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Journal

American journal of ophthalmology, 130(2)

ISSN

0002-9394

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Publication Date

2000-08-01

DOI

10.1016/s0002-9394(00)00585-7

Peer reviewed

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-

See also pp. 203–208.

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. (Am J Ophthalmol 2000;130:197–202. © 2000 by Elsevier Science Inc. All rights reserved.)

THE FAMILIAL OCCURRENCE OF POSTERIOR RETINAL pole drusen was first described by Doyme¹ 100 years ago in a British family in which the disease later became known as Doyme 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. Doyme 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 (MCMD1).⁹

Accepted for publication Nov 23, 1999.

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This work was supported by the Howard Hughes Medical Institute, Boulder, Colorado (Dr Han), and funds from the National Institutes of Health Core Grant and from Research to Prevent Blindness to Wilmer Eye Institute (Dr Zhang), Fight for Sight research division of Prevent Blindness America, Schaumburg, Illinois (Dr Kniazeva), and Foundation Fighting Blindness, New York, NY (Dr Traboulsi).

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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 μ l of whole blood was used for chromosomal DNA isolation with the QIAamp Blood Kit (QIAGEN, Valencia, CA). Three μ l (approximately 20 ng) of the DNA solution was combined in 10- μ l polymerase chain reaction mix with 1X polymerase chain reaction Buffer (Boehringer Mannheim, Indianapolis, Indiana), 1.5 mM $MgCl_2$ (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 -(^{32}P) 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.

RESULTS

THE DISEASE PHENOTYPE IN THIS NORTH AMERICAN FAMILY (Figure 1) is characterized by fine drusen that are most conspicuous in the macula (Figure 2) but that are also detected in the peripheral fundus of some patients. Overall, young adults with drusen had normal vision, whereas older patients such as I-2 and II-3 had atrophic macula in both eyes that reduced vision. There were two notable exceptions to this pattern of clinical findings in this pedigree. The proband (IV-4) presented at 3 years of age with a few drusen and bilateral atrophic macular changes. There has been no apparent progression of the macular abnormalities, and her visual acuity at age 8 years is 20/40 in each eye. Her first cousin (IV-1) was found to have bilateral round atrophic macular lesions at the age of 6 months. He was re-examined at 18 months of age and found to fix and follow small targets well, with no evidence of significant visual impairment. There was no change in the appearance of the macular lesions, except for the appearance of one druse in the right eye on the temporal edge of the atrophic lesion. The companion article by Stefko and associates¹¹ gives a complete description of clinical findings and additional fundus photographs.

Linkage analysis for markers linked to the malattia leventinese/Doyne honeycomb retinal dystrophy locus on chromosome 2 revealed negative LOD scores for D2S123 and D2S378 ($Z_{D2S123} = -2.23$ and $Z_{D2S378} = -2.73$ at a recombination fraction = 0.1). An initial candidate locus-directed genome scan demonstrated no significant linkage to the loci for Sorsby fundus dystrophy on chromosome 22,⁸ peripherin/retinal degeneration slow (RDS) on chromosome 6,¹⁴ cone-rod dystrophy (CORD7) on chromosome 6,¹⁰ age-related macular degeneration on chromosome 1,⁷ and known loci for dominant and recessive Stargardt macular dystrophy.¹⁵⁻¹⁹ A random genome-wide screen using microsatellite markers spanning the 22 autosomes with a step of approximately 24.2 cM (Research Genetics, Inc, Huntsville, Alabama) revealed a positive LOD score for marker D6S1056 ($Z_{D6S1056} = 2.14$ at $\theta = 0.10$). Family members were then genotyped for 15 additional markers in the vicinity of D6S1056. The order of the markers was determined according to genetic resource from the Marshfield Medical Research Foundation. Two-point linkage analysis was performed using the VITESSE program to test the hypothesis of linkage (Table 1). Two markers showed evidence of linkage: D6S1644 with a LOD score of 3.59 at θ equal to 0.05 and D6S249 with a LOD score 3.17 at a θ of 0.0. D6S249 is located in the vicinity of the North Carolina macular dystrophy locus (MCDR1).^{9,20-22} Additional markers were added to the framework map to better refine the regions of linkage. Three-point linkage analysis was performed using the VITESSE program to generate LOD scores between adjacent markers. The interval LOD scores were consistent with the two-point results, with two intervals of lod scores greater

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.

FIGURE 2. Fundus photograph of macular drusen in the proband's aunt (III-1). Vision was normal. The drusen are very fine and arranged in a circular pattern in the fovea.

TABLE 1. Two-Point LOD Scores Between Dominant Drusen and Short Tandem Repeat Markers on Chromosome 6q

Marker	LOD score at 0						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D6S1609	-1.53	0.46	1.10	1.26	1.17	0.84	0.43
D6S1652	1.16	1.16	1.14	1.07	0.84	0.56	0.26
D6S1644	-0.70	3.22	3.59	3.46	2.84	2.01	1.05
D6S1613	-0.71	1.27	1.85	1.94	1.72	1.26	0.67
D6S1570	-0.57	-0.50	-0.28	-0.11	0.02	0.02	-0.01
D6S1056	-1.45	1.50	2.06	2.14	1.88	1.37	0.72
D6S249	3.17	3.10	2.80	2.42	1.61	0.80	0.23
D6S1671	-1.18	1.48	2.03	2.10	1.80	1.27	0.62
D6S283	-0.19	-0.07	0.72	1.07	1.16	0.94	0.53

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).¹²

In order to identify candidate genes in our family, we performed a search of the Human Transcript Map.^{23,24} 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-100.²⁵ 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.²⁶ The genes encoding subunits of the type C gamma-aminobutyric-acid receptor rho-1 (GABA ρ -1 and GABA ρ -2)^{27,28} 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 Doyme 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

FIGURE 3. Multipoint LOD scores in the study family. Genetic distance in centimorgans is shown on the abscissa; LOD score value is given on the ordinate. Arrows indicate positions of genetic markers. Location of the marker D6S1609 is used as a reference at distance 0. The disease loci mapped to the region are shown in boxes below.

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,²⁹ 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.

ACKNOWLEDGMENTS

The authors wish to thank J. Zawistowski (student, Molecular, Cellular, and Developmental Biology Department, Colorado University at Boulder) for excellent technical support and Thomas Albini for help with clinical examination of the patients. The generosity and cooperation of the family members who participated in the study is particularly appreciated.

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