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A New Missense Mutation in FANCA Gene Detected by Whole Exome Sequencing in a Case with Fanconi Anemia

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Abstract

Fanconi Anemia (FA) is a rare genetic disease identified by a mutation in any of THE 22 FA associated genes that are linked with bone marrow failure and immunodeficiency. Of all FA associated genes, the most frequent mutation has been reported in the FANCA gene worldwide, which is responsible for about 60- 65% of all cases. In this study, we presented a case with a new missense mutation in the FANCA gene among the Iranian population. Accordingly, bruising around the eyes as the first symptom was manifested in an around 10-years-old case, along with lung infection, and pancytopenia while normal serum immunoglobulin levels were also observed.

Keywords: Fanconi anemia, FANCA, lung infection, pancytopenia

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Introduction

More than nine decades ago, a family with three male children aged between 5 to 7 years old was described by Fanconi as follows: the patients with large blood cells, progressively declining blood counts, and skeletal abnormalities (1, 2). These patients were named Fanconi anemia (FA), who were then classified as bone marrow failure associated with primary immunodeficiency (3). Notably, patients with FA manifest pancytopenia, hyperpigmentation, skeletal malformation, small stature, urogenital abnormalities, and familial occurrence (2). However, delay in diagnosis has still remained as a major problem for the diagnosis of this disease by physicians. Accordingly, increasing awareness regarding different aspects

of the main disease defect on immunity among physicians is a necessary issue (2).

Cytogenetic analysis of FA patients revealed that the hypersensitivity of their chromosomal breakage and sensitivity of their DNA to both cytotoxic and clastogenic effects agents such as diepoxybutane and mitomycin C are the main properties of FA cells (4). Hence, for the first time, a diagnostic test based on the evaluation of chromosomal breakage was developed by Arleen Auerbach (5). By screening chromosomal patterns of FA patients, scientists have found genetic heterogeneities among them (4). In this regard, up to now, 22 genes have been identified in FA that play critical roles in repairing DNA interstrand crosslinks (ICL) (6, 7).

Accordingly, these genes are as follows: *FANCA, FANCB, FANCC, FAND1 (BRCA2), FANCD2, FANCE, FANCF, FANCG (XRCC9), FANCI, FANCJ (BRIP1), FANCL, FANCM, FANCN (PALB2), FANCO (RAD51C), FANCP (SLX4), FANCQ (ERCC4), FANCR (RAD51), FANCS (BRCA1), FANCT (UBE2T), FANCU (XRCC2), FANCV (MAD2L2)*, and *FANCW (RFWD3)* with recessive inheritance (6). In this report, we presented a case with a homozygous missense in the *FANCA* gene and FA symptoms such as pancytopenia and mild hepatomegaly.

Case Presentation

The patient was an 18–year- old boy from non-consanguineous healthy parents who was their second child. At the age 10 years old, he presented bruising around the eyes as the first presentation. He was firstly admitted due to lung infection and subsequently in his medical reports, severe stomach ulcers, anemia, nausea, jaundice, mild hepatomegaly, and severe anal atresia/stenosis have been mentioned. Regarding the obtained laboratory data, there was pancytopenia in complete blood counts with an increased level of C-reactive protein.

Serum immunoglobulin levels including IgM, IgG, IgA, and IgE were tested, which their results were within the normal range. No defects were seen in specific antibody responses to tetanus and diphtheria vaccines. Also, other laboratory and immunologic data of the patient are provided in **Table 1**. Moreover, computed tomography (CT) scan of the lung showed interstitial lung disease (ILD) caused by severe scars of the lung (**Figure 1**). Based on the clinical/para-clinical investigation, FA was suggested as a possible diagnosis for this patient. Finally, the genetic evaluation was performed using whole-exome sequencing (WES) technique. In this regard, the genetic confirmed a homozygous missense variant (c.522G>C) in the *FANCA* gene (p.Q174H) (**Figure2**). Unfortunately, the patient has passed away in the age of 18–year-old due to respiratory failure.

