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Location of the retinoblastoma susceptibility gene(s) and the human esterase D locus

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SUMMARY Retinoblastoma occurs with increased frequency in children born with a deletion of the long arm of chromosome 13. Recent reviews have noted that the region 13q14 is consistently deleted in documented cases. Prometaphase and late prophase banding allowed Yunis and Ramsay[1] to determine that a deletion in one patient included the sub-bands q14·12, q14·13, and q14·2, and a portion of q14·11 and q14·3. We report the results of similar cytogenetic techniques applied in the case of a 26 month old Caucasian female with unilateral retinoblastoma, moderate developmental delay, and subtle dysmorphology. Prometaphase banding of cultured skin fibroblasts revealed the karyotype: mos46,XX/46,XX,del(13)(q13·1q14·11). Only the sub-band q14·11 is deleted in both our patient and that of Yunis and Ramsay. The results are consistent with the localisation of the retinoblastoma susceptibility gene(s) in the sub-band 13q14·11.

Electrophoretic analysis and activity assays of red blood cell esterase D are consistent with hemizygous expression of that marker in our proband. Comparison with published esterase D analyses in families with retinoblastoma permits the assignment of the esterase D locus to that same sub-band, 13q14·11.

Retinoblastoma is a retinal tumour of early childhood, with an estimated incidence of 1 in 20 000 live births.[2] Most cases are sporadic, without a definable mode of inheritance. However, there are two types of retinoblastoma of genetic aetiology: (1) a predisposition to develop the tumour based on an autosomal dominant mode of inheritance with incomplete penetrance; and (2) a predisposition to develop the tumour in persons with a deletion of the long arm of chromosome 13. Recent reviews[1 3] of 13q deletion cases have noted that the region 13q14 is consistently absent. Prometaphase and late prophase banding techniques allowed Yunis and Ramsay [1] to determine that a deletion in one patient included the sub-bands q14·12, q14·13, and q14·2, and a portion of q14·11 and q14·3. We report the results of cytogenetic studies of a patient which permit further localisation of the retinoblastoma susceptibility region.

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Analyses of electrophoretic polymorphisms and variations in activity of esterase D have permitted assignment of the esterase D locus to the 13q14 region.[4] We report the application of this approach in our patient and the evidence for a more precise assignment for the human esterase D locus.

Case report

The proband is a 26 month old Caucasian female with unilateral retinoblastoma. She was the third child born to a 26 year old mother and a 29 year old father after a 40 week pregnancy remarkable for third trimester maternal weight loss and decreased fetal activity. Delivery was complicated by a relatively large head circumference (70th centile). Birth weight was 2950 g (25th centile). During the first 5 months of life there was a history of poor feeding, irritability, and listlessness, with subsidence of these problems after 5 months. At the age of 22 months the sclera of her right eye became discoloured. The diagnosis of retinoblastoma was made and the right eye was

enucleated. Retinoblastoma was confirmed histologically in the enucleated eye. No radiation or chemotherapy was undertaken. At that time, the left eye showed no indication of tumour formation, and currently (6 years) it remains uninvolved. No other family members have retinoblastoma.

At the age of 26 months her height (80·5 cm) and weight (10·4 kg) were both slightly below the 10th centile with a head circumference (49 cm) at the 60th centile. Craniofacial anomalies included prominent forehead, epicanthic folds, and a slightly flattened maxilla (fig 1). There was bilateral fifth finger clinodactyly, bilateral overlapping of the middle toes, and mild joint laxity. The proband was moderately developmentally delayed, most markedly in her speech. On formal testing (aged $4\frac{1}{2}$ years) she achieved a mental age of $2\frac{3}{4}$ years (Stanford Binet Intelligence Scale).

