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*Review article*

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## A newly defined X linked mental retardation syndrome associated with $\alpha$ thalassaemia

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In 1981 three northern European families were described in which a severely mentally retarded son also had haemoglobin H (Hb H) disease.<sup>1</sup> These findings were of interest because Hb H disease, a relatively severe manifestation of  $\alpha$  thalassaemia, is rare in northern Europeans although it is frequently seen in Mediterranean and Oriental racial groups in which it is not known to be associated with an increased frequency of mental retardation. Furthermore, whereas the common forms of Hb H disease are always inherited in a mendelian fashion, in these northern European families this appeared not to be so.

Hb H disease occurs when a greater than 50% reduction in synthesis of the  $\alpha$  globin chains of adult haemoglobin (Hb A,  $\alpha_2\beta_2$ ) results in the accumulation of excess  $\beta$  globin chains which form  $\beta_4$  tetramers (Hb H). The common mendelian forms of Hb H disease result from mutations of both allelic  $\alpha$  globin complexes, most commonly owing to deletions or less frequently to small rearrangements or point mutations. The  $\alpha$  globin complex is located close to the telomere of the short arm of chromosome 16, within band 16p13.3.<sup>2</sup>

By 1990, a total of 13 subjects with  $\alpha$  thalassaemia and mental retardation (ATR) had been identified and two distinct syndromes were delineated.<sup>3,4</sup> Eight patients had large (1 to 2 megabases) deletions of the tip of chromosome 16p; the clinical features of this so called ATR-16 syndrome were rather variable, in

part because some patients had additional chromosomal aneuploidy. By contrast, the five other patients (four boys and one XY female), in whom no deletion or other abnormalities of the  $\alpha$  globin complex could be detected, had a remarkably uniform phenotype, comprising severe mental retardation, characteristic dysmorphic facies, genital abnormalities, and an unusual, mild form of Hb H disease. It was proposed that these five 'non-deletion' cases represented a distinct syndrome that probably mapped to the X chromosome.

Evidence from two additional families<sup>5-7</sup> has subsequently strengthened the suggestion that these non-deletion cases represent a distinct and recognisable X linked syndrome which has been named the X linked ATR syndrome (ATR-X).<sup>7</sup> The three case reports in this issue consolidate the evidence for X linkage and extend knowledge of the clinical and haematological phenotype of the affected subjects. In addition, they provide some important, provisional observations on the phenotype of carriers of this disorder. This review summarises the findings in the 16 cases published to date.<sup>4-10</sup>

### **The clinical features of the ATR-X syndrome**

In all cases of the ATR-X syndrome mental handicap is severe, global, and apparent from an early age. Usually the pregnancy and delivery are without complications and the birth weight is normal. However, hypotonia and feeding difficulty are commonly apparent in the first few days of life and all developmental milestones are subsequently delayed. Sitting is usually delayed and, at best, walking starts in the third year; in four cases walking has not been achieved by adulthood. Speech is absent in all but one case<sup>10</sup> and even then is limited to a few words; comprehension is usually very poor. None has achieved more than partial bowel or bladder control.

Other abnormal neurological findings have been observed but none appears to be specific to this syndrome. Seizures occur in seven of the patients.

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Hearing and vision are usually normal, although strabismus has been observed in five, and in one case<sup>4</sup> visual impairment with prolonged visual evoked responses was noted. Although hypotonia often persists from birth some patients have developed spasticity. A CT brain scan has been performed in seven subjects and in two has shown cerebral atrophy.

The dysmorphic facies are characteristic and are less than typical in only one<sup>8</sup> out of the 16 cases reviewed. Usually there is telecanthus, epicanthic folds, a flat nasal bridge, and mid-facial hypoplasia. The nose is rather small and triangular and the nares anteverted. The mouth has been described as 'carp-like' with full lips; the tongue is usually enlarged and frequently protruding. The teeth are often abnormal; crowding may occur but a more common finding is wide spacing of the incisors. The ears are often affected, being described variously as small and simple, deformed, low set, or posteriorly rotated. Facial features appear to coarsen with age and the nasal bridge often becomes more pronounced. Head circumference is usually less than the 3rd centile for age.

Genital anomalies are usually seen. Testicular abnormalities range from small, high lying testes through cryptorchidism to severe dysgenesis. An inguinal hernia has been reported in association with an undescended testis.<sup>8</sup> The scrotum may be hypoplastic or shawl-like and the penis small. Hypospadias has been reported in two brothers.<sup>10</sup> At one extreme, a single case was observed with female external genitalia in association with abdominal dysgenetic testes.<sup>4</sup>

A wide range of skeletal abnormalities has been noted. Bone age may be delayed. A variety of minor abnormalities of the fingers and toes has been described and the digits may appear tapering. Kyphosis or scoliosis was reported in five cases and hemivertebra with a missing rib in a single case. Talipes

was noted in three cases. Short stature is a feature in half the cases. In some of these growth retardation has been apparent throughout life whereas in others it has become manifest at a later stage, for example, around the time of the pubertal growth spurt.

