Case reports

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Localisation of human α globin to 16p13.3→pter

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SUMMARY A female child with α thalassaemia trait, moderate mental retardation, and dysmorphic feaures has inherited an abnormal chromosome 16 complement as a result of the unbalanced segregation of a maternal balanced translocation. Cytogenetic analysis indicates that the patient is monosomic for 16p13.3→pter and trisomic for 10q26.13→qter. DNA studies show that the patient has not inherited either maternal α globin allele. This accounts for the α thalassaemia trait in the child and places the human α globin complex in band 16p13.3→pter.

The human α globin gene (HBA) cluster was initially localised to chromosome 16 by observing the specific pattern of cDNA-DNA hybridisation to human × mouse somatic cell hybrids.[1] Subsequently, this localisation has been refined to the distal segment (p13.11→pter) of the short arm of chromosome 16 by mapping of two cell lines with translocation breakpoints at 16p13.11.[2–4] However, there are conflicting reports of the precise localisation of HBA within this region; Nicholls *et al*[3] localised the cluster to p13.1, whereas others locate it distal to p13.1[4] and specifically within 16p13.3→pter.[5] We report here a child with α thalassaemia and developmental abnormalities who also has an unbalanced karyotype involving chromosome 16 that is informative for the localisation of the HBA complex.

Case report

The proband, a female aged three years, presented with developmental delay and mild hypochromic, microcytic anaemia (Hb 10.4 g/dl, MCV 68 fl, MCH 19·5 pg). Her father is Maltese and her mother is English: neither has any haematological abnormality. There is no consanguinity and no relevant family history.

Further haematological investigations of the child showed normal levels of Hb A_2 and Hb F. However, when her red cells were incubated with 1% brilliant cresyl blue, occasional cells containing Hb H (β_4) inclusions were identified; such cells are pathognomonic of α thalassaemia. The α/β globin chain synthesis ratio measured in vitro by incorporation of ^{3}H leucine[6] was 0·63. These results indicate that her microcytosis is the result of α thalassaemia trait. Similar studies on the parents gave entirely normal results.

The proband has mild dysmorphic features, notably a high forehead, telecanthus, and a broad, flat nasal bridge. She has moderate, global developmental delay; her Griffiths Quotient was 69 at the age of three years.

CYTOGENETIC FINDINGS

G banded chromosome preparations obtained from peripheral blood lymphocytes of the patient, her mother, and father were examined. The father appears normal (46,XY) whereas the mother carries a balanced reciprocal translocation: 46,XX,t(10;16)(q26.13;p13.3) (fig 1a). It was possible to locate these breakpoints unambiguously because of the patterns of banding in the regions involved. A pale band corresponding to part of 16p13.3 can clearly be seen on the derived chromosome 16, and there is no band on the distal segment of the long arm of chromosome 10 with which this pale band could be confused.

The child has inherited the derived chromosome 16 (lacking the region 16p13.3→pter) from her mother, but has two normal chromosomes 10 owing to unbalanced segregation of the maternal translocation chromosomes: 46,XX,−16,+der 16(10qter→q26.13::16p13.3→qter) (fig 1b). Thus, the child is

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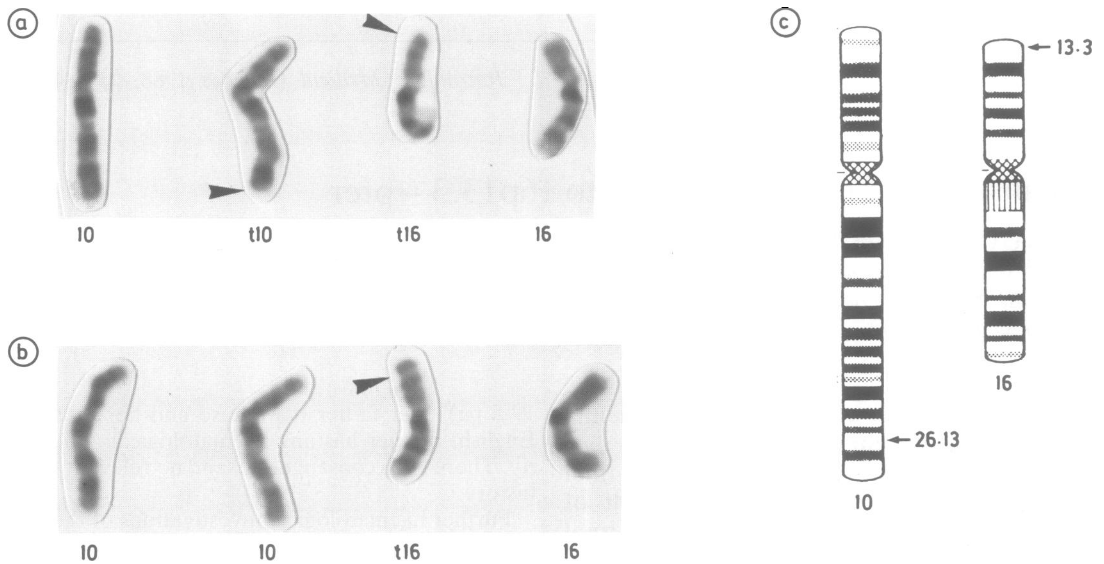

FIG 1 *(a) Partial karyotype of the proband's mother showing the balanced translocation (10;16) and normal chromosomes 10 and 16. The breakpoints are arrowed on the translocation chromosomes. (b) Partial karyotype of the proband with the derived translocation chromosome 16 but two normal chromosomes 10. (c) Ideograms of normal chromosomes 10 and 16 with the location of the breakpoints indicated.*

trisomic for 10q26.13→qter and monosomic for 16p13.3→pter.

