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SHORT REPORT

The Fanconi anemia DNA damage repair pathway in the spotlight for germline predisposition to colorectal cancer

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Colorectal cancer (CRC) is one of the most common neoplasms in the world. Fanconi anemia (FA) is a very rare genetic disease causing bone marrow failure, congenital growth abnormalities and cancer predisposition. The comprehensive FA DNA damage repair pathway requires the collaboration of 53 proteins and it is necessary to restore genome integrity by efficiently repairing damaged DNA. A link between FA genes in breast and ovarian cancer germline predisposition has been previously suggested. We selected 74 CRC patients from 40 unrelated Spanish families with strong CRC aggregation compatible with an autosomal dominant pattern of inheritance and without mutations in known hereditary CRC genes and performed germline DNA whole-exome sequencing with the aim of finding new candidate germline predisposition variants. After sequencing and data analysis, variant prioritization selected only those very rare alterations, producing a putative loss of function and located in genes with a role compatible with cancer. We detected an enrichment for variants in FA DNA damage repair pathway genes in our familial CRC cohort as 6 families carried heterozygous, rare, potentially pathogenic variants located in *BRCA2*/*FANCD1*, *BRIP1*/*FANCJ*, *FANCC*, *FANCE* and *REV3L*/*POLZ*. In conclusion, the FA DNA damage repair pathway may play an important role in the inherited predisposition to CRC.
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INTRODUCTION

Colorectal cancer (CRC) is the third most frequent neoplasm in the world and its average lifetime risk in the general population is ~5%.[1] There is some degree of familial aggregation in up to 35% of CRC patients, but the majority of the underlying germline predisposition factors remain still unidentified. The Mendelian CRC syndromes, with Lynch syndrome and familial adenomatous polyposis being the most common, correspond to 5% of total CRC cases and are mainly due to germline mutations in *APC*, *MUTYH* and the mismatch repair genes (ie, *MLH1*, *MSH2*, *MSH6*, *PMS2*). Recently, next-generation sequencing efforts in familial CRC have identified additional causative germline mutations in genes such as *POLE*, *POLD1* and *NTHL1*.[2–4]

Fanconi anemia (FA) is a very rare genetic disease with an incidence of 1–3 per 500 000 births and it causes bone marrow failure, congenital growth abnormalities and cancer predisposition. FA patients have chromosome fragility and hypersensitivity to drugs that induce DNA interstrand crosslinks (ICLs).[5] It corresponds to an autosomal recessive condition and it has been associated with germline mutations in 18 FA genes. Among them, monoallelic mutations in *FANCD1/BRCA2*, *FANCJ/BRIP1*, *FANCN/PALB2* and *FANCC* have also been linked to breast and ovarian cancer genetic predisposition.[6,7] The comprehensive FA DNA damage repair pathway requires the collaboration of 53 proteins and it is necessary to restore genome integrity by efficiently repairing damaged DNA, especially ICLs (Figure 1). ICLs affect both DNA strands impeding transcription and replication-fork progression and also complicating correct DNA repair as there is no unaffected template available.[8] Besides the link between FA genes and breast and ovarian cancer, some other genes not contributing to FA but part of the FA DNA damage repair pathway have additionally been involved in the same cancer predisposition and include *BRCA1*, *RAD51C* and *FANCM*.[9] Very recently, mutations in some FA DNA damage repair pathway genes have also been postulated to be the germline triggers in familial CRC cases, including *BRCA2*,[10,11] *FAN1*[12] and *BLM*.[13]

MATERIALS AND METHODS

We selected 74 CRC probands from 40 unrelated Spanish families (4 affected relatives from 1 family, 3 affected relatives from 8 families, 2 affected relatives

