<table>
<thead>
<tr>
<th>Title</th>
<th>Localization of the gene for X-linked recessive type of retinitis pigmentosa (XLRP) to Xp21 by linkage analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Musarella, MA; Burghes, A; AnsonCartwright, L; Mahtani, MM; Argonza, R; Tsui, LC; Worton, R</td>
</tr>
<tr>
<td>Citation</td>
<td>American Journal Of Human Genetics, 1988, v. 43 n. 4, p. 484-494</td>
</tr>
<tr>
<td>Issued Date</td>
<td>1988</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10722/42288">http://hdl.handle.net/10722/42288</a></td>
</tr>
<tr>
<td>Rights</td>
<td>This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.; American journal of human genetics. Copyright © University of Chicago Press.</td>
</tr>
</tbody>
</table>
Localization of the Gene for X-linked Recessive Type of Retinitis Pigmentosa (XLRP) to Xp21 by Linkage Analysis

M. A. Musarella,† A. Burghes,† L. Anson-Cartwright,† M. M. Mahtani,† R. Argonza,† L.-C. Tsui,† and R. Worton†

Departments of †Ophthalmology and †Genetics, The Research Institute, Hospital for Sick Children, Toronto

Summary

The X-linked recessive type of retinitis pigmentosa (XLRP) causes progressive night blindness, visual field constriction, and eventual blindness in affected males by the third or fourth decade of life. The biochemical basis of the disease is unknown, and prenatal diagnosis and definitive carrier diagnosis remain elusive. Heterogeneity in XLRP has been suggested by linkage studies of families affected with XLRP and by phenotypic differences observed in female carriers. Localization of XLRP near Xp11.3 has been suggested by close linkage to an RFLP at the locus DXS7 (Xp11.3) detected by probe L1.28. In other studies a locus for XLRP with metallic sheen has been linked to the ornithine transcarbamylase (OTC) locus mapping to the Xp21 region. In this study, by linkage analysis using seven RFLP markers between Xp21 and Xcen, we examined four families with multiple affected individuals. Close linkage was found between XLRP and polymorphic sites OTC (θ = .06 with lod 5.69), DXS84 (θ = .05 with lod 4.08), and DXS206 (θ = .06 with lod 2.56), defined by probes OTC, 754, and XJ, respectively. The close linkage of OTC, 754, and XJ to XLRP localizes the XLRP locus to the Xp21 region. Data from recombining in three of four families place the locus above L1.28 and below the Duchenne muscular dystrophy (DMD) gene, consistent with an Xp21 localization. In one family, however, one affected male revealed a crossover between XLRP and all DNA markers, except for the more distal DXS28 (C7), while his brother is recombined for this marker (C7) and not other, more proximal markers. This suggests that in this family the XLRP mutation maps near DXS28 and above the DMD locus.

Introduction

The X-linked recessive type of retinitis pigmentosa (XLRP) is considered to be the most severe clinical form of RP disorders. It accounts for 7%–30% of all cases and affects about 1 in 20,000 of the population (Jay 1972; Bird 1975; Boughman et al. 1980; Heckenlively 1983). In all genetic forms of RP, the classic ophthalmoscopic findings are midperipheral pigmentary changes, waxlike disks, and narrow retinal vessels. Although no phenotypic variation has been described in the retinas of affected males with XLRP, the carrier state has shown at least three retinal phenotypes: metallic sheen (tapetallike) reflex, peripheral pigmentary degeneration, and no fundus abnormality (Falls and Cotterman 1948; Francois 1962; Schapppert-Kimmiser 1963; Bird and Bloch 1970; Bird 1975; Fishman et al. 1986). The metallic sheen is seen in fundi of some XLRP carriers. Fundus and electoretinographic abnormalities can detect only a portion of carriers—and then only if the latter are tested in later stages of life. Carrier identification is, therefore, particularly problematic in young daughters of female carriers who are at risk of transmitting the disease to their sons but do not demonstrate any retinal abnormalities indicative of the carrier state.

