The *α-globin* gene cluster: genetics and disorders

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Introduction

Hemoglobin (Hb) is the oxygen-carrying protein packaged within circulating erythrocytes. It has been extensively studied in terms of its structure–function relationship, genetics and hereditary disorders. In 1949, Pauling and colleagues[1] described sickle cell anemia as the first molecular disease. Globin genes were the first to be cloned and to have their cis regulatory sequences identified and analyzed.[2] There are now more than 1000 known natural human globin gene mutations.[3] Collectively, α- and β-thalassemias and sickling disorders are the most common of all genetic diseases, assuming public health importance in many parts of the world today.[4] Hemoglobinopathy serves not only as a paradigm for hereditary disorders but is also of health care importance in Canada with its multicultural population.

Hemoglobins are tetrameric molecules composed of 2 α-globin-like chains (ζ or α) and 2 β-globin-like chains (ε, γ, δ or β), each of which contains a heme group. For example, fetal Hb (Hb F) is made up of $\alpha_2\gamma_2$, and adult Hb (Hb A) is made up of $\alpha_2\beta_2$. The *α-globin* gene cluster is located on the distal end of the short arm of chromosome 16, whereas the *β-globin* gene cluster is located on the short arm of chromosome 11. There is a sequential expression of different globin genes during embryonic and fetal development, such that different types of Hb predominate at different stages of gestation (Fig. 1).[2] Throughout the second and third trimesters of gestation, the major Hb is Hb F ($\alpha_2\gamma_2$). By six months af-

ter birth and thereafter, the major Hb is Hb A ($\alpha_2\beta_2$). *Alpha-globin* genes are expressed beginning early in fetal development, and therefore the effects of *α-globin* gene mutations are manifested throughout fetal and adult life. This is in contrast to mutations of the *β-globin* gene that exert their effect only after birth.

The *α-globin* gene cluster

The *α-globin* gene cluster contains 1 embryonic ζ- and 2 *α-globin* genes arranged in the order of 5′-ζ2-α2-α1-3′ on each chromosome 16 (Fig. 1). There are 4 pseudogenes within the *α-globin* gene cluster.[2] Since each individual has 2 chromosomes 16, there are usually a total of 4 functional *α-globin* genes. Overall, the combined production of α-globin chains from these 4 *α-globin* genes is approximately equivalent to that of the β-globin chains derived from the 2 *β-globin* genes on chromosome 11.

The number of *α-globin* genes per chromosome 16 can range from 0 to 4, owing to unequal crossing-over between misaligned *α-globin* gene clusters and other recombination events. Therefore the total number of *α-globin* genes an person may have can range from 0 to as many as 7 or 8.[5,6] These observations illustrate one example of human genetic diversity.

Whereas the *α2*- and *α1-globin* genes encode identical α-globin chains of 141 amino acid residues, the *α2-globin* gene accounts for twice the α-globin chains produced relative to the *α1-globin* gene, likely owing to the effect of different promoter sequences that are proximal to the coding sequences.[7] Conse-

quently, $\alpha2$-*globin* gene mutations generally are associated with more adverse effects than the same mutations on the $\alpha1$-*globin* gene.

About 40 Kb upstream of the α-*globin* gene cluster is a region known as HS-40, corresponding to a series of DNase hypersensitive sites and binding sites for transcription factors.[8] Its integrity is essential for α-*globin* gene expression, as amply demonstrated by its removal in several natural deletions that effectively silence the expression of α-*globin* genes downstream.[9] In such cases these people present as α-thalassemia carriers.

