ARTICLE

Development of primary early-onset colorectal cancers due to biallelic mutations of the *FANCD1/BRCA2* gene

Emilie Degrolard-Courcet^{1,15}, Joanna Sokolowska^{2,15}, Marie-Martine Padeano³, Séverine Guieu⁴, Myriam Bronner², Carole Chery², Fanny Coron⁵, Côme Lepage⁶, Caroline Chapusot¹, Catherine Loustalot³, Jean-Louis Jouve⁶, Cyril Hatem⁷, Emmanuelle Ferrant⁸, Laurent Martin¹, Charles Coutant³, Amandine Baurand⁵, Gérard Couillaud⁹, Alexandra Delignette¹⁰, Salima El Chehadeh⁵, Sarab Lizard¹¹, Laurent Arnould¹², Pierre Fumoleau⁴, Patrick Callier¹³, Francine Mugneret¹³, Christophe Philippe², Thierry Frebourg¹⁴, Philippe Jonveaux^{*,2,15} and Laurence Faivre^{*,5,15}

Fanconi anaemia (FA) is characterized by progressive bone marrow failure, congenital anomalies, and predisposition to malignancy. In a minority of cases, FA results from biallelic *FANCD1/BRCA2* mutations that are associated with early-onset leukaemia and solid tumours. Here, we describe the clinical and molecular features of a remarkable family presenting with multiple primary colorectal cancers (CRCs) without detectable mutations in genes involved in the Mendelian predisposition to CRCs. We unexpectedly identified, despite the absence of clinical cardinal features of FA, a biallelic mutation of the *FANCD1/BRCA2* corresponding to a frameshift alteration (c.1845_1846delCT, p.Asn615Lysfs*6) and a missense mutation (c.7802A>G, p.Tyr2601Cys). The diagnosis of FA was confirmed by the chromosomal analysis of lymphocytes. Reverse transcriptase (RT)-PCR analysis revealed that the c.7802A>G *BRCA2* variation was in fact a splicing mutation that creates an aberrant splicing donor site and results partly into an aberrant transcript encoding a truncated protein (p.Tyr2601Trpfs*46). The atypical FA phenotype observed within this family was probably explained by the residual amount of BRCA2 with the point mutation c.7802A>G in the patients harbouring the biallelic *FANCD1/BRCA2* mutations. Although this report is based in a single family, it suggests that CRCs may be part of the tumour spectrum associated with *FANCD1/BRCA2* biallelic mutations and that the presence of such mutations should be considered in families with CRCs, even in the absence of cardinal features of FA.

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INTRODUCTION

Fanconi anaemia (FA) is an autosomal recessive condition characterized by congenital abnormalities, progressive pancytopenia and a predisposition to leukaemia and solid tumours.^{1–3} Fifteen genes, defining 15 complementation groups, have been shown to be involved in FA.⁴ One of them, *FANCD1*, corresponds to the *BRCA2* gene, one of the main genes involved in autosomal dominant predisposition to breast and ovarian cancers. The proteins encoded by FA genes cooperate in the FA/BRCA pathway involved in the recognition and repair of damaged DNA.⁵ Disruption of FA/BRCA pathway leads to spontaneous and DNA cross-linking agents-induced chromosomal breakage.^{6,7}

In FA, there are typically several clinical stages related to age.^{8,9} At birth and during early childhood, only physical signs are usually

present with various severities, including typical but inconstant radial abnormalities. Aplastic anaemia is usually the first adverse event in patients with FA, occurring at a median age of 8–10 years. The diagnosis of FA is often made at this stage. Leukaemia occurs usually in teenagers and young adults, and solid tumours in the second decade. The more frequent solid tumours are head and neck and gynaecologic squamous cell carcinomas.^{10,11} Sub-typing of FA is clinically important considering the genotype/phenotype correlations. Indeed, some groups present a more severe phenotype than others. FA subtype D1 (FA-D1), due to biallelic *BRCA2* mutations and representing 3% of FA cases, is characterized by a more severe phenotype than that observed in the other groups, and FA-D1 patients often died in childhood.¹² Although FA-D1 patients display

