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Description of a rare β-globin gene mutation -28 (A>C) (HBB: c.-78A>C) in homozygosity state observed in a Syrian family: A case report

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Abstract

Background: β-Thalassemia (β-thal) is one of the most common inherited disorder characterized by a reduction (β+) or complete absence (β0) of β-globin chain synthesis. There are mutations affect transcriptional efficiency in 5′-flanking region of the gene, such as a modified TATA box to the beta globin gene.

Case presentation: We report a β-thal affected proband who had substitution mutation [-28 (A>C) beta+] (*HBB*:c.-78A>C) on the promoter of β-globin gene.

Conclusion: This mutation in homozygosity state was found in Syria for the first time, which leads to Beta-Thalassemia Major (β-TM) phenotype. It was associated with the elevation range of HbF and high HbA2 levels, with low red blood cell indices.

Keywords

-28 (A>C) mutation; Syria

Abbreviations

β-thal: β-Thalassemia; β-TM: Beta-Thalassemia Major.

Introduction

β-Thalassemia (β-thal) is one of the most common inherited disorder characterized by a reduction (β^+) or complete absence (β^0) of β-globin chain synthesis [1]. Molecular characterization of β-thal is substantial for the prevention of the disease in the society [2]. HbVar is one of the oldest and most estimated locus-specific databases, reported updates to >600 HbVar entries [3]. Most of the mutations are single

nucleotide substitutions or deletions, or insertions in the β-globin gene or its flanking sequences [4]. There are mutations affect transcriptional efficiency in 5′ -flanking region of the gene, such as a modified TATA box (ATAAAA to ATACAA) 5′ to the beta globin gene which previously reported in a Kurdish Jew depends on blood transfusion with homozygous genotype of β thalassemia [5]. In Syrian population, till date, the number of common detected mutations was 38 mutations [6]; additionally to rare mutations which identified also.

Here, we report a proband with β-thal caused by substitution mutation [-28 (A>C) beta+] (*HBB:*c.-78A>C) on the β-globin gene promoter. To the best of what we know, this is the first report which described the [-28 (A>C) / -28 (A>C)] genotype in Syrian family.

Case Presentation

A 26-year-old girl presented with clinical features resembling homozygous β^{+}-thal requiring regular blood transfusions since she was 4 years old. She has one sister and one brother, both are requiring regular blood transfusions since the sister was 4 years old and the brother was 2.5 years old, their parents are consanguineous. They originated from Aleppo province in north region of Syria.

Capillary Electrophoresis (CE) was performed on the blood of proband before blood transfusion. Furthermore, 2.5 mL of blood was drawn from each family member. Complete Blood Count (CBC) and (Sequencing) were done for each family member. Relevant clinical history of the patient and the family members were recorded.

Genomic DNA was isolated from peripheral blood from the patients using the QIAamp DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Purified DNA was run on a 1.0% agarose gel. The quality and quantity of the DNA was determined spectrophotometrically (NanoVue™; GE HealthCare, Freiburg, Germany).

Direct DNA sequencing of the whole human HBB gene was carried out on an ABI PRISM 310-DNA Analyzer (Applied Biosystem, CA, USA) as previously reported [6,7]. Specific primers [F: 5'-GAA GTC CAA CTC CTA AGC CAG TGC C-3', R: 5'-CGA TCC TGA GAC TTC CAC ACT GAT GC-3'] were used for the first reaction to amplify 784 bp of the 5' untranslated region (5'UTR), exon 1, intron 1, exon 2 and intron 2. Primers [F: 5'-CAA TGT ATC ATG CCT CTT TGC ACC-3', R: 5'-ATG CAG GAT AAG CAA ATG GGT AGT G-3'] were used for the second reaction to amplify 845 bp of the intron 2 region neighboring exon 3, exon 3 and of the 3' untranslated region (3'UTR) [8].

Detection of Xmn-I locus was performed with polymerase chain reaction–restriction fragment length (PCR-RFLP) technique with specific primers and restriction enzyme Xmn-I [9]. Reverse hybridization assay (α-Globin StripAssay® 4–160; ViennaLab Diagnostics Gmb Vienna, Austria) which covers 21 of α-thal mutations was used according to the manufacturer's instructions.

Sequencing was carried out using BigDye® Terminator (version 3.1) sequencing kit (Applied Biosystems, Foster City, CA, USA) and genetic analyzer data collection software (ABI 310 DNA Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) as previously reported [7].

The proband, her sister and brother are on a regular transfusion regimen at an average of 20-day intervals. At the age of 48 months, they presented with nausea, pallor, and reduced appetite. The Hb levels were 7.4, 8.7, 9.3 g/dL, respectively. The mean corpuscular volume (MCV) values were 73.1, 80.8, 77.3 fL, respectively. And the mean corpuscular hemoglobin (MCH) values were 24.6, 21.9, 24pg, respectively. They had a Spleenectomy at age 8 for proband and at age 2 for her sister, proband's brother has no Spleenectomy yet.

Molecular and hematological data for the family members are described in the Table 1.

The chromatogram of the proband revealed the presence of [-28 (A>C) beta+] (*HBB:*c.-78A>C) mutation, in homozygcity, resulting in a β^{+}/β^{+}- thalassemia Major phenotype.

