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Review

The Alpha Thalassaemias

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Abstract. Recent work in the α thalassaemia field has started to provide some indication of the mechanisms involved in the very high frequency of the different forms of α thalassaemia among the populations of tropical countries, and, at the same time, is starting to define at least some of the mechanisms for its remarkable phenotypic heterogeneity. These diseases continue to provide extremely valuable models for the better understanding of the regulation of the α globin genes, and for human molecular pathology in general. The much less common disorders, ATR-16 and ATR-X are also providing valuable information about the spectrum of molecular lesions associated with different forms of mental retardation and about the molecular mechanisms involved in their varying phenotypes.

Keywords. Thalassaemia, globin genes, globin synthesis, population genetics, malaria.

Introduction

The inherited disorders of haemoglobin, the haemoglobinopathies, are the commonest monogenic diseases. It has been estimated that approximately 7 % of the world's population are carriers, and that 300,000 to 500,000 babies are born with severe homozygous or compound heterozygous forms of these conditions each year [1]. They consist of the structural haemoglobin variants, haemoglobins S, C and E in particular, and disorders that result from the defective synthesis of the α or β globin chains that constitute adult haemoglobin, the α and β thalassaemias. These diseases occur at a particularly high frequency in populations of the tropical belt or in countries with large immigrant populations from this region [2].
In the case of α thalassaemia there are two other less common forms that are not restricted to populations in which the haemoglobinopathies occur at high frequencies. First, there is the heterogeneous syndrome of α thalassaemia associated with mental retardation. Second, there is the well-defined association between α thalassaemia and certain forms of myelodysplasia and related haematological disorders, particularly in the elderly.
The α thalassaemia field has been extensively reviewed [3–5]. Here, we focus on recent information regarding its population genetics and dynamics, molecular pathology, and what is known about the complex interactions between phenotype and genotype in the many and heterogeneous forms of the condition.

Genetic control of human haemoglobin

The different oxygen requirements during embryonic, fetal and adult life are reflected in the synthesis of different haemoglobins at each stage of development. They all have the same general tetrameric structure, however, consisting of two different pairs of globin

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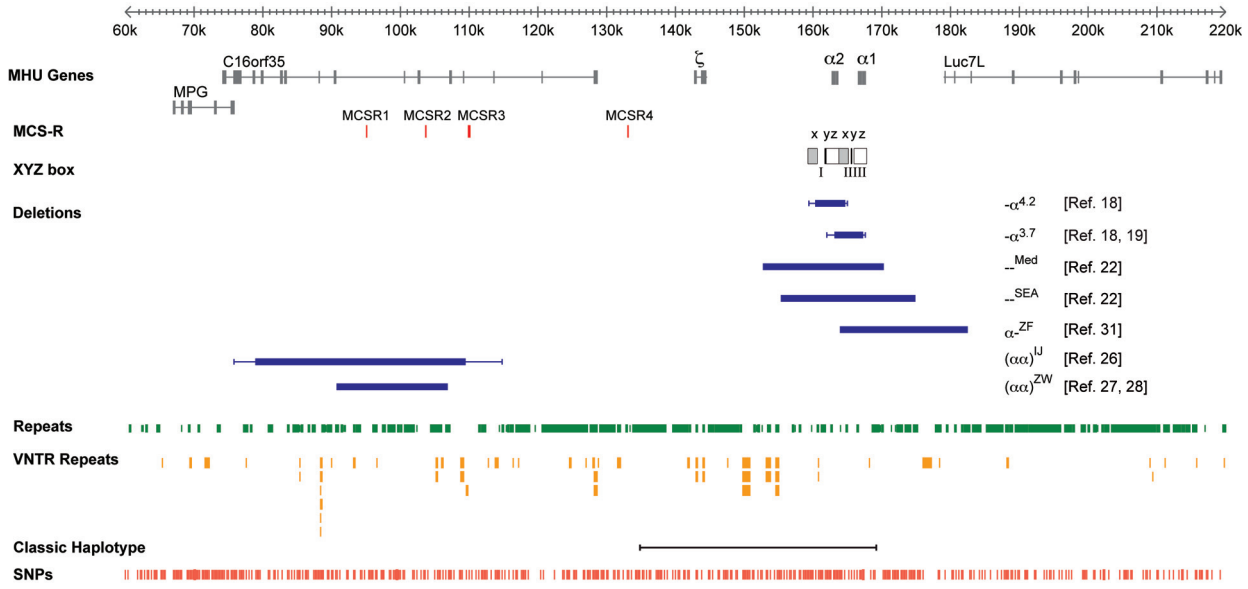

