

Hemoglobin D/ β -thalassemia and β -thalassemia major in a Saudi family

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ABSTRACT

The present report describes the clinical, hematological and molecular characteristics in a family with unique interaction between 3 different mutations discovered during routine workup for bone marrow transplantation. In this report, complete hematological and molecular studies were performed for a large Saudi family. The family consisted of parents and 9 children, which revealed that the father is compound heterozygous for hemoglobin (Hb) D Punjab/ β -thalassemia, the mother is a carrier for β -thalassemia and 3 of their children are transfusion dependent β -thalassemia. Two of the children are compound heterozygous for Hb D Punjab/ β -thalassemia like the father but with different genotype. The other 2 children have Hb D Punjab traits while 2 other children have β -thalassemia traits. Although, compound heterozygous for Hb D/ β -thalassemia has been well described in the literature, our report emphasizes the importance of careful analysis of the electrophoresis results and the usefulness of molecular studies in premarital screening and other screening hemoglobinopathy programs.

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Thalassemia syndromes usually result from molecular defects (mutations) that either reduce or completely abolish the synthesis of one or more of the polypeptide chains of the hemoglobin (Hb) molecule. β -thalassemia major is usually a serious disease that results from inheritance of one mutation from both parents or inheritance of a different mutation from each parent (compound heterozygous). Considerable variations in the severity of clinical picture result from the type of β -chain variant, the β -thalassemia mutation and the residual amount of β -chain. Patients with complete absence of β -chains (β^0) usually have more severe clinical picture than patients with some residual β -chain (β^+). Interaction between β -thalassemia and other hemoglobinopathy can result in a spectrum of disease severity.¹ Hemoglobin-D Punjab (β 121) glutamic acid (Glu) \rightarrow glutamine (Gln) was the

third abnormal human hemoglobin to be discovered.² Hemoglobin D-Los Angeles (D-Punjab) is primarily found in Northern India although it was first described in a family of mixed English-Irish and American-Indian ancestry.³ Subsequently, it has been reported in several other ethnic groups including Arabs.^{4,7} Several variants of Hb D have been reported including Hb D *iran* (β 22), Hb D *ouled Rabah* (β 19) and Hb D *Isadan* (β 87). Hemoglobin-D had been reported in 4 forms; heterozygous (Hb D trait), homozygous Hb D and in combination with β -thalassemia and sickle hemoglobin (Hb S).^{5,6} Co-inheritance of Hb D and β -thalassemia had been reported previously in a Saudi family.⁷ Patients with Hb S-D disease are clinically similar to sickle cell anemia but less severe than the homozygous sickle cell anemia. The co-existence of Hb D-Punjab and β -thalassemia

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both (δ) and (θ) is a rare phenomenon, however, they had been reported from different world regions.^{5,8} The condition is fairly mild and usually no more severe than -thalassemia minor. Hereby, we are reporting co-existence of Hb D Punjab and -thalassemia that when inherited in association with another -thalassemia mutation resulted in 3 children of the same family with -thalassemia requiring frequent blood transfusion.

Case Report. We report an 11-year-old, Saudi male (II-4) from the Western province of Saudi Arabia who is completely healthy with no previous history of blood transfusion, medical and surgical illness. He was seen in pre-bone marrow transplantation (BMT) clinic for evaluation as a possible donor for his siblings who were diagnosed transfusion dependent -Thalassemia. The clinical examination did not reveal significant findings. His hematological work up is summarized in (Figure 1). The stained blood film revealed mild anisopoikilocytosis with microcytic hypochromic anemia and normal white blood cells and platelet counts. Hemoglobin electrophoresis with separation and quantitation of various Hbs was carried out by -Thalassemia short program of high performance liquid chromatography (HPLC) from (Bio-rad Variant). It showed high levels of Hb D (92.5%) with normal Hb A2 (3.3%) and absence of Hb A1. Sickle solubility test was negative, and serum ferritin was within normal range. Family studies of both parents and the other 8 children are also summarized in (Figure 1). This showed that red blood cell indices and Hb electrophoresis of the mother (I-2) and 2 of the children (II-1), (II-7) were compatible with -thalassemia trait. The father (I-1) and one of the children (II-8) had mild anisopoikilocytosis with hypochromic (91.2%) and microcytic anemia (89.6%) with a high level of Hb D and high Hb A2 with absence of Hb A1. These findings were suggestive of compound heterozygous Hb D and -thalassemia trait. However, homozygous Hb D was not completely ruled out. The father and these 2 children had moderate reticulocytosis. The hematological studies of the twin sisters (II-6a), (II-6b) were consistent with Hb D trait. The laboratory studies of the other 3 children who have a clinical picture of -thalassemia major could not be interpreted with accuracy due to the frequent blood transfusion.

Molecular studies by -globin gene amplification and sequencing were carried out for all members of the family to confirm the diagnosis and define the genotype. Three mutations were found in the family in addition to a polymorphic base pair. The first mutation is a single nucleotide substitution in the codon 30 (AGG-ACG), this mutation is affecting

this codon and the donor site of intron 1(IVS-1) resulting in a transcription of 10 codon in the intron 1 and a termination afterward. The second mutation is a 4 base pair deletion (TTCT) affecting the entire codon 41 and one nucleotide of the codon 42 leading to a frame shift and a termination after 18 codons. The third mutation is affecting the codon 121, which is known as Hb D-Punjab mutation.

