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Association Between Germline Mutations in *BRF1*, a Subunit of the RNA Polymerase III Transcription Complex, and Hereditary Colorectal Cancer

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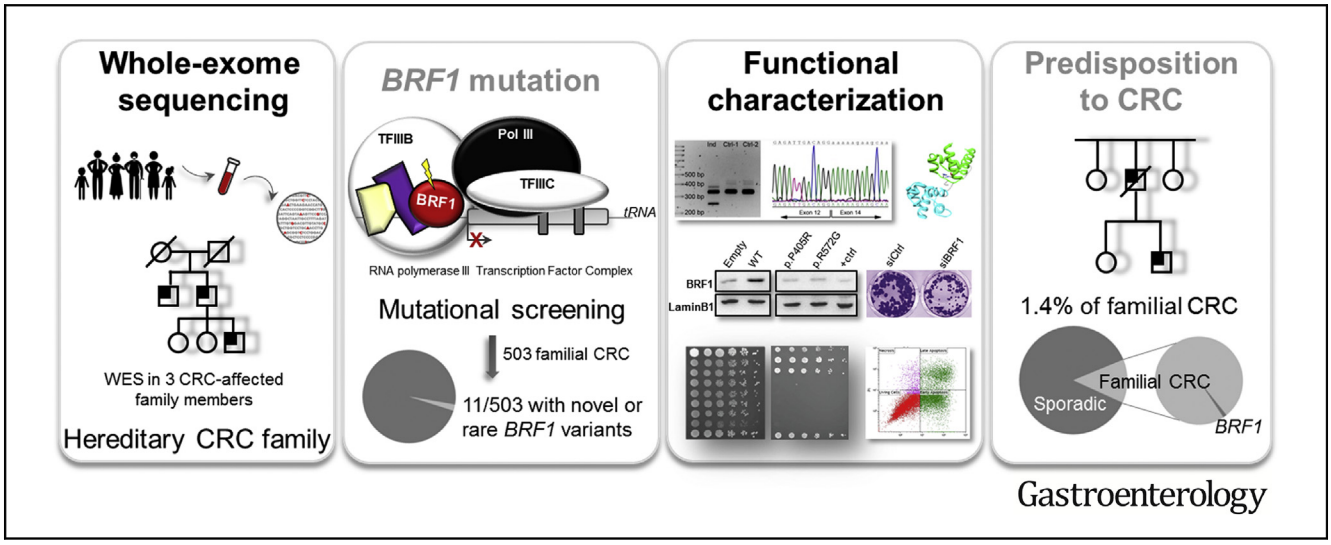

BACKGROUND & AIMS: Although there is a genetic predisposition to colorectal cancer (CRC), few of the genes that affect risk have been identified. We performed whole-exome sequence analysis of individuals in a high-risk family without mutations in genes previously associated with CRC risk to identify variants associated with inherited CRC. **METHODS:** We collected blood samples from 3 relatives with CRC in Spain (65, 62, and 40 years old at diagnosis) and performed whole-exome sequence analyses. Rare missense, truncating or splice-site variants shared by the 3 relatives were selected. We used targeted pooled DNA amplification followed by next generation sequencing to screen for mutations in candidate genes in 547 additional hereditary and/or early-onset CRC cases (502 additional families). We carried out protein-dependent yeast

EDITOR'S NOTES

BACKGROUND AND CONTEXT

Much of the genetic predisposition to colorectal cancer remains unexplained. The identification of genes associated with hereditary colorectal cancer will facilitate the management of families and individuals carrying pathogenic mutations.

NEW FINDINGS

Findings from whole-exome sequencing of a high-risk colorectal cancer family, followed by mutational screening in a large familial colorectal cancer series and functional assays, suggests that Germline heterozygous mutations in *BRF1* may be responsible for at least 1.4% of unexplained familial colorectal cancer cases.

LIMITATIONS

The identification of additional mutation carriers and evaluation of the phenotypes in large case and control populations will determine the clinical characteristics (tumor spectrum) and penetrance of the *BRF1*-associated syndrome.

