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Review

Investigation of a number of rare deletional mutations in the alpha globin gene cluster

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Abstract

Alpha thalassemia is one of the most common genetic diseases in the world. This disease is prevalent in various parts of the world, such as India, the Middle East, Africa, and many other countries. Several clinical conditions can result from mutations. In the condition where only one of the alpha globin genes is expressed, hemoglobin H disease (Hb H) occurs. Alpha thalassemia trait and silent carrier are milder forms of the disease, caused by the deletion of one and two alpha globin genes, respectively. Several mutations result in the deletion of alpha-globin. Seven common deletional mutations include -$\alpha^{4.2}$, -$\alpha^{3.7}$, -$(\alpha)^{20.5}$, --^{MED}, --^{SEA}, --^{Fil}, and --^{THAI}. The deletional mutations -$\alpha^{4.2}$ and -$\alpha^{3.7}$ remove only one of the alpha globin genes, while others remove both α1 and α2 globin genes from the gene cluster. Nowadays, laboratories identify these mutations using the Gap PCR method and other advanced methods. In addition to these mutations, some deletional mutations are found only in certain families or certain regions.

Keywords: Alpha thalassemia, deletional mutation, globin, hydrops fetalis

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Alpha thalassemia is a genetic disease with autosomal recessive inheritance, characterized by a reduction or lack of production of alpha globin chains. These alpha globin chains are components of fetal and adult hemoglobin. Unlike beta thalassemia, which is caused by non-deletion mutations, most known types of alpha thalassemia involve the deletion of one or two alpha globin genes on chromosome 13.3p16 [1, 2]. Both non-deletional and deletional mutations cause alpha thalassemia. Many non-deletional mutations of the alpha globin gene result from nucleotide substitutions. The most common deletional mutations are monogenic deletions of -$\alpha^{3.7}$ and -$\alpha^{4.2}$ in Asia and bigenic deletions --^{SEA} and --^{FIL} in Southeast Asia, -$(\alpha)^{20.5}$ and --^{MED} in Mediterranean regions, and --^{THAI} in Taiwan [3, 4]. Several other deletional mutations have been identified in recent years. In this article, we review eight of these deletional mutations. These deletions have a high prevalence and are seen in different proportions in different parts of the world. However, some deletional mutations are seen only in cases or in much smaller numbers.

Alpha Thalassemia

The alpha globin cluster at its locus includes duplicated alpha genes, an embryonic α-like gene (ζ2), three pseudogenes (Ψζ1, Ψα2, Ψα1), and a gene with an undetermined function (θ1). These genes and pseudogenes are arranged in the order 1ζ2-Ψζ1-Ψα2-Ψα1-α2-α1-θ1 [5–7]. Alpha globin is an essential subunit of human hemoglobin from the sixth fetal week to adulthood. Fetal hemoglobin (α2γ2) and adult hemoglobin (α2β2) are produced by the combination of two chains of alpha globin with two chains of gamma and beta globin, respectively [8, 9]. Based on how many of the alpha globin chain genes are deleted, the clinical symptoms of alpha thalassemia vary from mild anemia to severe symptoms. If one, two, three,

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or four of the alpha globin genes are deleted, alpha thalassemia trait, silent carrier state, hemoglobin H disease, and Hydrops fetalis syndrome occur, respectively. The frequency of alpha thalassemia has been studied in different parts of the world, with varying frequencies in different regions of countries such as Iran, Morocco, China, and Syria [10–13].

Alpha Thalassemia Phenotypes

Alpha thalassemia phenotypes are divided into four categories: Alpha thalassemia silent carrier, Alpha thalassemia trait, Hemoglobin H disease (Hb H), and Hydrops fetalis.

Table 1 shows the characteristics of alpha thalassemia types.

Classification of Alpha Thalassemia Based on the Number of Deleted Genes

Different types of deletional mutations with different frequencies have been investigated in various parts of the world [14–19]. Some of these mutations cause the deletion of one of the two alpha globin genes, while others cause the deletion of both genes [14–21].

Single Gene Deletions

The most common deletional mutations in alpha thalassemia delete one of the genes of the alpha globin chain, which leads to a mild alpha thalassemia phenotype (-α/αα). Reciprocal recombination, which occurs between highly similar regions called Z-boxes, leads to the deletion of 3.7 kb, which only includes one alpha gene ($-\alpha^{3.7}$). In contrast, recombination between the mismatched X-boxes results in the deletion of 4.2 kb ($-\alpha^{4.2}$). These recombinations lead to the creation of chromosomes containing three alpha globin genes [22, 23].

Double Gene Deletions

Several α0 defects that delete both alpha globin genes have been observed in different populations and are named according to their geographic region. These deletions include Southeast Asian ($--^{SEA}$), Mediterranean ($--^{MED}$), Filipino ($--^{FIL}$), Thai ($--^{THAI}$), and 20.5 kb type ($-(\alpha)^{20.5}$) [24, 25]. Partial or complete deletion of both alpha globin genes in the cis state leads to the absence of alpha globin chain synthesis in the body. Homozygotes with these deletions have hydrops fetalis syndrome. Many deletions also remove ζ and α globin genes. Individuals in the heterozygous state appear to develop normally, but survival is unlikely for individuals in the homozygous state even in the early months or days of pregnancy, as neither α2γ2 nor ζ2γ2 hemoglobin can be produced. It is worth mentioning that rare deletions causing α0 thalassemia delete the regulatory region 40–50 kb upstream of the alpha globin gene cluster, leaving the alpha globin chain genes intact.

