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SHORT COMMUNICATION

Clinical characteristics and genetic subtypes of Fanconi anemia in Saudi patients

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We reviewed our institutional experience from 2011 to 2015 on new cases of Fanconi anemia (FA). Ten unrelated cases were diagnosed during this period. Four patients with severe aplastic anemia (SAA) had c.2392C > T (p.Arg798*) *BRIP1/FANCJ* mutation. Another child with SAA had novel c.1475T > C (p.Leu492Pro) *FANCC* mutation. One individual with SAA and acute myeloid leukemia had c.637_643del (p.Tyr213Lysfs*6) *FANCG* mutation. Three patients presented with early onset of cancer, two had *BRCA2* mutation c.7007G > A (p.Arg2336His) and one had a novel c.3425del (p.Leu1142Tyrfs*21) *PALB2* mutation. Another infant with c.3425del *PALB2* mutation had clonal aberration with partial trisomy of the long arm of chromosome 17. Mutations in FA downstream pathway genes are more frequent in our series than expected. Our preliminary observation will be confirmed in a large multi-institutional study.

Keywords Fanconi anemia, *BRIP1*, *BRCA2*, *PALB2*, *FANCC*, *FANCG*

Introduction

Fanconi anemia (FA) is an inherited bone marrow failure and cancer predisposition syndrome that is inherited mostly as autosomal recessive. The distinctive finding in FA is the hypersensitivity of cells to DNA interstrand cross linking agents, such as diepoxybutane (DEB) or mitomycin C (MMC) resulting in chromosomal aberrations. Mutations in at least 18 genes were described to be associated with FA, eight of these genes are required for FANCD2 and FANCI monoubiquitination (1,2). The clinical presentation is widely variable. About two-thirds of patients have physical anomalies and the risk of bone marrow failure and cancer increases with age (3).

FA is the most frequent indication for bone marrow transplantation among patients with inherited bone marrow failure syndromes in Saudi Arabia (4). However, the epidemiology of FA and frequency of different subtypes in our population are not known. In this study, we described the clinical presentation and molecular pattern of 10 unrelated Saudi FA patients. The previously described truncating mutation c.2392C > T (p.Arg798*) in *BRIP1* was the most frequent among our 10 patients. We also identified novel mutations in *PALB2* and *FANCC* genes.

Patients and methods

Saudi patients with abnormal chromosomal fragility test diagnosed in the period from 2011 to 2015 were enrolled in this study. The following clinical data were obtained through chart review and patient/family interview: age, gender, phenotype, presence of aplastic anemia, presence and type of cancer,

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and family history of cancer. The study was approved by our institutional review board.

Genomic DNA was extracted from patient's peripheral blood leukocytes using standard Qiagen kit. AmpliSeq™ Fanconi anemia genes panel was customized to include 15 FA genes and sequencing was performed using Ion Torrent next generation sequencing (Life Technologies). The panel included: *BRCA2*, *BRIP1*, *FANCA*, *FANCB*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*, *FANCG*, *FANCI*, *FANCL*, *FANCM*, *PALB2*, *SLX4*, and *XRCC2*. All mutations were confirmed by Sanger sequencing using polymerase chain reaction (PCR). PCR products were cleaned-up using ExoSAP-IT (USB Products Affymetrix), and sequenced using BigDye Terminator v.3.1 Cycle Sequencing chemistry kits (Life Technologies) on an ABI 3730 DNA Analyzer.

Novel mutations were not present in 1000 genome, dbSNP142, and whole exome data from 50 non-FA Saudi controls. We used MutationTaster software to predict the pathogenicity of novel variants (5). Binomial probabilities were determined using STATA 13.1 (StataCorp, College Station, TX), and significant P values were ≤0.05.

Results

A total of ten unrelated FA patients were enrolled in the study. The pattern of genetic mutations and clinical features are summarized in Tables 1 and 2. All patients had a history of consanguinity and all mutations were homozygous. The most frequent mutation was the truncating mutation in *FANCJ/BRIP1* c.2392C > T (p.Arg798*) which occurred in 4 patients (40%) (6). The mean age of onset of severe aplastic anemia in *BRIP1*-FA patients was 6 (range, 4–8 years) with no evidence of leukemia or solid tumor and no family history of cancer. All had growth retardation, microcephaly, café au lait spots, and renal anomalies. The four patients were unrelated and belong to different large tribes.

