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'-CTAGGAGATGCTATGCAC-3' and 5'-GTTATCTAGTGTCAAGACATT-3'.

The sequences for the primers flanking the HPRT locus are 5'-AGCCACAGATAGTATACTGCTGCT-3' and 5'-CTCTGCCAGATAAGTAGTATG-3'.

The 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, 0.5 μmol/l of each dNTP, 0.8 μl 10-pM-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 Taq 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 0.25% 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 0.139 and between HPRT and XLP to be 0.06-0.110. 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 arise by mispairing in the dinucleotide repeat region during PCR.

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**Figure 1** Segregation of haplotypes in this XLP family (modified from reference 6, published with permission of European Journal of Pediatrics). Alleles for the two proximally flanking markers DXS94 (Xq42) and DXS11 (Xq42-q25) are coded 1 and 2. For the two PCR based markers DXS424 and HPRT used in this family alleles are coded as 1,2:3,4 in order of increasing size. The boxed haplotype cosegregated with XLP. Recombination events are indicated by asterisks.

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**Figure 2** Partial pedigree of the XLP family with the healthy father (III-9, lane A), the mother, who is an obligate carrier for XLP (III-8, lane B), one dead boy with the XLP phenotype (IV-4, lane C), and the male fetus (IV-6, lane D). The mother (III-8, lane B) was found to be heterozygous at both loci (for HPRT alleles 2 and 3, and for DXS424: alleles 1 and 2). Both the male fetus (lane D) and the dead boy with XLP (lane C) had received allele 3 at the HPRT locus and allele 2 at the DXS424 locus. This indicates that the fetus carried 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 arise by mispairing in the dinucleotide repeat region during PCR.
A variant of Wiskott-Aldrich syndrome with nophrophatosis is linked to DXS255

Wiskott-Aldrich syndrome (WAS,IMD MIM 301000) is an X linked recessive disorder characterised by eczema, thrombocytopenia 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 DXS225 locus that is detected with the hypervariable probe M272. A variant of WAS (MIM 314000) with eczema, thrombocytopenia with small platelets, raised serum IgA, and a virtually intact immune system, a mild neuropathy, and normal life expectancy has been reported. 

Guynet et al. also reported the results of prenatal diagnosis in two cases 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. 

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 DXS225 with the subjects available. The alleles were arbitrarily numbered 1 to 4 and the results were analysed by the LINKED computer program. The disorder maps to this region as well 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.

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### Table 1: Clinical details of the family

| II-4 | Well, no bleeding disorder |
| II-7 | 28 years, died, severe bleeding disorder |
| II-8 | 15 years, proband of the report in 1932 died of severe haemorrhage |
| III-1 | 9 months, easy bruising and at the age of 6 years had severe bleeding after dental work |
| III-2 | 34 years, osteomyelitis of right femur requiring surgical intervention |
| III-3 | 35 years, splenectomy and since has had a normal platelet count |
| III-6 | 37 years, subtotal colectomy and ileostomy for ulcerative colitis |
| III-9 | 39 years, mesangio proliferative glomerulonephritis, started on dialysis, and subsequently has had a renal transplant |

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### Table 2: Summary of the two point lod score

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V Schuster, S Seidenspinner and H W Kreth

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