Molecular Basis of α-Thalassemia in Bahrain

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Objectives: This study was designed to delineate the molecular lesions, on DNA level, that lead to α-thalassemia in the population of Bahrain.

Methods: Various polymerase chain reaction (PCR)-based methodologies were involved, namely, differential PCR amplification, PCR-restriction fragment length polymorphism (PCR-RFLP), and direct PCR-amplified genomic DNA sequencing.

Results: Five α-thalassemia determinants were identified. These include three deletional type, the rightward 3.7 kilobase (kb) deletion, the leftward 4.2 kb deletion, and the pentanucleotide deletion in 5' splice donor side of intron I in α2-globin gene (GGTGAGG→GG----), and two nondeletional α-thal determinants, the Saudi type polyadenylation (polyA) signal mutation in the α2-globin gene (AATAAA→AATAAG), and the Turkish type polyA signal mutation (AATAAA→AATGAA), also in α2-globin gene.

Conclusion: Three α-thalassemia mutations, the Saudi type polyA signal mutation, the pentanucleotide deletion and the rightward 3.7 kb deletion, account for 97% of all α-thalassemia determinants in Bahrain.

Recommendations: A well-tailored genetic counseling approach, supported by molecular studies, is advised for family members affected with α-thalassemia, and in particular for carriers of the polyA signal mutations.

Normal individuals have four α globin genes (αα / αα); each two located in a tandem within the α-like globin gene-cluster, which is arranged in the order 5'-ζ-Ψζ-ψ α - α 2- α 1-0-3' on the short arm of chromosome 16. α thalassemia syndromes, the most common single gene disorder in the world, result from the inheritance of functionally competent three, two, one or no α globin genes and characterised hematologically by a reduced or absent output of α-globin chains. Deletion of a single gene [(−α /); α°-thalassemia] from a chromosome is the most common variant observed throughout the tropical and subtropical regions of the world, where falciparum malaria is or has been endemic. Major deletions removing both genes from a single chromosome [(−−/); αα°-thalassemia] and point mutations affecting expression of both α genes, (αa° /) or (αα° /), have also been described. Overall, these mutations are classified as α± or α° thalassemia to indicate, respectively, a reduced or absent output of α globin chain from the affected chromosome. The two α globin genes on a single chromosome are not functionally equivalent, the expression of 5’ (upstream) α2 globin gene is 2-3 times that of the 3’ (downstream) α1 globin gene. The single gene deletional alleles of the α globin gene cluster [(−α°3.7/) and (−α°4.2/), respectively, 3.7 and 4.2 kb deletions] contribute to more than 50% of the expression level of intact chromosome (αα /) suggesting a transcriptional compensation by the intact gene. On the contrary, in the case of non-deletional alleles ((αα° /) there appears to be no associated elevation in expression of the remaining functional gene. Consequently these thalassemic mutations occurring in the predominantly expressed α2 globin gene are much more easily recognised at the phenotype level than those in α1 globin gene.

The genotype combination of these various alleles results in a wide spectrum of phenotypic categories that can be broadly designated as, α thalassemia trait, hemoglobin (Hb) H disease and Hb Bart’s hydrops foetalis syndrome. The latter two represent the major clinical syndromes of α thalassemia, while the former spans the clinical and hematological gap between normal individuals and HbH disease.

The Bart’s hydrops foetalis syndrome is common in Southeast Asia and reported sporadically in the Mediterranean regions, while HbH disease is not uncommon in both regions including the Middle East. Bahrain has a very high frequency of α-thalassemia as judged by a cord blood screening for hemoglobin Bart’s. This study covered 10,327 newborns and revealed that elevated levels of Hb Bart’s were observed in 24% of the studied samples. HbH disease is common in this island and exhibits a wide spectrum of clinical severity. Moreover, both of sickle cell disease and β thalassemia are quite prevalent in this country, hence it is important to assess accurately the molecular basis of α thalassemia in the population of Bahrain; not only for diagnostic purposes, but also to understand the phenotypic variability of the interacting hemoglobinopathies.

METHODS

Fifty-six unrelated individuals and their relatives attending Salmaniya Medical Complex were included in this study. Diagnostic criteria of α-thalassemia include: reduced mean corpuscular volume (MCV), reduced mean corpuscular hemoglobin (MCH), low Hb A2 and/or the presence of Hb Bart’s or HbH on electrophoresis and clinical phenotype of HbH disease.