The gene responsible for XLRP appears to map on the short arm of the X chromosome, but uncertainty exists as to its exact location. The initial report by Bhatthacharya et al. (1984) of close linkage of XLRP with DXS7 (probe L1.28), which maps to Xp11.3, gave a

Received March 9, 1988; revision received May 20, 1988.
Address for correspondence and reprints: Maria A. Musarella, M. D., F.R.C.S.(C), Department of Genetics, Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. © 1988 by The American Society of Human Genetics. All rights reserved. 0002-9297/88/4304-0016$02.00
maximum likelihood estimate of 3 centimorgans (cM) from DXS7 (95% confidence limits 0–15 cM). However, three reports have suggested that there may be a site for XLRP at Xp21, more distal from the centromere (Nussbaum et al. 1985; Francke et al. 1985; Denton et al. 1988). Nussbaum et al. (1985) found that in one large pedigree the locus for XLRP with metallic sheen in the heterozygote segregated with the ornithine transcarbamylase (OTC) locus located at Xp21 and that four crossovers were detected between the DXS7 and XLRP loci. Analysis of four phase-known meioses informative for DXS7, XLRP, and OTC suggested that XLRP with metallic reflex in the heterozygote maps distal to DXS7 (Xp11) and closer to the OTC locus (Xp21).

In support of this more distal location for XLRP, a patient, BB, has been described with four X-linked disorders; RP, Duchenne muscular dystrophy (DMD), McLeod phenotype, and chronic granulomatous disease (CGD). His karyotype revealed an interstitial deletion of Xp21. The DXS84 locus (probe 754) was found to be deleted, but the more centromeric OTC gene was not, suggesting that XLRP maps distal to OTC on the chromosome (Francke et al. 1985). DMD, McLeod phenotype, and CGD have all been mapped to Xp21. The DMD gene has been cloned, and its map location at Xp21.1–21.2 is unequivocal (Ray et al. 1985; Monaco et al. 1987; Burghes et al. 1987). The CGD locus has been mapped to Xp21.1 and cloned on the centromeric side of the DMD locus (Baehner et al. 1986; Royer-Pokora et al. 1986). The locus for the McLeod phenotype has been established between the CGD and DMD loci by analysis of patients with Xp21 deletions (Berтель森 et al. 1988). This leaves XLRP as the remaining locus to be verified as mapping within the BB deletion.

In an attempt to further define the locus for XLRP we report here the results of linkage analysis with seven loci on the X chromosome that span the region between Xcen and Xp21 in four Canadian XLRP families of different ethnic origins.

Material and Methods

Family Studies

Four families in which XLRP is segregating were ascertained through the ophthalmic genetic clinics at The Hospital for Sick Children in Toronto. Figures 1–4 illustrate the four pedigrees, F20, F13, F1, and F11, respectively. Kindreds F11 and F13 are of British descent, F20 is of Italian ancestry, and F1 is of Scottish origin. Detailed family histories and pedigrees were obtained through personal interviews with family members in each kindred. Available family members underwent careful ocular examinations—including Goldman perimetry, dark adaptometry, and electroretinography (ERG)—by one of us (M.A.M.). The criteria used for identifying affected males and female carriers and for establishment of X linkage have been previously described (Bird 1975; Deutman 1977; Wright et al. 1983). Females at risk were taken to be XLRP heterozygotes if they had abnormal electrodiagnostic testing with either (1) ophthalmoscopic changes consistent with the carrier state or (2) a normal fundus (Fishman et al. 1986). In some instances the carrier status of females was inferred if the pedigree structure showed them to be obligate heterozygotes. Males above the age of 13 years with no symptoms, normal examination, and a normal ERG were classified as unaffected.

On ophthalmoscopic examinations, only carriers of F13 demonstrated the tapetal reflex (metallic sheen) in their retinas. The other carriers had variable degrees of retinal pigment epithelium atrophy and pigmentary migration to the inner retinal layers. Several of the affected males in this pedigree F13 had a milder form of XLRP. Several in their thirties still maintain functioning visual acuities and drive during the day. In the other three families the affected males were legally blind before age 30.

DNA Analysis

Peripheral venous blood was collected in EDTA and used as a source of leukocyte DNA. In certain instances, heparinized blood was collected for establishing permanent lymphoblastoid lines by Epstein-Barr virus transformation (Wilson et al. 1982).

