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***GREM1* germline mutation screening in Ashkenazi Jewish patients with familial colorectal cancer**

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Summary

Background—A 40 kb ancestral germline duplication upstream of the *GREM1* gene was reported in Ashkenazi families with hereditary mixed polyposis syndrome (HMPS).

Objective—Assess the contribution of the *GREM1* mutation to familial colorectal cancer (CRC) in Ashkenazim.

Methods—Jewish Ashkenazi individuals (n = 472 155 males, 317 females) were genotyped for the *GREM1* duplication, 194 with CRC, 131 had other cancer types (endometrial, pancreatic and ovarian) that show a syndromic association with CRC, and 147 were cancer-free with a suggestive family history of CRC.

Results—One mutation carrier was found who fulfills the Amsterdam criteria for Lynch Syndrome (LS). The prevalence of this mutation amongst LS Ashkenazim is 0.7%.

Conclusion—If validated in additional studies it seems rational to recommend to look for the *GREM1* founder mutation in Ashkenazi individuals with multiple colorectal polyps and/or fulfill the criteria for LS.

Introduction

In about 25% of CRC patients and individuals with multiple colonic polyps a familial clustering is noted, suggestive of inherited predisposition. Yet only in a minority of these cases can germline mutations in the Mendelian CRC genes be detected. There are several known CRC syndromes affecting up to 5% of incident CRC cases: LS and familial

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Declaration of interest

None.

adenomatous polyposis are the most common ones with a defined phenotype (reviewed by Jaspersion *et al.*, 2010 and Patel & Ahnen 2012). Other rarer syndromes include a host of colonic polyposis syndromes that are clinically hallmarked by multiple colonic polyps (sometimes with distinct histological or phenotypic features) usually detected at a young age and associated with a substantially increased risk of CRC and other cancer types (reviewed by Jaspersion *et al.*, 2010 and Patel & Ahnen 2012). Mutations in several genes have been reported to underlie these mixed polyposis syndromes: *LKB1/STK11* (Peutz-Jeghers syndrome); *MUTYH* (*MUTYH* associated polyposis, MAP and some cases of serrated polyposis syndrome – SPS); *SMAD4* and *BMPRIA* (in juvenile polyposis); and *PTEN* and *GREM1* (HMPS) (reviewed by Omundsen & Lam, 2012). In HMPS, the only causal mutation known to date affecting the *GREM1* gene is an ancestral 40 kb duplication upstream of the *GREM1* gene that is specifically present in individuals of Ashkenazi origins (Jaeger *et al.*, 2012).

Ancestry-specific mutations in other cancer susceptibility genes have been described in the Ashkenazi ethnic group. Examples include breast and ovarian cancer predisposition genes *BRCA1* (c.185delAG) and *BRCA2* (c.61774delT) (Roa *et al.*, 1996), and CRC genes *APC* (p.Ile1307Lys) (Woodage *et al.*, 1998), *MSH2* (p.Ala636Pro) (Foulkes *et al.*, 2002) and *MSH6* (c.3984dup4) (Goldberg *et al.*, 2010). Of these, I1307 K**APC* and 6174delT**BRCA2* have been shown to be founder mutations (Greenwood *et al.*, 2010). Since the *GREM1* HMPS duplication is also likely to be a Jewish Ashkenazi founder 40 kb mutation, its contribution to the burden of familial CRC in the Ashkenazi population was evaluated in the present study.

Materials and methods

Study participants

Eligible participants were all Jewish Ashkenazi individuals counselled at the Oncogenetics Unit, the Institute of Human Genetics at the Sheba Medical Center, Tel-Hashomer, Israel from 1 January 2008–31 December 2012. All participants were referred for counselling either because they were either diagnosed with colorectal or other LS-associated cancer types under age 50 years, or were diagnosed with CRC and had at least two affected first- or second-degree relatives (on the same parental lineage) with CRC at any age, fulfilled the Bethesda guidelines, had 30 or more adenomatous polyps initially diagnosed under age 45 years or were healthy but with at least three first- and/or second-degree relatives with colon or other LS-associated tumors on the same parental side.