Figure 1. The human α globin cluster (5'ζ-α2-α1–3') surrounded by widely expressed genes (*MPG, C16orf35 and Luc7L*) on chromosome 16 (16p13.3). Below this the multispecies conserved elements (MCSs) are shown. The X, Y and Z boxes are the regions of duplication which play a part in the generation of the common α^+ thalassaemias, as discussed in the text. The most common deletions causing α^+ ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) and α^0 ($--^{Med}$ and $--^{SEA}$) thalassaemia are shown as horizontal bars as are the unusual deletions $(\alpha\alpha)^{IJ}$, $(\alpha\alpha)^{ZW}$ and α^{-ZF} and two deletions that remove the MCS elements. For a full catalogue of all deletions that cause α thalassaemia see ref [4]. At the bottom of the Figure the positions of common repeats, VNTRs and SNPs are shown. The region containing all SNPs and VNTRs corresponding to the classical haplotype used in population studies (as discussed in the text) is illustrated as a thin horizontal line.

chains, each attached to one haem molecule. Adult and fetal haemoglobins have α chains combined with β chains (Hb A, $\alpha_2\beta_2$), δ chains (Hb A_2, $\alpha_2\delta_2$) and γ chains (Hb F, $\alpha_2\gamma_2$). In embryos, α-like chains called ζ chains combine with γ chains to produce Hb Portland ($\zeta_2\gamma_2$) or with ε chains to make Hb Gower 1 ($\zeta_2\varepsilon_2$), while α and ε chains form Hb Gower 2 ($\alpha_2\varepsilon_2$).

The α-like and β-like globin chains are regulated by clusters of genes on chromosomes 16 and 11, respectively. The α-like gene cluster is located close to the telomere of chromosome 16 (16p13.3). The functional genes are arranged along the chromosome in the order telomere-ζ-α2-α1-centromere surrounded by widely expressed genes (Fig. 1). Upstream of the α cluster there are four highly conserved non-coding sequences, or multispecies conserved sequences (MCS), called MCS-R1 – R4 that are thought to be involved in the regulation of the α-like globin genes (Fig. 1). Of these elements, only MCS-R2 (also known as HS-40) has been shown to be essential for α globin expression [6]. As haemopoietic progenitors commit to the erythroid lineage and begin to differentiate to form mature red cells, key erythroid transcription factors and various co-factors progressively bind to the upstream elements and the promoters of the α-like globin genes. Finally, RNA polymerase II is recruited both to the upstream regions and the globin promoters as transcription starts in early erythroid progenitors. At the same time, the upstream elements and promoters of the globin genes interact with one another via the formation of chromatin loops [7].

As well as the globin genes and regulatory regions, the α globin gene cluster contains many single nucleotide polymorphisms (SNPs) some of which alter restriction enzyme sites. In addition, there are regions containing variable numbers of tandem repeats (VNTRs) (Fig. 1). Studies of the different arrangements of these polymorphisms and VNTRs in various population samples have made it possible to define a series of α globin-gene haplotypes (classical haplotypes, Fig. 1) that have been of considerable value in an analysis of both the evolutionary aspects of the gene clusters and in defining the origins of many of the α-thalassaemia mutations [8].

