

Original articles

The need to screen all retinoblastoma patients for esterase D activity: detection of submicroscopic chromosome deletions

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SUMMARY Roughly 5% of all patients with retinoblastoma carry a constitutional chromosome deletion on the long arm of chromosome 13, which confers a prezygotic predisposition to tumour development. As offspring of deletion carriers have a 50% risk of inheriting the predisposition locus it is important to identify deletion carriers. The site of the esterase D gene to the often deleted region offers an objective means of deletion identification. The chromosomes of a patient with unilateral retinoblastoma, previously supposed to have a normal karyotype, were re-examined after the discovery that his red blood cells contained reduced activities of esterase D. A small sub-band deletion was found in chromosome region 13q14. These findings emphasise the importance of measurements of esterase D in all patients with retinoblastoma, even those with an apparently normal karyotype.

Retinoblastoma is an intraocular tumour of children that affects roughly 1 in 20 000 live births.¹ There are both sporadic and hereditary forms. In the United Kingdom about 20–24% of cases are familial.² In about 5% of patients a prezygotic predisposition to tumour formation is associated with a somatic deletion, of variable length, from the long arm of one copy of chromosome 13.^{1 3} When the deletion is large there are usually associated congenital abnormalities, including severe mental retardation, and the patients do not reproduce. In patients with small deletions, however, the associated abnormalities may be less severe or absent⁴ and offspring have roughly a 50% chance of inheriting the deletion and also developing the tumour.

Esterase D is found in most human tissues, but its function is unknown. The gene for esterase D has been localised to region 13q14⁵ and patients with retinoblastoma carrying a deletion have 50% esterase D activity in their cells.² Determination of red blood cellular esterase D activities in a large population of patients with retinoblastoma led to the identification of patients with reduced enzyme activities, some of whom had not previously been diagnosed as patients with the deletion.² In this report we present details of one of these cases with a fairly small chromosome deletion that had escaped

detection until measurements of esterase D were made.

Materials and methods

Chromosome analysis. Peripheral blood samples were obtained in heparinised tubes. 0.2 ml of blood was cultured in 5 ml of Roswell Park Memorial Institute medium supplemented with 20% newborn calf serum and 0.2 ml of phytohaemagglutinin (Wellcome, Beckenham, Kent) for 72 hours. After this time vinblastine sulphate (0.1 µg/ml) was added for 20 minutes and the cells harvested and treated with 0.07M potassium chloride for 10 minutes. Lymphocytes were then fixed in three changes of 3:1 methanol:acetic acid, and chromosome preparations were made using standard air drying techniques. Subsequent chromosome banding was performed by the modified trypsin-Giemsa method described by Cowell.⁶

Enzyme analysis. Esterase D activities were measured in red blood cells essentially as described elsewhere.² The hydrolysis of 4-methylumbelliferyl acetate to 4-methylumbelliferone was followed fluorimetrically. Washed red cells were lysed by freezing/thawing and 10 µl of the lysate diluted 1:30

in 10mM sodium acetate pH 5.5. From this stock solution 10 μ l was added to 2.98 ml of 10mM sodium acetate at 25°C and the reaction begun by the addition of 10 μ l of 10mM 4-methylumbelliferyl acetate directly into the cuvette. The increase in fluorescence was measured over two minutes using an excitation wavelength of 322 nm and an emission wavelength of 450 nm.

Haemoglobin concentrations were measured by diluting 50 μ l of the original lysate 1:40 in neutral Drabkin's solution and reading the optical density at 415 nm. The enzyme activity was calculated as the relative production of 4-methylumbelliferone per gram of haemoglobin as described previously.²

Results

Case report. The patient, a boy, was born in 1982 at 36 weeks' gestation after a normal pregnancy and delivery. The head was large at birth with a circumference of 38 cm (over the 97th centile) and birth weight was 3400 g (50th centile). A squint was noticed at 10 months of age, darkening of the right iris at 1 year, and enlargement of the right eye at 13 months. At 16 months the diagnosis of right retinoblastoma was made and was immediately followed by enucleation. No abnormality of the left eye was found. Histopathological examination revealed a rosette forming retinoblastoma within the globe and no involvement of the cut end of the optic nerve. At a recent examination, when the child was aged 3, there was no recurrence of tumour and the left eye remained normal.

