

Mapping the X-linked lymphoproliferative syndrome

(immunodeficiency/Epstein-Barr virus/restriction fragment length polymorphism)

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ABSTRACT The X-linked lymphoproliferative syndrome is triggered by Epstein-Barr virus infection and results in fatal mononucleosis, immunodeficiency, and lymphoproliferative disorders. This study shows that the mutation responsible for X-linked lymphoproliferative syndrome is genetically linked to a restriction fragment length polymorphism detected with the DXS42 probe (from Xq24–q27). The most likely recombination frequency between the loci is 4%, and the associated logarithm of the odds is 5.26. Haplotype analysis using flanking restriction fragment length polymorphism markers indicates that the locus for X-linked lymphoproliferative syndrome is distal to probe DXS42 but proximal to probe DXS99 (from Xq26–q27). It is now possible to predict which members of a family with X-linked lymphoproliferative syndrome are carrier females and to diagnose the syndrome prenatally.

Epstein-Barr virus (EBV)-associated diseases—contagious mononucleosis, nasopharyngeal carcinoma, and Burkitt lymphoma—cause considerable morbidity and mortality. After EBV infection B lymphocytes are efficiently transformed into persistently proliferating lymphoblastoid cells. These cells can be killed by other proliferating cells that are evoked in an immune response. Infection of children is usually asymptomatic, but nonimmune adults develop infectious mononucleosis. EBV-induced lymphoproliferation is fatal in some new world primate species and in a small number of human subjects with both inherited and acquired immunodeficiencies. One such defect results from a mutation of the X chromosome.

This X-linked lymphoproliferative (XLP) syndrome mutation in some way prevents the immune system of an affected male from making an appropriate response to EBV infection (1–4). Before EBV infection occurs, males with an XLP mutation have normal cellular and humoral immune responses (5) and respond normally to bacterial and viral infections other than EBV. After EBV infection, about 75% of males with XLP develop fatal infectious mononucleosis, with liver destruction often the immediate cause of death. Boys with XLP who survive EBV infection have defects in humoral and cellular immunity and a high incidence of lymphoma (4–6). The interaction between EBV and B lymphocytes appears normal in affected males, but EBV-induced activation of anomalous killer T cells may cause the lymphoreticular cytopathology that leads to symptoms (5).

Restriction fragment length polymorphism (RFLP) markers are being used to map genetic disease loci and to construct the human genetic map (7). In this report we describe the use of X chromosome RFLPs to study a family with XLP. The discovery of RFLPs flanking the XLP locus will enable diagnosis prior to infection and may prove an important step toward understanding XLP at the molecular level.

METHODS

XLP Family. X-linked lymphoproliferative syndrome was diagnosed previously in this family and reported in detail (8). At least 19 of the males have died of clinical syndromes triggered by Epstein-Barr virus infection. Immunological and virological studies on two of the males before and during EBV infection have been reported (5).

RFLP Analysis. Lymphoblastoid cell lines were produced from the blood of each subject using EBV. Cells were lysed in 1% lauryl sarcosine/1% Tris base/0.5% EDTA-free acid and incubated with a final concentration of 100 µg of Pronase per ml. DNA was extracted with phenol/chloroform, precipitated with ethanol, and dissolved in 0.05% Tris base/0.005% EDTA-free acid. Two micrograms of DNA from each cell line was digested with an appropriate endonuclease (*Msp* I for probe DXS3; *Bgl* II for probe DXS42; and *Sst* I, for probe DXS99), electrophoresed in 0.6% agarose gels, and blotted onto nitrocellulose. The blots were hybridized for 16 hr at 37°C in 50% formamide with 0.1 µg of probe DNA labeled to $\approx 10^7$ dpm with [α - 32 P]dATP. The blots were washed in 0.08% NaCl/0.1% NaDodSO₄/0.08% Tris base/0.02% H₃PO₄ at 60°C and autoradiographed with an intensifying screen at –70°C.

RFLP probes used and their locations are as follows: DXS143 (p22–pter), DXS85 (p22), DXS9 (p22), DXS16 (p22), DXS43 (p22), DXS41 (p22), DXS84 (p21), OTC (p21), DXS7 (p11.3), DXS14 (p11–cent), DXYS1 (q13–q21), DXS3 (q21–q22), DXS87 (q21–q24), DXS17 (q21–q22), DXS11 (q24–q27), DXS42 (q24–q27), DXS19 (q26–q27), DXS37 (q26–q27), DXS100 (q26–q27), DXS51 (q27–qter), DXS10 (q26), DXS99 (q26–q27), F9 (q26–q27), DXS15 (q28), F8 (q28) and DXS52 (q28).

