

Defining the polyposis/colorectal cancer phenotype associated with the Ashkenazi *GREM1* duplication: counselling and management recommendations

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Summary

Hereditary mixed polyposis is a genetically heterogeneous, autosomal dominant condition with adenomatous, hyperplastic and juvenile polyps. We conducted a comprehensive clinical evaluation of a large Ashkenazi Jewish family with this phenotype and performed extensive genetic testing. As seen in one previous report, a 40 kb duplication upstream of *GREM1* segregated with the polyposis/colon cancer phenotype in this kindred. Our study confirms the association of *GREM1* with mixed polyposis and further defines the phenotype seen with this mutation. This gene should be included in the test panel for all Jewish patients with mixed polyposis and may be considered in any Ashkenazi patient with unexplained hereditary colon cancer when mutations in other hereditary colon cancer genes have been ruled out.

Introduction

Familial syndromes involving polyposis and colon cancer underlie 5% of colon cancer cases. Medical surveillance, and in some cases prophylactic surgery, can reduce morbidity and mortality in affected individuals (Gala & Chung, 2011). While clinical data are indispensable to the diagnostic workup, a definitive diagnosis normally rests on genetic testing. In a substantial number of patients presenting with familial colorectal cancer no underlying genetic defect is detected (Lindor, 2009).

Hereditary mixed polyposis syndrome (HMPS) is a genetically heterogeneous, autosomal dominant condition characterized by adenomatous, hyperplastic, and in some cases, juvenile polyps (Whitelaw *et al.*, 1997). *BMPRIA* and *MUTYH* mutations account for a small number of cases (Boparai *et al.*, 2008; Cheah *et al.*, 2009; O’Riordan *et al.*, 2010), but the underlying genetic defects remain unknown in the majority. Recently an unusual genetic alteration, a 40 kb duplication in the 3’ end of the *SCG*

gene upstream of *GREM1*, was found among a series of Ashkenazi Jewish HMPS families (Jaeger *et al.*, 2012). This duplication causes overexpression of *GREM1*, which is a negative regulator of BMP signalling. The pathogenesis of polyps in HMPS likely overlaps with juvenile polyposis syndromes caused by inactivating mutations in genes of the BMP pathway.

There are limited data on the prevalence of *GREM1* mutations in colon cancer families. Based on specific testing for this rearrangement it appears to be extremely rare among unselected colon cancer cases (Jaeger *et al.*, 2012) and among non-Ashkenazi patients with multiple hyperplastic polyps (Clendenning *et al.*, 2013). This gene has not previously been included in targeted panels for hereditary polyposis or Lynch syndrome. Even in more comprehensive tests, such as whole-exome sequencing, the usual analysis focuses on point mutations and small indels, and would miss a 40 kb duplication. Analysis of *GREM1* for upstream duplications and other activating mutations among a large set of polyposis and colon cancer cases will be required in order to determine its attributable risk among patients with these phenotypes.

With the identification of the *GREM1* duplication, a more complete and less biased description of the

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spectrum of malignant and non-malignant clinical features in Ashkenazi HMPS can be compiled by examining non-proband members of families who test positive for the mutation. We conducted a comprehensive clinical evaluation of a large Ashkenazi Jewish family presenting with mixed polyposis and early-onset colon cancer. Extensive genetic testing revealed the *GREM1* upstream duplication. Our findings further support the association between *GREM1* and HMPS, and help define phenotypic features of this condition.

Materials and methods

Subjects

A large HMPS kindred was ascertained through a 21-year-old proband with metastatic colon cancer and his 23-year-old sister who presented for genetic counselling. Written informed consent was obtained from 18 family members who provided blood or saliva samples. Among these subjects seven underwent a complete examination by a medical geneticist, full body skin examination by a dermatologist, and a full ophthalmologic exam. Medical records and pathology reports were sought from all affected family members and were used to determine polyp counts and histopathology. This work was reviewed and approved by the Yale University Institutional Review Board.

Genetic testing

Whole-exome sequencing was performed by the Yale Center for Genome Analysis following procedures available at <http://ycga.yale.edu/about/writeups.aspx>. Briefly, sheared genomic DNA was captured on a Nimblegen, SeqCap EZ Exome version 2 following the manufacturer's protocols (Roche/NimbleGen). Captured libraries were sequenced on the Illumina GAII as 75-bp single-end reads. Image analysis and base calling was performed by Illumina pipeline versions 1.3 and 1.4 with default parameters. Variant calling used an integrated pipeline that employed SAMtools and BWA.

