Novel variants in Iranian individuals suspected to have inherited red blood cell disorders, including bone marrow failure syndromes

Due to their rarity, heterogeneity and overlapping clinical and hematologic phenotypes, the diagnosis of rare types of anemia including inherited bone marrow failure syndromes is not straight forward, although this challenge has been helped by high-throughput technologies such as whole exome sequencing (WES) in recent years.[1] In this study, we used WES to identify the cause of congenital severe anemia in the affected member/members of four unrelated Iranian families (families I-IV) (Figure 1) with an inconclusive diagnosis. The affected members were suspected to have red blood cell (RBC) disorders, including bone marrow failure syndromes, based on their clinical and hematologic information (*Online Supplementary Table S1,* Figure 2). All probands were dependent on RBC or platelet transfusion on a regular or irregular basis and had unaffected parents with no family history of severe anemia in previous generations. WES was performed in the probands and tertiary analysis was focused on 281 genes related to RBC disorders and bone marrow failure syndromes (*Online Supplementary File*). The pathogenicity of the candidate variants was predicted using the American College of Medical Genetics (ACMG) guidelines.[2] Sanger sequencing was used to confirm the WES data and family studies *(Online Supplementary Figures S1-S5*). Five variants in *RPL5, RUNX1, RPS26, ADA2* and *CDAN1* genes, associated with different types of inherited bone marrow failure syndromes were identified, three of which were new (Table 1, Figure 1).

The first proband (P-I) was a 6-year old boy with a congenital thumb abnormality (Figure 2A) and severe anemia. After presenting with a low hemoglobin concentration (<8.5 g/dL) and later frequent bruising and nose bleeding associated with severe thrombocytopenia in the first year of his life, he started to receive initially RBC and later platelet transfusions at irregular intervals (every 15 days to 2 months). Fanconi anemia was ruled out by chromosomal breakage analysis. Hypocellular marrow (Figure 2B), with 60-65% cellularity and usual composition of hematopoietic elements, was reported in his bone marrow examination. After performing WES, we identified a known mutation in a ribosomal protein (RP)-encoding gene *RPL5*, together with a new variant in the *RUNX1* gene, both in a heterozygous state. These variants were absent in the proband's unaffected family members, including his parents and siblings, suggesting that they were *de novo* variants (Table 1, Figure 1). Thumb abnormality has been reported in 38% of cases of Diamond Blackfan anemia (DBA), including those with *RPL5* mutations.[3] The *RPL5* variant observed here has been previously reported in a *de novo* or sporadic status, with a range of other physical abnormalities, including myelomeningocele, cleft palate, facial dysmorphism, flat thenar eminence, grouped carpal bones, short stature and partial anomalous pulmonary venous return.[4,5] Mutations in *RUNX1* are associated with familial platelet disorder with a predisposition to myelodysplasia and/or acute myeloid leukemia.[6] *RUNX1*-

