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LETTER TO THE EDITOR

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Is *BRCA2* involved in early onset colorectal cancer risk?

Less than 10% of hereditary colorectal cancers (CRC) can be explained by well-defined syndromes, including Lynch syndrome, familial adenomatous polyposis, MUTYH-associated polyposis, and hamartomatous polyposis.[1] *BRCA2* codes for a DNA repair protein involved in homologous recombination. Heterozygous germline pathogenic variants in *BRCA2* are involved in hereditary breast and ovarian cancer (HBOC) syndrome and biallelic pathogenic variants are responsible for Fanconi anemia (FA), characterized by congenital abnormalities, chromosome instability, bone marrow failure and predisposition to cancer.[2] Some conflicting studies suggest the implication of BRCA2 in CRC risk.[3] Here we report a family presenting early-onset CRC associated with biallelic *BRCA2* variants but without signs of FA.

The proband, patient III-3 (Figure 1A), presented a colorectal adenocarcinoma at 37 years old. Two years later, colonoscopic investigation revealed a second colorectal adenocarcinoma and one sessile serrated adenoma. Immunohistochemistry analyses showed normal expression of the MMR proteins MLH1, MSH2, MSH6 and PMS2. His brother, patient

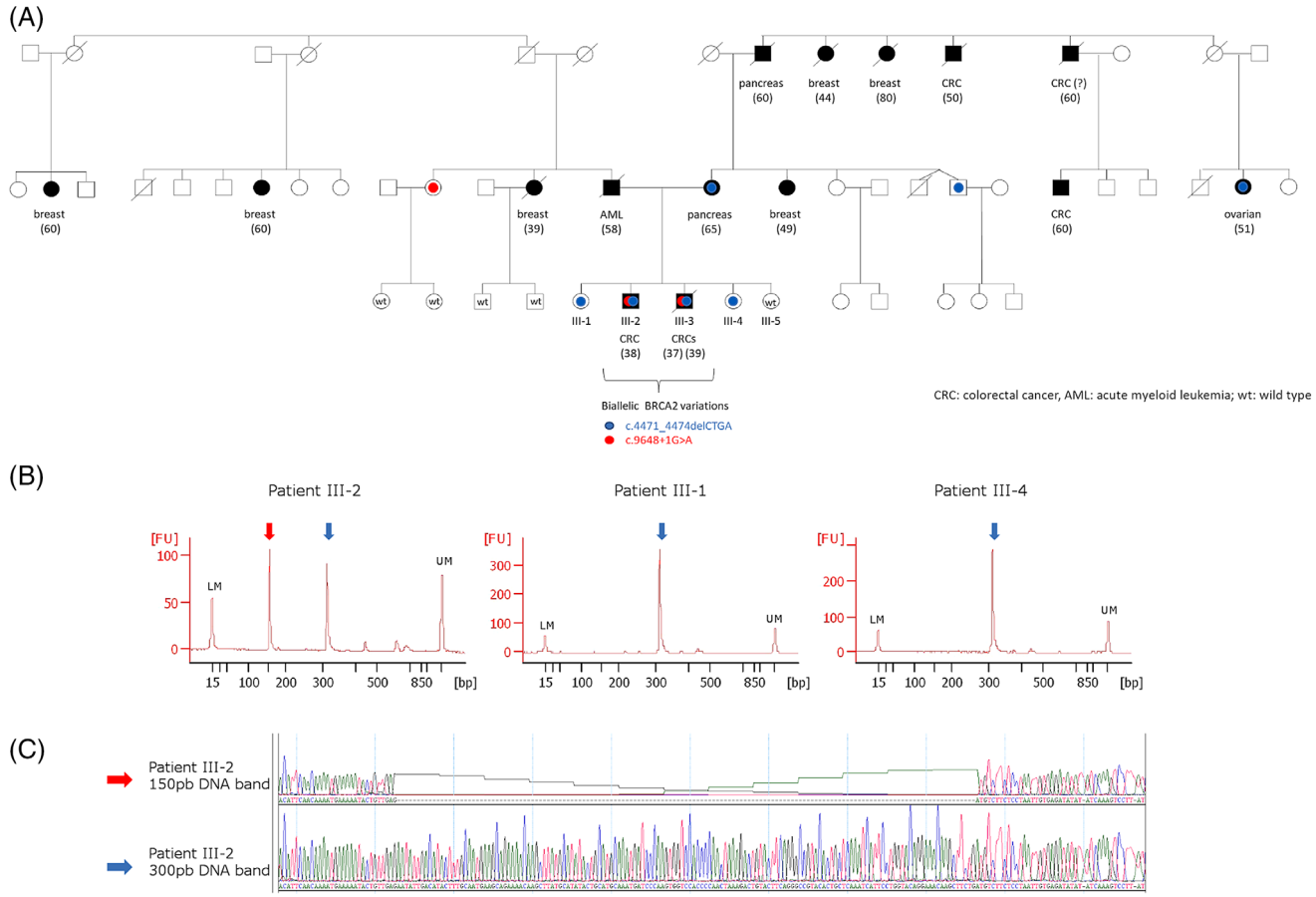

FIGURE 1 A, Closed symbols indicate patients affected with cancer. Open symbols indicate healthy individuals. The type of cancer and age at presentation are given in brackets. Blue circle represents c.4471_4474del variant and red circle represents the c.9648 + 1G > A. B, RNA was extracted from blood of patient III-3 and his sisters III-1 and III-4. RT-PCR analysis was performed with primers mapping to exons 25 and 27, and PCR products were separated by Bioanalyzer electrophoresis. The sizes of the DNA marker (M) are indicated to the left. LM: lower marker, UM: upper marker. C, Each RT-PCR product from patient III-3 was gel-purified and analyzed by Sanger sequencing. The 297-bpb and corresponds to the reference BRCA2 transcript and the 150-bp band corresponds to a BRCA2 transcript lacking exon 26 [Colour figure can be viewed at wileyonlinelibrary.com]

III-2, presented at 38 years of age a sigmoid adenocarcinoma with 17 adenomatous colorectal polyps. Retrospective clinical examination did not reveal any pathogenic features of FA. Chromosome-breakage analysis revealed no chromosomal breaks and exchanges typical of FA, particularly radial configurations from DEB-treated cultures.