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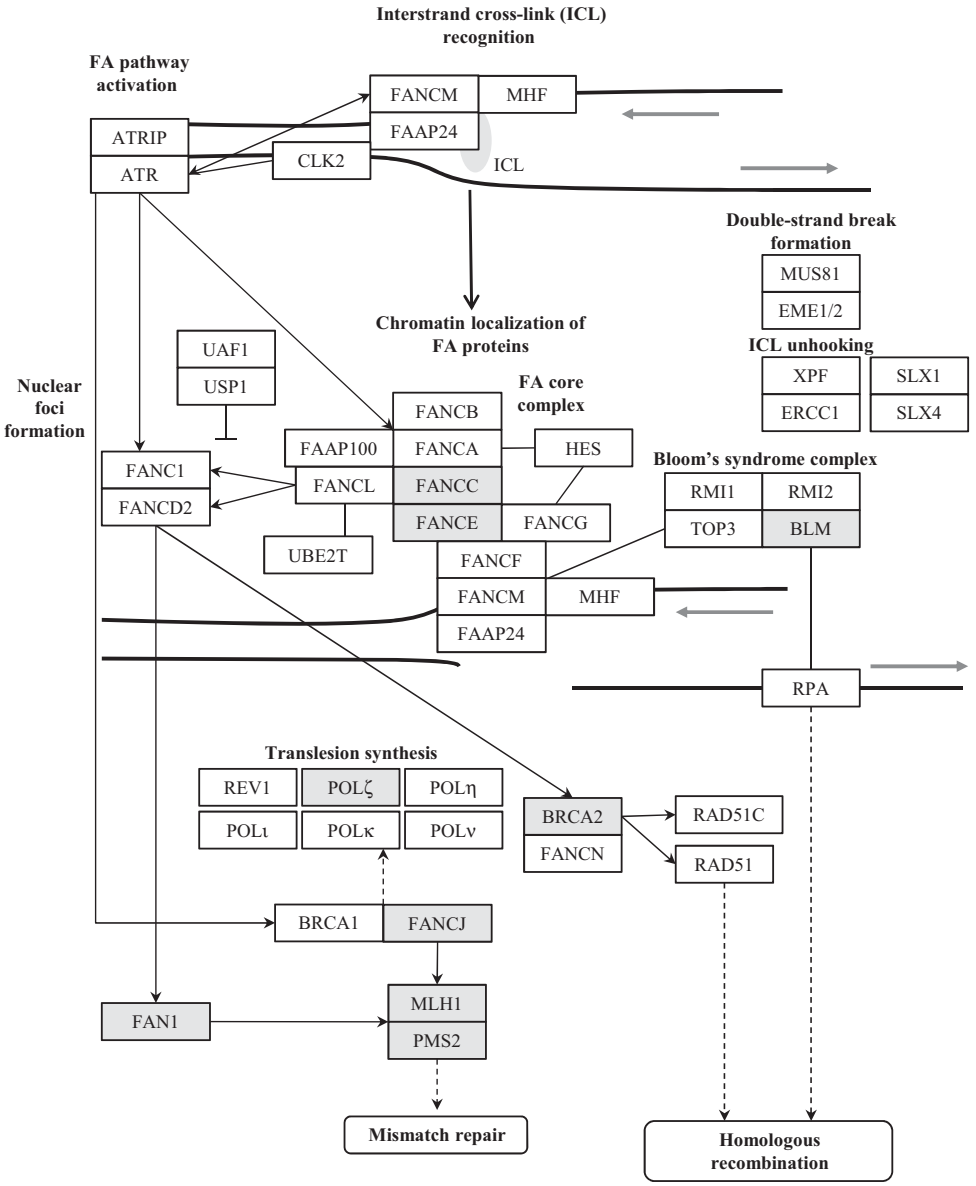

Figure 1 The Fanconi anemia (FA) DNA damage repair pathway. Genes linked to colorectal cancer (CRC) predisposition by the present report and previous evidence are shaded in gray (adapted from KEGG database, http://www.genome.jp/kegg/pathway.html).

from 15 families and 16 CRC unrelated patients) with strong CRC aggregation compatible with an autosomal dominant pattern of inheritance and without point mutations or large rearrangements in the most common hereditary CRC genes (*APC*, *MUTYH* and mismatch repair genes). Families were selected based on the following criteria: ≥ 3 relatives with CRC, ≥ 2 consecutive affected generations and at least one CRC diagnosed before the age of 60 years. This study was approved by the institutional ethics committee of each participating hospital and written informed consent was obtained at CRC diagnosis.

