

EDUCATION SESSION 9: THALASSEMIA



Molecular Defects in α Thalassemia

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The hemoglobin A (Hb A) molecule consists of two pairs of polypeptide chains, α and β globin, inside which resides the haem that is responsible for oxygen delivery to the tissues. Deficiency of globin chains results in anemia, and this group of hereditary anemias is known as thalassemia (thal). Since Hb A comprises 96% of circulating Hbs in the adult, there are thus two main types of thal: α and β thal. Thal affects 1–20% of the population in various parts of South-east Asia and poses a serious health problem.

In this region, α thal is mainly due to gene deletion, and its clinical severity depends on the number of α genes deleted. The α -like globin genes are located on the short arm of chromosome 16 and arranged in order of their expression, from the ζ gene, which is expressed in embryonic life, followed by 3 pseudo-genes (which are non-functional) and the duplicated α genes ($\alpha 2$ and $\alpha 1$). The $\theta 1$ gene at the 3' end has no known function.

The molecular pathology of α thal is now quite well understood. Normal individuals have 2 α genes on each chromosome 16 ($\alpha\alpha/\alpha\alpha$). The loss of 1 or 2 genes (α thal 2 and α thal 1, respectively) does not manifest any clinical symptoms, while loss of 3 genes (Hb H disease) gives rise to moderate anemia and α thal intermedia picture. Complete deletion of all 4 α genes (homozygous α thal 1, Hb Barts hydrops fetalis) is incompatible with late intrauterine or neonatal life without transfusion.

Deletion of one α gene

The α globin genes along with two pseudogenes are arranged in 4 kb tandem homology blocks. In the $\psi\alpha 1$, $\alpha 2$ and $\alpha 1$ gene region, there are X, Y and Z homology blocks. With mispairing of the homologous sequences in the Z and X blocks followed by unequal cross-over, gene deletion occurs. Single α gene deletion of the rightward type involves the Z block and deletes 3.7 kb of DNA while the leftward type involves the X block and 4.2 kb is lost.¹

Deletion of two α genes

Unequal crossover is also the mechanism for the occurrence of α thal 1, where both α genes (in cis) are lost on the same chromosome. The end result is a chromosome that has the ζ

and $\psi\zeta$ genes from one piece, with $\theta 1$ from another piece (**Figure 1**), deleting both $\alpha 2$ and $\alpha 1$ genes. This SEA deletion extends approximately 20.5 kb beginning from around the $\psi\zeta$ to beyond the 3' end of the $\theta 1$ gene.² Other types of deletions also involve the entire ζ - α gene cluster, such as in -- FIL and -- THAI,^{3,4} and some rarer types remove the upstream control element region (HS-40) only,⁵ with the two α genes intact but not being transcribed.

The loss of one α gene (α thal 2) gives a silent carrier state with no clinical or hematological change and loss of two α genes (α thal 1) results in a mild anemia only. However, if both parents were α thal 1, their risk of having a homozygous α thal 1 (Hb Barts hydrops fetalis) fetus would be 25%. This fetus would either die in utero in late gestation or within a few minutes of birth. Maternal morbidity and mortality may also occur.⁶ Thus prenatal diagnosis for pregnancies at risk should be advocated. α thal 1 couples can be easily identified at the antenatal clinic by their low MCV of < 80 fl and presence of occasional H-like inclusions when red blood cells are incubated with brilliant cresyl blue. Prenatal testing can be performed by either PCR-amplification of the α thal 1 and normal chromosomes⁷ or detecting α genes in fetal DNA by standard Southern blotting technique.⁸

Deletion of three α genes

Loss of 3 α genes results in Hb H disease, which is an α thal of intermediate severity. Patients have Hb levels of 7–11 g/dl, low MCV and splenomegaly. The majority of cases of Hb H disease are due to an α thal 1 chromosome and co-inheritance of either a rightward $-\alpha$ ^{3,7} or leftward $-\alpha$ ^{4,2} deletion,^{9,10} but some 20–60% of cases in Hong Kong, Thailand and Guangxi, respectively, may be due to co-inheritance of α thal 1 and the non-deletion of α gene defect.^{10–12} **Table 1** lists examples of some non-deletion α thal defects. Many of these mutations are similar to those found in β thal. Those that affect the initiation codon and others such as nonsense and frameshift mutations result in early termination of translation and absence of normal protein product. There are splice-site mutations¹⁶ that cause abnormal mRNA. Termination codon mutations¹⁷ such as α Constant Spring, α Koya

