LETTERS TO THE EDITOR

Prenatal diagnosis of X-linked lymphoproliferative disease using multiplex polymerase chain reaction

X-linked lymphoproliferative disease (XLP) is characterised by a selective immunodeficiency to the Epstein-Barr virus (EBV). Primary EBV infection in male infants with the defective XLP gene leads to severe or fatal infectious mononucleosis (51%), acquired hypogammaglobulinaemia (31%), hyper-immunoglobulinaemia M (6%), aplastic anaemia (5%), and malignant lymphoma (26%). Recently, the XLP mutation has been mapped to the distal long arm of the X chromosome at Xq25-q26 using different polymorphic DNA markers. Moreover, in three XLP families different chromosomal deletions in Xq25 have been detected.

We report here on the prenatal diagnosis of XLP by multiplex polymerase chain reaction (multiplex PCR). XLP was diagnosed in a German family, in which more than two boys had died from overwhelming infectious mononucleosis (fig 1). When a 32 year old woman, who was an obligate carrier for XLP, became pregnant again, she and her husband requested prenatal diagnosis for XLP. Chorionic villus biopsy (CVS) was performed at 13 weeks of gestation. The fetus was found to be a 46,XY male by cytogenetic analysis.

Genomic DNA was prepared from whole blood of several family members as well as from CVS and then analysed by multiplex polymerase chain reaction (PCR) using simultaneously two polymorphic markers which flank the XLP gene locus proximally (DXS424, chromosomal map location Xq24-q25) and distally (HPRT, chromosomal map location Xq26.1), respectively. The primer sequences flanking the DXS424 locus are 5’-CTAGTGGGCCCAGGCTACGAC-3’ and 5’-TTATCAGTGTCAAGACATTC-3’.

The sequences for the primers flanking the HPRT locus are 5’-ATGCCACAGATAAGACAATTC-3’. Amplified polymorphic HPRT and DXS424 alleles have a size of 263-299 and 126-142 base pairs, respectively.

Amplification reactions were carried out in a total volume of 50 μl containing 200 ng of genomic DNA, 1 μmol/l of both forward and reverse primers for DXS424 and HPRT, 200 μmol/l of each dNTP, 0.3 μl 32P-dCTP (3000 Ci/mmol, Amersham Buchler, Braunschweig, FRG), 10 mmol/l Tris-HCl, 50 mmol/l KCl, 1.5 mmol/l MgCl2, and 2.5 units of Tag DNA polymerase (Boehringer, Mannheim). Initial denaturation was for five minutes at 94°C. Subsequently, 25 cycles with denaturation for two minutes at 94°C, annealing for one minute at 58°C, and extension for two minutes at 72°C were performed in a Perkin-Elmer/Cetus thermocycler. A total of 5 μl of the final product was mixed and diluted with 5 μl formamide loading buffer (Merck, Darmstadt) containing 2-25% bromophenol blue and 0-25% xylene cyanol, and denatured for 10 minutes at 80°C. A total of 5 μl was electrophoresed on a 6% acrylamide/bisacrylamide/8-3 mol/l urea sequencing gel (Roht, Karlsruhe) until the tracking dye (bromophenol blue) reached the bottom of the gel. The gel was dried and subjected to autoradiography (Kodak XAR-5) for 16 hours.

As shown in fig 2, the male fetus (IV-6) had received from the mother (III-8), who is an obligate carrier, the same alleles for DXS424 and HPRT as a son with the XLP phenotype who had died (IV-4). The probability of a recombination between the proximal marker DXS424 and XLP has been estimated to be approximately 0.13th and between HPRT and XLP to be 0.06-0.1. Therefore, the probability of double recombinations of both markers, which would result in a misdiagnosis, is less than 1%. Thus, the fetus carries the XLP mutation with a probability of >99%. The healthy father (lane A) had allele 1 at the HPRT locus and allele 2 at the DXS424 locus. The extra bands seen for marker DXS424 probably arose by mispairing in the dinucleotide repeat region during PCR.