By examining the chromatogram of her parents, it was revealed that her father and mother are heterozygotes for this mutation [-28 (A>C)]. This mutation was reported to the HbVar database. It has been accepted with HbVar ID as 768, and recorded with HGVS nomenclature as *HBB*: c.-78A>C. The chromatogram of the proband is shown in Figure 1. On the other hand, the results of the α-thal test for the parents, proband's brother and sister revealed that none of the 21 common mutations covered by the kit were present. The result of PCR/RFLP indicates that, in our case, the *Xmn*I polymorphism at –158 to the $^{G}\gamma$-globin gene was absent (*Xmn*I [–/–]) for the proband and her family also.

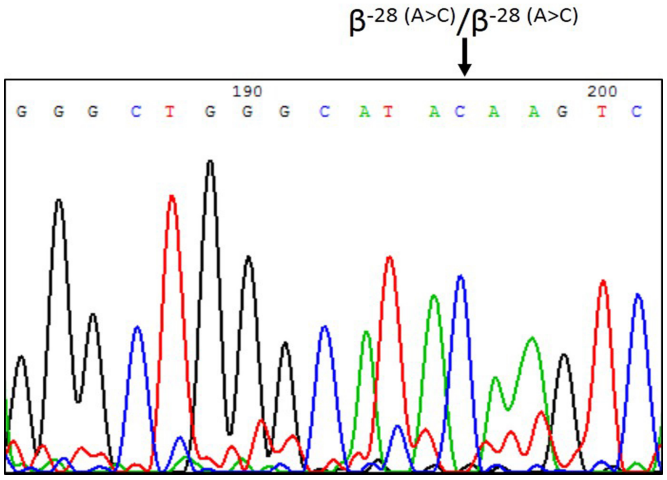

Figure 1: The chromatogram of the proband. Analysis of direct sequencing show the PCR fragment on the β-globin gene, the arrow indicates the A>C substitution at position -28 in the proband.

Table 1: The Hematological and Molecular Data of the Studied Family Members.

Parameters	Father	Mother	Propand's sister	Propand's brother	Propand
Sex-age (years)	M-56	F-48	F-17	M-8	F-26
Hb (g/dL)	13.7	11.2	8.7	9.3	7.4
RBC (10^{6}/μ L)	6.35	5.54	3.54	3.88	3.40
MCV (fL)	69.9	66.4	80.8	77.3	73.1
MCH (pg)	21.6	20.2	24.6	24	21.9
MCHC (g/dL)	30.9	30.4	30.4	31	29.9

Hb A1 (%)	92.1	92.4	30	62.1	51.8
Hb A2 (%)	5.9	5.6	5	4.5	8.9
Hb F (%)	2	2	65	33.4	39.3
α Genotype	αα/αα	αα/αα	αα/αα	αα/αα	αα/αα
β Genotype	$\beta^{A}/\beta^{-28\ (A>C)}$	$\beta^{A}/\beta^{-28\ (A>C)}$	$\beta^{-28\ (A>C)}/\beta^{-28\ (A>C)}$	$\beta^{-28\ (A>C)}/\beta^{-28\ (A>C)}$	$\beta^{-28\ (A>C)}/\beta^{-28\ (A>C)}$

Discussion

This [-28 (A>C)] mutation was inherited maternally and paternally. The −28 A>C mutation had been barely described in the scientific literature. The first report which described this allele published in the year 1982, it was about two Kurdish Jews patients with β-TM (Beta Thalassemia Major) were studied, that affects the TATA box in the HBB gene promoter which reducing the amounts of mRNA [5]. The next paper determined the genotype of the patients of same group, which were compound heterozygotes for this mutation of the TATA box [10]. Then in 1992, Basak et al. [11] studied the mutations in the HBB gene in Turkish patients suffering from β-TI (Beta Thalassemia Intermedia) and BTM. In these patients the −28 A>C mutation was rare but the authors did not establish the genotype (homozygous or compound heterozygous) or the phenotype linked with the mutation. Then in 1996, Perea et al. [12] described a Mexican family with β-TM caused by −28 A>C mutation and a CD11 –T frame shift compound heterozygosis. In the year of 2005, two reports about molecular epidemiology were published about the Middle East populations where the−28 A>C mutation seems to be a rare mutation. Adekile et al. [13] described Iraqi patient who had a compound heterozygote for the −28 A>C and the IVS-II-1 G>A mutations. This patient showed a β-TI phenotype. The mild clinical appearance was linked to a−158 C>T polymorphism of the γ-globin gene promoter with elevation in HbF together with α-gene deletion. In the same year, Darwish et al. [14] report one patient with −28 A>C mutation in homozygote type out of 148 beta thalassemia patients from Palestine. Unfortunately, the phenotype of that patient was not mentioned. It is known that Turkey and Iraq have a considerable percentage of Kurdish population.

In 2009, Gamarra et al. [15] reported a 21-year-Old Spanish male with β-thal major due to compound heterozygosis for Cd 39 C>T/-28 A>C. This allele has not been observed in any other Latin American populations.

Conclusion

In this study, the [−28 A>C] mutation was observed for the first time in a Syrian family and it leads to β-TM phenotype in homozygous genotype, and it was associated with the elevation range of HbF between 33 to 65 g/dL and high HbA_2 levels also with low red blood cell indices for the proband and her brother and sister. This mutation in this region may be resulted from old migrations in Syria. This requires further corroboration with larger sample size.

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