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Research Article

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Chromosomal analysis and BRCA2*617delT/88delTG and BRIP1 (c.2392C>T) mutations of Fanconi anemia in Iranian family, and its correlation to breast cancer susceptibility

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ABSTRACT

*Fanconi anemia (FA) is a rare disease, but it is the most common among the inherited bone marrow failure syndromes. In the present study, one family diagnosed with Fanconi anemia was examined. There was a 22-year-old female in this family who was diagnosed with both breast cancer and with Fanconi anemia. The chromosomal breakage in karyotyping was compatible with Fanconi anemia. Chromosomal analysis of mean breaks and rearrangements were calculated. The BRCA2*617delT/88delTG and the BRIP1 (c.2392C>T) mutations which are associated with Fanconi anemia and breast cancer were investigated. In order to, genomic DNA was extracted from blood samples collected from the case with both breast cancer and Fanconi anemia, from her brothers, her sisters and her parents, followed by Polymerase Chain Reactions to detect the BRCA2*617delT/88delTG and BRIP1 (c.2392C>T)mutations of exons 11 and17. In chromosomal analysis, four cases with a mean of 41.33 breaks and rearrangements (SEM of ±1.2) were observed in the culture of the prob and, yielding an average of 0.686 breaks per metaphase, while only an average of 0.03 breaks per metaphase was detected in the control group.The results of DNA sequencing and data analysis showed that there was no variation between the individuals in this family forBRCA2*617delT/88delTG and BRIP1 (c.2392C>T)mutations after alignment to the nucleotide sequences. Investigation of other mutations linked to the BRCA pathway such as FANCJ, BRCA1 and RAD51C/FANCO, or the use whole-exome sequencing for the further investigation of the disease are recommended.*

Keywords: Fanconi Anemia, Breast Cancer, BRCA2, BRIP1, Chromosomal Breakage.

INTRODUCTION

Although Fanconi anemia (FA) is a rare disease, it is the most common inherited aplastic anemia. It is characterized by multiple congenital abnormalities, developmental anomalies, cellular sensitivity to the DNA cross-linking agents, progressive bone marrow failure, and cancer susceptibility [1].Characteristic features of FA include short stature, caféaulait spots, small eyes, mental retardation and skeletal anomalies [2].

The average age at diagnosis is 6.5 and 8 years for boys and girls respectively [3]. Mutations have been identified in at least 15 different genes that are involved in the pathogenesis of Fanconi anemia [4].

Due to the defective DNA repair, complications of Fanconi anemia may include leukemia in approximately 10% of patients, liver tumors in 5% and cancers (except leukemia and liver tumors) in approximately 5% of cases. In

addition, clinical and radiologic investigation of children with Fanconi anemia resulted in the determination of survival factors, prognostic factors and other complications of disease were determined [5].

There are many different genes that can cause Fanconi anemia, and the condition has been broken into different subtypes including A, B,C, D1(BRCA2), D2,E, F, G, I, J, L, M, N, O and P[20].85% of patients belong to the subtypes A (approximately 60%), C (10-15%) and G (10%), and a minority of patients (approximately15%) is distributed over the other 12 subtypes [6]. Methodof inheritance for all subtypes of Fanconi anemia is autosomal recessive, expect for Fanconi anemia-B which is X-linked [6]. If both parents carry a mutation in the same FA gene, each of their children has a 25% chance of inheriting the defective gene from both parents [7].

Increased expression of some genes due to polymorphism may increase the risk of breast cancer. It has been also reported that the recognized mutations that may increase the risk of breast cancer within susceptible families are quite rare: BRCA1, BRCA2, BRIPI, RAD50, PTEN, ATM, TP53, CHEK2, CDH1 and MSH6[8]. Studies have revealed that some of the breast cancer susceptibility genes are associated with Fanconi anemia [2, 9 and 10].Although the majority of the diagnoses of these breast cancers are sporadic, approximately 5% to 10% of these cases are associated with the inheritance of breast cancer susceptibility genes such as BRCA1, BRCA2, PTEN, CDH1, CHEK2, TP53 and ATM [11].

