ORIGINAL ARTICLE

Colorectal cancer risk variants on 11q23 and 15q13 are associated with unexplained adenomatous polyposis

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ABSTRACT

Background Colorectal adenomatous polyposis is associated with a high risk of colorectal cancer (CRC) and is frequently caused by germline mutations in *APC* or *MUTYH*. However, in about 20–30% of patients no underlying gene defect can be identified. In this study, we tested if recently identified CRC risk variants play a role in patients with >10 adenomas.

Methods We analysed a total of 16 SNPs with a reported association with CRC in a cohort of 252 genetically unexplained index patients with >10 colorectal adenomas and 745 controls. In addition, we collected detailed clinical information from index patients and their first-degree relatives (FDRs).

Results We found a statistically significant association with two of the variants tested: rs3802842 (at chromosome 11q23, OR=1.60, 95% CI 1.3 to 2.0) and rs4779584 (at chromosome 15q13, OR=1.50, 95% CI 1.2 to 1.9). The majority of index patients (84%) had between 10 and 100 adenomas and 15% had >100 adenomas. Only two index patients (1%), both with >100 adenomas, had FDRs with polyposis. Forty-one per cent of the index patients had one or more FDRs with CRC.

Conclusions These SNPs are the first common, low-penetrant variants reported to be associated with adenomatous polyposis not caused by a defect in the *APC*, *MUTYH*, *POLD1* and *POLE* genes. Even though familial occurrence of polyposis was very rare, CRC was over-represented in FDRs of polyposis patients and, if confirmed, these relatives will therefore benefit from surveillance.

INTRODUCTION

Adenomatous polyps are the primary precursor lesions of colorectal cancer (CRC).[1] Patients with large numbers of adenomas (polyposis) have a high likelihood of carrying an inheritable high-penetrance germline defect, particularly those patients diagnosed at a young age. Germline mutations in *APC* and *MUTYH* are commonly found in patients with an adenomatous polyposis phenotype. More recently, mutations in the *POLD1* and *POLE* genes were also shown to be a cause of polyposis.[2] Nevertheless, a substantial number (ie, an estimated 20–30%, also depending on polyp count and age at diagnosis) of patients remain unexplained. Some susceptibility of these unexplained cases may be attributable to the SNPs that are known to increase CRC risk (table 1). Eight of these SNPs were recently shown to be over-represented in CRC-free patients with few (mean=2) adenomatous polyps.[3]

The main aim of our study was to investigate whether CRC-risk SNPs are associated with disease in patients with more than 10 colorectal adenomas but without a known causative mutation, that is, no germline mutations in the *APC*, *MUTYH*, *POLD1* and *POLE* genes. In addition, the phenotype in index patients and their first-degree relatives (FDRs) was evaluated.

METHODS

Patients

Patients were genotyped in a consecutive series of 1216 index patients tested for APC and/or MUTYH germline mutations at the Laboratory for Diagnostic Genome Analysis (LDGA) in Leiden, from 1986 to January 2009. Informed consent was given for further research at initial blood withdrawal. Exclusion criteria were less than 10 adenomas, low-quality DNA samples, a phenotype with predominantly hyperplastic polyposis or missing clinical information on the histopathology of the polyps.

Clinical data were collected from the Netherlands Foundation for the Detection of Hereditary Tumors (NFDHT) and from clinical genetics departments in the Netherlands. Collected data included date of birth, gender, date of diagnosis of polyposis, cumulative number of polyps counted at colonoscopy or in excised bowel, location and histology of polyps, presence of duodenal polyps, information on CRC, polyps/CRC in first-degree family members, date of last contact and status at last contact. The control cohort was described previously[4] and comprised a total of 745 controls for which both gender and age at blood sampling was known were included.

