

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

### 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.<sup>[1]</sup> 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].<sup>[2]</sup> The FANCC 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).<sup>[3]</sup> 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.<sup>[4,5]</sup> The majority of (about 85%) FA patients have mutations in the *FANCA*, *FANCC*, and *FANCG* genes but are altered in various populations.<sup>[6]</sup> *FANCA* gene is located on chromosome 16q24.3 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%.<sup>[7,8]</sup> 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.<sup>[9,10]</sup> 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.<sup>[11,12]</sup> 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* ( $\alpha$ IIb) or *ITGB3* ( $\beta$ 3) 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.<sup>[13,14]</sup> In this disorder, the platelet contains defective or small amounts of integrin  $\alpha$ IIb $\beta$ 3 at the platelet surface, which is a glycoprotein receptor for fibrinogen.<sup>[15]</sup> The platelets

Samira Negahdari<sup>1</sup>,  
Mina Zamani<sup>1,2</sup>,  
Tahereh Seifi<sup>1,2</sup>,  
Sahar Sedighzadeh<sup>1,2</sup>,  
Neda Mazaheri<sup>1</sup>,  
Jawaher Zeighami<sup>1</sup>,  
Alireza Sedaghat<sup>1,3</sup>,  
Alihossein Saberi<sup>1,4</sup>,  
Mohammad Hamid<sup>1,5</sup>,  
Bijan keikhaei<sup>6</sup>,  
Ramin Radpour<sup>7,8</sup>,  
Gholamreza  
Shariati<sup>1,4</sup>,  
Hamid Galehdari<sup>6</sup>

<sup>1</sup>Narges Genetics Diagnostic Laboratory, Ahvaz, Iran,

<sup>2</sup>Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran,

<sup>3</sup>Health Research Institute, Diabetes Research Center,

Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran,

<sup>4</sup>Department of Genetics,

Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran,

<sup>5</sup>Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran,

<sup>6</sup>Health Research Institute, Research Centre of Thalassemia and Hemoglobinopathies, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran,

<sup>7</sup>Tumor Immunology, Department for BioMedical Research (DBMR), University of Bern, Bern, Switzerland,

<sup>8</sup>Department of Medical Oncology, Inselspital, University of Bern, Bern, Switzerland

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: [reprints@medknow.com](mailto:reprints@medknow.com)

**How to cite this article:** Negahdari S, Zamani M, Seifi T, Sedighzadeh S, Mazaheri N, Zeighami J, et al. Identification of three novel mutations in the *FANCA*, *FANCC*, and *ITGA2B* genes by whole exome sequencing. *Int J Prev Med* 2020;11:117.

### Access this article online

#### Website:

[www.ijpvmjournal.net/www.ijpvm.net](http://www.ijpvmjournal.net/www.ijpvm.net)

#### DOI:

10.4103/ijpvm.IJPVM\_462\_19

#### Quick Response Code:



**Address for correspondence:**

Prof. Hamid Galehdari,

Health Research Institute, Research Centre of Thalassemia and Hemoglobinopathies, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

E-mail: [galehdari187@yahoo.com](mailto:galehdari187@yahoo.com)

Dr. Gholamreza Shariati,

Narges Genetics Diagnostic Laboratory, Ahvaz, Iran; Department of Genetics, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran.

E-mail: [shariatig@yahoo.com](mailto:shariatig@yahoo.com)

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.<sup>[16,17]</sup>

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	RP11-801152, 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	FANCI	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, R51H3, RAD51L2	17q23
SLX4 (84464)	SLX4 structure-specific endonuclease subunit	FANCP	BTBD12, MUS312	16p13.3
ERCC4 (2072)	Excision repair cross-complementation group 4	FANCP	RAD1, XPF, ERCC11	16p13.12
RAD51 (5888)	RAD51 recombinase	FANCR	BRCC5, HRAD51, HsRad51, HsT16930, MRMV2A, RECA	15q15.1
BRCA1 (672)	Breast cancer 1	FANCS	BRCA1, BRCC1, BROVCA1, IRIS, PNCA4, PPP1R53, PSCP, RNF53	17q21.31
UBE2T (29089)	Ubiquitin-conjugating enzyme E2T	FANCT	HSPC150	1q32.1

Figure 1: Human FANC genes<sup>[18]</sup>

[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].

