Homozygosity Mapping of the Achromatopsia Locus in the Pingelapese

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Summary

Achromatopsia, or total color blindness (also referred to as "rod monochromacy"), is a severe retinal disorder characterized clinically by an inability to distinguish colors, impaired visual acuity in daylight, photophobia, and nystagmus. Inherited as an autosomal recessive trait, achromatopsia is rare in the general population (1: 20,000–1:50,000). Among the Pingelapese people of the Eastern Caroline Islands, however, the disorder occurs at an extremely high frequency, as recounted in Oliver Sacks's popular book *The Island of the Colorblind*: 4%–10% of this island population have the disorder and ~30% carry the gene. This extraordinary enrichment of the disease allele most likely resulted from a sharp reduction in population in the late 18th century, in the aftermath of a typhoon and subsequent geographic and cultural isolation. To obtain insights into the genetic basis of achromatopsia, as well as into the genetic history of this region of Micronesia, a genome-wide search for linkage was performed in three Pingelapese kindreds with achromatopsia. A two-step search was used with a DNA pooling strategy, followed by genotyping of individual family members. Genetic markers that displayed a shift toward homozygosity in the affected DNA pool were used to genotype individual members of the kindreds, and an achromatopsia locus was identified on 8q21–q22. A maximal multipoint LOD score of 9.5 was observed with marker D8S1707. Homozygosity was seen for three adjacent markers (D8S275, D8S1119, and D8S1707), whereas recombination was observed with the flanking markers D8S1757 and D8S270, defining the outer boundaries of the disease-gene locus that spans a distance of <6.5cM.

Introduction

Achromatopsia (total color blindness; MIM 216900) is a hereditary disorder of the retina characterized by absence or malfunction of the retinal cones, the specialized sensory cells responsible for normal trichromatic color vision (Sharpe and Nordby 1990). In addition to total color blindness, incomplete achromatopsia exists, in which only two of the three cone pigment types are absent or defective (for a review, see Wissinger and Sharpe 1998). One form of incomplete achromatopsia is the blue cone monochromatism, an X-linked disorder caused by mutations in photoreceptor pigment genes (Lewis et al. 1987; Nathans et al. 1989). By contrast, complete congenital achromatopsia is inherited as an autosomal recessive disease, and genetic heterogeneity is suspected. The first locus for achromatopsia was mapped to 2p11–q12 in an inbred Iranian Jewish kindred (Arbour et al. 1997). Recently, a candidate gene, *CNGA3*, encoding the α-subunit of the cone photoreceptor cGMP-gated cation channel, was assigned to this locus (Wissinger et al. 1998), and missense mutations in highly conserved residues have been described in five families from Germany, Norway, and the United States with total color blindness (Kohl et al. 1998). Homozygous mutations were present in two of the families; compound heterozygous mutations were present in the remaining three families (Kohl et al. 1998).