The 40 kb duplication upstream of *GREM1* was detected by PCR using the primers described previously (Jaeger *et al.*, 2012). Each PCR reaction contained 7 μ l ddH₂O, 2.5 μ l PC2 buffer, 2.5 μ l dNTP, 1.25 μ l DMSO, 10 μ l 5M betaine, 0.2 μ l of a mixture of KlenTaq (15 parts) and Pfu DNA polymerase (1 part), and 0.5 μ l each of forward and reverse primers, both at 10 μ M. PCR was carried out in an Eppendorf Mastercycler using cycling conditions: 96 °C (30 sec), 55 °C (30 sec) and 72 °C (40 sec) for 35 cycles. Products were visualized on a 3% agarose gel stained with 5% ethidium bromide.

Results

Clinical report

Medical records and pathology reports obtained from 18 family members established a three-generation pedigree with multiple cases of mixed polyposis and/or colorectal cancer (Fig. 1). The 21-year-old proband had a history of one serrated adenoma, one tubulovillous adenoma, one tubular adenoma and eight hyperplastic polyps as well as metastatic colon cancer. His 23-year-old sister was found on colonoscopy to have one tubulovillous adenoma, one tubular adenoma and two hyperplastic colon polyps. She elected to have a total colectomy because of her brother's outcome.

Among 18 family members for whom medical records were available, at least one polyp was present in 13, all in the paternal lineage. The proband's mother was unaffected and had no significant family history of colorectal cancer or polyps.

Detailed pathology records obtained from seven individuals indicated that the majority of polyps were tubular or hyperplastic. In addition, there were five serrated polyps among three individuals. Two individuals each had one tubulovillous adenoma. Pathology reports detailing the type(s) of polyps in six other family members were sought but were not available. As part of this study, seven affected family members underwent detailed clinical evaluation. A full examination by a medical geneticist did not reveal any major or minor developmental anomalies. An ophthalmology exam including indirect ophthalmoscopy showed no lesions. A full body skin exam by a dermatologist did not demonstrate any unusual skin findings.

Genetic testing

At the time the family was ascertained, the affected brother had already undergone full sequencing of *APC*, which showed no mutation, and full sequencing of *MUTYH*, which showed a variant of unknown significance (P451A) found to be inherited from his unaffected mother. Immunohistochemistry of his colon tumor for MLH1, MSH2 and MSH6 showed no loss of these proteins. Prior to his death he contributed a blood sample for research as part of this study. Whole-exome sequencing identified no mutations in genes known or suspected to be associated with predisposition to colon polyps and colon cancer including *APC*, *AXIN*, *BMPRIA*, *CTTNB1*, *MLH1*, *MSH2*, *MLH3*, *MSH6*, *PMS1*, *PMS2*, *PTEN*, *SMAD4* and *TP53*. The previously identified variant in *MUTYH* was again seen.

During the course of the genetic evaluation the 40 kb duplication upstream of *GREM1* was reported in Ashkenazi Jewish families with HMPS (Jaeger *et al.*,