Sequencing, raw data analysis and variant filtering was performed as previously described for a subset of 42 patients.[14] In this regard, it should be noted that this previous cohort was completed with 31 additional new CRC patients, corresponding to 11 new families and 5 new cases in previously analyzed families, totaling 74 CRC probands from 40 families. Briefly, germline DNA whole-exome sequencing (WES) used the HiSeq2000 platform (Illumina, San Diego, CA, USA) and SureSelectXT Human All Exon for exon enrichment V4 (Agilent, Santa Clara, CA, USA). Mean coverage was $>95\times$ in all samples and 51 Megabases was the target size that required ~ 4 Gigabytes of sequencing per sample. After sequencing and data analysis, variant prioritization selected only those very rare alterations (0–0.1%), shared by individuals sequenced from the same family, producing a putative loss of function and located in genes with a role compatible with cancer (Supplementary Table 1). Variants were validated by Sanger sequencing (GATC Biotech, Köln, Germany) and segregation analysis of the prioritized variants was performed in additional affected family members (CRC and advanced adenoma) when constitutive DNA was available. Genetic variants have been submitted to the ClinVar database (http://www.ncbi.nlm.nih.gov/clinvar/; accession numbers SCV000262600, SCV000262601, SCV000262602, SCV000262603, SCV000262604 and SCV000262605). In addition, somatic loss of heterozygosity (LOH) was studied by Sanger sequencing or microsatellites in tumor DNA of patients (one per family) carrying the selected variants when possible. DNA was extracted from a percentage of tumor cells of 70–80% in most cases.

RESULTS AND DISCUSSION

The aim of our study was to find candidate germline predisposition variants by performing exome sequencing in a cohort of familial CRC

patients compatible with an autosomal dominant inheritance and without an alteration in the previously known hereditary CRC genes in order to facilitate genetic counseling and correctly address prevention strategies. Our preliminary results for a subset of CRC patients were previously published[14] but additional family members and new families were whole-exome sequenced more recently and the present report corresponds to the analysis of the complete cohort of 74 samples.

Interestingly, our data revealed heterozygous, rare, potentially pathogenic variants in six families located in genes belonging to the FA DNA damage repair pathway including *BRCA2*/*FANCD1*, *BRIP1*/*FANCJ*, *FANCC*, *FANCE* and *REV3L*/*POLZ* after data analysis and variant prioritization (Table 1 and Figure 2). All six prioritized variants were validated by Sanger sequencing (Supplementary Figure 1) and segregation analysis was correct in family members (CRC and advanced adenoma). It is also interesting to point out that among the 1–57 variants identified by WES that remained after filtering in each family, the genetic variants finally prioritized corresponded to the best candidate for genetic predisposition to CRC (not reported or with a very low frequency $<0.01\%$ in external control exome data sets including a Spanish database, potential loss-of-function variant affecting residues highly conserved in evolution, previous implication with cancer predisposition and correct segregation). It is important to highlight that three of the variants corresponded to frameshift alterations and three to missense variants. The former are expected to truncate the protein and, therefore, are likely pathogenic, whereas for the latter pathogenicity is plausible but has not been proven with functional studies. On the other hand, the identified missense variants comply with several criteria to be potentially deleterious and two of them (those in *BRCA2* and *FANCE*) that fall in protein domains where pathogenic mutations have been previously reported in FA or familial breast and ovarian cancer patients in the ClinVar database or the FA mutation database (http://www.rockefeller.edu/fanconi/). In addition, when comparing with a publically available genetic variation control data set (Exome Aggregation Consortium), we detected a clear enrichment for putative loss-of-function variants in these FA DNA damage repair pathway genes in our familial CRC cohort, when considering nonsense, canonical splice site, frameshift and missense variants with a likely pathogenicity prediction (CADD >15, Combined Annotation Dependent Depletion, CADD, http://cadd.gs.washington.edu/) and a genotype frequency $<0.01\%$ (6/40, 15% *vs* 2546/60 706, 4.2%; χ^2 test, uncorrected for multiple testing P-value $=0.0025$), or only nonsense, canonical splice site and frameshift with a genotype frequency $<0.01\%$ (3/40, 7.5% *vs* 306-/60 706, 0.5%; χ^2 test, uncorrected for multiple testing P-value <0.0001). It is also worth mentioning that in 5 of the 6 families carrying the reported variants, other neoplasms besides CRC were present with an age of onset <60 years, including breast cancer, endometrial cancer, prostate cancer, lung cancer, leukemia and gastric cancer. No relevant clinical or histopathological characteristics were detected among variant carriers, although a small sample size could be precluding the detection of such correlation.