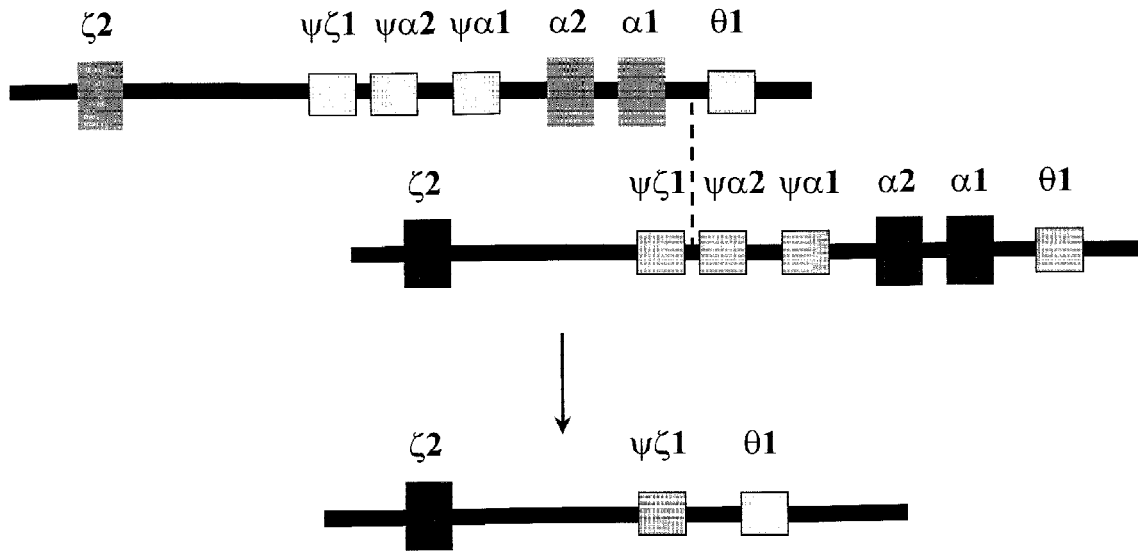


Figure 1. Unequal crossover between two chromosomes as the mechanism for the occurrence of the α thal 1 genotype (--SEA).

Table 1. Types of non-deletion α thalassemia defects.

Mutant Type	Genetic Defect	Origin
(I) Nonfunctional mRNA		
A. Initiation codon		
1	$\alpha 2$ ATG \rightarrow ACG	Mediterranean
2	$\alpha 2$ ATG \rightarrow GTG	Black
3	$\alpha 2$ ATG \rightarrow A-G	Vietnamese
B. Nonsense		
Codon 116	$\alpha 2$ ATG \rightarrow TAG	Black
C. Frameshift		
Codon 30/31	$\alpha 2$ ATG \rightarrow -- G	Black
Codon 39-41	$\alpha 2$ Δ 9 bp + 8 nt	Jewish
D. Termination codon (codon 142)		
1 α Constant Spring	$\alpha 2$ TAA \rightarrow CAA	Chinese
2 α Koya Dora	TAA \rightarrow TCA	India
3 α Icaria	$\alpha 2$ TAA \rightarrow AAA	Mediterranean
4 α Seal Rock	TAA \rightarrow GAA	Black
(II) RNA Processing		
A. Splice Junction		
IVS-1 donor site	$\alpha 2$ Δ 5 nt Ggtgagg Gg-----	Mediterranean
B. Polyadenylation		
1	$\alpha 2$ AATAAA \rightarrow AATAAG	Saudi Arabian
2	$\alpha 2$ AATAAA \rightarrow AATA--	Indian
3	$\alpha 2$ AATAAA \rightarrow AATGAA	Turkish
4	$\alpha 2$ Δ 16 bp 3'UTR •••-ATAAA	Arabian
(III) Unstable Globins		
1 α Quong Sze	$\alpha 2$ Codon 125 (CTG \rightarrow CCG)	Chinese
2 α Hong Kong 1	$\alpha 2$ Codon 30 (Δ GAG)	Chinese
3 α Hong Kong 2	$\alpha 2$ Codon 59 (GGC \rightarrow GAC)	Chinese
4 α Evanston	$\alpha 2$ Codon 14 (TGG \rightarrow AGG)	African
5 α Suan Dok	$\alpha 2$ Codon 109 (CTG \rightarrow CCG)	SE Asian
6 α Petah Tikvah	$\alpha 2$ Codon 110 (GCC \rightarrow GAC)	Middle Eastern