Germline mutations in BRCA1 or BRCA2,some genes encode for BRCA1- and BRCA2- interacting proteins, PALB2 and FANCJ are DNA repair genes associated with hereditary breast cancer and also with Fanconi anemia. Also, the inheriting of biallelic mutations in some breast cancer genes isa consequence of Fanconi anemia, and is associated with the complementation groups BACH1/FANCJ/BRIP1, PALB2/FANCA and BRCA2/FANCD1 [12, 13].

The FANCD1/BRCA2 protein is one of the Fanconi anemia proteins that is also a breast/ovarian cancer susceptibility protein[14]. BRCA1 is another breast/ovarian cancer susceptibility protein that cooperates with Fanconi anemia proteins in a DNA repair pathway which is required for resistance to DNA interstrand crosslinks[15].Some studies reported that biallelic mutations in BRIP1/BACH1 (FANCJ) and PALB2 (FANCN) cause rare subtypes of Fanconi anemia[10, 16,].Mutations of BRIP1 (FANCJ) have also been identified in patients with the early onset of breast cancer, and as PALB2binds directly to the BRCA2/FANCD1 protein, this might account for the strong clinical resemblance between FANCN and FANCD1 patients [10, 16]. Mutations of the FANCJ gene (BRIP1 helicase) have been found in some families with potentially inherited breast/ovarian cancer, including 1396C→T, 658C→T, 2392C→T, 2636G→A, 718G→A. 2778G→A, 2945T→G, 3552C→T, 3968A→C and 4049C→T[17].

Offit et al (2003) examined four kind reds afflicted with Fanconi anemia for the presence of the BRCA2 mutation [18]. Koren-Michowiz et al (2005) investigated 100Ashkenazi women with known BRCA1 and BRCA2 mutations who were also screened for the Fanconi anemia mutation IVS+4A→T, and their results revealed that there is an increased prevalence of the Fanconi anemia mutation carriers in the specific population studied when compared with the general Ashkenazi population[19].

The objective of this aspect of the paper is to estimate the chromosomal analysis of mean breaks and rearrangements. Then to consider the BRCA2*617delT/88delTG and BRIP1 (c.2392C>T) mutations associated with Fanconi anemia and breast cancer in a family that was diagnosed with Fanconi anemia.

