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Features of Patients With Hereditary Mixed Polyposis Syndrome Caused by Duplication of GREM1 and Implications for Screening and Surveillance

Lieberman S^{1*}, Walsh T^{2*}, Schechter M³, Adar T³, Goldin E³, Beerl R¹, Sharon N⁴, Baris H⁴, Ben Avi L⁵, Half E⁶, Lerer I⁵, Shirts BH⁷, Pritchard CC⁷, Tomlinson I⁸, King, MC², Levy-Lahad E^{1,9}, Peretz T¹⁰, Goldberg Y^{10§}

1. Medical Genetics Institute, Shaare Zedek Medical Center, Jerusalem, Israel
2. Departments of Medicine and Genome Sciences, University of Washington, Seattle, WA, USA
3. Gastroenterology Institute, Shaare Zedek Medical Center, Jerusalem, Israel
4. Genetics Institute, Rambam Medical Center, Jerusalem, Israel
5. Department of Human Genetics and Metabolic Diseases, Hebrew University-Hadassah Medical Center, Jerusalem, Israel
6. Gastroenterology Institute, Rambam Medical Center, Jerusalem, Israel
7. Department of Laboratory Medicine, University of Washington, Seattle, WA, USA.
8. Oxford Centre for Cancer Gene Research and Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
9. Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, Israel
10. Sharett institute of Oncology, Hebrew University-Hadassah Medical Center, Jerusalem, Israel

* Contributed equally to this work

§ Corresponding to Yael Goldberg, Sharett institute of Oncology, Hebrew University-Hadassah Medical Center, Jerusalem, Israel
yaelgo43@gmail.com

All authors declare that they have no conflicts of interest

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Abstract

Hereditary mixed polyposis syndrome is a rare colon cancer predisposition syndrome caused by a duplication of a non-coding sequence near the gremlin 1, DAN family BMP antagonist gene (GREM1) originally described in Ashkenazi Jews. Few families with GREM1 duplications have been described, so there are many questions about detection and management. We report 4 extended families with the duplication near GREM1 previously found in Ashkenazi Jews; 3 families were identified at cancer genetic clinics in Israel and 1 family was identified in a cohort of patients with familial colorectal cancer. Their clinical features include extra-colonic tumors, onset of polyps in adolescence, and rapid progression of some polyps to advanced adenomas. One family met diagnostic criteria for Lynch syndrome. Expansion of the hereditary mixed polyposis syndrome phenotype can inform surveillance strategies for carriers of GREM1 duplications.

KEY WORDS: HMPS1; CRC; colon cancer susceptibility, polyposis

Report

Hereditary mixed polyposis syndrome (HMPS1) was originally described in an Ashkenazi Jewish (AJ) family whose members had multiple polyps of more than one histological type (adenomas, hyperplastic and juvenile) and/or individual polyps with overlapping histological features (atypical juvenile with admixed histologic features)¹⁻⁴. The underlying cause was a 40kb duplication upstream of *GREM1* at chr15:30,752,231-30,792,051 (hg19), leading to increased and ectopic expression of *GREM1* in the colonic mucosa. Excess GREM1 protein suppresses bone morphogenetic protein⁴, which in mouse models allows epithelial cells to retain stem cell-like properties, form ectopic crypts, become neoplastic and ultimately cancerous⁶.

Additional reported cases of *GREM1* duplications include more AJ families with the 40kb duplication¹⁻⁷, a family with 16kb duplication in the *GREM1* regulatory region⁸, and a patient with early-onset colorectal cancer (CRC) with a large duplication encompassing the entire *GREM1* gene⁹. However, large genomic alterations at the *GREM1* locus are not routinely identified, because the non-coding region upstream of *GREM1* is not included in most targeted panels, and whole exome sequencing misses duplications in non-coding regions.

Given the paucity of published descriptions of *GREM1*-associated HMPS, management of affected families is challenging. We report four families with the 40kb AJ *GREM1* upstream duplication, who present previously unreported clinical features (Figure 1, Table 1). These families further support the causal relationship between *GREM1* alteration and HMPS1, and help define and extend phenotypic features of this condition.

