

Short communication

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New insertions-induced Hb H disease

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Abstract

In spite of high incidence of α -thalassemia as the most common inherited disorder of hemoglobin (Hb) production in Southeast Asia, the role of point mutations in this aspect not still well known. This fact can lead to missing rare variants of α -thalassemia mutations by the routine screening, which they may need to be screened for possibility of causing Hb H disease. In this study we found two insertions in alpha1 gene which cause to Hb H disease. One of the insertions, 108/109, is a new findings and another one, codon 44, is the mutation which has been followed for the first time. These new molecular findings about changes in α -globin production which results in decreased of hemoglobin (Hb) value, have high-impact clinical importance.

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Keywords

Alpha-thalassemia,
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 α -globin gene

Introduction

Alpha-thalassemia is one of the most common inherited hemoglobin disorders, characterized by reduced or absent production of alpha globin chains due to the alpha globin genes mutations or deletions (Alberly et al., 2014). The alpha globin gene cluster is located on chromosome 16pter-p13.3; consist of one embryonic zeta globin and 2 alpha globin genes,

HBA1 and HBA2. There are 4 alpha globin genes in each cell due to existence of 2 chromosomes 16 in every one (Bain et al., 2011; Hartevelde et al., 2010). Types of alpha thalassemia are result from the loss of some or all of these genes. Deletion or defect of only one alpha globin gene usually has no significant hematologic effects, as cells still produce some normal hemoglobin. These variants are called silent carriers with few or no health problems. Subjects with two residual functional alpha genes,

either in cis (- /-alpha alpha) or in trans (- alpha/-alpha) states, show borderline anemia, with microcytic and hypochromic red blood cells which is called the alpha-thalassemia trait (alpha trait) (Higgs, 2001). Third condition occurs when three alpha globin gene have been deleted or inactivated and just one residual functioning alpha globin gene has left (- -/- alpha). This hereditary disorder is known as Hb H disease because of presence of an excess beta 4 tetramers which called hemoglobin H (Hb H). Hb H is unstable and mostly precipitates inside the older red cells, which are prematurely destroyed in the spleen. Affected individuals usually show moderate to severe anemia by marked microcytosis and hypochromic cells which is variable in clinical and hematological severity (Origa et al., 2007). As patients are generally variable in clinical and hematological severity, correct DNA genotyping in alpha thalassemia is vital. For point mutations, or small deletion/insertion mutations, the diagnosis is often based on direct nucleotide sequencing of the PCR-amplified product of alpha1 and alpha2 globin gene (Lorey et al., 2001; McBride et al., 2001; Ma et al., 2001; Viprakasit et al., 2002). In this article, we report two α -globin1 gene mutations which one of them is a novel mutation (108/109) whereas another one (codon 44) was previously just mentioned by Bayat et al, (2013) as well as describe their importance in cause to Hb H disease.

Material and methods

Through the screening for thalassemia in our center, individuals with slightly decreased hematological values, with hypochromic microcytic anemia, low MCV (<80.0 fL) and MCH (<27.0 pg), who have a persistent anemia, after initially iron therapy, are referred for molecular analysis. Genomic DNA was extracted from peripheral blood cells, using standard salting out method (11). The gap-PCR was then applied to detect the most common α -thal deletions including $-\alpha^{3.7}$, $-\alpha^{4.2}$ and $-\alpha^{20.5}$ as a primary screening test (12, 13), followed by β -globin gene DNA sequencing to assure lack of the silent β thalassemia mutations, using the ABI PRISM™ 377-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

α -Globin Gene DNA Sequencing

Patients with no α deletions and β mutations, through gap-PCR and alpha sequencing methods, were initially subjected to $\alpha 2$ -globin gene DNA sequencing and for those with no α^2 mutation, α^1 globin gene was sequenced. A 922 bp fragment of the $\alpha 2$ -globin gene was amplified using the following primers (Table 1).

Table 1. Primers used to amplify the $\alpha 1$ - and $\alpha 2$ -Globin Genes.

Primer Sequence (5'>3')
F: GCTCCGCGCCAGCCAGCCAATGAG
R: CATGTGTGTCCCAGCTGCTGTC

Results and Discussion

The PCR products amplified from the patients were sequenced after purification. The c5 and c2 were found as the most frequent mutation in $\alpha 2$ and $\alpha 1$ globin genes, respectively. Also, two new heterozygous insertions, which both are located in alpha1 gene, were found in two patients (Fig 1& 2). One mutation is a +C insertion at codon 44 (CGCTAT to CAT) that first patient showed heterozygote statues of codon 44 insertion accompanied with a 20.5 deletion (Figure 1), while another one is related to 108/109 codon (CGCTAT to CAT), which the second case showed heterozygote statues of new108/109 insertion accompanied with a 3.7 deletion (Figure 2).