RESULTS

Fig. 1 shows the pedigree of the family studied. Over 40 family members were tested with over 20 RFLPs distributed along the X chromosome (data not shown). The DXS42 locus (also known as 43-15) had the smallest number of recombinants with the XLP phenotype, whereas two RFLP markers near DXS42 showed a recombination rate consistent with their location on the genetic map of the X chromosome (9, 10). The approximate locations of these polymorphic probes are DXS3 (Xq21–q22), DXS42 (Xq24–q27), and DXS99 (Xq26–q27). Table 1 shows the genotypes of all relevant family members examined for these three polymorphic probes.

Fig. 2 shows a portion of the experimental data for the DXS42 RFLP. The rare 9-kilobase (kb) allele is coinherited with the XLP mutation in every case except for individual IV-21 (indicated in Fig. 2 by an arrow). The common (6-kb) allele has a frequency of 80% in the general population, and

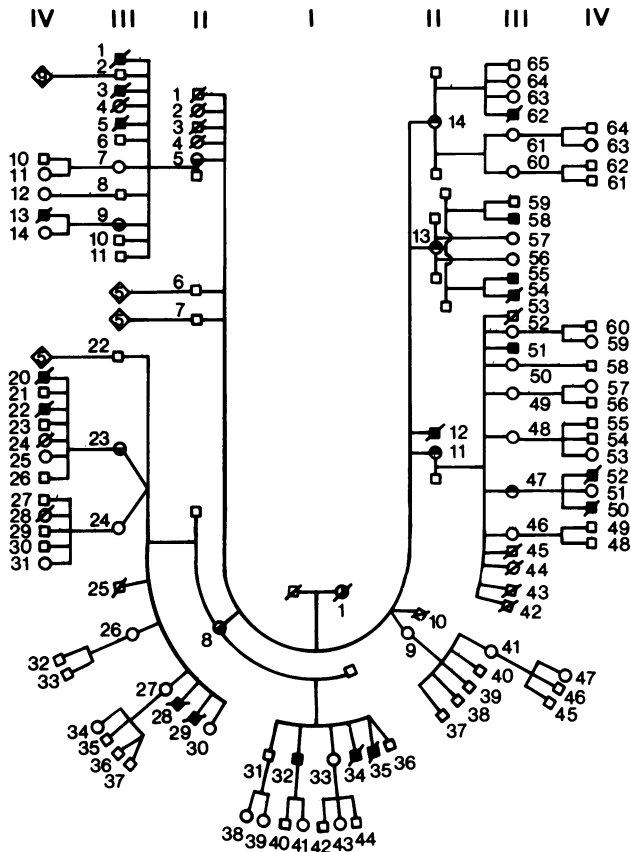


FIG. 1. Pedigree of the family under investigation. □, Male; ■, male affected; ○, female; ●, female carrier; ◇, uninformative offspring; slashed symbols indicate deceased.

all of the fathers in the kindred possess the common 6-kb allele. Their daughters carrying the XLP mutation have both the common 6-kb and rare 9-kb alleles.

A microcomputer version of the LIPED (11) program was employed to calculate the probability of genetic linkage between the DXS42 genotype and the XLP phenotype. (The genotypes of individuals I-1 and II-13 and the fathers of individuals II-5, III-9, III-23, and III-47 were deduced from the genotypes of their parents and children. Also individuals III-37, III-38, III-39, III-40, and IV-10 were considered unaffected, because they have survived beyond the usual age at which primary EBV infection occurs, and they have no symptoms of XLP.) The most likely frequency of recombination between the markers was 4%, and the corresponding logarithm of the odds ratio (lod) was 5.26.

The logarithm of the odds ratio at various recombination fractions for DXS3, DXS42, and DXS99 are shown in Table 2. The odds ratio for DXS42 and XLP is displayed in Fig. 3. The likelihood that the two markers exhibit 4% recombination is 180,000 times the likelihood that the markers exhibit 50% recombination (i.e., are unlinked). Fig. 3 shows that the probability of linkage at any distance from 1% to 10% is also very high (>90,000 to 1). The area under the probability curve from 0 to 10 recombination units (centimorgans) accounts for 75% of the total area, and the area between 0 and 15 centimorgans is 92% of the total area. Thus, the chance that XLP and DXS42 are <10 centimorgans apart is three times the chance that they are >10 centimorgans apart. There is only an 8% chance that the loci are >15 centimorgans apart.

