# Identification of a Novel Locus on 2q for Autosomal Dominant High-Grade Myopia

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**Purpose.** Myopia, or nearsightedness, is a visual disorder of high and growing prevalence in the United States and in other countries. Pathologic high myopia, or myopia of ≤−6.00 D, predisposes individuals to retinal detachment, macular degeneration, cataracts, and glaucoma. Autosomal dominant (AD) nonsyndromic high-grade myopia has been mapped to loci on 18p11.31, 12q21-q23, 17q21-q23, and 7q36. This is the report of significant linkage to a novel locus on the long arm of chromosome 2 in a large, multigenerational family with AD high-grade myopia.

**Methods.** The family contains 31 participating members (14 affected). The average spherical refractive error for affected individuals was −14.46 D (range, −7.25 to −27.00). Before a genome screening was undertaken, linkage to intragenic or flanking markers for the myopic genetic syndromes of Stickler syndrome types I, II, and III; Marfan syndrome; and juvenile glaucoma were ruled out. In addition, no linkage was found to the known AD high-grade myopia loci listed above. A full genome screen of the family was performed with 382 microsatellite markers with an average intermarker distance of 10 cM. SimWalk2 software was used for multipoint linkage analysis based on an AD model with a penetrance of 90% and a disease allele frequency of 0.01.

**Results.** Fine-point mapping with an additional nine custom-made and five commercial markers yielded a maximum two-point lod score of 5.67 at marker *D2S2348*. Results of multipoint analysis indicate that the 1-unit support intervals for this new locus spans approximately 9.1 cM from (238.7 to 247.8 cM) on the chromosome 2 genetic map at q37.1.

**Conclusions.** A novel locus for AD high-grade myopia has been determined, providing further evidence of genetic heterogeneity for this disorder. (*Invest Ophthalmol Vis Sci.* 2005;46:2300–2307) DOI:10.1167/iovs.04-1423

Myopia is the most common eye disorder and is found in approximately 25% of the adult population in the United States.[1–5] High-grade myopia of 6.00 D or greater severity, also termed pathologic myopia, is a major cause of legal blindness in many developed countries, with a prevalence of 1.7% to 2.1% in the general population of the United States.[6–9] High-grade myopia is especially common in Asia.[10–12] Comparative prevalence rates from different countries show considerable variability, but confirm that myopia affects a significant proportion of the population in many countries.[2,8,10–13]

Determining the role of genetic factors in the development of nonsyndromic common myopia has been hampered by the high prevalence, genetic heterogeneity, and clinical spectrum of this condition. Despite the impediments inherent in mapping genes for a complex common disorder such as myopia, considerable progress has been made in the past few years. An X-linked recessive form of myopia has been mapped and was designated the first myopia locus, *MYP1*.[14] Reanalysis of this Danish pedigree (*MYP1*) and another X-linked pedigree of Danish descent suggests that this disease locus involves a cone dysfunction and not simple myopia.[15] We have also studied several medium to large multigenerational families with AD high-grade myopia and found significant linkage at 18p11.31 (*MYP2*), 12q23.1-q24 (*MYP3*), and 17q21-q23 (*MYP5*).[16–18] Suggestive linkage for AD high-grade myopia was found on chromosome 7 at q36 (*MYP4*).[19] Two recent studies have determined loci for common myopia: one in dizygotic twins with linkage to chromosome 11 at p13 in the *PAX6* gene region[20] and another in Ashkenazi Jews with linkage to chromosome 22 at q12 (*MYP6*).[21]

Herein, we provide further evidence for the genetic heterogeneity of high-grade myopia by excluding myopia loci at 18p, 12q, 17q, 7q, 11p, and 22q. We report significant linkage to a novel AD locus on the long arm of chromosome 2 in a large, multigenerational family. The proband was examined at 8 weeks of age and was found to have a highly myopic refractive error. He was provided spectacle correction at 10 months of age and had a spherical refractive error of approximately −17.00 D. In this family, a variable spectrum of spherical myopic refractive error was observed in 14 individuals, with a range of −7.25 to −27.00 D. Two positional candidate genes, S-antigen (*SAG*) and diacylglycerol kinase delta (*DGKD*), were screened for sequence variants in this family.

## Methods

### Subject Data Collection

A large U.S. family of northern European extraction (Pedigree MYO-056) with 31 consenting members (14 affected) participated in the study. The study was approved by the Institutional Review Board of The Children's Hospital of Philadelphia and adhered to the tenets of the Declaration of Helsinki. This family was chosen based on the presence of numerous male and female family members and successful multiple generations with high-grade myopia, suggesting an AD mode of inheritance. Individuals with a spherical refractive error ≤−6.00 D and a history of myopia onset before 12 years of age were considered affected. No participants had known ocular disease or insult that could predispose to myopia or any known genetic syndrome associated with high-grade myopia. A comprehensive ophthalmic examination and blood collection was performed by one of the authors (TLY), as previously described.[16] In most instances, participants declined kera-

