



Case Report

Premarital Counseling on the Alpha Thalassemia Allele *HBA2*:c.*94A>G

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Abstract: The mutation *HBA2*:c.*94A>G (AATAAA>AATAAG; rs63751269) is a 3'-UTR (3 prime untranslated region) single-nucleotide substitution in the polyadenylation (PA) signal of *HBA2* ($\alpha^{PA:A \rightarrow G}$). This pathogenic (CADD score, 14.92) variant is sporadic in the Arabian Peninsula. It results in inefficient mRNA processing, transcription termination, and possibly using an alternate cryptic downstream polyadenylation signal. As a result, the allele α^T (or α^T -Saudi) poses challenges in premarital counseling with respect to fetal risk of hemoglobin H disease. Homozygous *HBA2*:c.*94A>G ($\alpha^T \alpha / \alpha^T \alpha$) results in moderate-to-severe microcytosis (mean red cell volume, MCV, 55 to 65 fL), reflecting markedly impaired hemoglobin synthesis (hemoglobin H disease). Homozygous rightward $-\alpha^{3.7}$ (a 3804-neocleotide deletion allele, NM_000517.4:c.[−2_−3delAC; $-\alpha^{3.7}$]), on the other hand, results in mild microcytosis (MCV, 70 to 75 fL, alpha-thalassemia trait). Thus, *HBA2*:c.*94A>G is more damaging than $-\alpha^{3.7}$. Consistently, the value of MCV in compound heterozygosity, *HBA2*:c.*94A>G and $-\alpha^{3.7}$, is 65 to 70 fL. We report here a healthy couple who presented for premarital counseling on their hemoglobinopathy. The man has homozygous *HBA2*:c.*94A>G ($\alpha^T \alpha / \alpha^T \alpha$), and the woman has compound heterozygous ($-\alpha^{3.7} / \alpha^T \alpha$, also annotated as: $-\alpha^{3.7} \alpha / \alpha^T \alpha$). As a result, the genotype of their offspring would be that of the father ($\alpha^T \alpha / \alpha^T \alpha$) or the mother ($-\alpha^{3.7} / \alpha^T \alpha$). The counseling was mainly based on the benign phenotypes of the parents. As both were asymptomatic and their anemia was clinically insignificant, they proceeded with the marriage.



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1. Introduction

Alpha thalassemia (MIM#604131) is an inherited blood condition in which one or more of the *HBA2* (hemoglobin-alpha locus 2, MIM#141850) or *HBA1* (hemoglobin-alpha locus 1, MIM#141800) genes are defective. The normal genotype consists of two copies of *HBA2* and two copies of *HBA1*, (one copy each on the two homologous chromosome 16). *HBA2* and *HBA1* share the same coding sequence but differ in the introns as well as the 5' and 3' UTR, thereby affecting gene expression [1]. For example, *HBA2* encodes 2–3 times more protein than *HBA1*.

The four forms of alpha thalassemia are as follows. (1) The silent carrier (three functional genes), causing a slightly low MCV (75 fL to 80 fL). (2) The alpha thalassemia trait (two functional genes), causing a more pronounced microcytosis (MCV, 70 ± 5 fL). When *HBA2* and *HBA1* on a single chromosome are deleted (or inactivated) the designation is cis deletion (α^0 or null allele). This form is prevalent in Southeast Asia and the Mediterranean population, and carries fetal risk for severe alpha-thalassemia. When *HBA2* or *HBA1* on a single chromosome is deleted, the designation is trans deletion (α^+). There are two types of deletional alpha-thalassemia. The 3.7 kb deletion (rightward type; $-\alpha^{3.7}$; HBVAR#1076) is found in the Arabian Peninsula, Africa, and the Mediterranean region (illustrated in

Figure 1B). The 4.2 kb deletion (leftward type, $-\alpha^{4.2}$) is found in Southeast Asia and Pacific Islands. (3) Hemoglobin H disease (one functional gene, e.g., offspring of one parent with α^0 thalassemia trait and one with silent carrier). Importantly, the unpaired β -chain aggregates, known as hemoglobin H (not to be confused with ‘hemoglobin H disease’), are more soluble than the unpaired α -chain aggregates found in β -thalassemia. Thus, individuals with hemoglobin H disease have moderate rather than severe anemia. (4) Hemoglobin Bart’s hydrops fetalis syndrome, i.e., four defective genes (e.g., in the offspring of parents with the α^0 thalassemia trait), is incompatible with life [2].