EXPERIMENTAL SECTION

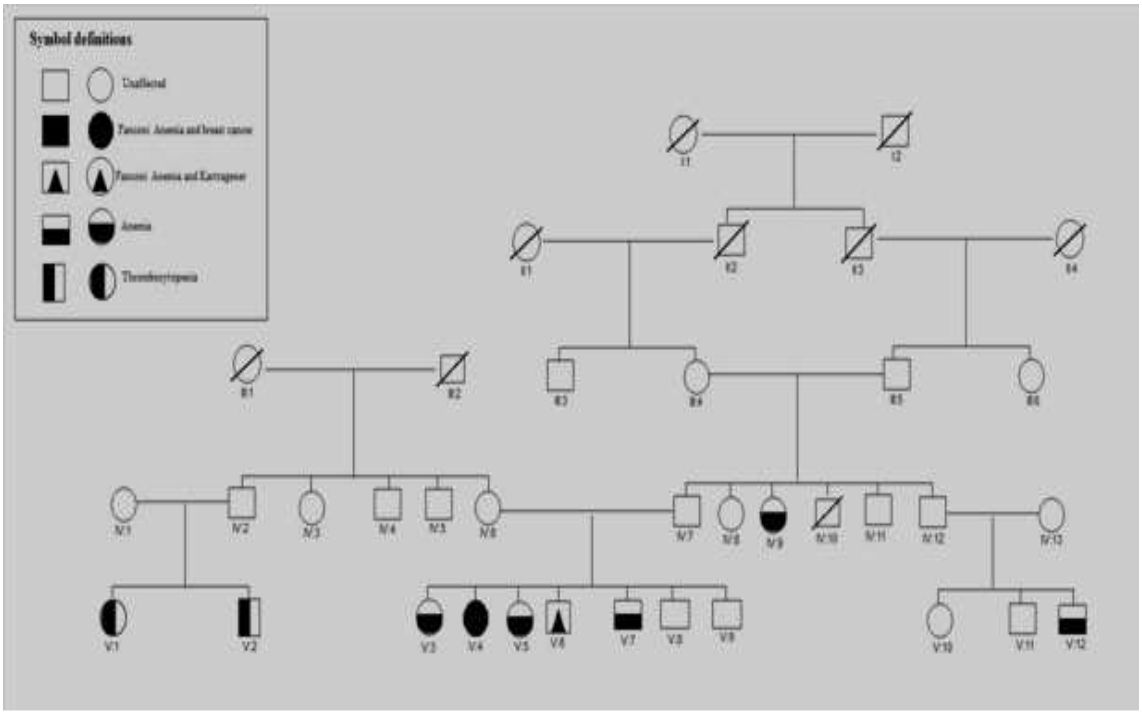
Case report

The family described in this report has seven members, four of whomare known cases of Fanconi anemia. The pedigree of this family is shown in Figure1.

Chromosomal Analysis

Fanconi anemia was diagnosed in the four individuals using the chromosomal breakage test. Bone marrow karyotyping of four chromosomal spreads were studied from routine cultures, 60 spreads from cultures prepared with the addition of Mitomycine C (Vidal, France), and these were compared with 60 spreads from age-related normal controls. G-banding was performed with the Olympus BX43 microscope (Tokyo, Japan) and at 450-500 band resolution. The co-occurrence of breast cancer and Fanconi anemia was observed in a 22-year-old female in this family.

Figure 1. Pedigree of the family diagnosed with Fanconi anemia



DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from blood samples collected from the female with breast cancer and Fanconi anemia, her parents without Fanconi anemia, her brothers and her sisters with Fanconi anemia using a commercial DNA isolation kit, the AccuPrep Genomic DNA Extraction Kit (Bioneer, South Korea), in accordance with the manufacturer's protocols. After measuring the DNA concentration and its purity with a Nanodrop 2000 spectrometer (Thermo Scientific, USA), the genomic DNA was diluted to a final concentration of50 ng/µl indH_2O and was stored at 4°C until subsequent experiments took place. PCR amplification was accomplished in a thermal cycler T-Personal (Biometra, Germany) in a final volume of 50µl. The PCR mix contained 5µl buffer 10X, 1.5 mM MgCl2, 1µl of dNTPs (10 mM each), 1.25µl of each primer (10 pM), 50 ng genomic DNA, 1.25U of Taq DNA polymerase and 36.75µl ofddH_2O (Cinna Gen, Tehran, Iran)in 35 cycles. PCR conditions for all fragments were initial denaturation at 95°C for 5 minutes, denaturation at 95°C for 30seconds, annealing at 59°C and at 61°C for 30 seconds for amplifying BRCA2*617delT/88delTG and BRIP1 (c.2392C>T)respectively,extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes.The list of primers used in the identification of the BRCA2*617delT/88delTG and BRIP1 (c.2392C>T) mutations and their product lengths are shown in Table 1. Amplified PCR fragments were separated by electrophoresis in a 1% agarose gel, then stained with ethidium bromide and viewed under a UV light.

Table1. Sequences of forward and reverse primers used in the identification of the BRCA2*617delT/88delTG and BRIP1 (c.2392C>T) mutations

Sequence	Primer name	Product Length
F-5'- GGTTGTTACGAGGCATTGGAT-3'	**BRCA2*617delT/88delTG**	
R-5'- AGTGAAGACTATGCTCAGTTCTG-3'		**607bp**
F-5'- AGCAGAGATTACCAGACTGGATAA-3'	**BRIP1(c.2392C>T)**	
R-5'- TTCACTCCACTTACCTACCAAGG-3'		**695bp**

DNA Sequencing and Data Analysis

The PCR amplicons were analyzed on agarose gel to verify size. The resulting amplicons were sequenced from both directions. The detection of the mutation within the amplification products of the BRCA2 and BRIP1 genes were carried out by Bioneer standard sequencing methods, (BioneerInc, South Korea). Then the sequences were edited and 600bp and 680 bp of the results sequences respectively were analyzed for BRCA2*617delT/88delTG and for BRIP1 (c.2392C>T) mutations. Sequence Aliments were analyzed using the CLC Main Workbench 5.5.

