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Lowe Oculocerebrorenal Syndrome: DNA-based Linkage of the Gene to Xq24-q26, Using Tightly Linked Flanking Markers and the Correlation to Lens Examination in Carrier Diagnosis

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Summary

The Lowe syndrome (LS), or oculocerebrorenal syndrome, has been studied using DNA-based linkage analysis, and the findings have been correlated with the result of a thorough ophthalmologic examination. It was found that the LS gene was linked to markers in the Xq24-q26 region and that the locus DXS42 was the most closely linked marker, giving a LOD score of 3.12 at zero recombination distance. Combined with earlier data, this forms the basis for carrier detection and prenatal diagnosis by using tightly linked flanking markers. A summary of our and other data suggests that the loci DXS17, DXS11, DXS87, and DXS42 are located on the proximal side, and DXS86 and DXS10 on the distal side of the Lowe locus. In isolated cases of LS the question of whether the mother is a carrier of the mutation arises. It was found that a lens examination with slit-lamp illumination and a count of the total number of lenticular opacities is a reliable method of ascertaining the carrier state.

Introduction

The Lowe oculocerebrorenal syndrome (LS) is an X-linked hereditary disease characterized by growth retardation, severe mental retardation, hypotonia, progressive renal tubular dysfunction with acidosis, rickets, aminoaciduria, and proteinuria and the eye abnormalities of congenital cataract and glaucoma (Lowe et al. 1952). Although several biochemical abnormalities have been found (Yamashina et al. 1983), the basic metabolic defect is unknown. The disease is inherited as an X-linked recessive trait; i.e., it almost exclusively affects males. There have been a few reports of female patients with LS (Svorc et al. 1967; Hodgson et al. 1986), one of them a girl with a chromosomal de novo X/autosome translocation.

Carriers of the LS gene are known to have lens opacities, which do not give any clinical symptoms (Cibis et al. 1986). In earlier studies these opacities could not be used alone to identify, for the purpose of genetic counseling, females carrying the LS gene, since lenticular opacities occur in a normal adult population (Cibis et al. 1986). Genetic counseling has been limited by the lack of a method of performing prenatal diagnosis.

The aim of the present study was to further map the LS gene by use of RFLPs and by DNA-based linkage analysis in order to establish the most closely linked polymorphic markers and, by the use of those markers, to introduce the possibility of prenatal diagnosis and increase the accuracy of carrier testing. The objective of this study was also to correlate the DNA-based findings with the findings of the eye examination. The report of a girl with LS and a de novo translocation in Xq25 (Hodgson et al. 1986) suggests that the responsible gene is located close to Xq25, and most of the probes were picked around that region. Recently, Silver Reilly and co-workers (Silver et al. 1987; Silver Reilly et al. 1988) have also reported that the LS gene is linked to Xq24-q26 and have shown that it is possible to use RFLPs to detect carriers. Since the LS individuals suffer from vitamin D–resistant rickets, and since the gene for X-linked hypophosphatemic rickets is mapped at

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Xp22 (Thakker et al. 1987), a few probes closely linked to Xp22 were used. Several hereditary monogenic diseases have been found to be chromosomal deletion syndromes (Francke et al. 1985), and for that reason a cytogenetic analysis was performed using high-resolution banding of the chromosomes from individuals with LS and from their mothers.

The investigation was mainly based on a large 6-generation family in which nine male patients with LS were known. In this family, 34 individuals were investigated. Another 3-generation family, with two affected boys, was also included in the study.

Material

Two families were used in the study. A pedigree of family 1 is presented in figure 1. It is a 6-generation family with nine affected male individuals, three of whom are still alive. The females included in the linkage study were characterized as carriers or noncarriers based on two criteria: (1) a position in the pedigree that makes them obligate carriers and (2) the results of an eye examination (table 3, fig. 1). Blood was drawn from 34 of the family members for the DNA-based study. Eye examination was carried out on 18 of the female family members, who were obligate carriers or could be carriers of the LS gene. Family 2 consists of nine individuals from 3 generations with two affected boys, one of them dead owing to renal failure. From the remaining eight individuals blood samples were taken for the DNA-based investigation.

After DNA analysis, it was possible to deduce the genotype for several of the dead family members. Because of unknown paternity, one woman had to be excluded from the calculations. Because of advanced age, two individuals could not be ophthalmologically examined, and in the calculations it was not stated whether they are carriers. In family 2, ophthalmologic examination was not performed because of low age of two of the four females.