The four newly identified families carrying the 40kb duplication in *GREM1* are shown in Figure 1. Families 1, 2, and 3 were identified through cancer genetic clinics, and family 4 was identified from a cohort of patients with familial CRC.

Family 1. The proband (III-2) had a colonoscopy at age 41 due to family history of polyposis. It revealed two tubular adenomas and a large sessile rectal polyp with intra-mucosal carcinoma. A year later, eight additional polyps were removed. The proband's cancerous polyp was microsatellite stable (MSS). Tumor molecular testing showed two commonly observed somatic mutations in *KRAS* and *TP53* and two additional somatic mutations in cancer related genes; *PTPN11* and *MSARCB1* (table 1). No *APC* or *BRAF* mutations were identified. Multi-gene germline testing revealed the 40kb *GREM1* duplication, and no other variants related to cancer predisposition. The proband's father (II-2), who also carried the *GREM1* duplication, had multiple adenomatous polyps, an abdominal desmoid tumor,

prostate cancer, and duodenal adenocarcinoma. His grandfather (I-2) had preventive colectomy due to hundreds of polyps. His paternal aunt (II-5) and uncle (II-6) had multiple polyps; two paternal uncles (II-1, II-4) had prostate cancer but no polyps. Among confirmed carriers of the *GREM1* duplication, three younger siblings (III-3, III-5, III-7) had polyps, and the proband's 13 year old daughter (IV-4) had a small tubulovillous adenoma (TVA). One carrier sister (III-6), age 33, did not have polyps. No polyps were found in upper endoscopy of the proband or his siblings.

Family 2. The proband (II-2) had colonoscopies since age 50, with 20-30 polyps found in each. The majority were hyperplastic, with a few adenomas, one with high-grade dysplasia. After 10 years of annual colonoscopies, he underwent subtotal colectomy. Multi-gene testing detected the 40kb *GREM1* duplication, and no other relevant variants. The proband's father (I-4) and two sons (III-2 and III-3) had colonic polyps. One son (III-3) had multiple hyperplastic polyps in his twenties. A cousin (II-1) died of CRC at 50.

Family 3. The proband (III-1) underwent colonoscopy at age 43 due to rectal bleeding. Several polyps were identified, including a 5cm TVA with low-grade dysplasia. Multi-gene testing detected the 40 kb *GREM1* duplication and no other relevant variants. The proband's mother (II-2) had 5 polyps after age 70. GI and endometrial cancers were reported in maternal great aunts (I-1 and I-2).

Family 4. To assess the contribution of the *GREM1* duplication to CRC in the AJ population, targeted genotyping of the 40kb duplication was undertaken in a series of 184 AJ patients (85 males, 99 females) referred for genetic counseling between 2010 and 2015 due to personal or family history of polyposis or CRC. 142 had familial CRC, with mean age at diagnosis of 56.4 ± 13.2 years (range 24–85); the remaining 42 patients had colonic polyps, either >5 polyps or at age <50 . The *GREM1* duplication was identified in one patient, a female (II-1) now age 84, who was diagnosed with metachronous CRC at ages 45 and 49. Her brother died of CRC at 45, and two maternal cousins developed CRC at ages 45 and 47. The proband did not carry the Ashkenazi founder mutations in *BRCA1*, *BRCA2*, *MSH2*, and *MSH6*.