Achromatopsia is a very rare condition in the general population, affecting 1:20,000–1:50,000 people (Francois 1961; Jager 1972). In marked contrast, it occurs in 4%–10% of the Pingelapese people of the Eastern Caroline Islands in Micronesia (Brody et al. 1970; Carr et al. 1971; Hussels and Morton 1972; Sacks 1997). The remarkably high frequency of the disease allele is probably related to a sharp reduction of the population to <20 individuals by a destructive typhoon in ~1780. These historic circumstances, in conjunction with geographic isolation and strong religious convictions, led to intermarriage and the spread of the disease allele. Equal sex distribution of patients and results of segregation analysis strongly suggest that the disease follows an autosomal recessive mode of inheritance (Hussels and Morton 1972). Disease symptoms are detectable within the first few months of life, and the clinical course varies
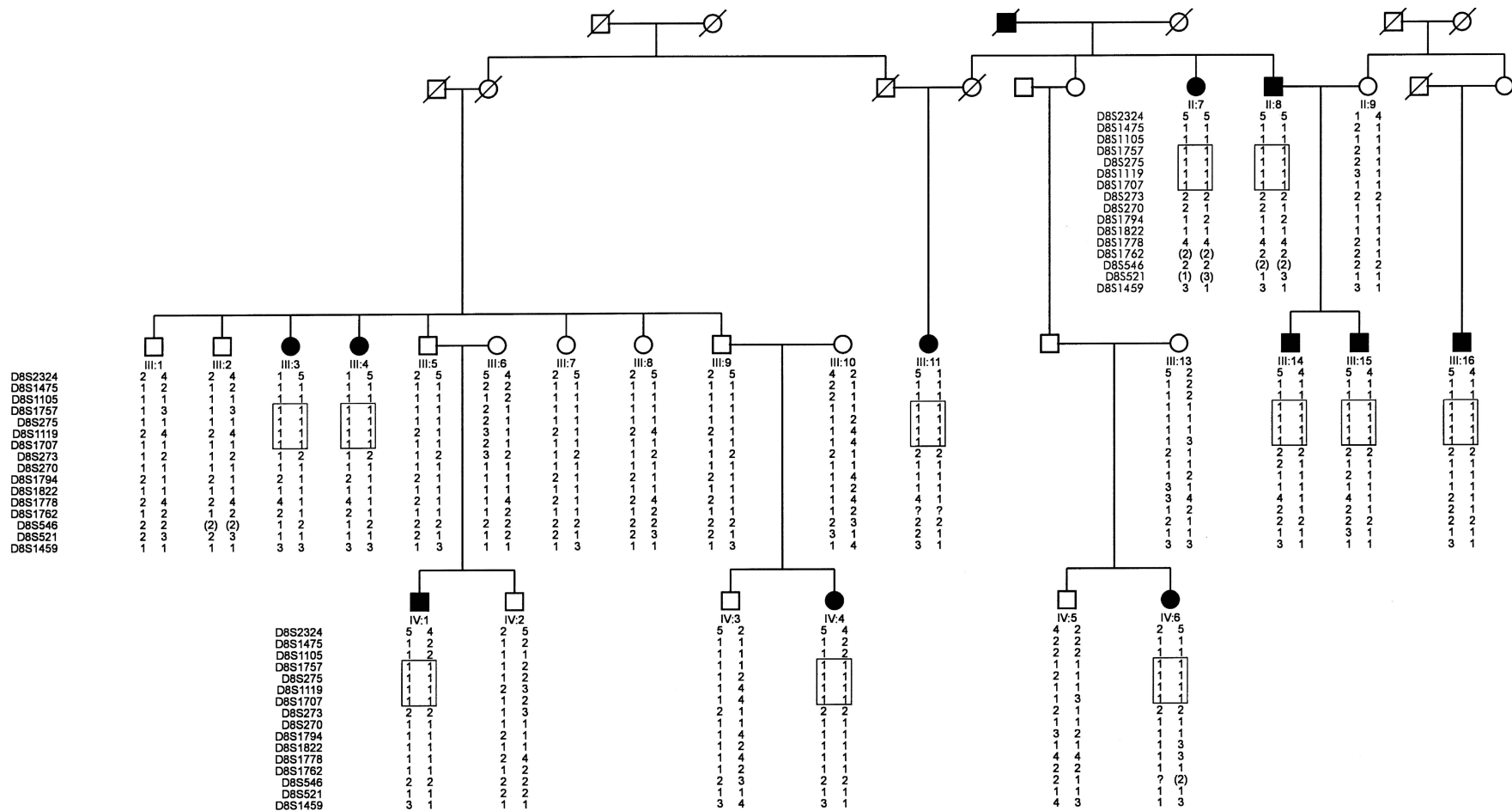
Figure 1 Pedigrees of three Pingelapese families with achromatopsia. Blackened symbols represent affected individuals. Haplotypes for the most closely linked STRPs are shown below each symbol. The markers showing homozygosity in each family appear in rectangles. Parentheses indicate inferred genotypes. A question mark (?) indicates an unknown allele.
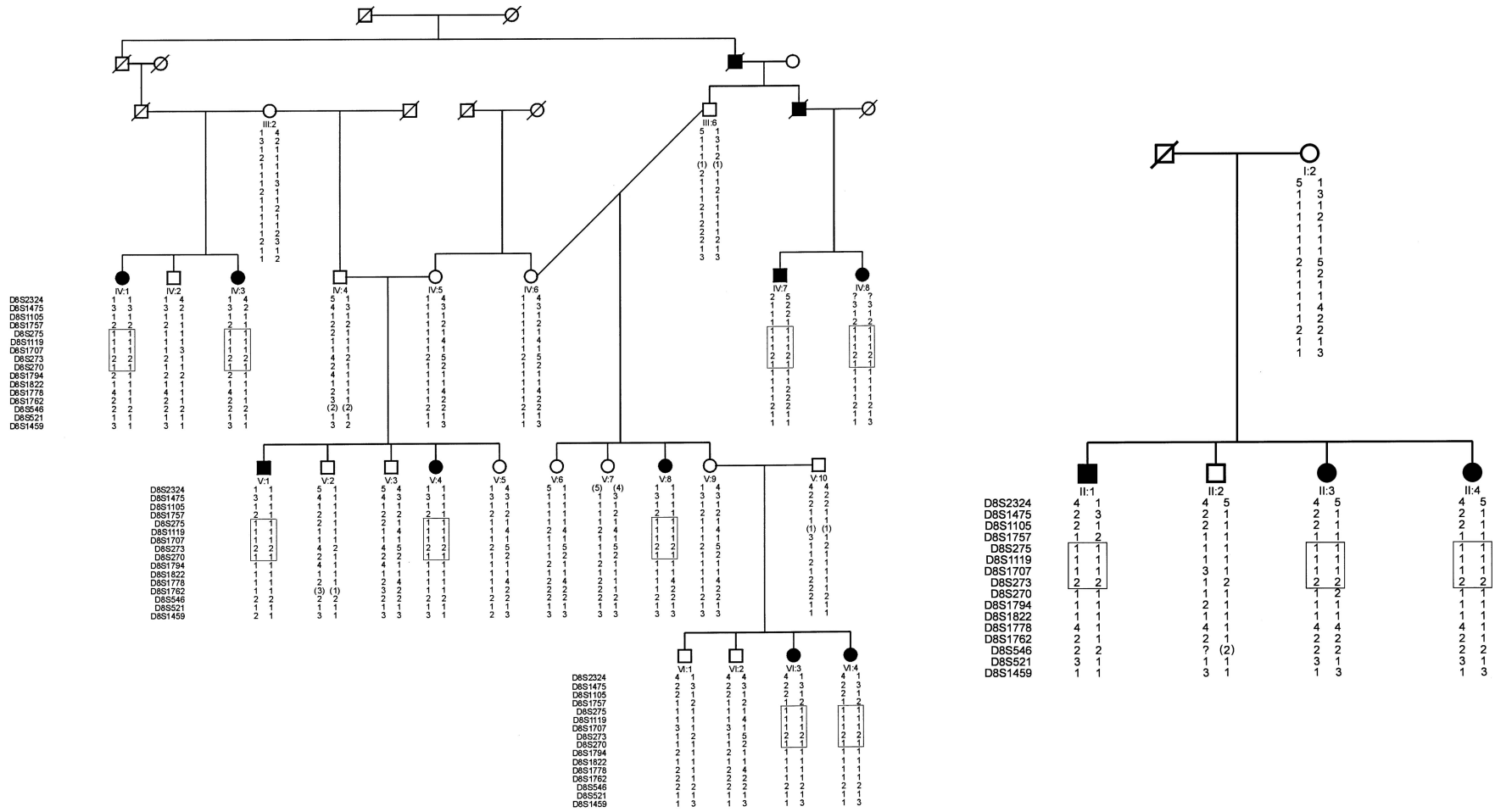
Figure 1 *(continued)*