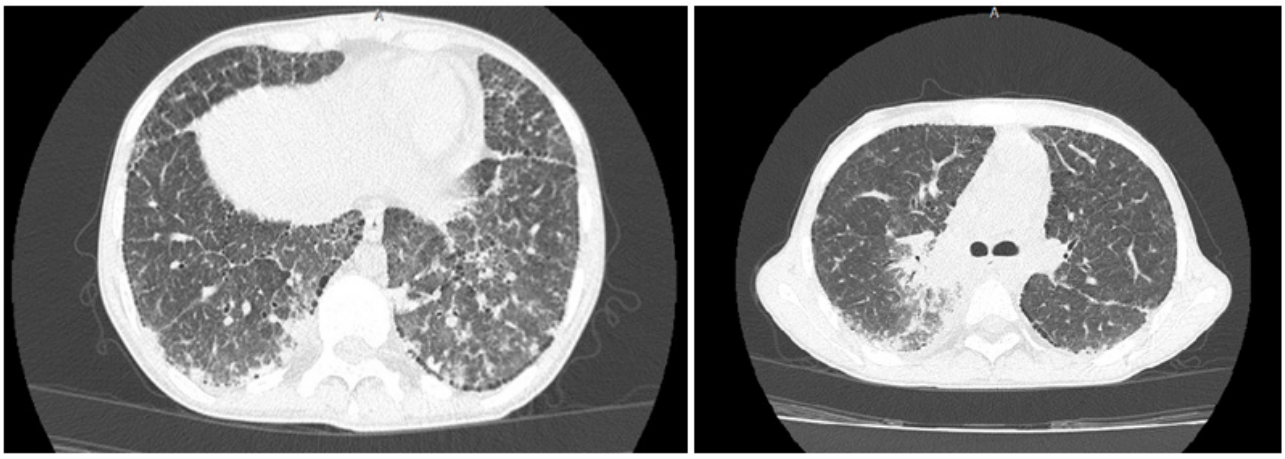

Figure 1. Computed tomography (CT) scan of the patient's lung showed interstitial lung disease (ILD) that caused severe scars of lung.

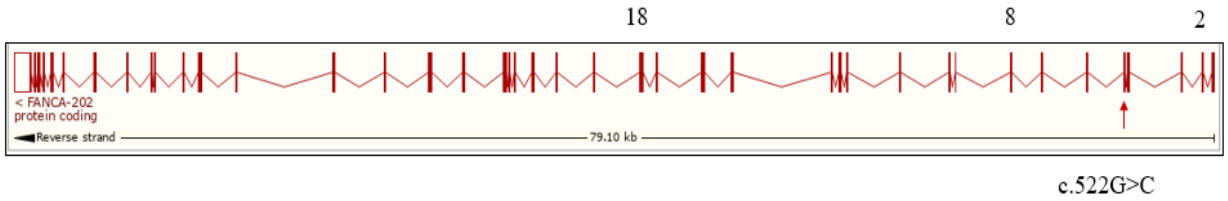

Figure 2. Map of mutation in FANCA gene. A homozygous missense variant (c.522G>C) in FANCA gene (p.Q174H)

Table1. Laboratory and immunologic data of the patient

Laboratory test	Patient	Reference Intervals
Complete blood count		
White blood cells (10^3/µl)	1.38↓	4 – 11
Red blood cells (10^6/µl)	1.97↓	4.5 – 6.2
Hemoglobin (gr/dl)	6.4 ↓	13 – 17
Hematocrit (%)	22.3↓	40 – 50
M.C.V (fl)	113.2 ↑	80 – 100
M.C.H (pg)	32.5	27 – 32
M.C.H.C (g/dl)	28.7	31.5 – 34.5
R.D.W-CV (%)	29.3	11.6 – 14
R.D.W-SD (fl)	115.9	34 – 54
Platelets (10^3/µl)	65 ↓	150 – 410
Erythrocyte sedimentation rate 1h	104	0 – 20
CRP	92.3	0 – 10
RBC Morphology		
Anisocytosis	(++)	
Hypochromia	(+)	
Macrocyte	(+)	
Ovalocyte	(+)	
Schistocyte	(+)	
Serum immunoglobulins		
IgM (mg/dl)	43	Adults: 40 – 230
IgG (mg/dl)	1558	700 – 1600
IgA (mg/dl)	203	Adults: 70 – 400
IgE (mg/dl)	66.1	Adults: up to 87
Vaccine antibodies		
St. Pneumonia Ab (IgG) (mg/dl)	173.8	<3.3 is negative 3.3 – 11: Intermediate >11 is positive
St. Pneumonia Ab (IgG2) (mg/dl)	82	<1.1 is negative – 3.4: Intermediate >3.4 is positive
Tetanus Ab (IgG) (IU/ml)	0.7	Non immune: <0.1 To be controlled after 1-2 years: 0.1 – 1.0 To be controlled after 2 – 4: 1.0 – 5.0 To be controlled after 4 – 8: >5.0
Diphtheria Ab (IgG) (IU/ml)	0.29	Basic immunization recommended: <0.01 Booster vaccination recommended: 0.01 – 0.1 Good immunity >0.1