In addition to the α-*globin* genes and their cis regulatory sequences there are important transcriptional factors encoded by genes unlinked to the globin gene clusters. These factors are pivotal in regulating gene expression by binding to the α-*globin* gene promoter or HS-40 sequences, or both, interacting with other DNA-binding proteins, or altering chromatin structure. A good example is the *ATRX* gene on chromosome Xq13.3. Mutations of this gene cause marked down-regulation of α-*globin* gene expression, plus severe mental disability (see below).[10]

Alpha-globin chain variants

There are now approximately 300 known natural α-*globin* gene mutations.[3] Many of the point mutations lead to variant α-globin chains of no clinical significance. However, if the mutations alter critically positioned amino-acid residues, the variant α-globin chains might lead to unstable hemoglobins and hemolytic anemia, such as Hb Bibba (codon 136 CTG→CCG or leucine→proline); high oxygen affinity hemoglobins and erythrocytosis, such as Hb Chesapeake (codon 92 CGG→CTG or arginine→leucine) or methemoglobins and cyanosis, such as Hb M-Boston (codon 58 CAC→TAC or histidine→tyrosine). All of the known α-*globin* gene mutations, including their relevant clinical and laboratory summaries, are tabulated on a Web database (http://globin.cse.psu.edu) that is amenable to queries and retrieval.

Alpha-thalassemia

Alpha-thalassemia is caused by α-*globin* gene mutations that result in deficient or absent α-globin chain production.[9] The common mutations are deletions, involving only 1 α-*globin* gene, both α-*globin* genes in tandem, or the entire ζ-α-*globin* gene cluster. Alpha-thalassemia can also be caused by point mutations that interfere with either transcription or translation, such as Hb Constant Spring ($\alpha2$ codon 142 TAA→CAA or termination→glutamine). Some mutations that give rise to highly unstable α-globin chain variants, such as Hb Quong Sze ($\alpha2$ codon 125 CTG→CCG or leucine→pro-
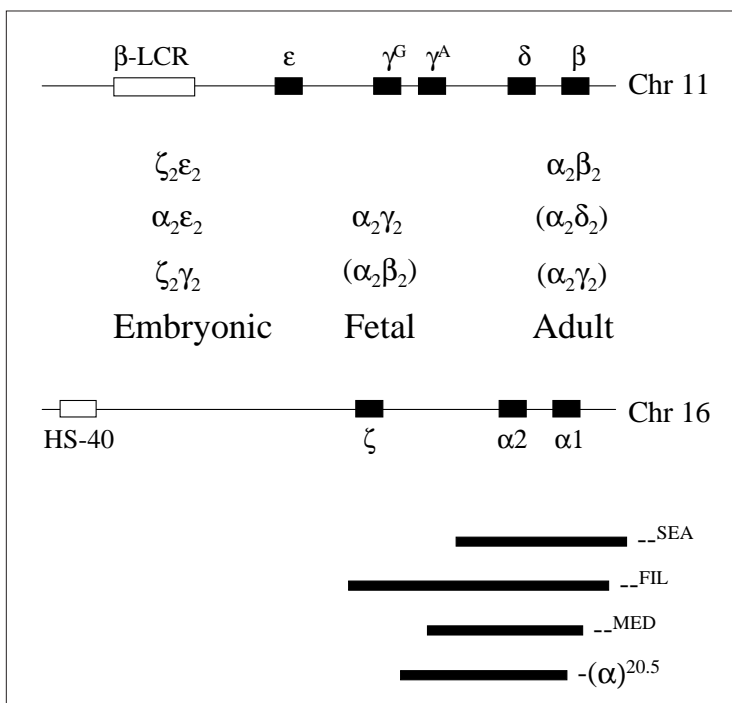

Fig. 1: Schematic representation of the β-**globin** gene cluster on chromosome 11p15.5, and α-**globin** gene cluster on chromosome 16p13.3. Closed boxes indicate the approximate locations of the functional genes, and open boxes depict the essential distal cis acting regulatory domains, β-locus control region (LCR) for the β-**globin** gene cluster, and HS-40 for the α-**globin** gene cluster. The predominant hemoglobins at each stage of development are noted, with the minor species indicated in parentheses. Also shown are the most common α^0-thalassemia deletions in Southeast Asia [--^{SEA} and --^{FIL}] and Mediterranean [--^{MED} and -(α)$^{20.5}$] regions. Note that the (--^{FIL}) deletion removes the entire ζ-α-**globin** gene cluster.

line), may also result in an α-thalassemic phenotype.

