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A Novel Locus for Leber Congenital Amaurosis Maps to Chromosome 6q

To the Editor:

Leber congenital amaurosis (LCA) (MIM 204000/204100) is a clinically and genetically heterogeneous retinal disorder that occurs in infancy and is accompanied by profound visual loss, nystagmus, poor pupillary reflexes, and either a normal retina or varying degrees of atrophy and pigmentary changes (Leber 1869, 1871; François 1968). The electroretinogram (ERG) is extinguished or severely reduced (Franceschetti 1954). LCA is largely a recessive disease, although autosomal dominant pedigrees have been identified (Sorsby et al. 1960; Heckenlively 1988). To date, three genes for LCA have been identified and sequenced: retinal guanylate cyclase (*GUCY2D*) on chromosome 17p13; retinal pigment epithelium protein (*RPE65*) on chromosome 1p31; and cone-rod homeobox (*CRX*) on chromosome 19q13.3. One additional locus has been identified on chromosome 14q24 (Stockton et al. 1998). We show evidence for linkage to chromosome 6q11-16 in a multigenerational kindred of Old Order River Brethren. The disease gene maps to a 23-cM interval flanked by DNA polymorphic markers D6S1551 and D6S1694, with a maximum two-point LOD score of 3.38 (recombination fraction [θ] zero) at D6S391. Two candidate genes on chromosome 6 were screened for mutations: gamma aminobutyric acid rho1 and rho2 (*GABRR1* and *GABRR2*) at 6q14-21 (Cutting et al. 1992), and interphotoreceptor matrix proteoglycan (*IMPG1*) at 6q13-15 (Gehrig et al. 1998).

The incidence of LCA is 3 in 100,000 persons and accounts for $\geqslant$5% of all inherited retinal dystrophies (Perrault et al. 1996). Clinical and genetic heterogeneity have been demonstrated (Wardenburg 1961; Camuzat et al. 1996). The phenotype has been associated with familial juvenile nephronophthisis and cone-shaped epiphyses (Saldino-Mainzer syndrome) and with kidney disease (Senior-Loken syndrome), osteoporosis, metabolic diseases, and neurological abnormalities (Loken et al. 1961; Senior et al. 1961; Dekaban 1969; Mainzer et al. 1970; Ellis et al. 1984).

The first locus for LCA was mapped to 17p13 with the use of homozygosity mapping in consanguineous families of North African descent (Camuzat et al. 1996). Mutations in the retina-specific guanylate cyclase gene (*RETGC 1*), on chromosome 17p13, involved in phototransduction, were subsequently identified (Perrault et al. 1996). Mutations in *RPE65* on chromosome 1p31, specific to the retinal pigment epithelium involved in retinoid metabolism, were reported in patients with LCA, thus establishing a second gene (*LCA2*) for this heterogeneous disease (Marlhens et al. 1997). The photoreceptor-specific homeobox gene *CRX,* on chromosome 19q13.3, has been implicated as the third gene, since mutations were demonstrated (Freund et al. 1998). A novel locus on chromosome 14q24 (*LCA3*) was identified in consanguineous Saudi Arabian families (Stockton et al. 1998).

We studied a consanguineous family belonging to the Old Order River Brethren, a religious isolate originating in eastern Pennsylvania. The Old Order River Brethren descended from the Swiss, who emigrated to America in the 1750s in pursuit of religious freedom (Breckvill 1972). The kindred includes three affected individuals in two related sibships (fig. 1) who were initially evaluated at the Johns Hopkins Center for Hereditary Eye Diseases (JHCHED) and who are being followed annually. The patients presented with visual acuities in the order of 20/100–20/400, nystagmus, high hypermetropia, poor pupillary reflexes, and normal fundi. Progressive hypermetropia and increasing peripheral retinal mottling, of varying degree, were noted. The ERG was abolished. Review of other systems was unremarkable. We report a novel locus for LCA (*LCA5*) in this pedigree, on chromosome 6q11-16, by linkage analysis and homozygosity mapping.