Alpha 2 and Alpha1 -globin gene DNA sequencing was performed respectively to distinguish related mutations. Of all the point mutations were found in $\alpha 2$ and $\alpha 1$ globin genes, the c5 was the most frequent mutation in $\alpha 2$ and c2 in $\alpha 1$ globin gene. Furthermore, we had 2 cases with two new heterozygous insertions including, codon 44 insertion accompanied with a 20.5 deletion and 108/109 insertion accompanied with 3.7 deletion.

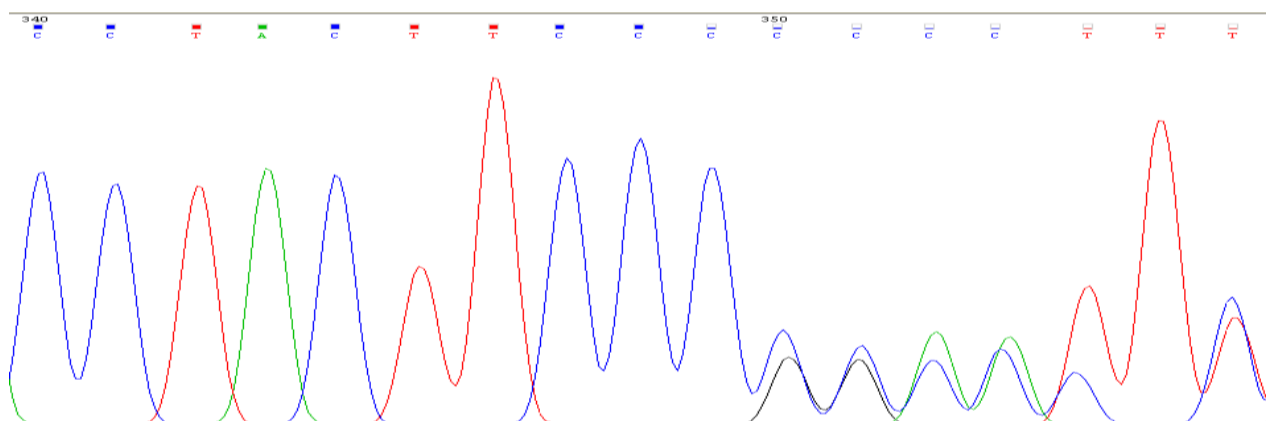


Figure 1. Sequencing result of PCR amplicons from patient with heterozygote statuses of codon 44 insertion accompanied with a 20.5 deletion.

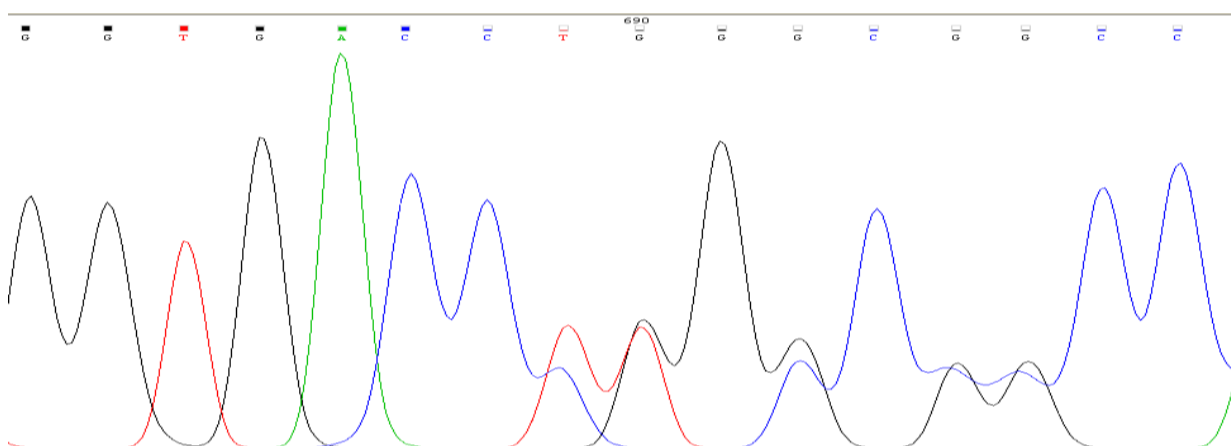


Figure 2. Sequencing result of PCR amplicons from patient with heterozygote statuses of new 108/109 insertion accompanied with a 3.7 deletion.

It is seemed that some point mutations like c44 and 108/109 due to they don't alter blood index significantly, ignored for analysis and they may not be identified by routine screening. So, sequencing for alpha thalassemia gene for cases with borderline indexes seems to be necessary.

Conflict of Interest

The author declare no conflict of interest

Ethical Considerations

Ethical principles in writing this article have been observed in accordance with the guidelines of the National Ethics Committee and the Committee on Publication Ethics (COPE).

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