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Homozygosity Mapping of a Gene Responsible for Gelatinous Drop–like Corneal Dystrophy to Chromosome 1p

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Summary

Gelatinous drop–like corneal dystrophy (GDLD) is a rare autosomal recessive disorder characterized clinically by grayish corneal deposits of amyloid and by severely impaired visual acuity. Most patients require corneal transplantation. To localize a gene responsible for GDLD, we performed linkage analysis of 10 consanguineous Japanese families with a total of 13 affected members. Homozygosity mapping provided a maximum LOD score of 9.80 at the D1S2741 marker locus on the short arm of chromosome 1. Haplotype analysis further defined the disease locus within a region of ~2.6 cM between D1S2890 and D1S2801.

Introduction

Gelatinous drop–like corneal dystrophy (GDLD; MIM 204870) is a rare autosomal recessive disease. It is characterized by the deposition of amyloid material in the subepithelial space of the cornea and was first reported by Nakaizumi (1914). Clinical manifestations appear in the 1st decade of life and include blurred vision, photophobia, and foreign-body sensation. Later, raised, yellowish-gray gelatinous masses severely impair visual acuity, and lamellar keratoplasty is required for most patients (Smolin 1994). Unfortunately, the same condition often develops in the transplanted cornea within several years, and additional keratoplasty is needed. The incidence of this disease is estimated to be ~1/300,000 in Japan, whereas only a few cases have been reported in Western countries (Ramsey et al. 1972; Weber and Babel 1980).

In recent years, positional-cloning approaches have led to the discovery of a large number of genes responsible for hereditary disorders. Although mutant genes responsible for some corneal dystrophies have been identified, the molecular basis of GDLD remains unknown. In an effort to localize a GDLD gene, we undertook a genomewide scan, using microsatellite markers to detect regions showing frequent homozygosity among 13 affected offspring of 10 consanguineous Japanese marriages. In this way, we mapped the disease locus to the short arm of chromosome 1.

Patients and Methods

Patients

The pedigree structures of the Japanese families participating in this study are shown in figure 1. The parents in each family are first cousins. All participating family members were examined and diagnosed by slit-lamp biomicroscopy. Individuals whose corneal surface had a mulberry appearance (fig. 2) were diagnosed as affected. From each participant, 20 ml of peripheral blood was drawn, with informed consent. Genomic DNA was extracted from white cells by standard methods (Grimberg et al. 1989).

Genotyping

A genomewide scan was performed with 63 fluorescently labeled microsatellite markers (Linkage Mapping Set; Perkin-Elmer) distributed at intervals of ~10 cM (Weissenbach et al. 1992; Gyapay et al. 1994). Each PCR was carried out in a 15-μl reaction mixture containing 20 ng of genomic DNA, 20 pmol of each primer, 1.0–2.5