The most common gastrointestinal problem is regurgitation of food which has been a troublesome feature in several cases. This may usually be a behavioural problem but severe gastro-oesophageal reflux requiring fundoplication occurred in one case.<sup>4</sup> Constipation is frequent; though not severe in the majority of cases, faecal impaction and rectal prolapse are known to have occurred in one case. Other systems may also be involved. Umbilical hernia was present in two out of four affected boys in one family.<sup>10</sup> Single cases have also been reported of a perimembranous ventricular septal defect,<sup>8</sup> unilateral renal agenesis with contralateral hydronephrosis,<sup>9</sup> and small joint swelling.<sup>5</sup>

In summary (table), the hallmarks of the ATR-X syndrome are severe mental retardation, characteristic facies, and genital anomalies. While this comprises a recognisable phenotype confusion might occur with other conditions. In particular, there is some overlap of clinical features with the Coffin-Lowry, Angelman, FG, and Smith-Lemli-Opitz syndromes and the coarse appearance may superficially resemble some of the lysosomal storage disorders; it may be important to exclude the ATR-X syndrome whenever these diseases present in an atypical fashion. Fortunately, the diagnosis of the ATR-X syndrome can be confirmed or refuted by haematological testing.

#### Subjects with the ATR-X syndrome have an unusually mild form of Hb H disease

It was apparent from the original report that patients with the ATR-X syndrome have a milder type of Hb H disease (with less hypochromia and lower

*The clinical features in 16 subjects with the ATR-X syndrome*

	Patients*															
	PT	SW	TH	NE	PE	DE	GA	MC	RH	DH	CR	DO	PA	JA	DA	RA
Severe mental retardation	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Normal birth weight†	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neonatal hypotonia	+	+	+	+	+	NA	+	NA	+	+	NA	+	+	-	+	+
Seizures	+	+	+	-	-	+	-	+	-	-	+	-	-	-	-	+
Characteristic facies	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Microcephaly‡	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-
Genital abnormalities	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Skeletal abnormalities	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+
Short stature§	NA	+	+	-	NA	+	-	+	+	-	+	+	+	+	+	+

\* PT, SW, TH, NE, and PE are the patients described in reference 4. DE is the proband in reference 5. GA is the proband in reference 6. RH, DH, and MC are cases 1, 2, and 3 in reference 8. DO is the proband (IV:2) and CR is case 2 (III:7) in reference 9. PA, JA, DA, and RA are cases 1 to 4 respectively in reference 10.

†+ indicates birth weight within 2 SD of mean for gestational age.

‡+ indicates OFC less than 2 SD of the mean for age.

§+ indicates height less than 2 SD of the mean for age.

NA indicates data not available.

levels of Hb H) than that found in the mendelian forms of Hb H disease.<sup>4</sup> However, since these original cases were all ascertained haematologically it is likely that they represent the severe end of the haematological spectrum of the ATR-X syndrome.

Subsequent cases, identified initially by their characteristic dysmorphic facies and associated clinical abnormalities,<sup>6-10</sup> have included some subjects with the ATR-X syndrome who have normal (case 1 in reference 10) or only marginally abnormal (case 3 in reference 8) haematological indices (figs 1 and 2). Hb H inclusions (fig 3) can always be shown after incubation of red cells with 1% brilliant cresyl blue (BCB), but the proportion of cells in which they are found varies widely, from 0.8% to 40%. The level of Hb H detected electrophoretically has ranged from 0% to 6.7% and is correlated with the proportion of cells in which Hb H inclusions can be found. Hb H is not detectable by electrophoresis when the proportion of Hb H positive cells falls below 3%. The variation in the haematological findings noted between unrelated cases may also be seen within the same pedigree.<sup>8,10</sup> This variation appears to be independent of iron status or age.