Taking into account our results and previous evidence,[10–13] our report draws the attention to the fact that the FA DNA damage

Table 1 Description of the 6 genetic variants belonging to the Fanconi anemia DNA damage repair pathway detected in a cohort of 40 Spanish colorectal cancer families

Family	*Gene*	*RefSeq*	*Genetic variant*	*Genotype frequency (ExAC, EVS, CSVS)*	*In silico*	*Family phenotype (age <60)*	*Cancer-AA carriers*	*LOH*	*Domain/region*	*OMIM*
FAM6	*BRCA2/FANCD1*	NM_000059.3	c.7759C>T p.(L2587F)	5/60676 0/6503 0/572	5/5	CRC, gastric	2/2	INC[a]	Interaction with DSS1	Breast and ovarian cancer, FA
FAM20	*BRCA2/FANCD1*	NM_000059.3	c.4963delT p.(Y1655fs*15)	0/60706 0/6503 0/572	FS	CRC, breast cancer, endometrial cancer, prostate cancer, lung cancer, leukemia	2/2	INC[a]	—	Breast and ovarian cancer, FA
H463	*BRIP1/FANCJ*	NM_032043.2	c.1702_1703delAA p.(N568fs*9)[b]	0/60706 0/6503 0/572	FS	CRC, gastric cancer	1/1	INC[a]	—	Breast and ovarian cancer, Spanish FA J family[c]
FAMN4	*FANCC*	NM_000136.2	c.591_592dupC p.(L199fs*12)	0/60706 0/6503 0/572	FS	CRC, adenomas	2/2	N	—	Breast cancer, pancreatic cancer, FA
FAM40	*FANCE*	NM_021922.2	c.598C>T p.(R200C)	0/60706 1/6503 0/572	5/5	CRC, breast cancer	2/2	NA	Interaction with FANCC	FA, esophageal and gastric cancer
FAM3	*REV3L/POLZ*	NM_002912.4	c.559A>T p.(R187W)	0/60706 0/6503 0/572	5/5	CRC, prostate cancer, adenomas	4/4	Y	—	Lung cancer, chromosomal instability

Abbreviations: AA, advanced adenoma; CRC, colorectal cancer; FA, Fanconi anemia; FS, frameshift; INC, inconclusive; LD, linkage disequilibrium; LOH, loss of heterozygosity; N, no; NA, not available; RefSeq, reference sequence; Seg, segregation; Y, yes.
Genotype frequency: presence or absence in external control exome data sets (ExAC (exome aggregation consortium), EVS (exome variant server) and CSVS (CIBERER Spanish variant server)).
In silico: number of deleterious predictions by bioinformatics tools used (CADD, PolyPhen, SIFT, PhyloP and LRT).
Cancer-AA carriers: number of cancer/advanced adenoma cases within the family that carry the variant.
LOH: depletion of the wild-type allele in tumor DNA in comparison with the germline.
Domain/region: the protein domain or region where the variant is located.
OMIM: OMIM database information including previous hereditary cancer involvement.
[a]Minimal LOH was observed.
[b]Previously reported in Esteban-Jurado *et al*.[14]
[c]Same variant in our CRC cohort and in the Spanish FA J family.

