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A novel *HBA2* gene conversion in *cis* or *trans*: "*α12* allele" in a Saudi population

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ABSTRACT

Thalassemia and sickle cell disease are the most prevalent hemoglobin disorders in the populations of Dammam, Al-Qatif and Al-Ahsa regions in the Eastern Province of Saudi Arabia where our study cases originated. Increased HbF can modify these disorders. Direct sequencing of the *HBA2* and *HBA1* genes from 157 Saudi subjects revealed a new *HBA2* gene conversion in *cis* or *trans* in 5.7% of the total. We refer to this new *HBA2* gene convert as an *α12* (*HBA12*) allele due to its combination of *α1* (*HBA1*) and *α2* (*HBA2*) sequences. Three genotypes, homozygous ($-\alpha^{3.7}_{12}/\alpha_1\alpha_{12}$), heterozygous ($\alpha_1\alpha_2/\alpha_1\alpha_{12}$) and hemizygous ($\alpha_1-^{4.2}/\alpha_1\alpha_{12}$) for the *α12* allele were observed. The majority of individuals who were positive for the *α12* allele had a reduction in the percentage of HbA_2. Further studies are necessary to evaluate the possible effect of these changes on globin gene expression.

Introduction

Sickle cell disease (SCD), α-thalassemia (α-thal) and β-thalassemia (β-thal) are the most common monogenic genetic disorders in Saudi Arabia. The disorders are highly concentrated along the coastal belt in the Eastern Province, especially in Jubail, Qatif, Dammam and Hofuf, with varying clinical features [1–6]. A number of studies have analyzed the variety of globin gene mutations in Saudi Arabia [5,7–13] and neighboring countries [14–16]. In the Eastern Province of Saudi Arabia the incidence of α-thal is as high as 45% [5,7,13]. The most prevalent mutations described in Saudi Arabia are $\alpha^{3.7}$ single gene deletion, poly A mutation (41%) and MED double gene deletions [4,5,8]. A recent study by Akhtar et al. [5] on the α-thal mutations among transfusion dependent β-thal patients from the Eastern Province of Saudi Arabia utilized the α-globin strip assay kit (Vienna Lab Diagnostic GmbH) and single tube multiplex PCR [17]. These techniques can identify only 16.4% of the known mutations. We sought to identify point mutations of *HBA2* and *HBA1* through direct DNA sequencing. During the course of this study, investigators identified a new gene conversion in the *HBA2* gene, termed *α12* (*HBA12*). Gene conversion, involves the unidirectional transfer of genetic material from a donor sequence to an acceptor [18]. Gene conversion is common between the two homologous α-globin genes [19]. We also studied family members of the six probands with *α12* to confirm the inheritability and phenotypic association. Here we report the identification of a new *α12* allele, coinherited genotypes and its impact on phenotypes.

Materials and methods

We enrolled 157 subjects in our study, consisting of 91 β-thal patients, 19 SCD patients, 8 sickle cell trait cases and 39 controls from Al-Ahsa, Jubail, Qatif and Dammam. The study was approved by the Institutional Review Board and Committee for Biological and Medical Ethics, University of Dammam. After obtaining signed informed written consent, blood samples were obtained from the 157 subjects. The study also enrolled the parents of five probands with the *α12* allele. In addition, blood samples for one proband, whose parents are deceased, were obtained through her husband and daughter. Hematological parameters were measured using a Coulter Micro Diff II (Beckman Coulter, CA, USA) and a VARIANT™ II Hemoglobin Testing System (Bio-Rad Laboratories, CA, USA).

