European Journal of Medical Genetics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: www.elsevier.com/locate/ejmg

Homozygous loss of function *BRCA1* variant causing a Fanconi-anemia-like phenotype, a clinical report and review of previous patients

Bruna L. Freire[a,b,1], Thais K. Homma[a,b,1], Mariana F.A. Funari[b], Antônio M. Lerario[c], Aline M. Leal[d], Elvira D.R.P. Velloso[d], Alexsandra C. Malaquias[a,e], Alexander A.L. Jorge[a,b,*]

[a] *Unidade de Endocrinologia Genética (LIM25) e Laboratório de Endocrinologia Celular e Molecular, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil*

[b] *Unidade de Endocrinologia do Desenvolvimento, Laboratorio de Hormonios e Genetica Molecular (LIM42), Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil*

[c] *Department of Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, MI 48109, USA*

[d] *Laboratorio de Citogenetica, Unidade de Hematologia, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Sao Paulo, SP, Brazil*

[e] *Unidade de Endocrinologia Pediatrica, Departamento de Pediatria, Irmandade da Santa Casa de Misericórdia de São Paulo, Faculdade de Ciencias Medicas da Santa Casa de Sao Paulo, SP, Brazil*

ARTICLE INFO

Keywords:
BRCA1
Fanconi anemia
FANC genes
FANCA genes
Genomic instability

ABSTRACT

Background: Fanconi Anemia (FA) is a rare and heterogeneous genetic syndrome. It is associated with short stature, bone marrow failure, high predisposition to cancer, microcephaly and congenital malformation. Many genes have been associated with FA. Previously, two adult patients with biallelic pathogenic variant in Breast Cancer 1 gene *(BRCA1)* had been identified in Fanconi Anemia-like condition.

Clinical report: The proband was a 2.5 year-old girl with severe short stature, microcephaly, neurodevelopmental delay, congenital heart disease and dysmorphic features. Her parents were third degree cousins. Routine screening tests for short stature was normal.

Methods: We conducted whole exome sequencing (WES) of the proband and used an analysis pipeline to identify rare nonsynonymous genetic variants that cause short stature.

Results: We identified a homozygous loss-of-function *BRCA1* mutation (c.2709T > A; p. Cys903*), which promotes the loss of critical domains of the protein. Cytogenetic study with DEB showed an increased chromosomal breakage. We screened heterozygous parents of the index case for cancer and we detected, in her mother, a metastatic adenocarcinoma in an axillar lymph node with probable primary site in the breast.

Conclusion: It is possible to consolidate the FA-like phenotype associated with biallelic loss-of-function *BRCA1*, characterized by microcephaly, short stature, developmental delay, dysmorphic face features and cancer predisposition. In our case, the WES allowed to establish the genetic cause of short stature in the context of a chromosome instability syndrome. An identification of *BRCA1* mutations in our patient allowed precise genetic counseling and also triggered cancer screening for the patient and her family members.

1. Introduction

Fanconi Anemia (FA) (OMIM #227650) is a rare and heterogeneous genetic syndrome, inherited in an autosomal recessive trait or X-linked (Fanconi anemia, complementation group B) (Wu, 2016). It is associated with short stature, bone marrow failure, high predisposition to cancer, microcephaly and congenital malformation (Petryk et al., 2015). In the cellular level, FA patients share underlying defects in their ability to process DNA lesions that interfere with DNA replication, what increases cellular sensibility to DNA interstrand crosslinks (ICLs) agents, such as diepoxybutane (DEB) (Cantor and Brosh, 2014). There are 21 identified genes (*FANCA* to *FANCV*) associated with FA and FA-like phenotypes, encoding proteins that participate in the DNA repair (Wu, 2016; Schneider et al., 2015; Gueiderikh et al., 2017; Bogliolo and Surrallés, 2015). The pathway in which these genes are part of, is also known as the Fanconi Anemia/BRCA pathway (Solomon et al., 2015).

Recently, biallelic pathogenic *BRCA1* (OMIM #604370) variant has been identified in two adult patients with short stature, microcephaly, developmental delay and cancer predisposing. Both patients with Fanconi Anemia–like phenotype (FA-like) were compound

* Corresponding author. Faculdade de Medicina da USP (LIM-25). - Av. Dr. Arnaldo, 455 5º andar sala 5340, CEP 01246-903, Sao Paulo, SP, Brazil.
E-mail address: alexj@usp.br (A.A.L. Jorge).

[1] B.L. Freire and T.K. Homma equally contributed to the article.

https://doi.org/10.1016/j.ejmg.2017.11.003
Received 5 September 2017; Received in revised form 3 October 2017; Accepted 8 November 2017

heterozygous for pathogenic variants in *BRCA1* (Domchek et al., 2013; Sawyer et al., 2015). In the present study, we report a Brazilian female child, with a nonsense homozygous *BRCA1* variant identified during the investigation of syndromic short stature by whole exome sequencing (WES). These findings consolidate that biallelic loss-of-function *BRCA1* variants cause FA-like.

2. Patient and methods

2.1. Clinical report

The index case is a 2.5-year-old Brazilian female with a proportionate severe short stature [height standard deviation score (SDS) = −6.1], low body mass index (−4.6 SDS) and microcephaly (head circumference SDS = −7.9). The patient is the only child of health consanguineous (third cousins) Brazilian parents with normal height (mid parental height SDS 1.2). She had intrauterine growth retardation and was born full term with birth weight of 1630 g (−4.4 SDS), birth length of 39.5 cm (−5.6 SDS) and head circumference of 25 cm (−7.7 SDS). At birth, patient was diagnosed with atrial sept defect, which has been corrected surgically. She had failure to thrive and neurodevelopmental delay. Physical examination showed additional dysmorphisms: thickened earlobe; long eyelashes, full upper lip, big teeth, anteverted nostrils, bilaterally clinodactyly and hyperchromic spots on trunk and feet (Fig. 1a; 1b). Routine screening tests for short stature were normal, including normal blood count, IGF-1, karyotype, skeletal survey and abdominal ultrasound. She was evaluated by a geneticist without specific clinical diagnosis. For this reason, the patient was selected for WES analysis.