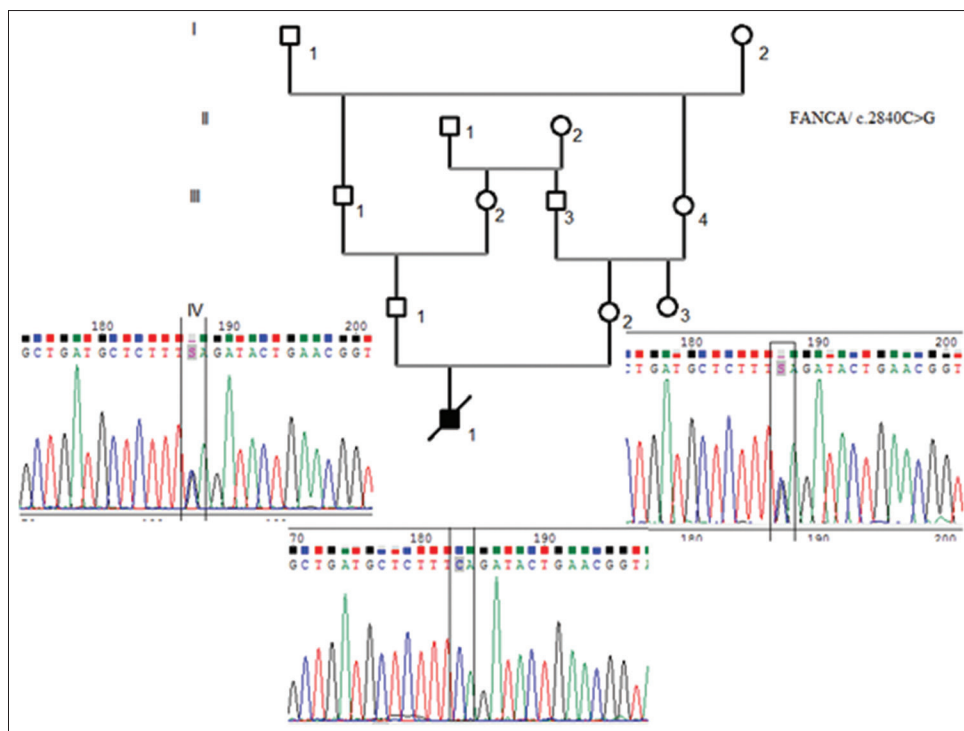


Figure 2: Pedigree and chromatograms of the affected dead child and his parents (FANCA/c.2840C>G)

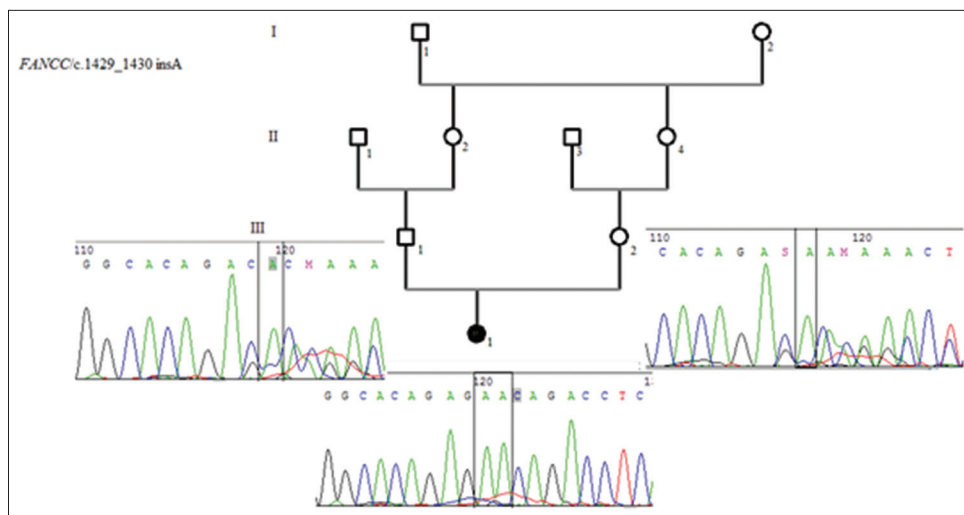


Figure 3: Pedigree and chromatograms of the affected child and her parents (FANCC/c.1429\_1430 insA)

**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 Muscle weakness, prolonged bleeding and clotting time, normal size and number of platelets