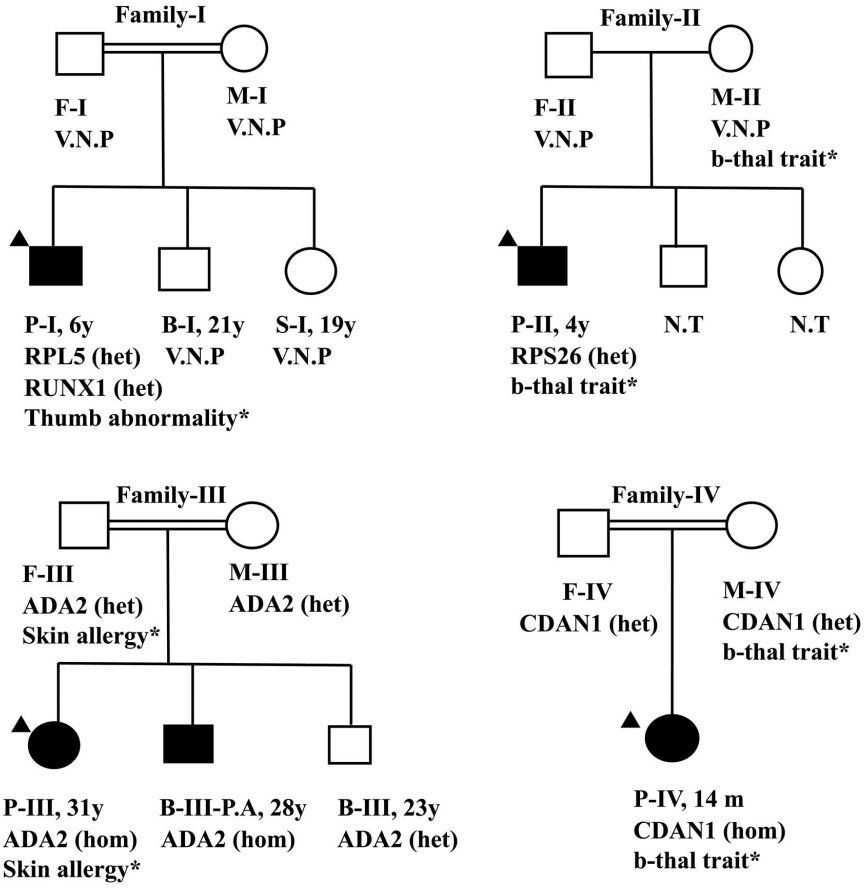

Figure 1. Pedigrees for families I-IV. F: father; M: mother; B: brother; S: sister; U: unaffected; A: affected; P.A: probably affected; b-thal: β-thalassemia; y: years old, m: months old; het: heterozygous; hom: homozygous; N.T: member not tested; V.N.P: variant not present; *:other phenotypes important to note.

related thrombocytopenia has been mostly reported in cases with a family history of this condition. There is a report of a *RUNX1* mutation suspected to be *de novo*, together with another somatic *RUNX1* mutation, in a patient with severe congenital thrombocytopenia, who subsequently developed a high-grade myelodysplastic syndrome.[7] The proband was suggested to have co-existence of DBA and a non-familial platelet disorder.

Table 1. Variants identified in families I-IV.

Proband	I		II	III	IV
Gene	*RPL5* (NM_000969.5)	*RUNX1* (NM_001754.4)	*RPS26* (NM_001029.3)	*ADA2 (CECR1)* (NM_017424.2) (NM_001282225.2)	*CDAN1* (NM_138477.2)
Genomic change	chr1:93299193, c.169_172delAACA, p.Asn57fs	chr21:36231877 T>C	chr12:56436389A>C	chr22:17684643A>G	chr15:43021261C>T
cDNA change	c.169_172delAACA	c.509-2A>G	c.181+3A>C	c.563T>C	c.2605G>A
Protein change	p.Asn57Glufs	NO RECORD	NO RECORD	p.Leu188Pro	p.Val869Met
Exon	3/8	5/8	2/3	4/10	19/28
Variant type	frameshift_variant	splice_acceptor, intronic	splice_region_variant, intronic	missense	missense
Co-segregation with phenotype	AD, Probably *de novo*	AD, Probably *de novo*	AD, Probably *de novo*	AR	AR
VAF for probably *de novo* variants	0.30	0.38	0.58	Not applicable	Not applicable
Zygosity in proband	Het	Het	Het	Hom	Hom
Variant in other family members					
Unaffected mother	V.N.P	V.N.P	V.N.P	Het	Het
Unaffected father	V.N.P	V.N.P	V.N.P	Het	Het
Unaffected brother	V.N.P	V.N.P	Not Tested	Het	–
Unaffected sister	V.N.P	V.N.P	Not Tested	–	–
Affected brother	–	–	–	Hom	–
Affected sister	–	–	–	–	–
Allele frequency in public databases					
dbSNP	NO RECORD	NO RECORD	NO RECORD	rs760102576	rs370895637
1KG	NO RECORD	NO RECORD	NO RECORD	NO RECORD	NO RECORD
ExAC	NO RECORD	NO RECORD	NO RECORD	AF: 0.000008781	AF: 0.00003295
gnomAD	NO RECORD	NO RECORD	NO RECORD	AF: 0.000007086	AF: 0.00003535
Iranome	NO RECORD	NO RECORD	NO RECORD	NO RECORD	NO RECORD
Bioinformatics predictions					
SIFT	NO RECORD	NO RECORD	NO RECORD	Damaging	Damaging
Polyphen2_HDIV	NO RECORD	NO RECORD	NO RECORD	Damaging	Damaging
Polyphen2_HVAR	NO RECORD	NO RECORD	NO RECORD	Damaging	Probably damaging
Mutation taster	NO RECORD	Disease causing	Disease causing	Disease causing	Disease causing
CADD v1.4	34	31	13.72	25.9	28.8
dbscSNV_ADA_SCORE	NO RECORD	1	0.3136	NO RECORD	NO RECORD
dbscSNV_RF_SCORE	NO RECORD	0.918	0.29	NO RECORD	NO RECORD
Disease Databases					
Clinvar	Pathogenic (Last evaluated: Jan 23, 2018)	NO RECORD	NO RECORD	Uncertain significance (Last evaluated: June 16, 2016)	NO RECORD
HGMD public	CD086184	NO RECORD	NO RECORD	NO RECORD	CM023036
Splice site prediction (NN splice)	Not applicable	Donor site prediction S=0.99	Acceptor site predictions S= 0.83	Not applicable	Not applicable
ACMG prediction	Pathogenic [PVS1,PM2, PM6]	Pathogenic [PVS1, PM2, PP3,PM6]	Uncertain significance [PM2, PM6, BP4]	Likely pathogenic [PM1, PM2, PP2, PP3]	Likely pathogenic [PM1, PM2, PP3, PP5]

AD: autosomal dominant; AR: autosomal recessive; VAF: variant allele frequency; Hom: homozygote; Het: heterozygote; -: no such relationship existed; V.N.P: variant not present.