High-molecular-weight leukocyte DNA was isolated from 20 ml of whole blood as described by Kunkel et al. (1982). For RFLP analysis, 5 μg of DNA were digested with appropriate restriction endonucleases by using 3–5 units/μg DNA for 6 h under appropriate buffer and temperature conditions. The resultant restriction fragments were separated by electrophoresis for 19–22 h at a constant voltage of 4.5 V through 0.6% agarose gels and transferred in 2 × SSC to Hybond N membranes (Amersham) by the method of Southern (1975). Prior to transfer the agarose gels were placed in denaturing solution (0.5 N NaOH, 1.5 M NaCl) for 40 min followed by 60 min of neutralization (1.5 M NaCl; 0.2 M Tris, pH 7.5; 1 mM EDTA, pH 8.0). The blots were hybridized for 18 h at 42°C in Blotto (5 × SSC; 0.5% Carnation skim milk powder; 0.5% SDS; 10% dextran sulfate; 40% formamide; 0.2 M so-
### Table 1

**RFLP Loci and Probes Used between Xp21-X Centromere**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Probe</th>
<th>Location</th>
<th>Enzyme</th>
<th>Alleles</th>
<th>Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS28</td>
<td>C7</td>
<td>Xp21.3</td>
<td>EcoRV</td>
<td>7, 5, 8</td>
<td>.85, .15</td>
</tr>
<tr>
<td>DXS164</td>
<td>pERT87-30</td>
<td>Xp21.2</td>
<td>BglII</td>
<td>30, 8</td>
<td>.63, .37</td>
</tr>
<tr>
<td>DXS164</td>
<td>pERT87-15</td>
<td>Xp21.2</td>
<td>TaqI</td>
<td>3.3, 3.5</td>
<td>.67, .33</td>
</tr>
<tr>
<td>DXS206</td>
<td>XJ1.1</td>
<td>Xp21.1-21.2</td>
<td>TaqI</td>
<td>3.1, 3.8</td>
<td>.72, .28</td>
</tr>
<tr>
<td>DXS206</td>
<td>XJ5.1</td>
<td>Xp21.1-21.2</td>
<td>SphI</td>
<td>24, 17</td>
<td>.20, .80</td>
</tr>
<tr>
<td>DXS84</td>
<td>754</td>
<td>Xp21.1</td>
<td>PstI</td>
<td>11, 9</td>
<td>.62, .38</td>
</tr>
<tr>
<td>DXS84</td>
<td>754-11</td>
<td>Xp21.1</td>
<td>EcoRI1</td>
<td>4.2, 2.2</td>
<td>.84, .16</td>
</tr>
<tr>
<td>OTC</td>
<td>OTC</td>
<td>Xp21.1</td>
<td>BamHI</td>
<td>18, 5.2</td>
<td>.75, .25</td>
</tr>
<tr>
<td></td>
<td>HO731</td>
<td>Xp21.1</td>
<td>MspI</td>
<td>6.6, 6.2</td>
<td>.61, .39</td>
</tr>
<tr>
<td>DXS7</td>
<td>L1.28</td>
<td>Xp11.3</td>
<td>TaqI</td>
<td>12, 9</td>
<td>.68, .32</td>
</tr>
<tr>
<td>DXS14</td>
<td>58-1</td>
<td>Xp11-cen</td>
<td>MspI</td>
<td>4, 2.5</td>
<td>.65, .35</td>
</tr>
</tbody>
</table>

Diium phosphate, pH 6.7, and 200 μg denatured salmon sperm DNA/ml (Johnson et al. 1984).

The DNA probes used in this study are purified from the vector by gel electrophoresis and by NACS column chromatography (BRL). Purified probes were radiolabeled by nick-translation (Rigby et al. 1977) or random priming (Feinberg and Vogelstein 1983) to a specific activity of $10^8$–$10^9$ dpm/μg. The probe concentration for each blot was $2.5 \times 10^5$ dpm/ml. The blots were washed at 42°C in 2 × SSC, 0.1% SDS for 30 min, followed by one 30-min wash in 0.2 × SSC, 0.1% SDS at 60°C. Blots were exposed to Kodak XAR film at −70°C for 1–3 days with a DuPont Cronex® Lightning Plus intensifying screen.

