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BRIEF REPORT

Germline Mutations in *FAN1* Cause Hereditary Colorectal Cancer by Impairing DNA Repair

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Identification of genes associated with hereditary cancers facilitates management of patients with family histories of cancer. We performed exome sequencing of DNA from 3 individuals from a family with colorectal cancer who met the Amsterdam criteria for risk of hereditary nonpolyposis colorectal cancer. These individuals had mismatch repair-proficient tumors and each carried nonsense variant in the FANCD2/FANCI-associated nuclease 1 gene (*FAN1*), which encodes a nuclease involved in DNA inter-strand cross-link repair. We sequenced *FAN1* in 176 additional families with histories of colorectal cancer and performed in vitro functional analyses of the mutant forms of FAN1 identified. We detected *FAN1* mutations in approximately 3% of families who met the Amsterdam criteria and had mismatch repair-proficient cancers with no previously associated mutations. These findings link colorectal cancer predisposition to the Fanconi anemia DNA repair pathway, supporting the connection between genome integrity and cancer risk.

Keywords: Lynch Syndrome; Genetic Risk Factor; Susceptibility; DNA Mismatch Repair.

Familial aggregation of colorectal cancer (CRC) is one of the strongest risk factors for CRC. Germline mutations in the DNA mismatch repair (MMR) genes, *EPCAM*, *APC*, *MUTYH*, *POLE*, *POLD1*, *GREM1*, *SMAD4*, *BMPR1A*, *STK11*, and *PTEN* cause hereditary forms of CRC.[1–3] However, part of the observed heritability and familial aggregation of the disease is yet to be explained.

With the aim of identifying new hereditary CRC genes, we sequenced the exomes of 3 cancer-affected members of a high-risk, Amsterdam I MMR-proficient, CRC family (Figure 1*A*, Family 1). Of 32 unreported or rare (minor allele frequency <1%) nonsynonymous variants shared by all affected relatives (Supplementary Table 1), a nonsense mutation in *FAN1*, c.141C>A (p.C47*) deserved our attention, as the coded protein, FANCD2/FANCI-associated nuclease 1 (MIM# 613534), is involved in interstrand cross-link repair (Fanconi anemia [FA]) and interacts with MMR components, such as MLH1, PMS2 and PMS1, thus playing a role in maintaining genome integrity.[4–8] The identified *FAN1* mutation had not been reported previously (NHLBI GO Exome Sequencing Project [ESP], 1000 Genomes Project) or found in 1648 alleles of Spanish origin, including

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Abbreviations used in this paper: CRC, colorectal cancer; FA, Fanconi anemia; MMC, mitomycin C; MMR, DNA mismatch repair; TCGA, The Cancer Genome Atlas.

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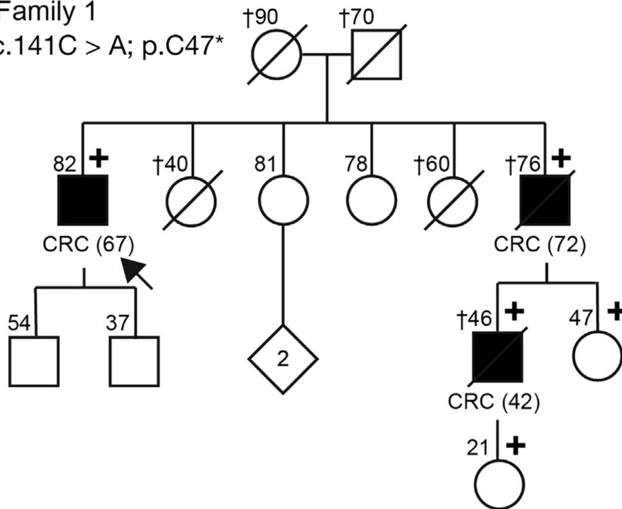