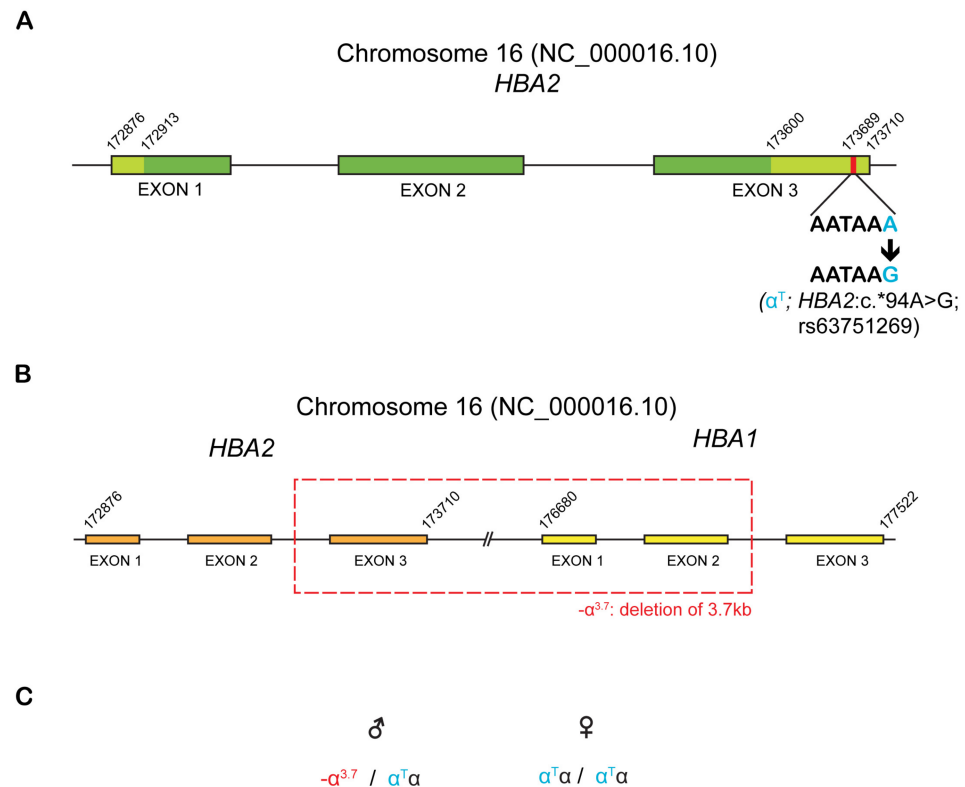


Figure 1. (A) Human *HBA2* gene located on chromosome 16 (NCBI RefSeq Accession: NC_000016.10) between 172876–173710. The three exons of *HBA2* are shown as green blocks. Light green in exon 1 represents 5' UTR, and in exon 3, it represents the 3' UTR. The polyadenylation signal (AATAAA) is located between 173689 and 173694. In the α^T variant (*HBA2*:c.*94A>G; rs63751269), this sequence is altered to AATAAG. (B) Human *HBA2* and *HBA1* genes are located on human chromosome 16 (NCBI RefSeq Accession: NC_000016.10) between 172876–173710 and 176680–177522, respectively. The three exons of *HBA2* are shown as orange blocks, while the three exons of *HBA1* are shown as yellow blocks. The $-\alpha^{3.7}$ variants involves a deletion of 3.7 kilobases as indicated by the red dashed box. (C) Genotypes of the studied couple.