RESULTS AND DISCUSSION

The chromosomal and clinical results showed, using the chromosomal breakage test, that there was Fanconi anemia in this family (Figure2). In chromosomal analysis, four cases of this family members with a mean of 41.33 breaks and rearrangements (SEM of ±1.2) were observed in the culture of the proband, yielding an average of 0.686 breaks per metaphase; only an average of 0.03 breaks per metaphase was detected in the normal control group. Breakages of more than 10 fold in comparison to the control are clinically significant. The four cases were diagnosed with multiple breaks and so were consistent with Fanconi anemia. Moreover, three cases in the family were also investigated for chromosomal aberrations, and the cytogenetic analysis revealed that there were 3.66 (SEM of ± 0.8819) with no chromosomal aberration (Figure 3).

Figure 2.Cytogenetic report indicated chromosomal breakage syndrome

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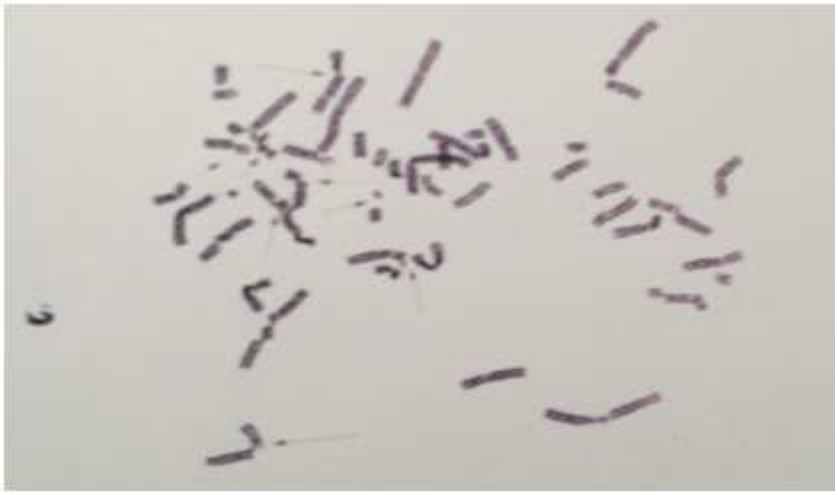
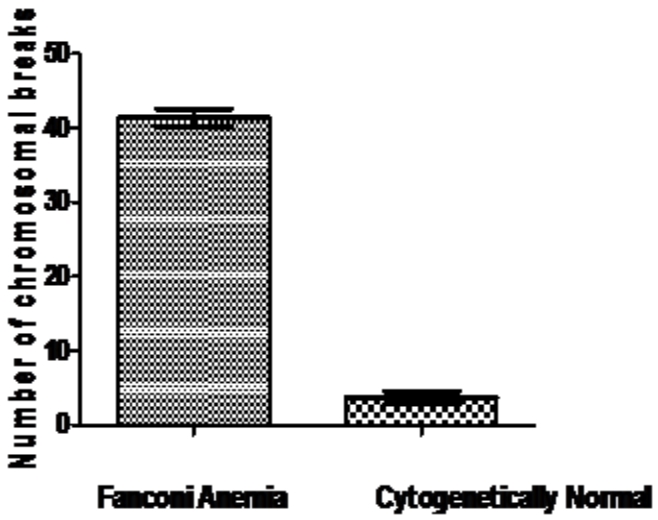
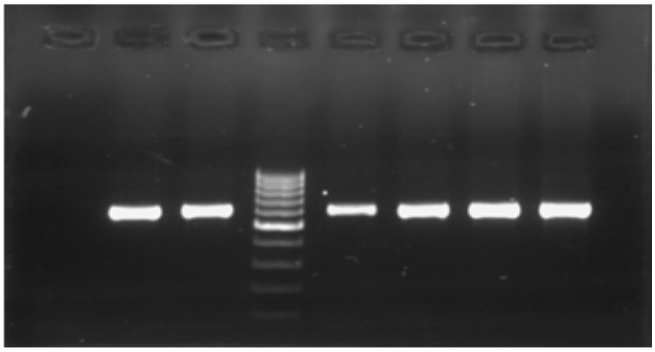


Figure 3. 41.33 breaks and rearrangements (SEM ± 1.2) were observed in the culture of the proband, yielding an average of 0.686 breaks per metaphase; while only average of 3.66 breaks per metaphase detected in other cases



PCR amplified the 607bp and 695bp fragments of the BRCA2*617delT/88delTG and BRIP1 (c.2392C>T) respectively. The fragmentsamplified by Polymerase Chain Reaction are shown in Figure4 and Figure5.