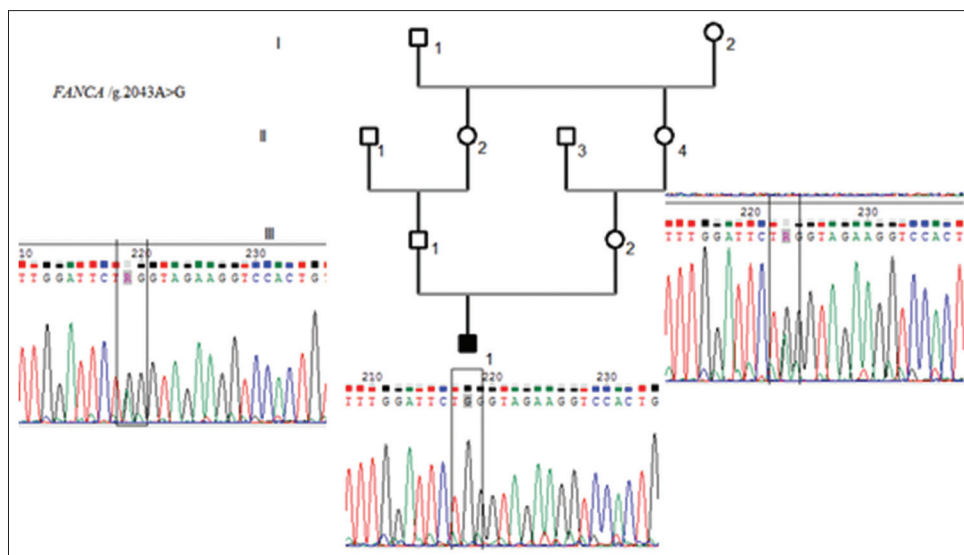


Figure 4: Pedigree and chromatograms of the affected child and his parents (*FANCA*/g.2043A>G)

### 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  $\times 100$  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	<i>FANCA</i> (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	<i>FANCA</i> (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	<i>FANCC</i> (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	<i>ITGA2B</i> (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

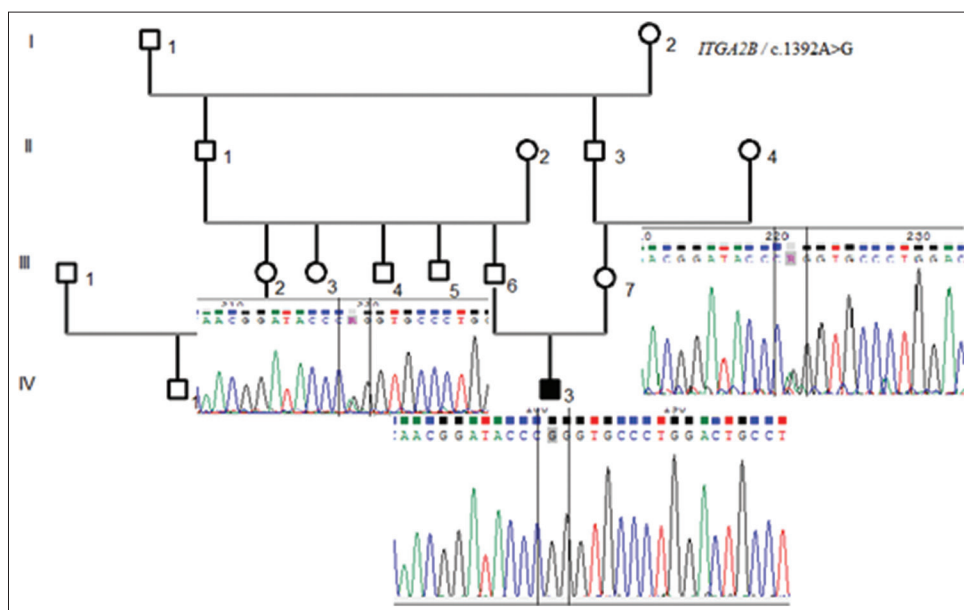


Figure 5: Pedigree and chromatograms of the affected child and his parents (*ITGA2B*/c. 1392A > G)

▼ 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	acggataccoggGT	81.29	52.34	NA	Site broken -35.81			
281	181	Donor	CAGgtgcc	CGgtgcc	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	CAGgtgcc	5.77	CGgtgcc	0.66	-88.86					

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.<sup>[19]</sup> Various studies have reported that there is a *FANCA* mutation in about 60% of patients with Fanconi disease.<sup>[1,19,20]</sup> Several types of indels and point mutations have been reported in patients with Fanconi disease.<sup>[2,7,21,22]</sup> 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.<sup>[20]</sup> 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.<sup>[4]</sup>

After *FANCA*, the mutation in *FANCC* is the most commonly diagnosed mutation in patients with FA.<sup>[23,24]</sup> 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.<sup>[25]</sup>

*FANCC* gene mutations are abundant in Ashkenazi Jews and the Japanese people.<sup>[26,27]</sup> 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.<sup>[28]</sup> There are also several reports showing the function of *FANCC* as an intracellular antioxidant to reduce the activity of reductase.<sup>[29,30]</sup>

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.<sup>[31,32]</sup> 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).<sup>[33-35]</sup> 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.<sup>[36]</sup>

## 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.

## Acknowledgments

The authors are indebted to the patients and family members for their cooperation.

