Original Article

# Identification of Three Novel Mutations in the *FANCA*, *FANCC,* and *ITGA2B* Genes by Whole Exome Sequencing

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## Abstract

**Background:** Various blood diseases are caused by mutations in the *FANCA*, *FANCC,* and *ITGA2B* genes. Exome sequencing is a suitable method for identifying single-gene disease and genetic heterogeneity complaints. **Methods:** Among families who were referred to Narges Genetic and PND Laboratory in 2015-2017, five families with a history of blood diseases were analyzed using the whole exome sequencing (WES) method. **Results:** We detected two novel mutations (c.190-2A>G and c.2840C>G) in the *FANCA* gene, c. 1429dupA mutation in the *FANCC* gene, and c.1392A>G mutation in the *ITGA2B* gene. The prediction of variant pathogenicity has been done using bioinformatics tools such as Mutation taster PhD-SNP and polyphen2 and were confirmed by Sanger sequencing. **Conclusions:** WES could be as a precise tool for identifying the pathologic variants in affected patient and heterozygous carriers among families. This highly successful technique will remain at the forefront of platelet and blood genomic research.

**Keywords:** *Blood platelets, congenital abnormalities, DNA, Fanconi anemia, sequence analysis*

## Introduction

Fanconi anemia (FA; MIM no. 227650) is an autosomal or X-linked recessive genetic disorder that causes bone marrow failure.[1] The mutations in several *FANCC* (A, B, C, D1, D2, E, F, G, I, J, L, M, N, O, *P* and Q) genes are associated with this disorder. It is estimated that one of every 300 people carry the mutation in *FANCC* genes [Figure 1].[2] The FANC proteins FANCA, B, C, E, F, G, and L constructed the complex known as the FA central complex. The FA complex triggers FANCD2 and FANCI proteins (ID complex).[3] Subsequently, these two proteins bring DNA repair proteins to the place of interstrand cross-linkers (ICLs), so the cross-link will be detached and replication of DNA can come to an end.[4,5] The majority of (about 85%) FA patients have mutations in the *FANCA*, *FANCC*, and *FANCG* genes but are altered in various populations.[6] *FANCA* gene is located on chromosem16q24.3 that is about 80 kb length with 43 exons and shows a much higher mutational frequency than other FA genes, which accounts for 60-65%.[7,8] FA complementation group A is an autosomal recessive disease with various clinical symptoms, such as small stature and low birth weight, renal abnormalities, skeletal malformations, hyperpigmentation of skin (Cafe-au-lait spots), hypogonadism and infertility, mental retardation, and hematological problems. These patients have an increased risk of certain cancers.[9,10] The FA complementation group C is prompted by mutations in the *FANCC* gene located on chromosome 9q22.32. FA complementation group C associated with anemia, leukopenia, thrombopenia, cardiac, renal and limb malformations, dermal pigmentary changes, and susceptibility to the development of malignancies.[11,12] Glanzmann's syndrome is a hereditary genetic disorder with severe platelet failure, which has a long bleeding time and a normal platelet count. Mutations in *ITGA2B* (αIIb) or *ITGB3* (β3) genes that are located on chromosome 17 (q21-22) may lead to Glanzmann's syndrome type I or type II with an autosomal recessive inheritance.[13,14] In this disorder, the platelet contains defective or small amounts of integrin αIIbβ3 at the platelet surface, which is a glycoprotein receptor for fibrinogen.[15] The platelets



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of patients with this condition due to the lack of fibrinogen-binding sites do not have the ability to bind to fibrinogen. As a result, the bleeding time will be significantly longer.[16,17]

In this study, we used the whole exome sequencing (WES) method in five patients with a history of clinically different anemia to identify the disease-causing mutations. Thus, we consider WES as a well-organized method to compete with a traditional molecular diagnosis of blood and other genetic disorders.