Genotyping

The *APC* and *MUTYH* genes had been tested as published previously.[5 6] Hotspot mutations for *POLE* (p.L424V) and *POLD1* (p.S478N) were

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Table 1 Summary results for the tested SNPs in 252 patients with unexplained adenomatous polyposis and 745 healthy controls

Chr	SNP	Base-pair position†	Allele*		MAF		p Value	OR (95% CI)	Gene	Reference
			Minor	Major	Patients	Controls				
1q41	rs6691170	222045446	T	G	0.378	0.346	0.194	1.1 (0.9-1.4)	*DUSP10 CICP13*	32
1q41	rs6687758	222164948	G	A	0.228	0.197	0.141	1.2 (0.9 to 1.5)	*DUSP10 CICP14*	32
3q26.2	rs10936599	169492101	T	C	0.237	0.244	0.745	1.0 (0.8 to 1.2)	*MYNN*	32
8q23.3	rs16892766	117630683	C	A	0.086	0.086	0.998	1.0 (0.7 to 1.4)	*TRPS1 EIF3H*	33
8q24.21	rs6983267	128413305	G	T	0.575	0.530	0.078	1.2 (1.0 to 1.5)	*POU5F1 MYC*	34
10p14	rs10795668	8701219	A	G	0.290	0.324	0.154	0.9 (0.7 to 1.1)	*KRT8P16 TCEB1P3*	33
11q23	rs3802842	111171709	C	A	0.355	0.256	0.00002	1.6 (1.3 to 2.0)	*C11orf93*	11
12q13.13	rs7136702	50880216	T	C	0.357	0.327	0.222	1.1 (0.9 to 1.4)	*TBX3 UBA52P7*	32
12q13.13	rs11169552	51155663	T	C	0.260	0.264	0.876	1.0 (0.8 to 1.2)	*DIP2B ATF1*	32
14q22.2	rs4444235	54410919	C	T	0.444	0.437	0.763	1.0 (0.8 to 1.3)	*RPS3AP46 BMP4*	12
15q13	rs4779584	32994756	T	C	0.256	0.187	0.001	1.5 (1.2 to 1.9)	*SCG5 GREM1 FMN1 CRAC1*	35
16q22.1	rs9929218	68820946	A	G	0.280	0.278	0.921	1.0 (0.8 to 1.3)	*CDH1*	12
18q21.1	rs4939827	46453463	C	T	0.486	0.491	0.864	1.0 (0.8 to 1.2)	*SMAD7*	14
19q13.1	rs10411210	33532300	T	C	0.090	0.092	0.918	1.0 (0.7 to 1.4)	*RHPN2*	12
20p12.3	rs961253	6404281	A	C	0.386	0.375	0.661	1.0 (0.9 to 1.3)	*TARDBPL BMP2*	12
20q13.33	rs4925386	60921044	T	C	0.297	0.316	0.423	0.9 (0.7 to 1.1)	*LAMA5*	32

*For SNP rs6983267, the minor allele originally reported (G) is the most frequent allele, both in our cases and controls.
†The SNP positions are according to Human Build 36.3.
Chr, chromosome; MAF, minor allele frequency.

determined for all cases using an in-house developed KASPar assay (primers available upon request). Next, those cases without *APC*, *MUTYH* or the *POLD1* or *POLE* hotspot mutations were selected for genotyping the 16 SNPs listed in table 1. Anonymised samples of leucocyte DNA from these cases and controls were tested using the Competitive Allele-Specific PCR (KASPar) assay as described previously.[7] All KASPar genotyping assays were performed by KBiosciences, UK.

Statistical analysis

All statistical analyses were carried out using PLINK,[8] including calculations of allele differences between cases and controls, genotype call rate per sample and per SNP, deviations from Hardy–Weinberg equilibrium (HWE), as well as multiple test correction. Genotypes of cases and controls were compared using a basic χ^2 allelic test. SNP profiles of specific subcohorts are available upon request. Multiple test correction was done using false discovery rate estimation.[9]

RESULTS

Genotype

Of the 1216 index patients surveyed, 252 patients fulfilled our inclusion criteria for SNP genotyping. We analysed 16 SNPs and compared allele frequencies in our mutation-negative cohort and in the control population (table 1). The call rates were above 99%. No deviations from HWE were observed. The association was strongest for SNPs rs3802842 ($p=2.0\times10^{-5}$; OR=1.60, 95% CI 1.3 to 2.0) and rs4779584 (p=0.001; OR=1.50, 95% CI 1.2 to 1.9) that remained significant after adjustment for multiple testing (p=0.001 and 0.026, respectively).