Genotyping for the predominant Jewish mutations in the *MSH2* and *MSH6* genes

All participants underwent genotyping for the predominant Ashkenazi Jewish mutations in the *MSH2* gene (A636P) and the *MSH6* gene (3984Dup4) as previously described (Barak *et al.*, 2010). Individuals with multiple polyps were additionally genotyped for the predominant mutations in the *MUTYH* gene (p.G396D and p.Y179C) as previously described (Pitroski *et al.*, 2011).

GREM1 duplication mutation genotyping

Genotyping of the 40 kb duplication upstream of the *GREM1* gene region was performed as previously described (Jaeger *et al.*, 2012) by using PCR primers that map to a unique segment of DNA intercalated between the duplicons and generate a specific PCR product of 190 bp. As a control, a region upstream of *GREM1* was used, which generates a product of 435 bp in all individuals. All positive mutation carriers were confirmed by sequencing.

Results

Study population

A total of 472 individuals were included in the study, 155 males and 317 females. Overall, 402 (85.1%) were of Ashkenazi descent in both parental lineages, and the rest ($n = 70$) were of mixed Ashkenazi/non-Ashkenazi origin, but the CRC history could be traced to the Ashkenazi parent's side of the family. There were 194 participants affected with CRC (94 males and 100 females) with mean age (\pm SD) at diagnosis of 54 ± 13 years, range 20–88 years, median 56 years; 131 individuals were non-colorectal tumor patients [with endometrial ($n = 96$, mean age (\pm SD) at diagnosis 56.5 ± 12.24 years, range 30–78 years, median 59 years), pancreatic ($n = 13$, mean age (\pm SD) at diagnosis 61 ± 8.4 years, range 44–74 years, median 60 years) or ovarian ($n = 22$, mean age (\pm SD) at diagnosis 53.6 ± 11.8 years, range 29–75 years, median 55 years) carcinomas] whose family history were consistent with an inherited predisposition to cancer but did not fulfill all of the Amsterdam criteria for LS (Giardiello *et al.*, 2014).

Additional 147 cancer-free individuals with a strong and suggestive family history of CRC: having two first- or one first- and one second-degree relatives with CRC with at least one of the affected family members diagnosed under age 50 years (39 males mean age at counselling 40.2 ± 11.6 years, range 25–72 years, median 38 years; 108 females mean age at counselling 47.05 ± 13.1 years, range 25–81 years, median 45.5 years). Overall, of the 325 participants who were affected with cancer, 138 were from families that fulfilled the Amsterdam criteria for LS.

Genetic analysis

None of the participants were found to harbour any of the tested mutations in *MSH2*, *MSH6* and *MUTYH*. In one of the participants, the *GREM1* HMPS duplication mutation was found. This individual was a male CRC patient, diagnosed at age 56 years, of maternal Ashkenazi and paternal Russian, non-Jewish origins. Notably, in none of the pathological or colonoscopic reports is there any mention of colonic polyps, although the possibility exists that existing polyps were simply not reported. His family history is noted for a mother with CRC at age 60 years, a maternal grandmother who was diagnosed with CRC at 49 years and a father with gastric cancer diagnosed at 52 years of age.

Discussion

The current study suggests that the HMPS 40 kb duplication upstream of the *GREM1* gene is present at low rates in Jewish Ashkenazi individuals with evidence of a familial

predisposition to CRC. Based on the current study, we estimate that the duplication is present in 0.7% (1/138) of Jewish Ashkenazi individuals with strict clinical LS criteria. Importantly, the individual who was found to harbour the mutation in the current study does not have any clinical evidence of the colonic polyp features suggestive of HMPS. As noted earlier it is still possible that existing polyps were simply not recorded or reported.

The genetic screen for the *GREM1* duplication only identifies carriers of the specific mutation present in HMPS patients. All the evidence based on previous haplotyping analysis and the low prior probability that the specific duplication would arise multiple times are consistent with this mutation representing another example of a founder effect in the Ashkenazi population. The relatedness of this ethnic group has been known and established for many years but was recently supported by a study of the genome in 128 unrelated Ashkenazim (Carmi *et al.*, 2014).

In conclusion, if the results are validated in a larger study that includes more individuals with multiple polyps and/or LS, it would make sense to look for the *GREM1* 40 kb duplication in Ashkenazi individuals who have multiple colorectal polyps and/or who fulfill the criteria for LS in this ethnic group. However, based on the results of the present study such recommendation seems premature.

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