The prevalence of α^+ thalassemia trait is high in the UAE. In one study, 49% of the newborns had *HBA2* or *HBA1* defects; the majority were $-\alpha^{3.7}$ [3]. In other studies, the prevalence of α^+ thalassemia trait ranged from 15% to 20% [4,5]. Thus, $-\alpha^{3.7}$ is the most common variant in the region, while the non-deletional variant *HBA2*:c.*94A>G is less frequent [6–8].

The four hemoglobin chains (α , β , γ , δ) form three normal types: A (2α with 2β , 93% to 97%), A2 (2α with 2δ , 1.5% to 3.5%), and F (2α with 2γ , 21.5% to 3.5%). Since the latter two hemoglobins do not contain β chains, their percentages increase in the beta-thalassemia trait, while A2 is normal in the alpha-thalassemia trait. Thus, accurate determination of A2 is crucial.

Premarital screening and counseling programs are well established in the UAE. They mainly aim to prevent beta-thalassemia major and sickle cell disease. Less frequently, α^0 thalassemia and *HBA2*:c.*94A>G also produce challenges with respect to assessing fetal risk. There is no fetal risk with respect to α^+ thalassemia, which is most common. We present here a healthy couple with moderate-to-severe microcytosis associated with alpha-thalassemia. They came for counseling on fetal risk of hemoglobin H disease.

2. Case

A healthy couple was seen for premarital counseling on their hemoglobinopathy. The results of their investigation are shown in Table 1. They were asymptomatic and learned about their anemia only during premarital screening.

Table 1. Characteristics of α -thalassemia in the studied couple.

	Female Proband ⁽¹⁾	Male Proband ⁽²⁾
Hemoglobin, g/L	115	115
MCV, fL	62	64
RDW, %	19.0	26.9
Ferritin, μ g/L	154	Not done
Hemoglobin A, %	97.8	76
Hemoglobin A2, %	2.2	0.8
Hemoglobin F, %	0	0
Hemoglobin H, %	0	23.2
Genotype *	$-\alpha^{3.7}/\alpha^T\alpha$	$\alpha^T\alpha/\alpha^T\alpha$
Interpretation	Alpha-thalassemia, compound heterozygous, deletional plus non-deletional variants	Hemoglobin H disease, homozygous non-deletional variant (<i>HBA2</i> :c.*94A>G)
Phenotype markers	MCV less than homozygous α^+ thalassemia, normal A2, and undetected hemoglobin H	MCV less than homozygous α^+ thalassemia, low A2, and high hemoglobin H

⁽¹⁾ Father’s hemoglobin was 163 g/L and MCV 72.8 fL. Mother’s hemoglobin was 128 g/L, MCV 75.8 fL, hemoglobin A = 96.8%, A2 = 2.9%, and F = 0.3%. Their predicted genotypes were $\alpha^T\alpha/\alpha\alpha$ and $-\alpha^{3.7}/\alpha\alpha$, respectively. ⁽²⁾ Father’s hemoglobin was 163 g/L and MCV 72.8 fL. Mother’s hemoglobin was 121 g/L, MCV 60.7 fL, and ferritin 121 μ g/L. Their predicted genotypes were $\alpha^T\alpha/\alpha\alpha$ and $\alpha^T\alpha/\alpha^T\alpha$ (the same as her son), respectively. * Beta globin gene sequencing was not requested.

They both had moderate-to-severe microcytosis (MCV <65 fL). The woman had mild anemia, normal serum ferritin, and normal hemoglobin analysis. MLPA (multiplex PCR assay) analysis showed heterozygous $-\alpha^{3.7}$. Sequencing analysis of *HBA2* and *HBA1* revealed heterozygous *HBA2*:c.*94A>G. Thus, her genotype was compound heterozygosity ($-\alpha^{3.7}/\alpha^T\alpha$), the common deletional variant (NM_000517.4:c.[−2_−3delAC; $-\alpha^{3.7}$], CA16602246, Variation ID: 38636, VCV000038636.3) and the less common non-deletional variant [NM_000517.6(*HBA2*):c.*94A>G; rs63751269], as shown in Table 1 and Figure 1C.