Figure 4. The 607 bp fragment of BRCA2*617delT/88delTG amplified by Polymerase Chain Reaction, in which the molecular marker is M100 (gene Fanavaran co., Tehran)



Figu5. The 695 bp fragment of BRIP1 (c.2392C>T) amplified by Polymerase Chain Reaction, in which the molecular marker is M100 (gene Fanavaran co., Tehran)

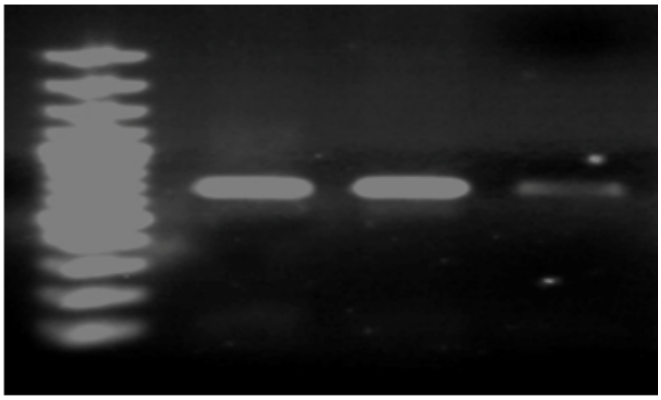
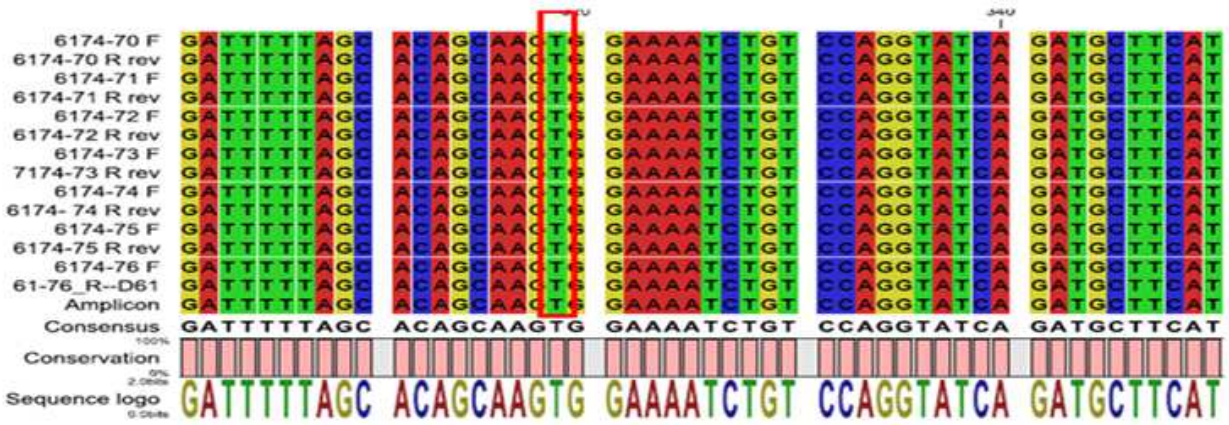


Figure 6. Sequences aligned for BRCA2*617delT/88delTG mutation in the patient and her family