Materials and methods

Peripheral blood cultures were established in commercial chromosome kits (Difco, Detroit), according to the manufacturer's instructions, but employing the culture modification for high resolution analysis of chromosomes of Yunis.[5] At 72 hours of culture, a cell cycle S period block was induced by adding Amethopterin (Sigma Chemical Company, St Louis) to 0·1 μmol/l final concentration. Seventeen hours later thymidine was added to 0·02 mmol/l final concentration, releasing the induced S period block. After 2 hours' additional incubation, colchicine was added to a final concentration of 0·07 μg/ml. The cell cultures were harvested in the usual manner 10 minutes later and microscope slides were prepared. These were heated for 1 hour on a 60°C slide warmer, then stained in trypsin-Giemsa solution (GTG). Chromosomes were examined under the microscope at ×800 and ×1250, and on photographs at ×2000 and ×4000 final enlargement. The nomenclature throughout the present report is that of ISCN (1981).[6]

Electrophoresis of esterase D was performed according to a modification of the method of Hopkinson *et al.*[7] For esterase D activity, the fluorescent method described by Sparkes *et al*[8] was used.

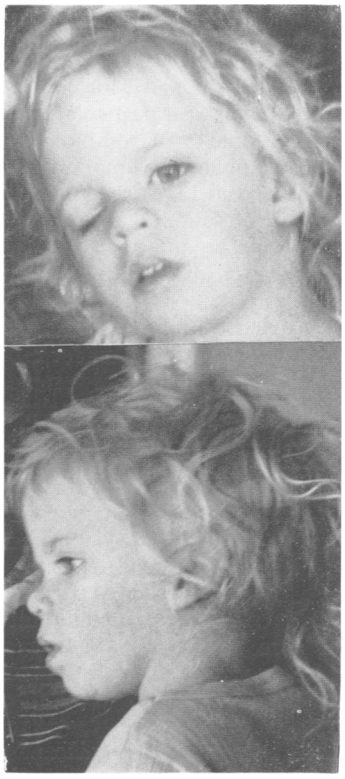

FIG 1 *The proband aged 26 months.*

Results

Metaphase banding performed on the proband's peripheral lymphocytes showed mosaicism for an interstitial deletion of 13q: 46,XX/46,XX,del(13)(q12q14). Of 60 cells, 56 showed the deletion. The proband's parents and sibs are cytogenetically normal, based on results obtained by the same method.

To define the deleted region more precisely, the proband's cultured skin fibroblasts were studied with prometaphase banding. These results were analysed based on ideograms of GTA banded human prometaphase chromosomes,[6] with the following assessment: 46,XX/46,XX,del(13)(q13·1q14·11) (fig 2). The percentage mosaicism was not calculated in fibroblast preparations.

Red blood cell esterase D activities were in the normal range[4] for the mother (68·6 units) and father (76 units) of our patient. The proband's activity of 38·9 units was 51 to 57% that of her parents. The electrophoretic phenotype of all three family members was type 1–1. Reduced activity on electrophoresis in the case of the proband, taken together with the quantitative assay data, permitted the final designation of the proband's electrophoretic pattern as 1–0.[4]

Discussion

It is important to note that our proband exhibited

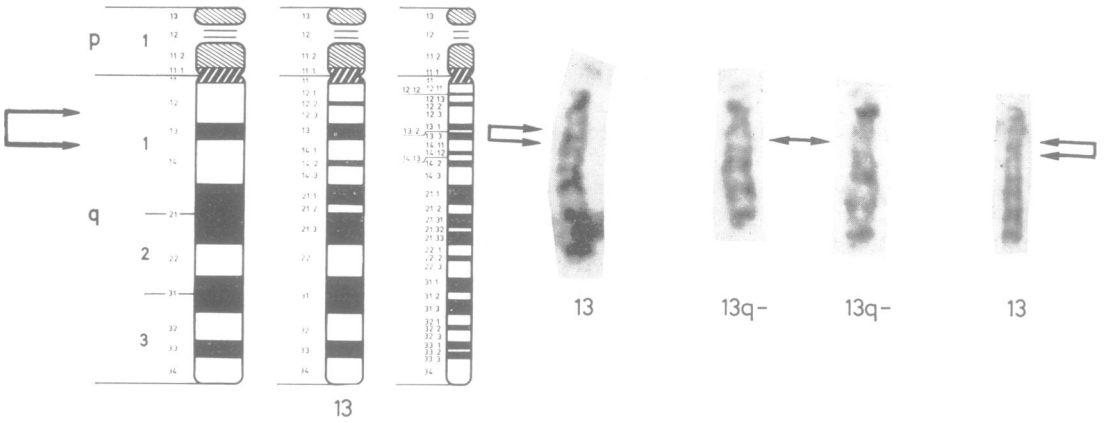

FIG 2 *Normal and deleted chromosomes 13 studied with prometaphase banding. Ideogram is from reference 6.*