Dora, etc. all result in an unstable α globin chain. In addition, a number of polyadenylation mutations¹⁸ have been characterized. Many of the non-deletion defects involve the $\alpha 2$ gene, the expression of which exceeds that of the $\alpha 1$ gene by a ratio of 3:1.¹⁹ This may account for the fact that patients with non-deletional HbH disease have a more severe phenotype than those with deletional HbH. Recent studies on HbH patients have shown that while there was little difference between patients with leftward or rightward α gene deletion, the Hb and MCV levels were lower in patients with non-deletional HbH defects. Other hematological indices such as splenomegaly, hepatomegaly and iron overload, as evidenced by serum ferritin level and magnetic resonance imaging (MRI) of the liver, were also worse in this group, despite the fact that these patients were not transfusion-dependent.²⁰ Two cases of severe non-deletion HbH resulting in hydrops fetalis had also been described. The molecular defect in one was a ζ - α thal 1 deletion with co-inheritance of an $\alpha 2 \Delta$ codon 30 defect. This resulted in a hydropic infant who died at birth. The second case was an α thal 1 with an $\alpha 2$ codon 59 (G→A) mutation.²¹ In this case, the fetus showed hydropic changes at 18 wks gestation, was given intrauterine transfusion at 22 wks, and remained transfusion-dependent since birth.

α thal - mental retardation

Apart from deletion of α genes, which causes decreased or absent globin chain synthesis, disturbances of α globin production may result from gene deletion at the telomere of chromosome 16, as in ATR-16 syndrome, or mutations on the X chromosome, as in ATR-X syndrome.

ATR-16²² is associated with moderate to severe mental retardation in two-thirds of cases and mild mental retardation in 10% of cases. Varying loss of DNA from the tip of chromosome 16 results in the following: deletion of the terminal 250 kb gives rise to α thal, deletion of up to 2000 kb

is associated with α thal, mental retardation and a variety of other congenital abnormalities; further loss of another 300 kb leads additionally to polycystic kidney disease and tuberous sclerosis (**Figure 2**).

ATR-X syndrome is an X-linked disorder comprising severe psychomotor retardation, facial dysmorphism, genital abnormalities and α thal.²³ It results from diverse mutations of the XH2/XNP gene that map to Xq13.3. The complex ATR-X phenotype suggests that XH2 when mutated, it down-regulates the expression of several genes, one of which is the α globin genes. This suggests that the XH2 protein may be a global transcriptional regulator.

Advances in molecular biology have helped in elucidating the molecular mechanism of a thal which can be used as a model for other single gene disorders.

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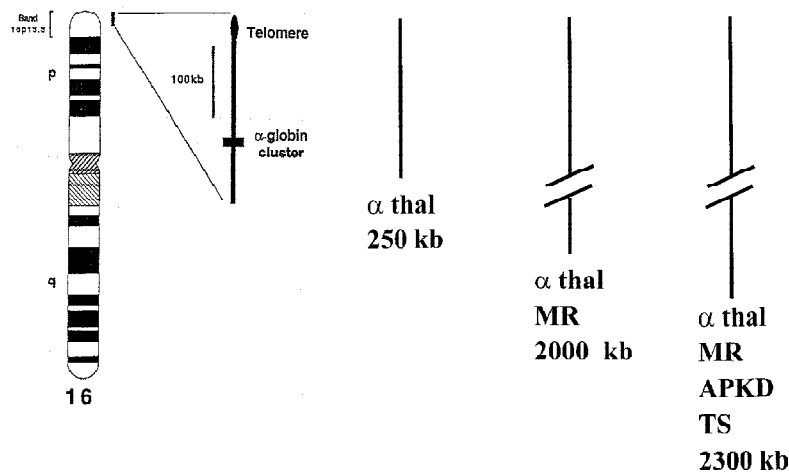


Figure 2. Deletions associated with the ATR-16 syndromes

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