Together, these four families include 16 carriers of the 40kb *GREM1* duplication. Previously unreported clinical features among carriers include extra-colonic tumors, occurrence of polyps as early as adolescence, and rapid polyp growth. We were struck by the overlap of these clinical presentations with those of Familial Adenomatous Polyposis (FAP) and even with Lynch syndrome. In family 1, a patient with the *GREM1* duplication, but normal *APC* sequence, presented with hundreds of polyps, a desmoid tumor, and duodenal adenocarcinoma, all typical of FAP, whereas extra-colonic tumors were not previously reported in HMPS. Tumor molecular testing detected commonly observed somatic mutations in *KRAS* and *TP53*. In the same family, a TVA was observed in a 13 year old girl. While in

most HMPS1 families, ages at onset of polyps are in the late twenties or older, polyps at ages of 10, 16 and 18 have been reported³. Some polyps in patients in family 1 grew aggressively, with 3 patients developing advanced adenomas within 2 years of prior colonoscopy (Table 1). Polyposis is the prominent feature in all previously reported HMPS1 patients and in three of the four families described here. However, family 4 fulfills Amsterdam criteria for Lynch syndrome, with CRC at young age as the prominent feature. Thus, the conventional distinction between polyposis and non-polyposis syndromes may not apply to HMPS1. In other polyposis syndromes, extensive clinical experience helped define diagnostic criteria and shape clinical guidelines for screening and prevention¹⁰⁻¹². This report extends the knowledge of phenotypic spectrum of HMPS1. Current NCCN recommendations for *GREM1* carriers are to begin colonoscopies at age 25-30, repeated at 2-3 year intervals (1-2 years if polyps are detected)¹¹. Our observations, coupled with a previous report of young age of polyp onset³ and rapid polyp growth, suggest earlier screening initiation, perhaps in early adolescence. The presence of extra-colonic features suggests further tailoring based on family-history, similar to the approach in other colon cancer predisposition syndromes. Given the phenotypic variability associated with the AJ *GREM1* founder mutation we suggest testing for this mutation in AJ patients with suspected Lynch, FAP or other polyposis syndromes. In addition, multi-gene panels targeting polyposis and hereditary CRC should include analysis of the entire *GREM1* locus, so as to detect duplications and other copy number variants in non-coding sequence in persons of any ancestry. Casting a wider net in *GREM1* testing will increase diagnosis of inherited polyposis and CRC, and may improve prevention and care for mutation carriers.

Figure 1. Four Ashkenazi Jewish families segregating the 40kb duplication at *GREM1*. Colonic manifestations and other cancers are indicated. Genotypes are indicated as N for normal (no duplication) and V for variant (presence of the duplication).

