OPEN ACCESS

SHORT REPORT

Colorectal cancer genetic variants are also associated with serrated polyposis syndrome susceptibility

Coral Arnau-Collell,[1] Yasmin Soares de Lima,[1] Marcos Díaz-Gay ,[1] Jenifer Muñoz,[1] Sabela Carballal,[1] Laia Bonjoch,[1] Leticia Moreira,[1] Juan José Lozano,[2] Teresa Ocaña,[1] Miriam Cuatrecasas,[3] Aranzazu Díaz de Bustamante,[4] Antoni Castells,[1] Gabriel Capellà,[5] Luis Bujanda,[6] Joaquin Cubiella,[7] Daniel Rodríguez-Alcalde,[8] Francesc Balaguer,[1] Clara Ruiz-Ponte,[9] Laura Valle,[5] Victor Moreno,[10] Sergi Castellvi-Bel [1]

For numbered affiliations see end of article.

Correspondence to
Dr Sergi Castellvi-Bel, Genetic predisposition to gastrointestinal cancer Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Rosselló 153, 08036 Barcelona, Spain; sbel@clinic.cat

Received 13 June 2019
Revised 10 January 2020
Accepted 22 January 2020

ABSTRACT

Background Serrated polyposis syndrome (SPS) is a clinical entity characterised by large and/ormultiple serrated polyps throughout the colon and increased risk for colorectal cancer (CRC). The basis for SPS genetic predisposition is largely unknown. Common, low-penetrance genetic variants have been consistently associated with CRC susceptibility, however, their role in SPS genetic predisposition has not been yet explored.

Objective The aim of this study was to evaluate if common, low-penetrance genetic variants for CRC risk are also implicated in SPS genetic susceptibility.

Methods A case-control study was performed in 219 SPS patients and 548 asymptomatic controls analysing 65 CRC susceptibility variants. A risk prediction model for SPS predisposition was developed.

Results Statistically significant associations with SPS were found for seven genetic variants (rs4779584-*GREM1*, rs16892766-*EIF3H*, rs3217810-*CCND2*, rs992157-*PNKD1*/*TMBIM1*, rs704017-*ZMIZ1*, rs11196172-*TCF7L2*, rs6061231-*LAMA5*). The *GREM1* risk allele was remarkably over-represented in SPS cases compared with controls (OR=1.573, 1.21–2.04, p value=0.0006). A fourfold increase in SPS risk was observed when comparing subjects within the highest decile of variants (≥65) with those in the first decile (≤50).

Conclusions Genetic variants for CRC risk are also involved in SPS susceptibility, being the most relevant ones rs4779584-*GREM1*, rs16892766-*EIF3H* and rs3217810-*CCND2*.

To cite: Arnau-Collell C, Soares de Lima Y, Díaz-Gay M, *et al*. *J Med Genet* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jmedgenet-2019-106374

INTRODUCTION

Colorectal cancer (CRC) is currently one of the most common neoplasms in developed countries, and it represents the second most fatal malignancy after lung cancer.[1] This neoplasm is developed from different precursor lesions, including conventional adenomas and serrated polyps. This heterogeneity drives the progression to carcinoma through distinct pathways.[2] While most CRC progress through the adenoma–carcinoma sequence, serrated polyps, previously known as hyperplastic polyps, are also considered precursor lesions with an alternative pathway to CRC.[3] The term serrated polyp refers to a lesion with a serrated or 'sawtooth' appearance of the colonic crypts at microscopy. On the other hand, the risk of developing CRC is influenced by both environmental and genetic factors. Part of this germline CRC predisposition is already known including both rare, high-penetrance and common, low-penetrance genetic variants.[4] Genome-wide association studies (GWAS) have been conducted since 2007 and have identified ~130 common, low-penetrance genetic variants associated with CRC risk.[5]

Interestingly, prior studies have evaluated the association between GWAS-identified SNPs linked to CRC susceptibility and polyp subtypes, including adenomas, serrated polyps and advanced/non-advanced polyps but not the association with serrated polyposis syndrome (SPS).[6–9]

SPS is a condition characterised by the development of large and/or multiple serrated polyps throughout the colon and increased risk for CRC. The prevalence of CRC in patients with SPS has been estimated to range between 15% and 35%.[3 10] In order to help in identifying this clinical entity, the WHO established in 2010 the following criteria defining SPS as the presence of (I) at least five serrated polyps proximal to the sigmoid colon, of which two measure at least 10 mm in diameter and/or (II) any number of serrated polyps occurring proximal to the sigmoid colon in an individual who has a first-degree relative with SPS and/or (III) more than 20 serrated polyps spread throughout the colon.[11] Despite recent developments in sequencing technologies, the genetic aetiology of SPS remains largely unknown. The only proposed gene for germline SPS predisposition is *RNF43*, which would explain a small proportion of SPS cases.[12–15]

Keeping in mind that serrated polyps are considered CRC precursor lesions and considering that SPS confers a relatively high risk for CRC, the current study aims to test if common, low-penetrance genetic variants for CRC risk are also involved in the predisposition to SPS.