The subjects for lens examination consisted of 18 obligate or possible carriers from family 1, varying in age from 15 years to 74 years.

Methods

Laboratory Methods

DNA extraction, restriction-enzyme cleavage, Southern blotting, nick-translation of probes, hybridization, washing, and autoradiography were performed according to standard procedures (Southern 1975; Kunkel et al. 1977; Maniatis et al. 1982).

Probes

Polymorphic probes defining 10 loci on the X chromosome were used (table 1; Aldridge et al. 1984; Boggs and Nussbaum 1984; Mulligan et al. 1985; Schmeckpeper et al. 1985; Oberlé et al. 1986).

Lens Examination

The pupils in both eyes were dilated, maximally, using 1% cyclopentolate (Cyclogyl®) and 10% epinephrine (Neosynephrin®) topically. The lenses were examined in a corneal microscope with an attached slit-lamp illumination (Haagstreit™ 900). Punctate opacities in the lens cortex (i.e., outside the clear central zone as seen in the slit-lamp) were counted, quadrant by quadrant. Other lens opacities were recorded as well.

Karyotyping

Chromosome preparations were made from peripheral blood cultures from the patients with LS and their mothers. High-resolution G-banding (Yunis and Lewan-

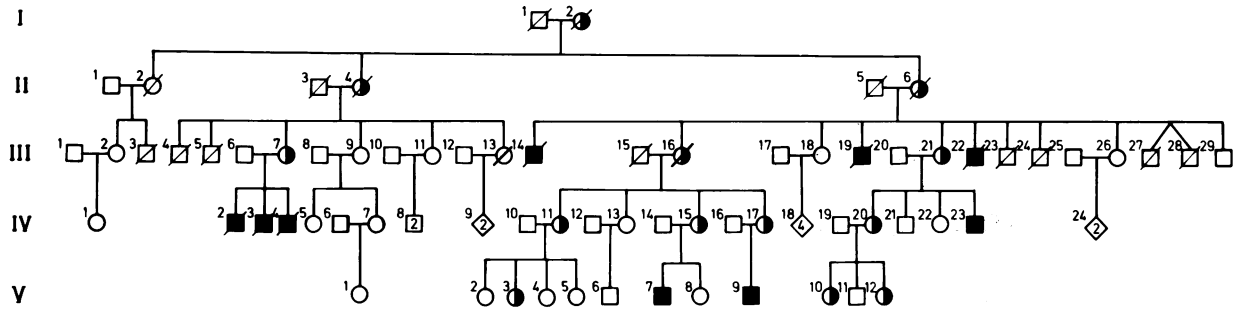

Figure 1 Pedigree of 5 of 6 generations in family 1 with LS. The family includes nine affected males, three of them still alive.

Table 1

The 10 Probes Used in the Study

Locus[a]	Probe	X Region	Source	Reference
DXS41	99-6	Xp22.1–p22.2	L. M. Kunkel	Aldridge et al. 1984
DXS43	D2	Xp22.1–p22.2	L. M. Kunkel	Aldridge et al. 1984
DXS72	X65H7	Xq13–q22	B. J. Schmeckpeper	Schmeckpeper et al. 1985
DXS17	S21	Xq21.3–q22	K. E. Davies	Aldridge et al. 1984
DXS87	A13:RI	Xq21–q24	ATCC	D. J. Shaw[b]
DXS11	22-33	Xq24–q26	L. M. Kunkel	Aldridge et al. 1984
DXS42	43-15	Xq24–q26	L. M. Kunkel	Aldridge et al. 1984
DXS10	6A-1	Xq26	R. Nussbaum	Boggs and Nussbaum 1984
DXS86	St1	Xq26–q27	J.-L. Mandel	Oberlé et al. 1986
DXS100.....	45h	Xq26–q27	B. N. White	Mulligan et al. 1985

[a] As defined by HGM9.

[b] Deposited at the ATCC by Duncan Shaw, Section of Medical Genetics, College of Medicine, University of Wales, Cardiff.

dowski 1983) was performed in all the LS individuals and obligate carriers.