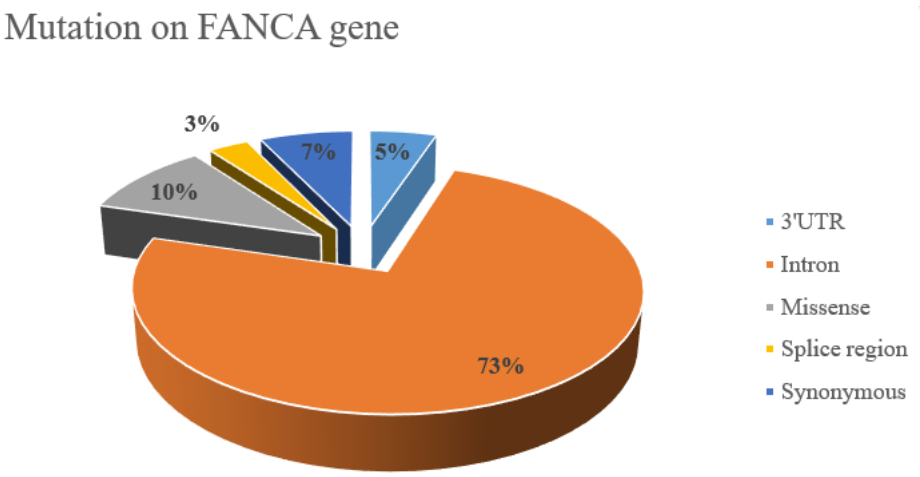

Figure3. Types of mutations on the FANCA gene. FANCA mutations' pie chart shows that the most frequent mutations occurred in introns in the Iranian population based on the Iranome database. Missense mutations is accounted for 10% of all.

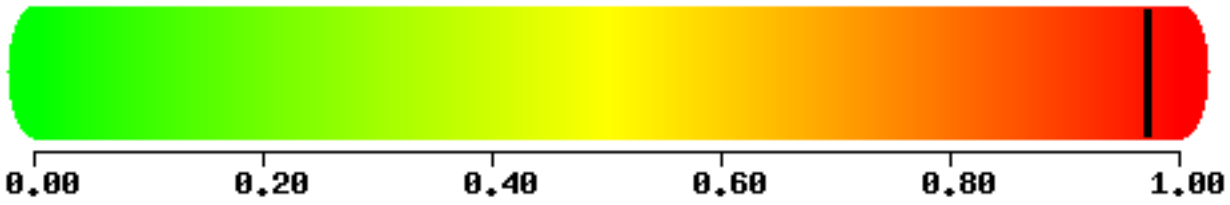

Figure 4. Predicting the effect of missense mutation on the related protein function using PolyPhen database. A homozygous missense variant (c.522G>C) mutation in FANCA was predicted to be probably damaging with score 0.972 (sensitivity: 0.60, specificity: 0.93)

Table2. Diversity missense of FANCA gene in Iranian population based on Iranome database

Variant	Transcript Consequence	Protein Consequence	Allele Frequency
16:89805074 C / T	c.4303G>A	p.Ala1435Thr	0.000625
16:89805301 G / C	c.4249C>G	p.His1417Asp	0.000625
16:89805311 C / G	c.4239G>C	p.Lys1413Asn	0.000625
16:89805324 C / T	c.4226G>A	p.Arg1409Gln	0.000625
16:89805373 C / T	c.4177G>A	p.Val1393Met	0.000625
16:89805382 C / T	c.4168G>A	p.Gly1390Ser	0.000625
16:89805644 T / A	c.4064A>T	p.His1355Leu	0.000625
16:89805672 C / T	c.4036G>A	p.Ala1346Thr	0.000625
16:89806477 C / T	c.3859G>A	p.Val1287Ile	0.00875
16:89813093 G / C	c.3412C>G	p.Leu1138Val	0.005625
16:89815122 T / C	c.3293A>G	p.Glu1098Gly	0.00125
16:89816193 C / T	c.3184G>A	p.Gly1062Arg	0.000625
16:89818581 G / A	c.3031C>T	p.Arg1011Cys	0.0025
16:89825107 G / C	c.2859C>G	p.Asp953Glu	0.00375
16:89831461 A / G	c.2615T>C	p.Met872Thr	0.000625