Venous blood samples were obtained from 27 family members of the Old Order River Brethren community and a cheek brush sample was obtained from an infant (individual 29). Consents were obtained in accordance with regulations of the Johns Hopkins Medical Institutions' Joint Committee on Clinical Investigation. DNA was isolated from whole blood by means of the QIAamp Blood Kit (Qiagen), according to the manufacturer's in-

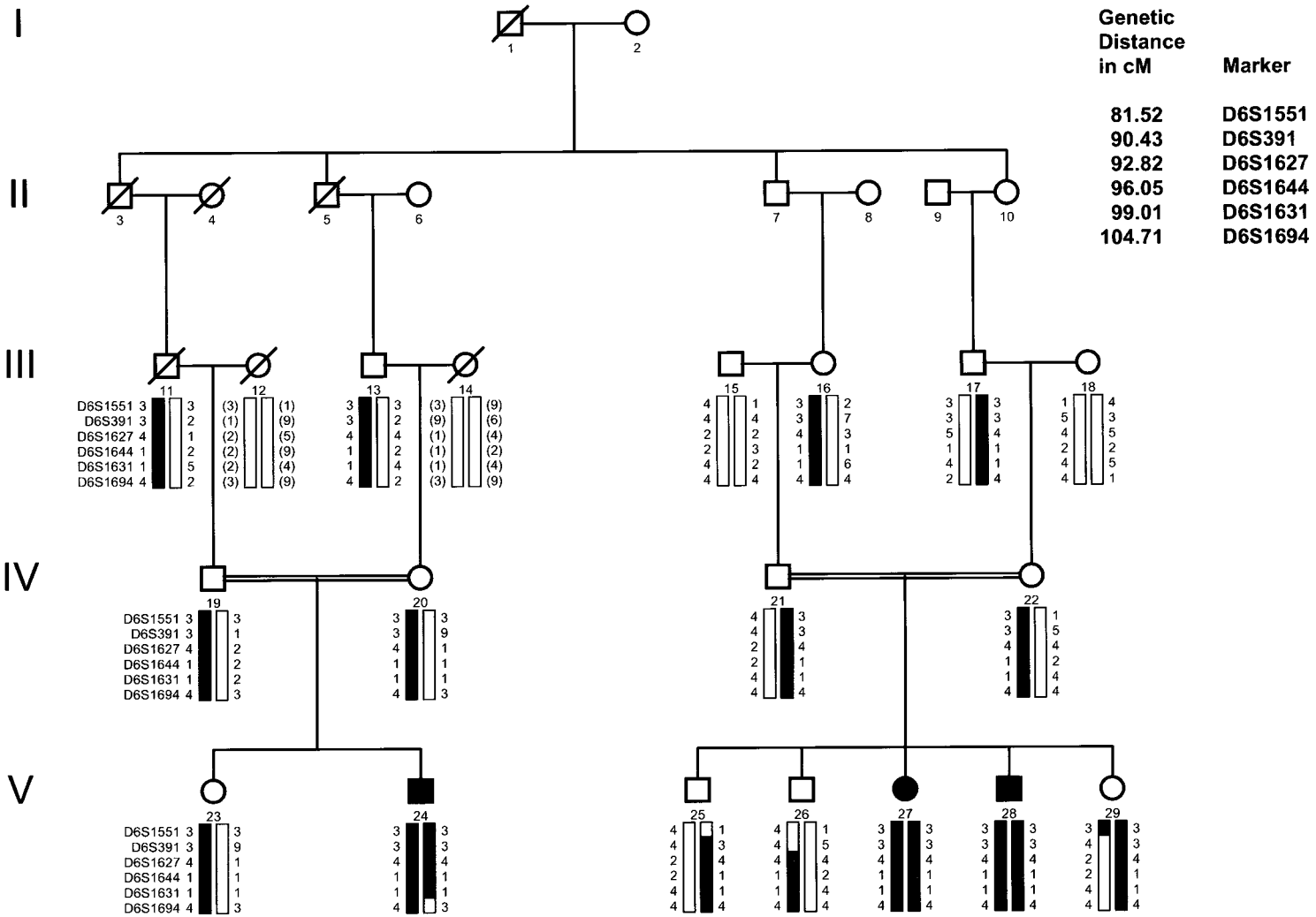

Figure 1 Pedigree of family with LCA. Blackened symbols represent affected individuals. Blackened bars indicate disease-linked haplotype. Genotype (in parentheses) is inferred. Markers are listed in order, from centromere to telomere. Critical recombination events are noted in individuals 29 and 24 at markers D6S1551 and D6S1694, respectively.

structions. The alkali method was used to obtain DNA from the single cheek sample.

Initially, the affected members and their first-degree relatives were screened to exclude linkage to the regions of the previously described genes involved in LCA on chromosomes 1, 17, and 19. The screen was then extended, by use of the whole-genome 8A multiplex version of markers spaced at 20 cM (Research Genetics). A region of homozygosity was identified on chromosome 6q. Further analysis, with additional markers in all potentially significant family members, was undertaken. Marker information was obtained from the Genome Database.

PCR-based genotyping, with fluorescent labeled markers, was performed by means of the Applied Biosystems 373 automated DNA sequencer. PCR reactions were performed in a 9600 Perkin Elmer thermocycler, and the PCR products were checked for amplification with a 3% agarose gel (Saiki et al. 1988).

The amplified PCR product was genotyped by means of the automated DNA sequencer. GENESCAN ANALYSIS 2.0.0 and GENOTYPER version 1.1 software were used, to size the PCR products and to analyze the data. Allele sizes were scored by two independent observers.