DISCUSSION

Precise localization of the XLP locus is not yet possible. Aldridge et al. (12) localized DXS42 to Xq24-qter. Drayna

Table 1. RFLP genotype in a family carrying XLP

| Individual | Genotype at loci | | | XLP status |
|------------|------------------|-------|-------|------------|
| | DXS3 | DXS42 | DXS99 | |
| I-1 | (cc) | cr | cr | + |
| II-5 | cr | cr | cr | + |
| III-6 | r | c | c | - |
| III-7 | cr | cc | cc | ? |
| III-8 | r | c | c | - |
| III-9 | cc | cr | cr | + |
| III-10 | r | c | c | - |
| II-8 | cr | cr | cc | + |
| III-22 | r | c | c | - |
| III-23 | cr | cr | cr | + |
| IV-21 | r | r | r | - |
| IV-22 | r | r | c | + |
| IV-23 | c | c | r | - |
| IV-26 | c | c | r | ? |
| III-24 | cc | cc | cr | ? |
| IV-27 | c | c | r | - |
| III-31 | r | c | c | - |
| III-32 | r | r | c | + |
| III-33 | cr | cr | cr | ? |
| IV-42 | c | r | c | ? |
| IV-44 | c | c | r | ? |
| II-9 | cr | cc | cc | ? |
| II-11 | cr | cr | cc | + |
| III-46 | cr | cc | cc | ? |
| IV-49 | c | c | c | - |
| III-47 | cc | cr | cc | + |
| IV-52 | c | r | c | + |
| III-48 | cr | cc | cc | ? |
| IV-54 | r | c | c | - |
| IV-55 | r | c | c | - |
| III-50 | cr | cc | cc | ? |
| IV-58 | c | c | c | - |
| III-51 | c | r | c | + |
| III-52 | cc | cr | cc | ? |
| IV-60 | c | c | c | ? |
| III-54 | c | r | c | + |
| III-55 | c | r | c | + |
| II-14 | cr | cr | cr | + |
| III-65 | r | c | c | - |

The individuals are designated by their number in Fig. 1. The more commonly found RFLP allele is designated c, and the more rarely found allele is designated r. Genotypes bracketed by parentheses are assumed rather than observed or deduced. Females who are designated + in the XLP column have at least one son with XLP. Males designated by a ? have not yet been infected by EBV, and females with a ? have either seronegative or healthy sons. Males with a - in the XLP column have been infected by EBV without consequence.

and White (9) reported close linkage between DXS42 and RFLP markers in the Xq26-q27 region. Markers in Xq28 were only loosely linked to these markers. This leads to the conclusion that DXS42 is from region Xq24-q27. From Table 1 we see that a recombination occurred between XLP and DXS42 in the egg cell that became individual IV-21. That same recombination is detected with DXS3 but not with DXS99. DXS3 is from the Xq13-q22 region (11), whereas DXS99 is from the Xq26-q27 region (10). The simplest interpretation of these data is that one recombination event took place distal to the DXS42 and proximal to the XLP locus (which is proximal to DXS99). Both DXS42 and XLP would lie in Xq24-q27. The possibility that XLP is proximal to DXS42 is at least 30 times less likely, because it requires three crossovers between DXS3 and DXS99. Knowledge of the location of XLP is an important step in characterizing the gene that is defective in XLP. Only genes that map between DXS42 and DXS99 are likely to be the gene that is defective

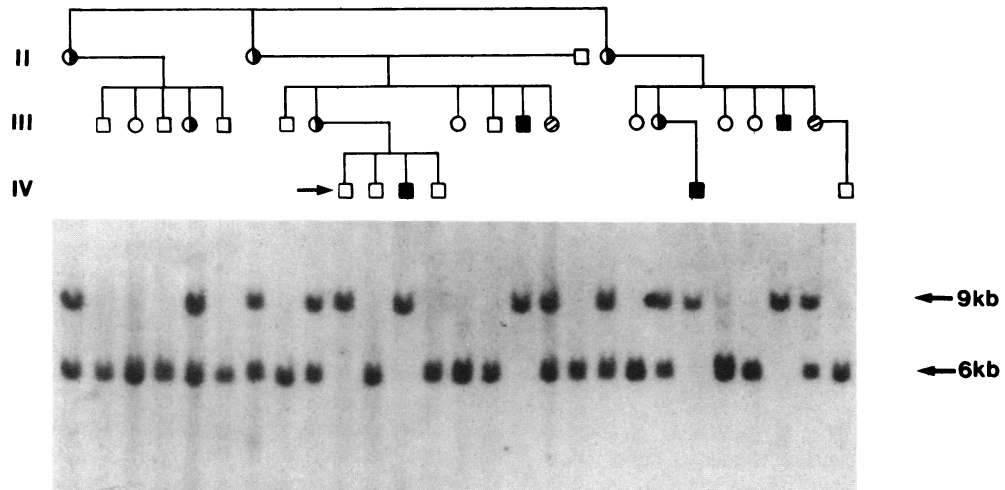


FIG. 2. Autoradiograph showing the DXS42 RFLP for several members of the family of Fig. 1. The pattern for each individual is shown directly under the corresponding symbol. Males with XLP are represented by darkened squares, obligate carrier females are represented by half-darkened circles, and females predicted to be carriers are represented by circles enclosing diagonal lines. The square marked by the arrow indicates individual IV-21 (Fig. 1), who shows a recombination event between the DXS42 locus and the XLP mutation.

in XLP. Of those genes that map between DXS42 and DXS99, the most attractive candidates lie <10 centimorgans from DXS42. This region comprises only 4% of the X chromosome genetic map.