Genomic DNA was extracted from the blood samples of all subjects (Promega, USA). DNA samples were analyzed using the α-globin strip

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assay kit (Vienna Lab Diagnostics GmbH, Austria) to identify the common α-thalassemia deletions such as 3.7 single gene deletion, MED double gene deletion, 20.5 kb double gene deletion, 4.2 single gene deletion, THAI double gene deletion, SEA double gene deletion, FIL double gene deletion, and anti-3.7 gene triplication. A single tube multiplex PCR as described earlier [17,20] was also performed simultaneously to confirm the α-globin gene deletions using the primers listed in Supplementary Table 1. *HBA1*, *HBA2* and *HBB* genes were amplified individually as described [21,22] in Bio-Rad MyCycler™ using amplification primers listed in Supplementary Table 1. All the primers were synthesized from Applied Biosystems (Life Technologies Corporation, USA). The purified (using a QIAquick PCR Purification Kit, Qiagen, Germany) amplicons were cycle sequenced using a BigDye Terminator Cycle Sequencing Kit (Life Technologies Corporation, USA) with sequencing primers listed in Supplementary Table 1. The purified cycle sequenced products were electrophoresed using POP 7 in a Genetic Analyzer 3500 (Life Technologies Corporation, USA). Data were analyzed using the Applied Biosystems DNA Sequencing Analysis Software Version 5.4. MAFFT version 7 was used for multiple sequence alignment.

Results and discussion

Sanger sequencing of *HBA2* and *HBA1* from our subjects revealed a *HBA2* gene conversion in *cis* or *trans*. We refer to this new *HBA2* gene conversion as an *α12* (*HBA12*) allele due to its combination of *α1* (*HBA1*) and *α2* (*HBA2*) sequences (Figs. 1, 2 and Supplementary Fig. 1).

The standard *α1* and *α2* sequences (Globin Gene Server NG_000006.1) and *α12* homozygous sequences ($\alpha_1\alpha_{12}/\alpha_1\alpha_{12}$) were aligned in MAFFT version 7. All the subjects with the *α12* allele have regions such as a 3′ promoter, exon 1, IVSI (intervening sequence I), exon 2 and 5′ IVSII of *HBA1* (−6 to 581 bp) and the 3′ enhancer of *HBA2* (774 bp onwards) gene sequences. There was no evidence for the 3′ IVSII, exon 3′ and 5′ enhancer regions from the donor (*HBA1*) or acceptor (*HBA2*) gene. The nucleotide 6 upstream of the cap is a "C" in *HBA1* (NG_000006.1 coordinate 37537) and a "G" in *HBA2* (NG_000006.1 coordinate 33733). The nucleotide −6 upstream of the cap in *HBA2* was substituted with the *HBA1* specific sequence (G→C). The *HBA2* sequence in IVSII, 55 "T" [coordinate 34247] was replaced with the *HBA1* sequence "G" in the *α12* allele. The *HBA2* sequence in IVSII, 119 "G" [coordinates 34311] was replaced with the 8 bp *HBA1* sequence "5′ CTCGGCCC 3′" in the *α12* allele (Figs. 1, 2 and Supplementary Fig. 1).

The new gene convert, *α12* allele was observed in 9 (5.7% of the study population) study subjects. One subject (sickle cell trait) was homozygous ($\alpha_1\alpha_{12}/\alpha_1\alpha_{12}$); one hemophilia A subject with a sickle cell trait was hemizygous ($\alpha_1\text{-}/\alpha_1\alpha_{12}$) and 7 (2 SCD patients, 2 β-thalassemia major, and 3 controls) were heterozygous ($\alpha_1\alpha_2/\alpha_1\alpha_{12}$). A study of the family members revealed that one parent was a carrier for the *α12* allele (Supplementary Fig. 2).

The homozygous *α12* allele was co-inherited with the most common $\alpha^{3.7}$ deletion (Supplementary Fig. 3 and Table 1). Subjects heterozygous for the *α12* allele were negative for α-globin gene deletions and $\alpha\alpha\alpha^{3.7}$ triplications, as concluded by a single tube multiplex PCR analysis, and these results were confirmed by an α-globin strip based Southern blot analysis (Table 1). A hemizygous *α12* allele was observed due to an $\alpha^{4.2}$ deletion (Supplementary Fig. 3 and Table 1).

To understand the influence of the *α12* allele on phenotype, the subjects with the *α12* allele were categorized into six groups: Group 1, HbS carrier; Group 2, β-thal carrier; Group 3, β-thal major and α-thal carrier (αα/α-); Group 4, SCD and α-thal carrier (αα/α-); Group 5, HbS carrier and α-thal carrier (αα/α-) and Group 6, normal (without any globin defects). Irrespective of the group, the majority of individuals who were positive for the *α12* allele had low HbA_2 (Table 1; Fig. 3). There was a significant reduction in the percentage of HbA_2 in the first five groups.