Psychomotor development was initially thought to be slow, but at 2 years the development quotient was 94. At 3 years the child was able to speak in sentences and was thought to be developing within the normal range.

Macrocephaly persisted from birth (at 3 years head circumference was 54.7 cm, which is above the 97th centile), while height and weight were still at the 50th centile. The head growth, however, remained parallel to the 97th centile from birth. There were no other dysmorphic features. The parents and an elder brother had no eye disorders. A recurrence risk of 2% for siblings and 6% for offspring was given at that time, based on estimations of Carlson and Desnick⁷ and Fuhrman and Vogel.⁸

Chromosome and enzyme analysis

In December 1983 the karyotype analysis of the patient was reported as being normal. Six months later the red cell esterase D value was shown to be 48% of normal activities, indicating deletion of one copy of the esterase D gene. Subsequent chromosome analysis revealed a small sub-band chromo-

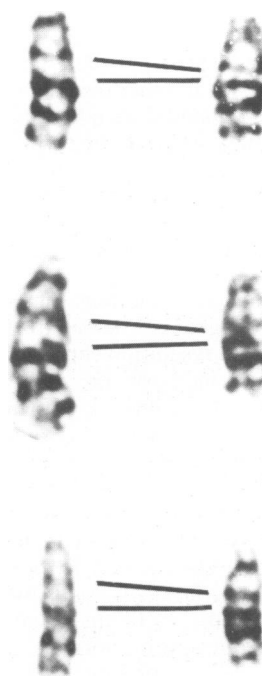


Figure Chromosome 13 homologues from the patient showing a small sub-band deletion within region 13q14. The normal chromosome is shown on the left in each case and the deletion chromosome is on the right.

some deletion (Fig. 1), tentatively described as 13q14.1-q14.3. Analysis of the parents showed no reduction in esterase D activities and no chromosome abnormality. The deletion in this instance thus represented a sporadic event, as is the case with the vast majority of 13q deletions associated with retinoblastoma.

Discussion

This report illustrates the importance of carrying out determinations of red cell esterase D routinely in patients with both unilateral and bilateral retinoblastoma because standard chromosome analysis is not always sufficiently sensitive for detection of small chromosome deletions. The findings have serious implications for this patient as the true recurrence risk in his offspring is roughly 50% instead of the 6% quoted before the detection of the deletion.

The importance of esterase D measurements was recently emphasised by Cowell *et al* where eight other deletions, four of which had not previously been recognised, were detected in a large series.²

Contrary to a popular belief that it is only the cases with bilateral tumours that have a genetic origin, we have shown that 66% of deletion cases have unilateral tumours.² In other instances chromosome deletions in the proband were inherited as a result of the deletion being carried in the form of a balanced chromosome translocation in one of the parents.⁹⁻¹³ Thus it is important that the chromosomes of parents of patients with 13q deletions are also analysed. In the case presented here the parents were karyotypically normal.

Small chromosome deletions in patients with retinoblastoma might be detected if they were analysed using high resolution chromosome banding techniques. Such methods are time consuming, often subjective, and technically difficult, however, in contrast to determinations of esterase D, which, although requiring more sophisticated machinery to carry out the tests, are reliable, quick, and easily subjected to quality control. One criticism of measurement of esterase D is that those deletions that do not include the esterase D locus will escape detection. To date, however, only two such cases have been reported.^{14 15} In these patients, whose esterase D activities were normal, the deletions must have involved a proximal breakpoint that occurred between the esterase D and retinoblastoma loci. Both patients had characteristic dysmorphic features and severe mental retardation, however, which are usually associated with a chromosome deletion and would in themselves warrant chromosome analysis. It is important to note that patients with smaller chromosome deletions, such as the one reported here, are not always mentally retarded and also that not all mentally retarded patients with retinoblastoma have chromosome deletions. (Cowell JK. Manuscript in preparation.)