When screening for the ATR-X syndrome, therefore, normal haemoglobin level and red cell indices do not exclude the condition nor does the inability to show Hb H electrophoretically. The most sensitive test is the demonstration of Hb H inclusions in red

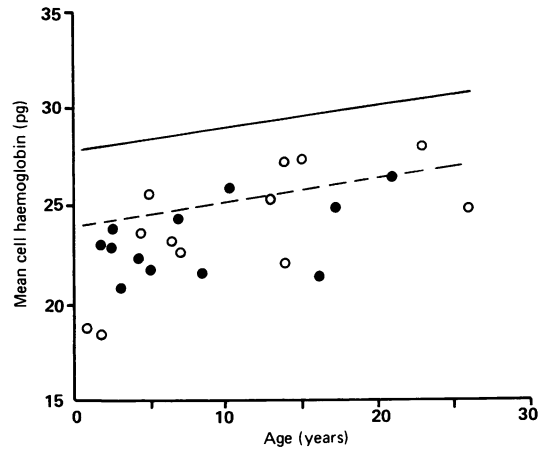


Figure 2 MCH levels in subjects with the ATR-X syndrome at various ages. Annotations as for fig 1.

cells after incubation with 1% BCB. This examination is best performed on a fresh sample of blood and the efficacy with which Hb H inclusions can be found may depend on the batch and freshness of the preparation of BCB used. We have found that incubation is best performed at room temperature; incubation at 37°C may occasionally induce very rare artefactual inclusions similar to those seen in  $\alpha$  thalassaemia (see reference 9 for example). However, this situation will not be confused with the appearance in affected boys in whom Hb H inclusions can be shown in many red cells (greater than one per high power field).

### The evidence for an X encoded syndrome

Analysis of the  $\alpha$  globin complex in patients with the ATR-X syndrome has indicated no abnormality.<sup>4</sup>

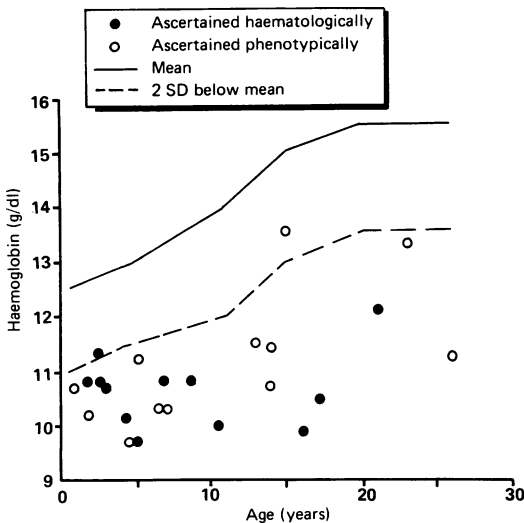


Figure 1 Haemoglobin levels in subjects with the ATR-X syndrome at various ages. Solid line indicates the mean and dashed line 2 SD below the mean.<sup>11</sup> Results are divided into those cases that were ascertained haematologically or phenotypically. For any subject only one result within each consecutive 5 year period is given and no results below the age of 6 months are included.

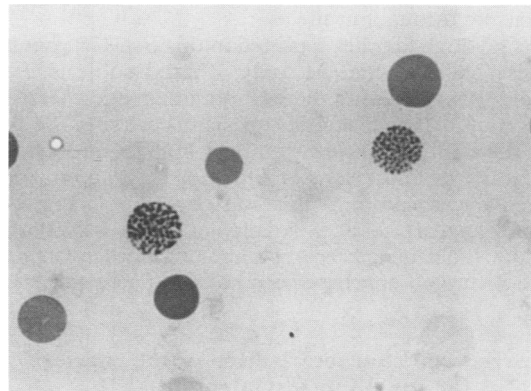


Figure 3 Photomicrograph of the peripheral blood of case 1 in reference 8 with the ATR-X syndrome showing some cells containing Hb H inclusions.

Further evidence that the defect is not located within the  $\alpha$  globin complex has come from analysis of a family with four affected brothers.<sup>10</sup> The parental  $\alpha$  globin 3'HVR alleles can be seen to assort independently in the four boys.

Since expression of the  $\alpha$  globin genes, as judged by mRNA analysis and protein synthesis, is reduced to less than 50% of normal<sup>14</sup> it seemed possible that both alleles were down regulated by a common *trans* acting mechanism. To analyse this situation further the maternal and paternal chromosomes 16 from one patient were isolated in *trans* specific (human  $\times$  mouse erythroleukaemia cell) hybrids.<sup>4</sup> Human  $\alpha$  globin synthesis was readily detected in both hybrids and furthermore both expressed  $\alpha 1$  and  $\alpha 2$  mRNA in a manner indistinguishable from that of a hybrid containing a normal chromosome 16. The simplest explanation for these findings is that expression of all four  $\alpha$  genes in patients with the ATR-X syndrome is down regulated by a new abnormality in a *trans* acting factor that interacts with the regulatory sequences of the  $\alpha$  globin cluster. Because expression of the  $\alpha$  globin genes appears normal in the mouse erythroleukaemia cells, it is presumed that the genetic defect is complemented by the mouse background.