## Methods

### Samples and study design

Among families who were referred to Narges Genetic and PND Laboratory in 2015-2017, four families from Khuzestan with a history of blood diseases were analyzed

| Gene symbol (NCBI gene ID) | Gene name | FANC symbol | Gene symbol synonyms | Chromosomal location |
|---|---|---|---|---|
| *FANCA* (2175) | Fanconi anemia complementation group A | *FANCA* | *FA, FA-H, FA1, FAA, FACA, FAH, FANCH* | 16q24.3 |
| *FANCB* (2187) | Fanconi anemia complementation group B | *FANCB* | *FA2, FAAP90, FAAP95, FAB, FACB* | Xp22.31 |
| *FANCC* (2176) | Fanconi anemia complementation group C | *FANCC* | *RP11-80I15.2, FA3, FAC, FACC* | 9q22.3 |
| *BRCA2* (675) | Breast cancer 2 | *FANCD1* | *RP11-298P3.4, BRCC2, BROVCA2, FACD, FAD, FAD1, FANCD, GLM3, PNCA2, XRCC11* | 13q12.13 |
| *FANCD2* (2177) | Fanconi anemia complementation group D2 | *FANCD2* | *FA-D2, FA4, FACD, FAD, FAD2, FANCD* | 3p25.3 |
| *FANCE* (2178) | Fanconi anemia complementation group E | *FANCE* | *FACE, FAE* | 6p21.22 |
| *FANCF* (2188) | Fanconi anemia complementation group F | *FANCF* | *FAF* | 11p15 |
| *FANCG* (2189) | Fanconi anemia complementation group G | *FANCG* | *FAG, XRCC9* | 9p13 |
| *FANCI* (55215) | Fanconi anemia complementation group I | *FANCI* | *KIAA1794* | 15q26.1 |
| *BRIP1* (83990) | BRCA1 interacting protein C-terminal helicase 1 | *FANCJ* | *BACH1, OF* | 17q23.2 |
| *FANCL* (55120) | Fanconi anemia complementation group L | *FANCL* | *FAAP43, PHF9, POG* | 2p16.1 |
| *FANCM* (57697) | Fanconi anemia complementation group M | *FANCM* | *FAAP250, KIAA1596* | 14q21.3 |
| *PALB2* (79728) | Partner and localizer of BRCA2 | *FANCN* | *PNCA3* | 16p12 |
| *RAD51C* (5889) | RAD51 paralog C | *FANCO* | *ROVCA3, RS1H3, RAD51L2* | 17q23 |
| *SLX4* (84464) | SLX4 structure-specific endonuclease subunit | *FANCP* | *BTBD12, MUS312* | 16p13.3 |
| *ERCC4* (2072) | Excision repair cross-complementation group 4 | *FANCQ* | *RAD1, XPF, ERCC11* | 16p13.12 |
| *RAD51* (5888) | RAD51 recombinase | *FANCR* | *BRCC5, HRAD51, HsRad51, HsT16930, MRMV2A, RECA* | 15q15.1 |
| *BRCA1* (672) | Breast cancer 1 | *FANCS* | *BRCAI, BRCC1, BROVCA1, IRIS, PNCA4, PPP1R53, PSCP, RNF53* | 17q21.31 |
| *UBE2T* (29089) | Ubiquitin-conjugating enzyme E2T | *FANCT* | *HSPC150* | 1q32.1 |

**Figure 1: Human *FANC* genes[18]**

[Figure 1-4]. This study was confirmed by the Medical Ethics Committee of Ahvaz University of Medical Sciences. The informed consent form was completed and signed by all members of the family who entered the study [Table 1].