Two-point linkage analysis was performed by use of the MLINK option of the FASTLINK program, version 5.1 (Lathrop et al. 1984; Cottingham et al. 1993.) In this pedigree, LCA was analyzed as an autosomal recessive trait with complete penetrance, with an assumed allele frequency of .0032. A total of 40 microsatellite markers (Research Genetics) on chromosome 6 were analyzed, to determine the minimum region containing the new gene (Lander and Botstein 1987). Marker allele frequencies were estimated by means of the Genetic Analysis System, version 2.0 (Young), and GCONVERT (Duffy). The final LOD scores were computed by means of the allele frequencies generated by the GCONVERT program. Recombination frequencies for males and females were assumed to be equal. All inbreeding loops in the family were disconnected for computational reasons (Ott 1991) (fig. 1).

The GENEHUNTER program was used to perform multipoint linkage analysis against a fixed map of 17 informative markers, with an assumed equilibrium between marker and test loci (Kruglyak et al. 1996). These markers were selected from the original 40 microsatellite markers because they were highly polymorphic and their relative orders and map distances were well estimated in public databases. The comprehensive genetic map of the Center for Medical Genetics, Marshfield Medical Research Foundation, provided the sex-averaged genetic distances for the markers noted in figure 1. The same map provided the order for 37 markers. The remaining three markers were placed by means of the Genome Location Database. Subsequently, the genetic framework map from the Center for Medical Genetics, Marshfield Medical Research Foundation, reduced the 37 markers to 23, indicating that some markers occurred at identical positions. Thus, in the analyses in which multiple markers had identical positions, the markers with reduced information content were dropped in favor of those with better information content.

Multipoint LOD scores were computed with the same model described above for two-point analysis. Multipoint nonparametric linkage (NPL) scores were also computed with the use of only the affected individuals. Because of the inherent limitation on pedigree size in the GENEHUNTER program, the large pedigree was trimmed and broken into two separate units, accounting for some potential loss of power in the analysis. The data were examined for regions of allelic homozygosity in the affected individuals (Dib et al. 1996). Haplotype analysis was used to further define the interval containing the disease locus (Lathrop et al. 1985).

