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Familial cavitory optic disc anomalies: Identification of a novel genetic locus

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Abstract

PURPOSE—To identify the chromosomal location of the gene involved in the pathogenesis of cavitory optic disc anomalies in a large pedigree with autosomal dominant inheritance of disease.

DESIGN—Linkage analysis of a pedigree affected with cavitory optic disc anomalies.

METHODS—Optic disc photographs were examined for the presence of cavitory optic disc anomalies. Sixteen affected family members and one obligate carrier were identified and studied with linkage analysis using both microarrays of single nucleotide polymorphisms (SNPs) and short tandem repeat polymorphism (STRP) markers.

RESULTS—Multipoint linkage analysis of SNP genotypes yielded a maximum nonparametric LOD score of 21.7 with markers located on chromosome 12q. Linkage was confirmed with 16 STRP markers in the 12q region. A maximum two point LOD score of 4.06 ($\Theta=0$) was obtained with marker D12S1700. The disease interval defined by observed recombinants is 9.1 cM, which corresponds to 13.5 Mbp. Three candidate genes (*GDF-11*, *NEUROD4*, and *WIFI*) in the chromosome 12q locus were evaluated as possible disease-causing genes. No mutations were detected in the coding sequence of these genes.

DISCUSSION—The discovery of the chromosomal location of a gene responsible for cavitory optic disc anomalies is a key step in identifying the genetic basis of this condition and may ultimately provide important insight into the pathogenesis of more common optic nerve diseases such as normal tension glaucoma and primary open angle glaucoma.

INTRODUCTION

Excavation of the optic disc is the chief feature of some diseases of the optic nerve including adult-onset conditions which are progressive (the glaucomas) and congenital malformations

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of the optic nerve (optic pits, optic nerve coloboma, and morning glory disc anomaly) which are stationary. Optic pits, optic nerve coloboma, and morning glory disc anomaly have a similar appearance of the optic disc and are associated with a high frequency of serous retinal detachments. Consequently, these congenital conditions have been collectively referred to as cavitory optic disc anomalies.^{1, 2}

Optic pits are focal depressions in the optic disc in which the normal tissue of the optic nerve is absent. Most optic pits are located in the temporal optic disc, however, they may also be seen centrally. Patients with optic pits may have excellent vision, however, schisis or splitting of the retina and serous retinal detachments of the macula frequently develop and are associated with severe vision loss. While the majority of optic pits are sporadic, autosomal dominant inheritance of this condition has been reported.³

Ocular colobomas are malformations due to defects in the closure of the optic cup fissure during embryogenesis, which may lead to structural defects in an inferonasal location involving the iris, ciliary body, lens, choroid, and optic nerve. If the optic nerve is involved in the coloboma, it is characterized by deep excavation of the optic disc and is frequently associated with serous retinal detachments of the macula and significant vision loss. Ocular colobomas are often part of “syndromes” which include other ophthalmic and systemic abnormalities. Many of these coloboma syndromes are hereditary and a subset of cases is caused by known genes⁴ including *PAX6* (OMIM 607108),⁵ *SHH* (OMIM 600725),⁶ *MAF*,⁷ and *CHX10* (OMIM 142993).⁸ Although most cases involve multiple ocular structures, coloboma of the optic nerve may occur without involvement of any other ocular tissues. Isolated optic nerve coloboma is generally sporadic, however, familial cases have demonstrated autosomal dominant inheritance of this condition.⁹ No genes for isolated optic nerve coloboma have been identified.

The term “morning glory disc anomaly” was first used by Kindler,¹⁰ to describe a constellation of abnormal optic nerve features that have the appearance of a morning glory flower. The optic disc in this condition is enlarged and deeply excavated with an abnormal collection of centrally located glial tissue. Retinal vessels originating from the ciliary circulation radiate from the edge of the disc in an anomalous pattern. The optic disc has a funnel-shape and is surrounded by an elevated annulus of abnormal chorioretinal pigmentation. Morning glory disc anomaly is associated with an increased risk of retinal detachment and poor visual acuity. Most cases of morning glory disc anomaly are unilateral and sporadic, however a single bilateral case has been associated with a *PAX6* mutation.⁵

While the majority of the different types of cavitory optic disc anomalies occur sporadically, rare cases have been reported in which optic pits, optic nerve colobomas, and morning glory disc anomaly have been observed within the same family.^{1, 2, 11} The gene associated with this heritable form of cavitory anomalies of the optic disc is unknown. In this study, a large family affected with a range of isolated cavitory anomalies of the optic disc was studied with genetic linkage analysis to identify the chromosomal location of the disease-causing gene.