¹Service d'Anatomie et Cytologie Pathologiques, Pole Technique et biologie CHU Dijon, Dijon, France; ²Laboratoire de Génétique et INSERM U-954, CHU Nancy, Université de Lorraine, Nancy, France; ³Département de Chirurgie, Centre de lutte anti-cancereux Georges François Leclerc, Dijon, France; ⁴Département d'oncologie médicale, Centre de lutte anti-cancereux Georges François Leclerc, Dijon, France; ⁵Centre de Génétique, Hôpital d'Enfants, CHU Dijon et Université de Bourgogne, Dijon, France; ⁶Service d'Hépatogastro-entérologie, CHU "Bocage Central", Dijon, France; ⁷Hépatogastro-entérologie, Clinique Drevon, Dijon, France; ⁸Service d'Hématologie Clinique, Hôpital d'Enfants, CHU Dijon, Dijon, France; ⁹Service de Pédiatrie 1, Hôpital d'Enfants, CHU Dijon, Dijon, France; ¹⁰Service de Radiologie, Centre de lutte anti-cancereux Georges François Leclerc, Dijon, France; ¹¹Biologie Moléculaire, Centre de lutte anti-cancereux Georges François Leclerc, Dijon, France; ¹²Anatomopathologie, Centre de lutte anti-cancereux Georges François Leclerc, Dijon, France; ¹³Service de Cytogénétique, Pole Technique et biologie, CHU Dijon, Dijon, France; ¹⁴InsERM U1079 et Département de Génétique, CHU de Rouen, France

¹⁵These authors contributed equally to this work.

*Correspondence: Professor P. Jonveaux, Service de cytogénétique et génétique moléculaire, CHU de Nancy, Rue du morvan, 54511 Vandoeuvre-les-Nancy, Cedex, France. Tel: +33 3 83 15 37 71; Fax: +33 3 83 15 37 72; E-mail: p.jonveaux@chu-nancy.fr or Professor L. Faivre, Centre de Génétique Hôpital d'Enfants, CHU Dijon, 14 rue Gaffarel, 21079 Dijon, Cedex, France. Tel: +33 3 80 29 53 13; Fax: +33 3 80 29 32 66; E-mail: laurence.faivre@chu-dijon.fr

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less frequent bone marrow suppression and less frequent skeletal abnormalities, they present, as compared with the other FA patients an unusually high spontaneous chromosome aberration rate and early-onset malignancies including acute myeloid leukaemia (AML), Wilms' tumour and medulloblastoma.

Here, we report a remarkable family harbouring a biallelic *BRCA2* mutation and presenting with multiple malignancies including primary colorectal cancers (CRCs).

Clinical reports

Patient 1 (III: 4) is a 33-year-old female, the first of four children born from healthy unrelated parents (Figure 1 and Supplementary Figure). No birth defect or developmental abnormalities were noticed at birth. She had an uneventful childhood. She has a healthy 12-year-old boy. She presented at 31 years of age with stomach aches progressing over 3 months with night sweating. Gastric fibroscopy with biopsies revealed an intense infiltration of CD20 and bcl-6 expressing cells, corresponding to a diffuse large B-cell lymphoma of the stomach (Ann Arbor Classification). Clinical examination and a PET scan showed cervical lymph nodes compatible with another localization of the lymphoma. The treatment of this stage III B-cell lymphoma consisted of eight courses of chemotherapy (R-CHOP: rituximab–cyclophosphamide–doxorubicin–vincristine) including a cross-linker agent. No severe chemotherapy toxicity was noticed. Complete remission was obtained after the seventh course of chemotherapy. During the first systemic screening, scintigraphic examination showed an abdominal hyperfixation. The colonoscopic investigation revealed in the caecum, the ascending colon and sigmoid, three tumours that corresponded to three pT2N0 colorectal adenocarcinomas, which lead to colectomy. Molecular and immunochemistry analyses of the tumours showed no RER phenotype and a normal expression of the MSH2, MLH1, MSH6 and PMS2 MMR proteins. These tumours had, therefore, a microsatellite stable (MSS)phenotype. At the age of 35, four years after lymphoma and CRCs onset, a colonoscopic follow-up revealed a new rectal tumour that corresponded to a pT3N0 adenocarcinoma. The patient underwent a laparoscopic colectomy. Prophylactic hysterectomy with bilateral salpingo-oophorectomy was performed at the same time. Retrospective clinical examination revealed in the patient café-au-lait spots (CALs).

Patient 2 (III: 5) is a female patient and the second child of the family. She also had a dizygotic twin sister (patient 3 III: 6, Figure 1). No birth defect or developmental abnormalities were noticed at birth, and she had an uneventful childhood. At 27 years of age, she presented with recurrent constipation and left iliac fossa pain.