Readers who wish to learn more about the molecular and cell biology of the α gene cluster are referred to more extensive reviews [3,4].

Classification of the common α thalassaemias

The α thalassaemias are classified broadly into the α^0 thalassaemias, in which no α chains are produced from the affected chromosome, and the α^+ thalassaemias in which there is a reduced output of α chains from the particular chromosome [5]. The normal α genotype can be represented as αα/αα. The homozygous and heterozygous states for α^0 thalassaemia, which most

commonly result from deletions of both of the linked α globin genes, are represented as --/-- and --/αα, respectively. The α^+ thalassaemias may result from the deletion of one of the linked pairs of α globin genes or from a mutation (T) that inactivates one of the pair. The homozygous and heterozygous states for the deletion forms are represented as -α/-α and -α/αα, respectively. Similarly, those for the non-deletion forms of α^+ thalassaemia are represented as $\alpha^T\alpha/\alpha^T\alpha$ and $\alpha^T\alpha/\alpha\alpha$, respectively. It follows, therefore, that when we speak of an 'α thalassaemia gene' what we are often referring to is a haplotype; that is, the state of function of both of the linked α globin genes.

Once the molecular pathology of either the deletion or non-deletion forms of α thalassaemia has been determined it is useful to add a superscript to define the origin of the mutation. For example, there are particularly common forms of α^0 thalassaemia due to different deletions in South East Asia or the Mediterranean region; heterozygous states for these conditions can therefore be represented as $--^{SEA}/\alpha\alpha$ or $--^{MED}/\alpha\alpha$ (Fig. 1). As discussed later, while these are the commonest forms of α thalassaemia, there are many other mutations that result in this condition that do not fit easily into this general classification.

As the result of severe α chain deficiency in fetal life excess γ chains are synthesised which form γ_4 tetramers which form a viable haemoglobin called Hb Bart's; in adult life excess β globin genes also form a viable tetramer with the structure β_4, or HbH. Due to their very high oxygen affinity Hbs Bart's and H are not functional haemoglobins, and HbH is unstable and precipitates in older red cell populations. The two main groups of clinical disorders that result from the interaction of these different forms of α thalassaemia reflect the homozygous state for α^0 thalassaemia, which is not compatible with survival and is called the Hb Bart's hydrops fetalis syndrome, and the compound heterozygous state for α^0 and α^+ thalassaemia, which results in a disorder of variable severity called HbH disease. Both these conditions are highly heterogeneous and there are many exceptions to these general rules about their pathogenesis.

Distribution and population genetics

Overall, the α thalassaemias follow a similar distribution to the β thalassaemias, extending from sub-Saharan Africa through the Mediterranean region and Middle East, to the Indian sub-continent and East and South East Asia [5].

The α^0 thalassaemias reach their highest frequency in South East Asia, where in some regions more than 10% of the population are carriers. They also occur commonly in the Eastern Mediterranean islands and mainland populations but have only been reported sporadically in the Middle East and Indian sub-continent.

The deletion forms of α^+ thalassaemia occur at a much higher frequency right across the tropical belt. In this region the carrier frequency ranges from 2 – 70% and some populations, the Tharu people of southern Nepal and those of the northern coasts of Papua New Guinea for example, have carrier frequencies of 80% or more. The spectrum of mutations that underlie the α thalassaemias vary widely in these different populations, indicating that they have almost certainly arisen locally and that their frequency has expanded by natural selection together with some degree of founder effect and gene drift. There is strong evidence that selection reflects past or present exposure to malaria. Case-control studies in Papua New Guinea and Africa have shown that both the homozygous and heterozygous states for α^+ thalassaemia offer highly significant protection against the serious complications of malaria due to *Plasmodium (P) falciparum* [9–11]. Interestingly, studies in Vanuatu and Papua New Guinea have shown that young children with α^+ thalassaemia are more prone to infection with the malarial parasite *P. vivax* which causes a milder form of malaria; since there is cross immunity between the two common species of human malarial parasites it has been suggested that prior infection with *P. vivax* may reduce the severity of subsequent *P. falciparum* infection [12]. However, since *P. vivax* infection is extremely rare in sub-Saharan Africa this protective effect would only be mediated in certain Asian populations.

