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A New Locus for Dominant Drusen and Macular Degeneration Maps to Chromosome 6q14

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PURPOSE: To report the localization of a gene causing drusen and macular degeneration in a previously undescribed North American family.

METHODS: Genetic mapping studies were performed using linkage analysis in a single family with drusen and atrophic macular degeneration.

RESULTS: The clinical manifestations in this family ranged from fine macular drusen in asymptomatic middle-aged individuals to atrophic macular lesions in two children and two elderly patients. We mapped the gene to chromosome 6q14 between markers D6S2258 and D6S1644.

CONCLUSIONS: In a family with autosomal dominant drusen and atrophic macular degeneration, the gene maps to a 3.2-cM region on chromosome 6q14. This locus appears to be distinct from, but adjacent to, the loci for cone-rod dystrophy 7 (CORD7) and North Carolina macular dystrophy (MCDR1). Future identification of the gene responsible for the disease in this family will provide a better understanding of macular degeneration.

T HE FAMILIAL OCCURRENCE OF POSTERIOR RETINAL pole drusen was first described by Doyne1 100 years ago in a British family in which the disease later became known as Doyne hereditary retinal dystrophy. Autosomal dominant inheritance of a similar disorder, now referred to as malattia leventinese, was later reported by Vogt2 in a Swiss family. Doyne hereditary retinal dystrophy and malattia leventinese are characterized by subretinal deposits in the central and peripheral retina and by the development of macular degeneration and visual loss with advancing age. Despite some clinical differences, the two disorders are currently recognized as being identical at the genetic level. This conclusion was reached after the mapping of both to the same region on chromosome 2p,3–5 and the subsequent cloning of the gene and characterization of the same mutation in all patients.6 Another dominant form of age-related macular degeneration with drusen was mapped to chromosome 1q25-q31 in a US family.7 Other retinal dystrophies with drusen include Sorsby fundus dystrophy,8 and North Carolina macular dystrophy.9

We have identified a North American family with what appears to be a unique form of autosomal dominant drusen and atrophic macular degeneration. Using genetic linkage analysis we excluded the locus for dominant drusen on chromosome 2p. A genome-wide search revealed positive linkage of the trait to chromosome 6q14, between the loci for cone-rod dystrophy 7 (CORD7)10 and North Carolina macular dystrophy (MCDMD1).9

See also pp. 203–208.
METHODS

INDIVIDUALS WERE INVITED TO PARTICIPATE IN A STUDY to identify the gene responsible for drusen and macular degeneration in their family. Informed consent was obtained and ophthalmologic examinations, fundus photography, and collection of blood samples were undertaken during home and office visits. Details of the clinical evaluation of this family form the subject of a companion article by Stefko and associates in this issue of the JOURNAL.

Six hundred µl of whole blood was used for chromosomal DNA isolation with the QIAamp Blood Kit (QIAGEN, Valencia, CA). Three µl (approximately 20 ng) of the DNA solution was combined in 10-µl polymerase chain reaction mix with 1X polymerase chain reaction Buffer (Boehringer Mannheim, Indianapolis, Indiana), 1.5 mM MgCl₂ (Boehringer Mannheim, Indianapolis, Indiana), 0.2 mM dNTPs (Boehringer Mannheim, Indianapolis, Indiana), 0.5 U Taq-polymerase, and 0.8 mM each forward and reverse primers (Research Genetics, Inc, Huntsville, Alabama). Forward primer was labeled with γ-(32P) ATP. The samples were subjected to 5 minutes denaturation at 95 C followed by 30 cycles of amplification reaction; (94 C, 30 seconds, 63 C, 20 seconds, 72 C, 30 seconds), and 5 minutes of extended elongation at 72 C.

All DNA samples were analyzed with polymorphic short tandem repeat markers spanning 22 autosomes with a step of approximately 24.2 cM (Research Genetics, Inc, Huntsville, Alabama). In most cases multiplex polymerase chain reaction was performed. After amplification, polymerase chain reaction fragments were separated on a denaturing 6% polyacrylamide gel and visualized by autoradiography.

Sequencing analysis of the candidate genes encoding subunits of the type C gamma-aminobutyric-acid receptor rho-1 (GABAc rho-1 and GABAc rho-2) was performed with the AmpliCycle Sequencing Kit (Perkin Elmer, Foster City, CA), followed by electrophoresis on a 6% polyacrylimide gel and visualization by autoradiography. Haplotypes of the individuals were constructed manually according to the order of short tandem repeat markers from the Marshfield Medical Research Foundation database (http://www.marshmed.org/genetics).12 Haplotypes were reconstructed whenever possible for individuals whose DNA samples were not available.