Considering the familial history, she underwent a colonoscopy that revealed in the ascending colon a tumour corresponding to a moderately differentiated pT1N0 MSS adenocarcinoma. The patient underwent a colectomy. After 2 years, the patient discovered, by self-examination in the right breast, a tumour that corresponded to a pT1bN0M0 infiltrative ductal carcinoma, positive for progesterone receptor and negative HER2 staining. The patient underwent mastectomy, followed by hormone therapy. Retrospective clinical examination revealed CALS, mild microcephaly, enophthalmia and abnormal shape of ears. The serum AFP level was at 169 µg/l ($N < 10$ µg/l), consistent with the levels observed in FA patients. The hemogram, including the mean cell volume, remained normal.

Patient 3 (III: 6) was the twin sister of patient 2 and died at 5 years of age from an AML. At birth, measurements were < -1 standard deviation (SD) for weight and size and < -1.5 SD for occipital frontal circumference. No congenital abnormalities had been noticed. At 5 years of age, an AML was diagnosed. Bone marrow failure or myelodysplasia were not reported before the development of AML. Before the initiation of chemotherapy, a complete blood-cell count had revealed a white blood-cell count of $45\,900/\text{mm}^3$, haemoglobin 8.8 g/dl , mean cell volume 95 fl (within normal range for age) and the platelet count was $9 \times 10^9/\text{l}$. Blood and bone marrow were infiltrated with 77% and 85% of blastic cells, respectively. The treatment included daunorubicin ($200\text{ mg/m}^2/\text{j}$), and aracytine ($200\text{ mg/m}^2/\text{j}$). The first chemotherapy course was complicated by prolonged aplasia, leading to severe mucositis, disseminated fungal infection, and bacteremia, which resulted in death of the patient, 2 months after initial diagnosis. Microcephaly (< -2 SD) had been noticed in her clinical file at the time of AML diagnosis.

As indicated in the Supplementary Figure, the patients also had another 20-year-old cancer-free sister (III: 7). The father of the proband (II: 7) had a negative colonoscopic examination at 57 years of age. One of his sisters (II: 6) died from breast cancer at the age of 50 years. The mother of the proband (II: 8) had a negative mammography and a negative colonoscopy at 58 years of age. Her father (I: 3) died at 62 years of age of a liver cancer. The primary or secondary origin of this cancer was unknown.

MATERIALS AND METHODS

Mutational analysis of the *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6*, *TP53* and *MYH* genes

Genomic DNA was extracted from peripheral blood samples, after informed consent was obtained for each patient. Point mutation screening of the *BRCA1* and *BRCA2* genes was performed in patient 2 (III: 5), using HRM/sequencing

Figure 1 Tree of the reported family. Closed symbols indicate patients affected with FA. Open symbols indicate patients unaffected with FA. The type of cancer and age at presentation (in brackets) are given under the symbol. BRCA2 mutations are added next to the symbol. Wt: wild type.

(*BRCA2*: NM_000059.3). Screening for large rearrangements of the *BRCA1* and *BRCA2* genes was performed using multiplex ligation-dependent probe amplification. Segregation analyses in the proband family for the mutations of *BRCA2* were performed by exon 10 and 16 sequencing. Sequencing of the *MLH1*, *MSH2*, and *MSH6* genes, screening for large rearrangements of *MSH2* and *MLH1* using multiplex ligation-dependent probe amplification, sequencing of the *MLH1* promoter and determination of the *MLH1* methylation status were performed in patients 1 (III: 4) and 2 (III: 5). Sequencing and screening for large rearrangements of *TP53* and direct sequencing of *MYH* were performed in Patient 1(III: 4).

In silico prediction tools

To analyse the impact of *BRCA2* c.7802A>G variant on splicing, we used the Alamut decision-support software (Interactive Biosoftware, Rouen, France) that includes the splice site prediction by neural network (NNSplice),¹³ splice site finder,¹⁴ Max-EntScan,¹⁵ ESE finder¹⁶ and human splicing finder¹⁷ algorithms. This software also includes three protein-function prediction tools: Align-GVGD, (<http://agvgd.iarc.fr/>), SIFT (<http://sift.jcvi.org/>), and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>).

Splicing study of the *BRCA2* c.7802A>G variant

Total RNAs were isolated from the PaxGen blood RNA tube, using the RNeasy mini kit (Qiagen, Courtaboeuf, France). RNA was reverse-transcribed using random primers (Superscript (Life Technologies, Saint Aubin, France), Invitrogen (Life Technologies)) and *BRCA2* cDNA was amplified, using primer F: 5'-T CACAAAGTGTGAAGAAGAACCTT-3', primer R: 5'-AGATGCCTAAGCCCA-GAAA-3', corresponding to exons 14 and 17, respectively. RT-PCR products were electrophoresed on agarose gel, purified using the NucleoSpin Extract II kit (Macherey-Nagel, SARL, Hoerd, France) and sequenced using the BigDye Terminator kit (Applied Biosystems, Life Technologies).