Since the DXS42 and XLP genetic markers cosegregate in about twenty-four out of twenty-five meiotic events, it is possible to ascertain carrier status when one knows the DXS42 genotype of a female. Assuming 4% recombination, any female in this family who possesses the 9-kb allele is 96% certain to also possess the XLP mutation. Furthermore, her sons can be diagnosed before they are exposed to EBV. From data shown in Table 1, for example, we predict that individual III-52 is a carrier and that her seronegative son (IV-60) did not inherit XLP. We also predict that individual IV-26, who has not yet been infected with EBV, did not inherit XLP from his carrier mother. When a flanking marker such as DXS99 is employed in addition to DXS42, the accuracy of diagnosis becomes 99%. Individual III-33 has a 99% chance of being a carrier of XLP. Study of her two young seronegative sons indicates that one (IV-44) is unaffected, whereas the other (IV-42) has inherited XLP. We are currently administering gamma globulin to this male in an attempt to prevent primary EBV infection until an effective EBV vaccine is available.

Diagnosis of XLP prior to infection with EBV has been impossible until now. Some investigators (13) have concluded that females with abnormal EBV antibody profiles are

carriers, but in this family there is no correlation between carrier status and EBV antibody titer. A previous study suggested that males with XLP have a defect in antibody synthesis following immunization with bacteriophage ϕ X174 (14). Three at-risk individuals showed a poor antibody response. However, the mothers of the three boys (III-46 and III-48) do not carry XLP based on their RFLP genotypes, and the boys are still healthy over 5 years after infection with EBV. Thus, an abnormal antibody response to bacteriophage ϕ X174 is not diagnostic of XLP.

Our expectation that the mutation that causes XLP in other families has the same molecular basis (and therefore is at the same location) must be confirmed before diagnosis can be certain in every case. Therefore, we invite referral of other families with XLP for genetic studies.

Note Added in Proof. Individual II-7 was found to have the 6-kb allele at the DXS42 locus. This increases the odds ratio to 347,000:1 in favor of linkage at 4% recombination.

Table 2. Linkage analysis of loci defined by three molecular probes and the X-linked lymphoproliferative locus

| Recombination fraction | DXS3 Xq21-q22 | DXS42 Xq24-q27 | DXS99 Xq26-q27 |
|------------------------|------------------|-------------------|-------------------|
| 0.05 | 0.73 | 5.24 | 0.37 |
| 0.10 | 1.33 | 4.99 | 0.91 |
| 0.15 | 1.54 | 4.57 | 1.13 |
| 0.20 | 1.56 | 4.07 | 1.19 |
| 0.25 | 1.48 | 3.51 | 1.15 |
| 0.30 | 1.31 | 2.88 | 1.04 |
| 0.35 | 1.08 | 2.20 | 0.86 |
| 0.40 | 0.78 | 1.48 | 0.61 |
| 0.45 | 0.42 | 0.73 | 0.34 |

Summary of the logarithm of the odds for possible linkage of XLP with loci DXS3, DXS42, and DXS99 at 5% recombination intervals. Shown for each of the three loci are the approximate locations of each on the human X chromosome (7).

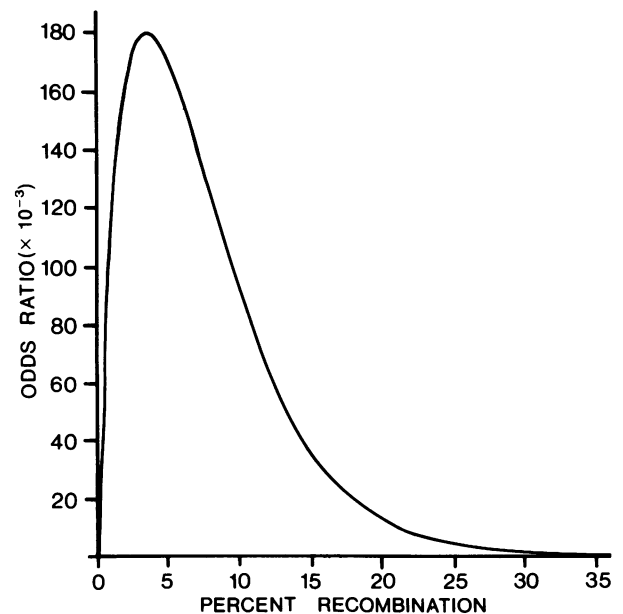


FIG. 3. Plot showing odds of linkage between XLP and DXS42 at various recombination frequencies. LIPED was employed to calculate the logarithm of the odds ratio.

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