Occurrence of the $\alpha$ thalassaemia-mental retardation syndrome (non-deletional type) in an Australian male

M P Harvey, A Kearney, A Smith, R J Trent

Abstract
The rare association of $\alpha$ thalassaemia and mental retardation has been described previously. Molecular studies of the $\alpha$ globin cluster in these cases have been heterogeneous, with some patients having large deletions while in others the $\alpha$ globin complex appears to be intact (non-deletional). The non-deletional cases form a distinct group whose features include severe mental retardation, haematological changes of haemoglobin H (Hb H) disease, developmental defects, and unusual patterns of inheritance. To date, five cases have been described with non-deletional $\alpha$ thalassaemia-mental retardation. We present here a further example of a young male of Northern European origin who appears to have the non-deletional form of the disease. Clinical features included severe mental retardation, Hb H disease, and developmental defects similar to those reported previously. DNA mapping, including pulsed field electrophoresis, showed no evidence of deletions within the $\alpha$ globin cluster. Karyotypic analysis indicated an increase in random breakage, which has been observed previously in one case of deletional $\alpha$ thalassaemia-mental retardation. Profuse Hb H bodies and Hb H on electrophoresis were consistent with Hb H disease. However, the latter was present at a relatively low level (1-6%) and, as well, the mean corpuscular volume (82.8 fl)

and mean corpuscular haemoglobin (26.4 pg) were surprisingly high. Our findings are compared to other cases described with the non-deletional Hb H-mental retardation syndrome.

The human $\alpha$ globin cluster is located on the terminal band of the short arm of chromosome 16, 16p13.3.¹ ² The genes are arranged in order of developmental expression, with embryonic zeta ($\xi$) at the 5' end, followed by the three pseudogenes $\psi_1\xi$, $\psi_\alpha 2$, and $\psi_\alpha 1$, the duplicated adult genes $\alpha_2$ and $\alpha_1$, and the incompletely characterised $\theta$ at the 3' end. The $\alpha$ thalassaemia syndromes result from reduced expression of one to four of the normal diploid complement of $\alpha$ globin genes. In contrast to the $\beta$ thalassaemias, most of the $\alpha$ thalassaemias result from deletions, with point mutations occurring much less commonly. Persons with one gene deletion ($-a^+/-a^0\alpha\alpha$) may have normal haematological parameters. Deletions of two $\alpha$ globin genes (either $-a^+/-a^+-\alpha$ or $-a^+/-\alpha$) produce a mild, asymptomatic, hypochromic, microcytic anaemia. Three gene deletions ($-/-\alpha\alpha$) give the phenotype of Hb H disease. Clinically, Hb H disease may vary from asymptomatic to a severe, blood transfusion dependent anaemia. Typically, prominent hypochoytic microcytic changes of the red cells are found. On incubation of the blood with supravital dyes, such as methylene blue, profuse Hb H bodies are seen. These are the result of precipitation of $\beta$ chain tetramers secondary to unbalanced globin chain synthesis. The association of $\alpha$ thalassaemia and mental retardation has been reported previously, often with the accompanying features of facial abnormalities, hypogonadism, and other developmental defects.³-⁵ Recently, Wilkie et al.⁶ ⁷ have shown that two distinct subgroups can be identified within the $\alpha$ thalassaemia-mental retardation syndrome. One group (eight cases) have extensive deletions within 16p13.3, with variable somatic defects. A second group of five karyotypic males have

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no demonstrable deletions, severe mental impairment, and a more homogeneous pattern of associated abnormalities. This report describes the clinical, cytogenetic, and molecular features found in an additional male patient with the non-deletional \( \alpha \) thalassaemia-mental retardation syndrome, and compares the findings with the previously described cases.