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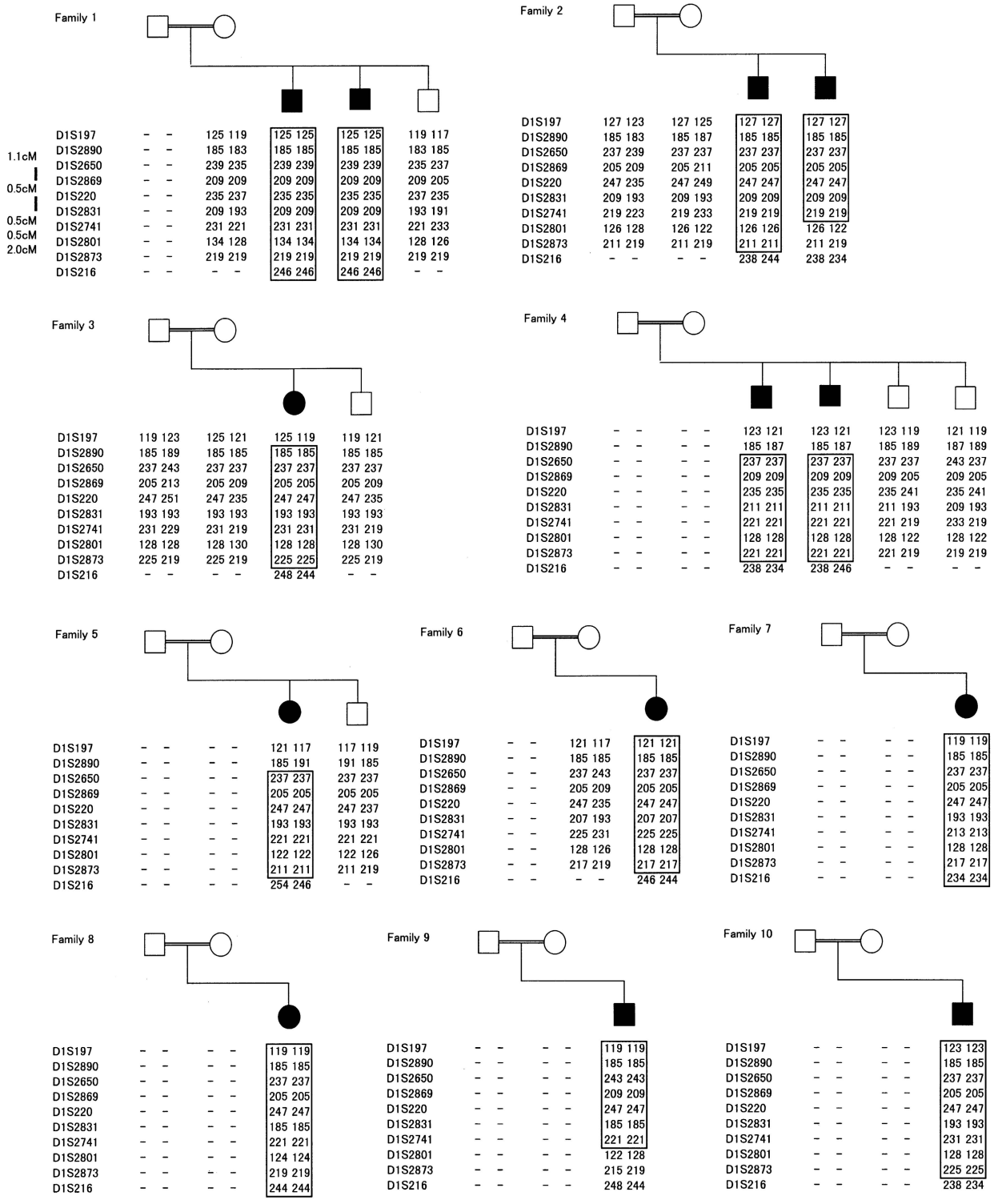

Figure 1 Pedigree and haplotype data for 10 GDLD families. All parents in these marriages are first cousins. Genotypes unavailable because of lack of DNA are denoted by dashes (–). The homozygous region of each affected individual is boxed.