We identified one novel mutation in *FANCC* c.1475T > C (p.Leu492Pro) in a child who presented with severe aplastic anemia that started at the age of 4 years and is currently transfusion dependent. There was no evidence of leukemia or MDS on bone marrow assessment. This variant is predicted to be disease causing by MutationTaster.

The only adult patient in our series (Table 1) had *FANCG* mutation and presented with acute myeloid leukemia at age 21 soon after being diagnosed with severe aplastic anemia.

The two children with *BRCA2* c.7007G > A mutation shared similar phenotype and had early onset of cancer. They were not closely related but from the same tribe. The cancer types among those patients included T cell acute lymphoblastic leukemia, rhabdomyosarcoma, and neuroblastoma. There was a family history of ovarian cancer. Two children had a novel homozygous truncating mutation in *PALB2* c.3425del (p.Leu1142Tyrfs*21). One of those presented with multiple congenital anomalies and concurrent metastatic Wilms tumor and stage I neuroblastoma in the first year of life. There was a strong family history of cancer of unknown type. The other child with *PALB2* mutation had multiple café au lait spots and peripheral blood karyotype showed mosaic duplication of the long arm of chromosome 17 from band q21.2. The family declined bone marrow exam. Her sister had FA and died secondary to acute myeloid leukemia. The *PALB2* c.3425del mutation is predicted to be disease causing by MutationTaster.

None of the 5 mutations described here were present in whole exome data from 50 Saudi non-FA controls indicating suggesting a possible low frequency of these mutations in the Saudi population, but this number of controls is too small to permit any conclusion. We compared the frequency of the observed genetic subtypes in Saudi to the expected rate based on data from Europe and North America using a binomial probability test as shown in Table 3 (1,7). Variants in FA downstream pathway genes, particularly *BRIP1*, are significantly more frequent in Saudi patients. The lack of *FANCA* mutations in our cases series is noteworthy, in contrast with a previous report in Arabs (8).

Discussion

The present series of ten Saudi FA patients showed that *BRIP1* (*FANCJ*) was the most frequent FA subtype (40%) at our institution, compared with about 2% in Europeans (7). BRIP1, also termed BACH1, is a DNA helicase that interacts with the tumor suppressor protein BRCA1 (9). The truncating mutation p.Arg798* was initially described in 2005 and was associated with lack of expression of BRIP1 protein. Cells deficient in this protein were not capable of repairing double-stranded DNA breaks by homologous recombination (6,9,10). All four patients with mutated *BRIP1* developed severe aplastic anemia. They shared a similar phenotype with the previously published report (10).

Biallelic *BRCA2/FANCD1* mutations were associated with early onset of cancer (11–14). Our two patients with homozygous *BRCA2* mutation (p.Arg2336His) presented with aggressive metastatic tumors and leukemia in the first two years of life. This mutation was previously reported in a child with FA and acute myeloid leukemia (11). There was a strong family history of cancer in both patients.

Biallelic mutations in *PALB2* cause Fanconi anemia (FANCN) and are also associated with cancer in early childhood (15). PALB2 binds to BRCA2 and promotes its DNA repair and tumor suppressor effects (16,17). PALB2 is also necessary for the interaction between BRCA1 and BRCA2 (18). Several studies reported the association between *PALB2* monoallelic mutations and breast or pancreatic cancers (19–22). Our two patients had a novel homozygous frameshift mutation resulting from one base pair (T) deletion at c.3425del (p.Leu1142Tyrfs*21). This mutation is located within exon 13 in the area of WD40-like repeats structural motifs, the site of BRCA2, RAD51, and Polη interaction with PALB2. Monoallelic loss of function mutations adjacent to this mutation were linked to breast cancer risk (23).

The severity of FA in individuals with *FANCC* mutations is variable (3). Our patient had a novel mutation in *FANCC* gene c.1475T > C (p.Leu492Pro) that was associated with early onset severe aplastic anemia and physical abnormalities consistent with a severe phenotype. The *FANCG* c.637_643del mutation described in one individual in our series, who was originally from Saudi Arabia, was frequent among FA patients from sub-Saharan Africa (24). This variant was associated with acute myeloid leukemia in our patient that was preceded by SAA consistent with prior observations of increased