In order to disentangle whether the identified association for these two SNPs was based on polyposis or CRC, we compared allele frequencies in index patients with (n=96) and without CRC (n=156) and in our control population. The association for SNPs rs3802842 (p=0.0037; OR=1.5 (95% CI 1.1 to 1.9)) and rs4779584 (p=0.023; OR=1.4 (95% CI 1.0 to 1.9)) remained statistically significant for patients without CRC, that is, with a polyposis-only phenotype. In addition, the 14 other SNPs were not significantly associated with CRC.

Phenotype

The clinical description of the 252 patients is listed in table 2. The majority of cases (63%) had a cumulative polyp count of between 10 and 50 (with a mean age at diagnosis of 53 years), 21% showed between 50 and 100 polyps, while 15% of the cases had more than 100 polyps ('carpeted', 'numerous', 'classic polyposis' definitions included). Histopathologic examination showed all or a vast majority of polyps as adenomas in 208 cases (83%), while 44 cases (17%) displayed a mixed phenotype of predominantly adenomas with some hyperplastic polyps. Our cohort included 64 patients that carried both SNPs. These patients did not appear to have a more exacerbated phenotype (ie, 41/160 (26%) in the 10–50 group, 10/53 (19%) in the 50–100 group and 13/39 (33%) in the >100 polyp count group).

CRC was found in 96 patients (38%) at a mean age of 53 years. A substantial number of patients (17%) had two or more synchronous or metachronous CRCs (n=16), and three (n=1) or even four (n=1) synchronous CRCs were also seen. In terms of location, 48 CRCs were found distal to the splenic flexure (left-sided CRC), 23 CRCs were right sided and 9 patients had both left-sided and right-sided tumours. The exact tumour location was unknown for 16 CRCs. A total of 81 patients (32%) underwent upper gastrointestinal endoscopy. Fifteen patients (19%) had duodenal adenomas, and one patient had both duodenal and gastric polyps.

On investigation of family history, 143 (57%) index patients had close relatives with colorectal tumours; 104 patients (41%) had one or more FDRs with CRC, diagnosed at a mean age of 60 years (table 2). A steep increase in CRC incidence for FDRs was observed after the age of 30 (figure 1). Moreover, 103 patients (41%) had one or more FDRs with polyps. A family history with strong phenotypes was present in only two families, and in addition to index patients with >100 adenomas, one

Table 2 Clinical details of three categories of index patients: 10–50 adenomas, 50–100 adenomas and >100 adenomas

Panel A

Index					Personal history					
Adenomas (n)	Number		Mean age	Men (%)	CRC		Men (%)	Age at CRC	Duodenal adenoma	
10–50	160	63%	53 (29–82)	63	59	37%	56	53 (26–75)	7/20	35%
50–100	53	21%	51 (13–73)	57	18	34%	72	55 (37–73)	1/17	6%
>100	39	15%	44 (4–80)	56	19	49%	58	51 (29–80)	7/44	16%
Total	252	100%	51 (4–82)	60	96	38%	59	53 (26–80)	15/81	19%

Panel B

Family history

Adenomas (n)	FH		FDRs with CRC (n)	Age at CRC	FDRs with polyps (n)	Age
10–50	95	59%	72	61 (32–85)	68	55 (30–85)
50–100	30	57%	20	62 (44–91)	21	55 (26–78)
>100	18	46%	12	56 (17–79)	14	51 (15–70)
Total	143	57%	104	60 (17–91)	103	55 (15–85)

Panel A: Clinical details of the index patients. Number of patients, percentage and mean age at diagnosis; number and percentage of male patients; number, percentage, sex distribution and age at diagnosis of CRC in polyposis patients; number of patients with duodenal adenomas/patients who underwent upper gastrointestinal endoscopy.
Panel B: Clinical data of the first-degree relatives (FDRs) of the index patients. Number and percentage of index patients with a positive family history (FH), that is, FDRs with CRC and/or multiple adenomas (ie, <10 polyps).
CRC, colorectal cancer.

sister had >50 adenomas and the brother of the second case had >100 adenomas. The remaining index patients all had FDRs with low polyp counts (ie, less than 10 adenomas).