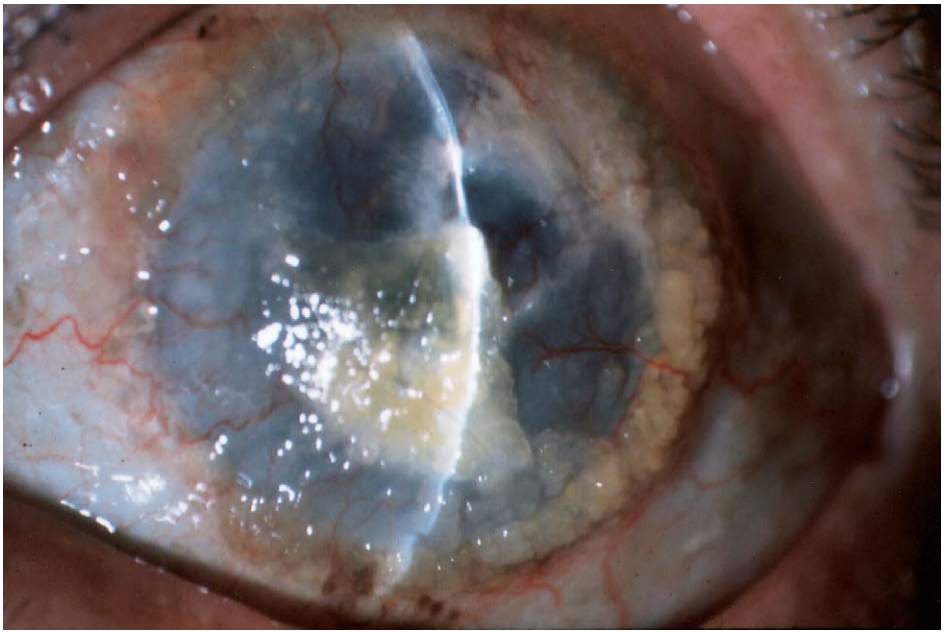

Figure 2 Phenotype of GDLD. The corneal surface has a mulberry appearance.

mM $MgCl_2$, 1 × reaction buffer (Takara), 200 μM of each dNTP, and 1.0 unit of EX *Taq* polymerase (Takara). Samples were amplified in 35 cycles of 20 s at 94°C, for denaturing, 20 s at optimum annealing temperature, and 20 s at 72°C for extension, in a GeneAmp 9600 thermocycler (Perkin-Elmer). Alleles were separated by electrophoresis on a 4% denaturing polyacrylamide gel for 2 h in a model 377 DNA sequencer (Perkin-Elmer). Genescan analysis software was used to determine allele sizes.

We selected seven additional markers on chromosome 1p (D1S2890, D1S2650, D1S2869, D1S2831, D1S2741, D1S2801, and D1S2873) for fine mapping (Gyapay et al. 1994). For genotyping, one of the PCR primers for each marker was end-labeled with $\gamma[^{32}P]$-ATP by T4 polynucleotide kinase. PCR conditions were the same as those used for the fluorescently labeled markers. Alleles were separated on 6% denaturing polyacrylamide gels at 1,700–2,200 V for 3–5 h and were visualized by exposure to x-ray film for 5–12 h.

Linkage analysis

Linkage analysis was performed with FASTLINK software, version 4.0P (Lathrop et al. 1984; Cottingham et al. 1993), under the assumption of autosomal recessive inheritance with complete penetrance and a gene frequency of .001, on the basis of allele frequencies in $\geqslant$64 chromosomes from unrelated healthy Japanese. Recombination frequencies were assumed to be equal in males and females.

Linkage-Disequilibrium Analysis

For each consanguineous family, a disease chromosome was identified and used for linkage-disequilibrium analysis, except for the five loci D1S197, D1S2890, D1S2801, D1S2873, and D1S216, since we were unable to define the disease-carrying chromosome. An assessment of allelic association was performed by comparison of allele frequencies in the GDLD chromosome versus those in $\geqslant$64 chromosomes from unrelated healthy Japanese. The 2 × n table χ^2 test was performed.

Results

After examining 63 markers distributed throughout the genome, we found that all 13 affected members and none of the 11 unaffected members of the 10 consanguineous GDLD families studied were homozygous at the D1S220 locus on the short arm of chromosome 1. Using additional markers flanking the D1S220 locus to confirm the evidence of linkage, we found that all of the affected individuals were homozygous for two additional markers (D1S2831 and D1S2741) on the proximal side of D1S220 and also for two markers on the distal side (D1S2869 and D1S2650; see fig. 1). Table 1 summarizes the results of pairwise linkage analysis at each of 10 marker loci on chromosome 1p. Five markers revealed no recombination, with LOD scores of 4.40–9.80; the maximum score of 9.80 was obtained at the D1S2741 locus. Haplotype analysis (see fig. 1) indicated that two patients from family 4 and one patient from