There are differences between the observation (*α12*) in the present study and the *α212* patch work by Law et al. [19]. They reported that the exon 3 in *α12* was not from *HBA2*. However, our study does not show any evidence that the 3′ IVSII, exon 3 and 5′ enhancer regions in *α12* are from *HBA2* (Fig. 2 and Supplementary Fig. 1). The nucleotide −6 upstream of the cap in *HBA2* has been substituted with an *HBA1* specific sequence (G→C). This observation revealed the existence of a 3′ promoter, exon 1, IVSI, exon 2 and 5′ IVSII of *HBA1* in the *α12* allele. It was evident from the sequence homology that the 3′ enhancer (774 bp onwards) sequences were from *HBA2*. A complete electropherogram based diagram is pictured in Fig. 2. The *HBA2* sequence in IVSII, 119 "5′ G 3′" [coordinates 34311] was replaced with the 8 bp *HBA1* sequence "5′ CTCGGCCC 3′" in the *α12* allele. The insertion of an 8 bp *HBA1* sequence "5′ CTCGGCCC 3′" in the heterozygous ($\alpha_1\alpha_2/\alpha_1\alpha_{12}$) subjects resulted in a false frame shift mutation (Supplementary Fig. 4). Hence, the unidirectional sequencing techniques cannot be used to reveal the real mutations in the 3′ IVSII, exon 3 and enhancer regions of *HBA12* in heterozygous ($\alpha_1\alpha_2/\alpha_1\alpha_{12}$) subjects. Bidirectional sequencing is mandatory to identify the sequential defects in exon 3 and its splicing site of *HBA12* in the study population.

The new non-allelic gene conversion was observed in 15 subjects (9 probands and 6 family members). Three genotypes, homozygous ($-\alpha^{3.7}_{12}/\alpha_1\alpha_{12}$), heterozygous ($\alpha_1\alpha_2/\alpha_1\alpha_{12}$) and hemizygous ($\alpha_1\text{-}^{4.2}/\alpha_1\alpha_{12}$) for the *α12* allele were observed. The effect of the changes on α-globin gene expression from the *α12* allele is undefined at present, except for the reduction in the HbA_2. There were no significant changes in the MCV (Supplementary Fig. 5).

The high percentage of consanguinity in the study population is the reason for the high prevalence of the newly discovered *HBA12* allele.

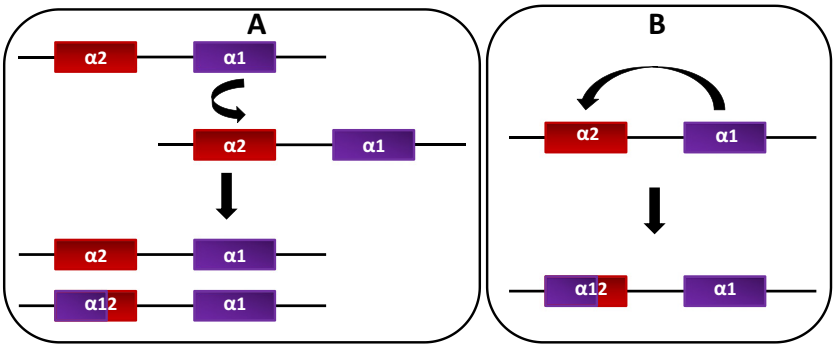

Fig. 1. A. Non-allelic (or interlocus) gene conversion in *trans*, shown as an event occurring between paralogous *HBA1* and *HBA2* sequences (represented as brown and violet boxes) that reside on sister chromatids or on homologous chromosomes. B. Non-allelic gene-conversion events in *cis* (between non-allelic *HBA1* and *HBA2* gene copies that reside on the 16th chromosome). *HBA2* gene-conversion events, which are depicted in A and B, are virtually indistinguishable from each other.
Modified from Chen et al. [18].