In the hypothetical case that a healthy XLP negative HLA identical subject existed in the family was refused by the parents. At the 16th week of gestation a therapeutic abortion was therefore performed. Multiplex PCR analysis of DNA extracted from the umbilical cord confirmed that the fetus carried the XLP mutation. This result was compared with conventional analysis of DNA restriction fragment length polymorphisms (RFLPs) using Southern blot techniques, multiplex PCR with highly polymorphic markers is less time consuming and uses less DNA and in many cases is more informative. Generally, XLP has a poor prognosis with a lethality of 77% in affected boys at 10 years of age. Thus, in the case of an affected male fetus, prenatal diagnosis of XLP offers the possibility of preventing this disastrous disease. Recently, the defect in patients with XLP has been corrected by transplantation of either allogeneic cord blood stem cells or
bone marrow.2,11 Prenatal DNA diagnosis will not only identify males at risk, but also unaffected males and females. These may be potential donors of cord blood stem cells or, later in life, of bone marrow, provided that they are HLA identical. The structure of the XLP gene and the function of its coded protein are still unknown. However, cloning of the entire candidate region using YAC walking technology will permit the identification of the defective gene in the near future. This may allow a direct and a 100% accurate prenatal diagnosis of XLP and may finally offer the opportunity of gene therapy.

This work was supported by the Deutsche Forschungsgemeinschaft (DFG Schu 560/2-3). We wish to thank Dr S Ulhaas, Institute of Human Genetics, University of Bonn, for supplying DNA from chorionic villus biopsy.

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8. Luty D, Ouyang Z, Ledbetter DH, Ledbetter S, Liit M. Five polymorphic microsatellite loci within the X chromosome region Xq25-q26 of a family with X-linked lymphoprolifera


A variant of Wiskott-Aldrich syndrome with nephropathy is linked to DXS255

Wiskott-Aldrich syndrome (WAS, IMD2 MIM 301000) is an X linked recessive disorder characterised by eczema, thrombocytopaenia with small platelets, multiple infections, and susceptibility to malignancy; T and B lymphocyte deficiencies are common. Affected males usually die in the first decade of life though there are exceptions. WAS has been mapped to Xp11.2 and is closely linked to the DXS2255 locus that is detected with the hypervariable probe M27P. A variant of WAS (MIM 314000) with eczema, thrombocytopenia with small platelets, raised serum IgA, and a virtually intact immune, a mild nephropathy, and normal life expectancy has been reported. Gütenberger et al. reported the results of renal biopsies from three males from the same family. A brief description of the family is given in table 1 and the figure. This family presented to the Genetics Clinic at Guy's Hospital when IV-2 was pregnant. The family was reported in 1932, and the proband was included in a later report. A family of the disease is given in table 1 and the figure. The clinic picture is similar to the variant of WAS reported previously. The disease appears to be uniform in the family; affected subjects have easy bruising from an early age and therefore clinically normal males with no history of a bleeding disorder were considered to be normal. A computer simulation of a linkage study showed it might be possible to confirm linkage to DXS2255 with the subjects available. The alleles were arbitrarily numbered 1 to 4 and the results were analysed by the LIPED computer program.12 The results (table 2) confirm that the condition in this family is linked to the DXS2255 locus though the confidence limits for the recombination fraction are wide (0–0.18). This disorder maps to the same region as WAS suggesting that it may be allelic with the classical form of WAS. Alternatively it may be caused by a mutation in a closely linked gene. The resolution of these two possibilities may be possible with the isolation of the gene mutated to cause WAS.13

Table 1 Clinical details of the family

| II-4 | Well, no bleeding disorder  |
| II-7 | 28 years, died of bleeding disorder |
| II-8 | 15 years, proband of the report in 1932 died of haemorrhage |
| III-1 | 9 months, easy bruising and at the age of 6 years had severe haemorrhage after dental work |
| III-4 | 24 years, osteomyelitis of right femur requiring surgical intervention |
| III-5 | 30 years, infections requiring prophylactic antibiotics. He has alopecia which appears to be connected with his WAS and his blood count and renal and liver function are normal. He has avoided hospitalisation as bleeding has not really been a problem |
| III-3 | Childhood, allergic eczema, asthma, thrombocytopenic purpura, and recurrent middle ear infection |
| III-10 | Easy bruising |
| I-V | Thrombocytopenia and easy bruising |
| V-3 | Thrombocytopenia, no evidence of immunodeficiency |

Table 2 Summary of the two point lod score

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doi: 10.1136/jmg.32.9.756

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