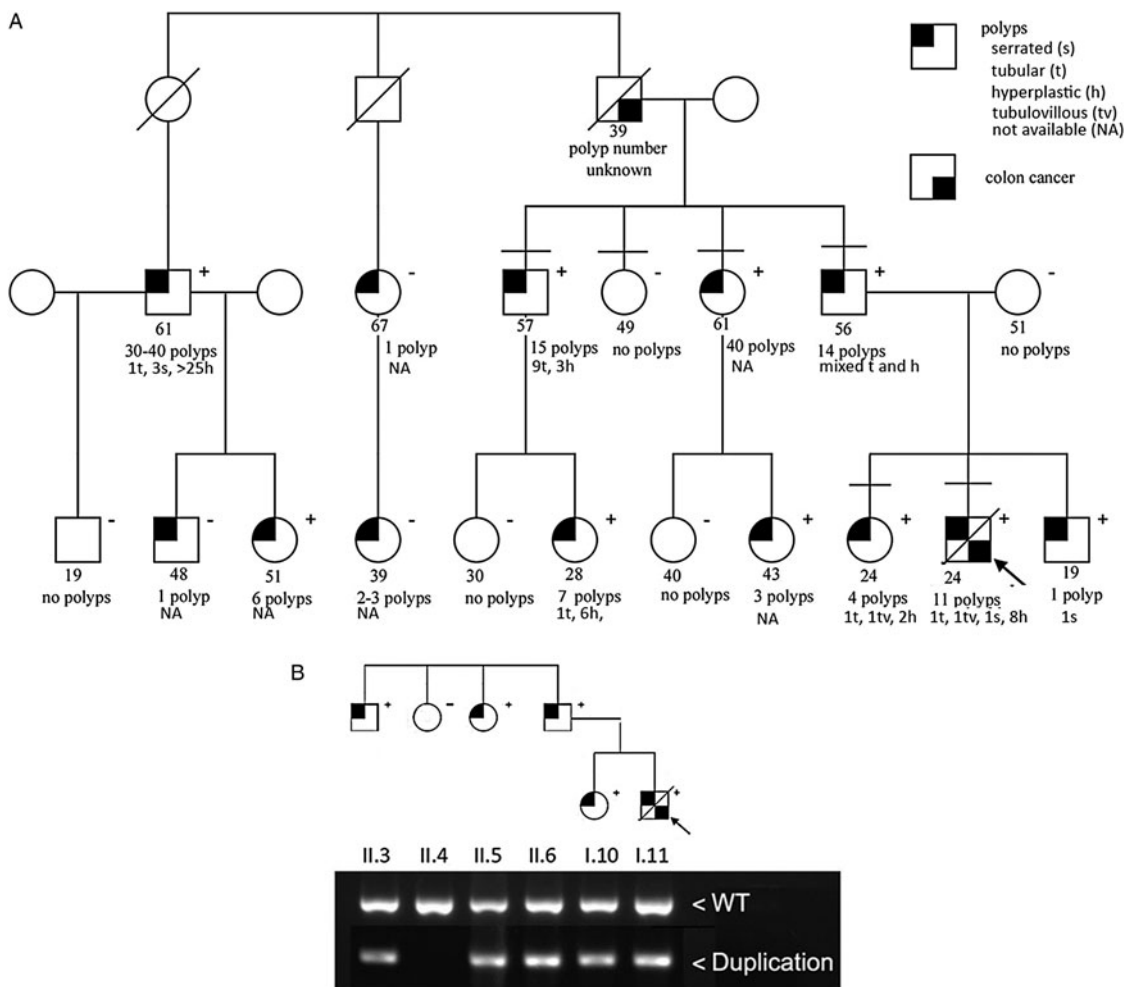


Fig. 1. (a) Pedigree showing age, cancer and polyp phenotype, and presence (+) or absence (-) of a 40 kb duplication upstream from *GREM1*. Symbols with horizontal bars above them indicate family members also shown in (b). (b) PCR product from wild type (WT) region upstream of *GREM1* and an aberrant product produced with PCR primers that flank a duplication breakpoint (Jaeger *et al.*, 2003).

2012). Published primer sequences were used to test members of the current kindred for the duplication (Fig. 1).

Correlation between GREM1 test results and clinical findings

Among 10 family members testing positive for the *GREM1* mutation, polyps were seen in all 10. Detailed pathology was available for seven of these individuals. With the exception of one 19-year-old, who had only one serrated polyp, all others had a mixture of tubular and hyperplastic polyps with several family members also having serrated polyps and tubulovillous polyps. The number of polyps ranged from one in a 19-year-old to 40 in a 61-year-old (mean polyp number 14.1). Notably, multiple polyps were seen in every affected family member over the age of 20.

Among eight family members testing negative, three had one or more polyps (mean polyp number

0-625). No polyps were seen in individuals below the age of 30 and only one individual younger than 40 had any polyps.

Discussion

We performed clinical evaluation and genetic testing of an Ashkenazi Jewish kindred presenting with features of HMPS and found segregation of the 40 kb duplication upstream of *GREM1* with polyp formation and colon cancer. This study confirms results of the single previous research study showing an association between the *GREM1* duplication and HMPS in Ashkenazi Jewish patients (Jaeger *et al.*, 2012).