The following retina-specific genes on chromosome 6 were evaluated for the presence of disease-causing mutations: the *GABRR1* and *GABRR2* genes on chromosome 6q14.1-21 (Cutting et al. 1991, 1992) and the *IMPG1* gene on chromosome 6q13-15 (Gehrig et al. 1998) (fig. 2).

GABRR1 and *GABRR2* are assumed to have arisen by gene duplication and share a 50% homology with each other. GABA is a neuroinhibitory transmitter mediating fast synaptic inhibition by activating chloride channels. *GABRR1* is expressed largely in the retina; *GABRR2* is expressed primarily in the brain. *GABRR1* expression in the developing retina suggests its possible role as a candidate gene. In exons 1 and 4, polymorphic changes were identified. This did not change in the amino acid. These polymorphic changes were also identified in the normal population. No sequence changes were noted in *GABRR2*.

IMPG1, a novel gene encoding a major proteoglycan of the interphotoreceptor matrix, is expressed in the retina by both rods and cones and maps to 6q13-15; it was considered a further candidate gene for LCA (Gehrig et al. 1998). No significant changes were noted after the 17 coding exons of this gene were sequenced.

Linkage of LCA in the Old Order River Brethren was found at chromosome 6q11-16, supported by statistically significant two-point LOD scores with maximum LOD score (Z_{max}) 3.38 ($\theta = 0$) at D6S391 (table 1). Haplotype analysis of recombination events localizes the disease locus to a region of 23 cM, flanked by D6S1551 and D6S1694, thus identifying a new locus for LCA. Critical recombinant events were observed at marker D6S1551 in individual 29, who is unaffected, and at marker D6S1694 in individual 24, who is affected, defining the centromeric and telomeric boundaries, respectively. A common haplotype covers the region in all

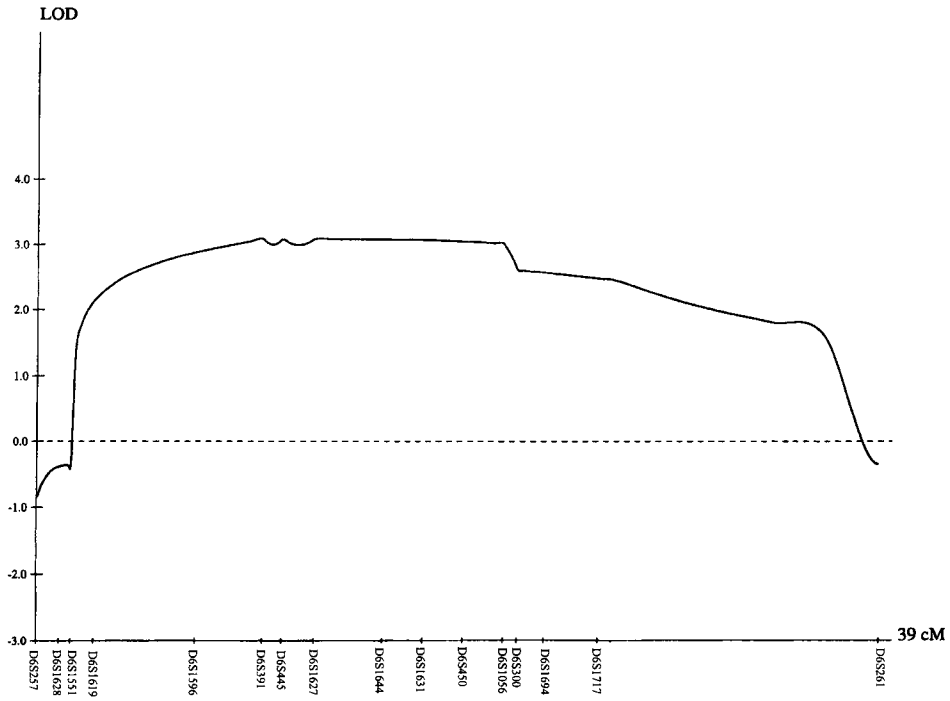

Figure 2 Results of multipoint LOD score analysis using the GENEHUNTER program. The centromere is located toward the left of the graph. Maximum LOD scores were obtained between markers D6S391 and D6S450.

the affected individuals (fig. 1). Homozygosity of other highly informative markers across the candidate region was noted.