Variant	Transcript Consequence	Protein Consequence	Allele Frequency
16:89833583 A / G	c.2567T>C	p.Leu856Ser	0.000625
16:89836359 G / A	c.2390C>T	p.Ala797Val	0.000625
16:89836363 A / C	c.2386T>G	p.Ser796Ala	0.000625
16:89836654 C / A	c.2236G>T	p.Ala746Ser	0.003125
16:89842176 C / G	c.1874G>C	p.Cys625Ser	0.000625
16:89846323 C / T	c.1669G>A	p.Val557Met	0.000625
16:89849458 G / C	c.1523C>G	p.Thr508Arg	0.000625
16:89858365 A / G	c.1195T>C	p.Cys399Arg	0.000625
16:89858418 G / A	c.1142C>T	p.Thr381Met	0.000625
16:89862388 A / G	c.932T>C	p.Ile311Thr	0.004375
16:89862413 T / G	c.907A>C	p.Ser303Arg	0.000625
16:89869722 C / T	c.737G>A	p.Gly246Glu	0.003125
16:89874756 G / A	c.542C>T	p.Ala181Val	0.00125
16:89877438 C / T	c.325G>A	p.Val109Met	0.000625
16:89877446 C / G	c.317G>C	p.Gly106Ala	0.00125
16:89882386 C / G	c.88G>C	p.Val30Leu	0.00125
16:89882956 G / T	c.68C>A	p.Ala23Asp	0.00646
16:89883000 G / C	c.24C>G	p.Asn8Lys	0.0006452

Discussion

FA is a rare genetic disease with the prevalence of 1 out of every 136,000 newborns (8), which can be defined as a DNA repair deficiency syndrome caused by a mutation in FA genes (9, 10). In addition, it is characterized by the early onset of symptoms including congenital abnormalities, hematological manifestations, and a predisposition to malignancies (9). Moreover, hyperinsulinemia has been reported to affect 72% of all FA patients (11). Due to nonspecific clinical manifestations, it is difficult to diagnose FA based on these different clinical manifestations (10). For this reason, performing molecular genetic testing is essential for confirming the diagnosis of FA as well as specifying the subtype of the disease from other bone marrow failures (12, 13). Herein, we described a FA patient who deceased at the age of 18 years old. Our case manifested bruising around the eyes along with lung infection, pancytopenia, and normal serum immunoglobulin levels. Also, WES was performed for this patient and showed a missense mutation in the *FANCA* gene.

The FA genes are characterized for their roles in DNA damage repair (14), especially for DNA interstrand crosslink (ICL) repair (6). Except for *FANCB* that is located on the X chromosome and is X-lined recessive, other FA genes are autosomal recessive (12). Up to now, mutations of 22 FA genes have been identified to play critical roles in FA (15). Furthermore, the mutations in *FANCA*, *FANCC*, *FANCG,* and *FANCG2* genes were reported as the most frequent mutations among FA patients worldwide (12). Moreover, it has been indicated that FA genes play important roles in other disorders such as cancers, infertility, hyperinsulinemia, infertility, and endocrine abnormalities (2, 11, 16). Hence, different clinical manifestations in FA could be related to the different roles in FA genes.

Among all genes found in FA that were mentioned earlier, mutations in *FANCA* (also named *FA, FA1, FAA, FACA, FANCA_HUMAN, FAH, and FANCH*)

are the most frequent ones among FA patients that are responsible for 60- 65% of the cases worldwide (12). The *FANCA* is located at 16q24.3 on chromosome 16 and has 43 exons that code a relatively large protein with 1455 amino acids (17, 18). Increasing studies on *FANCA* mutation have shown diversity in types of mutations including insertions, deletions, nonsense, missense, splicing, and frameshift. In this regard, the *FANCA* gene is considered as a highly polymorphic gene (12). In the case of the present study, WES detected a homozygous missense variant (accounted for nearly 10% of all mutations in *FANCA* in the Iranian population (**Figures 2 and 3**) c.522G>C in the *FANCA* gene. Accordingly, this variant has occurred at the last nucleotide of the exon. In terms of the ACMG guidelines, this variant can be classified as a variant uncertain significance (VUS). To predict whether an amino acid change in the protein affects protein function, we used SIFT, PolyPhen, and Mutation taster databases. Based on our analysis of the PolyPhen database, this mutation was predicted to be probably damaging with a score of 0.972 (sensitivity: 0.60, specificity: 0.93) (**Figure4**).

Conclusions

The *FANCA* is a large gene containing 43 exons that resulted in a distinct set of private mutations in FA patients (19). In this study, we provided a diversity missense of the *FANCA* gene in the Iranian population based on the Iranome database (**Table2**) (http://www.iranome.ir/). Hence, more precisely studying different mutations in *FANCA* is necessary to achieve an accurate diagnosis. Furthermore, evaluating parents and sibling genomes could also be useful for more goals, including genetic counseling for families, a better understanding of the disease processes, and finally for exploring new therapeutic options.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgment

Written informed consents were obtained from the patients`s legal guardians for publication of this case report and its accompanying information.

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