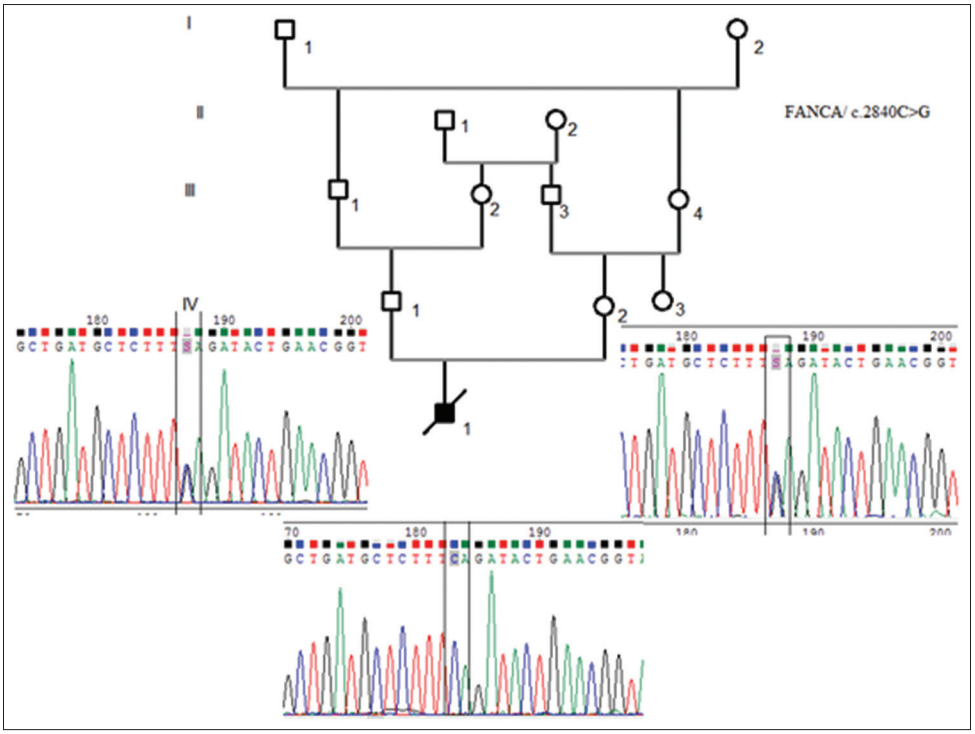



**Figure 2: Pedigree and chromatograms of the affected dead child and his parents (FANCA/c.2840C>G)**

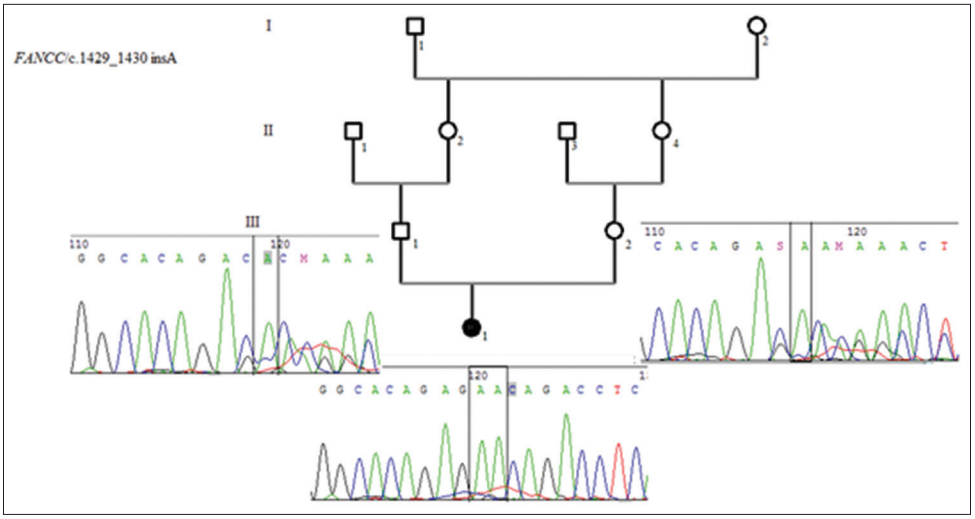



**Figure 3: Pedigree and chromatograms of the affected child and her parents (*FANCC*/c.1429_1430 insA)**

**Table 1: Characteristics of patients in the current study**

| Patient ID | Consanguinity | Age | Sex | Clinical synopsis |
|---|---|---|---|---|
| 33417 | yes | 32-years | Female | Pregnant woman with a history of a dead girl due to Fanconi anemia |
| 35307 | yes | 8-years | Female | Growth retardation, MR, strabismus, microcephaly, low platelets |
| 34916 | yes | 9-months (dead fetus) | Female | Gastrointestinal malformations, cardiovascular problem, lack of ears, lack of fingers |
| 43979 | yes | 1-year | Male | Referred for Glanzmann's syndrome<br>Muscle weakness, prolonged bleeding and clotting time, normal size and number of platelets |

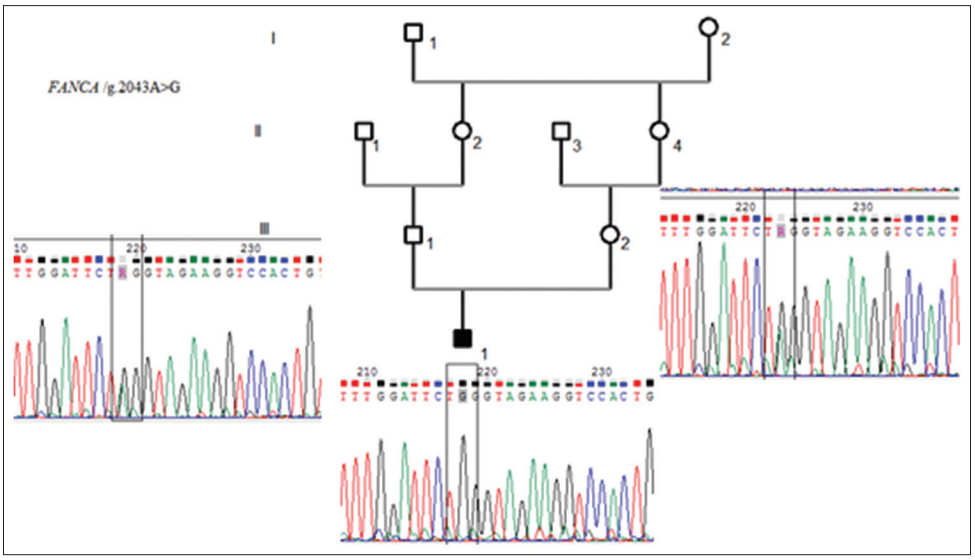



**Figure 4: Pedigree and chromatograms of the affected child and his parents (*FANCA*/g.2043A>G)**

### DNA sample isolation

To obtain genomic DNA, 10 mL of peripheral blood (PB) was collected in ethylenediamine tetraacetic acid (EDTA)-containing tubes from the patients. Deoxyribonucleic acid (DNA) was extracted from PB-derived leukocytes of the family members using the standard salting out protocol. The quality of the DNA was evaluated using the NanoDrop spectrophotometer (ASP-2680, ACTGene Inc., NJ, USA).

### Whole exome sequencing and data analysis

The WES was performed by the Illumina-HiSeq 2000 genome analyzer platform by × 100 depth of sequencing and 5Gb output (CNAG; Macrogen Company). Among the variants detected, all previously reported mutations and probable pathogenic variants including novel non-synonymous ones were confirmed by Sanger sequencing. The variants were queried against the public version of the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/). The prediction of variant pathogenicity has been done using Mutation taster and Human Splicing Finder (HSF). We used PredictSNP and PolyPhen-2 for the prediction of protein functions in mutated forms. The allele frequency of each variation was detected using the Genome Aggregation Database (gnomAD), the Exome Aggregation Consortium (ExAC) and 1000 Genome project (http://www. 1000genomes.org/), and the exome database located at our laboratory (including the results of 800 Iranian WES).

