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

Maryam Neishabury,1 Maghsood Mehri,1 Zohreh Fattahi,1 Hossein Najmabadi1,2 and Azita Azarkeivan3
1Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran; 2Kariminejad-Najmabadi Pathology & Genetics Center, Tehran and 3Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

Correspondence: MARYAM NEISHABURY - mneisha@gmail.com
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Supplemental information

Supplementary methods

Families and patients

Four Iranian Families (Families I-IV), (Figure 1; Supplementary Table 1) with at least one affected individual, with inconclusive diagnosis were referred from various provinces to “Adult Thalassemia Clinic, Blood Transfusion Research Centre, High Institute for Research and Education in Transfusion Medicine”, Tehran, Iran, as a referral centre. Based on previous clinical and haematological data (Supplementary Table 1; Figure 2) they were suspected to have rare types of red blood cell disorders, including bone marrow failure syndromes. Therefore, after informed consent, they became candidate for Whole Exome Sequencing (WES). Five ml blood samples were collected from each individual and DNA was extracted using standard salting out procedure.

Whole Exome Sequencing and bioinformatic prediction of pathogenicity of the variants

Qualitative and quantitative evaluation of DNA was performed using standard techniques, Whole Exome Sequencing was performed using (HiSeq400 sequencer, Sure Select V6-Post by Macrogen Inc.) Mapping reference hg19 from UCSC (original GRCh37 from NCBI, Feb. 2009) was used to address the variants. Synonymous and intronic variants and variants with higher than 1% frequency were excluded by filtering. Remaining variants were filtered using a list of 281 anemia related genes (Supplementary Table 2). For preparing this list we used the information provided by Viapath red cell gene panel (196 genes), http://www.viapath.co.uk/our-tests/red-cell-gene-panel, and the bone marrow failure syndrome panel (128 genes) of blueprint genetics (https://blueprintgenetics.com/tests/panels/hematology/anemia-panel/. We also tested our data for recently published genes/variants in the field of bone marrow failure syndromes, Diamond Blackfan Anemia and Congenital Dyserythropoietic anemia (References 1,3 and 8 in the main text).

Variants identified, Bioinformatic algorithms, Population and disease related databases that were used for variant analysis are presented in Table 1. These included a human genome variation database of eight major ethnic groups that live in Iran and neighbouring countries in the Middle East, Iranome. The American College of Medical Genetics (ACMG) guidelines were used to predict the pathogenicity of the variants.
Sanger sequencing

The PCR primers for Sanger sequencing (Supplementary Table 3) were designed by primer 3 or manually to amplify the variant regions by Polymerase Chain Reaction (PCR). Chromatograms relating to each family are presented in Supplementary Figures 1-5.

Ethical Considerations

This research was conducted based on the guidelines of ethical committee of the University of Social Welfare and Rehabilitation Sciences, Tehran, Iran, in accordance with Helsinki declaration.

Supplementary Reference

1. Akbari MR, Fattahi Z, Beheshtian M, et al., A human genome variation database of eight major ethnic groups that live in Iran and neighbouring countries in the Middle East. ASHG Annual Meeting, October 2017, Orlando-USA.

<table>
<thead>
<tr>
<th>Family</th>
<th>WBC (10^3/µl)</th>
<th>RBC (10^6/µl)</th>
<th>HGB (g/dL)</th>
<th>HCT (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>PLT (10^3/µL)</th>
<th>RBC morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>8.2</td>
<td>3.79</td>
<td>9.70</td>
<td>29.7</td>
<td>78.4</td>
<td>25.6</td>
<td>52.7</td>
<td>14</td>
<td>Hypochromia, Anisocytosis</td>
</tr>
<tr>
<td>II</td>
<td>7.23</td>
<td>2.19</td>
<td>6.1</td>
<td>17.6</td>
<td>80.2</td>
<td>27.7</td>
<td>34.5</td>
<td>400</td>
<td>Normal</td>
</tr>
<tr>
<td>III</td>
<td>2.4</td>
<td>2.29</td>
<td>6.0</td>
<td>19.0</td>
<td>83.0</td>
<td>26.2</td>
<td>31.6</td>
<td>235</td>
<td>Anisocytosis</td>
</tr>
<tr>
<td>IV</td>
<td>11.3</td>
<td>3.39</td>
<td>9.6</td>
<td>27.9</td>
<td>82.3</td>
<td>28.3</td>
<td>34.4</td>
<td>490</td>
<td>Hypochromia, Anisocytosis, Poikilocytosis, Microcytosis</td>
</tr>
</tbody>
</table>

Bone marrow Reports

I Bone marrow aspirate and trephine biopsy: hypocellular marrow (60-65% cellularity) with usual composition of haematopoietic elements

II Bone marrow aspirate and trephine biopsy: hypocellular marrow (40-45% cellularity) with usual composition of haematopoietic elements

III Bone marrow aspirate and trephine biopsy: hypercellular marrow (95% cellularity), Increased megakaryocytes

IV Not performed

Other genetic investigations prior to NGS

I Alpha and beta globin genotyping: Normal
Chromosomal breakage analysis for Fanconi Anemia: Normal

II Alpha and beta globin genotyping: IVSI-110 mutation in beta globin gene in the proband and his mother

III Chromosomal breakage analysis for Fanconi Anemia: Normal
Copy number analysis (BCR-ABL to ABL): Normal
V617F mutation in JAK2 gene: Ruled out

IV Alpha and beta globin genotyping: IVSI-5 mutation in beta globin gene in the proband and her mother

Supplementary Table 1. Clinical and haematological information collected prior to this study.
### Supplementary Table 2. A set of 281 genes related to red blood cell disorders and bone marrow failure syndromes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRPL</td>
<td>CGACGAGGTACTGTCACCTTT</td>
<td>TACCAAGTTTCCAGGTTCCA</td>
</tr>
<tr>
<td>RUNX1</td>
<td>AAATTCCGAGGTGTTGTCA</td>
<td>GCAAAGTTTGGCTTTACGG</td>
</tr>
<tr>
<td>RPS26</td>
<td>ACAGTTTTCCATCCTGTCG</td>
<td>CACTCTGCCCAGAAAAGAAT</td>
</tr>
<tr>
<td>ADA2</td>
<td>GGGGAGGGGATTTAAGCACG</td>
<td>GCAGGTGTTGTCCTGAGACACT</td>
</tr>
</tbody>
</table>

### Supplementary Table 3. Primer sequences of the variants

### Supplementary figure legends

**Supplementary figure 1.** Chromatograms for *RPL5* variant in family I. P: proband, M: mother, F: father, S: sister, B: brother.

**Supplementary figure 2.** Chromatograms for *RUNX1* variant in family I.
Supplementary figure 3. Chromatograms for RPS26 variant in family II.

Supplementary figure 4. Chromatograms for ADA2 variant in family III. (P.A: probably affected).

Supplementary figure 5. Chromatograms for CDAN1 variant in family IV.
Supplementary figure 2
Supplementary figure 3
Supplementary figure 4
Supplementary figure 5