subtle dysmorphic features, including only a few of the characteristic findings in reported cases with larger deletions.[1 2 9] Because of this, chromosomal analysis might not have been performed without the clinical diagnosis of retinoblastoma. In numerous reported cases which have undergone karyotyping since the seminal study of Lele *et al*,[10] both metaphase[9 11–23] and prometaphase[1 19] banding techniques have been used. Unilateral[4 24 25] and bilateral[25] retinoblastoma have been reported with mosaicism for deletions of chromosome 13. Based on accumulated cytogenetic evidence, Francke[3] and Yunis and Ramsay[1] noted the consistent deletion of band 13q14.

A comparison of our results with case 2 of Yunis and Ramsay[1] (a small interstitial deletion of 13q associated with retinoblastoma) is instructive. The proband of Yunis and Ramsay shows a deletion involving all of sub-bands 13q14·12, 13q14·13, and 13q14·2 and adjacent portions of 13q14·11 and 13q14·3. Our proband's deletion spans the sub-bands 13q13·1 to 13q14·11, leaving intact the sub-bands 13q14·12, 13q14·13, 13q14·2, and 13q14·3. The region 13q14·11 remains as the only sub-band deleted in both our patient and the proband of Yunis and Ramsay.[1]

The comparative findings are consistent with localisation of the retinoblastoma susceptibility gene(s) to the sub-band 13q14·11. The results are also consistent with previous attempts at such localisation, except for two reports,[22 26] each involving translocations with breakpoints at 13q12 and intact 13q14 bands. Davison *et al*[22] suggest that a second genetic element may be influencing the susceptibility to retinoblastoma in their proband. Nichols *et al*[26] reported studies of a proband with retinoblastoma and a 13q;Xp translocation, previously reported by Cross *et al*.[18] The report of Nichols *et al*[26] suggests that the 13q14 band remained intact in the same segment translocated to Xp, but that inactivation of the derivative chromosome predisposed the patient to retinoblastoma. This interpretation is supported by analysis of this patient's cells when fused to mouse cells.[27]

In related studies of familial (dominant) retinoblastoma, Knight *et al*[28] and Morten *et al*[29] found discordant segregation of a particular chromosome 13 and retinoblastoma in a total of six of seven families. The fundamental assumption, namely that the deleted sub-band in 13q− retinoblastoma is also the location of the mutation in autosomal dominant retinoblastoma, may be questioned on the basis of such studies. However, meiotic crossing over with a high recombination fraction between the centromere and the q14 locus, as proposed by the authors,[28 29] may be an alternative explanation for their results. Such a proposal is supported by high recombination frequency estimates independently derived from chiasma distribution data along the long arm of chromosome 13.[30]

It has previously been demonstrated that human esterase D, a polymorphic genetic marker, maps in the same 13q14 region as the locus for predisposition to retinoblastoma.[4] Important evidence for such close linkage came from studies of the expression of esterase D in patients with retinoblastoma and deletions of chromosome 13. That approach is extended in the present work. Both the half-parental activity and the 1–0 electrophoretic pattern of esterase D are consistent with hemizygous expression of esterase D in our proband. Significantly, esterase D activity in the critical proband (case 2) of Yunis

and Ramsay[1] was also half that of the parents (family 4 of reference 4). Accordingly, as the region 13q14·11 is the only sub-band deleted in both our patient and the Yunis and Ramsay proband, we contend that the esterase D locus is indeed quite closely linked to the retinoblastoma susceptibility locus in sub-band 13q14·11. This contention is supported by recent family studies demonstrating close linkage of inherited retinoblastoma susceptibility and esterase D.[31] Such close linkage should prove valuable for genetic counselling and antenatal diagnosis, and in studies of familial retinoblastoma segregating as a Mendelian trait.

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