**Case report**
The proband was born in 1968 to parents who were said to be of normal intelligence and unrelated. Pregnancy was complicated by severe pre-eclampsia, requiring induction at 35 weeks and instrumental delivery. His birth weight was 2272 g (50th centile for 35 weeks’ gestation). At the time of delivery, his mother was aged 18 and his father 19. Shortly after delivery, the mother’s urine was found to be positive for tuberculosis. There was no evidence of infection in the proband. At 9 months, he was investigated because of poor milestones. He was found to have a flat nasal bridge, epicanthic folds, hypertelorism, and bilateral undescended testes. His head circumference was 44.5 cm (3rd to 10th centile) and inner canthal distance was 3.2 cm (>97th centile). Subsequently, the proband has remained profoundly mentally retarded, with failure of development of speech and inability to walk unsupported.

There was one other sib, a male, born in 1971, who died in childhood. The latter was also profoundly mentally retarded and said to have a similar physical appearance, but no other details are available. From the age of 10, the proband was institutionalised. His parents separated and were then lost to further contact. There is no record of any normal children. Extensive metabolic and cytogenetic testing failed to show a cause for his mental retardation. Recurrent urinary tract infections and grand mal seizures have been a feature over the years. In 1986, at the age of 18, swelling of the small joints of both hands and deterioration in hand grip were noted. Antinuclear antibodies, rheumatoid factor, serum hepatitis B surface antigen (HBsAg), and biochemical analysis were normal and a hypochromic microcytic anaemia was found. At present, at the age of 21, his height is 150 cm (<3rd centile), weight 44.5 kg (<3rd centile), and head circumference 51 cm (<2nd centile), with an inner canthal distance of 3.5 cm (75th to 97th centile) (figure). He has a mild thoracic scoliosis, uncorrected left sided talipes equinovarus, and small, soft descended testes, high in the scrotum.

**HAEMATOLOGICAL FINDINGS**
Peripheral blood was obtained for haematological study, cytogenetic analysis, and the establishment of EBV transformed lymphoblastoid lines. Blood count and haematological indices were measured on a Coulter model S Plus cell counter. The haematological findings are summarised in table 1. There was evidence of a hypochromic microcytic anaemia, as well as iron deficiency. Results, after successful therapy with oral iron supplements, are also listed in table 1. Profuse Hb H bodies (5 to 7%) were present after two hours’ incubation with new methylene blue. There was no increase in the number of Hb H bodies after overnight incubation, and there was no evidence of an unstable haemoglobin on either isopropanol or heat instability testing. On cellulose acetate Hb electrophoresis (Hb EPG), Hb H was estimated to be

<table>
<thead>
<tr>
<th>Haemoglobin (g/dl)</th>
<th>Before iron</th>
<th>After iron</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.3</td>
<td>11.3</td>
<td>12.1</td>
<td>13.0–18.0</td>
</tr>
<tr>
<td>White cell count (&lt;10^9/l)</td>
<td>8.1</td>
<td>8.1</td>
<td>4.0–11.0</td>
</tr>
<tr>
<td>Platelet count (&lt;10^9/l)</td>
<td>295</td>
<td>295</td>
<td>150–500</td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
<td>1.8</td>
<td>1.8</td>
<td>0.2–2.0</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>77.6</td>
<td>77.6</td>
<td>76–96</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>24.8</td>
<td>24.8</td>
<td>20–27</td>
</tr>
<tr>
<td>Hb A(_2) (%)</td>
<td>2.1</td>
<td>2.1</td>
<td>1.5–3.7</td>
</tr>
<tr>
<td>Kleihauer test</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>Hb H bodies (%)</td>
<td>5–7</td>
<td>5–7</td>
<td>0</td>
</tr>
<tr>
<td>Hb EPG</td>
<td>Faint Hb</td>
<td>Faint Hb</td>
<td>1–6% Hb H</td>
</tr>
<tr>
<td>Serum ferritin ((\mu)g/l)</td>
<td>10</td>
<td>70</td>
<td>20–300</td>
</tr>
<tr>
<td>Serum B12 (ng/l)</td>
<td>483</td>
<td>483</td>
<td>200–800</td>
</tr>
<tr>
<td>Serum folate ((\mu)g/l)</td>
<td>&gt;20</td>
<td>&gt;20</td>
<td>3.2–24</td>
</tr>
</tbody>
</table>