Chromosome breakage analysis

Metaphase cells from peripheral blood lymphocytes of patient 2 were G-banded after brief pretreatment in 25% pancreatin (Gibco, Life Technologies), followed by counterstaining with Wright stain. Sixty-five G-banded metaphase cells from the baseline and sixty-five metaphase cells from mitomycin (MMC) conditions were evaluated for chromosomal abnormalities, as previously described.⁷

RESULTS

In this family presenting with multiple primary early-onset CRCs, analysis of *MSH2*, *MLH1*, *MSH6*, *MYH*, *BRCA1* and *TP53* genes revealed no germline alteration abnormality. In patient 2, early-onset breast cancers led us to analyse the *BRCA* genes. This analysis unexpectedly revealed two *BRCA2* mutations, corresponding to a nonsense mutation c.1845_1846delCT (p.Asn615Lysfs*6) within exon 10 (Figure 2) and a missense variation c.7802A>G (p.Tyr2601Cys) within exon 16 (Figure 3). Segregation analysis in the family confirmed that each mutation was inherited from one parent. Both mutations were also found in the older sister (patient 1), who had developed two early-onset cancers. Pre-symptomatic testing revealed that the younger sister III: 5 carried the maternal *BRCA2* c.1845_1846delCT mutation. In contrast to the maternal c.1845_1846delCT, which has previously been reported in the French database (http://www.umd.be/BRCA2/4DACTION/DMD_EX1/10/) of the Groupe Génétique et Cancer (GGC) and is deleterious, the c.7802A>G variant, paternally inherited, was of unknown significance. It has not been reported in the GGC French mutation database, neither in the 1000 Genomes project (<http://browser.1000genomes.org/>) nor in dbSNP (built 137). This variation is predicted to result in a missense mutation (p.Tyr2601Cys) and *in silico* predictions, AGVGD (Class C55); Polyphen (score 1 probably damaging); SIFT (predict non-tolerated), classified this variant as deleterious at the protein level. Nevertheless, this genetic variation is located four nucleotides before the end of exon 16 and the different splicing prediction algorithms predicted the creation of an aberrant splicing donor site, located four nucleotides upstream of the physiological 5' splicing site (human splicing finder scores of 74,1 *versus* 74,7). We therefore analysed, by RT-PCR performed on RNA extracted from patient 2 peripheral blood, the impact on splicing of the c.7802A>G variant. As indicated in Figure 4, sequencing of the RT-PCR products showed the presence of three different transcripts: Transcript A corresponded to the expected RNA expressed from the mutant *BRCA2* allele carrying the c.1845_1846delCT mutation (Figure 5a). Transcripts B and C derived from the allele harbouring the

Figure 2 Forward and reverse *BRCA2* sequence with the exon 10 mutation c.1845_1846delCT p.Asn615Lysfs*6.

Figure 3 Forward and reverse *BRCA2* sequence with the missense variant c.7802A>G p.Tyr2601Cys in exon 16.

Figure 4 *In silico* analysis using Alamut software: appearance of a *de novo* 5' splicing site (GU), located four nucleotides upstream of the physiological 5' splicing site, suggesting a splice anomaly. The strength of this *de novo* 5' splicing site is equivalent to the physiological 5' splicing site, with scores of 74,1 versus 74,7 according to the human splicing finder.

substitution c.7802A>G and, as indicated by the bioinformatics prediction, transcript C was characterized by a skipping of the four last nucleotides of exon 16, owing to the creation of an aberrant splice donor site: r.7802_7805delauag. This aberrant transcript was predicted to result in a truncated protein: p.Tyr2601Trpfs*46. Comparison of the sequencing electropherogram peak heights suggested that transcripts A, B and C represent, respectively, 50%, 25% and 25% of the transcripts expressed in the blood from the patient (Figure 5b and c).

In peripheral blood from patient 2, the cytogenetic analysis revealed 41% of cells with aberrations (27/65) and one triradial

figure, confirming the diagnosis of FA. In the presence of a DNA cross-linker agent (MMC), an exceptionally high number of MMC-induced breaks per cell (100%) and several triradial figures were observed.