The maximum multipoint LOD score was 3.10 between D6S391 and D6S450, a 9.5-cM interval (fig. 2). The multipoint NPL score was significant, with $P <$ .004 for a 23-cM region. Sequencing of candidate genes *GABRR1* and *GABRR2* and *IMPG1* revealed polymorphic changes only.

LCA in the Old Order River Brethren, a highly inbred community, maps to a 23-cM interval on chromosome 6q11-16, as defined by linkage analysis and homozygosity mapping. Since this population is genetically isolated, and since LCA is quite rare, we presume that a single common ancestor was a carrier for this recessive trait (Lander and Botstein 1987). The large size of the region of homozygosity in the family and the history of migration indicate the recency of the mutation in the population (fig. 1).

LCA in this pedigree was not associated with multisystem abnormalities. Renal function remains normal. Neurological and hepatic function were within normal limits. The patients are of normal stature and intelligence. Neither photophobia nor photoattraction was reported in infancy, although pressing on the globes (the digito-ocular phenomenon of Franceschetti-Bamatter) played a prominent part in childhood behavior (Franceschetti 1954). Visual dysfunction, nystagmus, and the digito-ocular phenomenon were noticed in early infancy. A high hyperopic refractive correction was noted in all the patients (Wagner et al. 1985). Ophthalmoscopic examination in infancy revealed normal fundi, but in childhood, attenuated retinal vasculature with a varying degree of pigmentary changes was noticed. Electroretinography showed a markedly reduced response in the affected individuals. Vision has been stable in all affected members of the family who have been followed clinically at JHCHED.

Genetic studies have identified a large region on chromosome 6q responsible for several retinal dystrophies (Small et al. 1992, 1993, 1997; Stone et al. 1994; Kelsell et al. 1995, 1998; Sauer et al. 1997; Griesinger et al. 1998; Rabb et al. 1998; Ruiz et al. 1998). *LCA5* lies in the overlapping region of autosomal recessive RP at 6cen-q16 (Ruiz et al. 1998), progressive bifocal chorioretinal dystrophy (*PBCRA*) at 6q12-21 (Kelsell et al. 1995), North Carolina macular dystrophy at 6q14-16.2 (Small et al. 1993), Stargardt-like dominant macular degeneration (*STGD3*) at 6q13 (Stone et al. 1994; Griesinger et al. 1998), and dominant cone-rod dystrophy (*CORD7*) at 6q13-15 (Kelsell et al. 1998) (fig. 3). The occurrence of multiple loci so closely spaced in the genome could indicate the presence of a number of retinal genes in continuum, since the phenotype of all these

retinal dystrophies, their ophthalmologic appearance, age at onset, and the extent and pattern of visual loss are varied. On the other hand, like the *ABCR* gene mutations that cause autosomal recessive retinitis pigmentosa (Martinez-Mir et al. 1997, 1998), juvenile and late-onset fundus flavimaculatus (Allikmets et al. 1997*a*), cone-rod dystrophy (Cremers et al. 1998), age-related macular disease, and recessive Stargardt disease (Kaplan et al. 1993; Gerber et al. 1995, 1998; Allikmets et al. 1997*b*), leading to distinct phenotypes (Lewis et al. 1999), it is possible that a single large gene in the proximal centromeric portion of the long arm of chromosome 6 could cause a myriad of retinal dystrophies, LCA being the most severe.

Perhaps *LCA5* is allelic with *STGD3, RP25, CORD7, MCDR,* and *PBCRA*. It is conceivable that mutations in different sites cause different structural alterations in the predicted protein, predisposing to varying phenotypes (Rozet et al. 1998).