little from case to case (Brody et al. 1970; Carr et al. 1971; Hussels and Morton 1972). The clinical features include complete inability to discriminate between colors, diminished visual acuity in daylight, photophobia, horizontal pendular nystagmus, and gradually developing cataracts (Brody et al. 1970; Carr et al. 1971; Hussels and Morton 1972; Sharpe and Nordby 1990).

A genomewide search for linkage was performed in three Pingelapese kindreds affected with achromatopsia. A two-step search was used with a DNA pooling strategy, followed by genotyping of individual family members. DNA pooling has been successfully used to map genes for several recessive disorders (Sheffield et al. 1994; Nystuen et al. 1996; Scott et al. 1996; Wang et al. 1997; Brennan et al. 1998; Parvari et al. 1998; Stockton et al. 1998) and has proved especially effective when used in families from genetically isolated populations. This strategy relies on the assumption that in genetically isolated kindreds affected with an autosomal recessive disorder, both disease alleles originate from a common founder (Sheffield et al. 1995). Genetic markers that displayed a shift toward homozygosity in the affected DNA pool (manifested as a reduction in the number of alleles in the affected versus the unaffected parental and sibling control pool) were used to genotype available members of all three kindreds (fig. 1A–C). Our genotyping of individual family members established significant linkage between the disease phenotype and markers localized on chromosome 8q21–q22.

Subjects and Methods

Three Pingelapese families residing in Pohnpei (capital of the Federated States of Micronesia) participated in this study. Each participating subject (or in the case of minors, the responsible adult) signed an informed consent form, as approved by the Rockefeller University's Institutional Review Board and the corresponding board for the Federated States of Micronesia. Blood samples from a total of 63 affected and unaffected individuals were collected. Detailed family relationships were delineated by in-person interview, and pedigrees were constructed. All affected individuals had a medical history of achromatopsia and displayed classic clinical symptoms, described in detail elsewhere (Brody et al. 1970), including complete absence of color vision, photophobia, low visual acuity in daylight, and horizontal pendular nystagmus.

DNA was extracted from whole blood by means of standard nonorganic procedures (Grimberg et al. 1989), and DNA concentration was determined by spectrophotometry readings at 260 nm. The samples were diluted to 40 ng/μl in a total volume of 1 ml in 10 mM Tris, 0.2 mM EDTA, pH 7.3. The samples were then used as templates in control PCR DNA amplification

reactions to confirm equal amplification of each individual sample prior to pooling. Equivalent amounts of DNA from 11 individuals from family 1 (fig. 1A) were combined into two separate pools: (1) affected patients and (2) unaffected or carrier parents and siblings. A set of 387 fluorescently labeled simple-tandem–repeat polymorphic (STRP) markers, with an average heterozygosity of .76 and an average spacing of 10cM (CHLC [Cooperative Human Linkage Center] Human Screening Set/ Weber version 8A; Research Genetics), was used for PCR amplification. Reactions included 20 ng pooled DNA in 67 mM Tris-HCl, pH 8.8; 16 mM $(NH_4)_2SO_4$; .001% Tween-20; 1.5 mM $MgCl_2$; 200 μM dCTP, dGTP, dATP, and dTTP; 2.5 pmol each primer; and 0.2 U *Taq* polymerase in a final volume of 10 μl. Samples were subjected to 30 cycles of amplification (94°C × 45 s; 57°C × 45 s; and 72°C × 60 s), and the products were separated by electrophoresis onto 6% denaturing polyacrylamide gels (7.7 M urea) on ABI 373/377 DNA sequencers. Allele sizes for all samples were called by ABI GENO-TYPER (1.1.1.), with respect to TAMRA GeneScan 350 and 500 size standards. The electropherograms showing allele traces were inspected for allele differences among the two pools. To genotype individual samples, PCR amplification was used in an identical manner, with the exception of radiolabeling one primer by means of ^{32}P γ-ATP, and exposing the gels on autoradiographic film (Kodak).