Linkage Analysis

The program package LIPED (Ott 1974) was used in an updated version (Oct. 1987) as were LIPED MAX (Oct. 1987) and VACO3 (July 1987), also from J. Ott. The frequency of the LS gene was estimated to be 0.5×10^{-3}, and the RFLP allele frequencies were as published (see references in table 1). Calculations were performed assuming a mutation rate for LS of 5×10^{-6} and a penetrance of .94 in the female heterozygote (Silver Reilly et al. 1988).

Results

The DNA examination revealed that the locus DXS42 is most closely linked to the gene responsible for LS, giving a peak LOD score of 3.12 at 0% recombination (table 2). DXS42 is mapped to Xq24-q26 (Aldridge et al. 1984). The result of the linkage analysis is thus in agreement with findings of a translocation breakpoint at Xq25 in a female LS patient (Hodgson et al. 1986). The locus DXS86 showed no recombination either but gave a lower LOD score. The other loci that mapped centromeric and telomeric to Xq25 showed recombinations with regard to the LS gene. The LOD scores

Table 2

Linkage Analysis of Loci Defined by 10 Moleuclar Probes and the X-Linked LS, with the LOD Scores at Different Recombination Fractions

		Recombination Fraction							
Locus	X Region	.00	.01	.05	.10	.15	.20	Z_{max}	$\hat{\theta}$
DXS41	Xp22.1-p22.2	.63	.63	.57	.52	.46	.40		
DXS43	Xp22.1-p22.2	−16.56	−11.35	−4.60	−3.14	−2.27	−1.66		
DXS72	Xp13-q22	−.66	−.65	−.45	−.31	−.22	−.15		
DXS17	Xq21.3-q22	−4.65	−.67	2.52	2.81	2.78	2.60	2.82	.117
DXS87	Xq21-q24	−1.06	1.21	2.60	2.55	2.37	2.13	2.60	.065
DXS11	Xq24-q26	−.88	1.11	2.86	3.01	2.92	2.71	2.99	.099
DXS42	Xq24-q26	3.12	3.11	2.85	2.56	2.27	1.95	3.12	.000
DXS10	Xq26	−12.17	.70	2.26	2.32	2.19	1.97	2.33	.084
DXS86	Xq26-27	2.13	2.13	1.96	1.78	1.60	1.41	2.13	.000
DXS100	Xq26-q27	−104	−15.46	−4.63	−2.74	−1.74	−1.11		

NOTE.—Z_{max} = maximum LOD score; $\hat{\theta}$ = maximum likelihood recombination distance.

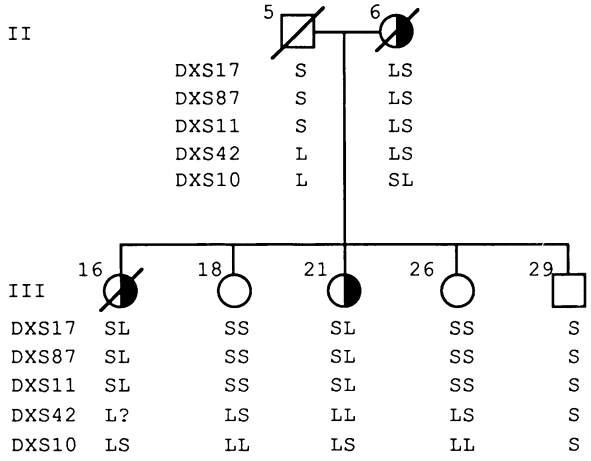

Figure 2 Condensed pedigree of part of the second and third generations of family 1 with LS. L = large allele; S = small allele; ? = unknown allele. In III:29 a recombination event has occurred between DXS10 and the Lowe locus. The individual has inherited the S allele that is linked to the mutated Lowe gene in the family.