Detailed analysis of the deletional mutation $-(\alpha)^{20.5}$ reveals the importance of providing a more precise description of the 5' end of this deletion from the alpha globin genes. This is especially crucial because different phenotypes are associated with defects in the alpha globin gene cluster. The first cases of alpha-α0 thalassemia deletion, which deleted the entire alpha globin gene set, described a rare condition characterized by Hb H disease and congenital mental retardation [26]. While the $--^{SEA}$ deletional mutation is the most common cis deletion in alpha globin chains in Southeast Asia, several other deletions have also been reported in other scientific papers. $--^{THAI}$ and $--^{FIL}$ deletional mutations are common among some Southeast Asian populations. These common deletional mutations, which were first described by Fischel Ghodsian and colleagues, cover about 30–38 kilobases and delete the zeta2 globin gene as well as alpha globin genes located on chromosome 16 [27].

New Deletional Mutations

$-\alpha^{6.9}$ deletion

This deletion mutation, first observed in China, causes the deletion of the α2 gene but leaves the α1 gene intact (NG-000006.1.29785–36746 del 6962bp). The researchers who found this mutation initially identified alpha thalassemia by examining the phenotype of the subjects. Then, using the Multiplex Ligation-dependent Probe Amplification (MLPA) method, they evaluated the new mutation, and through DNA sequencing and bioinformatics analysis, confirmed the mutation's existence [28]. If this deletional mutation is combined with a single deletion, it causes alpha thalassemia trait, and if it is combined with a double deletional mutation, it causes Hb H disease.

$--^{27.2}$ deletion

In a study, researchers in China discovered this new mutation after examining a young woman in pre-pregnancy screening who had hypochromic and microcytic hematological features [29]. This mutation causes the deletion of both alpha globin genes located on chromosome 16, and if it is combined with another double deletional mutation, it causes hydrops fetalis.

Table 1. Characteristics of alpha thalassemia types

Name of disease	Number of deleted genes	Genetic condition of the disease	Severity of clinical symptoms
Silent carrier	One	-α/αα	Usually mild or no symptoms
Trait	Two	--/αα	
-α/-α	Usually mild anemia		
Hb H	Three	--/-α	mild to severe
Hydrops fetalis	Four	--/--	Severe symptoms

$(\alpha\alpha)^{ZRX}$ deletion and $--^{336}$ deletion

These deletions, which cause the deletion of both alpha globin chains, were observed in two Chinese families. In the patient with the $--^{336}$ deletion, a spontaneous mutation was identified, as neither parent had this mutation. Both carriers had symptoms of microcytic hypochromia, a characteristic of people with α^0-thalassemia [30].

$--^{GB}$ deletion

This deletion mutation, first observed in Malaysia, results in the deletion of both genes of the alpha globin chain (α^0-thalassemia). Most individuals with this mutation are of Malay descent. The main risk of this mutation, when accompanied by a single or double deletion, is Hb H disease or hydrops fetalis, respectively [31].

$(\alpha\alpha)^{FJ}$ deletion

This deletion mutation, which caused the deletion of 91.5 kilobases in the alpha globin gene cluster in a family from China, deletes both α1 and α2 genes. In areas with a high frequency of alpha thalassemia, this mutation should be investigated [32].

$-\alpha^{6.3}$ deletion and $-\alpha^{27.6}$ deletion

These mutations, identified in two Chinese patients, delete one of the alpha globin chains (α+-thalassemia). Like single deletion mutations such as $-\alpha^{3.7}$ and $-\alpha^{4.2}$, they are accompanied by double deletion mutations, causing Hb H disease [33].

$--^{CR}$ deletion

This deletion mutation, found in Thailand, deletes 44.6 kilobases of the alpha globin gene cluster. There is limited information on the effects of this mutation, especially in combination with other types of thalassemia. Hypochromic and microcytic blood phenotypes have been observed in carriers [34].

$--^{14.9}$ deletion

This mutation, discovered in China, deletes both alpha globin chains. Affected individuals had abnormal blood parameters, and after screening for 23 types of common mutations, this specific mutation was identified [35].

107 kilobase deletion

In a patient suffering from mild anemia, screening for thalassemia was performed by Gap-PCR and PCR-reverse dot blot methods. Following additional investigations, this deletional mutation was identified [36].

Common Diagnostic Methods in Identifying Deletional Mutations in Alpha Thalassemia Disease

For a long time, methods such as loop-mediated isothermal amplification, Multiplex Ligation-dependent Probe Amplification (MLPA), and single-tube multiplex polymerase chain reaction (PCR) have been used worldwide in the diagnosis of alpha thalassemia. However, new methods with greater speed and accuracy are also needed, as they can identify many genetic mutations in a short period of time [37]. In addition to traditional methods that have been used for years by diagnostic centers, new methods have also been introduced in recent years [38–40].

Conclusion

Considering the high prevalence of alpha thalassemia worldwide, as well as the complications of this disease, particularly in cases of Hb H disease and hydrops fetalis, it is advisable to screen individuals suspected of having this condition. Following diagnosis, genetic counseling should be provided to inform patients about the risks in pregnancy and prevent the birth of infants with severe alpha thalassemia. In areas where rare mutations are present, these mutations should also be studied.

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