2.2. Molecular-genetic analysis

This study was approved by the local ethics committee and a parental written informed consent was obtained before initiating the genetics studies. DNA sample was extracted from peripheral-blood leukocytes using standard procedures. The proband underwent WES according to previously published protocols (Sawyer et al., 2015; de Bruin et al., 2016). Briefly, the library was constructed with SureSelect Human All Exon V6 Kit (Agilent Technologies, Santa Clara, USA) according to the manufacturer's instructions. The exome library was sequenced on a HiSeq 2500 plataform (Illumina, San Diego, USA) with the use of Hiseq SBS V4 cluster generation and sequencing kit (Illumina, San Diego, USA) running on paired-end mode. Reads were aligned to the hg19 assembly of the human genome with the bwa-mem aligner. Duplicated reads were flagged with the bammarkduplicates tool from biobambam2 (Tischler and Leonard, 2014). Variant calling was performed with Freebayes and the resulting VCFs were annotated with ANNOVAR (Wang et al., 2010).

Based on the family pedigrees indicating consanguinity (Fig. 1c), the exome data were screened for homozygous variants in the patient, in addition to having a minor allele frequency (MAF) of 0.1% in our in house sequencing data sets and in public databases (genomAD, http://gnomad.broadinstitute.org/and Abraom http://abraom.ib.usp.br/). Silent mutations were excluded and we inspected visually the candidate variants using the Integrative Genomics Viewer (IGV). The assessment of gene function was performed using the Online Mendelian Inheritance in Man (OMIM) and the PubMed databases. Sanger sequencing was performed to validate the candidate variant identified.

2.3. Chromosome breakage study with diepoxybutane (DEB)

Heparinized blood sample was obtained from patient for testing chromosomal instability. Peripheral blood lymphocytes were stimulated with phytohemagglutinin (Vitrocell) in two 72 h cultures, consisting in 2 mL de blood and 5 mL of culture medium (RPMI 1640, fetal bovine serum, penicilyn and L-glutamin). In one of these cultures, after 24 h, the DNA cross-linking agent DEB (Aldrich Chemical Company, Inc) at the final concentration 0.1 μg/mL was added. The other untreated culture was used as a control. Harvesting was done according to current protocols. Slides were stained with Wright's eosin methylene blue solution for analysis of chromosomal aberrations. For each culture, 40 metaphases were scored by the numbers of rearrangements, gaps

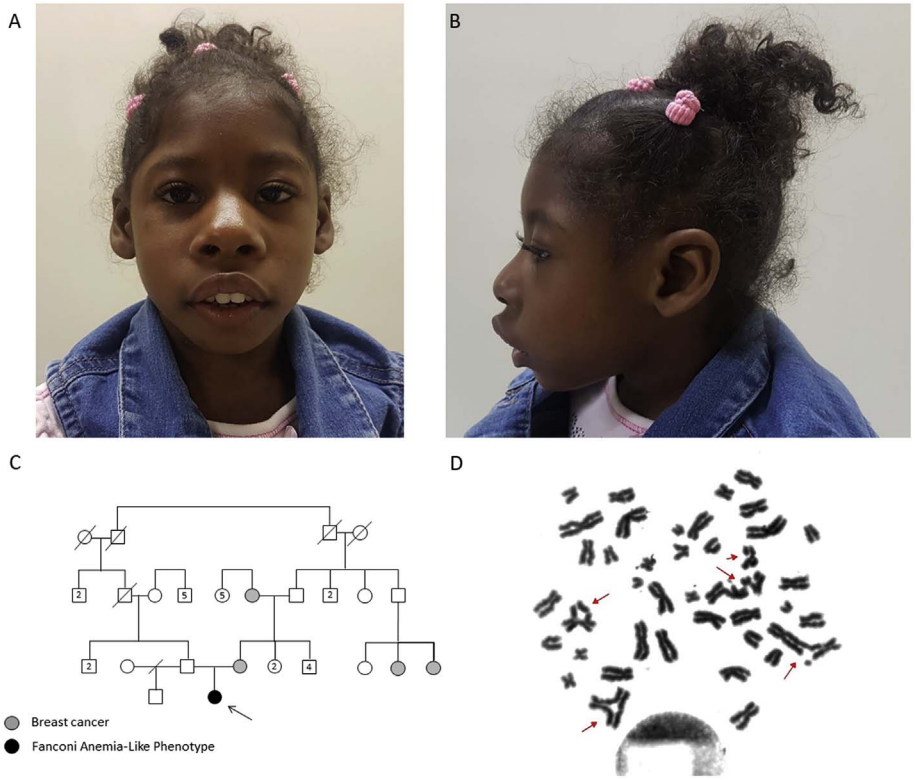

Fig. 1. (a, b) The patient displays facial dysmorphism, including microcephaly, thickened earlobe; long eyelashes, microphthalmia, upper lip full, big teeth, anteverted nose, bilaterally clinodactyly. **(c)** Pedigree of the family with *BRCA1*: c.2709T > A (p.Cys903*). Circles indicate females and squares indicate males. Slashes indicate death. An arrow indicates the proband. Shading indicates breast cancer. **(d)** Cytogenetic study with diepoxybutane showed chromosomal breakage.