Alpha-thalassemia is one of the most common single-gene disorders, owing to a selective advantage of carriers against *Plasmodium falciparum* infection. As a result, α-thalassemia is most prevalent among people from regions of the world where malaria is or has been endemic. For example, the carrier frequencies for single *α-globin* gene deletion among Afro-Americans and Sri Lankans are 30% and 16% respectively.[11,12] In Northern Thailand, the carrier frequency for the 2 *α-globin* gene deletions of the Southeast Asian type ($--^{SEA}/\alpha\alpha$) is 14%.[13] With population migrations in recent decades, α-thalassemias of clinical significance are now encountered much more frequently in North America.

Alpha-thalassemia silent carrier and trait

People who have a single *α-globin* gene inactivated either by deletion or point mutation almost always have a normal blood counts and are well. They are known to be α-thalassemia silent carriers (Table 1).[14]

People who have 2 defective *α-globin* genes, with 1 defective gene on each chromosome 16 (e.g., -α/-α), or 2 defective genes on 1 chromosome 16 (e.g., --/αα), usually have microcytosis but normal or borderline low Hb levels. They are asymptomatic and are said to have α-thalassemia trait (Table 1).[14] An accurate diagnosis in these people is clinically important, so as to avoid potentially harmful iron supplement therapy and unnecessary investigations

for gastrointestinal bleeding, for example. Furthermore, they and other appropriate family members need to be informed and counselled in regard to potential reproductive risks for conceiving fetuses with Hb Bart's hydrops fetalis syndrome (see below).

Hemoglobin H disease

People who have loss or inactivation of 3 *α-globin* genes caused by either deletions (-α/--) or a combination of deletion and point mutation ($\alpha^{Th}\alpha/--$ or $\alpha\alpha^{Th}/--$) have only 1 functional *α-globin* gene. There is a marked excess of β-globin chains that form the homotetramer (β_4), known as Hb H, hence, Hb H disease (Table 1).[15,16] They have moderate to severe anemia but seldom require transfusions except possibly during infection, ingestion of oxidant drugs or compounds, and during pregnancy. In general, the degree of anemia in Hb H patients with deletion of 2 *α-globin* genes plus a non-deletional *α2-globin* gene mutation such as Hb Constant Spring ($\alpha^{CS}\alpha/--$) is more severe than those with deletions removing 3 *α-globin* genes (-α/--).

The natural history of Hb H disease, particularly during infancy and childhood, has not been well documented. A recent report on more than 100 patients with Hb H disease suggests that there may be greater morbidity than previously appreciated.[15] Many children with Hb H disease had a growth rate below the third percentile. The majority of adult

Table 1: Alpha-thalassemia syndromes			
Clinical syndrome	*Alpha-globin* genotype	Clinical and laboratory findings	Reproductive significance *
Normal	αα/αα	Clinically well	None
Alpha-thalassemia — silent carrier	-α/αα $\alpha^{Th}\alpha/\alpha\alpha$ or $\alpha\alpha^{Th}/\alpha\alpha$	Normal Hb level. Normal or borderline low MCV. Clinically well	Hb H disease
Alpha-thalassemia trait	-α/-α $\alpha^{Th}\alpha/-\alpha$ or $\alpha\alpha^{Th}/-\alpha$	Normal or borderline low Hb level Low MCV. Clinically well	Hb H disease
	--/αα	Normal or borderline low Hb level. Low MCV. Clinically well	Hb H disease. Hb Bart's hydrops fetalis
Hb H disease	-α/-- $\alpha^{Th}\alpha/--$ or $\alpha\alpha^{Th}/--$	Moderate anemia. Low MCV. Usually not transfusion dependent	Hb H disease. Hb Bart's hydrops fetalis
Hb Bart's hydrops fetalis	--/--	Severe anemia and hypoxia in utero. Fetal death in 2nd or 3rd trimester or death within hours after birth. Risk of severe maternal complications.	Not applicable

*Potential risk of fetus inherited with these α-thalassemia syndromes, depending on partner's α- *globin* genotype.
Hb = hemoglobin, α^{Th} = non-deletional α-thalassemia mutation involving either *α2-* or *α1-globin* gene, MCV = mean corpuscular volume.

patients had iron overload unrelated to transfusion history, and some had hepatic cirrhosis or cardiac dysfunction. Hypersplenism and cholelithiasis were other complications.