METHODS

The study was approved by the University of Iowa’s Institutional Review Board and informed consent was obtained from study participants. Sixteen clinically affected family members had eye examinations (visual acuity, slit lamp examination, indirect ophthalmoscopy and retinal biomicroscopy) performed by one of the authors (RAH, MDM, or LMJ). Throughout the study, the clinicians remained masked to the genotypic data. Patients were included in the linkage analysis if: 1) they were found to have cavitory optic nerve abnormalities including optic pits, atypical optic nerve coloboma, or morning glory

disc anomaly; or 2) they were obligate carriers, by virtue of having offspring with cavitary optic disc anomalies. Blood samples were obtained from all of the affected family members, obligate carriers, and spouses of affected patients with children. Seven to ten milliliters of blood were obtained from each patient in EDTA-containing glass tubes. DNA was prepared from the blood using a non-organic method.¹²

Pedigree members were first genotyped with short tandem repeat polymorphism (STRP) genetic markers flanking genes previously identified as ocular coloboma genes including *PAX2* (OMIM 167409), *PAX6*, and *SHH*. Genotyping with STRP genetic markers was conducted using standard methods as previously described.¹³ A genome-wide scan was next performed with Affymetrix microarrays (GeneChip Human Mapping 10K Array Xba 2.0, Affymetrix, Santa Clara, CA) which interrogate 10,204 single nucleotide polymorphisms (SNPs). Sample processing and labeling were performed using the manufacturer's instructions. The arrays were hybridized, washed, and scanned in the University of Iowa DNA core facility. Array images were processed with GeneChip DNA Analysis software.

Micro-array data were analyzed and multipoint non-parametric LOD scores were calculated using the Genespring GT software package (Agilent Technologies, Palo Alto, CA). Pairwise linkage analysis was performed with the MLINK and LODSCORE programs as implemented in the FASTLINK (v2.3) version¹⁴, ¹⁵ of the LINKAGE software package.¹⁶ Penetrance and disease gene frequency were set to 99% and 0.1% respectively. Multipoint linkage analysis was conducted using the VITESSE software package¹⁷ and parts of the LINKAGE software package (LINKMAP). The positions of 8 markers were obtained from the Marshfield map (ncbi.nlm.nih.gov/mapview/) and were held fixed while the disease locus was moved through the map in a stepwise fashion. Multipoint LOD scores were calculated using data from nearest 4 markers as the position of the disease locus was varied.

Due to variable expressivity of the disease, only affected patients and informative spouses were included in the linkage analysis. For the data given in figure 2, the allele frequencies were assumed to be equal for each marker. The true population allele frequencies for each marker could not be reliably estimated from the small number of spouses in the pedigree. In order to show that the assumption of the equal allele frequencies would not significantly affect our linkage results, we recalculated the LOD scores using allele frequencies for the "affected" allele of two of the most tightly linked markers (D7S1700 and D7S1702) ranging from 0.01 to 0.5. The Z_{max} for D7S1700 was 2.86 and the Z_{max} for D7S1702 was 2.09 when the "affected" allele frequency was arbitrarily set to 50%. In the 4 spouses that were studied, the actual frequencies of the "affected" alleles of D7S1700 and D7S1702 were much lower than 50%. In this small sample, the frequency of the "affected" of D7S1700 was 37.5% and the frequency of the "affected" allele of D7S1702 was 12.5%, suggesting that our use of equal allele frequencies for D7S1700 (14%) and D7S1702 (14%) were reasonable.

DNA samples from two affected family members and from two normal control subjects were tested for mutations in candidate genes (*GDF11*, OMIM 603936; *NEUROD4*; and *WIFI*, OMIM 605186) using bi-directional sequencing of PCR products that encompass the entire coding sequence. Sequencing was performed using dye-terminator chemistry on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA). PCR amplification was performed with a standard protocol¹⁸ using primer sequences that are available on request. Potential mutations were identified by comparing the DNA sequence of the affected family members and normal control subjects. Similarly, the DNA sequences of the affected family members were compared with NCBI reference sequences (*GDF11*, NM_005811; *NEUROD4*, NM_021191; *WIFI*, NM_007191). Identified sequence variations were evaluated as potential disease-causing mutations using standard criteria.¹⁹

RESULTS

The clinical features of the cavitory optic nerve anomalies in a large family (Figure 1) are described in detail in an accompanying report (Honkanen et al., accompanying manuscript). The optic disc phenotype observed in this family has features that lie within the spectrum of optic pits, optic nerve colobomas, and morning glory disc anomaly (Figure 2). Seventeen members of this pedigree have a cavitory optic nerve anomaly. DNA samples from 16 of these family members and 1 obligate carrier were subsequently studied with linkage analysis using a stepwise approach.