### Sanger sequencing

After confirming the PCR results using electrophoresis on 1% agarose gel, polymerase chain reaction (PCR) products were sent for Sanger sequencing. Primers were designed by Oligo software (Oligo, USA) according to the sequences of each gene obtained from gene bank. Primer sequences were available upon request. All the identified mutations were confirmed via Sanger sequencing with an ABI Prism 3700 instrument (Applied Biosystems, CA, USA). The outcomes were evaluated using the Chromas Lite 2.1.1 software and then compared with the reported gene sequence using the BLASTN program.

## Results

Using the WES method, coding regions, splice site, frameshift variants, indels, and a part of the intronic region were analyzed. We removed all the common variants (minor allele frequency, MAF >1%) and reported homozygote individuals for c.2840C>G and c.1392A>G variants in *ITGA2B* were zero (n = 0). We did not find g.2043A>G variants in *FANCA* gene, c. 1429_1430 ins A frameshift variant in *FANCC* gene in gnomAD, ExAC, and 1000G databases. Finally, after the filtering steps, the genes were compared with those that were identified by examining the texts as candidate genes in blood diseases [Table 2]. Sanger sequencing confirmed the existence of the detected mutations in patients and family members. Other family members were also screened for the mutation [Figure 2-5]. We used HSF software to predict the potential splice sites c. 1392A > G variant in *ITGA2B* gene [Figure 6].

## Discussion

WES is a suitable method for identifying single-gene disease and genetic heterogeneity complaints. This technology has created genetic studies that allow the sequencing of human pathogenic variants in a relatively short time and low cost. In the present study, we identified a previously reported frameshift homozygous single nucleotide variation c.2840C>G (HGMD ID: CM970493) in the dead child of a couple who was subjected for the prenatal diagnosis (PND) test. In addition, a novel nonsense

**Table 2: Location, predicted pathogenicity of the variation in the patients and HGMD report for each change**

| Patient | Gene (GenBank accession number)/ Genotype/Inheritance | Type | DNA Change/ Location | Protein Change | Mutation taster score | PredictSNP2/ PolyPhen2 prediction | Reported in HGMD Database |
|---|---|---|---|---|---|---|---|
| 33417 | *FANCA* (NM_000135.2)/ Hetrozygous/Autosomal recessive | Single base exchange/ frameshift | c. 2840C>G/ Exon 29 | p.S947fs | Disease causing , prob: 1 | Not provided score 0.027/AA change leads to a stop codon | Reported (HGMD ID: CM970493) |
| 35307 | *FANCA* (NM_000135.2)/ Homozygous/Autosomal recessive | Single base exchange | g. 2043A>G/ Intron 2-3 | no AA changes | Disease causing , prob: 1 | Neural , score 0.16/ no AA changes | Unreported |
| 34916 | *FANCC* (NM_000136)/ Homozygous/Autosomal recessive | Frameshift insertion | c. 1429_1430 insA/Exon 4 | p.T477Nfs | Disease causing , prob: 1 | -/Benign score 0.05 | Unreported |
| 43979 | *ITGA2B* (NM_000419.3)/ Homozygous/Autosomal recessive | Single base exchange/ splice region | c. 1392A>G/ Exon13 | no AA changes | Disease causing , prob: 1 | Deleterious score 0.03/no AA changes | Unreported |