Clearly there must be other protective mechanisms involved [13,14]. There is no evidence for a reduced rate of invasion or growth of *P. falciparum* in red cells of individuals with α^+ thalassaemia [13]. However, it has been found that these cells consistently bind more malaria immunoglobulin than normal red cells and that α thalassaemic red cells infected with parasites appear to be more susceptible to phagocytosis *in vitro* and are less able than normal red cells to form rosettes, an *in vitro* phenomenon whereby uninfected cells bind to infected cells. It has also been found that complement receptor 1 (CR1) expression, which is required for rosette formation, is reduced on α thalassaemia red cells. Recently, detailed statistical analyses of the patterns of profound anaemia due to *P. falciparum* malaria have suggested that the significant increase in the red-cell count of those who are homozygous for α^+ thalassaemia may be at least in part protective against this often-lethal complication [15]. Overall, it appears that the protective effect of α thalassaemia against malaria is mediated both at the cellular and immune

levels, a pattern that is emerging in the case of other inherited haemoglobin disorders that have come under selection by malaria, notably Hbs S and C [13,14].

Recent studies in Africa have emphasized the complexity of the population genetics of these conditions [16]. While both α thalassaemia and the heterozygous state for the sickle cell gene offer considerable protection against the severe complications of *P. falciparum malaria*, in individuals who inherit both polymorphisms the protective effect is completely lost. This elegant example of negative epistasis, as well as underlining the complexity of the interactions of protective polymorphisms, also offers some valuable information about the potential cellular protective mechanisms involved. For example, the co-inheritance of α thalassaemia in those with the sickle-cell trait results in a highly significant reduction of the level of HbS in their red cells, suggesting that a critical level of HbS is required for protection against *P. falciparum* malaria.

As in some of the other haemoglobinopathies, relatively high levels of α thalassaemia have been observed in populations in which malaria has never, as far as is known, been prevalent. For example, gene frequencies of 1 % to 15 % have been reported in parts of the South West Pacific, from Fiji in the west to Tahiti and beyond in the east, and in the Micronesian atolls. However, it has been found that almost 100 % of α^+ thalassaemia in these regions can be accounted for by a single mutation that had previously been defined in Vanuatu [17]. Furthermore, this mutation occurs on the common Vanuatuan α-globin gene haplotype, an observation which provides strong evidence that the occurrence of the α thalassaemia gene in these non-malarious areas is the result of population migration and genetic drift.

Molecular pathology

The linked α-globin genes are embedded within two highly homologous 4kb duplication units, the sequence identity of which appears to have been maintained throughout evolution by gene conversion and unequal crossing over events. These regions are divided into homologous subsegments, X, Y and Z, by non-homologous elements, I, II and III (Fig. 1). Reciprocal recombination between Z segments, which are 3.7kb apart, produces chromosomes with only one α gene ($-\alpha^{3.7}$, rightward deletion, Fig. 1) that causes α thalassaemia and others with three α genes ($\alpha\alpha\alpha^{anti3.7}$). Since the crossover events in the Z box can occur in different sites, several different forms of $-\alpha^{3.7}$ deletions can be generated. Similarly, recombination events between the X boxes, which are 4.2kb apart give rise to an α thalassaemia determinant ($-\alpha^{4.2}$, leftward deletion, Fig. 1) and an $\alpha\alpha\alpha^{anti4.2}$ chromosome [18, 19]. Recombination events can give rise to chromosomes with up to five α genes. Since it is only the α thalassaemia genes that have come under selection, chromosomes with increased numbers of α genes occur at a low frequency in many populations. In addition to these extremely common forms of α^+ thalassaemia, a few result from other rare deletions in the α gene cluster. Recent work suggests that these reciprocal recombination events occur during mitosis in the germ line and that the frequencies of -α and ααα arrangements in sperm are in the order of $1-5x10-^5$ [20]. Given the high frequency of these events it has been suggested that in populations in which there is no α thalassaemia, there must be selection against -α/-α and ααα/ααα homozygotes [21].