A subset of fetuses that have inherited deletion of 2 α-globin genes on 1 chromosome 16 plus a non-deletional $\alpha2$-globin gene mutation on the other chromosome 16 ($\alpha^{Th}\alpha$/--) have very severe anemia and hypoxia in utero. They present with edema, developmental abnormalities and even death, similar to Hb Bart's hydrops fetalis syndrome (see below). Several non-deletional $\alpha2$-globin gene mutations have been implicated in the Hb H hydrops fetalis syndrome, all of which result in highly unstable α-globin chain variants.[17,18] The molecular and pathophysiologic mechanisms to account for this serious disorder, in contrast to the relatively benign clinical course of most patients with Hb H disease, are currently not well understood.

Hemoglobin Bart's hydrops fetalis syndrome

The most severe form of α-thalassemia is homozygous α^0-thalassemia, or the Hb Bart's hydrops fetalis syndrome (Table 1).[14,19] The affected fetuses have inherited deletions removing all α-globin genes. In utero, the γ-globin chains form γ_4 homotetramers, also known as Hb Bart's that are incapable of oxygen delivery to tissues. Fetuses that are homozygous for deletions that remove both the embryonic ζ- and α-globin genes (e.g., --^{FIL}/--^{FIL}) cannot make any functional Hb and are thought not to survive beyond early embryonic life.[20] Conversely, affected fetuses harbouring at least 1 intact embryonic ζ-globin gene (e.g., --^{SEA}/--^{FIL} or --^{SEA}/--^{SEA}) synthesize small quantities of functional Hb Portland 1 ($\zeta_2\gamma_2$), which are sufficient to sustain life into the second and third trimesters of gestation. Unfortunately, the amounts of these hemoglobins are insufficient to keep pace with the remarkable growth and development of the fetus, especially during the third trimester. Ultimately, the affected fetuses succumb to hypoxia and heart failure either in utero or shortly after birth.

As a consequence of hypoxic insults beginning during embryogenesis and of extramedullary erythropoiesis, affected fetuses often have developmental defects involving many organ systems including the central nervous system.[14,19] In addition, there is an increased rate of serious maternal complications, such as pre-eclampsia and hemorrhage in these pregnancies, likely contributed by placentomegaly.[14,19]

There are sporadic reports in the literature of survival of newborn infants who had no functional α-globin gene. They were given in utero or perinatal transfusions. These surviving infants must be maintained on a regular transfusion and iron chelation program similar to those children having β-thalassemia major.[14,19] A recent review concluded: "Current attempts to treat this condition are associated with an unknown risk of rescuing infants with multiple, often severe developmental abnormalities. Until these problems have been adequately addressed and solved, further human experimentation should be avoided. Given the serious obstetric risk to the mother of an affected fetus, it seems most prudent to advise early termination of pregnancy in all cases."[14]

Alpha-thalassemia associated with mental retardation

During the past decade, 2 unusual syndromes have been recognized consisting of α-thalassemia often manifested by Hb H disease, mental retardation and other developmental abnormalities. One group of patients is characterized by extensive deletions involving the terminal 2 Mb of the short arm of chromosome 16, which encompasses the α-globin gene cluster. Mental retardation found in this ATR-16 syndrome is thought to be caused by deletion of one or more genes involved in development of the central nervous system.[10] Accordingly, detailed mapping of deletion end points in these patients has been carried out in an attempt to identify genes of interest. So far, none has been found in the most telomeric 350 Kb of chromosome 16p, including the 200 Kb region proximal to the α-globin gene cluster.[21] Characterization of the more extensive deletions in ATR-16 patients is in progress.