IMPACT

If these findings are confirmed in larger familial colorectal cancer cohorts, and when the associated lifetime risks estimated, *BRF1* mutation carrier families will benefit from a clinical management based on carrier status and personalized risk assessment.

growth assays and transfection studies in the HT29 human CRC cell line to test the effects of the identified variants. **RESULTS:** A total of 42 unique or rare (population minor allele frequency below 1%) nonsynonymous genetic variants in 38 genes were shared by all 3 relatives. We selected the *BRF1* gene, which encodes an RNA polymerase III transcription initiation factor subunit for further analysis, based on the predicted effect of the identified variant and previous association of *BRF1* with cancer. Previously unreported or rare germline variants in *BRF1* were identified in 11 of 503 CRC families, a significantly greater proportion than in the control population (34 of 4300). Seven of the identified variants (1 detected in 2 families) affected *BRF1* mRNA splicing, protein stability, or expression and/or function. **CONCLUSIONS:** In an analysis of families with a history of CRC, we associated germline mutations in *BRF1* with predisposition to CRC. We associated deleterious *BRF1* variants with 1.4% of familial CRC cases, in individuals without mutations in high-penetrance genes previously associated with CRC. Our findings add additional evidence to the link between defects in genes that regulate ribosome synthesis and risk of CRC.

Keywords: Colon Cancer; Familial Colorectal Cancer; Protein Translation; Mechanism.

Hereditary forms of colorectal cancer (CRC) are caused by germline mutations or epimutations in *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*, *APC*, *MUTYH*, *GREM1*, *SMAD4*, *BMPR1A*, *STK11*, and *PTEN*.[1] Recently, additional familial CRC genes, with more or less evidence of causality and each explaining a small fraction of familial cases, have been identified through the application of whole-genome and whole-exome sequencing (WES) or genome-wide copy number techniques.[2] Among these genes are *POLE*, *POLD1*, *NTHL1*, *MSH3*, and *RNF43*, responsible for CRC and polyposis syndromes.[3–5] Despite these advances, much of the observed heritability and familial aggregation of the disease remains unexplained.

With the aim of identifying novel causal genes for CRC predisposition, we carried out WES in 3 affected members of an Amsterdam I mismatch repair (MMR)-proficient CRC family, followed by sequencing of the candidate genes in a large series of genetically unexplained CRC families and confirmation of the functional impairment of the identified variants.

Methods

Patients and Samples

Family 1. Family 1, of Spanish origin (White), had 3 family members affected with CRC and 1 member diagnosed with early-onset breast cancer. One of the CRC-affected individuals was first-degree relative of the other two, and the patient with breast cancer, daughter of one of the patients with CRC (Figure 1). The CRC of III.1 was diagnosed at age 62, and corresponded to a pT2pN0pM0 grade 2 microsatellite-stable tumor located in the sigmoid colon. The CRC diagnosed in III.2 at age 65 corresponded to a pT3pN1pM0 grade 2 microsatellite-stable tumor (unreported location in the colon). The rectal tumor developed at age 40 by individual IV.3 was pT3pN0pM0 grade 2 and showed normal expression of MLH1, MSH2, MSH6, and PMS2, assessed by immunohistochemistry. The breast cancer (pT1pN1pM0) of IV.2 was diagnosed at age 38 and was estrogen and progesterone receptor-positive. This patient had not been tested for *BRCA1/BRCA2* mutations. Sanger sequencing of the exons and exon-intron boundaries of *MLH1*, *MSH2*, *MSH6*, and *PMS2* in individuals III.2 and IV.3, and the study of large rearrangements by multiplex ligation-dependent probe amplification confirmed the absence of germline mutations in these 4 genes. The family was recruited through the Genetic Counselling Unit of the Hereditary Cancer Program of the Catalan Institute of Oncology, IDIBELL. The members of the family who participated in the study signed a specific informed consent for this project (IDIBELL Ethics Committee Approval PR073/12).

Validation series. *BRF1* mutation screening was performed in 151 Amsterdam-positive MMR-proficient unrelated

*Authors share co-first authorship.

Abbreviations used in this paper: CRC, colorectal cancer; LOH, loss of heterozygosity; MAF, minor allele frequency; miRNA, microRNA; MMR, DNA mismatch repair; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RNA Pol III, RNA polymerase III; rRNA, ribosomal RNA; RT, reverse transcription; siBRF1, siRNA *BRF1*; siC, siRNA control; siRNA, small interfering RNA; SNP, single nucleotide polymorphism; TBP, TATA-binding protein; TFIIIB, transcription factor complex of the RNA Pol III; UTR, untranslated region; WES, whole-exome sequencing; 3D, 3-dimensional.

Most current article

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