Blood was collected in EDTA vacutainers and hematologic indices were obtained by an automated cell counter. Hemoglobin electrophoresis was performed on cellulose acetate at alkaline pH (Helena Laboratories, Texas, USA). Further investigations involved the technique of cation exchange high-performance liquid chromatography (HPLC) for quantitation of Hb A2 and for assessing the presence of HbH.
DNA analysis involved typing of the common deletional forms (\(\alpha^{-3.7}\) and \(\alpha^{-4.2}\)) by a PCR-based methodology as described previously\(^{11,12}\) and by using sample controls for which genotype was previously confirmed through classical technique of Southern blotting hybridisation. Detection of the pentanucleotide deletion allele (\(a^{Hph}\alpha;\ GGTGAGG \rightarrow GG----\))\(^{13}\) was performed by a PCR-RFLP based approach\(^{14}\). The polyadenylation signal mutations in \(\alpha\) 2 globin gene, either the Saudi type (\(\alpha^{TSaudi};\ AATAAA \rightarrow AATAAG\))\(^{15}\) or the Turkish type (AATAAA \(\rightarrow\) AATGAA)\(^{16}\), were explored by direct nucleotide sequencing of the \(\alpha\) 2 globin gene-specific PCR product\(^{14}\) by using the dideoxy termination method. Further detection of the Saudi type polyA signal mutation was done by using a recently described methodology\(^{17}\). The initiation codon mutation (\(\alpha^{NcoI}\alpha;\ CCATGG \rightarrow CCACGG\)) was explored as described before\(^{14}\).

**RESULTS**

All the studied cases had low MCV (51.8-76.5 fl) and MCH (15.9-26.1 pg) with low Hb A\(_2\) (0.9-2.4) except two individuals with concurrent \(\beta\)-thalassemia or sickle cell trait. The age range of the patients was 14 days to 78 years.

<table>
<thead>
<tr>
<th>(\alpha)-Thalassemia Allele</th>
<th>No. of Chromosome (n=97)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-\alpha\ (3.7\ kb))</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>Type I</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Type II</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>(-\alpha\ (4.2\ kb))</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>(\alpha^{Hph}\alpha)</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>(\alpha^{TSaudi}\alpha)</td>
<td>51</td>
<td>53</td>
</tr>
<tr>
<td>(\alpha^{TTurkish}\alpha)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

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*Figure 1. Direct nucleotide sequencing for the detection of the Saudi type polyadenylation signal mutation in \(\alpha\) 2 globin gene*

The overall frequency of the different \(\alpha\)-thalassemia determinants in this population is presented in Table 1. Strikingly the highest frequency (53%) of all the encountered \(\alpha\) thalassemic alleles is the nondeletional type \(\alpha\)-thalassemia, represented essentially by the Saudi type poly(A) signal mutation (Fig.1). The allele with pentanucleotide deletion at the 5’ end of intervening sequence I (IVSI) in \(\alpha\) 2 globin gene represented at a frequency of 12%, while the Turkish type poly(A) signal mutation was found in a compound heterozygous state in one individual with HbH disease. The deletional type - \(\alpha^{3.7}\) allele is the second most common mutation (32%) and almost 90% corresponds to the type I - \(\alpha^{3.7}\) allele (type I and II are differentiated by the exact sites of breakpoints of the deletion\(^{11,12}\)).
Table 2. Hematological features of the α-thalassemia genotype combinations encountered in Bahrain.

<table>
<thead>
<tr>
<th>Genotype Combination</th>
<th>No. Hb* (g/dl)</th>
<th>RBC* (x10^{12}/l)</th>
<th>MCV* (fl)</th>
<th>MCH* (pg)</th>
<th>MCHC* (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>- α^{3.7}/α α</td>
<td>7</td>
<td>11.56 (1.8)</td>
<td>4.95 (1.0)</td>
<td>71.0 (9.2)</td>
<td>22.6 (3.4)</td>
</tr>
<tr>
<td>- α^{3.7}/- α^{3.7}</td>
<td>10</td>
<td>11.09 (2.3)</td>
<td>4.71 (1.1)</td>
<td>73.4 (5.9)</td>
<td>23.6 (1.7)</td>
</tr>
<tr>
<td>- α^{4.2}/α α</td>
<td>2</td>
<td>9.8 &amp; 12.3</td>
<td>4.84 &amp; 4.71</td>
<td>60.3 &amp; 76.5</td>
<td>20.2 &amp; 26.1</td>
</tr>
<tr>
<td>- α^{3.7}/- α^{4.2}</td>
<td>2</td>
<td>13.2 &amp; 8.9</td>
<td>5.67 &amp; 4.16</td>
<td>69.9 &amp; 64.3</td>
<td>23.3 &amp; 21.4</td>
</tr>
<tr>
<td>- α^{4.2}/α Hph α</td>
<td>6</td>
<td>10.0 (1,1)</td>
<td>4.76 (0,4)</td>
<td>64.7 (5,2)</td>
<td>21.0 (1,6)</td>
</tr>
<tr>
<td>α Hph α / α α α Hph</td>
<td>3</td>
<td>10.9 (1)</td>
<td>6.25 (0,3)</td>
<td>54.6 (0,9)</td>
<td>17.4 (0,8)</td>
</tr>
<tr>
<td>α Hph α / α TSaudi α</td>
<td>2</td>
<td>12.3 &amp; 14</td>
<td>6.8 &amp; 5.91</td>
<td>64.6 &amp; 64.8</td>
<td>20.6 &amp; 20.8</td>
</tr>
<tr>
<td>α TSaudi α / α TSaudi</td>
<td>3</td>
<td>10.9 (1,1)</td>
<td>5.8 (0,3)</td>
<td>59.4 (4,3)</td>
<td>18.9 (0,9)</td>
</tr>
<tr>
<td>α TSaudi α / α TTurkish</td>
<td>26</td>
<td>8.6 (0,7)</td>
<td>4.8 (0,5)</td>
<td>58.5 (5,1)</td>
<td>18.0 (1,6)</td>
</tr>
<tr>
<td>α TSaudi α / TSaudi α</td>
<td>1</td>
<td>9.1</td>
<td>4.8</td>
<td>63.1</td>
<td>19</td>
</tr>
<tr>
<td>- α^{3.7}/α TSaudi α</td>
<td>11</td>
<td>10.3 (1,5)</td>
<td>5.4 (0,5)</td>
<td>61.4 (2,7)</td>
<td>19.5 (1,1)</td>
</tr>
</tbody>
</table>