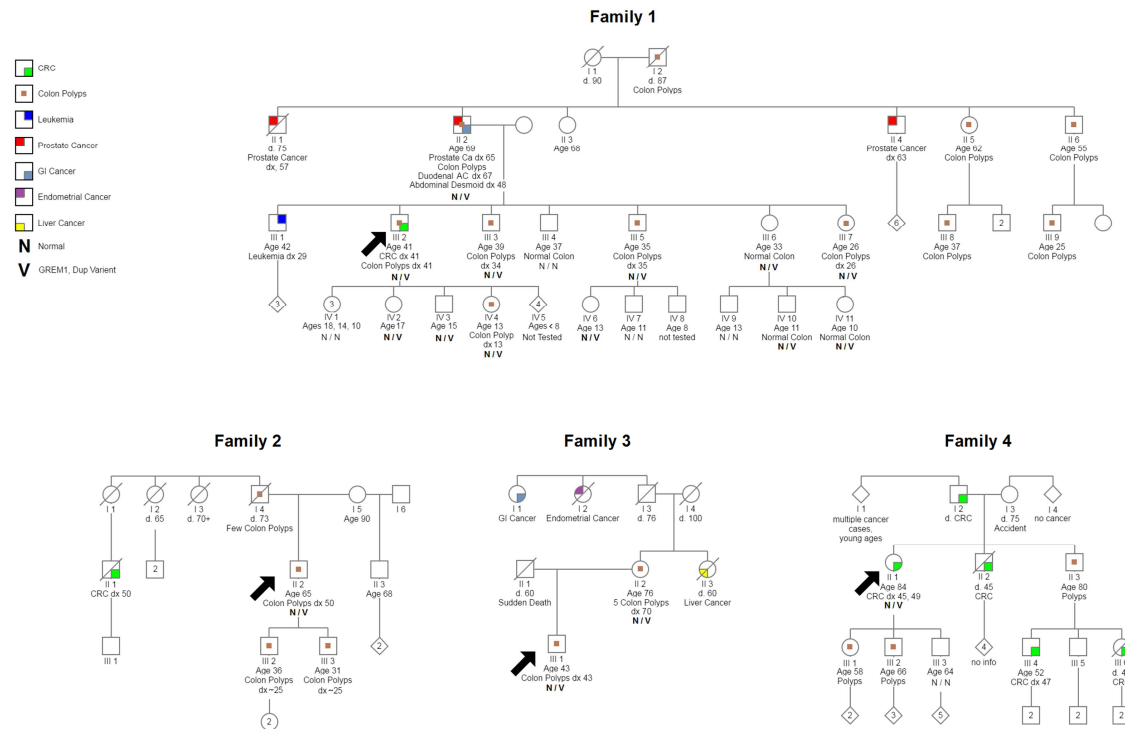
References

1. Thomas HJ, et al. Am J Hum Genet 1996;58(4):770-6.
2. Whitelaw SC, et al. Gastroenterology 1997;112:327-34.
3. Rozen P, et al. Am J Gastroenterol 2003;98:2317-20.
4. Jaeger E, et al. Nature Genet 2012;44:699-703.
5. Ziai J, et al. Genet Res (Cambridge) 2016 Mar 7;98:e5.
6. Davis H, et al. Nature medicine 2015;21:62-70
7. Laitman Y, et al. Genet Res (Cambridge) 2015 May 20;97:e11.
8. Rohlin A, et al. Genes Chromosomes Cancer 2016;55:95-106.
9. Venkatachalam R, et al. Int J Cancer 2011;129:1635-42
10. Lieberman DA, et al. Gastroenterology 2012;143 (3):844-57
11. National Comprehensive Cancer Network. Genetic/Familial High Risk Assessment: Colorectal (Version 1. 2016)
https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf Accessed 2016
12. Syngal S, et al. Am J Gastroenterol 2015;110(2):223-62

Table 1: Colonic manifestations in GREM1 40 kb duplication carriers

Family	Patient	Sex ^a	Age at first colonoscopy /Age at first polyp diagnosis	Polyps at first screening (N)	Polyps at repeat screening within 2 years (N)	Advanced adenoma within 2 years (Y\N ^b)	>5 Polyps in single examination (Y\N ^b)	HGD polyps (Y\N ^b)	Total polyps (N)	A/H/J/I polyps ^c	Cancer diagnosis	Molecular pathology (Gene: mutation (protein), % ^f)
1	II2	M	45/45	NA ^d	<5	Y	Y	N	>50	Multiple A/one documented H	I. Desmoid dx.48 II. Prostate dx. 65 III. Duodenal adenocarcinoma dx.67	
1	III2	M	41/41	3	5	Y	Y	Y	12	9A/1H/ 1 SSA/1 I	CRC dx.41	TP53: c.818G>A (p.R273H), 17% 11% KRAS: c.35G>A (p.G12D), 31% 30% PTPN11C: 215C>T (p.A72V), 19% 16% SMARCB1: C.215C>A (p.T72K).19% 19%
1	III3	M	34/34	14	3	N	Y	Y	20	14A /1H/1J Other NA	-	
1	III5	M	35/35	3	4	Y	N	N	?	4A, 2I, 1 H	-	
1	III6	F	33/-	0	0	0	N	N	0	-	-	
1	III7	F	26/26	2	NR ^e	NR	N	N		2A	-	
1	IV2	F	-/-	-	-	-	-	-	-	-	-	
1	IV3	M	15/-	0	NR	NR	N	N	0	-	-	
1	IV4	F	13/13	1	NR	NR	N	N	1	1A	-	
1	IV6	F	13/-	0	NR	NR	N	N	0	-	-	
1	IV10	M	11/-	0	NR	NR	N	N	0	-	-	
1	IV11	F	10/-	0	NR	NR	N	N	0	-	-	
2	II2	M	50/50	20	>20	N	Y	N	>>50	Multiple H/ some A	-	
3	III1	M	43/43	4	NR	NR	N	Y	7	3A, 4H	-	
3	II2	F	70/70	3	2	N	N	NA	5	NA	-	
4	II1	F	NA	NA	NA	NA	NA	NA	NA	NA	I. CRC dx.45 II. CRC dx.49	