MCH=mean corpuscular haemoglobin; MCV=mean corpuscular volume; EPG=electrophoretogram.
Table 2 A summary of the clinical and haematological findings in the reported cases of α-thalassaemia-mental retardation (non-deletional type).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Case</th>
<th>Haematological findings</th>
<th>Phenotype</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>3, 8, 11</td>
<td>'PT' in 11, Male age 17 y</td>
<td>Hb H 6-7% MCV 90 fl MCH 25 pg</td>
<td>Microcephaly, hypertelorism, seizures, cryptorchidism, flat face and nasal bridge</td>
<td>Mother had phenotypic evidence of --α/α. father normal</td>
</tr>
<tr>
<td>3, 11</td>
<td>'SW' in 11, Male age 8 y</td>
<td>Hb H 2-5% MCV 70 fl MCH 22 pg</td>
<td>Microcephaly, hypertelorism, seizures, cryptorchidism, flat face and nasal bridge, epicanthic folds</td>
<td>As for previous patient</td>
</tr>
<tr>
<td>11</td>
<td>'TH' in 11, Male age 2 y</td>
<td>Hb H 1% MCV 74-3 fl MCH 23-8 pg</td>
<td>Microcephaly, hypertelorism, seizures, cryptorchidism, flat face and nasal bridge, epicanthic folds, unilateral talipes</td>
<td>Normal α/β globin chain synthesis ratios in parents</td>
</tr>
<tr>
<td>11</td>
<td>'NE' in 11, phenotypic female age 4 y</td>
<td>Hb H 0-7% Male karyotype (46,XY) MCV 69-7 fl MCH 21-7 pg</td>
<td>Female external genitalia, abdominal tests, microcephaly, hypertelorism, epicanthic folds, flat nasal bridge</td>
<td>Haematology normal in parents, α/β globin chain synthesis ratios not done</td>
</tr>
<tr>
<td>11</td>
<td>'PE' in 11, male age 2 y</td>
<td>Hb H 3-5% MCV 74 fl MCH 22-9 pg</td>
<td>Hemivertebra, small ears, cryptorchidism, convergent squint, hypoplastic teeth</td>
<td>Parents had normal haematology and α/β globin chain synthesis ratios</td>
</tr>
<tr>
<td>Present report</td>
<td>Male age 21 y</td>
<td>Hb H 1-6% MCV 82-8 fl MCH 26-4 pg</td>
<td>Microcephaly, unilateral talipes, hypertelorism, late descent of testes, flat nasal bridge, epicanthic folds</td>
<td>Parents not available</td>
</tr>
</tbody>
</table>

1·6%. An α/β ratio of 0·54 was consistent with either a two gene deletion (−α/−α or −/−αα) or Hb H disease (−/−α) 12 Red cell glutathione peroxidase, glucose 6 phosphate dehydrogenase, triose phosphate isomerase, phosphoglycerokinase, and glutathione reductase levels were normal.

**GENE MAPPING**

DNA was prepared from 10⁸ EBV transformed lymphoblastoid cells 13 digested with restriction endonuclease enzymes (Boehringer Mannheim) according to the manufacturer's instructions, electrophoresed on 0·8% agarose gels, and transferred by Southern blotting to nylon (Hybond, Amersham) using established techniques. 14 α and ε probes were gifts of Dr D R Higgs, MRC Molecular Haematology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford. DNA was digested with the enzymes BamHI, BglII, EcoRI, SacI, and HindIII and probed with α and ε probes. There was no evidence of deletions or rearrangements within the α globin cluster. The finding of two polymorphic bands with the SacI digest, hybridised with the ε probe, showed that both α globin loci were present. 15

DNA for pulsed field electrophoresis was prepared from fresh lymphoblastoid cells, and the restriction endonuclease digestions were performed according to the method of Smith et al. 16 Specimens were then run by transverse alternating field electrophoresis (Geneline™, Beckman) according to the manufacturer's instructions. Conditions were chosen to give maximum resolutions in the 200 to 800 kb range. Digests with NotI, PvuI, XhoI, SacII, BglII, MluI, SalI, ClaI, and SfiI were probed with the α and ε probes. No deletions were detected when DNA from the proband was electrophoresed in parallel with DNA from normal controls.