The second proband (P-II) was a 4-year old boy who had β-thalassemia trait, inherited from his carrier mother *(Online Supplementary Table S1*, Figure 1). He presented with anemia on the 25th day of his life and started receiving RBC transfusions at 30-day intervals. Hypocellular marrow (Figure 2C) with 40-45% cellularity was noted in his bone marrow examination. Following WES, a new variant in another known RP involved in DBA, *RPS26* was observed in this proband. This variant was absent in his parents (Table 1, Figure 1). As for *RPL5*, *de novo RPS26* variants have been previously reported in DBA cohorts.[8,9] No physical abnormalities were observed in this proband. In Doherty's report, only three out of 11 cases with a *RPS26* mutation had physical abnormalities.[9] Coexisting DBA with β-thalassemia trait, which could make the diagnosis more challenging, has been previously reported in a Turkish family.[10] It is important to note that among DBA cases with mutated RP genes, patients with *RPS26* mutations have been reported to show the poorest response to steroid therapy.[11] DBA has been described as one of the inherited bone marrow failure syndromes related to RP dysfunction. Today, mutations in at least 19-26 RP genes are suggested to be involved in DBA pathogenicity. Fifty-five percent of DBA cases are due to sporadic or *de novo* mutations and half of the patients show physical abnormalities.[3,8]

The proband in the third family (P-III) was a 31-year old young woman with a probably affected brother (B-III-P.A). These siblings both had short episodes of severe anemia and received RBC transfusions during the first year of their lives. The childhood episode of anemia was successfully treated in both by steroid therapy. P-III had a second episode of severe anemia after 26 years of age. Showing a poor response to steroid therapy, she again started to receive RBC transfusions at 10- to 20-day intervals. Bone marrow biopsy findings, including a hypercellular marrow (Figure 2D) with more than 95% cellularity and increased megakaryocytes, suggested a probable myeloproliferative disorder. However, copy number analysis (BCR-ABL to ABL) and V617F mutation analysis in the *JAK2* gene did not detect any mutation. Chromosome breakage analysis ruled out Fanconi anemia in this individual (*Online Supplementary Table S1*). After failure of these diagnostic approaches, WES was performed for this proband, which identified a new variant in the *ADA2* gene in a homozygous state. This variant was also observed in her probably affected brother (B-III-P.A) in a homozygous state. Her unaffected parents and unaffected brother were carriers of this variant (Table 1, Figure 1).