After linkage to loci containing genes already associated with ocular coloboma was excluded (data not shown), a genome-wide scan for linkage was conducted by genotyping DNA samples from nine of the affected family members with microarrays of SNPs. Analysis of the SNP data identified a region of chromosome 12q with a maximum nonparametric multipoint LOD score of 21.7 with a p-value of 0.00097. All nine affected pedigree members were found to share an allele of each of the 49 consecutive SNPs in this locus. This 12q locus is 14.6 Mbp wide and is defined by the centromeric SNP, rs725029, and the telomeric SNP, rs722748.

The chromosome 12q linkage was confirmed by genotyping all 17 affected pedigree members with 16 STRP markers in this region (Figure 3). LOD scores over 3.0 were obtained from six STRP markers and a maximum LOD score of 4.06 ($\Theta=0$) was obtained with marker D12S1700. The analysis of patients with recombination events near the linked interval is also shown in figure 3. These recombination events indicate that the disease-causing gene lies within the 9.1 cM (13.5 Mbp) interval between markers D12S1618 (centromeric) and D12S1702 (telomeric).

Multipoint analysis was performed with the genotypic data from 8 STRP markers located within the 9.1 cM locus (D12S1618, D12S1586, D12S1632, D12S1644, D12S329, D12S1726, D12S1700, and D12S1702). This analysis reveals a peak LOD score of 6.27 centered on marker D12S1726 (data not shown). The LOD-1 confidence interval²⁰ is 5.1 cM.

The chromosome 12q locus, as defined by recombination events, contains 245 genes. Three of these genes (*GDF11*, *NEUROD4*, and *WIFI*) were considered good candidates for causing cavitory optic nerve defects based on their expression in the eye and presumed function in development and tissue differentiation. DNA from two affected family members was tested for mutations in the coding sequence of these three genes with bi-directional sequencing. No disease-causing mutations were discovered.

Discussion

Similarities in the optic disc appearance and clinical course associated with optic pits, optic nerve colobomas, and morning glory disc anomaly have been recognized suggesting that these conditions are part of a spectrum of disease (cavitory optic disc anomaly).² Several large pedigrees demonstrating autosomal dominant cavitory optic disc anomalies have been reported.^{2, 11} We now report the genetic basis of the cavitory optic disc anomalies in one such pedigree. Our linkage study indicates that a single gene on chromosome 12 is capable of causing the cavitory optic disc anomalies in this pedigree. This disease-causing gene lies within a 9.1 cM (13.5 Mbp) locus that contains 245 known genes.

Three of the genes in the chromosome 12q locus (*GDF11*, *NEUROD4*, and *WIFI*) were considered promising candidates for causing cavitory optic disc anomalies due to their function and expression pattern. Gene differentiation factor 11 (*GDF11*) is a member of the

TGF- β superfamily of genes and is expressed in the retina during retinal ganglion cell differentiation prior to closure of the optic fissure.^{21, 22} *GDF11* regulates cell fate during retinal neurogenesis and controls the development of several retinal cell types, including retinal ganglion cells.²² Neurogenic differentiation factor-4 (*NEUROD4*) encodes a basic helix-loop-helix (bHLH) transcription factor that is expressed in the eye and has a role in the development and differentiation of the retina.^{23, 24} WNT inhibitory factor-1 (*WIFI*) encodes a secreted protein that is expressed in tissues of the eye and regulates development of the retina by binding proteins involved in the WNT signaling pathway.^{25, 26} Affected members of the cavitory optic disc anomaly pedigree were tested for disease-causing mutations in the coding sequences of these genes (*GDF11*, *NEUROD4*, and *WIFI*) with DNA sequencing. No disease-causing mutations were detected (data not shown).