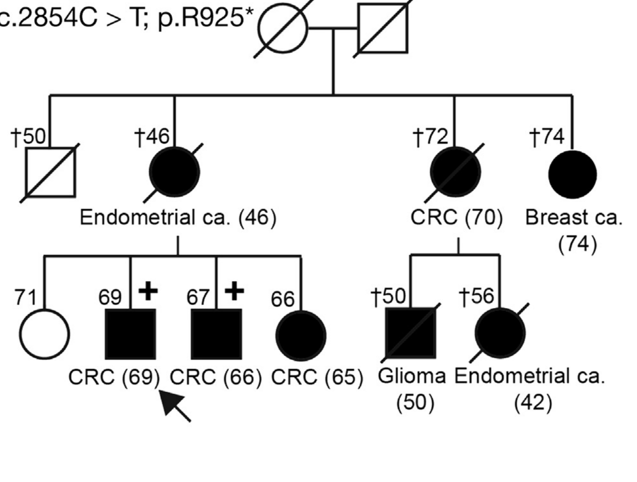

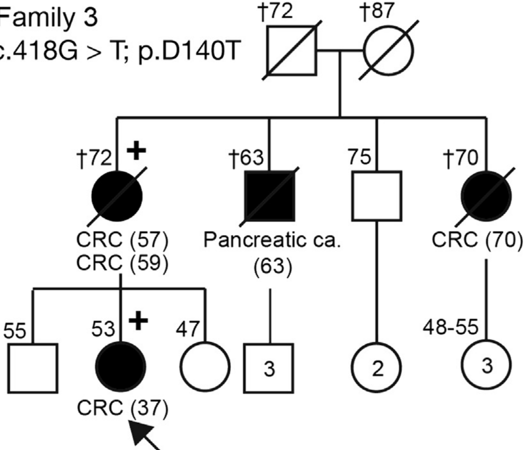

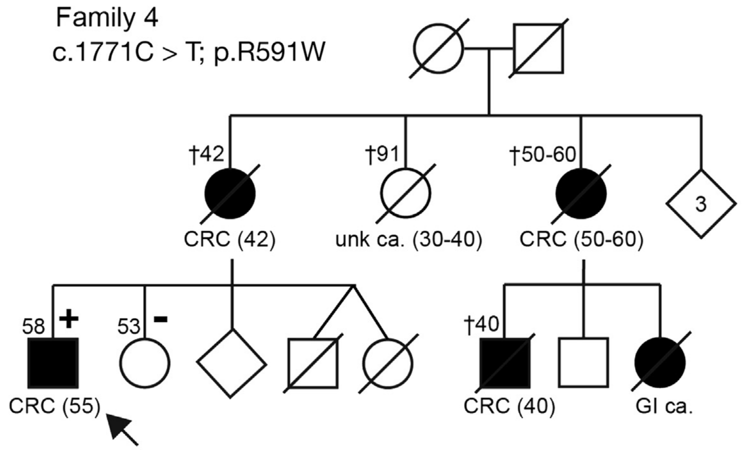

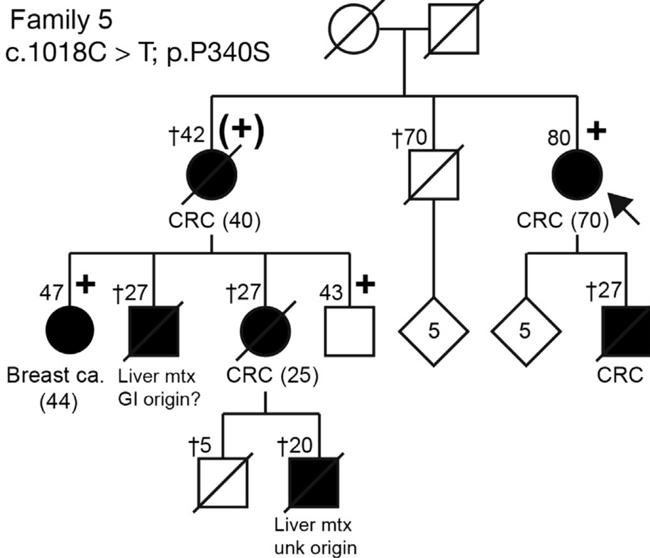

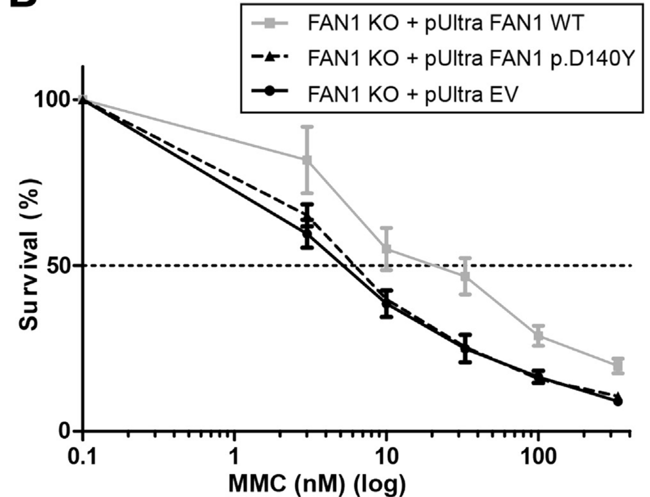

Figure 1. (*A*) Pedigrees of the families with germline *FAN1* mutations. *Filled symbol*, cancer; +, mutation carrier; *(+)*, obliged mutation carrier; −, wild-type; *arrow*, index case. Ages at information gathering or at death, when available, are indicated on the *top-left corner* of each individual's symbol. Ca., cancer; GI, gastrointestinal; mtx, metastasis; unk, unknown location. (*B*) MMC sensitivity assay with the *FAN1* knockout HEK293T cell line stably transfected with a pUltra empty vector (EV), the vector with wild-type *FAN1* (WT), and the vector with c.418G>T (p.D140Y)-mutated *FAN1*.