Informed consent was obtained for each patient and principles outlined in the Helsinki Declaration were followed. Study ethics approval was obtained on July 11, 2019 (CECIC Rhône-Alpes-Auvergne, Grenoble, IRB 5921). Multigene panel analysis was performed on DNA extracted from peripheral blood of patients III-3 and III-2 and did not reveal any variant in the major genes known to be involved in CRC risk (*MMR*, *APC/MUTYH*, *POLD1/POLE*, *BMPR1A/SMAD4*) and in three additional genes recently described in CRC risk (*NTHL1*, *GREM1* and *RNF43*). Two *BRCA2* (*LRG_293*) variants were observed: one causal c.4471_4474del; p.(Leu1491Lysfs*12) in exon 11 and one of unknown significance, c.9648 + 1G > A, a splice site variant in intron 26.

RT-PCR analysis of patient RNA revealed that the c.9648 + 1G > A variant resulted in skipping of the second to last exon (exon 26), leading to an in-frame deletion of 46 amino acids (r.9502_9648del; p.Asn3168_Leu3216del) (Figure 1B). Minigene analysis showed that exon 26 skipping is total for this allele (Figure 1C). Three other variants causing skipping of exon 26 were previously reported as pathogenic or likely pathogenic for HBOC in the ClinVar database and this isoform was not listed in the naturally occurring alternate splicing events.[4] However, loss of exon 26 does not seem to impact the tertiary structure and *BRCA2* exon 26 is not a highly conserved region. BRCA2 protein without exon 26 could thus represent a partially functional isoform. The fact that both brothers declared early-onset CRC may indicate a role for the splice variant. Degrolard-Courcet et al (2014)[5] already described a family of CRC predisposition with a *BRCA2* pathogenic variant associated in *trans* with a variant leading to a partial splicing defect. This study and ours suggest that these splice variants could be hypomorphic, representing low-penetrance or disease-modifier alleles. The constitutional absence of any fully functional BRCA2 allele may also result in unexpected pleiotropic effects.

In conclusion, multigene panel testing can reveal genetic predisposition, irrespective of the familial clinical phenotype. Our observations suggest that biallelic *BRCA2* variants could be implicated in familial CRC inheritance but this has to be confirmed in large scale studies.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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