**Linkage Analysis**

The seven DNA marker loci examined are DXS28 (C7) (Bakker et al. 1985); DXS164 (pERT-87) (Kunkel et al. 1986); DXS206 (XJ1) (Ray et al. 1985); DXS84 (754) (Kofker et al. 1985); OTC (Horwich et al. 1984); DXS7 (L1.28), a gift from P. Pearson (Wiesacker et al. 1984); and DXS14 (58-1) (Bruns et al. 1984). The polymorphic probes used to detect the RFLP and their location are described in table 1. The order of the alleles in each haplotype follows the currently accepted order of the loci on the short arm of the X chromosome: centromere–L1.28 (DXS7)–OTC (OTC)–754 (DXS84)–XJ (DXS206)–pERT87 (DXS164)–C7 (DXS28)–telomere (Goodfellow et al. 1985; Ray et al. 1985; Kunkel et al. 1986). In family 20 (fig. 1), two additional probes, p20 (Wapenaar et al. 1987) and J66 (Ginjaar et al. 1987), were used to define the site of recombination in individual II-6. The latter two probes were gifts from G.-J.B. van Ommen and map within the DMD gene between DXS164 and DXS28.

Linkage analysis was carried out on an IBM-PS/2 using the computer program LIPEP (Ott 1974) and ILINK (Lathrop et al. 1986) for pairwise and multilocus analysis, respectively. For LIPEP, the frequency of the XLRP gene in the general population was taken to be .0001 (Bunker et al. 1984). The RFLP allele frequencies used are previously published values and are shown in table 1. In the multilocus analysis, the spontaneous mutation rate was taken as zero and penetrance was assumed to be 100%. Confidence limits were derived from the z = 1 method proposed by Conneally et al. (1985). In the analysis the two pERT87 probes, 87-30 and 87-15 (DXS164), were treated as one, as were the XJ probes, XJ1.1 and XJ5.1 (DXS206), and the 754 probes, 754 and 754-11 (DXS84). The maximum likelihood recombination distance, $\theta_{max}$, between the loci was chosen as the value of the recombination distance, $\theta$, that maximizes the odds ratio.

**Results**

The results of the RFLP analysis are summarized in the four pedigrees (figs. 1–4). Individuals for whom the XLRP status was unavailable or uncertain are identified in the figure captions. Results of the two-point maximum likelihood estimates for recombination fractions between XLRP and the seven RFLP loci are shown in table 2. For each RFLP locus, values for $\theta_{max}$ and the lod score at $\theta_{max}$ are given. The 90% confidence limits on $\theta_{max}$ are given for lod scores greater than 3.
Table 2

Linkage Data Between the XLRP Locus and Each Marker Locus on Xp

<table>
<thead>
<tr>
<th>Locus</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>.00</th>
<th>.01</th>
<th>.05</th>
<th>.10</th>
<th>.15</th>
<th>.20</th>
<th>.25</th>
<th>.30</th>
<th>.35</th>
<th>.40</th>
<th>θ&lt;sub&gt;max&lt;/sub&gt;</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS28 (C7)</td>
<td>2</td>
<td>199.98</td>
<td>170</td>
<td>14</td>
<td>.71</td>
<td>.89</td>
<td>.89</td>
<td>.80</td>
<td>.63</td>
<td>.42</td>
<td>.20</td>
<td>.18</td>
<td>.91</td>
</tr>
<tr>
<td>DXS164 (pERT87)</td>
<td>3</td>
<td>198.08</td>
<td>431</td>
<td>.53</td>
<td>.72</td>
<td>1.21</td>
<td>1.36</td>
<td>1.32</td>
<td>1.14</td>
<td>.89</td>
<td>.58</td>
<td>.21</td>
<td>1.36</td>
</tr>
<tr>
<td>DXS206 (XJ)</td>
<td>3</td>
<td>97.30</td>
<td>2.13</td>
<td>2.55</td>
<td>2.49</td>
<td>2.30</td>
<td>2.04</td>
<td>1.74</td>
<td>1.41</td>
<td>1.05</td>
<td>.69</td>
<td>.06</td>
<td>2.56</td>
</tr>
<tr>
<td>DXS84 (754)</td>
<td>3</td>
<td>95.68</td>
<td>3.73</td>
<td>4.08</td>
<td>3.91</td>
<td>3.60</td>
<td>3.21</td>
<td>2.77</td>
<td>2.27</td>
<td>1.74</td>
<td>1.17</td>
<td>.05</td>
<td>4.08</td>
</tr>
<tr>
<td>OTC (OTC)</td>
<td>4</td>
<td>93.90</td>
<td>5.47</td>
<td>5.65</td>
<td>5.28</td>
<td>4.75</td>
<td>4.13</td>
<td>3.45</td>
<td>2.72</td>
<td>1.98</td>
<td>1.24</td>
<td>.03</td>
<td>5.69</td>
</tr>
<tr>
<td>DXS7 (L1.28)</td>
<td>3</td>
<td>197.78</td>
<td>1.46</td>
<td>.37</td>
<td>.92</td>
<td>1.08</td>
<td>1.06</td>
<td>.95</td>
<td>.76</td>
<td>.53</td>
<td>.20</td>
<td>.10</td>
<td>1.09</td>
</tr>
<tr>
<td>DXS14 (58-1)</td>
<td>3</td>
<td>299.97</td>
<td>-5.53</td>
<td>-2.23</td>
<td>-99</td>
<td>-.38</td>
<td>.04</td>
<td>.14</td>
<td>.21</td>
<td>.16</td>
<td>.22</td>
<td>.33</td>
<td>(.27</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of informative families.
<sup>b</sup> Number of scored meioses.
<sup>c</sup> Number of recombinants.
<sup>d</sup> θ<sub>max</sub> if germinal mosaic occurred in family 1.