Two-point and multipoint LOD scores were calculated with MLINK and LINKMAP, respectively, of the LINKAGE 5.1 computer program package (Lathrop et al. 1984). The linkage analyses were carried out under an autosomal recessive mode of inheritance, in which the disease phenotype is fully penetrant with no phenocopies. The recombination fraction was assumed to be equal for male and female subjects. The disease frequency was set to .26, which reflects the disease-allele frequency for achromatopsia in the people of Pingelap. The marker-allele frequencies were estimated from the data by means of both observed and reconstructed genotypes from founders within the pedigrees. For multipoint linkage analysis, map distances were obtained from the Marshfield Map (Broman et al. 1998). SIM-WALK (Weeks et al. 1995) was used to generate the haplotypes with the highest likelihood.

Results and Discussion

Genetic studies on the three Pingelapese families initially involved an analysis of markers on chromosome 2p11–q12, where the first locus for achromatopsia was mapped, in an inbred Iranian Jewish kindred (Arbour et al. 1997). Two-point maximum likelihood calculations between chromosome 2 markers and the disease phenotype failed to reveal any evidence for linkage.
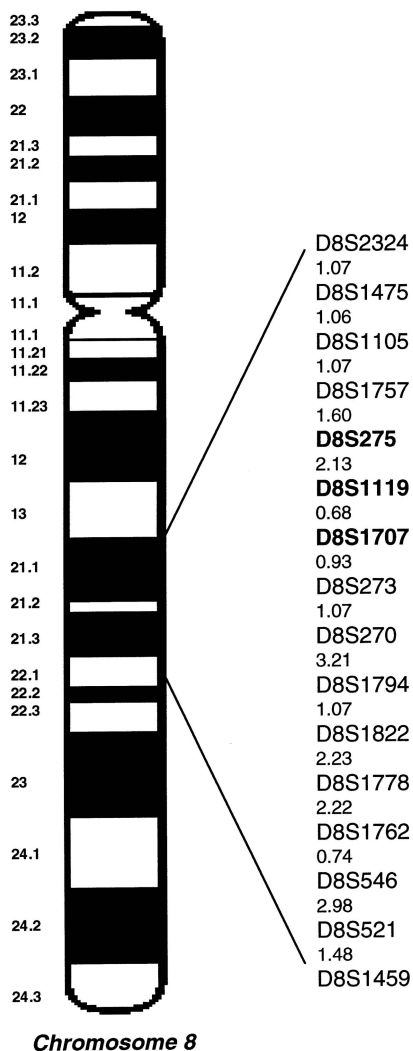
D8S2324
1.07
D8S1475
1.06
D8S1105
1.07
D8S1757
1.60
D8S275
2.13
D8S1119
0.68
D8S1707
0.93
D8S273
1.07
D8S270
3.21
D8S1794
1.07
D8S1822
2.23
D8S1778
2.22
D8S1762
0.74
D8S546
2.98
D8S521
1.48
D8S1459

Chromosome 8

Figure 2 Genetic map location of linked markers on chromosome 8 defining the achromatopsia locus. Recombination distances are shown in centimorgans (cM). The disease interval, determined on the basis of recombination events, is flanked by markers D8S1757 and D8S270 (6.4cM). The region of homozygosity is defined by markers D8S275, D8S1119, and D8S1707 (indicated in boldface).

More recently, a disease gene, *CNGA3*, was identified from this locus (Kohl et al. 1998). SSCP analysis, as well as RFLP analysis, of PCR-amplified fragments did not reveal any novel or previously described mutations in the *CNGA3* gene among the Pingelapese affected individuals.

Failure to confirm the previously described linkage on chromosome 2 prompted us to initiate a genomewide linkage analysis by means of a DNA pooling technique (Sheffield et al. 1995). DNA samples were divided into two pools on the basis of phenotype: the affected pool consisted of all 11 affected members from family 1, and the unaffected pool consisted of an equal number of

Table 1

Two-Point LOD Scores between the Achromatopsia Locus and Chromosome 8 Markers in All Three Pingelapese Families

