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Identification of a rare synonymous beta globin variant, *HBB*: c.60C>T in an Afghan Family as a benign variant

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ABSTRACT

Beta thalassemia is a common autosomal recessive disorder. In this study, we report a rare beta globin gene variant, *HBB*: c.60C>T, identified in an Afghan Family.

Sequencing of a 30 year old pregnant woman and her children showed that this synonymous variant, when present alongside other pathogenic HBB mutations, does not affect beta globin production. In the proband, hematological findings were not consistent with a beta thalassemia minor phenotype. Although this variant has been reported in Clinvar as a variant of uncertain significance (VUS), our findings support its classification as likely benign.

1. Introduction

Thalassemia is a kind of hereditary genetic disorder that is caused by deletions or mutations in globin genes and result in impaired globin gene synthesis (α and β chains) (Chauhan et al., 2023).

Beta thalassemia is one of the important sub-classification of thalassemia and is a common cause of microcytosis and /or hypochromatosis as a result of defective βeta goblin chain production (Santos et al., 2024). It has a high prevalence among Asian, Indian, Middle Eastern and Mediterranean populations (Wang et al., 2024). Many different mutations in the beta globin gene have been reported so far (Duzkale et al., 2013). However new rare variants are still reported (Wang et al., 2024). Here we describe a beta globin synonymous mutation *HBB*: c.60C>T in an Afghan Family as a benign variant on the basis of hematological indices in three patients in one family. This variant has been reported uncertain significance in one clinical testing due to the possible effect on splicing based on Clinvar (ClinVar. [VCV001083541.9], 2025).

2. Case report

A 30-year-old pregnant woman originating from Afghanistan (proband) was referred to the AL-Zahra Medical genetic laboratory, Isfahan University of medical sciences, for beta globin gene mutation screening to assess her carrier status and the couple's reproductive risk (prenatal diagnosis). Hematological analysis showed mild microcytic (MCV=77.4 fl), hypochromic (MCH=24.9 pg) anemia with borderline in hemoglobin level (11.8 g/dl), normal MCHC (32.3 g/dl), normal RBC count ($4.73x10^6$ µl) and a normal HbA2 level (2.6%). Based on proband's hematological profile and normal HbA2 level, she was not considered a carrier for Beta thalassemia and the mild decrease in MCV, MCH and hemoglobin was due to her pregnancy and the presence of one 3.7 kb deletion in *HBA2* gene.

Her husband was identified as a beta thalassemia carrier based on hematological indices and an elevated HbA2 level (5.5%).

Initially ARMs-PCR was performed to identify the underlying beta thalassemia mutation in proband's husband. The gel electrophoresis result clearly indicate that the husband is heterozygous for the IVSI-5 mutation, confirming the molecular basis of his beta thalassemia phenotype (Supplementary Fig. 1).

Based on screening program subsequently Sanger sequencing was carried out in the proband to confirm the absence of any pathogenic mutations that might contribute to a severe Beta thalassemia phenotype in future off spring. Sanger sequencing analysis revealed that the proband was heterozygote for *HBB*: c.60C>T (Supplementary Fig. 3).

To further characterize the presence of *HBB*: c.60C>T variant in proband's husband and confirm the ARMs-PCR findings, Sanger sequencing also was conducted in her husband (Supplementary Fig. 2).

Abbreviations: HBB, Hemoglobin Subunit Beta; HBA2, Hemoglobin Subunit Alpha 2; Hb, Hemoglobin; MCV, Mean corpuscular volume; MCH, Mean corpuscular hemoglobin; RFLP, Restriction fragment length polymorphism; CADD, Combined annotation-dependent depletion; DANN, Defense Associations National Network.

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Sequencing result confirmed the presence of IVSI-5 mutation in heterozygous state and absence of *HBB*: c.60C>T variant in the husband.

Since the presence of *HBB*: c.60C>T variant in the proband was not associated with any clinical or hematological features of Beta thalassemia minor, we hypothesized that this variant might be benign.