Deletional events are also by far the commonest cause of the α^0 thalassaemias. While many of them are quite rare, some occur at relatively high frequencies in particular regions, $--^{MED}$ and $-^{SEA}$, for example [22]. The underlying deletions either completely or partially delete both α globin genes. In many cases the embryonic ζ–globin genes are also deleted. Following the completion of the DNA sequence of 16p13.3 and beyond, it has been now possible to define the full extent of many of these deletions. They can be subdivided into those which lie entirely within the α globin gene cluster and those that extend beyond the cluster to include its flanking regions. More extensive deletions associated with monosomy for a large segment of 16p13.3 result in the syndrome of α thalassaemia and mental retardation (see later section).

A detailed analysis of several different deletion forms of α^0 thalassaemia have shown that they frequently result from illegitimate or non-homologous recombination events, often involving the dispersed family of Alu repeats [23]. In addition, sequence analysis of the junctional regions of the deletions have revealed other interesting features including palindromes, direct repeats, regions of weak homology, and the frequent occurrence of the motif GAGG. Some deletions involve complex rearrangements that introduce new pieces of DNA bridging the two break points. It has recently been shown that these complicated rearrangements may result from slippage and strand switching at sites of microhomology during DNA replication [24].

The α^0 thalassaemias can also result from deletions of the upstream α-globin regulatory elements; although the α genes remain intact they may be completely inactivated by lesions of this kind. Since the first description of a deletion of this type [25], which

removes 62kb including the HS-40 (MCS-R2) element, more than 20 patients with α^0 thalassaemia have been described with deletions involving this region (see examples $(\alpha\alpha)^{IJ}$ and $(\alpha\alpha)^{ZW}$ in Fig. 1 [26–28]). Subsequent experiments in transgenic mice suggest that HS-40 (MCS-R2) is the major regulatory element that is lost in these arrangements [29]. Like the deletions that remove the α globin genes, at least some of these deletions seem to result from recombination events between partially homologous Alu repeats and subtelomeric rearrangements.

In addition, there have been single case reports of other novel deletions that result in the phenotype of α^0 thalassaemia. In one case a deletion completely removed the α2 gene and the 3' break point lay within the promoter of the linked α1 gene (Rugless et al., in preparation). In another case, a deletion involving the α1 gene also inactivated the intact linked α2 gene. Subsequent studies indicated that this particular deletion juxtaposes a down-stream gene *(Luc7L)* next to the structurally normal α2 gene (α^{-ZF} in Fig. 1). Remarkably, it was demonstrated that transcription of anti-sense mRNA from *Luc7L* through the α2 gene was responsible for methylation of the associated CpG island and silencing of α globin expression, a hitherto unreported mechanism underlying human genetic disease [30, 31].

Non-deletion types of α thalassaemia

Since the first description in 1977 [32], at least 69 forms of non-deletion α thalassaemia have been reported, 46 in the α2 gene, 17 in the α1 gene, and 5 on a -α chromosome ($-\alpha^T$) [4]. It is interesting to compare the relative paucity of non-deletion forms of α thalassaemia with the β thalassaemias, in which in excess of 200 different mutations have been reported affecting almost every stage of gene expression. Gene deletions are very rarely found as the cause of β thalassaemia yet, as we have seen, there are many different deletion forms of α thalassaemia that reach extremely high gene frequencies. Although, as described earlier, there are possible explanations for the relatively high frequency of the molecular events that lead to the deletion forms of α thalassaemia, the reasons for these discrepancies in the underlying mutations that cause these two extremely common diseases are still far from clear.