## Financial support and sponsorship

This study has been funded by Narges Genetic and PND Laboratory, Ahwaz, Iran.

## Conflicts of interest

There are no conflicts of interest.

**Received:** 14 Dec 19

**Accepted:** 27 Mar 20

**Published:** 06 Aug 20

## References

1. Moghrabi NN, Johnson MA, Yoshitomi MJ, Zhu X, Al-Dhalimy MJ, Olson SB, *et al.* Validation of Fanconi anemia complementation Group A assignment using molecular analysis. *Genet Med* 2009;11:183-92.
2. Kee Y, D'Andrea AD. Expanded roles of the Fanconi anemia pathway in preserving genomic stability. *Genes Dev* 2010;24:1680-94.
3. Kottmann MC, Smogorzewska A. Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. *Nature* 2013;493:356-63.
4. Ameziane N, Sie D, Dentro S, Ariyurek Y, Kerkhoven L, Joenje H, *et al.* Diagnosis of fanconi anemia: Mutation analysis by next-generation sequencing. *Anemia* 2012;2012:132856.
5. Bogliolo M, Schuster B, Stoepker C, Derkunt B, Su Y, Raams A, Trujillo JP, *et al.* Mutations in ERCC4, encoding the DNA-repair endonuclease XPF, cause Fanconi anemia. *Am J Hum Genet* 2013;92:800-6.