For these four families XLRP was found to be tightly linked to genetic markers in Xp21, OTC, and DXS84 (754), with θ<sub>max</sub> of .03 (90% confidence limits of θ .002–0.15) and .05 (90% confidence limits of θ .002–.22), respectively. RFLP locus DXS206 (XJ), located at Xp21, also shows linkage to XLRP with a θ<sub>max</sub> of .06.

Each of these markers showed only one recombination with XLRP. This occurred in individual II-6 (fig. 1) of family 20. In this affected male there was an apparent recombination between XLRP and all markers tested except for DXS28 (C7) (fig. 1), suggesting that in this family the XLRP mutation maps near DXS28 and above the DMD gene markers (pERT 87-15, XJ1.1, and XJ5.1). Two additional DMD gene probes, J66 and p20, which lie between DXS28 and DXS164, were used to further characterize the limits of the recombination in this patient and to seek a junction fragment that might suggest the possibility of a cytogenetically undetectable inversion. No anomalous bands were seen in this individual or his mother, but individual II-6 was found to be recombined for probes J66 and p20. One of his affected brothers, II-4, carries the opposite recombinant chromosome, recombinant for DXS28 but not for the more proximal markers, suggesting a map location for XLRP below DXS28. Thus, in this family the mutation appears to lie between DXS28 and the DMD locus, a result inconsistent with either previously published linkage data or the fact that the deletion in BB—a male affected with DMD, CGD, McLeod phenotype and RP—does not extend above the DMD locus.

In family 13 (fig. 2) two apparent crossovers were detected between the centromeric locus DXS14 (58-1) and XLRP. These were seen in affected males III-3 and III-7. Three additional recombinations detected in fe-

Figure 1

Segregation of RFLP alleles with XLRP in family 20. The summary of the probes and alleles is given above in the same order as the data presented along the sides of the chromosome stick figures. When phase is known, alleles in coupling are placed on the same side of the vertical line. The mother, I-1, is heterozygous for XLRP, C7, J66, p20, pERT87-15, XJ5.1, 754, OTC, L1.28, and 58-1. Asterisks (*) mark the loci that are recombined with XLRP. Son II-6 is recombinant (*) for J66, p20, pERT87-15, XJ5.1, 754, OTC, L1.28, and 58-1. Another son, II-4, is a recombinant for C7 (*). Daughter II-1, aged 40 years, at time of detailed ophthalmic evaluation, has been scored as normal.
Figure 2  Segregation of RFLP alleles with XLRP for family 13. In this pedigree the carriers have the metallic sheen or tapetal reflex and some males appear to have a milder form of XLRP. Carriers are partially informative for pERT87-35, XJ1.1, XJ5.1, 754, OTC (MspI polymorphism, 6.6/6.2), and 58-I. None of the carriers were informative for L1.28 probes. Clinical status of individuals III-5, III-6, III-9, IV-1, IV-2, IV-3, and IV-5 is unknown. Male IV-3 has inherited the same affected X chromosome as his affected brother, IV-4, but no ophthalmological examination has been performed on him. Females III-5 and III-9 also appear to have inherited the X chromosome with the XLRP mutation from their carrier mother. The status of these females and their sons—IV-1, IV-2, and IV-5—is also unknown. Since males in this pedigree still have functioning vision at age 30, diagnosis may not have been made or may be delayed in these individuals of questionable status. The alleles of these seven individuals were not included in the lod calculations.

males III-5 and III-9 and in male IV-5 were not considered in the calculations, owing to their questionable XLRP status. There were no detectable recombinations between XLRP and either OTC or the more distal probes. DXS7 (L1.28), however, was not informative in this family. Therefore, in this family the XLRP gene maps telomeric to DXS14.