DISCUSSION

We report in this study a family presenting a remarkable history of multiple primary cancers, including CRCs, lymphoma and breast cancer, due to biallelic *BRCA2* mutations and revealing unexpectedly the diagnosis of FA. This report is the first describing the association of early-onset CRCs with FA and, therefore broadens the phenotypic

In FA, all the genotype/phenotype correlation studies indicate that the FA-D1 group, like the FA-N group, can be distinguished from the other groups by a more severe phenotype and a high mortality rate in early childhood.^{12,18} Table 1 gives a review of the 30 patients from 24 families with biallelic *BRCA2* mutations and whose phenotype had previously been described in details family.^{12,19–28} Among these patients, the females and males were equally affected and the mean age of FA diagnosis was 1.9 years (range, 0.1–5.2 years). Most of the patients were diagnosed as FA because of their typical physical features (68%). Ten patients had severe congenital malformations (cardiac, brain or intestinal abnormalities), including five VATER associations (Vertebral defects, Anal atresia, Tracheoesophageal fistula with esophageal atresia, Renal, and Radial dysplasia). Myelodysplasia or bone marrow failure was reported in eight patients. Among the patients, 19 (63.3%) were dead at a mean age of 3.9 years (range 1.4–12 years). Among the 11 (36.6%) alive at the time of report, the mean age was of 5.84 years (range 0.8–30 years). Fourteen died because of the progression of malignancy and five died because of a severe toxicity of chemotherapy. Malignancies included brain tumours

Table 1 Review of manifestations in biallelic *BRCA2* mutation cases

ID	Malformations	Sex	MDS/BMF	Cancer (age in years)	Allele 1	Exon 1	Allele 2	Exon 2	Outcome	Diagnosis presentation (in years)	Reference
?	Microcephaly; esotropia; cerebellar hypoplasia; arachnoid cyst; abnormal radii and thumbs; clinodactyly; FTT; CLS	F	+	Bilateral Wilms' (1), myelodysplasia (2), medulloblastoma (2.5)	c.3264dupT p. (Gln1089Serfs*10)	11	c.8487 + 3A > G p.(Ile2778_Gln2829del)	19j	Dead (2.5) of disease	Onset of bilateral Wilms' tumour + physical findings (1)	25
?	Intestinal duplication cysts/mesenteric lymphangioma jejunum; FTT; microcephaly; CLS; ear anomalies; bilateral clinodactyly	M	+	Neuroblastoma (1.3) aplasia (1.5) AML (1.7)	c.1813dupA, p. (Ile605Asnfs*11)	10	c.631 + 2T > G p.(Gly173Serfs*19)	7i	Dead (2) of disease	Physical findings (1.3)	25
?	Holoprosencephaly; microcephaly; TEF; FTT; ear anomalies; hearing loss; pelvic kidneys; optic anomalies; polydactyly; CLS; congenital naevus	F	+	Differentiating neuronal neoplasm (1.7), MDS (2), AML (2.7)	c.2899_2900delCT, p.(Leu967Argfs*14)	11	c.7007G > A p.(Arg2336His)	13	Dead (2.7) of disease	Physical findings (0.2)	25
HSC62	Abnormal thumb	M	NA	None (30)	c.8488-1G > A r.8488_8491del p.(Trp2830_Lys2833del)	19j	c.8488-1G > A r.8488_8491del p.(Trp2830_Lys2833del)	19j	Alive (30)	NA	21
EUFA423	Pigmented, abnormal thumb	F	NA	Brain (3)	c.7464_7465insTA p. (Asp2489*)	15	c.9672dupA p. (Tyr3225Ilefs*30)	27	Alive (3)	NA	21
EUFA579	Pigmented, abnormal thumb	F	NA	AML (NA)	c.7007G > A p.(Arg2336His)	13	c.5609_5610delTCinsAG p. (Phe1870*)	11	Alive (NA)	NA	21
AP37P	Short, pigmented, CLS, abnormal thumb, Sprengel, midface hypoplasia	M	+	AML M2 (2)	c.8187G > T p. (Lys2729Asn)	18	c.8504C > A p.(Ser2835*)	20	Dead (3.9) of disease	Physical findings (2)	24
1A	Short CLS, pigmented, abnormal thumbs and radii, microcephaly, imperforate anus, epicanthal folds, micropenis, undescended testes, dislocated hips, hydronephrosis, abnormal hearing (VATER*)	M	NA	Brain-medulloblastoma or astrocytoma (4.9)	c.5946delT p. (Ser1982Argfs*22)	11	c.9207T > A p.(Cys3069*)	24	Dead (4.9) of disease	Physical findings (1.4)	27
1B	Short, abnormal thumbs, microcephaly, imperforate anus with rectovaginal fistula, slanted eyes, anomalous kidneys, small ear, hip dysplasia (VATER*)	M	NA	Brain-astrocytoma (2)	c.5946delT p. (Ser1982Argfs*22)	11	c.9207T > A p.(Cys3069*)	24	Dead (2) of disease	Physical findings (NA)	27
2	No exam reported	F	NA	Brain-medulloblastoma (4.5)	c.5946delT p. (Ser1982Argfs*22)	11	c.658_659delGT p.(Val220Ilefs*4)	8	Alive (4.5)	NA	27
3	No exam reported	F	NA	Brain-medulloblastoma (2.5)	c.5073dupA p.(Trp1692Metfs*3)	11	c.7669T > C p.(Ile2490Thr)	15	Alive (2.5)	NA	27
4	No exam reported	F	NA	Brain-medulloblastoma (3.5)	c.3922G > T p.(Glu1308*)	11	c.9196C > T p.(Gln3066*)	24	Alive (3.5)	NA	27
772/1	CLS, microcephaly, cardiac (patent ductus arteriosus)	M	-	Brain-medulloblastoma (2.3)	c.658_659delGT p. (Val220Ilefs*4)	8	c.8219T > A p.(Leu2740*)	18	Dead (2.5) of toxicity	Toxicity to chemotherapy (2.5)	20
772/2	CLS, abnormal facies, Epicanthus, clinodactyly	M	-	Wilms' (1.3), Brain-medulloblastoma (4.3)	c.658_659delGT p. (Val220Ilefs*4)	8	c.8219T > A p.(Leu2740*)	18	Dead (4.5) of toxicity	Onset of Wilms' tumour + familial history (1.3)	20
K2S1	Short, microcephaly	M	-	Wilms' (0.5), AML (2)	c.4648G > T p.(Glu1550*)	11	c.7529T > C p.(Leu2510Pro)	15	Dead (2) of toxicity	Toxicity to chemotherapy (2)	20
K2S2	Short, pigmented macules, bifid thumb, elfin facies, small palpebral fissures	F	-	T-ALL (5)	c.4648G > T p.(Glu1550*)	11	c.7529T > C p.(Leu2510Pro)	15	Dead (5) of toxicity	Familial history (at birth)	20
129/1	Short, CLS, FTT microcephaly, imperforate anus	NA	NA	AML (2.2)	c.631 + 2T > G p.(Gly173Serfs*19)	7i	c.631 + 2T > G p.(Gly173Serfs*19)	7i	Dead (5.2) of toxicity	Physical findings (NA)	20