The mutations that cause the non-deletional α thalassaemias, like those that cause β thalassaemia, act at different levels of gene regulation and expression. They include variants that involve RNA splicing, the poly(A) addition signal, initiation of mRNA translation, defective translation due to frame shifts and nonsense mutations, the production of extended α-globin variants due to chain-termination mutations, and highly unstable α-chain variants that are associated with the phenotype of α thalassaemia.

While many of the deletions that cause this form of α thalassaemia are predictable in their effect, many questions remain. For example, those that occur in the highly conserved sequence motif AATAAA have a variable effect on α gene expression. In some cases it appears that two mechanisms may be involved; a reduction in the amount of α2mRNA and, possibly, read-through of transcripts extending beyond the mutation site that may interfere with the expression of the linked α1 gene [33, 34]. However, this type of phenomenon is not known to occur in all the mutations of the poly(A) additional signal. Similar questions arise regarding the precise pathophysiology of the chain-termination mutations [35]. There are potentially nine single nucleotide variants of the termination codon TAA of the α2 globin gene. Two encode stop (non-sense) mutations while the others encode amino acids. The latter allow mRNA translation to continue to the next in-phase termination codon (UAA); in each case an α chain extended by 31 amino acids is produced. Of the six predicted variants, five have been described, each with a unique amino acid at α142. Of these, Hb Constant Spring (α142Gln) appears to be the commonest, reaching frequencies of up to 4% of the population in Thailand [5]. These elongated α chain variants do not appear to be unstable but several observations have suggested that α^{cs} mRNA is unstable, possibly due to inappropriate translation of the 3' non-coding region wherein the disrupted sequences are located [36, 37].

Quite recently, yet another non-deletional form of α thalassaemia has been described with a completely novel mutational basis [38]. In short, it results from the creation of a new promoter-like sequence between the α globin genes and their regulatory elements. It has been suggested that this new promoter may 'steal' the activity of the upstream regulatory elements away from the natural α globin promoters and hence down regulate their expression.

For a more detailed discussion of the non-deletion α thalassaemias the reader is referred to a recent review [4].

Phenotype/Genotype Relationships

If it is assumed that a critical excess of γ or β globin chains has to be produced before the resulting homotetramers, Hb Bart's (γ_4) and HbH (β_4) are present in significant amounts in red cells, then many forms of α thalassaemia can be viewed as being the

result of a straightforward gene dosage effect. Heterozygotes (-α/αα or $\alpha^T\alpha/\alpha\alpha$) have minimal haematological changes with no detectable HbH. Homozygotes for deletional variants (-α/-α) have no detectable HbH while those for non-deletional forms ($\alpha^T\alpha/\alpha^T\alpha$) have significantly higher though variable levels of HbH; both conditions have the phenotype of mild α thalassaemia trait with very low levels of HbH. Compound heterozygotes for α^+ and α^0 thalassaemia (-α/-- or $\alpha^T\alpha$/--) have the phenotype of HbH disease with 5 – 30% HbH, and homozygotes for α^0 thalassaemia (--/--) are stillborn with the Hb Bart's hydrops syndrome with approximately 80% Hb Bart's and no HbA. However, while these phenotypes are in general agreement with the concept of a gene dosage effect, in practice the situation is considerably more complicated.

One of the main reasons for the phenotypic diversity of the α thalassaemias is that the overall defect in α chain synthesis appears to be more severe in the non-deletional as compared with the deletional forms of α thalassaemia. This probably reflects the fact that when the α2 gene is deleted the output from the linked α1 gene appears to be increased, whereas if the α2 gene is inactivated by a point mutation such compensation may not occur. Thus the non-deletional forms of α^+ thalassaemia, on the whole, tend to have a more severe phenotype; in some cases the homozygous state may be associated with the phenotype of HbH disease [39]. Furthermore, there is at least some evidence that the forms of HbH disease that result from compound heterozygosity for non-deletional forms of α^+ thalassaemia and α^0 thalassaemia are, overall, more clinically severe than those that result from compound heterozygosity for a deletional form of α^+ thalassaemia and α^0 thalassaemia [40]. But this is not the only mechanism for the phenotypic diversity of HbH disease. In countries in which the latter form of HbH disease is common there is also considerable phenotypic heterogeneity, much of which remains to be explained.