Family 1 (fig. 3) has two recombinants occurring in two affected brothers, III-1 and III-2. They both demonstrate recombinations between XLRP and the loci DXS7 (L1.28) and DXS14 (58.1), placing the XLRP locus telomeric to DXS7. These recombinants occur within the same region with the probes available and could represent independent recombination events. However, another possibility is that they may be a single recombination in a gonadal stem line of their mother, resulting in germ-line mosaicism, as has been documented in several DMD families (Bakker et al. 1987; Darras and Francke 1987; Monaco et al. 1987). With the available markers it is not possible to distinguish between these two possibilities. For this reason these crossovers in family 1 were counted as one single and two independent recombinants. Table 2 includes lod scores and $\theta_{\text{max}}$ values for both possibilities.

In family II (fig. 4) the parents of individuals in generation III were not available for study. However, the genotypes of the females, III-1 and III-6, could be derived for the family pedigrees by minimizing the total number of possible recombinations. In generation III, it was predicted that there were a minimum of three recombinants for XLRP with DXS164, regardless of the marker configurations in the two females. In these three recombinants XLRP segregates with the markers. It appears that the mutation is proximal to the DMD gene (i.e., centromeric to DXS164 in fig. 4). There are two other apparent recombinants with DXS164 in IV-6 and V-6, and in each case the XLRP gene cosegregates with the proximal markers mapping the XLRP locus below the DMD region.
More significant than the lod score data are the meiotic recombinations that occurred in these families. The recombinants in family I map XLRP distal to DXS7 and are consistent with results from family 13, which two recombinants for probe 58-1 also position XLRP on the telomeric side of DXS14. In family 11 the recombinants localize the XLRP locus below DXS164. The recombinations in families 1, 13, and 11, therefore, place the XLRP locus between DXS7 and DXS164 (DMD region).

The results in family 20 are inconsistent with results obtained in the other families. Individual II-6 has a recombination between XLRP and seven of the probes tested, mapping the XLRP mutation in this family distal to the DMD locus. His brother, II-4, has a recombination at DXS28 (C7), placing the XLRP site below DXS28. However, several families have been reported with multiple phenotypes of adrenal hypoplasia (AH), glycogen kinase deficiency (GK), and DMD in various combinations and deletions of the probes C7, L1, and 754. Since the loci for AH and GK map between C7 and DMD and since none of the individuals with deletions of this region express the RP phenotype, the XLRP gene is not likely to be located between DXS28 and the DMD gene (Kousseff 1981; Bartley et al. 1986; Reinier et al. 1983; Patil et al. 1985; Wieringa et al. 1985; Francke et al. 1987; Kenrick et al. 1987). The deletion in patient BB, known to have RP, does not extend above the DMD locus (Koenig et al. 1987).

Evidence in favor of an XLRP locus centromeric to DXS84 (754) comes from DMD patients with deletions of DXS84 and with no detectable evidence of RP or CGD. One of these cases has been studied by pulsed-field gel electrophoresis (van Ommen et al. 1986) using cx5.4 (DXS48), an Xp21 probe centromeric to DXS84 (754). A junction fragment was detected in this patient, indicating a deletion breakpoint close to but above DXS148. In contrast, BB, known to have RP, is deleted for cx5.4. His deletion, unlike other large DMD deletions, extends considerably more centromeric. This would explain why only he has CGD and RP, while other patients with multiple phenotypes do not. To our knowledge, there is only one other patient reported with CGD and DMD (Kousseff 1981), but, unfortunately, ophthalmological examination was not reported. There have also been two other reports of RP occurring in patients with a muscular dystrophy phenotype (Unterrieh 1941; Reinberg 1950), but details are insufficient to draw firm conclusions.