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**TABLE 1.** Clinical Characteristics of Family MYO-056

| Subject | Gender | Affection Status | Age at Onset (y) | Age at Exam (y) | Refractive Error (OD, OS) | Axial Length (mm; OD, OS) | Keratometry (D; OD, OS) | Ocular History |
|---|---|---|---|---|---|---|---|---|
| 9 | F | A | 7 | UA | −18.00 sph, −18.00 sph | 29.30, 28.22 | 47.00/45.75, 48.50/ 46.87 | Amblyopia, strabismus |
| 10 | M | NA | 18 | 63 | −4.25 + 3.25 × 180, −2.75 + 1.50 × 175 | | | |
| 11 | F | NA | UA | 61 | +1.00 + 0.50 × 10, Plano | | | |
| 12 | M | NA | UA | 65 | +5.25 +0.50 × 130, +3.75 + 0.50 × 40 | | | |
| 13 | F | A | 2 | 36 | −16.00 + 1.00 × 140, −15.00 sph (pre-op) | | | Amblyopia, strabismus |
| 14 | M | NA | UA | 70 | Plano OU | | | |
| 16 | M | A | 4 | 71 | −14.75 + 2.00 × 150, −13.00 sph | | | Retinal detachment, cataracts, glaucoma |
| 19 | F | A | 4 | 57 | −9.00 + 1.75 × 150, −9.25 + 2.50 × 45 | | | |
| 21 | F | NA | UA | 48 | −0.25 + 0.25 × 170, Plano + 0.25 × 180 | | | |
| 22 | M | NA | UA | 58 | Plano OU | | | |
| 23 | F | A | 4 | 55 | −16.25 sph, −7.25 sph | 27.02, 27.30 | 49.25/48.12, 51.50/49.50 | Keratoconus (OS) |
| 26 | M | NA | 16 | 35 | −4.25 sph, −5.00 sph | | | |
| 27 | M | NA | UA | 36 | Plano OU | | | |
| 28 | F | NA | UA | 33 | Plano OU | | | |
| 29 | M | A | 3 | 34 | −11.00 − 1.00 × 65, −10.75 − 50 × 125 | | 44.75/45.25, 44.00/44.75 | |
| 30 | F | A | 1 | 19 | −14.75 + 4.50 × 100, −14.00 + 4.25 × 75 | | 43.50/44.75, 43.25/45.75 | |
| 31 | F | NA | 12 | 48 | −5.25 − 0.25 × 10, −4.50 − 0.25 × 5 | | | |
| 32 | M | A | 6 | 7 | −27.00 + 0.50 × 150, −25.00 + 2.00 × 45 | | 43.75/44.12 (OS) | Retinal detachment, cataracts |
| 34 | M | A | 4 | 26 | −13.00 + 1.50 × 110, −13.50 + 1.25 × 63 | | | |
| 35 | M | NA | UA | 31 | Plano OU | | | |
| 36 | M | A | 2 | UA | −7.75 + 3.50 × 79, −7.25 + 2.75 × 102 | | | |
| 37 | M | A | 1 | 27 | −15.00 sph, −15.00 sph | | | |
| 38 | F | NA | 7 | 29 | −3.00 + 1.50 × 25, −3.25 + 1.25 × 146 | | | |
| 39 | F | NA | UA | 26 | +0.50 sph, +0.50 sph | | | |
| 40 | M | NA | UA | 31 | Plano OU | | | |
| 41 | F | A | 4 | UA | −15.50 + 1.00 × 105, −15.25 + 0.50 × 59 | | | Strabismus |
| 42 | F | NA | UA | 28 | +1.50 sph, +1.25 sph | | | |
| 43 | M | NA | UA | 17 | −1.50 + 0.50 × 10, −1.75 sph | | | |
| 44 | M | A | 0.15 | 0.83 | −17.75 sph, −16.00 sph | | | |
| 45 | M | A | 1.5 | 2 | −15.00 sph, −15.00 sph | | | |
| 46 | M | NA | UA | 1 | +1.75 sph, +1.75 sph | | | |

The refractive errors of all control participants are also provided. A, affected; NA, not affected; UA, unavailable; M, male; F, female; C, control; OD, right eye; OS, left eye; P, plano; sph, sphere; OU, both eyes; mm, millimeters.