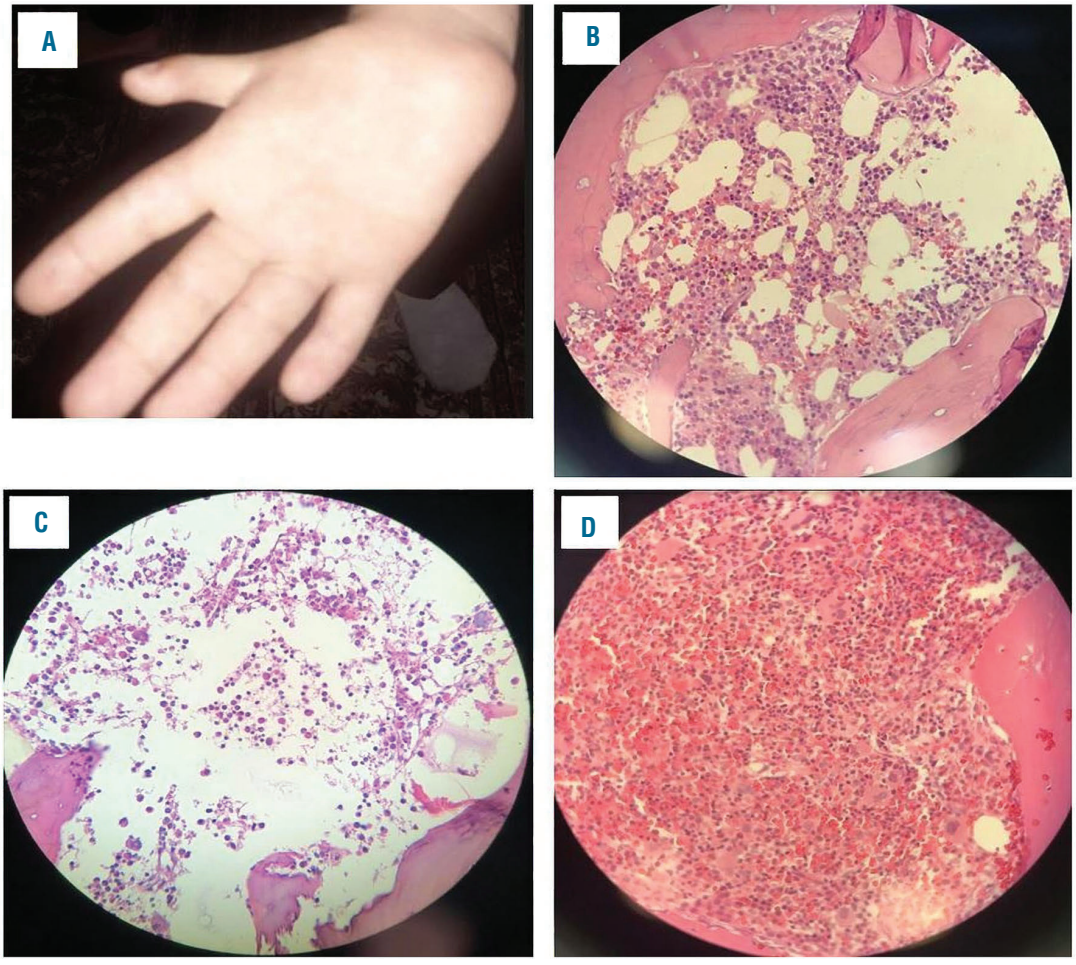

Figure 2. Phenotypic and/or trephine biopsy findings in probands I-III. (A) Thumb abnormality observed in P-I. (B) Hypocellular marrow observed in P-I. (C) Hypocellular marrow observed in P-II. (D) Hypercellular marrow with increased megakaryocytes observed in P-III.

Mutations in *ADA2* cause a rare condition described in recent years as "Deficiency in Adenosine Deaminase 2" (DADA2). Only 160 cases of DADA2 have been reported so far. This condition is associated with vasculopathy, immunodeficiency and bone marrow failure. Patients with DADA2 have a strikingly heterogeneous phenotype even among individuals carrying the same mutation and 55% of the cases have anemia.[12] DADA2 has been reported in some patients with pure red cell aplasia, which mimics DBA. Accordingly, a remarkable erythroid hypoplasia was observed in our patient's bone marrow biopsy (Figure 2D). It is not clear whether *ADA2* is involved in the same causal pathway as RP-related DBA genes.[8] P-III's currently anemia-free brother (B-III-P.A) has not had a second episode of anemia so far. It is worth considering that the proband (P-III) and her father (F-III), who is a carrier of an *ADA2* variant, both have presented skin allergy, possibly correlated to ADA2 deficiency.

The last case (P-IV) was a 14-month old girl, who had thalassemia trait inherited from her carrier mother (*Online Supplementary Table S1*, Figure 1). She presented with anemia at birth and started RBC transfusions at 5-week intervals. A known variant in the *CDAN1* gene in homozygous state was identified in this proband, while her parents were carriers of this variant (Table 1, Figure 1). Mutations in the *CDAN1* gene are associated with congenital dyserythropoietic anemia (CDA) type I. The various types of CDA are classified as a subtype of bone marrow failure syndromes, in which proliferation and maturation of the erythroid lineage is impaired. Based on the genes involved (*CDAN1, C15ORF41, SEC23B, KIF23*, and *KLF1*), CDA are classified into four groups. Depending on the type of CDA and the severity of the disease, treatment strategies for CDA include blood transfusion, chelation therapy, stem cell transplantation, splenectomy and interferon therapy.[13]

Patients with suspected RBC disorders, including bone marrow failure syndromes in this study, presented a remarkable heterogeneity. Further evaluation of the clinical and hematologic phenotypes of the patients, together with bioinformatic analysis could confirm the pathogenicity of the novel variants and diagnosis. The variant allele frequency of the heterozygote variants which were suggested to be *de novo* in our patients is shown in Table 1. However, due to unavailability of buccal tissue, we could not investigate the possibility of the somatic nature of these variants. As in our previous report, consanguinity of parents among patients with rare types of blood cell disorders is notable, since three of the four families were consanguineous.[14] Finally, as bone marrow failure syndromes could be associated with poor prognosis leukemia later in life, the diagnosis should be confirmed and close monitoring should be carried out. In addition, stem cell transplantation is suggested for patients who meet transplantation criteria.[15]

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