While the Hb Bart's hydrops fetalis syndrome usually results from homozygosity for α^0 thalassaemia, in a small number of cases it has been found to have the genotype --/$\alpha^T\alpha$, where the associated non-deletion α thalassaemia allele has been of the more severe variety. And despite the relative genetic uniformity of Hb Bart's Hydrops fetalis syndrome there is considerable phenotypic variation. For example, associated developmental abnormalities have been reported in up to 17% of cases and there is considerable variation in the pace and extent of abnormal development in fetal life [41]. Although these observations have been explained as being the result of variation in the severity of intra-uterine anaemia, this is difficult to prove and may not be the whole story [5]. In short, although there are reasonable correlations between phenotype and genotype in the α thalassaemias, many questions remain unanswered. Because of its very high frequency in many parts of Southeast Asia these questions are of considerable importance in relationship to HbH disease, particularly for genetic counselling, decisions about pre-natal diagnosis, and, in the future, the case for the application of novel forms of therapy.

The wide variety of clinical disorders that arise from interactions between the diverse α thalassaemia alleles and α or β structural haemoglobin variants is reviewed elsewhere [5].

α Thalassaemia and mental retardation

There are two distinct syndromes in which α thalassaemia is associated with mental retardation (ATR) [42–44]. The first, ATR–16, is characterised by large chromosomal rearrangements or sub-telomeric deletions that remove many genes from the short arm of chromosome 16. The second, ATR–X, is a complex phenotype which includes α thalassaemia, multiple developmental abnormalities and mental retardation due to mutations at a locus on the X chromosome, the product of which is a regulator of α-gene expression during development.

The definition of the ATR–16 syndrome was important because it provided the first evidence that mental retardation may result from cryptic, sub-telomeric rearrangements and truncations, an observation that was later found to underlie a significant proportion of unexplained mental retardation [45, 46]. The condition results from large chromosomal rearrangements that delete many genes from the short arm of chromosome 16. Conventional cytogenic analysis has revealed a number of different cytogenetic abnormalities, although in some cases they are not present and the condition has been identified by variation in the number of VNTRs within the α gene cluster. Using a combination of conventional cytogenetics, FISH and molecular analyses, a variety of chromosomal arrangements either inherited or *de novo* have been observed, including translocations, inversion/deletions and sub-telomeric truncations [26, 47, 48]. The associated chromosomal abnormalities which cause them vary widely, and it is not surprising that the phenotype of this condition also shows remarkable variability with respect to both the degree of mental retardation and associated developmental abnormalities.

By analysing the length and mechanism of these deletions it has been possible to obtain at least some information about the basis for the related phenotype. Small sub-telomeric deletions of up to ~900kb (associated with monosomy for 16p only) seem to be associated with a normal phenotype. Longer deletions (~900 – 2000 kb), may be associated with a variable degree of mental retardation, while deletions in excess of 2000kb, because they involve the loci responsible for tuberous sclerosis type 2 and polycystic kidney disease type 1, result in contiguous gene syndromes characterised by α thalassaemia together with these conditions [49, 50]. The precise nature of the genes that are lost in producing the ATR-16 syndrome remains to be determined.