*Mean (± SD).

Figure 2. B114 is the only exceptional case with HbH disease out of eleven individuals with the genotype of (-α^{3.7}/α^{TSaudi} α).

Ten different genotype combinations found in this study are presented in Table 2. Almost consistently, the only genotype responsible for HbH disease condition involves homozygosity for a single poly(A) signal mutation, or compound heterozygosity for two different poly(A) signal mutations, however, two exceptions exist. One out of eleven - α^{3.7}/α^{TSaudi} α genotype combination is associated with HbH disease (Fig.2), and the second case related to the genotype of - α^{3.7}/- α^{3.7}. In both of these two conditions, the possibility of having initiation codon mutation and pentanucleotide deletion in - α^{3.7} chromosome has been ruled out by using the restriction endonucleases Neo I and Hph I, respectively. On the other hand, the only single case of compound heterozygosity for the Saudi and Turkish type poly(A) signal mutations, has a typical phenotype of HbH disease. Other genotype combinations do not result in HbH disease. An example of the later is shown in Fig.3, where the propositus, homozygote for the poly(A) signal mutation, has HbH disease, while her father with compound heterozygosity for poly(A) signal mutation and pentanucleotide deletion does not have HbH disease.

Figure 3. Absence of HbH disease in the father, despite being a compound Heterozygote for poly A signal mutation and pentanucleotide deletion.
DISCUSSION

Salmaniya Medical Complex is the only public hospital in Bahrain and cases requiring specialised hematological work-ups are commonly referred to this center. Hence, the studied cases are fairly representative of the genotype spectrum of α-thalassemia in this population.

Our study revealed that five different alleles constitute the spectrum of α thalassemic mutations in Bahrain (Table 1). Two alleles, one deletional (-α²⁺/_) and the other nondeletional (αTsaudi) account for 85% of the alleles encountered in this population.

The most striking feature is the exceptionally high frequency (53%) of the αTsaudi mutation contrasting with the global distribution pattern. We cannot exclude the possibility of enrichment of αTsaudi allele due to recruitment bias. As noted in Table 2, the common basis for HbH disease in this island is the homozygosity for this allele. However, three cases do not fall in this category. The first one, a compound heterozygote for two different mutations but both in the polyA signal (αTsaudi and αTTurkish). Hb H disease phenotype in this case is understandable because polyA signal mutation is known to cause a more severe deficiency in α globin chain synthesis than the other nondeletional and single gene deletional alleles15,18.

The second exceptional genotype with HbH disease is a compound heterozygosity for - α³⁻ allele and αTsaudi. In order to produce HbH disease in this genotype, we predict, through family studies (Fig.2), that the - α³⁻ allele might bear an additional mutation. The third case, is a homozygous state for - α⁻ allele. Here again we postulate that one of the two - α³⁻ alleles might bear an additional thalassemic mutation as in the previous case. Molecular studies are in progress to decipher the linked mutation to such – α³⁻ allele.

We did not find HbH disease phenotype in all of three individuals with the genotype combination of (αHph α/ αTsaudi α) (Table 2), as has been claimed previously in a Kuwaiti study19. The phenotypic differences may be due to additional mutation in the Kuwaiti chromosomes inactivating one of the linked α₁ globin genes.

The Saudi type poly(A) signal mutation has been reported in 10% of the α thalassemic alleles in one Kuwaiti study20, while it is around 15% in the eastern part of Saudi Arabia, including the first case reported from this area in the Qatif population21. The geographically restricted prevalence of this mutation to the eastern part of Arabian Peninsula, might suggest a common origin of this mutation in the vicinity of this region, with subsequent spreading by gene flow and population movements18.

CONCLUSION

In conclusion, HbH disease in Bahrain is essentially due to homozygosity for αTsaudi mutation. The spectrum of mutations found in this island must facilitate targeted diagnostic procedures for α thalassemia as well as assessing the epistatic effect of α thalassemia in the prognosis of other hemoglobinopathies.

REFERENCES