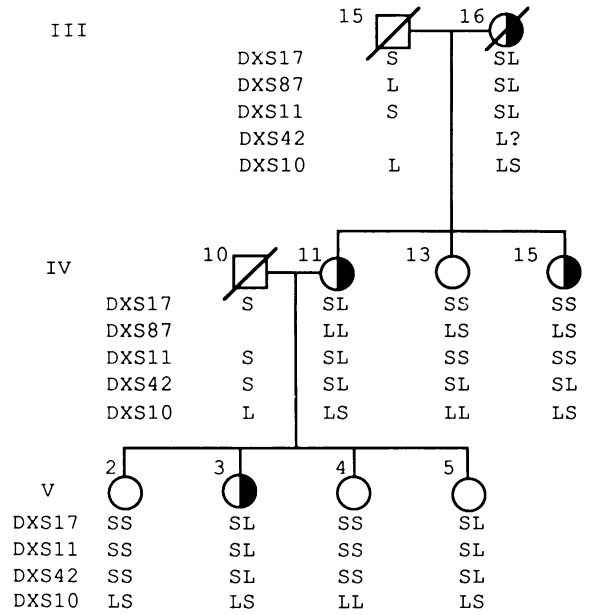

Figure 3 Condensed pedigree of part of the third, fourth, and fifth generations of family 1 with LS. L = large allele; S = small allele; ? = unknown allele. Since the phase of linkage in III:16 is known from her father (fig. 2; II:5), it is found that in the case of IV:15 a recombination event has occurred between the Lowe locus and the loci DXS17, DXS87, and DXS11. In another phase-known meiosis, V:2 inherited the DXS10 S allele linked to the mutated Lowe gene. Since she has a normal lens finding, it was concluded that a crossover had occurred. V:5 has the most normal lens finding of all the women examined, yet she has inherited the DXS42 L allele and the DXS10 L allele, which in this family are linked to the mutated Lowe gene. This means that she can represent either a nonpenetrant carrier or a double recombinant.

for the different probes at varying recombination distances are given in table 2.

An interesting observation was that in the meiosis giving rise to III:29 (fig. 2) a recombination has taken place between DXS10 and the LS gene. No recombination events between the LS gene and any of the loci DXS17, DXS87, DXS11, and DXS42 were observed. On the other hand, in the meiosis giving rise to IV:15 (fig. 3) a recombination event has occurred between the LS gene and the loci DXS17, DXS87, and DXS11 but not between DXS10 and the LS gene. This indicates that DXS17, DXS87, and DXS11 are located on one side of the LS gene and that DXS10 is on the other side. When the previous subchromosomal localization of the respective loci is taken into account, this means that DXS17, DXS87, and DXS11 are centromeric and that DXS10 is telomeric with regard to the LS gene. It is worth noting that the loci DXS41 and DXS43, known to map closely to the Xp locus for X-linked hypophosphatemic rickets (Thakker et al. 1987), do not show linkage to the LS gene. This indicates that rickets in LS is caused by a mechanism different from the one that causes classical X-linked hypophosphatemic rickets.

Lens Examination

The total numbers of punctate opacities from both lenses of each patient are given in table 3. The investigated women fell into two groups: one with few lenticular punctate opacities (<100; range 4–66) and one with many (>100; range 159–706). The patients with >100 opacities in both lenses also displayed a similar pattern; i.e., the opacities were located in the equatorial area of the lens. When correlated with the DNA-based study, all but one of the subjects (V:5; fig. 1) fell into the corresponding group, i.e., carrier or noncarrier. The examination revealed one subject, 74 years old, with bilateral senile cortical opacities and one, 24 years old, with very small central bilateral, nuclear congenital opacities.

Cytogenetic Investigation

The X chromosomes of the patients with LS and of their mothers did not show any detectable deletion when high-resolution G-banding was used. If any deletion is responsible for the syndrome it must thus be submicroscopic.

Discussion

This study has shown that the gene responsible for LS is closely linked to the locus DXS42 mapped to Xq24-

Table 3

Total Number of Punctated Lenticular Opacities from Both Lenses of Obligate and Potential Carriers of the LS Gene

Pedigree No.	Year Born	No. of Opacities
III:18	1913	66
III:21	1918	462
III:26	1925	30
IV:7	1934	44
IV:11	1932	532
IV:13	1940	33
IV:15	1941	413
IV:17	1948	220
IV:20	1942	706
IV:22	1946	30
V:1	1963	55
V:2	1958	10
V:3	1961	159
V:4	1964	13
V:5	1967	4
V:8	1972	20
V:10	1964	285
V:12	1970	236

NOTE.—More than 100 opacities is regarded as diagnostic for carriers of the LS gene.