Table 1 (Continued)

ID	Malformations	Sex	MDS/BMF	Cancer (age in years)	Allele 1	Exon 1	Allele 2	Exon 2	Outcome	Diagnosis presentation (in years)	Reference
357/1	Short, thumb, imperforate anus, FTT	NA	NA	AML (1.9)	c.7878G>C p. p.(Trp262Cys)	17	c.1813dupA p.(Ile605Asnfs*11)	10	Dead (2.8)	Physical findings (NA)	22
632/1	Short, CLS, dysplastic hips, pelvic kidney	F	NA	AML (3)	c. 631 + 1G>A p.(?)	7i	c.5682C>G p.(Tyr1894 ^a)	11	Dead (5)	Physical findings (NA)	22
632/2	Short, imperforate anus, hypoplastic thumb	F	NA	AML (1.8)	c. 631 + 1G>A p.(?)	7i	c.5682C>G p.(Tyr1894 ^a)	11	Alive (2.4)	Physical findings (NA)	22
800/1	Short, CLS, FTT, microcephaly, micropenis	M	NA	AML (0.9)	c. 631 + 2T>G p.(Gly173Serfs*19)	7i	c.4936_4939delGAAA p.(Glu1646Glnfs*23)	11	Dead (1.7) of disease	Physical findings (NA)	22
800/2	Short, CLS, FTT, microcephaly	M	+	Wilms' (0.8)	c. 631 + 2T>G p.(Gly173Serfs*19)	7i	c.4936_4939delGAAA p.(Glu1646Glnfs*23)	11	Alive (0.8)	Physical findings (NA)	22
900/1	Short, CLS, FTT	M	-	ALL (5.2)	c.2588dupA p. (Asn863Lysfs*18)	11	c.1114C>A p.(Asn372His)	10	Alive (6.6)	Physical findings (NA)	22
RB	Short, pigmented, CLS, microcephaly, cryptorchidism	M	NA	Wilms' (3.5), Brain-glioblastoma multiforme (9)	c.658_659delGT p.(Val220Ilefs*4)	8	c.5645C>A p.(Ser1882 ^a)	11	Dead (10.1) Of disease	NA	28
CB	Pigmented, CLS	M	NA	Wilms' (0.6), Brain-medulloblastoma (6), B-ALL (10)	c.658_659delGT p.(Val220Ilefs*4)	8	c.5645C>A p.(Ser1882 ^a)	11	Dead (12) Of disease	NA	28
SB1690CB	Hypermobile thumb,FTT, microcephaly, imperforate anus, deaf, renal dysplasia, midfacial hypoplasia (VATER ^a)	M	+	MSD (1.2) AML (2.1)	c. 631 + 2T>G p.(Gly173Serfs*19)	7i	c.3599_3600delGT p.(Cys1200 ^a)	11	Dead (2.3) Of disease	Physical finding (1.2)	25
PT2	Short, pigmented, CLS, adducted thumbs, microcephaly, sacral hemivertebra, ventricular septal defect, pelvic kidney, oesophageal atresia, micrognathia, CNS gyrations, congenital cataract (VATER ^a)	F	+	Wilms' (1), neuroblastoma (1.1), Brain-posterior fossa (1.4)	c.1311_1314delAGAT p.(Lys437Asnfs*22)	10	c.1311_1314delAGAT p.(Lys437Asnfs*22)	10	Dead (1.4) of disease	Physical finding (0.1)	19
NCI 1	Short, CLS, microcephaly, facial dysmorphism, abnormal thumbs, anterior anus, cloudy corneas, ectopic kidneys, delayed development, hydrocephaly (VATER ^a)	F	NA	Brain-medulloblastoma (3.1)	c.5946delT p.(Ser1982Argfs*22)	11	c.9196C>T p.(Gln3066 ^a)	24	Alive (3.1)	Physical findings (VACTER) (1.7)	12
?	No physical abnormality	M	NA	High grade neuroepithelial tumour (1.3)	c.5946delT p.(Ser1982Argfs*22)	11	c.658_659delGT p.(Val220Ilefs*4)	8	Dead (1.6) of toxicity	Familial history (Retrospectively)	23
12	No physical abnormality	M	+	Medulloblastoma (1.7)	c.5946delT p.(Ser1982Argfs*22)	11	c.658_659delGT p.(Val220Ilefs*4)	8	Alive (2)	Toxicity to therapy + age of cancer (2)	23