6. Bagby G. Recent advances in understanding hematopoiesis in Fanconi Anemia. *F1000Res* 2018;7:105.
7. Solomon PJ, Margaret P, Rajendran R, Ramalingam R, Menezes GA, Shirley AS, *et al.* A case report and literature review of Fanconi Anemia (FA) diagnosed by genetic testing. *Ital J Pediatr* 2015;41:38.
8. Hess J, Unger K, Orth M, Schötz U, Schüttrumpf L, Zangen V, Gimenez-Aznar I, *et al.* Genomic amplification of Fanconi anemia complementation group A (FancA) in head and neck squamous cell carcinoma (HNSCC): Cellular mechanisms of radioresistance and clinical relevance. *Cancer Lett* 2016;386:87-99.
9. Bogliolo M, Surrallés J. Fanconi anemia: A model disease for studies on human genetics and advanced therapeutics. *Curr Opin Genet Dev* 2015;33:32-40.
10. Solanki A, Mohanty P, Shukla P, Rao A, Ghosh K, Vundinti BR. FANCA gene mutations with 8 novel molecular changes in Indian Fanconi anemia patients. *PLoS One* 2016;11:e0147016.
11. Cerabona D, Sun Z, Nalepa G. Leukemia and chromosomal instability in aged Fanc<sup>c</sup>-/- mice. *Exp Hematol* 2016;44:352-7.
12. Sertorio M, Amarachintha S, Wilson A, Pang Q. Loss of fanc<sup>c</sup> impairs antibody-secreting cell differentiation in mice through deregulating the wnt signaling pathway. *J Immunol* 2016;196:2986-94.
13. Kulkarni B, Ghosh K, Shetty S. Second trimester prenatal diagnosis in Glanzmann's Thrombasthenia. *Haemophilia* 2016;22:e99-100.
14. Ghosh A, Kumar S, Chacko R, Charlu AP. Total extraction as a treatment for anaemia in a patient of Glanzmann's thrombasthenia with chronic gingival bleed: Case report. *J Clin Diagn Res* 2016;10:ZD11-2.
15. Nurden AT, Pillois X, Wilcox DA. Glanzmann thrombasthenia: State of the art and future directions. *Semin Thromb Hemost* 2013;39:642-55.
16. Maclachlan A, Watson SP, Morgan NV. Inherited platelet disorders: Insight from platelet genomics using next-generation sequencing. *Platelets* 2017;28:14-9.
17. Collier BS, Shattil SJ. The GPIIb/IIIa (integrin alphaIIb beta3) odyssey: a technology-driven saga of a receptor with twists, turns, and even a bend. *Blood* 2008;112:3011-25.
18. Dong H, Nebert DW, Bruford EA, Thompson DC, Joenje H, *et al.* Update of the human and mouse Fanconi anemia genes. *Hum Genomics* 2015;9:32.
19. Moghadam AA, Mahjoubi F, Reisi N, Vosough P. Investigation of FANCA gene in Fanconi anaemia patients in Iran. *Indian J Med Res* 2016;143:184-96.
20. Knies K, Schuster B, Ameziane N, Rooimans M, Bettecken T, de Winter J, *et al.* Genotyping of fanconi anemia patients by whole exome sequencing: advantages and challenges. *PLoS One* 2012;7:e52648.
21. Auerbach AD. Fanconi anemia and its diagnosis. *Mutat Res* 2009;668:4-10.
22. Centra M, Memeo E, d'Apolito M, Savino M, Ianzano L, Notarangelo A, *et al.* Fine exon-intron structure of the Fanconi anemia group A (FAA) gene and characterization of two genomic deletions. *Genomics* 1998;51:463-7.
23. Aftab I, Iram S, Khaliq S, Israr M, Ali N, Jahan S, *et al.* Analysis of FANCC gene mutations (IVS4+4A>T, del322G, and R548X) in patients with Fanconi anemia in Pakistan. *Turk J Med Sci* 2017;47:391-8.
24. Niedzwiedz W, Mosedale G, Johnson M, Ong CY, Pace P, Patel KJ, *et al.* The Fanconi anaemia gene FANCC promotes homologous recombination and error-prone DNA repair. *Mol Cell* 2004;15:607-20.
25. Esmail Nia G, Fadaee M, Royer R, Najmabadi H, Akbari MR. Profiling Fanconi anemia gene mutations among Iranian patients. *Arch Iran Med* 2016;19:236-40.
26. Gillio AP, Verlander PC, Batish SD, Giampietro PF, Auerbach AD, *et al.* Phenotypic consequences of mutations in the Fanconi anemia FAC gene: An International Fanconi Anemia Registry study. *Blood* 1997;90:105-10.
27. de Vries Y, Lwiwski N, Levitus M, Kuyt B, Israels SJ, Arwert F, Zwaan M, *et al.* A Dutch Fanconi anemia FANCC founder mutation in Canadian manitoba mennonites. *Anemia* 2012;2012:865170.
28. Pace P, Johnson M, Tan WM, Mosedale G, Sng C, Hoatlin M, *et al.* FANCE: The link between Fanconi anaemia complex assembly and activity. *EMBO J* 2002;21:3414-23.
29. Pang Q, Keeble W, Diaz J, Christianson TA, Fagerlie S, Rathbun K, *et al.* Role of double-stranded RNA-dependent protein kinase in mediating hypersensitivity of Fanconi anemia complementation group C cells to interferon gamma, tumor necrosis factor-alpha, and double-stranded RNA. *Blood* 2001;97:1644-52.
30. Joenje H, Patel KJ. The emerging genetic and molecular basis of Fanconi anaemia. *Nat Rev Genet* 2001;2:446-57.
31. Sandrock-Lang K, Oldenburg J, Wiegering V, Halimeh S, Santoso S, Kurnik K, *et al.* Characterisation of patients with Glanzmann thrombasthenia and identification of 17 novel mutations. *Thromb Haemost* 2015;113:782-91.
32. Poon MC, d'Oiron R, Zotz RB, Bindslev N, Di Minno MN, Di Minno G, *et al.* The international, prospective Glanzmann Thrombasthenia Registry: Treatment and outcomes in surgical intervention. *Haematologica* 2015;100:1038-44.
33. Jones ML, Murden SL, Bem D, Mundell SJ, Gissen P, Daly ME, *et al.* Rapid genetic diagnosis of heritable platelet function disorders with next-generation sequencing: Proof-of-principle with Hermansky-Pudlak syndrome. *J Thromb Haemost* 2012;10:306-9.
34. Westbury SK, Turro E, Greene D, Lentaigne C, Kelly AM, Bariana TK, *et al.* Human phenotype ontology annotation and cluster analysis to unravel genetic defects in 707 cases with unexplained bleeding and platelet disorders. *Genome Med* 2015;7:36.
35. Simeoni I, Stephens JC, Hu F, Deevi SV, Megy K, Bariana TK, *et al.* A high-throughput sequencing test for diagnosing inherited bleeding, thrombotic, and platelet disorders. *Blood* 2016;127:2791-803.
36. Buitrago L, Rendon A, Liang Y, Simeoni I, Negri A, ThromboGenomics Consortium, *et al.* AlphaIIb beta3 variants defined by next-generation sequencing: predicting variants likely to cause Glanzmann thrombasthenia. *Proc Natl Acad Sci U S A* 2015;112:E1898-907.