Where might the locus encoding such a factor lie? Circumstantial evidence favouring X linkage was presented in the initial report of the ATR-X syndrome.<sup>4</sup> All five original cases were 46,XY including one phenotypic female. It was also noted that occasional Hb H inclusions could be seen in one female sib who was otherwise entirely normal. Since then, as summarised by Wilkie *et al.*,<sup>7</sup> two recent papers have added further support to this conclusion. Harvey *et al.*<sup>5</sup> described the probable occurrence of two affected males in a sibship. Porteous and Burn<sup>6</sup> described a 6 year old boy, subsequently identified as having the ATR-X syndrome, whose dead maternal uncle had similar clinical features and had been anaemic throughout life.

The three families reported in this issue<sup>8-10</sup> extend these observations. All nine affected subjects are male. In each family there is more than one affected boy but male to male transmission is not seen in any of the families. In one family<sup>8</sup> a high frequency of miscarriage and deaths of known males in utero has been documented; in this and one other pedigree<sup>9</sup> there appears to be a paucity of normal liveborn males. To date no karyotypic abnormalities involving the X chromosome have been noted in this syndrome.

#### Provisional characterisation of the carrier status for the ATR-X syndrome

Being an X linked syndrome one might expect to find evidence for partial expression in some females in these families. Rare (1:1000 to 1:100 000) Hb H

inclusions were found in the red cells of some obligate carriers and female sibs. The presence of Hb H inclusions in such subjects provides unequivocal evidence that they are carriers of the ATR-X syndrome: care must be taken not to misinterpret artefactual inclusions (see above and reference 9). The converse is not the case; inclusions may not always be detected in obligate carriers. With one exception,<sup>8</sup> Hb H inclusions could only be found rarely in the red cells of female carriers (1% or less of the number observed in affected boys). One explanation for this might lie in the pattern of X inactivation in the haemopoietic cells of the female carriers. A markedly skewed pattern of X inactivation, in which the abnormal X chromosome was inactivated in the majority of red cell precursors, might be reflected by the relatively small number of red cells in which Hb H inclusions can be found. Preliminary studies of the methylation patterns of X chromosome loci in carriers for the ATR-X syndrome support this hypothesis and this may provide the basis for a more sensitive screening test for the identification of female carriers (unpublished data).

Dysmorphic features, including hypertelorism and midfacial hypoplasia, were noted in a mother and female sib who also exhibit occasional Hb H inclusions; in all other respects both are normal.<sup>10</sup> These features may be other manifestations of carrier status: variations in the phenotype of carriers may reflect the pattern of X inactivation in different tissues.

#### Conclusion

The ATR-X syndrome represents a distinct clinical/haematological entity with a subtle but recognisable spectrum of dysmorphism. We would recommend that boys with severe undiagnosed mental retardation and features suggestive of the ATR-X syndrome are tested by looking for the presence of cells containing Hb H inclusions after incubation with 1% BCB. For completeness, a normal  $\alpha$  globin genotype ( $\alpha\alpha/\alpha\alpha$ ) should be shown by DNA blotting to exclude the common inherited deletions that may cause  $\alpha$  thalassaemia.<sup>2</sup> Hb H inclusions in related females are indicative of carrier status but the test is insufficiently sensitive to identify all carriers.

The available evidence strongly suggests that the locus for this condition maps to the X chromosome. As previously discussed,<sup>4</sup> this locus possibly encodes a *trans* acting factor which modulates expression of the  $\alpha$  globin genes although at present we do not know at what level in the hierarchy of gene expression it may be involved. It has recently been shown that globin gene transcription is controlled by the interaction of tissue specific and ubiquitous factors<sup>12</sup> which are, themselves, modified via other *trans*

acting factors. Certainly a mutation in such a ubiquitous factor could account for the apparently unrelated features of the ATR-X syndrome. Similarly, a mutation which reduced the specificity of a tissue restricted factor could underly this syndrome. An alternative explanation is that a factor controlling  $\alpha$  globin expression is inactivated together with neighbouring genes on the X chromosome as part of a contiguous gene syndrome.

The identification of the three new pedigrees described in this issue, together with previously described families, offers the possibility of localising the region of the X chromosome containing the mutation underlying this syndrome. Developments in our understanding of the *trans* acting factors normally involved in controlling  $\alpha$  globin gene expression may lead us more specifically to the abnormality which may represent a new class of mutation causing dysmorphism and mental handicap.

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