The phenotype associated with ATR-X is usually characterised by more severe mental retardation and consistent developmental abnormalities than ATR-16. The gene for ATR-X, *ATRX*, is located on the X chromosome (Xq13.1-q21.1) [51]. Its product is a chromatin-associated protein which is a member of the SNF2 family of chromatin-remodelling proteins [52]. It has two highly conserved domains. At the N-terminus there is a plant homeodomain (PHD)-like zinc finger, while at the C-terminal half of the protein there is a helicase-adenosine triphosphatase (ATPase) domain. Currently, some 126 different constitutional mutations have been described, restricted mainly to the two main domains [53]. These mutations have been associated with changes in the pattern of methylation of several highly repeated sequences, including rDNA arrays, a Y-specific satellite and sub-telomeric repeats, observations that offer a link between the process of chromatin remodelling, DNA methylation and gene expression during development [54].

In general, the severe phenotype of patients with ATR-X syndrome is remarkably uniform, regardless of the mutations. However, as more cases have accumulated some correlations between the position of mutations and the phenotype have emerged [53]. For example, psychomotor impairment may be more severe in N-terminus mutations, while those affecting the C-terminus are more frequently associated with severe genital abnormalities. Similarly, the severity of the associated α thalassaemia, as assessed by the amount of HbH produced, also appears to reflect, at least in part, whether the associated mutations involve the N- or C-termini.

Despite considerable progress in defining the phenotype of ATR-X syndrome, the properties of the protein and the consequences of mutations, there is still little information about the function of this protein and, in particular, its role in α globin expression. A key issue that remains to be solved is how a protein that associates with heterochromatin and is required for a normal pattern of DNA methylation at repetitive DNA sequences influences the expression of euchromatic genes. It is of particular interest that the locus involved in this syndrome also appears to be involved in a considerable number of cases of X-linked mental retardation without an α thalassaemic phenotype.

α Thalassaemia associated with myelodysplasia

Acquired mutations in *ATRX* are found in the blood cells of patients with this syndrome, a pre-leukaemic disorder which occurs predominantly in elderly males although some clear examples in females have been recently observed [55–57]. Overall, the severity of the α thalassaemia and the associated level of HbH production is greater than that observed in the α thalassaemia mental retardation syndromes. Although fewer mutations have been defined than for ATR-X, they also appear to be divided more or less equally between the two major domains of *ATRX* [58, 59]. It is very unlikely that mutations of *ATRX* are involved in the pathogenesis of the myeloproliferative syndrome. Rather they are probably acquired as passenger mutations. What is not clear is why the same mutation in *ATRX* in cases of ATR-X syndrome causes a mild form of thalassaemia whereas when these mutations occur in the setting of a myeloproliferative disease they cause much more severe HbH disease. It seems likely that the *ATRX* mutation is somehow made more severe when it interacts with one or more of the common genetic or epigenetic mutations in a myeloproliferative disorder. Hence further analysis of these conditions may shed some important light on the molecular basis of some of the common forms of myelodysplastic syndrome.

Readers who wish to obtain more detailed information about the forms of α thalassaemia associated with mental retardation or myelodysplasia are referred to several recent reviews [53, 60].

Clinical Implications

The Hb Bart's hydrops syndrome is characterized by fetal death just before or at term associated with considerable maternal morbidity due to the increased frequency of eclampsia and post-partum problems due to a massively enlarged placenta. For this reason carrier detection and pre-natal diagnosis is carried out widely for this condition in many Asian countries [5]. A few babies with this condition have been rescued by intra-uterine transfusion but of course they are then

transfusion-dependent for the rest of their lives. Intrauterine bone-marrow transplantation has been tried in a small number of cases without success.

As mentioned earlier, HbH disease has a remarkably variable phenotype. Although pre-natal diagnosis for this condition is offered in some countries it would be much more acceptable were it possible to be able to predict in advance the clinical course of the illness; it is mild in most cases. Although, as discussed earlier, some progress has been made, much has still to be learnt, particularly about the reasons for the phenotype diversity of the deletional forms.

The global burden posed by the different forms of thalassaemia, including the α thalassaemias, and current approaches to the control and better management of these conditions in the developing countries is the subject of several recent reviews [2, 61, 62].

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