Prior studies of pedigrees affected with POAG, primary congenital glaucoma and juvenile onset open angle glaucoma (JOAG) have identified potential glaucoma susceptibility loci on chromosome 1q24–25 (GLC1A),²⁷ 2cen-q13 (GLC1B),²⁸ 3q21–24 (GLC1C),²⁹ 8q23 (GLC1D),³⁰ 10p15-p14 (GLC1E),³¹ 7q35-q36 (GLC1F),³² 5q21-q22 (GLC1G),³³ 15q11-q13 (GLC1H),³⁴ 9q22 (GLC1J),³⁵ 20p12 (GLC1K),³⁵ 2p22-p21 (GLC3A),³⁶ and 1p36 (GLC3B).³⁷ A small fraction of glaucoma cases have been strongly linked to these loci. At present, however, the vast majority of glaucoma cases have not yet been associated with any genetic loci and the role of the chromosome 12q locus in these cases is not known.

The chromosome 12q gene responsible for cavitory optic disc anomalies has potent effects on the development of the optic nerve. A defect in this gene is capable of causing deep excavations of the optic disc during development. It is plausible that one set of mutations in this gene cause cavitory optic disc anomalies while another set of mutations might be associated with adult-onset forms of optic nerve disease with disc excavation, such as normal tension glaucoma (NTG) and primary open angle glaucoma (POAG). There is some support for this hypothesis. Two members of the cavitory optic disc anomalies pedigree in this report exhibited progressive cupping of the optic nerve head, which is a nearly pathognomonic feature of glaucoma (Honkanen et al., submitted and ref38) Also, the genes associated with other early-onset optic nerve conditions such as primary congenital glaucoma³⁹ and dominant optic atrophy⁴⁰ have been implicated in the pathophysiology of adult-onset glaucoma. Mutations in a gene that causes primary congenital glaucoma (cytochrome P450 1B1) have been shown to influence the phenotype of some POAG patients.⁴¹ Similarly, the *OPAI* gene, which causes dominant optic atrophy (DOA), has also been investigated for a role in causing adult-onset glaucoma. While the classic feature of DOA is optic nerve head pallor, recent studies have shown that mutations in the *OPAI* gene may be associated with optic nerve head changes similar to those seen in POAG.⁴² Also, association studies have suggested that the *OPAI* gene might also be involved in POAG.⁴³ In addition to clarifying the pathogenesis of cavitory optic disc anomalies, the identification of the disease-causing gene in our pedigree might also provide insight into the biologic processes of other more common optic nerve diseases such as NTG and POAG.

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C. Contributions of the authors: Design and conduct of the study (JHF, SPS, VCS, EMS, WLMA); Collection, management, analysis, and interpretation of the data (JHF, RAH, SPS, LMA, MAE, DMD, LMJ, VCS, EMS, WLMA); Preparation, review, or approval of the manuscript (JHF, RAH, SPS, LMA, MAE, DMD, LMJ, VCS, EMS, WLMA)

Biography

John H. Fingert, M.D., Ph.D. is an Assistant Professor of Ophthalmology at the University of Iowa with research and clinical interests that focus on glaucoma and other inherited diseases of the optic nerve. Dr. Fingert received his medical and genetics degrees from the University of Iowa where he also completed an ophthalmology residency and fellowships in molecular ophthalmology and glaucoma.