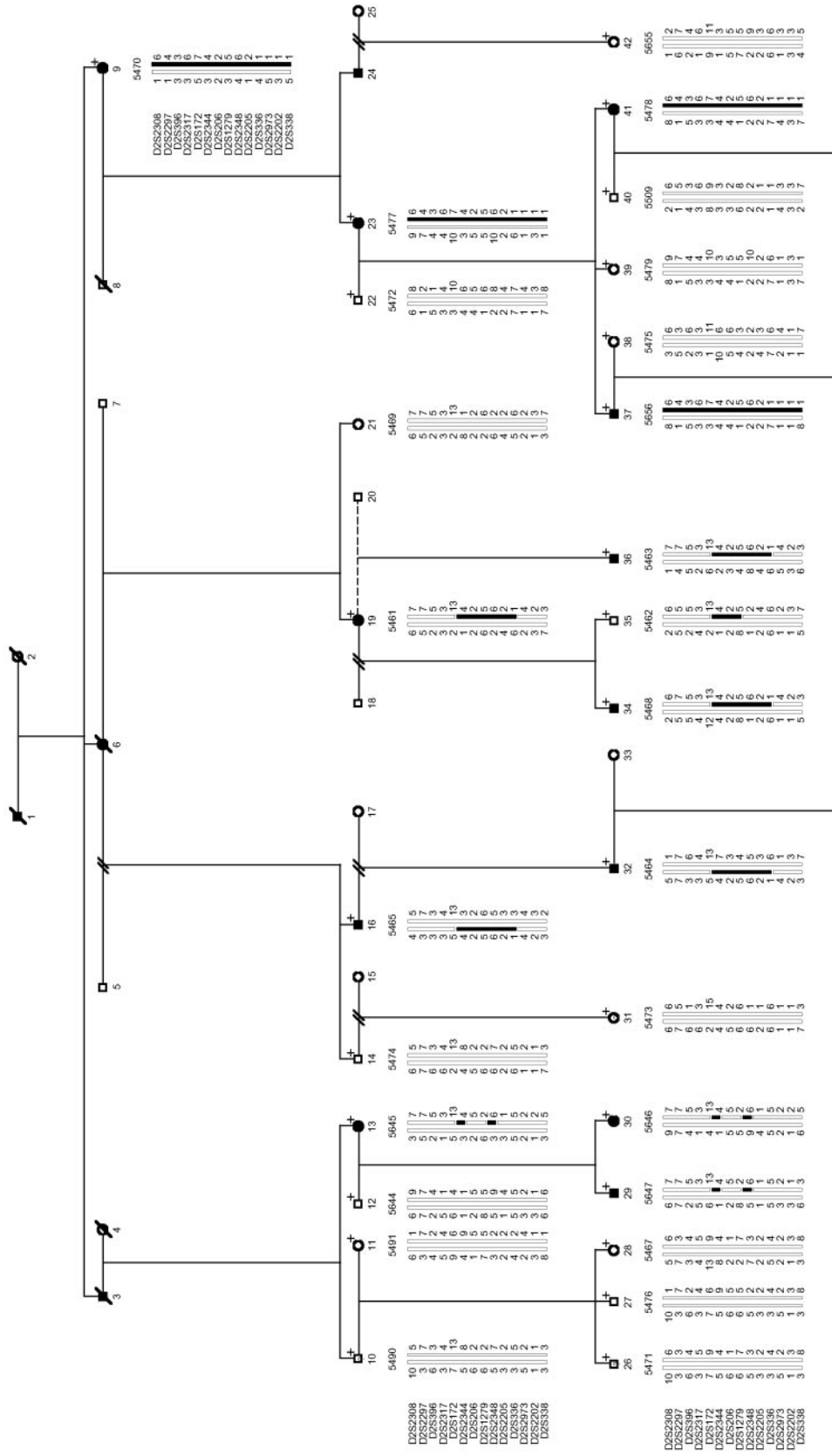

**FIGURE 1.** Pedigree MYO-056 with familial high-grade myopia. *Circles* and *squares* denote females and males, respectively; *filled symbols* denote affected individuals; *symbols with diagonal lines* denote deceased individuals. The alleles for the most informative polymorphic markers are shown for each studied individual. Haplotypes were constructed based on the minimum number of recombinations between these markers. *Filled columns*: the chromosome assumed to carry the inherited disease allele; *open columns*: normal haplotypes.

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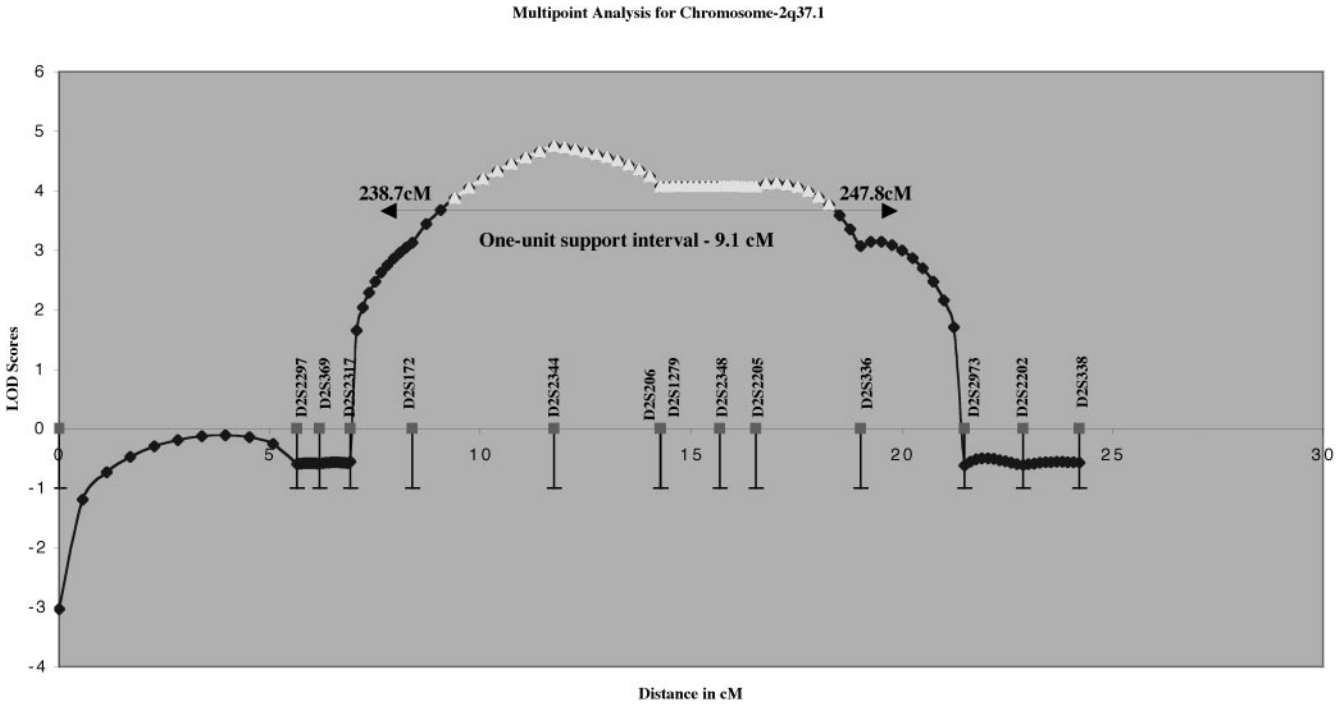


