Case reports

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

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Abstract

The rare association of α thalassaemia and mental retardation has been described previously. Molecular studies of the α globin cluster in these cases have been heterogeneous, with some patients having large deletions while in others the α 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 α 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 α globin cluster. Karyotypic analysis indicated an increase in random breakage, which has been observed previously in one case of deletional α 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 α 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 (ξ) at the 5' end, followed by the three pseudogenes $\psi\xi$, $\psi\alpha_2$, and $\psi\alpha_1$, the duplicated adult genes α_2 and α_1 , and the incompletely characterised θ at the 3' end. The α thalassaemia syndromes result from reduced expression of one to four of the normal diploid complement of α globin genes. In contrast to the β thalassaemias, most of the α thalassaemias result from deletions, with point mutations occurring much less commonly. Persons with one gene deletion ($-\alpha/\alpha\alpha$) may have normal haematological parameters. Deletions of two α globin genes (either $-\alpha/\alpha\alpha$ or $-\alpha/-\alpha$) produce a mild, asymptomatic, hypochromic, microcytic anaemia. Three gene deletions ($---\alpha$) give the phenotype of Hb H disease. Clinically, Hb H disease may vary from asymptomatic to a severe, blood transfusion dependent anaemia. Typically, prominent hypochromic 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 β chain tetramers secondary to unbalanced globin chain synthesis. The association of α thalassaemia and mental retardation has been reported previously, often with the accompanying features of facial abnormalities, hypogonadism, and other developmental defects.²⁻⁹ Recently, Wilkie *et al*^{10 11} have shown that two distinct subgroups can be identified within the α 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 α 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

The proband aged 21 years.

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 1 Haematological data on index case before and after iron therapy.

	Before iron	After iron	Normal range
Haemoglobin (g/dl)	11.3	12.1	13.0-18.0
White cell count ($\times 10^9/l$)		8.1	4.0-11.0
Platelet count ($\times 10^9/l$)		293	150-500
Reticulocyte count (%)		1.8	0.2-2.0
MCV (fl)	77.6	82.8	76-96
MCH (pg)	24.8	26.4	27-31
Hb A ₂ (%)	2.1	1.5	1.5-3.7
Kleihauer test	Normal	Normal	
Hb H bodies (%)	5-7	5-7	0
Hb EPG	Faint Hb H band	1.6% Hb H	0
Serum ferritin ($\mu g/l$)	10	70	20-300
Serum B12 (ng/l)		483	200-800
Serum folate ($\mu g/l$)		>20	3.2-24

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).

Reference	Case	Haematological findings	Phenotype	Family
3, 8, 11	'PT' in 11, Male age 17 y	Hb H 6.7% MCV 90 fl MCH 25 pg	Microcephaly, hypertelorism, seizures, cryptorchidism, flat face and nasal bridge	Mother had phenotypic evidence of $-\alpha/\alpha$, father normal
3, 11	'SW' in 11, Male age 8 y	Hb H 2.5% MCV 70 fl MCH 22 pg	Microcephaly, hypertelorism, seizures, cryptorchidism, flat face and nasal bridge, epicanthic folds	As for previous patient
11	'TH' in 11, Male age 2 y	Hb H 1% MCV 74.3 fl MCH 23.8 pg	Microcephaly, hypertelorism, seizures, cryptorchidism, flat face and nasal bridge, epicanthic folds, unilateral talipes	Normal α/β globin chain synthesis ratios in parents
11	'NE' in 11, phenotypic female age 4 y	Hb H 0.7% Male karyotype (46,XY) MCV 69.7 fl MCH 21.7 pg	Female external genitalia, abdominal testes, microcephaly, hypertelorism, epicanthic folds, flat nasal bridge	Haematology normal in parents. α/β globin chain synthesis ratios not done
11	'PE' in 11, male age 2 y	Hb H 3.5% MCV 74 fl MCH 22.9 pg	Hemivertebra, small ears, cryptorchidism, convergent squint, hypoplastic teeth	Parents had normal haematology and α/β globin chain synthesis ratios
Present report	Male age 21 y	Hb H 1.6% MCV 82.8 fl MCH 26.4 pg	Microcephaly, unilateral talipes, hypertelorism, late descent of testes, flat nasal bridge, epicanthic folds	Parents not available

1.6%. An α/β ratio of 0.54 was consistent with either a two gene deletion ($-\alpha/-\alpha$ or $-/\alpha\alpha$) or Hb H disease ($-/-\alpha$).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 10^8 EBV transformed lymphoid 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 ξ 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 *Bam*HI, *Bgl*II, *Eco*RI, *Sac*I, and *Hind*III and probed with α and ξ probes. There was no evidence of deletions or rearrangements within the α globin cluster. The finding of two polymorphic bands with the *Sac*I digest, hybridised with the ξ 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 (GenelineTM, Beckman) according to the manufacturer's instructions. Conditions were chosen to give maximum resolutions in the 200 to 800 kb range. Digests with *Not*I, *Pvu*I, *Xho*I, *Sac*II, *Bgl*II, *Mlu*I, *Sal*I, *Clal*, and *Sfi*I were probed with the α and ξ probes. No deletions were detected when DNA from the proband was electrophoresed in parallel with normal controls.

CYTOGENETIC ANALYSIS

Cytogenetic analysis was performed on 92 hour peripheral blood cultures set up in deprived medium (Iscoves'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.*⁵ Recently, Wilkie *et al.*^{10, 11} have extensively investigated these original three cases, four other cases described elsewhere,²⁻⁹ 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 α thalassaemia-mental retardation were a much more homogeneous group. The significant findings in these five cases of non-deletional α thalassaemia-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 forefoot 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 α^+ thalassaemia ($-\alpha/\alpha$). Hence the suggestion was made³ that the α^0 thalassaemic haplotype inherited from the father may represent a paternal germline mutation. As previously commented,¹¹ 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 α/β globin chain ratio of 0.54 is consistent, the MCV and MCH are surprisingly high.¹² This has been a conspicuous feature of the previously described cases of non-deletional α thalassaemia-mental retardation, suggesting a different mechanism from the commonly encountered cases of Hb H disease.¹¹ The increase in random chromosomal breaks in the proband is an interesting observation, and was described in a patient with deletional α thalassaemia-mental retardation.^{4, 5} 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 α globin locus.¹⁸ It is difficult to relate the finding of increased chromosomal fragility to α thalassaemia-mental retardation. The increased breakage in the deletional case may have predisposed to the apparent paternal germline 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 per-

formed 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.*¹¹ speculated whether the defect may in fact involve a gene on the X chromosome, coding for a transacting protein that regulates α globin expression. Some evidence already exists for gene(s) responsible for γ globin regulation on the X chromosome.^{19, 20} 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.

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