^aM, male; F, female; ^bY\N yes or no; ^cA, adenomatous; H, hyperplastic; SSA, sessile serrated adenoma; J, juvenile; I, inflammatory ; ^dNA, not available; ^eNR, not yet repeated ; ^f % of DNA sequences.



Supplement material - Subjects and methods:

Subjects: Patients were ascertained at three medical institutions in Israel. Clinical and pathology data was retrieved from medical records. This study was approved by the IRBs of all institutions. All participants provided written informed consent. The series of patients genotyped for the duplication were AJ individuals referred for genetic counselling between 2010-2015 at two institutions, either with a diagnosis of familial CRC, or with a diagnosis of colonic polyps; either multiple (>5) or at a young age (<50y).

Genetic testing: Testing for AJ founder mutations was performed as previously published¹.

Gene panel: Germline genomic DNA for each patient was hybridized using the BROCA panel for 65 genes known or suggested to harbor mutations leading to solid tumors². Each locus was fully sequenced to a depth of at least 100x, including exons, untranslated regions, introns, and 5-10 kb of intergenic sequence flanking the longest transcript. Sequencing was carried out and variants were evaluated as previously described³. Genomic deletions and duplications, including the duplication at the *GREM1* locus, were identified by analysis of BROCA sequence read depth, as previously described⁴. With the exception of the *GREM1* duplication, no potentially damaging variants were detected in any family in any of the 65 genes sequenced.

For each patient, the duplication in *GREM1* was confirmed and its exact breakpoints determined by PCR amplification and Sanger sequencing. PCR primers specific to the duplication, 5'-GGGCATCTTCTGGTCTCT-3' (forward) and 5'-AGTGAGACCTGGGGAAAG-3' (reverse), and control primers 5'-GGGCATCTTCTGGTCTCT-3' (forward) and 5'-CGACCGGGTCTTATGTATC-3' (reverse), were kindly provided by Emma Jaeger, Oxford University.

Tumor Testing: The tumor area was identified by a pathologist on an H&E slide. An appropriate region was selected from the corresponding unstained slide(s). Tumor cells consisted 60% of cells analyzed. Analysis has been done twice. NGS, based on an Ion Torrent system was done for identifying mutation, including ~2,800 COSMIC mutations in 50 cancer related genes. The detected regions are from the genes⁵: *ABL1*, *EZH2*, *JAK3*, *PTEN*, *AKT1*, *FBXW7*, *IDH2*, *PTPN11*, *ALK*, *FGFR1*, *KDR*, *RBI*, *APC*, *FGFR2*, *KIT*, *RET*, *ATM*, *FGFR3*, *KRAS*, *SMAD4*, *BRAF*, *FLT3*, *MET*, *SMARCB1*, *CDH1*, *GNA11*, *MLH1*, *SMO*, *CDKN2A*, *GNAS*, *MPL*, *SRC*, *CSF1R*, *GNAQ*, *NOTCH1*, *STK11*, *CTNNB1*, *HNFI1A*, *NPM1*, *TP53*, *EGFR*, *HRAS*, *NRAS*, *VHL*, *ERBB2*, *IDH1*, *PDGFRA*, *ERBB4*, *JAK2*, *PIK3CA*.

References:

1. Gabai-Kapara E, et al. *Proc Natl Acad Sci* 2014; 111(39): 14205-10.
2. http://tests.labmed.washington.edu/BROCA#BROCA_Gene_List Accessed Jan 2017
3. Shirts BH, et al. *Genet Med* 2016; 18: 974-981
4. Nord AS, et al. *BMC Genomics* 2011; 12: 184
5. <http://products.invitrogen.com/ivgn/product/4475346>.