**FIGURE 2.** Multipoint lod score data on the MYO-056 family for chromosome 2 at region q37 with marker order in *gray boxes* on the *x*-axis. Lod scores were plotted against the marker distance in centimorgans.

tometry and axial-length measurement of their eyes. Some affected individuals in this family had other ocular diseases, such as retinal detachment, strabismus, and glaucoma. Details of the ophthalmic examinations are summarized in Table 1.

## Screening for Known Candidate and Syndromic Loci

Initially, 11 candidate loci were screened by using flanking and intragenic microsatellite markers specific for the six known myopia loci mentioned earlier, as well as the five known myopic genetic syndromes of Stickler syndrome types I, II, and III; Marfan syndrome; and juvenile glaucoma, as described previously.[16–20]

## Genotyping

Genome screening was performed on pedigree MYO-056, with 382 microsatellite markers from a commercial set (Prism Linkage Mapping Set-MD10; Applied Biosystems, Inc. [ABI], Foster City, CA) with an average intermarker distance of 10 cM. The polymerase chain reaction (PCR) samples were prepared in 10-$\mu$L volumes in 96-well plates with 6 $\mu$L of PCR premix (True Allele; ABI), 1 $\mu$L primer, 1 $\mu$L sterile $H_2O$, and 2 $\mu$L of 30 ng/$\mu$L DNA. The conditions recommended by ABI were optimized for our thermocyclers (MJ Research Inc., Waltham, MA). Multiplexed PCR products (three or four combined amplicons, depending on the size range for adequate amplicon spacing) were electrophoresed on an automated DNA sequencer (Prism 377; ABI). The gel-file output was checked for correct lane tracking, and the allele size was determined on computer (GeneScan analysis software; ABI), and genotyping software (Genotyper; ABI) was used for automated allele calling and manual verification. After the initial genome screening, markers from a linkage mapping set (Linkage Mapping Set-HD5; ABI) with an average intermarker distance of 5 cM and additional custom-made markers with high heterozygosity were selected from publicly available genetic maps[22,23] were added in all regions with positive lod scores. The additional marker amplicons were run on a newer auto-

**TABLE 2.** Two-Point LOD Score Data of the MYO-056 Family for Chromosome 2q37 Microsatellite Markers

| Marker | 0.0 | 0.01 | 0.05 | 0.1 | 0.2 | 0.3 | 0.4 |
|---|---|---|---|---|---|---|---|
| *D2S2308* | −3.48 | −1.04 | 0.37 | 0.94 | 1.25 | 1.10 | 0.68 |
| *D2S2297* | 2.32 | 2.39 | 2.47 | 2.41 | 2.06 | 1.54 | 0.85 |
| *D2S396* | 0.98 | 1.12 | 1.63 | 1.87 | 1.78 | 1.34 | 0.71 |
| *D2S2317* | −1.07 | −0.69 | −0.02 | 0.35 | 0.61 | 0.58 | 0.37 |
| *D2S172* | −0.27 | −0.20 | −0.02 | 0.10 | 0.25 | 0.27 | 0.19 |
| *D2S2344* | 4.22 | 4.14 | 3.80 | 3.37 | 2.47 | 1.53 | 0.64 |
| *D2S1279* | 1.39 | 2.60 | 2.98 | 2.90 | 2.38 | 1.66 | 0.83 |
| *D2S206* | 1.20 | 1.29 | 1.44 | 1.45 | 1.26 | 0.93 | 0.51 |
| *D2S2348* | 5.67 | 5.57 | 5.18 | 4.67 | 3.57 | 2.39 | 1.14 |
| *D2S2205* | −0.28 | −0.23 | −0.05 | 0.12 | 0.28 | 0.28 | 0.18 |
| *D2S336* | 2.82 | 3.05 | 3.22 | 3.08 | 2.50 | 1.75 | 0.89 |
| *D2S2973* | −0.80 | 0.14 | 0.67 | 0.81 | 0.80 | 0.63 | 0.36 |
| *D2S2202* | 0.60 | 1.02 | 1.56 | 1.71 | 1.52 | 1.06 | 0.49 |
| *D2S338* | −1.11 | −0.70 | 0.07 | 0.49 | 0.80 | 0.76 | 0.48 |

0.9 penetrance, 0.001 phenocopy rate.