	LOD Score at $\theta =$						
Marker	.0	.01	.05	.10	.20	.30	.40
D8S2324	...	3.70	4.45	4.18	2.98	1.61	.49
D8S1475	...	−.82	.88	1.24	1.07	.64	.25
D8S1105	.64	.62	.56	.48	.32	.18	.07
D8S1757	...	−2.37	−.54	.02	.23	.15	.05
D8S275	1.76	1.71	1.53	1.31	.90	.53	.22
D8S1119	5.36	5.24	4.75	4.10	2.77	1.44	.38
D8S1707	2.48	2.42	2.16	1.83	1.20	.63	.18
D8S273	6.27	6.12	5.50	4.73	3.19	1.71	.53
D8S270	...	.93	1.42	1.44	1.12	.67	.24
D8S1794	3.64	3.55	3.16	2.69	1.77	.95	.32
D8S1822	.31	.30	.25	.20	.11	.05	.01
D8S1778	...	1.65	2.70	2.74	2.01	1.05	.29
D8S1762	...	−.06	1.09	1.33	1.12	.64	.21
D8S546	1.07	1.04	.94	.81	.53	.27	.08
D8S521	...	.98	1.60	1.67	1.30	.71	.18
D8S1459	...	−1.67	.53	1.16	1.17	.70	.21

unaffected or carrier parents and siblings from the same family. DNA pools were used as templates for PCR amplification with a set of 387 STRPs, spaced, on average, every 10cM in the genome (CHLC Human Screening Set/Weber version 8A; Research Genetics). Genotypes were generated by fluorescently labeled PCR amplification and subsequent polyacrylamide gel electrophoresis. Relative intensity of a given allelic band correlates with its frequency within the pooled DNA population, and on the basis of this assumption, allele distributions between affected and unaffected DNA pools were determined. STRPs not linked with the disease locus show similar distribution of band intensities in affected and unaffected DNA pools. A reduction in the number of alleles in the affected versus the unaffected pool is interpreted as a shift toward homozygosity and is an initial indication of linkage between the disease and the STRP. Individual members of all three Pingelapese kindreds were genotyped with markers from the autosomal loci that showed an apparent reduction in allele frequencies in the affected pool when compared with the unaffected parental and sibling pool. Two-point maximum likelihood calculations were performed, and statistically significant evidence for linkage with marker D8S1119 was found on chromosome 8q. Several markers in the vicinity of D8S1119 were subsequently analyzed (fig. 2), and the two-point LOD scores for all markers genotyped are displayed in table 1. The highest LOD score of 6.3, for marker D8S273, occurred at $\theta = 0$. The results of the multipoint linkage analysis are displayed in figure 3. In addition, in this region there were no recombination events with D8S275, D8S1119, D8S1707, and D8S273. Recombination events were observed with flanking markers D8S1757 and D8S270. These flanking markers
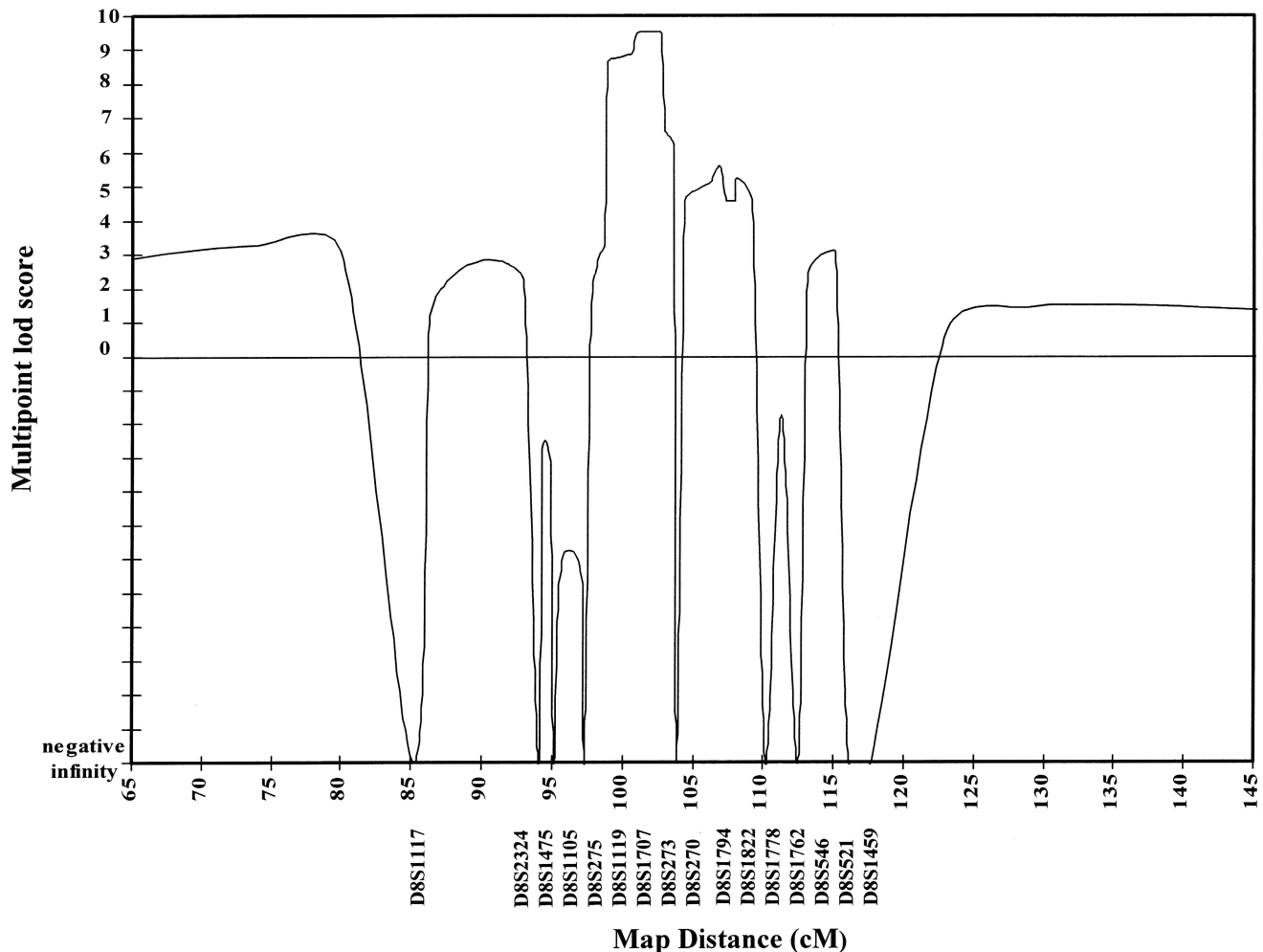
Figure 3 Results from the multipoint linkage analysis.