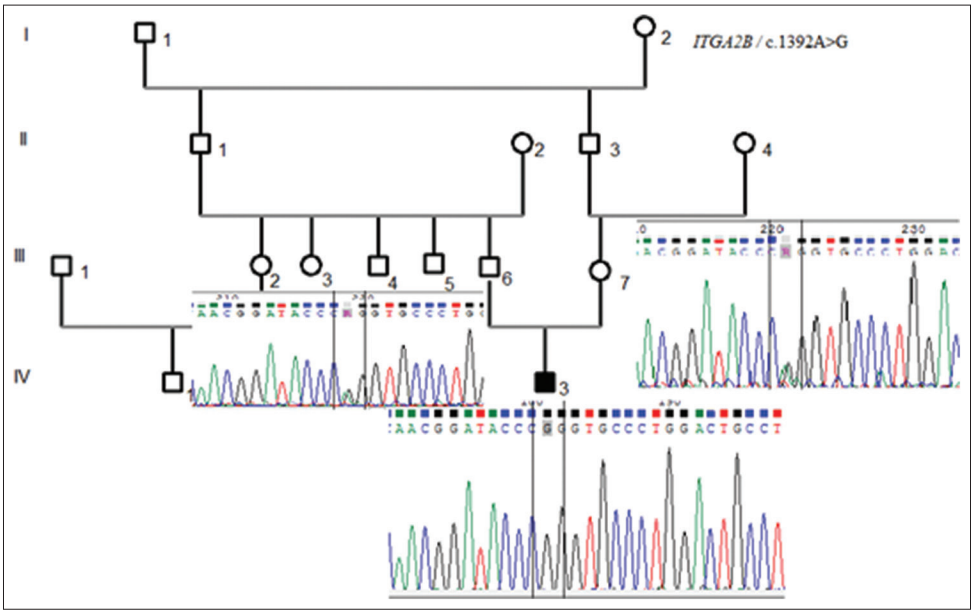
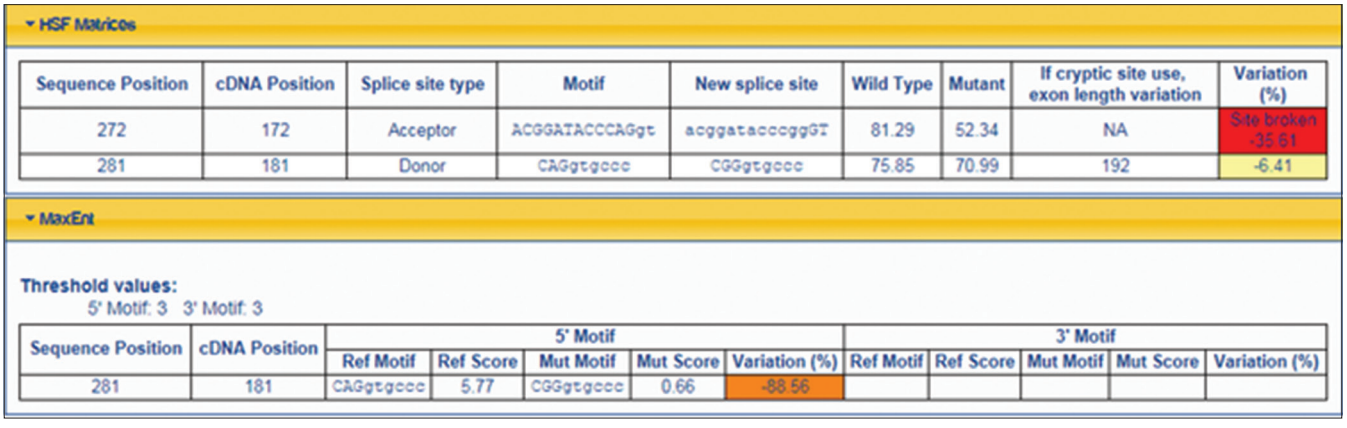



**Figure 5: Pedigree and chromatograms of the affected child and his parents (*ITGA2B*/c. 1392A > G)**



HSF Matrices

| Sequence Position | cDNA Position | Splice site type | Motif | New splice site | Wild Type | Mutant | If cryptic site use, exon length variation | Variation (%) |
|---|---|---|---|---|---|---|---|---|
| 272 | 172 | Acceptor | ACGGATACCCAGgt | acggataccc**g**GT | 81.29 | 52.34 | NA | Site broken -35.61 |
| 281 | 181 | Donor | CAGgtgccc | CGGgtgccc | 75.85 | 70.99 | 192 | -6.41 |

MaxEnt

Threshold values:
5' Motif: 3 3' Motif: 3

| Sequence Position | cDNA Position | 5' Motif | | | | | 3' Motif | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ref Motif | Ref Score | Mut Motif | Mut Score | Variation (%) | Ref Motif | Ref Score | Mut Motif | Mut Score | Variation (%) |
| 281 | 181 | CAGgtgccc | 5.77 | CGGgtgccc | 0.66 | -88.56 | | | | | |