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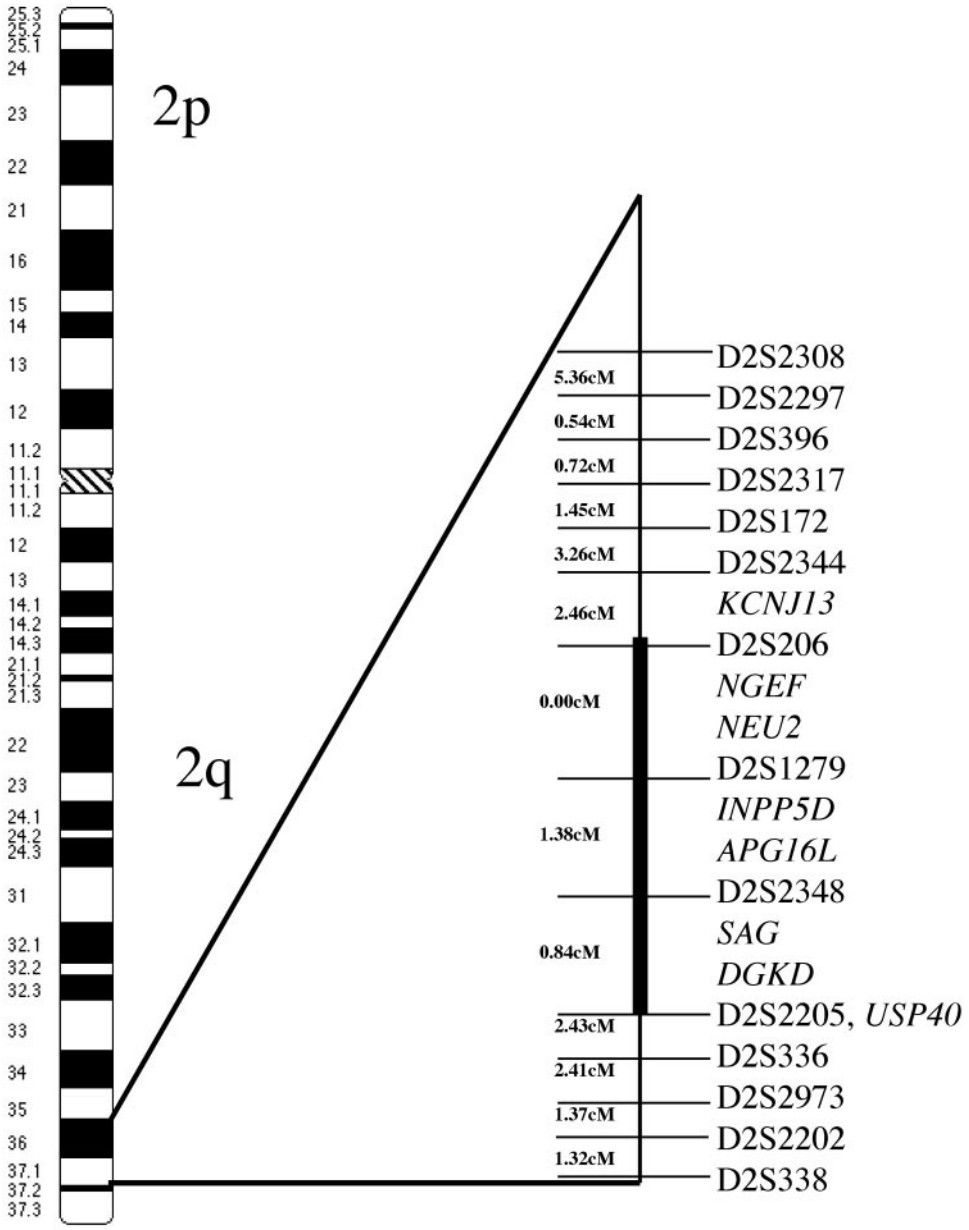


**FIGURE 3.** Chromosome 2 ideogram schematic of 14 microsatellite markers that map to chromosome 2, region q37.1. The mapping order and genetic distances (in centimorgans) were obtained from the Marshfield comprehensive genetic map of the human genome (see Appendix A). The *bold vertical segment* denotes the linked interval determined by haplotype analysis. Positional candidate genes are *italicized*.

mated DNA sequencer (Prism 3730; ABI) and gene-mapping software (GeneMapper, ver. 3.0; ABI) was used for automated allele calling and manual verification.