Table 1. Germline *FAN1* Mutations Identified in 176 MMR-Proficient Amsterdam-Positive CRC Families

Family	*FAN1* genetic variant	Protein prediction (score) PolyPhen-2 (HumDiv / HumVar)	SIFT	Condel	Structure prediction	Splicing prediction (HSF)	ICL repair status	Population MAF (%) (dbSNP/ESP)
1	c.141C>A (p.C47*)	—	—	—	**Protein truncation**	—	**Deficient**[a]	**0/0**
2	c.2854C>T (p.R952*)	—	—	—	**Protein truncation**	—	NP	**0.05/0**
3	c.418G>T (p.D140Y)	Benign (0.03/0.019)	**Damaging (0.04)**	**Deleterious (0.708)**	NI[b]	**New ESS, broken ESE**	**Deficient**[c]	**0/0**
4	c.1771C>T (p.R591W)	**Probably damaging (1/0.998)**	**Damaging (0)**	**Deleterious (1)**	**Protein destabilization**	**New ESS**	NP	**0/0.0154**
5	c.1018C>T (p.P340S)	Benign (0.221/0.024)	Tolerated (0.09)	Neutral (0.056)	NI[b]	No change	NP	**0/0**

NOTE. Evidence that supports the damaging nature of the variants is in bold type.
ESP, NHLBI GO Exome Sequencing Project; HSF, human splicing finder v.3.0; ICL, DNA interstrand cross-link; MAF, minor allele frequency; NI, not informative; NP, not performed.
[a]Supplementary Figure 1.
[b]D140 and P340 are located in a region predicted to have a disordered structure.
[c]Figure 1*B* and Supplementary Figure 3.

286 sporadic CRC patients. In vitro, the *FAN1*-deficient phenotype shows lower sensitivity to mitomycin C (MMC) than other FA genes.[9] Even so, heterozygous c.141C>A (p.C47*) cells showed higher sensitivity to relatively high doses of MMC (10–70 nM) than wild-type cells (Supplementary Figure 1).

Four additional unreported or rare genetic variants in *FAN1* were identified in 176 MMR-proficient Amsterdam-positive families: a truncating mutation, c.2854C>T (p.R952*), and 3 missense variants, c.418G>T (p.D140Y), c.1018C>T (p.P340S), and c.1771C>T (p.R591W). Mutation carrier status could be assessed in 15 members of the *FAN1*-mutated families: all cancer-affected (10 CRC and 1 breast cancer) and 3 unaffected 21-, 43-, and 47-year-old individuals were carriers, and 1 unaffected 53-year-old was a noncarrier (Figure 1*A*). No exonic or splice-site variants were identified in 71 MMR-proficient Bethesda CRC families, in the normal colonic mucosae of 42 Spanish sporadic CRC patients and of 100 CRC patients from The Cancer Genome Atlas (TCGA),[10] and in 250 Spanish individuals without CRC.[11] However, among the 6503 ESP individuals, a total of 10 nonsense, frameshift or splice-site *FAN1* variants with minor allele frequency <1%, were identified in 16 subjects (0.24%). Unfortunately, no information about personal or family history of cancer is available. The limited number of mutation carriers identified ($n = 14$), together with the ascertainment bias due to the study of mostly cancer-affected family members, hampers at this point the estimation of risks and penetrance.

The identification of 2 truncating mutations in *FAN1* prompted us to investigate whether the other 3 variants might also affect the protein function. In silico algorithms predicted damaging functional effects for p.R591W and p.D140Y (Table 1). p.R591W, located in an evolutionary conserved residue, is also predicted to destabilize the protein structure, being localized in an exposed loop that connects 2 α-helices in the vicinity of the DNA-binding (SAP) domain (Supplementary Figure 2). c.418G>T (p.D140Y) is located in the first traduced exon, which codes for the UBZ domain, essential for FAN1 localization to sites of damage.[7] Heterozygous c.418G>T (p.D140Y) cells showed similar sensitivity to MMC than heterozygous c.141C>A (p.C47*) (Supplementary Figure 3), suggesting functional implications for c.418G>T. To confirm this, we generated a *FAN1* knockout HEK293T cell line that recapitulated the MMC-sensitive phenotype observed in $FAN1^{-/-}$ cells (Supplementary Figure 4), and stably transfected it with wild-type *FAN1*, c.418G>T-mutated *FAN1* and the empty vector. The c.418G>T-transfected cell line showed the same level of sensitivity to MMC as the empty vector (Figure 1*B*) without affecting FAN1 protein expression (Supplementary Figure 5), strongly suggesting that the missense mutation causes an DNA interstrand cross-link repair defect.