The man (non-smoker) had moderate-to-severe anemia, low hemoglobin A2, and high hemoglobin H (23.2%). The results of his MLPA showed no deletion or duplication in the α -globin gene cluster. Sequencing analysis of *HBA2* and *HBA1* revealed homozygous *HBA2*:c.*94A>G ($\alpha^T\alpha/\alpha^T\alpha$), consistent with hemoglobin H disease (Table 1 and Figure 1C). This 3′-UTR variant in the polyadenylation (PA) signal of *HBA2* ($\alpha^{PA:A\rightarrow G}$) is pathogenic (CADD score, 14.92) [9,10]. Counseling was provided.

3. Discussion

Polyadenylation, or the addition of a poly(A)-tail, is an essential post-transcriptional modification of nearly all eukaryotic messenger RNAs (mRNA) [9–11]. This step is vital for the stability of mRNA in the cytoplasm. In humans, the highly conserved sequence motif AATAAA, the polyadenylation site, ensures efficient cleavage of the primary transcript and subsequent addition of the poly(A)-tail [12]. Variations in this signal sequence are rare and are typically associated with various disorders or the use of alternate polyadenylation

sites [13]. In *HBA2*, the canonical signal sequence is encoded in the third exon of the gene, 89 bases downstream of the translation stop codon (Figure 1). The mutation *HBA2*:c.*94A>G results in a change in the transcribed mRNA from AAUAAA to AAUAAG, resulting in inefficient mRNA processing, and in some instances, the use of an alternate cryptic downstream polyadenylation signal [10], as shown in Figure 1A. This variation thus has significant impact on the levels of the mature mRNA and translated gene product—hemoglobin α protein—produced from *HBA2*. Of note, *HBA2* encodes 2–3 times more protein than *HBA1* [14], which explains the greater impact of *HBA2* on alpha-thalassemia.

The phenotypes and genetic studies were all necessary for assessing the fetal risk of ‘hemoglobin H disease’ or ‘hemoglobin Bart’s hydrops fetalis syndrome’ in the studied couple. The results showed the predicted genotypes of the offspring were either ($\alpha^T\alpha/\alpha^T\alpha$) for the father or ($-\alpha^{3.7}/\alpha^T\alpha$) for the mother. As both were healthy and their anemia was clinically insignificant, they proceeded with the marriage. The determined genotypes are also helpful for other family members [15–17].

4. Conclusions

Individuals with homozygous *HBA2*:c.*94A>G ($\alpha^T\alpha/\alpha^T\alpha$) have moderate-to-severe microcytosis (MCV, 55–65 fL), moderate anemia (hemoglobin, 20–30 g/L lower than normal), high hemoglobin H, and low hemoglobin A2. Individuals with $-\alpha^{3.7}/\alpha^T\alpha$ have moderate microcytosis (MCV, 60–65 fL), mild anemia (hemoglobin, 10–20 g/L lower than normal), and normal hemoglobin analysis. Thus, the *HBA2*:c.*94A>G allele is more damaging than the ‘ $-\alpha^{3.7}$ deletion’. It decreases the expression of *HBA2* by 75% [10]. Furthermore, the fact that MCV is much lower in $-\alpha^{3.7}/\alpha^T\alpha$ compared to homozygous α^+ thalassemia ($-\alpha^{3.7}/-\alpha^{3.7}$) suggests that the expression of *HBA1* could be affected by *HBA2*:c.*94A>G. Studies (MLPA and sequencing analysis) of *HBA2* and *HBA1* are necessary for premarital counseling on alpha-thalassemia in the presence of moderate-to-severe microcytosis. The severity of the phenotype is also crucial for proper counseling.

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