DNA STUDIES

DNA obtained from peripheral blood mononuclear cells and EBV transformed lymphocytes from the mother, father, and child was cut in single digests with a variety of restriction enzymes. The DNA was then Southern blotted as previously described[7] and hybridised on separate occasions with α globin 3′HVR[8] and α globin 5′HVR probes.[9] These probes flank the α globin genes and are separated by 100 kb.

Using these probes, the proband was shown to have inherited one paternal allele at each locus, as expected. However, she did not inherit a maternal allele at either locus (fig 2). Thus the proband, who is monosomic for 16p13.3→pter, failed to inherit the α globin complex and at least 100 kb of the surrounding DNA from her mother. Paternity was confirmed by hybridising under less stringent conditions using the α globin 3′HVR probe to produce a minisatellite 'fingerprint' pattern (unpublished method).

Discussion

The data presented here are consistent with the localisation of the HBA complex to p13.3→pter proposed by Breuning *et al*,[5] based on analysis of the polymorphic marker (α globin 3′HVR) in a fetus monosomic for this region. As in that case, our patient is monosomic for p13.3→pter; analysis of two polymorphic regions around the α globin complex shows that she concurrently failed to inherit a maternal allele at either locus, thus indicating that the α globin complex lies within p13.3→pter.

Using a similar approach, Nicholls *et al*[3] previously showed that the HBA locus lies distal to the reported breakpoints (16p13.11) in two cell lines, GM2324 and GM6227. These findings were confirmed in two subsequent independent reports.[2,4] Reanalysis of the GM2324 cell line (data not shown) has indicated that the breakpoint could equally well be 16p13.3. This would explain the data of Harris *et al*,[2] which showed that 11 DNA sequences derived from chromosome 16 map distal to the GM6227 breakpoint (16p13.11), but proximal to the GM2324 breakpoint (16p13.11 or p13.3). Clearly, if the latter GM2324 breakpoint is correct, then the initial observations[3] would also place α globin in p13.3→pter.

The refinement of the localisation of HBA to 16p13.1, as proposed by Nicholls *et al*,[3] was based on the assignment of breakpoints within an inverted

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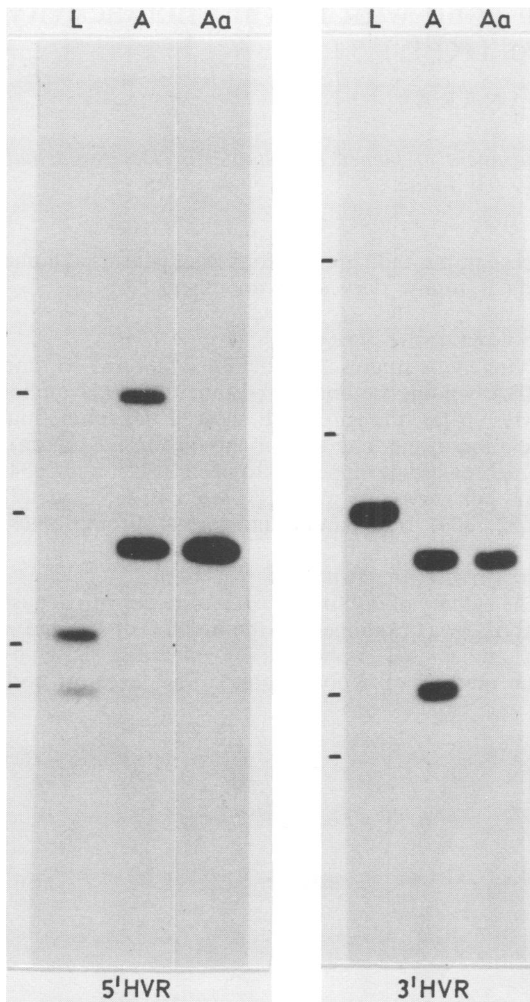

FIG 2 *RsaI digested DNA from the mother (L), father (A), and proband (Aa) hybridised to α globin 5'HVR and to α globin 3'HVR. Relevant size markers for λ DNA cut with HindIII are shown (6·5, 4·3, 2·3, 2·0 kb).*

chromosome 16,[10] which may be erroneous for reasons previously discussed.[4] In addition, the novel use of a biotinylated probe may have contributed to the misleading data produced.

Available genetic linkage data for the short arm of chromosome 16 also support a distal assignment for the HBA locus. All polymorphic sequences cloned from 16p lie proximal to the 3'HVR probe of the α globin locus.[11 12] Furthermore, the genes for phosphoglycollate phosphatase and adult polycystic kidney disease have also been shown to lie proximal to HBA.[13]

The mental retardation and dysmorphic features noted in this patient presumably result from her chromosomal imbalance. Some of her clinical features resemble those described for a duplication of part of the long arm of chromosome 10.[14] Several patients with α thalassaemia or haemoglobin H disease and mental retardation have been reported,[15] although this is the first report of an associated cytogenetic abnormality.

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