**CYTOGENETIC ANALYSIS**

Cytogenetic analysis was performed on 92 hour peripheral blood cultures set up in deprived medium (Isocyes's low folate and FdU), looking for the folate sensitive fragile site on 16p12, and analysed with high resolution techniques. 17 High resolution analysis to the 700 band stage failed to show an obvious deletion or translocation. Fragile site analysis showed nine random breaks in seven out of 55 cells on one occasion, and 22 breaks in 13 of 50 cells on a second occasion. This is higher than the normal (2 to 5%) for the laboratory. There was no pattern to the breaks, with some constituting common fragile sites. In particular, no fragile X chromosome was seen.

**Discussion**

The syndrome of Hb H-mental retardation was first
well defined by Weatherall et al. Recently, Wilkie et al have extensively investigated these original three cases, four other cases described elsewhere, as well as six unreported cases. Of this total of 13 cases, eight had extensive DNA deletions involving 16p13.3 (five had cytogenetic abnormalities), whereas five had no demonstrable deletions. Clinically, the five non-deletional cases of athalassaemia-mental retardation were a much more homogeneous group. The significant findings in these five cases of non-deletional athalassaemia-mental retardation plus the present case are summarised in table 2.

The cases described in table 2 have several features in common, which include facial, genital, and foot abnormalities, hypertelorism, seizures, and mild Hb H disease, all of which were present in our patient. In cases 'PT' and 'SW', the family studies are noteworthy, since the father was haematologically normal and the mother had evidence of heterozygous non-deletional a+ thalassaemia (αα/ααα). Hence the suggestion was made that the αβ thalassaemic haplotype inherited from the father may represent a paternal germ line mutation. As previously commented, all the cases described so far have occurred in chromosomal males, including the present example. The inability to perform family studies in the case of our proband was unfortunate, particularly given the history of a male sib with mental retardation and similar physical features.

Some of the haematological findings in the proband are intriguing. The profuse Hb H bodies and detectable Hb H on EPG are diagnostic of Hb H disease. Although the αβ globin chain ratio of 0.54 is consistent, the MCV and MCH are surprisingly high. This has been a conspicuous feature of the previously described cases of non-deletional athalassaemia-mental retardation, suggesting a different mechanism from the commonly encountered cases of Hb H disease. The increase in random chromosomal breaks in the proband is an interesting observation, and was described in a patient with deletional athalassaemia-mental retardation. Here, evidence of increased chromosomal breakage was found in the father as well. In both this case and our case, the breaks detected were randomly distributed. In the present case, despite an increased level of breakage, none was seen in 16p12 which is the rare fragile site adjacent to the a globin locus. It is difficult to relate the finding of increased chromosomal fragility to athalassaemia-mental retardation. The increased breakage in the deletional case may have predisposed to the apparent paternal germ line mutation observed.

The use of pulsed field electrophoresis, with its ability to map large fragments of DNA quickly, has provided the capacity to examine adjacent regions of chromosome 16p for microdeletions which may not be detectable on cytogenetic analysis. None was observed with the limited pulsed field electrophoresis performed in our patient. Given the inability to find any abnormality of 16p in the cases described so far, the unusual haematological findings, and the failure to identify any chromosomally female cases, Wilkie et al speculated whether the defect may in fact involve a gene on the X chromosome, coding for a transacting protein that regulates a globin expression. Some evidence already exists for gene(s) responsible for y globin regulation on the X chromosome. The history of another affected male sib in the present case would be consistent with this pattern of inheritance, and would be the first time that two males in a sibship have been affected. Linkage studies, using X chromosome probes in the available families, may shed some light on this intriguing syndrome.

We are grateful to Drs A O M Wilkie and D R Higgs for their helpful comments and for providing their unpublished data. We would also like to thank Dr A Lammi and Ms P Beale of the Department of Haematology, Royal Alexandra Hospital for Children, Camperdown, NSW, for performing red cell enzyme assays on this patient.

14 Old JM, Higgs DR. Gene analysis. In: Weatherall DJ, ed. The
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