MATERIALS AND METHODS

Study population

Our study comprised 219 Spanish patients ascertained in high-risk clinics for CRC that fulfilled

the SPS clinical criteria.[11] They included 130 men (59.36%) and 89 women (40.63%) aged between 20–78 (mean age 54.62±11.08 years old). Presence of conventional adenomas in SPS patients was frequent (around 70%). We also included 548 asymptomatic controls from the Barcelona CRC screening programme with a positive faecal immunochemical test but a negative colonoscopy showing no relevant findings related to CRC risk. The control group included 269 men (49.08%) and 279 women (50.92%), aged between 50–69 (mean age 59.49±5.57 years old). Cases and controls included in this study were negative for a family history of hereditary or familial CRC or adenomatous polyposis (ie, ≥2 first-degree relatives with CRC/adenomatous polyposis or one first-degree relative diagnosed before the age of 60). DNA was obtained from frozen peripheral blood by standard extraction procedures for all samples. *RNF43* mutations were excluded in the cases cohort. Mutations in other high-penetrance CRC genes such as *MUTYH* could not be ruled out although their contribution may be considered scarce since cases did not present a relevant family history for hereditary or familial CRC or adenomatous polyposis. Written informed consent was obtained from all individuals.

SNP genotyping and quality control

Sixty-five SNPs previously associated with CRC susceptibility were selected and genotyped in all available DNA samples. SNPs were selected from previous GWAS published before mid-2017 (www.ebi.ac.uk/gwas/, GWAS Catalogue). Genotyping was performed with the Biomark 96.96 Genotyping dynamic array (Fluidigm, San Francisco, CA, USA) and TaqMan assays (Thermo Fisher Scientific, Waltham, MA, USA). Data quality was assessed using the Fluidigm SNP Genotyping Analysis and PLINK softwares. Samples and SNPs with genotyping success rate below 90% were removed from subsequent analyses (including rs7229639, rs3764482). One SNP was monomorphic and was also eliminated (rs10904849). In order to further test for genotyping quality, five samples were duplicated and genotype concordance was 100%. Deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg equilibrium (HWE) was assessed by the χ^2 test (1df). Each SNP was in HWE (p value >0.01) in controls (data not shown), thereby excluding the possibility of genotyping artefacts and any hidden population stratification. After quality filtering, the final available dataset comprised 741 samples (215 cases and 526 controls) and 62 SNPs. The overall genotyping success rate in the remaining individuals was 99.07%.

Statistical analysis

Logistic regression analysis was used to evaluate the association between each SNP and SPS risk under an additive model. The total number of risk alleles was calculated for all samples and coded as 0, 1 or 2 for each SNP assuming an additive genetic effect. Two-sided t-test was applied to compare the mean number of risk alleles between cases and controls. Furthermore, an additive genetic risk score (GRS) was developed by using a general linear model to assess for genetic susceptibility. GRS was defined as the count of risk alleles across all available SNPs. An unweighted GRS was preferred. A weighted genetic risk score was also tested, using the originally published log-ORs as weights. Since the results were essentially the same, and the weights may be biassed due to the winner's course effect, we opted to use the unweighted score. All statistical tests were two-sided, and p values <0.05 were considered statistically significant. All analyses were performed using PLINK v1.09 (V.3.4.1). Bonferroni correction was used for multiple testing adjustment (α adjusted=$0.05/62=8.06\times10^{-4}$).

RESULTS

Association tests for individual SNPs

A total of 215 SPS patients and 526 controls were successfully genotyped for 62 SNPs previously linked with genetic susceptibility to CRC. First, the frequency of CRC risk alleles between SPS cases and controls was compared and those significantly enriched in the SPS cohort were detected. Results are shown in table 1. Seven CRC SNPs showed statistically significant associations with SPS (rs992157, rs16892766, rs704017, rs11196172, rs3217810, 4779584, rs6061231) and these genetic associations were in the same direction as previously reported for CRC susceptibility. Despite being not significant, most remaining SNPs (42/52), showed ORs in the same directions as those previously described in the literature for CRC susceptibility.

Genetic risk score model

The presence of higher number of CRC risk alleles in the SPS cohort when compared with controls was also evaluated. The distribution of risk by allele number for the 62 genotyped SNPs is displayed in figure 1, both for cases and controls. The distribution of risk alleles followed a normal distribution in both SPS cases and controls with a shift towards a higher number of risk alleles in affected individuals consistent with a cumulative impact of CRC risk alleles on SPS predisposition. The mean number of risk alleles in control individuals was 56.20 compared with 57.46 in SPS cases and there was a statistically significant difference in the mean number of risk alleles between SPS cases and controls (difference: −1.26; two-sided t-test p value=0.0016).

Next, a GRS was calculated for SPS cases and controls when carrying an increasing number of CRC risk alleles. The median number of risk alleles in controls, 56, was considered as reference. SPS cases and controls were grouped considering subjects carrying ≤45 risk alleles and ≥65 alleles, because of the small number of subjects at these extremes. The risk score was higher in the SPS group compared with the control cohort (OR=1.05, 95% CI 1.02 to 1.09, p value=0.0019). We observed that there was a twofold increase in SPS risk for subjects in the highest quintile of risk alleles (≥62) compared with those in the first quintile (≤53) (OR=2.16, 95% CI 1.29 to 3.63, p=0.0034). Additionally, a fourfold increase in SPS risk was detected for subjects in the highest decile of risk alleles (≥65), compared with those in the first decile (≤50) (OR=4.24, 95% CI 1.76 to 10.21, p value=0.0013). As shown in figure 1, the increase in risk per allele was linear, indicating the independent additive contribution of each allele to SPS risk.