Two-point and multipoint linkage analyses were performed using VITESSE.13 Analyses were done under the assumption that the disease allele frequency was 0.0001 and the mode of inheritance was autosomal dominant with a penetrance of 90%. Map order and genetic distances between all markers were obtained from the Marshfield Medical Research Foundation web site. Allele frequencies were set to be equal at each marker.
FIGURE 1. Autosomal dominant drusen with macular degeneration: pedigree and extended haplotypes. Squares = men; circles = women; filled symbols = affected; open = nonaffected; slashed symbols = deceased. Disease-associated haplotype is boxed. Dashes = lacking or equivocal results. Fifteen polymorphic short tandem repeats are shown in order on the genetic map of chromosome 6.
than 3 (data not shown). Because multipoint analysis is not possible using all 16 markers, a method of sliding windows using six markers at a time was used to cover the framework map. The results of seven-point LOD score analyses showed the importance of including as many markers as possible to calculate the most accurate LOD score information at each point on the framework map. The seven-point results (Figure 3) supported the two-point and three-point linkage results in the first region with $Z = 3.5$ (D6S1644), but not the result in the MCDR1 region with $Z = 1.1$ (D6S249).

The genotyping data obtained with short tandem repeat markers located in the region were used to construct the haplotypes and to detect recombination events (Figure 1). This analysis revealed two critical recombination events in individuals III-5 and III-12. The recombination events restrict the minimal disease locus between D6S2258 and D6S1644 in a 3.2-cM interval, 92.85 to 96.05 cM from the top of chromosome 6 (sex-average distance obtained from the Marshfield Medical Research Foundation database). In order to identify candidate genes in our family, we performed a search of the Human Transcript Map. Two unidentified transcripts and two known genes that are expressed in the retina have been mapped to the chromosomal region of interest. One of the transcripts (account number H92643) is the homologue of the human gene encoding apolipoprotein B-100. The other transcript’s product (account number H79994) shows a limited homology to human E1A-associated protein p300, which may function in cell cycle regulation. The genes encoding subunits of the type C gamma-aminobutyric-acid receptor rho-I (GABAc rho-1 and GABAc rho-2) were searched for point mutations. No deleterious sequence changes were found in the coding regions in either gene in affected individuals.

DISCUSSION

THE PHENOTYPE OF AUTOSOMAL DOMINANT DRUSEN WITH macular degeneration in this US family shares some similarities with malattia leventinese and Doyne hereditary retinal dystrophy that map to chromosome 2p and with North Carolina macular dystrophy that maps to chromosome 6. Two-point mapping allowed us to exclude the gene locus on chromosome 2p in our study family. No significant linkage was found to the known loci for other macular dystrophies, as described in the section on results. A maximal two-point LOD score, $Z(0.05) = 3.58$, was obtained for marker D6S1644. In view of the close position of marker D6S1644 to the North Carolina (MCDR1) locus, we performed extensive additional genotyping with markers in this region. Although we found a maximal LOD score of 3.17 at theta = 0 for marker D6S249 in the MCDR1 region, multipoint linkage analysis supported linkage results in the region of marker D6S1644 with $Z_{max} = 3.5$ but not in the MCDR1 region with $Z_{max} = 1.1$ (D6S249-D6S1617, Figure 3). Analysis of the constructed haplotypes spanning the chromosomal loci from D6S1609 to D6S283 revealed two recombination events in individuals III-5 and III-14. Based on these two recombination events, the disease interval was restricted to the region between markers D6S2258 and D6S1644 in a 3.2-cM region. This region maps to the 92.85 cM to 96.05 cM interval on the genetic map of the chromosome 6. The recombination in III-5 eliminates MCDR1 as the disease locus in the present family. The recombination in III-12 excludes CORD7 as the disease locus. Finally, we found that individual IV-6 carried the disease haplotype yet did not exhibit clinical signs of the disease. He may not have developed ocular signs of this disorder because of his young age or because of variable expression of the disease in this family. Alternatively, this finding can be explained as two
recombination events that occurred between markers D6S1004 and D6S1644.

The disease interval in the present family overlaps with that of Stargardt disease type 3 and a form of autosomal dominant macular dystrophy, raising the possibility that they may be allelic to one another. However the phenotype of the present family differs markedly from that of Stargardt disease type 3 and autosomal dominant macular dystrophy. For example, macular drusen were a hallmark of the disease in the present family, and retinal pigment epithelium atrophy and subretinal flecks are prominent features of Stargardt disease type 3 and autosomal dominant macular dystrophy.

The study of the genes that cause macular degeneration is essential for the understanding of the mechanisms of normal retinal function and of retinal diseases. Drusen are a hallmark of age-related macular degeneration, the leading cause of irreversible legal blindness in the elderly in the United States and other developed countries. The gene responsible for retinal degeneration in this family may play
a role in common and atypical forms of macular dystrophy in children and in age-related macular degeneration. The locus interval that we have defined is relatively small (3.2 cM), and further refinement for positional cloning should be possible with the development of more informative markers in the region.

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