span a 6.4-cM region. The highest multipoint LOD score of 9.5 occurred at 101.8cM, three unit-support–interval spans of 98.7cM–103.4cM (5.7cM).

In addition, examination of the haplotypes reveals that affected members from all three kindreds are homozygous for markers D8S275, D8S1119, and D8S1707. This common haplotype covers a region that is <5.3cM. The homozygous haplotype in family 1 spans D8S1757–D8S1707 (fig. 1A); in family 2, D8S275–D8S270 (fig. 1B); and in family 3, D8S275–D8S273 (fig. 1C). Although we could not identify consanguinity in the pedigrees we studied, Pingelapese people traditionally marry within their community, which would explain the observed shared haplotypes. On the basis of current available genetic- and radiation-hybrid maps, the region of homozygosity maps to 8q21–q22.

Linkage of achromatopsia to a chromosomal region is an essential first step in the identification of a disease-causing gene, which will provide valuable insights into the pathophysiology of this disorder. In addition, iden-

tification of disease-causing mutations and comparisons with achromatopsia families of different ethnic origins, segregating the same disease locus, might provide insights into the genetic history of this Micronesian population and facilitate the design and interpretation of other genetic studies in the region. In that respect, it is interesting to mention that the 8q21–q22 region was also recently implicated in an Irish kindred with achromatopsia (Milunsky et al. 1998).

Electronic-Database Information

The accession number and URLs for data in this article are as follows:

CHLC, http://www.chlc.org/ (for determining average spacing of markers)

Marshfield Map, http://www.marshmed.org/genetics/ (for determining average spacing of markers)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for achromatopsia [MIM 216900])

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