?	CLS	F	-	Lymphoma (31) Colon-Adenocarcinoma (31)	c.7802A>G p.(Tyr2601Cys, Tyr2601Trpfs*46)	16	c.1845_1846delCT p.(Asn615Lysfs*6)	10	Alive (34)	Familial history (34)	Current report
?	Microcephaly, CLS, exophthalmia	F	-	Breast cancer (29) Colon-Adenocarcinoma (27)	c.7802A>G p.(Tyr2601Cys, Tyr2601Trpfs*46)	16	c.1845_1846delCT p.(Asn615Lysfs*6)	10	Alive (31)	Breast Cancer (30)	Current report
?	Microcephaly, short stature	F	+	AML (5)	c.7802A>G p.(Tyr2601Cys, Tyr2601Trpfs*46)	16	c.1845_1846delCT p.(Asn615Lysfs*6)	10	Dead (5) of toxicity	Familial history (retrospectively)	Current report

Abbreviations: i, intron; NA, not available; F, female; M, male; ALL, acute lymphoblastic leukaemia; AML, acute myelogenous leukaemia; MDS, myelodysplasia; BMF, bone marrow failure; CLS, café-au-lait spots; FTT, failure to thrive; CNS, central nervous system; TEF, tracheoesophageal fistula.
^aVATER association includes vertebral anomalies, anal atresia, tracheoesophageal fistula with esophageal atresia, and abnormal radii.

(17 cases corresponding mainly to medulloblastoma), AML (13 cases), Wilm's tumours (eight cases), and acute lymphoblastic leukaemia (four cases). At 2 years of age, half of the patients (15/30) had already developed a cancer. At 5 years of age, all but one had developed at least one tumour, and in 11 cases (34%) multiple cancers occurred. In agreement with these data, Alter *et al*¹² estimated that, in FA patients, the cumulative risk of malignancy, at 5.2 years of age, is 97%. *BRCA2* is directly involved in DNA double-strand break DNA repair, by homologous recombination, which might explain the severity of the phenotype in FA-D1 group.⁵ It has been first speculated that individuals with *BRCA2* biallelic mutations will only be viable if they carried at least one 'hypomorphic' allele, such as a missense mutation or a truncating mutation close to the 5' terminus,²¹ but this was subsequently disproved.²⁸ The spectrum of mutations in FA-D1 patients is not consistent with the mutation spectrum observed in breast cancer families related to *BRCA2* mutations, suggesting that some combinations of deleterious mutations are not viable. Furthermore, and despite the high population prevalence of *BRCA2* heterozygous carriers of the c.6147delT mutation in the Ashkenazim, no homozygous carrier has been reported.²⁹ Table 1 shows only eight cases carrying one truncating mutation and one missense mutation. More than half of the patients had two truncating mutations (18/30; 60%). HSC62 was the only patient who reached adulthood without a history of cancer (Table 1), and he was homozygous for an in-frame deletion of four codons that might explain this mild phenotype.²¹