## Linkage Analysis

Mendelian error checking was performed with PedManager, ver. 0.9. Mega2, ver. 3.0,[24] was used to create the files needed for linkage analysis by using SimWalk2, ver. 2.89.[25–27] SimWalk2 was used for multipoint linkage analysis based on an AD model with 90% penetrance, 0.1% phenocopy rate, and a myopia gene frequency of 0.01. Two-point linkage analysis was performed with the MLINK program from the FASTLINK 4.0 software package[28–30] for the region of interest on chromosome 2 at q37. Marker allele frequencies were estimated based on our pedigree data. Sex-average genetic marker maps were used from the internet database of the Marshfield Center for Medical Genetics. (Internet locations of databases used in the study are provided in Appendix A).

## Positional Candidate Gene Mutation Screening

*SAG* contains 14 exons encoding a protein of 406 residues.[31,32] *DGKD* contains 30 exons and two transcript variants encoding two proteins of 1170 (*DGKD1*) and 1214 (*DGKD2*) residues.[33] We designed and optimized 15 novel sets of forward and reverse primer pairs for *SAG* and 31 for *DGKD*, which extended 50 to 200 bp beyond each intron–exon boundary (Appendix B). For each amplimer, PCR was performed on 150 ng of participant genomic DNA using *Taq* polymerase (Ampli*Taq* Gold; Roche Molecular Systems, Inc., Branchburg, NJ) at 55°C annealing temperature. Amplified products were separated by agarose gel electrophoresis and visualized by staining with ethidium bromide. Five highly myopic affected family members were screened, and four control subjects with either plano or hyperopic refractive error were obtained from family marry-ins, nonmyopic family members, and unrelated subjects. External control subjects were self-defined as white and were from the United States.

## Denaturing High Performance Liquid Chromatography

A DNA fragment analysis system (Wave) and associated software (Navigator; Transgenomic, Inc., Omaha, NE) were used for denaturing high performance liquid chromatography (DHPLC). A mixture of 15 $\mu$L of each amplicon from a subject's DNA was heated for 5 minutes at 95°C and then cooled to room temperature. An aliquot (5 $\mu$L) of the DNA

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**TABLE 3.** Observed Sequence Polymorphisms in the S-Antigen (*SAG*) and Diacylglycerol Kinase Delta (*DGKD*) Genes

| RefSNP ID | Position on NT_005120.15 | Wild Type | Observed | Sample No. (Bold = affected) | Exon | AA Change |
|---|---|---|---|---|---|---|
| SAG | | | | | | |
| Novel | 162234 | C | C/T | 5462 & 5472 | Intron 5 | |
| rs2304774 | 168503 | G | C/G, C/C | C009, 5462, **5464,** 5472 & **5657** | Intron 6 | |
| rs2304773 | 168574 | C | C/T | **5657** | At bp position 62 of exon 7 | Ala-Glu |
| rs13427703 | 180262 | C | C/G | **5657** | Intron 10 | |
| Novel | 183522 | | Possible AA insertion | C009, 5462, **5464,** 5472 & **5657** | Intron 11 | |
| rs1046974 | 188301 | G | G/A, A/A | C009, 5462, **5464,** 5472, 5509, **5470, 5477, 5656 & 5657** | At bp position 92 of exon 14 | Val-Ile |
| rs1046976 | 188302 | T | T/C | **5477** | At bp position 93 of exon 14 | Val-Ala |
| DGKD | | | | | | |
| Novel | 277918 | G | A/G | C009 | Intron 7 | |
| rs3214826 | 279846 | | A insertion | C009, 5462, **5464,** 5472 & **5657** | Intron 9 | |
| rs2924811 | 305521 | T | T/C | C009 | Intron 27 | |
| rs2242098 | 305739 | A | A/C | C009 | Intron 28 | |
| Novel | 305789 | C | C/T | C009 | Intron 28 | |
| Novel | 311026 | C | C/T | 5472 | 3′ UTR | |

The wild-type sequence is derived from the scaffold sequence NT_005120 of 2q37.1. Amino acid changes are for relevant splice variants. Affected individual sample numbers are in *bold.* A possible novel poymorphism was observed at position 183522 within *SAG* because of inconclusive sequencing data due to a poly-A stretch. C009, control; UTR, untranslated region; rs, public reference single nucleotide polymorphism (SNP) number from the dbSNP database.

mixtures was directly injected into a separation column. Each fragment was analyzed by using three partially denaturing temperatures, which were based on fragment-melting profiles. Sample amplicons exhibiting a heteroduplex pattern (with a shorter retention time than that of the normal control) were sequenced to confirm putative sequence variations. PCR products were purified (ExoSAP-IT; USB Corp., Cleveland, OH) and sequenced with dye terminator chemistry (BigDye Terminator, ver. 3.1 on a 3730 DNA Analyzer; ABI).