There are at least three possible explanations for the results observed in family 20. One possibility is that
there may be an inversion in the family that reverses the order of XLRP and DMD, placing XLRP next to DXS28. Although there is no cytogenetic evidence for such an inversion, it cannot be ruled out. A second possibility is that three crossovers could be responsible for the pattern of the markers. Another possibility is that the RP in this family may be autosomal recessive rather than X linked. However, this is unlikely, considering the severity of their clinical course and electrophysiological findings. Three of the four affected brothers have had severe visual loss prior to age 30, high myopia, and nonrecordable ERGs. The severity of their clinical findings is more compatible with the X-linked form of RP than with the autosomal recessive form. If the anomalous results in this family are excluded from the analysis, namely, that there is close linkage of XLRP to both OTC and DXS84; in fact, the number of OTC and DXS84 recombinants is reduced to zero and strengthens the main conclusion.

The locus for XLRP has previously been found to exhibit tight linkage to DXS7 (L1.28), which is thought to be located at Xp11 (Bhattacharya et al. 1984; Mukai et al. 1985; Friedrich et al. 1985; Clayton et al. 1987; Wright et al. 1987). The initial data of Bhattacharya et al. (1984) was based on four kindreds with typical XLRP and on another family with a variant of XLRP, choroidoretinal dystrophy. The maximum likelihood estimate of linkage distance was 3 cM (95% confidence limit 0–15 cM). Mukai et al. (1985), in a subsequent study of four informative kindreds, reported a 10-cM distance. When combined with Bhattacharya’s results, the distance was 5 cM, with a 90% confidence interval of 1.5–14 cM. A third study, using C-banding heteromorphism as a centromeric polymorphic marker, demonstrated significant linkage of XLRP to the centromere at an approximate distance of 11 cM (Friedrich et al. 1985).

The recombination fraction in the normal population for X-linked markers gives an estimated distance between DXS7 and DXS84 of approximately 12.5 cM (Drayna and White 1983). If the estimate of Bhattacharya et al. (1984) for the distance between DXS7 and XLRP is taken as being close to the upper 95% confidence limit of 15 cM, this distance would be compatible with an XLRP locus at Xp21. Other groups, including ours, have observed closer linkage to the probes in the Xp21 region. Nussbaum et al. (1985), using a large Latin-American kindred with XLRP in which the carriers demonstrate a metallic sheen or tape
tal reflex, found that four recombinants occurred be-
tween DXS7 and XLRP. These individuals were not recombined for OTC, indicating that the XLRP mutation is much closer to the OTC locus at Xp21. Denton et al. (1988) have reported linkage data in three large pedigrees segregating for XLRP with the tapetal reflex in the heterozygotes. Strongest linkage was found be-
tween the XLRP locus at OTC, with a maximum lod score of 10.64 at a recombination fraction of .00. Analysis of three-point crossovers from these three kindreds suggested that the XLRP locus is distal to DXS7 and proximal to DXS84.

Recently Wright et al. (1987) have addressed the question of a locus at Xp21 by performing multipoint linkage analysis with nine markers in 20 kindreds. Although the multilocus analysis indicates that the position of the XLRP locus is closer to DXS7 (lod = 9.35 at θ = .06 with DXS7 and 2.80 at θ = .16 with OTC), it is difficult to assess the information. It is not clear how many scorables meioses were analyzed for each probe, since the complete pedigree data are not given. The authors provide only one example in a single family of a crossover for DXS84, OTC, and XLRP and never comment on whether DXS7 and the more centromeric markers are informative or recombinant in this family. In addition, the mother’s chromosomes are inferred and the recombinations seen in the two daughters raise the possibility of nonpaternity. Even if the question of paternity is resolved, the recombinations in these daughters are identical and they could possibly be the result of germ-line mosaicism, as described for individuals III-2 and III-3 in family 1 of our study.

It appears at present that there is no clear distinction to the positioning of XLRP at Xp21 or Xp11. There are two possibilities for explaining the discrepancies in localizing the XLRP gene. One is that a single locus for XLRP exists in the short arm of the X chromosome, somewhere between DXS7 and DXS84, with the discrepant data due simply to the results of small sample size. To analyze this situation, a large number of XLRP families must be studied with multiple informative markers. This is currently in progress. XLRP family linkage data is being collected from major centers throughout the world for multipoint linkage analysis. Multilocus analysis is most useful when the data on a large number of families are available; however, for small data sets, such as that used in this study, it is often difficult to draw useful conclusions from this type of analysis. In such data sets the interlocus genetic distance may fail to reach significance or to generate meaningful maximum likelihood recombination estimates. Similarly, chance lack of informativeness may
yield recombination fractions of two adjacent intervals incompatible with that of the composite interval. Therefore, although visual inspection of the pedigrees indicates that XLRP maps telomeric to L1.28 and although two-point analysis indicates tight linkage of OTC and 754, multilocus linkage analysis was not helpful in identifying the most likely placement of XLRP among the set of marker loci.