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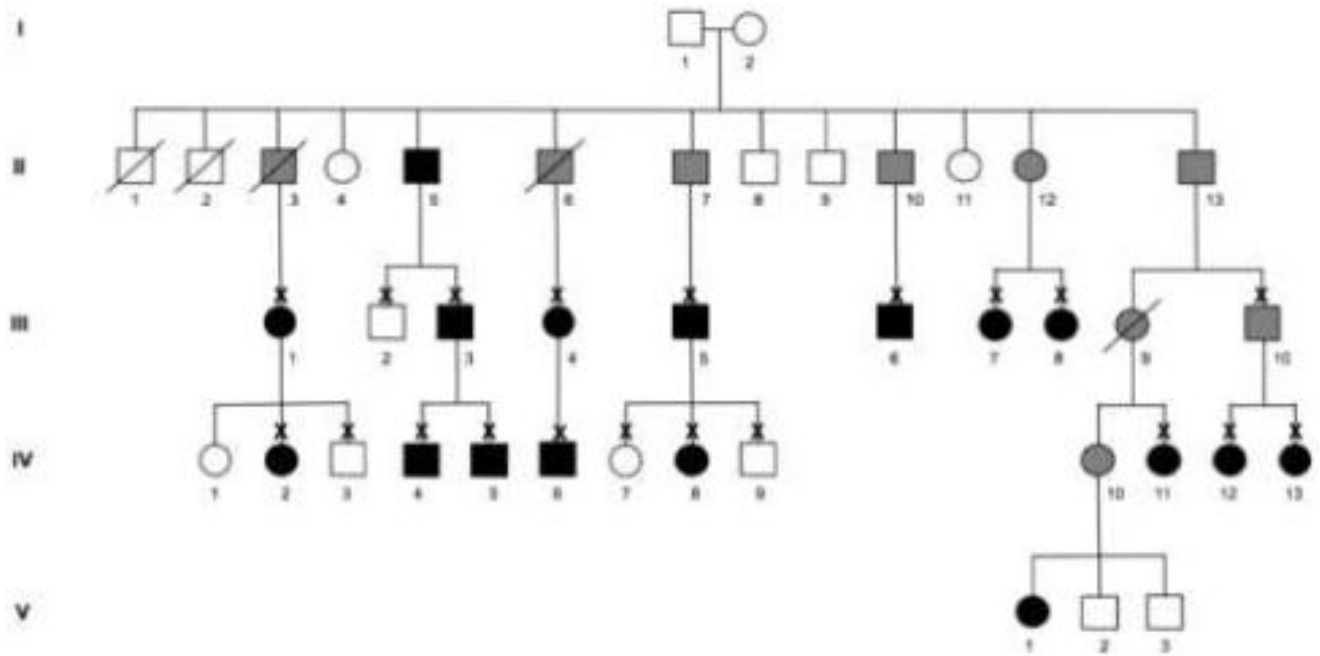


Figure 1. Pedigree affected with cavitory optic disc anomaly. Individuals found to be clinically affected with cavitory anomalies of the optic nerve including optic pits, optic nerve colobomas, or morning glory disc anomaly are represented by black symbols while unaffected individuals are depicted with open symbols. Family members who are obligate carriers are indicated with grey symbols. These family members were either unavailable for examination or they did not meet clinical criteria for having definite cavitory optic disc anomalies, however, they are obligate carriers by virtue of having offspring with cavitory optic disc anomalies. Individuals that are deceased are marked with a slash. Patients who were examined by the authors are indicated with an “X”.