## RESULTS

A large, multigenerational, U.S. family of northern European extraction with AD high-grade myopia (pedigree MYO-056) was identified and characterized (Fig. 1). DNA was available from 31 family members (14 affected). The average age of diagnosis of myopia in the affected individuals was 3.1 years (range, 10 months to 7 years), and the average spherical component refractive error of the affected individuals was −14.46 D (range, −7.25 to −27.00 D). The proband, individual 44, was initially evaluated with an off-axis cycloplegic refraction at 8 weeks of age and found to have a significant myopic refractive error of approximately −15.00 D. He received his first spectacle correction of −17.00 D at 10 months of age. Corneal thinning, lenticonus, and dislocated lens were absent in study participants. No evidence of linkage was observed in this family for the known AD myopia loci and syndromic myopia loci. The lod scores at $\theta = 0.0$ were as follows: *D18S63* (MYP2), −11.6; *D12S78* (MYP3), −5.71; *D7S798* (MYP4), −7.68; *D17S1290* (MYP5), 0.56; *D22S280* (MYP6), −7.83; *D11S904* (*PAX-6*), −9.69; *D15S648* (Marfan syndrome), −7.31; *D1S196* (juvenile open-angle glaucoma), −7.91; *D12S1620* (Stickler type 1), −5.64; *D1S2626* (Stickler type 2), −2.44; and D6S276 (Stickler type 3), −3.98.

The initial 10-cM genome screen did not show suggestive evidence of linkage, as no lod score above 2.00 was obtained. However, three chromosomal regions, on 2q, 3p, and 10p had lod scores >1.00. In each region, additional polymorphic markers were genotyped and analyzed by linkage analysis. The new markers for 3q and 10p gave lower lod scores, and multipoint analysis confirmed the exclusion of these regions. In contrast, the lod scores for an interval on 2q increased, showing significant evidence of linkage between markers *D2S172* and *D2S336* (Fig. 2). Two-point analysis identified significant linkage at markers *D2S2344* and *D2S2348* (Table 2). The maximum multipoint lod score was 4.75 for marker *D2S2344* (Fig. 2). Based on the 1-unit support intervals, the critical region for myopia susceptibility gene in this family spans approximately 9.1 cM (from 238.7 to 247.8 cM) on the chromosome 2 genetic map at 2q37.1 (Fig. 2).

Haplotype analysis revealed recombination events that could help narrow the critical region containing the gene to 2.22 cM, between markers *D2S1279* and *D2S2205* at 2q37.1 (Fig. 3). Biologically relevant candidate genes found between markers *D2S1279* and *D2S2205* at 2q37.1 were S-antigen (also known as S-arrestin; *SAG*) and diacylglycerol kinase delta (*DGKD*; 130 kDa). Mutation screening of *SAG* resulted in seven polymorphisms (three missense and four intronic), but none of these segregated with the affection status. *DGKD* mutation screening revealed six intronic polymorphisms in this family, again without affection status cosegregation (Table 3). Five polymorphisms were novel and have been submitted to the National Center for Biotechnology Information (NCBI) dbSNP database. Eight polymorphisms corresponded with previously reported single nucleotide polymorphisms (SNPs) in public databases. A pattern change was observed in exon 9 for all the samples within the *SAG* gene, apparently due to a poly-A stretch. Whether the pattern change was due to a novel polymorphism in the number of A's in this region (position 183522) could not be confirmed, because of difficulty in sequencing through that homopolymer stretch.

## DISCUSSION

In this study, we have identified a novel locus for AD high-grade myopia and provide additional evidence for genetic heterogeneity of this phenotype. Linkage to early-onset disorders associated with high-grade myopia (Marfan syndrome, juvenile glaucoma, and the Stickler syndromes) was excluded, to ensure that the family did not exhibit a mild phenotypic expression of these conditions limited to the ocular findings. Linkage to known candidate loci for AD myopia was also excluded.

After genome-wide screening and fine mapping were performed, the only region that showed evidence of linkage to the myopia trait in this family was on chromosome 2 at q37.1, with a maximum multipoint lod score of 4.75 at marker *D2S2344*.

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Two-point analysis identified significant linkage at markers *D2S2344* and *D2S2348*, although the two intervening markers (*D2S206* and *D2S1279*) show nonsignificant linkage because of the double recombination observed in affected individual 13. On the distal side of the affected haplotype, the boundary of the critical region can be set at marker *D2S2205*, as affected individual 13 and her two affected children, individuals 29 and 30, do not share the same allele with the other affected individuals for this marker. Note that the haplotype observed in affected individual 13 is also transmitted to her affected children. This must be the result of recombinations between markers *D2S2344* and *D2S206*, which are separated by 2.46 cM, and again between markers *D2S1279* and *D2S2348*, which are separated by 1.38 cM (Fig. 1). Although the data for each individual are consistent with the rules of Mendelian inheritance, the occurrence of a double recombinant in this small interval is unlikely. The order of the maps was checked in all the public databases (Généthon and Golden Path, University of California Santa Cruz [UCSC], Genome Bioinformatics, University of California Santa Cruz, CA.) including the sequence of the human genome from NCBI. We also repeated the genotyping analysis for the two intervening markers (*D2S1279* and *D2S206*) twice and obtained the same results.