The three other genes causing LCA are known to cause other phenotypically varied retinal dystrophies as well, raising the possibility of a similar situation in the *LCA5* gene. *GUCY2D* mutations (*LCA1*) have been identified in autosomal dominant cone-rod dystrophy (Kelsell et al. 1998), although *RPE65* (*LCA2*) mutations cause autosomal recessive retinitis pigmentosa as well as LCA (Morimura et al. 1998). Mutations in the cone-rod homeobox gene are now known to cause autosomal dominant cone-rod dystrophy, LCA, and late-onset dominant retinitis pigmentosa (Sohocki et al. 1998). It is currently possible to identify mutations of the known LCA genes in less than one-third of the patients with LCA (Dharmaraj et al. 1999). The isolation of another locus for this retinal disorder, *LCA5,* on chromosome 6q, will account for an additional proportion of patients with

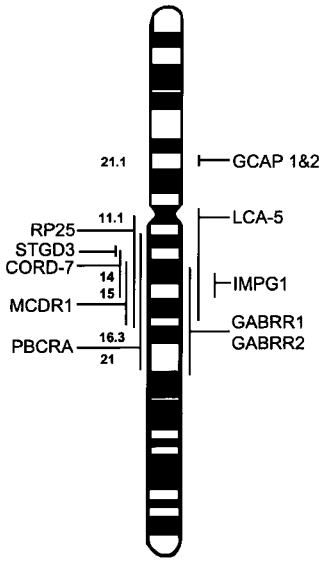

Figure 3 Chromosome 6 ideogram, showing the location of candidate genes screened and retinal disease loci in the region.

Table 1

Two-Point LOD Scores for Linkage between LCA and Chromosome 6 Markers

	LOD Score at θ =								
Order	.00	.01	.50	.10	.20	.30	.40	θ_{max}	Z_{max}
D6S257	−1.33	−.17	.67	.92	.93	.71	.39	.145	.97
D6S1628	−1.24	1.00	1.49	1.52	1.29	.93	.49	.084	1.53
D6S1658	−1.23	−.63	−.15	.04	.19	.18	.11	.239	.20
D6S430	−1.17	1.41	1.85	1.82	1.46	1.00	.51	.066	1.86
D6S1551	−1.32	.27	.82	.94	.88	.67	.37	.117	.95
D6S1619	1.20	1.17	1.03	.87	.59	.36	.17	.001	1.20
D6S1596	1.23	1.20	1.10	.96	.71	.47	.23	.001	1.23
D6S391	3.38	3.30	3.00	2.62	1.87	1.16	.55	.001	3.38
D6S1707	3.15	3.07	2.76	2.37	1.63	.98	.47	.001	3.15
D6S251	3.22	3.14	2.83	2.45	1.71	1.06	.50	.001	3.22
D6S445	2.97	2.89	2.59	2.23	1.56	.98	.48	.001	2.97
D6S1627	2.33	2.28	2.07	1.82	1.33	.87	.43	.001	2.33
D6S1644	2.20	2.15	1.93	1.66	1.17	.74	.35	.001	2.20
D6S1631	2.70	2.63	2.38	2.07	1.47	.93	.45	.001	2.70
D6S450	2.28	2.23	2.01	1.74	1.24	.79	.38	.001	2.28
D6S1056	1.47	1.42	1.22	1.02	.70	.46	.23	.001	1.47
D6S300	1.24	1.33	1.43	1.36	1.07	.71	.35	.050	1.43
D6S1716	.08	.10	.13	.14	.13	.11	.08	.090	.14
D6S1694	−.40	−.31	−.14	−.07	−.07	−.07	−.04	.848	.19
D6S1717	−.73	−.74	−.77	−.77	−.65	−.43	−.20	.827	.39
D6S261	−1.18	−.60	−.10	.10	.23	.21	.13	.231	.23

an identifiable gene mutation. Recruitment of additional families with LCA to further narrow the critical region is under way, and candidate gene analysis continues.