The results depicted in (Figure 1) showed that the father (I-1) is compound heterozygous for Hb D Punjab (codon 121 GAA-CAA) and -thalassemia allele (codon 41/42 TTCT) while the mother (I-2) is a carrier for -thalassemia allele (codon 30 AGG-ACG) and has a polymorphic site in the 3' un-translated region of the transcript. The index case (II-4) and his brother (II-8) are compound heterozygous for Hb D Punjab and -thalassemia allele (codon 30 AGG-ACG). One sister (II-1) and a brother (II-7) are carriers for -thalassemia allele (codon 41/42 TTCT). The twin sisters (II-6a and II-6b) are carriers for Hb D Punjab. The 3 siblings with the clinical picture of -thalassemia major (II-2, II-3 and II-5) are compound heterozygous for the -thalassemia mutations (codon 30 AGG-ACG) and (codon 41/42 TTCT).

Discussion. This case represents a unique co-existence of 3 different -globin gene mutations in one family. Although the co-existence of Hb D and -thalassemia is a rare event, it had been reported from various regions of the world with more prevalence in India and other Asian populations.⁶ In this report, we confirmed by molecular studies that the interaction between compound heterozygous conditions and -thalassemia carrier resulted in a severe form of -thalassemia major in the 3 children from the same family. Also, we confirm what had been reported before of the benign nature of the compound heterozygous of Hb D Punjab/ -thalassemia. The initial Hb electrophoresis of the index case that showed significant elevation of Hb D Punjab did not explain the clinical picture in the diseased siblings. So, further analysis of the phenotype in the rest of the family members revealed that the father and one of the offspring have identical hematological picture of the index case while the mother and 2 of the offspring have -thalassemia trait. These findings had raised the possibility that the index case is a compound heterozygous for Hb D and -thalassemia. To confirm the presence of -thalassemia trait in the index case depending only on electrophoresis was inadequate for 2 reasons: it is known that Hb A2 measurement by HPLC is affected by contamination with small amounts of Hb D.⁹ Secondly, it is known that some types of

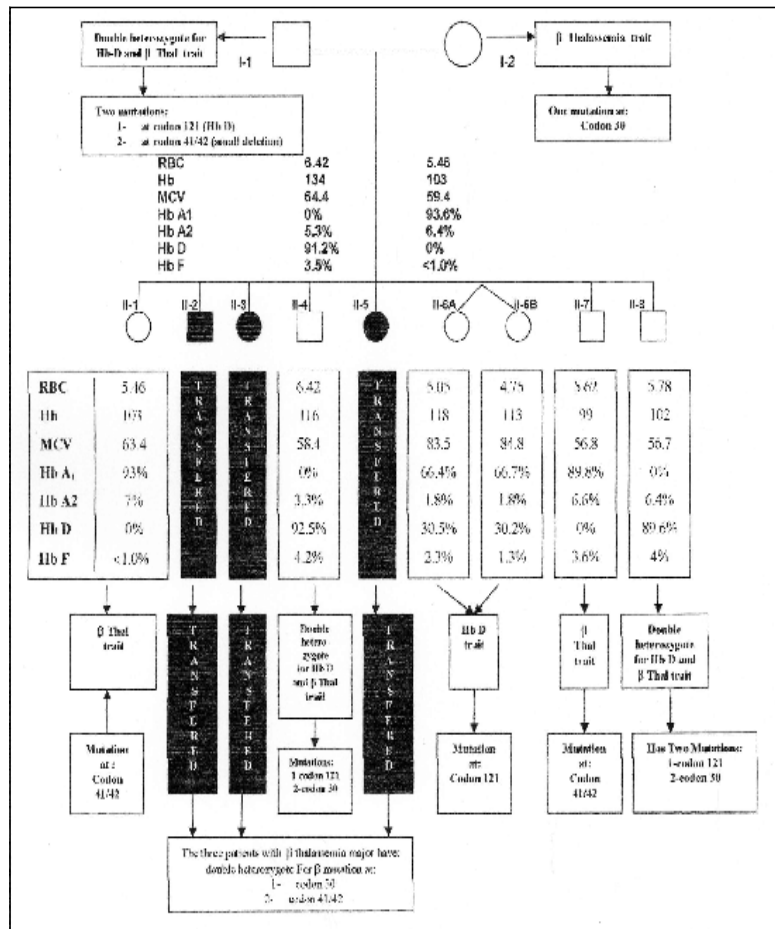


Figure 1 - Pedigree of the family with the hematological data and genotype. RBC - red blood cells, Hb - hemoglobin, MCV - mean corpuscular volume.

-thalassemia have normal Hb A2 levels.¹ The molecular analyses of the family revealed 2 mutations in addition to 121 (Hb D Punjab). Different mutations had been reported at codon 30. ° Thalassemia resulting from mutation in the Codon 30 (AGG-ACG) being reported previously in a Jewish family then in a Mauritian family and other ethnic groups.^{1,10} It is characterized in heterozygous by mild anemia with microcytosis and hypochromasia and normal Hb A2 (method of quantitation was not mentioned). The other mutation in this family involves 4 base pair deletions of the entire codon 41 and one nucleotide of codon 42 which had been reported in Chinese and Indians.¹⁰ It is characterized by mild microcytic, hypochromic anemia, with elevated Hb A2 and Hb F and absence of Hb A1. The amount of Hb F varies among the reported cases of Hb D/ -thalassemia. Where Hynes and Lehmann¹¹ reported a level can reach up to 4.2%, Huisman et al⁹ reported in his series levels varying between 1-7.5%. In our case, the level of Hb F was high in all cases with Hb D allele but more with Hb D/ -thalassemia than with Hb D trait. Hemoglobin A1 was absent in all the 3 cases of Hb D/ -thalassemia which confirms previous reports that both mutations (codon 30 AGG-ACG) and (codon 41/42 TTCT) can result in ° thalassemia.⁹

Our report presents an unusual interaction between 3 different gene mutations that if not detected early during the screening program can result in clinically severe cases. This report also demonstrated the possible impact of molecular studies on premarital hemoglobinopathy screening programs.

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