Table 1

Pairwise LOD Scores at 10 Loci on Chromosome 1p

Locus	LOD Score at Recombination Fraction of							Maximum LOD Score	Maximum Recombination Fraction
	.00	.01	.05	.10	.20	.30	.40		
D1S197	1.65	3.62	4.58	4.31	3.07	1.78	.72	4.58	.05
D1S2890	.16	1.62	2.26	2.10	1.40	.73	.25	2.26	.06
D1S2650	4.40	4.26	3.71	3.08	1.99	1.13	.48	4.40	.00
D1S2869	5.67	5.48	4.73	3.85	2.34	1.22	.45	5.67	.00
D1S220	8.36	8.11	7.13	5.95	3.77	2.01	.77	8.36	.00
D1S2831	8.88	8.66	7.76	6.55	4.57	2.73	1.20	8.88	.00
D1S2741	9.80	9.75	8.62	7.44	5.16	3.09	1.35	9.80	.00
D1S2801	$-\infty$	4.67	5.14	4.67	3.29	1.96	.86	5.17	.04
D1S2873	$-\infty$	5.48	5.94	5.45	3.96	2.43	1.10	5.97	.04
D1S216	$-\infty$	−8.27	−3.06	−1.21	−.06	.15	.09	.15	.31

family 5 were heterozygous at the D1S2890 locus proximal to D1S220 and that one patient from family 2 and one patient from family 9 were heterozygous at the D1S2801 locus distal to D1S220. These cases defined the critical region for GDLD, within a 2.6-cM interval between D1S2890 and D1S2801. Furthermore, we found that 8 (80%) of 10 disease chromosomes carried the 247-bp allele at the D1S220 marker locus, whereas only 5% of the control chromosome carried this allele. As summarized in table 2, a significant linkage disequilibrium between GDLD and the locus D1S220 ($\chi^2 = 36.24$; $P < .001$) was observed, indicating that the GDLD locus and the D1S220 locus are in very close proximity.

Discussion

Homozygosity mapping has proved to be a powerful method for the mapping of autosomal recessive diseases. One advantage of this strategy is that it requires only a small number of patients from consanguineous marriages. The very low incidence of GDLD makes it difficult to define the disease locus by means of linkage analysis in nonconsanguineous families. Using DNA from only 13 affected individuals among 10 consanguineous Japanese families, we successfully assigned the gene for GDLD to the D1S220 locus on the short arm of chromosome 1. Subsequent linkage and haplotype analyses with D1S220 and nine other microsatellite markers revealed that all 13 affected individuals were homozygous at four other marker loci: D1S2650, D1S2869, D1S2831, and D1S2741. This result localized the GDLD locus within a 2.6-cM interval between D1S2890 and D1S2801.

The serum amyloid P gene (SAP; Whitehead et al. 1988) and the transforming growth factor β–induced gene (βig-h3; Skonier et al. 1992) have been considered as candidate genes for GDLD. Although the amyloid deposit observed in the corneas of GDLD patients is of the SAP type (Mondino et al. 1981), the SAP gene is located on chromosome 1q12-q23; hence, our results excluded mutation of the SAP gene as the cause of GDLD in our panel of families. Similarly, βig-h3, which is responsible for lattice corneal dystrophy type I, another form of corneal amyloidosis (Munier et al. 1997), was also excluded as a candidate, because it lies on chromosome 5q31. We also excluded the 16q22 region, where the gene for macular corneal dystrophy has been mapped (Vance et al. 1996).

To our knowledge, only two genes, one encoding a serine-threonine kinase (Hanes et al. 1994) and the other an AMP-activated protein kinase (Beri et al. 1994), have been assigned to the interval between D1S2890 and D1S2801. Since the biological functions of these two enzymes are not known in detail, each could be a candidate for GDLD. The incidence of this disease is much higher in Japan and only a few cases of GDLD have been reported in other countries. This fact anticipates a founder mutation in strong linkage disequilibrium with close markers of the disease gene. In fact, our linkage-disequilibrium analysis has disclosed significant linkage disequilibrium between D1S220 and GDLD, indicating that the GDLD locus and the D1S220 locus are in very close proximity.

Table 2

Linkage-Disequilibrium Analysis

Locus	χ^2	P
D1S2650	3.32	.35
D1S2869	7.64	.37
D1S220	36.24	<.001
D1S2831	13.87	.18
D1S2741	17.42	.026

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Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for GDLD [MIM 204870])

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