**Figure 6: Human Splicing Finder (HSF) prediction result for 1392A>G variant identified in ITGA2B gene**

homozygous single nucleotide variation g.2043A>G was also identified in the splice acceptor of the FANCA gene and novel disease-causing and pathogenic homozygous c.1429_1430 ins a frameshift mutation in the FANCC gene. The result of in silico analysis using mutation taster, polyPhen-2, and PredictSNP2 show that these are

disease-causing and pathogenic variants. In addition, these variants are not present in our exome database or in the Population frequency databases including 1000G, ExAC, and GenomAD.

FANCA protein plays an important role in binding to the chromatin in the DNA repair process.[19] Various studies have reported that there is a *FANCA* mutation in about 60% of patients with Fanconi disease.[1,19,20] Several types of indels and point mutations have been reported in patients with Fanconi disease.[2,7,21,22] Knies *et al.* used WES to screen four FA patients to evaluate the efficiency of exome sequencing for FA genotyping and described WES as an appreciated and adequately safe method for the molecular analysis of FA gene's variants in competition with the traditional genetic testing/screening strategies.[20] Ameziane *et al.* showed that the WES method was able to recognize different mutations of *BRCA2*, *FANCD2*, *FANCI,* and *FANCL* genes in novel unclassified FA patients indicating that WES could be the first test to diagnose FA.[4]

After *FANCA*, the mutation in *FANCC* is the most commonly diagnosed mutation in patients with FA.[23,24] Fadaee *et al.* used NGS and multiplex ligation-dependent probe amplification for 48 Iranian families with FA patients and reported that NGS is the precise method for identification of a pathogenic mutation in FA patients.[25]

*FANCC* gene mutations are abundant in Ashkenazi Jews and the Japanese people.[26,27] Considering the severe sensitivity of FANCC-deficient cells, various studies suggested that FANCC protein has an important role in chromosome stability during the DNA repair process.[28] There are also several reports showing the function of *FANCC* as an intracellular antioxidant to reduce the activity of reductase.[29,30]

We could also identify a novel c.1392A>G mutation in the splice region of the *ITGA2B* gene from a patient presenting blood disorders, using the WES method. Frequencies of reported homozygote variants in the gnomAD browser and in our Exome database are counted as zero (n = 0). This variant was predicted as pathogenic using mutation taster and PredictSNP2 (93% disease). Since the c.1392A>G mutation makes no change in the amino acid sequence, the disease phenotype seems to be the result of a change and loss of mRNA splicing pattern. Analysis of c.1392A>G mutation in the splice region of *ITGA2B* gene showed that this mutation breaks the splice site and therefore is regarded as pathogenic [Figure 6]. Functional studies are necessary to assess the mechanism of disease development due to this splice region mutation more accurately. Glanzmann's disease has a high frequency in cultures with more rates of consanguineous marriage such as Indians, Iranians, and Iraqi Jews.[31,32] As clinical manifestations of blood disorders vary from person to person, many studies using NGS technology have been conducted to identify different causative mutations involved in inherited platelet disorders (IPDs).[33-35] Buitrago *et al.* identified 114 novel missense variants in *ITGA2B* and 68 novel missense variants in *ITGB3* genes by WES and reported that WES had 69-98% sensitivity in identifying Glanzmann mutations.[36]

## Conclusions

The WES method has a significant benefit such as high coverage and requires a low amount of genomic samples, which improves the sensitivity of mutation detection compared with traditional methods. Given that, most of our patients were reported in families with a history of consanguinity, WES could serve as a precise method for identifying the heterozygous carriers among the screened families for further investigations. This highlights the major impact of the WES method for screening and identification of platelet and blood-related genomic variants.

### Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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### Conflicts of interest

There are no conflicts of interest.

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