DISCUSSION

The present study provides new susceptibility loci for patients with colorectal adenomatous polyposis, defined here as a

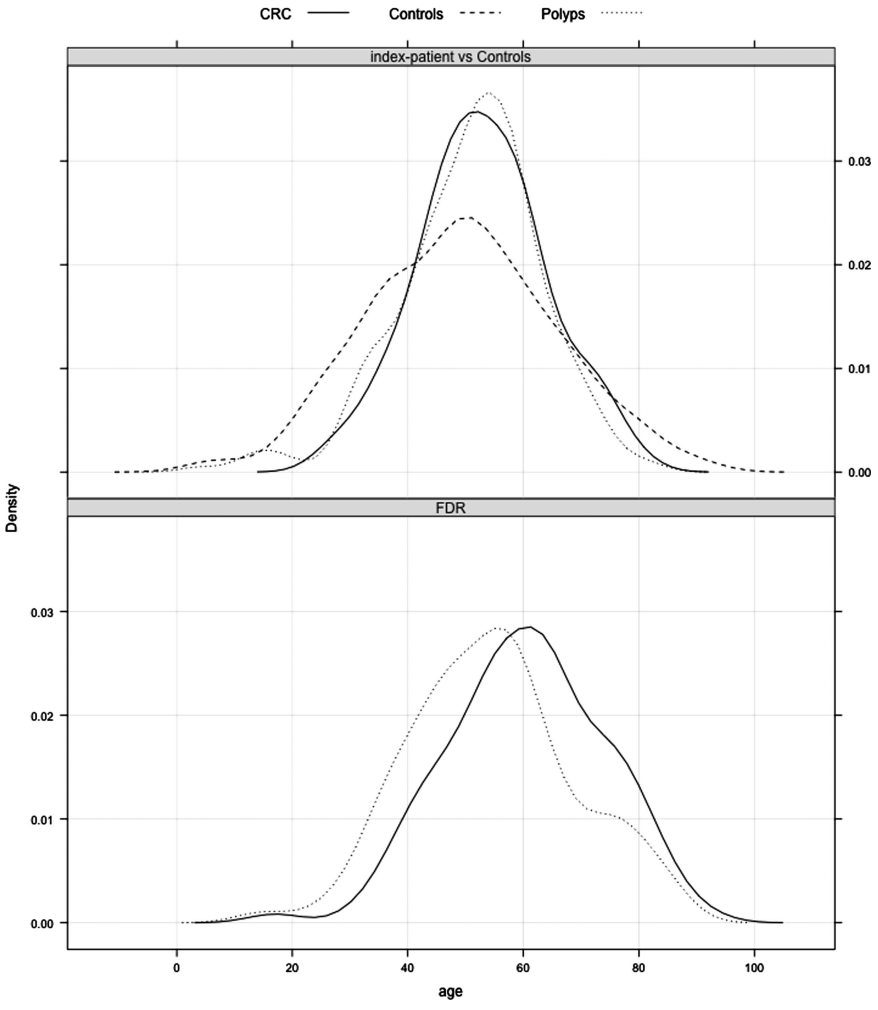

Figure 1 Age distribution as kernel density estimates. Age of controls at blood withdrawal, age of index patients at polyposis diagnosis (Polyps) and age of index patients at colorectal cancer (CRC) diagnosis. The lower part shows the age of diagnosis of polyps and CRC in first-degree relatives (FDRs).

cumulative total of more than 10 colorectal adenomas, without an identified causative germline defect. Variants rs3802842 (on chromosome 11q23.1) and rs4779584 (on chromosome 15q13) are the first common, low-penetrant risk variants reported in this type of patients. The ORs we found (1.60 and 1.50) are much higher than values reported for patients with a few adenomas or for patients with CRC (typically ORs of 1.1 to 1.2).[3 10–12] Our series is biased towards ascertaining index patients and their family members presenting with symptomatic CRC and is not a population-based series of polyposis patients. Therefore, prospective studies are needed to confirm our findings. Moreover, disentanglement of whether the identified association for these two SNPs was based on polyposis or CRC is difficult given how much these two phenotypes are interrelated. However, we found that the association still stood in the index patients with a polyposis-only phenotype, that is, after excluding the index patients with CRC. Therefore, even though the low-risk variants identified in the present study are unlikely to explain polyposis by themselves, they still might act as modifiers of so far unidentified high-risk factors or participate in a multifactorial cause of the phenotype.