Clinical features of HMPS include polyps confined to the colon and rectum in relatively low numbers, (typically 1-15 and rarely over 50) and colorectal cancer. Inheritance is autosomal dominant, and the average age at presentation is approximately 40 years. Polyp histology includes adenomatous, hyperplastic,

Table 1. *Clinicopathologic features of the major polyposis syndromes (Patel & Ahnen, 2012; Schreiber et al., 2005).*

Syndrome	Average age at presentation	Polyps			Extracolonic manifestations	Inheritance	Gene(s)
		Type	Typical number				
Hereditary mixed polyposis (HMPS)	41 years	Adenoma, hyperplastic, serrated adenoma	5–80		None	Autosomal dominant	<i>GREM1</i> , <i>BMPRI</i> , <i>MUTYH</i>
Hyperplastic polyposis syndrome	Variable	Hyperplastic, serrated adenomas	6–100+		None	Not defined	Unknown
Peutz–Jeghers syndrome	20–30 years	Hamartoma	10–100+		Skin, buccal mucosa, cancer of several other organs	Autosomal dominant	<i>STK11</i>
Juvenile polyposis syndrome	Variable, often in childhood	Juvenile, adenomas infrequent	50–200		Polyps can be found throughout gastrointestinal tract	Autosomal dominant	<i>BMPRIA</i> , <i>SMAD4</i> , <i>ENG</i>
Cowden syndrome	~40 years	Hamartoma	Variable		Breast, endometrial, thyroid, kidney, skin, CNS, eyes	Autosomal dominant	<i>PTEN</i>
Familial adenomatous polyposis (FAP)	16 years (classic form), 35 years (attenuated form)	Adenoma	Hundreds to thousands (classic)		Duodenum, stomach, bones, skin, CNS	Autosomal dominant	<i>APC</i>
MutYH-associated polyposis	Similar to attenuated FAP	Adenoma, hyperplastic	10–100		Duodenum, occasionally extra-gastrointestinal tumors	Autosomal recessive	<i>MUTYH</i>

and in some cases juvenile polyps (Whitelaw *et al.*, 1997), a clinical constellation distinct from other inherited polyposis or colorectal cancer syndromes (Table 1). Most families with *GREM1*-linked disease have adenomatous and hyperplastic polyps (Jaeger *et al.*, 2003). Juvenile polyps were seen in only one kindred (Whitelaw *et al.*, 1997). Similarly, histologic types in the current family included tubulovillous, hyperplastic, tubular or serrated adenomatous polyps. Juvenile polyps and even partial juvenile histology within a polyp were not reported in any family member. In affected individuals in the current kindred, the average polyp number was 14.1.

Cancer predisposition syndromes often have skin or eye manifestations (Harbour, 1999; Shah *et al.*, 2013). In a previous study (Whitelaw *et al.*, 1997) and in our detailed clinical evaluation no manifestations outside the gastrointestinal tract were observed and polyps were restricted to the colon.

Elements of the medical history in this family provide guidance on appropriate genetic counselling and management of this disease. It is not uncommon for patients to report a personal and/or family history

of mixed and multiple polyposis. Depending on the exact histology and number of polyps, standard genetic testing to establish a diagnosis and treatment plan may include *APC*, *MYH*, *PTEN*, *STK11*, *SMAD4*, *BMPRIA* and the genes associated with Lynch syndrome. Academic and private clinical laboratories offer large genetic panel testing that includes some or all of the above genes; however, these panels would not typically include the *GREM1* upstream duplication. Many laboratories now use whole-exome sequencing to examine large panels of genes, but the *GREM1* duplication would not typically be detected by this methodology because analysis of exome data usually focuses on point mutations and small indels. However, targeted examination of sequencing read numbers in the 3' end of *SCG* could be used to identify an excess in gene dosage among affected individuals (data not shown).

In Ashkenazi Jewish families with HMPS, *GREM1* testing should be the standard of care. A caveat of offering such testing is the limited data available on surveillance and risk reduction. The current study demonstrates that very early-onset colon cancer can

occur in *GREM1* families. For this reason, we suggest that baseline colonoscopy be offered to at-risk individuals carrying the mutation by age 18 or 5 years before the earliest age of onset, whichever comes first. It would seem reasonable to offer yearly stool guaiac tests and repeat colonoscopies every 5 years to family members with the mutation who have normal colonoscopies. In family members with polyps, a gastroenterologist should guide timing of colonoscopies. Although prophylactic colectomy certainly is an option, the cancer risk and typical age of onset in this syndrome seem similar to attenuated familial adenomatous polyposis, which is usually managed by surveillance (Gala & Chung, 2011).

In summary, our findings further document a relationship between the 40 kb duplication upstream of *GREM1* and HMPS in Ashkenazi Jews. Genetic counselling and appropriate molecular testing can lead to successful management of HMPS patients.

Declaration of interest

None.

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