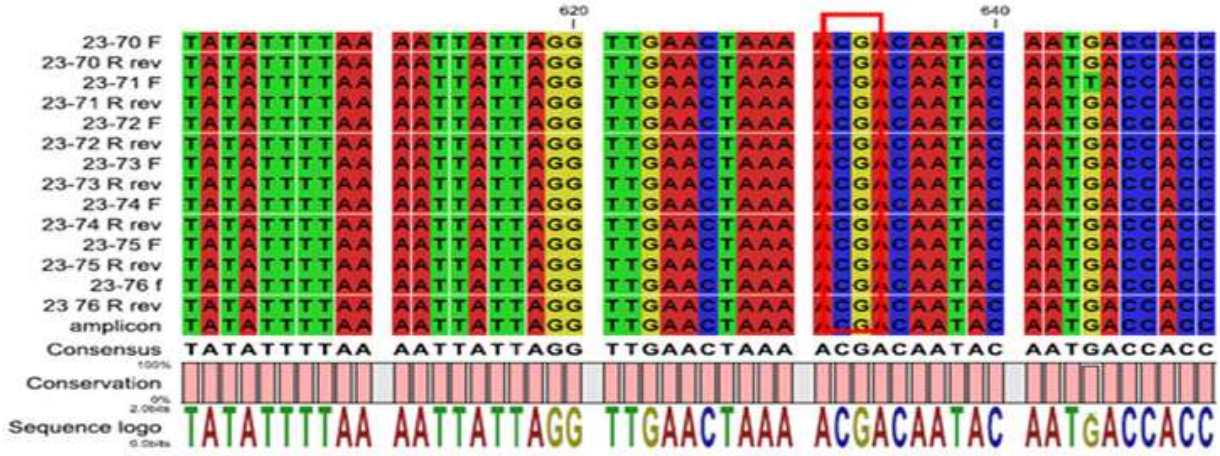
The results of DNA sequencing and data analysis showed that there was no variation between the individuals in this family forBRCA2*617delT/88delTG and BRIP1 (c.2392C>T) mutations when we aligned the nucleotide sequences (Figure 6 and S7).

To find the genetic and genomic gene links between breast cancer and Fanconi anemia, we should find Single Nucleotide Polymorphisms (SNP) that have an essential role in breast cancers, such as SNPs in the BRCA1, BRCA2, and BRIP1genes.

Among the SNPs above, we first chose the BRCA2*617delT/88delTG and BRIP1 (c.2392C>T) mutations. These gene fragments were amplified and then the sequencing results were compared. The sequencing results were compared with the reference gene.

The results from Offit et al. (2003) indicated that one kindred who was diagnosed with breast cancer (BRCA2*617delT/C3069X) compound heterozygotes also had Fanconi anemia and brain tumors; a second kindred, a child, was diagnosed with Fanconi anemia and BRCA2*617delT/88delTG compound heterozygotes; a third and fourth kindred, also a child were diagnosed with BRCA2*I2409T/5301insA and BRCA2*Q3066X/E1308X compound heterozygotes[18].

Figure 7. Sequences aligned for BRIP1 (c.2392C>T) mutation in the patient and her family



Knies et al. (2012) performed whole-exome sequencing in four FA patients to investigate the potential of this method for the diagnosis of Fanconi anemia, and their results showed mutations that included homozygous and heterozygous single base pair substitution and two base pair duplications in FANCJ, -D1or -D2[20]. The whole-exome sequencing method was also used to identify the affected genes in a boy with Fanconi anemia, and those results showed non-synonymous mutation(c.3971C>T,p.P1324l) and a new frame shift mutation(c.989_995del, p.H330LfsX2) in FANCA[16].

We didn't find any mutations. Thus the link between breast cancer and Fanconi anemia was not observed in the two SNPs that were investigated. For verification, the sequence of these gene fragments should be amplified for other family members and their genome results compared to the reference genome. Therefore, to find a link between breast cancer and Fanconi anemia, other genes should be investigated to find other impressions that we are currently doing this item to find SNPs associate with breast cancer and Fanconi anemia.

Recent studies that have reported an association between some breast cancer susceptibility genes and Fanconi anemia, as well as evidence of a strong link between Fanconi anemia and the BRCA pathway; therefore we can conclude that BRCA pathway mutations that cause breast cancer may cause some Fanconi anemia groups, although we didn't observe any mutations in which Fanconi anemia was associated with breast cancer. We need to investigate other mutations in the BRCA2 gene and also to study other mutations in the BRCA pathway such as BRIP1 that is also known as FANCJ, BRCA1 andRAD51C/FANCO[6], using whole-exome sequencing to identify the affected genes in this family.

CONCLUSION

In this study, we have investigated the BRCA2*617delT/88delTG and BRIP1 (c.2392C>T) mutations linked to Fanconi anemia in a certain family. We did not observe these mutations in these family members. We now should investigate other mutations of the BRCA pathway such as FANCJ, BRCA1 and RAD51C/FANCO, or use the whole-exome sequencing technique to investigate any association between Fanconi anemia and breast cancer in this family.

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