Genetic variations, which cause aberrant splicing, may represent up to 50% of all the deleterious mutations.³⁰ Depending on tissue localization and/or stage of development, pre-mRNA transcripts may be differentially spliced. We showed that the *BRCA2* c.7802A>G variant detected in this study produced 50% of transcripts harbouring a premature stop codon (r.7802_7805delauag) and 50% of transcripts with a p.Tyr2601Cys mutation. In patients 1 and 2 harbouring both *BRCA2* mutations and exhibiting an atypical FA phenotype mostly characterized by early-onset tumours, at least 75% of the *BRCA2* transcripts are predicted to code for a truncated protein and 25% for the p.Tyr2601Cys variant. The 2601 residue is located within the helical domain (located from 2478 to 2668 amino acid) of *BRCA2* in the C-terminal domain. The absence of classical FA features in patients 1 and 2 suggests that this variant protein either partially conserved the *BRCA2* function or had a normal function and that its amount is sufficient to prevent the appearance of bone marrow failure in early childhood and classical FA features. The development in patient 3 of early-onset AML, corresponding to a more severe phenotype, might be explained by a phenotypic variability of the mutation, which is commonly observed with splicing alterations. Despite identical *BRCA2* mutations, divergent phenotype and clinical courses within these siblings might be also explained by the differential genetic background, influences of modifier genes, environmental effects or chance effects.

The results of the cDNA sequencing suggest that the aberrant mRNAs A and C (RT-PCR on blood sample Paxgene RNA) are stable in our family (Figure 5). The explanation for this finding can be as follows: an exon-exon junction complex of proteins deposited approximately 20–24 nt upstream of exon-exon junctions during pre-mRNA splicing is considered to be a primary determinant of nonsense-mediated decay (NMD) in mammalian cells; and as a rule, NMD is generally triggered when the translation termination at a nonsense codon is located more than approximately 50–55 nt upstream of an exon-exon junction.³¹ In our patient, the aberrant mRNAs A and C are both containing a premature translation

termination codon (PTC) located in the area of approximately 50–55 nt upstream of an exon-exon junction and thus are not supposed to trigger NMD. For the mutant c.1845_1846delCT p.Asn615Lysfs*6, the PTC is located 55 nt before the end of the exon 10 (exon 10–exon 11 junction), and for the mutant c.7802A>G p.Tyr2601Cys, the aberrant transcript r.7802_7805delauag p.Tyr2601Trpfs*46 introduces the PTC approximately 35 nt before the end of the exon 17 (exon 17–exon 18 junction).

Finally, the development of early-onset CRCs in two FA patients might be indicative of an interaction between *BRCA2* and MMR proteins. Heterozygous mutations of the *MMR* genes constitute the genetic basis of the Lynch syndrome, the main cause of the Mendelian form of CRCs, and the development of tumours in Lynch syndrome results from the RER phenotype leading to somatic mutations within target genes containing repeats.^{32,33} Remarkably, homozygous inactivation of the *MMR* genes, corresponding to the mismatch-repair deficiency syndrome (CMMR-D), was subsequently identified in children or young adults presenting with early-onset brain tumours, leukaemia or lymphoma, malignancies of the digestive tract and CALS, and this phenotype spectrum was a first clinical indication that the *MMR* genes were also involved in DNA double-strand homologous recombination, an hypothesis that was later confirmed.²⁹ Several studies have already established an interaction between MMR and *BRCA2* proteins. The FANCD1 protein has been shown to interact with MLH1 and disruption of this interaction resulted in hypersensitivity to inter-strand cross-linking agents.³⁴ The pivotal FA protein FANCD2 was subsequently shown to interact with MSH2 and MLH1.³⁵ Our report indicates that the presence of biallelic *BRCA2* mutations should be considered in families with early-onset breast and CRCs, even in the absence of cardinal features of FA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

M-MP, SG, FC, CL, CH, EF, AD, GC, SEC, PF, LF, AB, CL, J-LJ provided clinical data. ED-C, JS, MB, CC, CC, LM, SL, LA, PC, FM, TE, PJ and LF contributed to conception and design, acquisition of data, and analysis and interpretation of data. ED-C, JS, TE, PJ, LF contributed to writing the manuscript.

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