The SNP rs3802842 appears to be involved in risk predisposition in hereditary forms of CRC and has previously been associated with early-onset CRC (diagnosed under the age of 50 years), low numbers of adenomas and familial aggregation of CRC.[4 13 14] Moreover, rs3802842 was also shown to be associated with CRC risk in female patients with Lynch syndrome.[15 16] Mechanistically, none of the transcripts (*FLJ45803*, *LOC120376*, *C11orf53* and *POU2AF1*) mapping to the rs3802842 region at chromosome 11q23 showed possible causative exonic mutations, and rs3802842 is currently annotated to an intron of an uncharacterised protein, C11orf93. While the impact of this SNP might be via non-coding effects on gene expression, a clear mechanistic explanation for this association remains elusive despite extensive research.[17]

A more plausible mechanistic explanation may be available for the SNP rs4779584, as its chromosome 15q13.3 locus encompasses the genes *SCG5*, *FMN1* and *GREM1*. A 40 kb duplication upstream of the *GREM1* gene was previously identified in a large Ashkenazi family with a mixed polyposis phenotype.[18] *GREM1* is a component of the TGF-b superfamily signalling pathway acting as a bone morphogenetic protein (BMP) antagonist in the colon. Increased *GREM1* expression is predicted to reduce BMP activity, a mechanism also found in juvenile polyposis.[18] Given the reported association of 15q13 SNP with a mixed polyposis phenotype, we compared the allelic counts in patients with adenoma and patients with a phenotype of predominantly adenomas and some hyperplastic polyps (table 3). However, we did not find an over-representation of the 15q13 SNP in the subset with both adenomas and hyperplastic polyps (p=0.5).

Table 3 Analysis of the rs4779584 SNP on 15q13 in patients with adenoma and patients with a phenotype of predominantly adenomas and some hyperplastic polyps

Phenotype	CC	TC	TT	Total
Adenoma	115	83	10	208
Mixed	24	15	5	44
Total	139	98	15	252

Phenotype

In our large cohort of patients with genetically unexplained polyposis, medical records were scrutinised in order to collect accurate phenotypic data. The majority of subjects exert a phenotype resembling that of attenuated adenomatous polyposis since they had a moderate number of polyps (ranging from 10 to 100) at an advanced age of diagnosis (mean age 52 years). The proportion of patients affected with duodenal adenomas was also in more agreement with *MUTYH*- (ie, 20%) than classic *APC*-associated polyposis (ie, 90%).[19 20] Previous studies on *APC* and *MUTYH* mutation-negative polyposis patients show a clinically heterogeneous disease, with scarce information on family members.[21–30]

Regarding family history, strong phenotypes (>50 adenomas) were present in only two families (1%), and 41% of the index patients had one or more FDRs with single or multiple adenomas (ie, <10 polyps). Moreover, CRC in FDRs was observed in 41% of the index patients. This observation mimics studies on hyperplastic polyposis, where even in the absence of hyperplastic polyposis an increased CRC risk for FDRs of patients was found and for whom surveillance is advised.[23 31]

Clinical implications

Based on the clinical evaluation of our cohort, the following surveillance guidelines for FDRs of polyposis patients without known cause may be considered. First, to exclude the presence of polyposis—and despite the rare occurrence in the relatives of the patients in our series—we would recommend performing colonoscopy in parents, siblings and offspring at an early age. Based on the endoscopic findings, decisions can be made on the need for follow-up examinations. Second, based on the high CRC incidence in FDRs, we would also recommend colonoscopic surveillance, even in the absence of a polyposis phenotype on first colonoscopy at an early age. Given the age distribution of CRC onset in FDRs (figure 1), we suggest that surveillance commence at age 30, with intervals of 5–6 years dependent on endoscopic findings.

In conclusion, this is the first study to report an association between common, low-penetrance genetic variants and an unexplained adenomatous polyposis phenotype. Future studies should explore the mechanistic role of these variants in the development of polyposis. Whereas the familial occurrence of polyposis in relatives was rare, almost half of FDRs developed CRC. If over-representation of CRC in FDRs of polyposis patients is confirmed in other studies, these relatives should undergo colonoscopic surveillance, even in the absence of a polyposis phenotype upon first colonoscopy.

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