q26, with a maximum LOD score of 3.12 at 0% recombination distance. The locus DXS86 also shows no recombination, but the family material was too small to give definite proof of linkage. In the large family 1, recombination events have occurred that make it likely that the loci DXS17, DXS87, and DXS11 are located centromeric and that DXS10 is located telomeric relative to the LS gene. This finding is of great importance in genetic counseling since the use of flanking markers makes the diagnosis much more reliable. If someone has inherited the haplotype known to be linked to the healthy gene, a double recombination has to have occurred for that person to have in fact inherited the mutated gene. The loci DXS42 and DXS86 did not show any recombination event. Thus, this study cannot answer the question of whether they are located centromeric or telomeric. In a recent report (Silver Reilly et al. 1988), the segregation of the translocation chromosomes, from the girl with LS (Hodgson et al. 1986), into different somatic cell hybrids was described. It was shown that the chromosome carrying the Xq25-qter region also contained DXS10, which is in agreement with our finding. This indicates that no major rearrangements have occurred near this locus. It was also shown that the chromosome with the region Xpter-q25 contained DXS42, which indicates that it can be used as a flanking marker on the centromeric side. DXS86 has been mapped to Xq26-q27 (Oberlé et al. 1986), which argues that it is located on the distal side of the LS gene and thus can be used as a flanking marker on that side. A summary of our and other data (Silver Reilly et al. 1988) suggests that the loci DXS17, DXS11, DXS87, and DXS42 are located on the proximal side and that DXS86 and DXS10 are on the distal side of the Lowe locus. A newly identified *BclI* polymorphism at the DXS42 locus (Silver Reilly et al. 1988) did not add any information to our material.

In the report by Silver Reilly et al. (1988), linkage analysis of LS was performed, identifying DXS42 and DXS10 as the most closely linked loci. No recombination was found between LS and DXS42, with a maximum LOD score of 6.63. The two studies taken together give a maximum LOD score of 9.75 at 0% recombination distance, providing very strong evidence for linkage. The study by Silver Reilly et al. showed close linkage between LS and DXS10, with a maximum LOD score of 7.07 at 3% recombination, but in our study we found a most likely recombination frequency of 8.4% (95% confidence limits 5.9%–10.9%, Z_{max} 2.33). This should be taken into consideration when DXS10 is used for genetic counseling.

Several attempts have been made to find a reliable way of determining the carrier state by lens examination. The results, however, have been conflicting, and the reasons for this are several. One reason is that punctate cortical opacities occur both in the normal group and in the carrier group (Gardner and Brown 1976). Another difficulty is that the time of development of the punctate opacities in both the normal population and the carrier group is unknown. For genetic counseling, it is of course most important to be able to diagnose the carrier state as early as possible. In our study, we could show that a very careful lens examination in slit-lamp illumination and a count of cortical punctate opacities divided the women into one group with <100 and one group with >100 opacities. Furthermore, the group with >100 opacities all displayed a typical pattern, with the opacities being located in the equatorial area of the lens. When these findings were correlated with the results of the DNA analyses, some observations were made. In family 1, one person, V:5 (fig. 1), had the smallest number of lens opacities of all women examined, although she had inherited both the DXS42 and DXS10 alleles linked to LS; i.e., she had inherited flanking markers linked to the mutated gene. This means

that she can represent either a nonpenetrant carrier or a double recombinant. Person V:2 of family 1 also had a normal lens status and had inherited the DXS42 allele linked to the "healthy" LS gene. On the other hand, she had inherited the DXS10 allele linked to the mutated LS allele. We conclude that a recombination event has occurred between the LS gene and DXS10 in the meiosis that gave rise to V:2, family 1. In all remaining women there was a positive correlation between the results of the lens examination and the RFLP findings. Women with <100 lenticular opacities had inherited markers linked to the healthy gene, and those with >100 lenticular opacities had inherited markers linked to the mutated gene. In two earlier studies (Cibis et al. 1986; Silver Reilly et al. 1988) all examined obligate carriers had a positive eye examination, which was also the case in our study, making a total of 26 obligate carriers, all showing a typical eye finding. When potential carriers were included (Silver Reilly et al. 1988), a penetrance of 94% was estimated. We also noted that the number of opacities increases with age, but in no case did the number reach 100 in women classified as noncarriers, and even in a young (17-year-old) carrier 236 lens opacities were found.

In conclusion, we have demonstrated that a combination of DNA investigation and eye examination is a powerful method for deciding whether a woman is a carrier of an LS mutation. This method is also applicable when the first case in a family occurs. We have also demonstrated a method for performing prenatal diagnosis using RFLP markers flanking the LS locus.

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