DISCUSSION

The association of 65 common, low-penetrance CRC susceptibility variants in SPS development was assessed in a cohort of 768 samples (219 SPS cases and 548 controls) in order to check if common, low-penetrance genetic variants for CRC risk were also involved in SPS genetic susceptibility. Our results showed statistically significant association of seven CRC genetic variants with SPS (rs992157, rs16892766, rs704017, rs11196172, rs3217810, rs4779584 and rs6061231).

Among the detected SPS genetic associations, the most significant corresponded to rs4779584. The T risk allele frequency was remarkably higher in SPS cases (25%) than in controls (17%) (OR=1.573, 1.21 to 2.04, p value=0.0006). This association remained statistically significant even after multiple testing

Table 1 Case-control association results obtained by logistic regression analyses

SNP*	Region	Nearby gene	Variant type	Risk allele	RAF cases/controls	OR	95% CI	P value
rs12080929	1p33	*SLC5A9*	Intronic variant	T	0.73/0.71	1.086	(0.85 to 1.39)	0.5093
rs72647484	1p36.12	*CDC42/WNT*	Intergenic variant	C	0.08/0.08	1.096	(0.72 to 1.66)	0.6656
rs10911251	1q25.3	*LAMC1*	Intronic variant	A	0.58/0.59	0.9767	(0.78 to 1.23)	0.8409
rs6691170	1q41	*DUSP10*	Intergenic variant	T	0.34/0.36	0.9123	(0.72 to 1.15)	0.4376
rs11903757	2q32.3	*NABP1*	Intergenic variant	C	0.13/0.15	0.8341	(0.61 to 1.15)	0.2653
rs992157	**2q35**	***PNKD/TMBIM1***	**Intronic variant**	**A**	**0.58/0.5**	**1.36**	**(1.09 to 1.7)**	**0.007454**
rs11676348	2q35	*CXCR2*	Intergenic variant	T	0.49/0.54	0.8345	(0.67 to 1.03)	0.09742
rs812481	3p14.1	*LRIG1*	Intronic variant	G	0.53/0.52	1.022	(0.82 to 1.28)	0.8472
rs35360328	3p22.1	*CTNNB1*	intergenic variant	A	0.14/0.14	0.9986	(0.72 to 1.38)	0.9931
rs10936599	3q26.2	*TERC*	Synonymous variant	C	0.78/0.75	1.174	(0.89 to 1.54)	0.2474
rs7136702	4q13.2	*LARP4/DIP2B*	Intergenic variant	T	0.32/0.34	0.9124	(0.72 to 1.16)	0.4501
rs3987	4q26	*NDST3*	Intergenic variant	G	0.45/0.46	0.9761	(0.78 to 1.22)	0.831
rs2736100	5p15.33	*TERT*	Intronic variant	A	0.5/0.52	0.9495	(0.76 to 1.19)	0.6472
rs647161	5q31.1	*PITX1*	Intronic variant	A	0.73/0.69	1.196	(0.94 to 1.52)	0.1458
rs1321311	6p1.2	*CDKN1A*	Intergenic variant	A	0.29/0.27	1.086	(0.84 to 1.4)	0.527
rs4711689	6p21.1	*TFEB*	Intronic variant	A	0.54/0.54	1.01	(0.81 to 1.27)	0.9282
rs11987193	8p12	*DUSP4*	Intergenic variant	C	0.7/0.74	0.8091	(0.63 to 1.05)	0.1055
rs16892766	**8q23.3**	***EIF3H***	**Intergenic variant**	**C**	**0.08/0.05**	**1.686**	**(1.09 to 2.62)**	**0.01959**
rs6469656	8q23.3	*EIF3H*	Intragenic variant	A	0.9/0.89	1.112	(0.78 to 1.58)	0.5505
rs6983267	8q24.21	*MYC*	Non-coding transcript variant/intronic variant	G	0.54/0.51	1.099	(0.88 to 1.37)	0.4035
rs719725	9p24	*TPD52L3/UHRF2*	Intronic variant	A	0.62/0.58	1.198	(0.95 to 1.51)	0.1215
rs10795668	10p14	*ARN5SP299/GATA3*	Intronic variant	A	0.3/0.33	0.8803	(0.69 to 1.12)	0.2995
rs704017	**10q22.3**	***ZMIZ1-AS1***	**Intronic variant**	**G**	**0.57/0.5**	**1.307**	**(1.04 to 1.64)**	**0.02218**
rs1035209	10q24.2	*ABCC2/MRP2*	Intergenic variant	T	0.16/0.16	0.9457	(0.7 to 1.28)	0.7188
rs11190164	10q24.2	*SLC25A28*	Intergenic variant	G	0.2/0.23	0.8207	(0.62 to 1.08)	0.1657
rs11196172	**10q25.2**	***TCF7L2***	**Intronic variant**	**A**	**0.11/0.17**	**0.648**	**(0.46 to 0.91)**	**0.01186**
rs12241008	10q25.2	*VTI1A*	Intronic variant	C	0.11/0.09	1.272	(0.87 to 1.86)	0.2129
rs4246215	11q12.2	*FEN1*	3'-UTR variant	T	0.3/0.33	0.8561	(0.67 to 1.09)	0.2136
rs174537	11q12.2	*MYRF*	Intronic variant	G	0.7/0.67	1.161	(0.91 to 1.48)	0.226
rs1535	11q12.2	*FADS2*	Intronic variant	A	0.69/0.66	1.168	(0.92 to 1.49)	0.206
rs174550	11q12.2	*FADS1*	Intronic variant	T	0.69/0.67	1.124	(0.88 to 1.43)	0.3422
rs3824999	11q13.4	*POLD3*	Intronic variant	G	0.53/0.49	1.146	(0.92 to 1.43)	0.2257
rs3802842	11q23.1	*COLCA1/COLCA2*	Intronic variant	C	0.26/0.26	0.9952	(0.78 to 1.28)	0.9696
rs2238126	12p13.2	*ETV6*	Intronic variant	G	0.14/0.16	0.8391	(0.61 to 1.15)	0.2764
rs10849432	12p13.31	*CD9*	Intronic variant	T	0.88/0.88	1.065	(0.75 to 1.52)	0.7295
rs11064437	12p13.31	*SPSB2*	Splice acceptor variant	C	1/0.99	2.453	(0.57 to 10.48)	0.2256
rs3217901	12p13.32	*CCND2*	Intronic variant	G	0.38/0.34	1.162	(0.92 to 1.47)	0.2046
rs10774214	12p13.32	*CCND2*	Intronic variant	T	0.37/0.35	1.125	(0.89 to 1.42)	0.3242
rs3217810	**12p13.32**	***CCND2***	**Intronic variant**	**T**	**0.12/0.08**	**1.686**	**(1.16 to 2.45)**	**0.006237**
rs11169552	12q13.12	*DIP2B/ATF1*	Intergenic variant	C	0.76/0.76	0.9557	(0.74 to 1.24)	0.7319
rs3184504	12q24.12	*SH2B3*	Mis-sense variant	C	0.58/0.56	1.066	(0.85 to 1.33)	0.5737
rs59336	12q24.21	*TBX3*	Intronic variant	T	0.54/0.52	1.092	(0.87 to 1.37)	0.4528
rs73208120	12q24.22	*NOS1*	Intronic variant	G	0.06/0.05	1.053	(0.64 to 1.74)	0.8393
rs4444235	14q22.2	*BMP4*	Intergenic variant	C	0.56/0.55	1.036	(0.83 to 1.3)	0.7544
rs1957636	14q22.2	*BMP4*	Intronic variant	T	0.41/0.4	1.044	(0.83 to 1.31)	0.7078
rs17094983	14q23.1	*RTN1*	Intergenic variant	G	0.86/0.86	0.9959	(0.72 to 1.38)	0.9803
rs4779584	**15q13.3**	***GREM1***	**Intergenic variant**	**T**	**0.25/0.17**	**1.573**	**(1.21 to 2.04)**	**0.0006432**
rs9929218	16q22.1	*CDH1*	Intronic variant	G	0.72/0.69	1.169	(0.91 to 1.49)	0.212
rs16941835	16q24.1	*FOXL1*	Intergenic variant	C	0.17/0.18	0.9193	(0.68 to 1.24)	0.5842
rs12603526	17q13.3	*NXN*	Intronic variant	C	0.01/0.01	1.368	(0.45 to 4.13)	0.5786
rs4939827	18q21.1	*SMAD7*	Intronic variant	T	0.56/0.55	1.063	(0.85 to 1.33)	0.5909
rs12970291	18q22.3	*TSHZ1*	Intergenic variant	A	0.04/0.03	1.127	(0.6 to 2.12)	0.7105
rs10411210	19q13.11	*RHPN2*	Intronic variant	C	0.87/0.87	0.9811	(0.71 to 1.36)	0.9099
rs2241714	19q13.2	*B9D2*	Mis-sense variant/2 kb upstream variant	C	0.67/0.65	1.096	(0.86 to 1.39)	0.4517