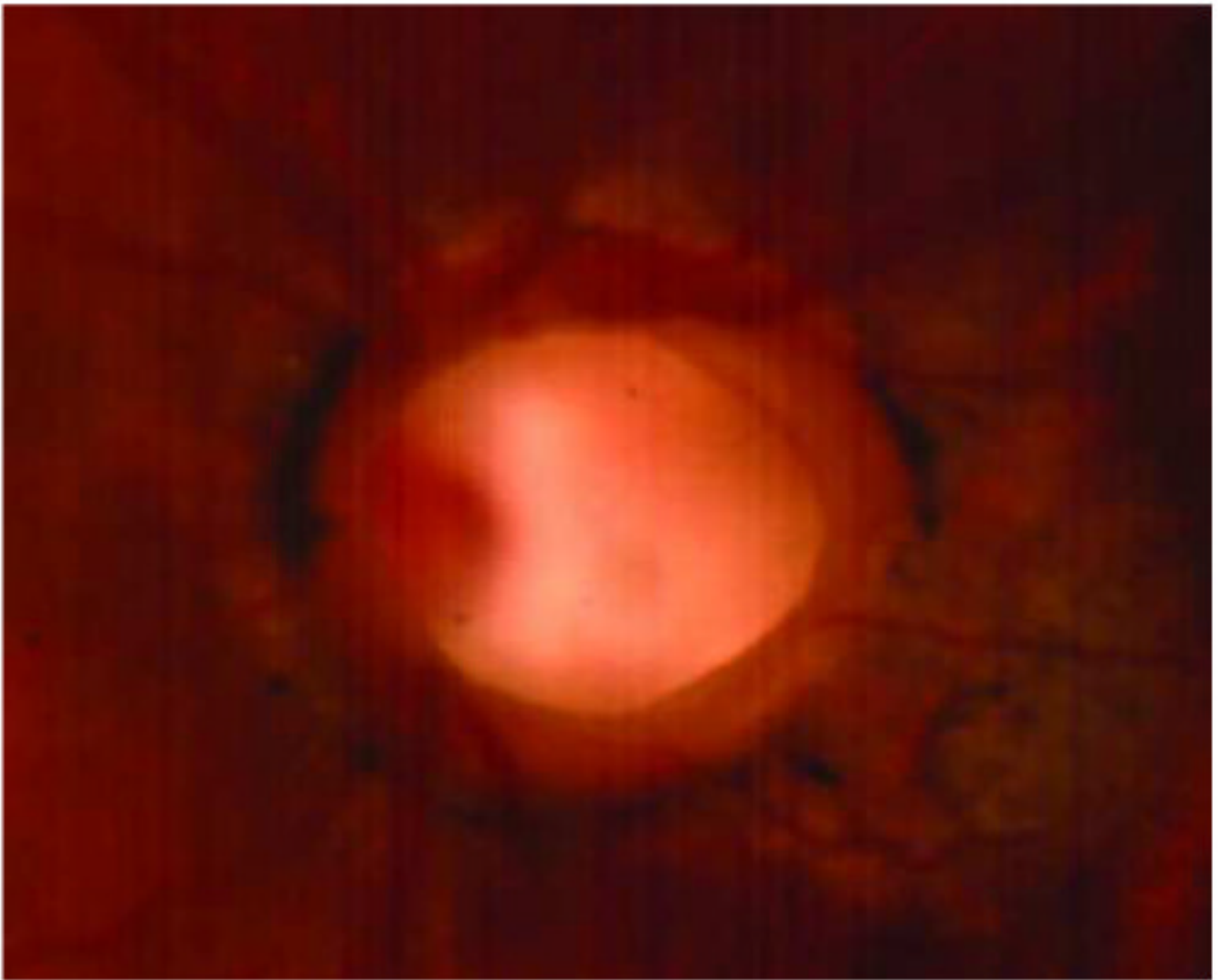




Figure 2.

Appearance of cavitory optic disc anomalies. Affected members of the cavitory optic disc anomaly pedigree have abnormal optic discs with features of optic pits, optic nerve colobomas, and morning glory disc anomaly. **A.** The left optic disc of patient IV-11 is deeply excavated and exhibits features of an optic nerve coloboma and optic pit. **B.** The right optic disc of patient IV-8 is anomalous and has some characteristics that are similar to that of the morning glory disc anomaly including abnormal, radial vessels and a central tuft of glial tissue.

Marker	Locus (bp)	Locus (cM)	Zmax (θ)	Recombinants				
				III-1	III-4	III-6	III-8	IV-11
D12S1618	52178914-52179156	68.16	0.53 (n=0.15)					
D12S1566	52433038-52433204	69.23	1.68 (n=0)					
D12S1632	54701662-54701899	71.61	2.75 (n=0)					
D12S1644	55791343-55791440	72.20	1.73 (n=0)					
D12S980	56710453-56710620	71.61	2.60 (n=0)					
D12S1298	57644124-57644337	75.17	2.87 (n=0)					
D12S1700	58303040-58303219	75.76	4.06 (n=0)					
D12S1056	59832288-59832566	75.17	0.53 (n=0)					
D12S1072	58843135-58843352	75.17	2.19 (n=0)					
D12S1662	59130788-59130993	75.17	3.25 (n=0)					
D12S1650	59222242-59222447	75.17	3.00 (n=0)					
D12S1726	61291062-61291236	75.17	3.67 (n=0)					
D12S329	61428510-61428668	74.58	3.50 (n=0)					
D12S1702	65849524-65849783	77.25	3.24 (n=0.043)					
D12S1291	66204650-66205052	78.14	1.63 (n=0.001)					
D12S313	66787301-66787441	79.93	1.32 (n=0.136)					

Figure 3. Cavitory optic disc anomalies linkage analysis: Two point linkage data and analysis of recombinant individuals. Sixteen genetic markers from the long arm of chromosome 12 are listed on the left of the figure with the most centromeric marker at the top. The physical position of the STRP markers is based on NCBI Build 36.1 of the human genome and the genetic position of the markers is based on the Marshfield map (<http://www.ncbi.nlm.nih.gov/mapview/>). The maximum LOD score (Zmax) is given for each marker as well as the recombination frequency at which the Zmax occurred. The patient designations correspond to those in Figure 1. A black box indicates that during the meiosis that gave rise to the individual (or that individual’s ancestor), an informative recombination event occurred between the marker and the disease gene. Parents were not available from any of the recombinant individuals. Consequently, it is not possible to identify those markers in which no recombinations occurred between the disease gene and the markers. The recombination events summarized in this figure suggest that the disease-causing mutations lie within the 9.1 cM (13.5 Mbp) interval bounded by D12S1618 and D12S1702.