repair pathway may play an important role in the inherited predisposition to CRC. It is also important to highlight that pleiotropy is becoming important in germline predisposition to cancer as a higher number of genes may be involved in the genetic predisposition to a broader spectrum of neoplasms, as evidenced by our results and others.[11] Finally, it could be hypothesized that defects in the FA DNA

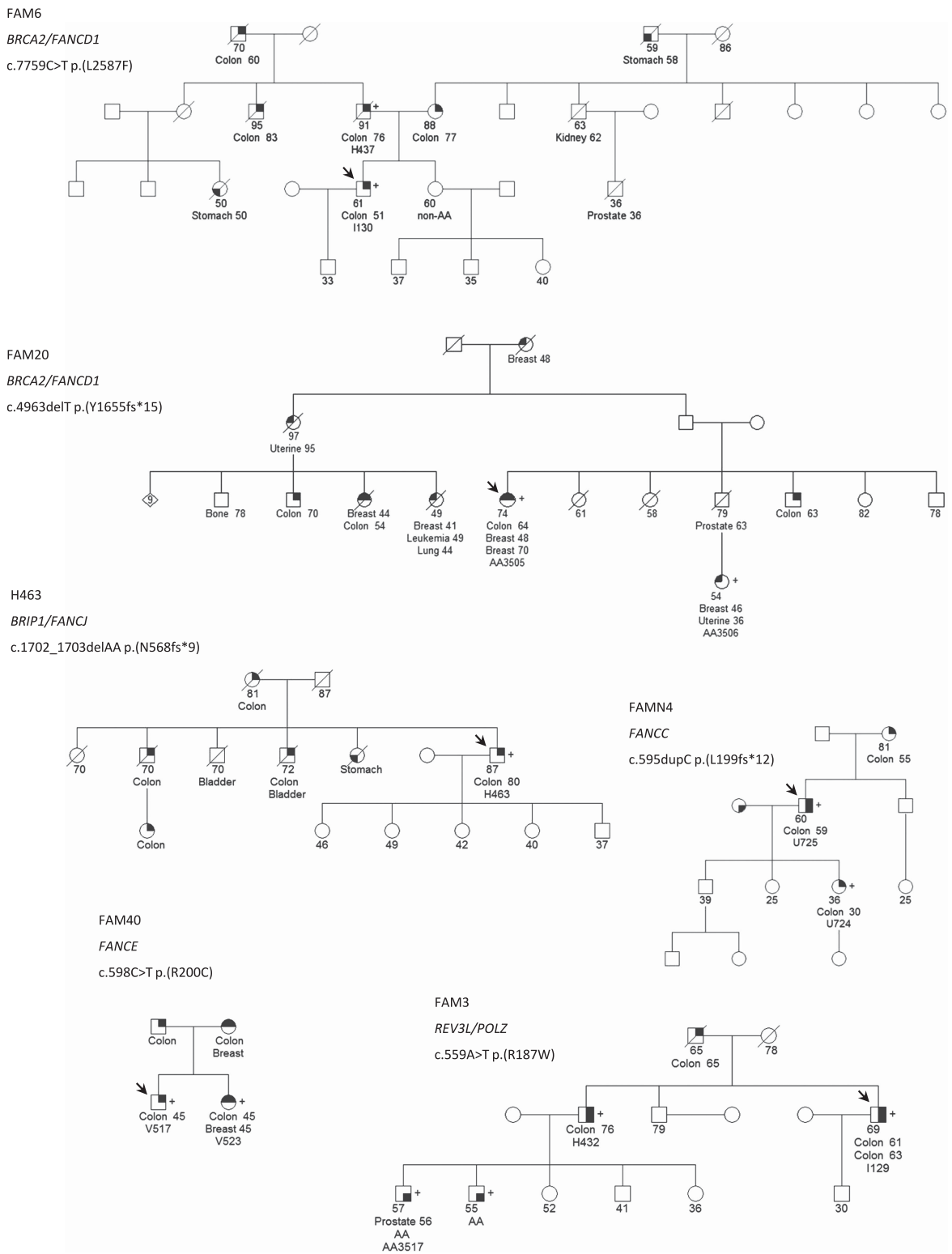

Figure 2 Pedigrees from families FAM3, FAMN4, FAM6, FAM20, FAM40 and H463 are shown. Filled symbol indicate affected for CRC (upper right quarter) adenoma/s (lower right quarter), stomach cancer (lower left quarter) and breast cancer (upper left quarter). Colon, breast, stomach, lung, prostate, kidney, uterine, leukemia and bladder refer to the type of cancer. AA/non-AA, advanced adenoma/nonadvanced adenoma; +, variant carrier. Index cases are indicated with an arrow.

damage repair pathway would affect correct homologous recombination and contribute to genome instability. However, the contribution to CRC predisposition of genetic variants in this pathway needs further investigation and collaborative efforts should be made in order to fully characterize it. If this involvement is further confirmed in additional familial CRC cohorts, it would become very relevant regarding the molecular genetic diagnosis for the hereditary forms of this neoplasm.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893–2917.
2 Short E, Thomas LE, Hurley J, Jose S, Sampson JR: Inherited predisposition to colorectal cancer: towards a more complete picture. *J Med Genet* 2015; **52**: 791–796.
3 Palles C, Cazier JB, Howarth KM *et al*: Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat Genet* 2013; **45**: 136–144.