Continued

Table 1 Continued

SNP*	Region	Nearby gene	Variant type	Risk allele	RAF cases/ controls	OR	95% CI	P value
rs1800469	19q13.2	*TGFB1*	2 kb upstream variant/0.5 kb downstream variant	G	0.67/0.65	1.102	(0.87 to 1.4)	0.4215
rs2423279	20p12.3	*HAQ1*	Intergenic variant	C	0.28/0.31	0.8595	(0.67 to 1.1)	0.2333
rs961253	20p12.3	*BMP2*	Intergenic variant	A	0.34/0.32	1.085	(0.86 to 1.37)	0.4941
rs4813802	20p12.3	*BMP2*	Regulatory region variant?	G	0.36/0.32	1.216	(0.96 to 1.54)	0.1046
rs6066825	20q13.13	*PREX1*	Intronic variant	A	0.6/0.58	1.113	(0.89 to 1.39)	0.3445
rs6061231	**20q13.33**	***LAMA5***	**Intergenic variant**	**C**	**0.78/0.72**	**1.412**	**(1.08 to 1.85)**	**0.01189**
rs4925386	20q13.33	*LAMA5*	Intronic variant	C	0.73/0.68	1.261	(0.98 to 1.62)	0.06688
rs5934683	Xp22.2	*SHROOM2*	Intronic variant	C	0.59/0.61	0.9276	(0.71 to 1.22)	0.5873