Another possibility that may also be tested by multipoint analysis given larger number of families is that of heterogeneity; there may be two XLRP loci—e.g., Xp1 and Xp21—on the short arm of the X chromosome. In fact, clinical evidence supports the presence of two distinct loci for XLRP. The frequency of XLRP is significantly different between populations in North America and Great Britain. In Great Britain XLRP comprises 25%–30% of all RP cases reported, while in the United States and Canada only 8% of RP is X linked (Jay 1972; Bird 1975; Fishman 1978; Macrae 1982). Heterogeneity is further supported by phenotypic differences observed in female carriers. Although only one of our pedigrees exhibits the tapetal reflex in the heterozygotes, there are reports of other pedigrees demonstrating segregation of XLRP with tapetal reflex with OTC (Nussbaum et al. 1985; Denton et al. 1988).

The best indication that there is a locus for XLRP located in Xp21 comes from the male patient BB, who has DMD, CGD, RP, and McLeod red cell phenotype. This deletion extends from the DMD locus toward the centromere to include the DXS84 locus but not the OTC locus (Francke et al. 1985). The loci for DMD, CGD, and McLeod phenotype have been mapped to Xp21 (Kunkel et al. 1985; Ray et al. 1985; Baechner et al. 1986; Bertelson et al. 1988). Although the diagnosis of XLRP has been questioned in the patient (Clayton et al. 1986; Wright et al. 1987), in our view the clinical and pathological findings seen in BB are highly consistent with the diagnosis of XLRP. Although there are no characteristic clinical features to distinguish the genetic phenotypes of RP, BB's early onset and severe involvement by age 15 are compatible with the X-linked type (Fishman 1978; Berson et al. 1980). The early detection (age 2 years) of his pigmentary retinopathy is probably due to the fact that he was examined by an ophthalmologist at age 2 for failure to thrive, at an age prior to onset of ocular symptoms. The salt-and-pepper pigmentary disturbance described in BB at age 2 is usually detected in the X-linked form of RP in the first decade of life (Tasman 1983). The only atypical feature associated with his RP was his refractive error, which was hyperopic rather than myopic. Myopia is found in 95% of patients with XLRP, compared with 12% of the normal population (Sieving and Fishman 1978). Histological examination of his 3-h postmortem retinas showed only cone photoreceptors and total absence of rod photoreceptors, in agreement with the ultrastructural findings in retinas from a similar-age patient with XLRP (Hurwitz et al. 1985; Szamier et al. 1979). The suggestion that the RP phenotype in BB is due to his CGD gene deletion (Wright et al. 1987) is unlikely, since the focal chorioretinal lesions described in some males with CGD were never seen in BB. Furthermore, the electroretinographic examinations of patients with CGD are almost always normal, while in BB no rod or cone responses were detectable (Marty et al. 1972; R. A. Pagon, personal communication). Thus, it would appear from the evidence that BB most likely had XLRP in addition to his DMD, CGD, and McLeod phenotypes. If this is the case, then there can be little question that a gene for XLRP exists in band Xp21, between DXS84 and the OTC gene. The results from three of our four families are entirely consistent with this conclusion.

In summary, the linkage data presently available do not exclude either the Xp21 region or the Xp1 region as a location(s) for XLRP genes. If accurate prenatal diagnosis and carrier detection is to be established, new families need to be studied and multipoint linkage analysis of all existing data needs to be performed. Such analysis is underway as a multicenter collaborative effort, and the results may help clarify the present available data in the linkage of the XLRP gene.

Acknowledgments

We thank Drs. J. L. Mandel, P. Pearson, and G.-J. B. van Ommen for the gifts of their probes. We are grateful to Drs. W. Macrae and J. Parker for referring the families to the Ocular Genetics Clinic, to Dr. H. Willard for his helpful discussions, and to S. Burghes for critical review of the manuscript. This research was supported by grants from the RP Eye Research Foundation of Canada and in part by a grant from The Atkinson Charitable Foundation.

References


X-linked Retinitis Pigmentosa