A second subset of patients with α-thalassemia and severe mental retardation does not harbour mutations of the α-globin gene cluster. Instead, this ATR-X syndrome is caused by mutations of *ATRX* gene on chromosome Xq13.3.[10] The *ATRX* gene encodes a peptide with ATPase and helicase domains. It

interacts with chromatin and therefore is expected to affect expression of many other genes during fetal development.[10] It is of interest that *ATRX* mutations downregulate α-*globin* gene expression but not β-*globin* gene expression. This is further evidence that the α- and β-*globin* gene clusters are contained within different chromosomal environments and are regulated differently.

Interaction with *β-globin* gene mutations

The degree of anemia in people with *β-globin* gene mutations can be modulated by their α-*globin* genotypes. For example, patients with sickle cell disease, that is those who are homozygous for sickle cell Hb (β codon 6 GAG→GTG or glutamic acid→valine), and concomitant α-thalassemia often have higher Hb levels and lower reticulocyte counts than those having a normal complement of 4 α-*globin* genes.[22] The presence of α-thalassemia leads to lower intracellular sickle cell Hb concentration, thus impeding its polymerization, and consequently less hemolysis. Paradoxically, increased Hb levels in these patients lead to increased blood viscosity and therefore more bony infarcts, retinopathy and episodes of pain.[22]

Some heterozygous carriers of β-thalassemia mutations are found to have triplicated or quadruplicated α-*globin* genes. They may be more anemic than those who are simple β-thalassemia heterozygotes, presumably because of increased imbalance of α- to β-globin synthesis.[23] The excess α-globin chains form intracellular precipitates, leading to ineffective erythropoiesis and hemolysis. Conversely, some patients who have inherited 2 β-thalassemia mutations plus α-thalassemia can have a milder phenotype.[24]

Carrier screening and prenatal diagnosis

Relative to most genetic diseases, the prevention of the devastating Hb Bart's hydrops fetalis syndrome through carrier screening, genetic counselling and prenatal diagnostic services should be straightforward because the mutational spectrum and ethnic distribution are well defined, and carriers can be readily identified using widely available and inexpensive screening protocols (i.e., blood counts and Hb analysis).

Hb Bart's hydrops fetalis syndrome is found almost always in couples of Southeast Asian ancestry. Occasional cases have been reported in Mediterranean populations. In practice, any adult person found to have a low erythrocyte mean corpuscular volume (MCV, <80 fL) without iron deficiency should be considered a carrier of thalassemia.[19] A history of previous hydropic infants in families of Southeast Asian or Mediterranean ancestry should alert the physician to the likely possibility of α-thalassemia. On the other hand, lack of a family history of anemia does not in any way exclude the possibility of a globin gene mutation being present in the family.

The most important diagnostic criteria to detect thalassemia carriers are microcytosis (MCV <80 fL) or hypochromia (mean corpuscular Hb [MCH] <27 pg). It is noteworthy that adult carriers of deletions removing 2 α-*globin* genes (e.g., --^SEA/αα) usually do not have significant anemia. Therefore, it is important that physicians caring for patients during their adolescent or reproductive years pay attention not only to the Hb levels, but also to the MCV and MCH levels.[19] Since 2000, MCV has been added to the antenatal forms for all pregnant women in Ontario.[25]

Hemoglobin analysis and quantification of Hb A_2 (α$_2$δ$_2$) should be performed as part of the routine laboratory investigation to diagnose thalassemia carriers. Carriers of β-thalassemia trait almost always have an elevated Hb A_2 level (>3.5%). If not, the person may be a carrier of α-thalassemia. It should be noted that people who have microcytosis and high Hb A_2 can be carriers of both β- and α-thalassemia.[19] In our laboratory, α-*globin* genotypes are determined for all people referred for investigation as possible thalassemia carriers. A recently developed ELISA test to detect embryonic ζ-globin chains in adults holds promise as a rapid and reliable screening test for carriers of the --^SEA/αα type of α-thalassemia deletion.[26]

Rapid and reliable polymerase chain reaction-based DNA diagnostic tests have been developed for common deletional and non-deletional mutations. Definitive genotyping results are needed for proper assessment of possible reproductive risks and genetic counselling. For couples at risk for pregnancies with severe hemoglobinopathy syndromes, including Hb Bart's hydrops fetalis syndrome, genetic counselling and prenatal diagnosis ought to be offered.