Association results for 216 SPS cases and 526 controls. Results are based on the reported risk allele from previous CRC GWAS. Statistically significant associations are denoted in bold (p value <0.05).
*SNPs with genotyping success rate below 90% were removed from subsequent analyses (including rs7229639, rs3764482). We also removed a monomorphic SNP (rs10904849).
CRC, colorectal cancer; GWAS, genome-wide association studies; RAF, risk allele frequency; SPS, serrated polyposis syndrome; UTR, untranslated region.

corrections. This SNP maps to chromosomal region 15q13.3, is intergenic and lies between *SCG5* and *GREM1*. It was previously suggested that this variant captures two independent association signals represented by rs16969681 and rs11632715 upstream of *GREM1*.[16] The rs16969681 variant lies upstream of *GREM1* and is close to a regulatory element that acts as an allele-specific *GREM1* enhancer. The rs16969681 risk allele differentially binds with a higher affinity the intestine-specific transcription factor *CDX2* and the Wnt effector *TCF7L2* producing a stronger *GREM1* expression and promoting tumourigenesis.[17] Noteworthy, a 40 kb duplication upstream of this gene was previously linked with increased *GREM1* expression in individuals with hereditary mixed polyposis.[18] This clinical entity shows some overlap with SPS since patients develop polyps of multiple and mixed morphologies including serrated lesions, Peutz-Jeghers polyps, juvenile polyps, conventional adenomas and CRC (OMIM # 601228). Moreover, this genetic variant had been linked with serrated polyps in previous studies.[7 9]

Additionally, our results show that rs16892766 at 8q23.3 is associated with SPS susceptibility. Previous fine mapping in this region and functional analysis identified rs16888589 as the potential effector of this association through increasing the expression of *EIF3H*, promoting carcinogenesis.[19] Besides, we also detected an association with SPS for rs3217810 at 12p13.32 located in the intron of *CCND2*, a cyclin involved in cell cycle G1/S transition.[20] Both SNPs had been previously linked with serrated polyps.[9]

Further novel associations with SPS were also detected in our cohort for rs992157 (2q35, *PNKD/TMBIM1*), rs704017 (10q22.3, *ZMIZ1*), rs11196172 (10q25.2, *TCF7L2*) and rs6061231 (20q13.33, *LAMA5*). Interestingly, rs11196172 was previously associated with a higher *TCF7L2* expression.[21] As

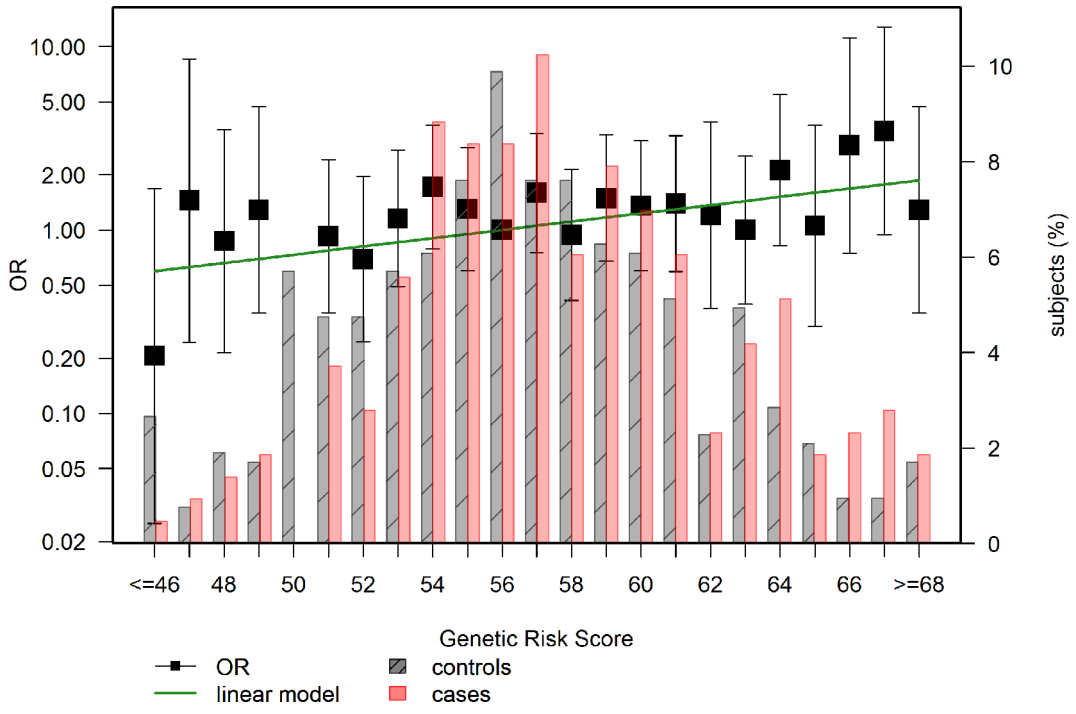

Figure 1 Genetic risk score. Distribution of risk by allele number for the 62 SNPs genotyped. The presence of multiple CRC risk alleles is displayed for SPS cases (bold bars) and controls (stripped bars). CRC, colorectal cancer; SPS, serrated polyposis syndrome

previously commented, high levels of activated *TCF7L2* increase *GREM1* expression. Therefore, the rs11196172 risk allele may also have an impact on *GREM1* expression. It is also important to highlight that the 20q13.33 region harbouring *LAMA5* may be suggested as relevant for SPS susceptibility since two variants from that locus showed either a significant (rs6061231) or borderline significant association (rs4925386).