A centromeric recombination event was noted between markers *D2S1279* and *D2S2348* in the third generation. Unaffected individual 35 had the same haplotype for markers proximal to *D2S2348* as did his affected siblings 34 and 36. This finding excludes this region from containing the disease gene, unless we assume that individual 35 is in fact a nonpenetrant carrier. We consider this unlikely, because individual 35 has no refractive error compared with his affected siblings (Table 1). The allele shared by all affected individuals for the highest lod score marker *D2S2348* is multiallelic (89% heterozygosity) and has 10 alleles in this family. Thus, it is highly unlikely that the affected allele is a common allele. This refines the critical region containing the gene to 2.22 cM, between markers *D2S1279* and *D2S2205* on chromosome 2 at q37.1 (Fig. 3).

A search for genes physically mapped between markers *D2S1279* and *D2S2205* revealed eight regulatory or structural genes, four hypothetical genes, and one cDNA clone (UCSC database). Among these are potassium channel, inwardly rectifying, subfamily j, member 13 (*KCNJ13*); neuronal guanine nucleotide exchange factor (*NGEF*); neuraminidase 2 (*NEU2*); inositol polyphosphate-5-phosphatase (*INPP5D*; 145 kDa); S-antigen (also known as S-arrestin; *SAG*); diacylglycerol kinase, delta (*DGKD*; 130 kDa); and ubiquitin-specific proteinase 40 (*USP40*). Of these, *SAG* and *DGKD* are biologically relevant candidate genes for this newly identified myopia locus, as both of these genes are expressed in human retina.[31–33]

*SAG* is a major soluble photoreceptor protein that is involved in desensitization of the photoactivated transduction cascade.[32] It is expressed in the retina and pineal gland and inhibits coupling of rhodopsin to transducin in vitro.[31,32] Mutations in this gene have been associated with Oguchi disease,[34] a rare autosomal recessive form of night blindness.

*DGKD* encodes a cytoplasmic enzyme that phosphorylates diacylglycerol to produce phosphatidic acid.[33] Diacylglycerol kinase (*DGK*) has many isozymes, all containing characteristic zinc finger structures, and plays an important role in cellular signal transduction.[35] *DGK* genes cause retinal degeneration in *Drosophila* and are considered candidate genes for mammalian eye diseases.[35] An isozyme of the *DGK* gene (*DGKG*) was predominantly expressed in human retina.[36] Alternative splicing of *DGKD* results in two transcript variants encoding different isoforms (*DGKδ1* and *DGKδ2*). Expression studies revealed limited expression of *DGKδ1*, but *DGKδ2* was ubiquitously expressed in all normal human tissues.[37] *SAG* and *DGKD* both are expressed in the retina and may influence the growth of the eye. Direct sequence screening of the coding regions of both genes did not reveal any myopia-implicated mutations.

Novel SNPs observed with both genes were submitted to the public SNP database (see Appendix A). Observed frequencies were submitted for known SNPs. Currently, we are investigating other genes in this region.

In summary, we have mapped a novel chromosomal locus for high-grade myopia. Mutational characterization of the remaining genes in this locus for high-grade myopia will provide additional insight into the molecular mechanisms underlying this most common form of visual impairment and into the regulation of eye growth. We also continue our work to identify other possible loci for myopia.

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## APPENDIX A

### Electronic Database Information

Accession numbers and Internet addresses for databases used in the study are as follows:

Généthon, French Association against Myopathies, Evry, France (for genetic markers and maps). http://www.genethon.fr/

Human Genome Mapping Project Resources Center, Cambridge, UK (MLINK and ILINK programs of FASTLINK, version 4.0). http://www.hgmp.mrc.as.uk/

Human Gene Nomenclature Committee, Center for Human Genetics, University College London, London, UK (for abbreviated gene name assignment). http://www.gene.ucl.ac.uk/cgi-bin/nomenclature/searchgenes.pl/

Human Genome Project Working Draft ("Golden Path"), UCSC Genome Bioinformatics, University of California Santa Cruz, CA. http://genome.ucsc.edu/

Marshfield Laboratories, Marshfield, WI (for genetic markers and maps). http://www.marshmed.org/genetics/

Mega2 Version 3.0 software (to prepare files for SimWalk2 linkage analysis). http://watson.hgen.pitt.edu/mega2.html

National Center for Biotechnology Information (NCBI), National Institutes of Health, Bethesda, MD (for BLAST searches, EST data, the Human Gene Map, and the UniGene and SAGE Collections). http://www.ncbi.nlm.nih.gov/

Online Mendelian Inheritance in Man (OMIM), NCBI, National Institutes of Health, Bethesda, MD, for accession numbers MYP1, MIM 310460; MYP2, MIM 160700; MYP3, MIM 603221; MYP4 MIM 608367; MYP5, MIM 608474; and MYP6, MIM 608908. http://www.ncbi.nlm.nih.gov/omim/

Public SNP repository. http://www.ncbi.nlm.nih.gov/SNP/

Simwalk2 Version 2.89 software (for multipoint linkage analysis). http://watson.hgen.pitt.edu/docs/simwalk2.html

The Whitehead Institute, Massachusetts Institute of Technology, Cambridge, MA (PedManager, pedigree data). http://www.genome.wi.mit.edu/ftp/distribution/software/pedmanager/

## APPENDIX B

### Primers Designed for Mutation Screening

Available online at http://www.iovs.org/cgi/content/full/46/7/2300/DC1.

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