The Ontario experience

The Province of Ontario has a population of 11 million people, with approximately 20% belonging to ethnic groups for which the carrier rates for globin gene mutations are high. Over the past decade, our laboratory has undertaken DNA-based analyses to diagnose globin gene mutations in more than 6000 Ontario residents. Of the approximately 2000 confirmed cases of α-thalassemia, more than half were heterozygous for deletions of 2 α-globin genes in tandem (--/αα). The Southeast Asian type of deletion (--^{SEA}/αα) accounted for most of these cases. The prevalence of the Filipino type of deletion (--^{FIL}/αα), removing both the ζ- and α-globin gene in tandem (Fig. 1) was approximately 10-fold less in our referral population. The remainder of the α-thalassemia cases were either heterozygous or homozygous for single α-globin gene deletions (-α/αα or -α/-α). A relatively minor proportion of cases involved triplicated or quadruplicated α-globin genes (ααα/αα or αααα/αα), or non-deletional α-thalassemia mutations (αThα/αα or ααTh/αα). In addition, we have diagnosed more than 200 cases of Hb H disease and about 40 cases of Hb Bart's hydrops fetalis syndrome.

The laboratory has also provided DNA testing for more than 4000 investigations of β-thalassemia or clinically significant β-globin chain variants (e.g., Hb S, Hb C, Hb E). As part of these investigations, we routinely screened for the common deletional forms of α-thalassemia. Overall, more than 20% of these people had concomitant α-thalassemia. Most of these were either heterozygous or homozygous for single α-globin gene deletions (-α/αα or -α/-α). Nevertheless, approximately 2% of the β-thalassemia and Hb E carriers were also heterozygous for the 2 α-globin gene deletion of the Southeast Asian type (--^{SEA}/αα). Many fewer were heterozygous for the Filipino type of deletion (--^{FIL}/αα). There are approximately 600 000 people of Southeast Asian origin living in Ontario. Given the carrier rates of α^0-thalassemia in these populations, it is estimated that annually there are 20 to 60 pregnancies at risk for Hb Bart's hydrops fetalis syndrome. Since 1989, our laboratory has undertaken prenatal testing for 85 pregnancies that were at 25% risk for Hb Bart's hydrops fetalis syndrome. Over the same period, there should have been several hundred pregnancies at risk for this devastating

syndrome in Ontario. These figures indicate that despite the availability of hospital-based genetic services in Ontario, only a small proportion of at-risk pregnancies are identified and the women provided with genetic counselling and diagnostic services.

Conclusions

Both the α- and β-globin gene clusters on chromosomes 16 and 11, respectively, are among the most thoroughly studied human genes. This review highlights the salient features of the regulation of α-globin gene expression, through cis regulatory sequences, and transacting factors coded by genes unlinked to the globin gene clusters. The variability of phenotypes in patients with identical or similar α-globin genotypes underscores the importance of genetic modifiers that can modulate the pathophysiology of these hereditary Hb disorders.[27] Future identification of these genetic loci can bring about better understanding of gene–gene interactions and possibly lead to novel therapeutic approaches.

Alpha-thalassemia is very common throughout the world, including North America. The correct diagnosis is important so as to prevent potentially harmful iron supplement therapy, prescription of oxidant drugs, and unnecessary investigations for other causes of anemia. Most important, screening for and identification of couples who are carriers of α^0-thalassemia is essential. These couples have a 25% risk in each pregnancy of conceiving a fetus with the devastating Hb Bart's hydrops felalis syndrome (fetal death in the second or third trimester and risk of serious maternal complications). Unfortunately, this public health care issue has been neglected in many jurisdictions, with catastrophic consequences for the affected families and significant associated health care costs. A concerted effort in education for practising physicians and the general public, and a coordinated program for community-based screening and counselling ought to be a high priority for present day maternal and child health care in Canada.[28]

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