Regarding the results of our polygenic risk model, the distribution of CRC risk alleles follows a normal distribution in both SPS cases and controls, with a shift towards a higher allele number in SPS cases. These results show an enrichment of CRC susceptibility variants in the SPS cohort, therefore suggesting those variants may play a role in mediating CRC risk through serrated lesions and SPS predisposition. A similar study from our group identified CRC risk variants related to advanced adenoma, implying that part of CRC risk is mediated through susceptibility to this other polyp type.[8]

It should be noted that this study corresponds to the first analysis of common, low-penetrance CRC risk variants in a SPS cohort. However, we are aware that our results are preliminary and this study has several limitations including limited sample size, lack of environmental data or no replication in an independent SPS cohort. Therefore, further studies are needed to confirm our findings. On the other hand, specific GWAS for SPS susceptibility or serrated polyps are needed to further characterise more precisely the germline predisposition architecture of this clinical entity.

In summary, our study suggests that some CRC risk genetic variants could also be involved in SPS susceptibility. The rs4779584 variant, near *GREM1*, seems to be the most important effector of the common, low-penetrance SPS susceptibility identified so far. Likewise, rs16892766, and rs3217810 could be relevant since they have been identified linked to serrated lesions in previous studies. There are potential implications of our study in the near future. CRC SNPs predisposing to SPS and new SPS SNPs could be used to identify a subgroup of individuals with increased disease risk and modulate population-based CRC-prevention measures.

Author affiliations
[1]Gastroenterology Department, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Hospital Clínic, Barcelona, Spain
[2]Bioinformatics Platform, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Barcelona, Spain
[3]Pathology Department, Hospital Clinic de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD) and Tumor Bank-Biobank, Hospital Clínic, Barcelona, Spain
[4]Genetics Unit, Hospital Universitario de Mostoles, Mostoles, Spain
[5]Hereditary Cancer Program, Catalan Institute of Oncology, Oncobell, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Barcelona, Spain
[6]Gastroenterology Department, Hospital Donostia-Instituto Biodonostia, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Basque Country University (UPV/EHU), San Sebastian, Spain
[7]Gastroenterology Department, Complexo Hospitalario Universitario de Ourense, Instituto de Investigación Sanitaria Galicia Sur, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Ourense, Spain
[8]Digestive Disease Section, Hospital Universitario de Mostoles, Mostoles, Spain
[9]Fundación Pública Galega de Medicina Xenómica, Grupo de Medicina Xenómica_USC, Instituto de Investigación Sanitaria de Santiago (IDIS), Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Santiago de Compostela, Spain
[10]Unit of Biomarkers and Susceptibility, Oncology Data Analytics Program, Catalan Institute of Oncology (ICO); Colorectal Cancer Group, ONCOBELL Program, Bellvitge Biomedical Research Institute (IDIBELL); Consortium for Biomedical Research in Epidemiology and Public Health (CIBERESP); Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, Spain

Correction notice This article has been corrected since it was published Online First. The name of author Miriam Cuatrecasas has been corrected.

Acknowledgements We are sincerely grateful to the patients, the Barcelona CRC screening program, the Functional Genomics and the Biobank core facilities of IDIBAPS Hospital Clínic, Biobanco Vasco para la Investigación/O+ehun-Hospital Donostia, the Genetics Unit at the Hospital Universitario de Móstoles and the Hereditary Cancer Program of the Catalan Institute of Oncology. The work was carried out (in part) at the Esther Koplowitz Centre, Barcelona.

Contributors CA-C, VM and SC-B planned the study. CA-C, YSdL, MD-G, JM, SC, LB, LM, JJL, TO, MC and SC-B conducted the study. CA-C, YSdL, MD-G, JM, SC, LB, LM, JJL, TO, MC, ADdB, LB, JC, DR-A, FB, CR-P, LV, VM and SC-B collected data. CA-C, YSdL, AC, GC, VM and SC-B interpreted data. CA-C and SC-B drafted the manuscript. The final draft of the manuscript was approved by all authors.

Funding CA-C, JM and JJL were supported by a contract from CIBEREHD. YSdL was supported by a fellowship (LCF/BQ/DI18/11660058) from 'la Caixa' Foundation (ID 100010434) funded EU Horizon 2020 Programme (Marie Skłodowska-Curie grant agreement no. 713673). LB was supported by a Juan de la Cierva postdoctoral contract (FJCI-2017-32593) and MD-G by a contract from Agència de Gestió d'Ajuts Universitaris i de Recerca, AGAUR, (Generalitat de Catalunya, 2018FI_B1_00213). CIBEREHD, CIBERER, CIBERESP and CIBERONC are funded by the Instituto de Salud Carlos III. This research was supported by grants from Fondo de Investigación Sanitaria/FEDER (14/00613, 16/00766, 17/00509, 17/00878), Fundación Científica de la Asociación Española contra el Cáncer (GCB13131592CAST), Spanish Ministry of Science, Innovation and Universities, co-funded by FEDER funds, (SAF2016-80888-R), PERIS (SLT002/16/00398, SLT002/16/0037, Generalitat de Catalunya), CERCA Programme (Generalitat de Catalunya) and Agència de Gestió d'Ajuts Universitaris i de Recerca (Generalitat de Catalunya, GRPRE 2017SGR21, GRC 2017SGR653, 2017SGR1282, 2017SGR723). This article is based upon work from COST Action CA17118, supported by European Cooperation in Science and Technology (COST). www.cost.eu.