Over 90% of patients with retinoblastoma survive the tumour, although blindness often results. If the tumour presents in an advanced form the treatment involves the removal of the eye. If this is not performed experience in the Third World, where treatment is minimal, has shown that the tumour will metastasise, usually down the optic nerve, and kill the patient. If tumours are detected early enough the consequences of the treatment may be less drastic. Cobalt implants, for example, often confine irradiation to the tumour and immediately adjacent areas and, depending on the site(s) of the tumour(s), vision may be only slightly impaired. Diagnosis of deletion carriers is often too late to help the affected individual as their deletions are only diagnosed at presentation. One patient seen by our group recently had severe developmental delay and was subsequently diagnosed as a 13q deletion carrier as a result. (Fitzsimmons J. Personal com-

munication.) Frequent, detailed examination of the retinas allowed detection of the tumours at a very early stage, and treatment will probably be effective. For those patients diagnosed as deletion carriers at presentation the management of the tumour is no different from any other retinoblastoma. For the children of these patients, however, it will be possible to determine prenatally whether they have inherited the deletion and, if so, either the pregnancy could be terminated or special attention given to the child during the early months in an attempt to detect and treat the tumours at an early stage. Tumours rarely recur after 5 years.

We suggest, in conclusion, that determination of esterase D is a powerful diagnostic tool for the detection of chromosome deletions in chromosome region 13q14 and should be part of the routine tests performed on patients with retinoblastoma. The same samples used for determination of enzyme concentration can also be used for enzyme phenotyping, which, in the familial form of the tumour, can also be used for prenatal diagnosis.¹⁶

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Twenty five years ago

Purpura fulminans in a newborn baby

R L Van Der Horst (Durban)—*Arch Dis Child* 1962;**37**:436–41

An Indian male infant, the third child of healthy parents, was found to have several raised red patches on both arms, right thigh, and left cheek immediately after a normal birth at home. Over the next three days these lesions became dark blue in colour, increased in size, and blistered in the centre. After admission to hospital new lesions appeared and merged into one another, more bullae developed, and in some areas such as the buttocks the skin became thickened, hard, and necrotic. On the fourth day of life the left lower limb was cold and indurated. It was purple in colour with a demarcation line above the ankle. No peripheral pulses were palpable. In spite of treatment with vitamin K and infusions of fibrinogen and fresh whole blood the infant's condition deteriorated with bleeding from nose and gums, the passage of tarry stools, and distension of the abdomen. The positive laboratory findings included lowered plasma fibrinogen, thrombocytopenia, and haemoglobin concentration down to 5·6 g/dl. Clot retraction was abnormal. Death occurred on the eighth day. At autopsy widespread thrombosis was found in veins, capillaries, and arterioles. There was extravasation of blood in the subcutaneous fat, large intestine, bladder, and perinephric fat. All the intracranial venous sinuses were thrombosed.

The author concluded that this disorder was essentially one of intravascular thrombosis. As the first child of the parents had died aged 9 days of a very similar illness the possibility of a genetically determined hereditary disorder was considered. From estimations of plasma fibrinogen concentrations in both parents (less than 40% of the mean) Van Der Horst suggested the presence of a congenital deficiency of fibrinogen in this family, although he seemed to consider that the cause of the purpura fulminans was more probably related to Shwartzman's phenomenon.

Comment. This was the first recorded case of purpura fulminans in a newborn infant. Several recent case reports have indicated, however, that the disease is a manifestation of disseminated intravascular coagulation and due to congenital absence of plasma protein C, which is one of the vitamin K dependent serine proteases. In its activated form it is a potential anticoagulant as well as a profibrinolytic agent. The disease is inherited as an autosomal recessive. Affected infants have protein C concentrations at zero, while in the heterozygous parents concentrations have been reported to be under 50% of normal. The cases so far reported have all ended fatally save for one Chinese infant in whom cryoprecipitate and fresh frozen plasma induced a remission that was successfully maintained by the administration of warfarin.¹ It is important to recognise purpura fulminans in the newborn as a distinct entity, quite separate from the cases of fulminating or necrotic purpura that have been reported in older children. J H HUTCHISON

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