal, 21 Duodenal, 28	
9	20	M	Sigmoid	Rectal bleeding	I			
10	21	M	Hepatic flexure	Anaemia	I			
11	22	M	Splenic flexure	Rectal bleeding, anaemia	II			
12	22 (d)	M	Caecum	Anaemia, 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 diarrhoea	I		Colorectal, 40	
16	24	F	Sigmoid	Not reported			Colorectal, 26	

\*Patient No 8 is the daughter of patient No15. d, deceased.

Am I, Amsterdam I criteria: three 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 the age of 50 years.

Am II, Amsterdam II criteria: three relatives with a hereditary non-polyposis colorectal cancer 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 the age of 50 years.

**Table 2** Molecular features of young colorectal cancer patients

Patient No	Immunohistochemistry			MMR germline testing	Other molecular genetic information
	MSI	MLH1	MSH2		
1	H	I	I	Homozygous <i>MLH1</i> mutation <sup>20</sup> Exon 18 codon 687 CGG>TGG Arg287Trp	Normal karyotype
2	H	NT	NT	<i>PMS2</i> mutation <sup>19</sup> Exon 5 codon 134,CGA→TGA Arg→stop	
3	H	I	D	<i>MSH2</i> mutation 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 <i>MSH2</i> deficient tumour, no germline mutation found
8*	NT	NT	NT	NT	Kin had MSI-H and <i>MLH1/MSH2</i> immuno intact tumour, no germline mutation found
9	H	I	D	<i>MSH2</i> mutation Exon 12 codon 636 G→C (1906G>C A636P)	
10	H	D	I	<i>MLH1</i> mutation Exon 19 codon 730 (4bp ins AACA) 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 <i>MLH1</i> promoter
13	NT	NT	NT	No mutation found ( <i>MLH1</i> and <i>MSH2</i> )	
14	NT	NT	NT	<i>MSH2</i> mutation 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 No 8 is the daughter of patient No 15.

NT, not tested; MMR, mismatch repair.

MSI, microsatellite instability—H, high frequency of microsatellite instability; S, microsatellite stable.

Immunohistochemistry: I, intact; D, deficient.

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 (that is, instability at 1–3 of 10 loci).<sup>7</sup>

### MSH2 and MLH1 protein expression in colon cancers

Immunostaining was performed using anti-MSH2 (clone FE11, 1/50; Oncogene Research Products, Cambridge, Massachusetts, USA) and anti-MLH1 (clone G168-728, 1/250; Pharmingen, San Diego, California, USA), as previously described.<sup>16</sup> 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 (Canadian Life Technologies, Burlington, Ontario, Canada), according to manufacturer's protocols.

**Table 3** Second cancers in seven 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

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.<sup>17</sup>

## RESULTS

### Patient demographics (table 1)

Of all probands with colorectal cancer referred to our registry between 1960 and 2003 (n = 1382), 16 were 24 years or younger at the time of initial diagnosis; eight subjects were younger than 18 years. Six of the 16 cases were male (38%).

Eight of the 16 family histories met the Amsterdam criteria for HNPCC, and two others satisfied the modified Amsterdam criteria.<sup>18</sup> 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 10 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 16 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 No 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 eight 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<sup>19</sup>). One patient was homozygous for a missense *MLH1* mutation. Her family has been reported in detail previously.<sup>20</sup>

Immunohistochemistry for *MLH1* and *MSH2* proteins was performed on tumours from eight 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,<sup>21</sup> and two tested children were found to have a normal karyotype.

Seven of the 16 (44%) patients developed a second cancer during follow up (table 3) (mean 12.8 (SD 12.4) years; range 0.08–30). The majority of second tumours (75%) were in the gastrointestinal tract (n = 8). Five patients developed two second cancers. Seventy one per cent of subjects (5/7 patients) who developed second cancers had high frequency microsatellite instability in their original colorectal tumours. 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 tumour was not tested for high frequency microsatellite instability. To date, six of 16 patients (38%) are deceased.

## DISCUSSION

The purpose of this report was 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.<sup>20</sup> 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 11 years of age. A sister (six years old) has a plexiform neurofibroma and other characteristics of neurofibromatosis type 1. Homozygous germline *MLH1* mutations associated with haematological malignancy and neurofibromatosis have been reported at an early age in occasional families with HNPCC.<sup>22</sup> 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 germline 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 probably did not have FAP. The youngest child (patient No 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 (patient Nos 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 have been identified to date. The tumour from the relative of patient No 7 had normal expression of *MSH2* and *MLH1* by immunohistochemistry. The relative of patient No 8 had a tumour which was identified as *MSH2* immunodeficient. These two families met the Amsterdam and modified Amsterdam criteria. Therefore, HNPCC is likely even though germline mismatch repair gene mutations were not found. The fourth patient (patient No 2) who had three adenomatous polyps developed a glioblastoma 12 years after the initial diagnosis of colorectal carcinoma. She had a *PMS2* mutation but no *APC* mutation.<sup>19</sup>

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 was 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 tumour 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 eight patients who met the Amsterdam criteria, three had germline HNPCC mutations. Two of five 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* promoter (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) and at a population based cancer registry (median age 31 years; range 14–35). Seventy per cent 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.<sup>23</sup>

In our series, colorectal cancer was seen in a parent of the affected child in seven of 16 families, with 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.<sup>24</sup> 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 five affected children with known or obligate HNPCC carrier status remain unaffected to date. One of the youngest probands (age 14 years) 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 (No 4) and three others (Nos 11, 12, and 16) in this series who did not have *MLH1* and *MSH2* mutations identified had *MYH* analysed by dHPLC and no mutations were identified<sup>25</sup> (data not shown).

Gafanovich *et al* evaluated paediatric patients with a variety of primary malignancies, including colorectal adenocarcinoma, who developed second tumours.<sup>26</sup> 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 extraintestinal 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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