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Genome Location Database, http://cedar.genetics.soton.ac.uk/pub/PROGRAMS/ldb (for placing markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for LCA [MIM 204000/204100])

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A Gene for an Autosomal Dominant Scleroatrophic Syndrome Predisposing to Skin Cancer (Huriez Syndrome) Maps to Chromosome 4q23

To the Editor:

Huriez syndrome (MIM 181600), also referred to as "sclerotylosis," is an autosomal dominant genodermatosis, characterized by the triad of congenital scleroatrophy of the distal extremities, palmoplantar keratoderma (PPK), and hypoplastic nail changes, that was first described in two large pedigrees from northern France (Huriez et al. 1968). Several additional families have since been described (Lambert et al. 1977; Fischer 1978; Shaw et al. 1978; Hamm et al. 1996; Kavanagh et al. 1997). The development of aggressive squamous cell carcinoma (SCC) of the affected skin is a distinctive feature of the syndrome, occurring in ~15% of affected individuals. SCC in Huriez syndrome is characterized by early onset, mostly in the third to fourth decade of life, and by early metastasis formation (Hamm et al. 1996). The pathogenetic mechanism of tumorigenesis in Huriez syndrome is unknown.

Linkage to the MN–blood-type locus on chromosome 4q28-q31 was reported initially and has subsequently been refuted (Delaporte et al. 1995; Kavanagh et al. 1997). We therefore embarked on a linkage analysis for Huriez syndrome with highly polymorphic microsatellite markers, beginning on chromosome 4. After informed consent was obtained, 22 affected and 35 unaffected members of one of the families first described by Huriez (family A) and a second family originating from the same region of northern France (family B) were included in the analysis. We calculated two-point LOD scores between each marker locus and Huriez syndrome, under the assumption of autosomal dominant inheritance with complete penetrance, a frequency of .0005 for the disease allele, and equal allele frequencies for each marker allele, using the LINKAGE version 5.21 software (Lathrop et al. 1984). With reference to the Human Gene Map (Schuler et al. 1996), we identified marker D4S424 within 2 cM of the glycophorin A gene (GYPA), which is the erythrocyte membrane protein that encodes the MN–blood-group receptors. D4S424 yielded a LOD score of -10.7 at recombination fraction (θ) 0.01. In the families under investigation, the MN–blood-type locus was therefore excluded as a candidate region for Huriez syndrome. Our finding is consistent with the exclusion of the MN locus in the first English family with Huriez syndrome (Kavanagh et al. 1997). In contrast, marker D4S1560 gave evidence for linkage, with a LOD score of 4.4 at $\theta = 0$. Subsequent analysis of additional flanking markers confirmed localization of the Huriez locus to this region, with the highest LOD score (Z_{max}) of 12.22 at $\theta = 0$, with D4S2380 (table 1). For fine mapping of the candidate region, additional microsatellite markers were selected and mapped on the high-resolution Stanford Human Genome Center TNG radiation hybrid panel. Relative distances in centirays (fig. 1) were determined with reference to the Stanford G3 panel (Stewart et al. 1997).

Under the more conservative assumption of incomplete penetrance, the candidate interval is defined by two recombination events in one affected individual (family A, member 6.1). Here, the 17-cM region is delimited centromerically by D4S395 and distally by D4S411. Under the assumption of complete penetrance, two additional recombination events in two unaffected probands further limit the Huriez locus (fig. 2): one in family A (member 4.11), localizing the gene telomeric to D4S1544, and the other in family B (member 2.5), localizing the gene centromeric to D4S2966. The Huriez locus is thus confined to an 8-cM region between D4S1544 and D4S2966. Haplotypes were constructed for both families by use of 16 markers between D4S2963 and D4S1564. Comparison between the disease alleles of the two families revealed three adjacent markers identical by state (IBS) (fig. 1). For two of these markers, D4S2973 and D4S1559, the shared allele is the most common allele found among all individuals, with relative frequencies of .87 and .58, respectively. There is no known relationship between the two families investigated. However, in view of the rarity of the condition