To determine the benign nature of *HBB*: c.60C>T variant and segregation of *HBB*: c.60C>T and IVSI-5 mutation to find Cis or Trans configuration of these two variants, Sanger sequencing was also performed on her three children, which revealed that C1 and C2 had inherited the IVSI-5 variant from the father and the *HBB*: c.60C>T variant from the mother, confirming their Trans configuration (SupplementaryFig. 4). Clinical evaluation and hematological indices of Two children (C1 and C2) demonstrated that, despite being compound heterozygotes for IVS-I-5 and *HBB*: c.60C>T, they exhibited a beta thalassemia minor phenotype without signs suggestive of Beta thalassemia intermedia or major. Sequencing data also revealed that C3 was normal for IVSI-5 mutation and *HBB*: c.60C>T variant, (Supplementary Fig. 5), this data were consistent with his normal hematologic parameter. (Hematological data of three children shown in Table 1.)

Taken together, although ClinVar has suggested that this variant affect splicing, the patient carries it in a heterozygous state with or without other pathogenic mutation in *HBB* gene shows thalassemia minor or normal phenotype respectively. These clinical features suggest that this variant may not significantly affect normal splicing, although functional validation is needed.

3. Discussion

Beta thalassemia is a heterogeneous autosomal recessive Mendelian disorder with more than 350 mutations have been reported up-to date (Taher et al., 2021; Rao et al., 2024).

Here, we demonstrate a new rare variant *HBB*: c.60C>T which is segregated in an Afghan family with one clinically unaffected individual. There are three clinical testing reports in Clinvar regarding the pathogenicity of this variant. One report in Clinvar classifies this variant as uncertain significance, mainly due to its low frequency and potential impact on splicing, suggesting a possible cryptic splice site gain.

One of the main challenges in variant classification is the uncertainty regarding its pathogenicity. Several approaches, including bioinformatics tools, insilico predictions, amino acid substitution effects, and segregation analysis in family are used to assess the clinical classification of variants (ClinVar.[VCV001083541.9], 2025).

Protein sequence analysis demonstrated that this change does not alter the amino acid sequence, and this variant is classified as a synonymous change. Bioinformatics tools and In-silico predictors like (Mutation Taster, CADD, and DANN) predicted this variant to be benign or likely benign. (Varsome. [Variant NM_000518.5 (HBB: c.60C>T]. Available at https://varsome.com/variant/hg19/HBB%3Ac.60C%3ET?annotation-mode=germline. Accessed originally on Jan 2025; information may have been updated since.) However despite its synonymous nature, Clinvar suggests it may potentially affect splicing. Therefore, segregation analysis is required. Segregation studies in this family demonstrated that the *HBB*: c.60C>T nucleotide change is not associated with any Beta thalassemia minor phenotype when present alone. Furthermore our findings that is extensively described in the result section, suggest that when this variant is inherited with other pathogenic mutation does not lead to any severe phenotype like Beta thalassemia major and thalassemia intermedia. Based on these findings, there is a clear genotype-phenotype correlation suggesting that *HBB*: c.60C>T variant, even when inherited with other pathogenic mutation (in this study it is inherited in Trans with IVSI-5 in two siblings), is likely benign.

According to the ACMG/AMP guidelines for variant interpretation, this variant can be classified as likely benign, based on the following criteria:

BP4 (multiple in-silico tools predicted no impact on the gene or protein function.), BS2 (the variant was observed in healthy individual without any relevant clinical symptoms, while the phenotype would be expected to manifest if the variant were pathogenic.), BP2 (the variant was observed in Trans configuration with a known pathogenic variant (IVSI-5) in two siblings, both showing a mild beta thalassemia minor phenotype.)

Together, these lines of evidence support the classification of the *HBB*: c.60C>T variant as likely benign.

To our knowledge, this is the first report of this variant in the published literature, including the results of molecular analysis in population.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' information

Not applicable.

CRediT authorship contribution statement

Zahra Taherian-Esfahani: Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Conceptualization. **Hamideh Namazi:** Visualization, Data curation.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study did not require approval from an institutional ethics committee. However, written informed consent was obtained from all patients prior to inclusion in the study.

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Table 1
Hematological indices of three children.

	MCV fl	MCH pg	MCHC g/dl	Hbg/dl	HbA HbA2	Hb F	RBCx10^6	age
C1	62	18.7	30.2	11.2	92.8 6.3	0.9	5.98	12
C2	61.4	19.5	31.8	10.5	90.1 5.8	4.1	5.40	5
C3*	81.9	29.0	35.4	13.8	97 3	-	4.76	10

* Third child was normal for IVS-I-5 and *HBB*: c.60C>T variant.

Declaration of competing interest

The authors declare that they have no competing (conflicts) interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humgen.2025.201481.

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