4 Weren RD, Ligtenberg MJ, Kets CM *et al*: A germline homozygous mutation in the base-excision repair gene NTHL1 causes adenomatous polyposis and colorectal cancer. *Nat Genet* 2015; **47**: 668–671.
5 Kitao H, Takata M: Fanconi anemia: a disorder defective in the DNA damage response. *Int J Hematol* 2011; **93**: 417–424.
6 Thompson ER, Doyle MA, Ryland GL *et al*: Exome sequencing identifies rare deleterious mutations in DNA repair genes FANCC and BLM as potential breast cancer susceptibility alleles. *PLoS Genet* 2012; **8**: e1002894.
7 Bogliolo M, Surrallés J: Fanconi anemia: a model disease for studies on human genetics and advanced therapeutics. *Curr Opin Genet Dev* 2015; **33**: 32–40.
8 Walden H, Deans AJ: The Fanconi anemia DNA repair pathway: structural and functional insights into a complex disorder. *Annu Rev Biophys* 2014; **43**: 257–278.
9 Economopoulou P, Dimitriadis G, Psyrri A: Beyond BRCA: new hereditary breast cancer susceptibility genes. *Cancer Treat Rev* 2015; **41**: 1–8.
10 Garre P, Martín L, Sanz J *et al*: BRCA2 gene: a candidate for clinical testing in familial colorectal cancer type X. *Clin Genet* 2015; **87**: 582–587.
11 Yurgelun MB, Allen B, Kaldate RR *et al*: Identification of a variety of mutations in cancer predisposition genes in patients with suspected Lynch syndrome. *Gastroenterology* 2015; **149**: 604–613.
12 Seguí N, Mina LB, Lázaro C *et al*: Germline mutations in FAN1 cause hereditary colorectal cancer by impairing DNA repair. *Gastroenterology* 2015; **149**: 563–566.
13 de Voer RM, Hahn MM, Mensenkamp AR *et al*: Deleterious germline BLM mutations and the risk for early-onset colorectal cancer. *Sci Rep* 2015; **5**: 14060.
14 Esteban-Jurado C, Vila-Casadesús M, Garre P *et al*: Whole-exome sequencing identifies rare pathogenic variants in new predisposition genes for familial colorectal cancer. *Genet Med* 2015; **17**: 131–142.

Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)