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by the corresponding institutional ethics committees.

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Marcos Díaz-Gay http://orcid.org/0000-0003-0658-0467
Sergi Castellvi-Bel http://orcid.org/0000-0003-1217-5097

REFERENCES

1 Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;2018:394–424.
2 Kalimuthu SN, Chelliah A, Chetty R. From traditional serrated adenoma to tubulovillous adenoma and beyond. *World J Gastrointest Oncol* 2016;8:805–9.
3 IJspeert JEG, Vermeulen L, Meijer GA, Dekker E. Serrated neoplasia—role in colorectal carcinogenesis and clinical implications. *Nat Rev Gastroenterol Hepatol* 2015;12:401–9.
4 Valle L, de Voer RM, Goldberg Y, Sjursen W, Försti A, Ruiz-Ponte C, Caldés T, Garré P, Olsen MF, Nordling M, Castellvi-Bel S, Hemminki K. Update on genetic predisposition to colorectal cancer and polyposis. *Mol Aspects Med* 2019;69:10–26.
5 Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, McMahon A, Morales J, Mountjoy E, Sollis E, Suveges D, Vrousgou O, Whetzel PL, Amode R, Guillen JA, Riat HS, Trevanion SJ, Hall P, Junkins H, Flicek P, Burdett T, Hindorff LA, Cunningham F, Parkinson H. The NHGRI-EBI GWAS catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Res* 2019;47:D1005–12.
6 Zhang B, Shrubsole MJ, Li G, Cai Q, Edwards T, Smalley WE, Ness RM, Zheng W. Association of genetic variants for colorectal cancer differs by subtypes of polyps in the colorectum. *Carcinogenesis* 2012;33:2417–23.
7 Burnett-Hartman AN, Newcomb PA, Hutter CM, Peters U, Passarelli MN, Schwartz MR, Upton MP, Zhu L-C, Potter JD, Makar KW. Variation in the association between colorectal cancer susceptibility loci and colorectal polyps by polyp type. *Am J Epidemiol* 2014;180:223–32.
8 Abulí A, Castells A, Bujanda L, Lozano JJ, Bessa X, Hernández C, Álvarez-Urturi C, Pellisé M, Esteban-Jurado C, Hijona E, Burón A, Macià F, Grau J, Guayta R, Castellví-