Five colorectal tumors developed by *FAN1* mutation carriers (3 c.141C>A and 2 c.418G>T) were available for somatic testing. Whole-exome sequencing of the Family 1 proband's tumor identified a total of 236 somatic mutations in transcribed sequences (Supplementary Table 2), with a mean mutation rate of 5/Mb, or 1.3/Mb for nonsynonymous

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changes. This mutation burden corresponds to that of nonhypermutant CRCs.[10] However, the mutation spectrum is characterized by an excess of T:A>G:C (10.5%) and C:G>G:C transversions (12.5%), both exceeding the 95th percentiles observed in nonhypermutant TCGA CRCs (Supplementary Figure 6). On the other hand, no clear evidence of somatic *FAN1* second hits was obtained: no loss of heterozygosity (0/5) or somatic mutation (0/3) (Supplementary Figure 7). In addition, neither loss of RNA expression of the wild-type allele nor reduction of expression of the FAN1 protein was observed in the tumor developed by a c.141C>A (p.C47*) carrier. However, FAN1 protein levels of normal colon mucosa from the *FAN1* c.141C>A carrier were lower than those of a wild-type individual (Supplementary Figure 8). These observations, together with the deficient DNA interstrand cross-link repair observed in lymphoblastoid cells from heterozygous mutation carriers (Supplementary Figures 1 and 3), suggest that *FAN1* haploinsufficiency might cause a bias toward a specific type of error due to defective DNA maintenance.

FAN1 interacts with MMR proteins and the nuclease function is required for fully functional MMR.[7,8,12] However, as MMR proficiency is an inclusion criteria in our study, tumors developed by *FAN1* mutation carriers showed microsatellite stability and/or normal expression of MMR proteins (Supplementary Figure 9). The fact that a plethora of nucleases, including FAN1, EXO1, and MRE11, can carry out the required nuclease activity for the MMR function[12] might explain the absence of MMR deficiency in *FAN1* mutation carriers' tumors.

FAN1 deficiency causes distinct milder phenotypes than other components of the FA pathway. Biallelic loss of *FAN1* does not cause FA, but karyomegalic interstitial nephritis (MIM# 614817), a very rare recessive disease (approximately 20 families reported so far) characterized by slow progressive renal failure that leads to end-stage renal disease before age 50 years.[13] Despite the lack of information on cancer history of monoallelic carriers, development of cancer at early ages has been described in 2 families: an autopsy performed in a 30-year-old individual with karyomegalic interstitial nephritis revealed a rectal adenocarcinoma, and another affected individual died of hepatocellular carcinoma at age 22 years.[14,15] Interestingly, although karyomegalic interstitial nephritis–associated biallelic mutations in *FAN1* localize toward the C-terminus of the protein, after the SAP domain, monoallelic mutations associated with hereditary CRC do not show preferential gene location (Supplementary Figure 10).[7]

Our findings implicate *FAN1* mutations in the inherited susceptibility to CRC. The analysis of larger familial CRC series will provide information about the prevalence of *FAN1* mutations (2.8% of Amsterdam-positive MMR-proficient families in our series) and allow the estimation of lifetime cancer risks for mutation carriers. Likewise, a thorough analysis of genetic and genomic alterations found in *FAN1*-associated tumors will clarify the underlying repair defects accumulated and, therefore, the mechanism of action of *FAN1* in colorectal carcinogenesis. Our findings further support the relationship between defective DNA repair and cancer predisposition, providing the first unequivocal evidence linking the FA pathway and CRC through *FAN1*, a bridge between FA and MMR DNA repair pathways.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.05.056.

References

1. Kastrinos F, et al. Cancer J 2011;17:405–415.
2. **Palles C, Cazier JB**, et al. Nat Genet 2013;45:136–144.
3. **Jaeger E, Leedham S**, et al. Nat Genet 2012;44:699–703.
4. **Kratz K, Schöpf B**, Kaden S, et al. Cell 2010;142:77–88.
5. **Liu T, Ghosal G**, et al. Science 2010;329:693–696.
6. MacKay C, et al. Cell 2010;142:65–76.
7. Smogorzewska A, et al. Mol Cell 2010;39:36–47.
8. Cannavo E, et al. J Biol Chem 2007;282:2976–2986.
9. **Trujillo JP, Mina LB**, et al. Blood 2012;120:86–89.
10. Cancer Genome Atlas Network. Nature 2012;487:330–337.
11. Quesada V, et al. Nat Genet 2011;44:47–52.
12. Desai A, Gerson S. DNA Repair (Amst) 2014;21:55–64.
13. **Zhou W, Otto EA**, et al. Nat Genet 2012;44:910–915.
14. Moch H, et al. Pathologe 1994;15:44–48.
15. Burry AF. J Pathol 1974;113:147–150.

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Conflicts of interest
The authors disclose no conflicts.

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