Bel S, Andreu M, PROCOLON research group. Genetic variants associated with colorectal adenoma susceptibility. *PLoS One* 2016;11:e0153084.
9 Hang D, Joshi AD, He X, Chan AT, Jovani M, Gala MK, Ogino S, Kraft P, Turman C, Peters U, Bien SA, Lin Y, Hu Z, Shen H, Wu K, Giovannucci EL, Song M. Colorectal cancer susceptibility variants and risk of conventional adenomas and serrated polyps: results from three cohort studies. *Int J Epidemiol* 2019:pii: dyz096.
10 Carballal S, Rodríguez-Alcalde D, Moreira L, Hernández L, Rodríguez L, Rodríguez-Moranta F, Gonzalo V, Bujanda L, Bessa X, Poves C, Cubiella J, Castro I, González M, Moya E, Oquiñena S, Clofent J, Quintero E, Esteban P, Piñol V, Fernández FJ, Jover R, Cid L, López-Cerón M, Cuatrecasas M, López-Vicente J, Leoz ML, Rivero-Sánchez L, Castells A, Pellisé M, Balaguer F. Colorectal cancer risk factors in patients with serrated polyposis syndrome: a large multicentre study. *Gut* 2016;65:1829–37.
11 Snover DC. Update on the serrated pathway to colorectal carcinoma. *Hum Pathol* 2011;42:1–10.
12 Gala MK, Mizukami Y, Le LP, Moriichi K, Austin T, Yamamoto M, Lauwers GY, Bardeesy N, Chung DC. Germline mutations in oncogene-induced senescence pathways are associated with multiple sessile serrated adenomas. *Gastroenterology* 2014;146:520–9.
13 Taupin D, Lam W, Rangiah D, McCallum L, Whittle B, Zhang Y, Andrews D, Field M, Goodnow CC, Cook MC. A deleterious RNF43 germline mutation in a severely affected serrated polyposis kindred. *Hum Genome Var* 2015;16.
14 Buchanan DD, Clendenning M, Zhuoer L, Stewart JR, Joseland S, Woodall S, Arnold J, Semotiuk K, Aronson M, Holter S, Gallinger S, Jenkins MA, Sweet K, Macrae FA, Winship IM, Parry S, Rosty C. Genetics of colonic polyposis study. Lack of evidence for germline RNF43 mutations in patients with serrated polyposis syndrome from a large multinational study. *Gut* 2017;66:1170–2.
15 Quintana I, Mejías-Luque R, Terradas M, Navarro M, Piñol V, Mur P, Belhadj S, Grau E, Darder E, Solanes A, Brunet J, Capellá G, Gerhard M, Valle L. Evidence suggests that germline *RNF43* mutations are a rare cause of serrated polyposis. *Gut* 2018;67:2230–2.
16 Tomlinson IPM, Carvajal-Carmona LG, Dobbins SE, Tenesa A, Jones AM, Howarth K, Palles C, Broderick P, Jaeger EEM, Farrington S, Lewis A, Prendergast JGD, Pittman AM, Theodoratou E, Olver B, Walker M, Penegar S, Barclay E, Whiffin N, Martin L, Ballereau S, Lloyd A, Gorman M, Lubbe S, Howie B, Marchini J, Ruiz-Ponte C, Fernandez-Rozadilla C, Castells A, Carracedo A, Castellvi-Bel S, Duggan D, Conti D, Cazier J-B, Campbell H, Sieber O, Lipton L, Gibbs P, Martin NG, Montgomery GW, Young J, Baird PN, Gallinger S, Newcomb P, Hopper J, Jenkins MA, Aaltonen LA, Kerr DJ, Cheadle J, Pharoah P, Casey G, Houlston RS, Dunlop MG. Multiple common susceptibility variants near BMP pathway loci GREM1, BMP4, and BMP2 explain part of the missing heritability of colorectal cancer. *PLoS Genet* 2011;7:e1002105.
17 Lewis A, Freeman-Mills L, de la Calle-Mustienes E, Giráldez-Pérez RM, Davis H, Jaeger E, Becker M, Hubner NC, Nguyen LN, Zeron-Medina J, Bond G, Stunnenberg HG, Carvajal JJ, Gomez-Skarmeta JL, Leedham S, Tomlinson I. A polymorphic enhancer near GREM1 influences bowel cancer risk through differential Cdx2 and TCF7L2 binding. *Cell Rep* 2014;8:983–90.
18 Jaeger E, Leedham S, Lewis A, Segditsas S, Becker M, Cuadrado PR, Davis H, Kaur K, Heinimann K, Howarth K, East J, Taylor J, Thomas H, Tomlinson I. Hereditary mixed polyposis syndrome is caused by a 40-kb upstream duplication that leads to increased and ectopic expression of the BMP antagonist GREM1. *Nat Genet* 2012;44:699–703.
19 Pittman AM, Naranjo S, Jalava SE, Twiss P, Ma Y, Olver B, Lloyd A, Vijayakrishnan J, Qureshi M, Broderick P, van Wezel T, Morreau H, Tuupanen S, Aaltonen LA, Alonso ME, Manzanares M, Gavilán A, Visakorpi T, Gómez-Skarmeta JL, Houlston RS. Allelic variation at the 8q23.3 colorectal cancer risk locus functions as a cis-acting regulator of EIF3H. *PLoS Genet* 2010;6:e1001126.
20 Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, Berndt SI, Bézieau S, Brenner H, Butterbach K, Caan BJ, Campbell PT, Carlson CS, Casey G, Chan AT, Chang-Claude J, Chanock SJ, Chen LS, Coetzee GA, Coetzee SG, Conti DV, Curtis KR, Duggan D, Edwards T, Fuchs CS, Gallinger S, Giovannucci EL, Gogarten SM, Gruber SB, Haile RW, Harrison TA, Hayes RB, Henderson BE, Hoffmeister M, Hopper JL, Hudson TJ, Hunter DJ, Jackson RD, Jee SH, Jenkins MA, Jia WH, Kolonel LN, Kooperberg C, Küry S, Lacroix AZ, Laurie CC, Laurie CA, Le Marchand L, Lemire M, Levine D, Lindor NM, Liu Y, Ma J, Makar KW, Matsuo K, Newcomb PA, Potter JD, Prentice RL, Qu C, Rohan T, Rosse SA, Schoen RE, Seminara D, Shrubsole M, Shu XO, Slattery ML, Taverna D, Thibodeau SN, Ulrich CM, White E, Xiang Y, Zanke BW, Zeng YX, Zhang B, Zheng W, Hsu L. Colon cancer family registry and the genetics and epidemiology of colorectal cancer Consortium. Identification of genetic susceptibility loci for colorectal tumors in a genome-wide meta-analysis. *Gastroenterology* 2013;144:799–807.
21 Zhang B, Jia WH, Matsuda K, Kweon SS, Matsuo K, Xiang YB, Shin A, Jee SH, Kim DH, Cai Q, Long J, Shi J, Wen W, Yang G, Zhang Y, Li C, Li B, Guo Y, Ren Z, BT J, Pan ZZ, Takahashi A, Shin MH, Matsuda F, Gao YT, JH O, Kim S, Ahn YO. Genetics and epidemiology of colorectal cancer Consortium (GECCO), Chan at, Chang-Claude J, Slattery ml; colorectal Transdisciplinary (CORECT) study, Gruber SB, Schumacher Fr, Stenzel SL; colon cancer family registry (CCFR), Casey G, Kim HR, Jeong JY, Park JW, Li HL, Hosono S, CHO SH, Kubo M, Shu XO, Zeng YX, Zheng W. large-scale genetic study in East Asians identifies six new loci associated with colorectal cancer risk. *Nat Genet* 2014;46:533–42.