ELECTRONIC LETTER

A novel locus on 19q13 associated with autosomal-dominant macular dystrophy in a large Greek family

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J Med Genet 2006;**43**:e57 (http://www.jmedgenet.com/cgi/content/full/43/12/e57). doi: 10.1136/jmg.2005.040188

Objective: To describe the clinical features of and genetic locus associated with autosomal-dominant macular dystrophy (MCDR5) in a large Greek family.

Methods: 26 members of a single family underwent clinical examinations and venepuncture. A genomewide linkage scan using 400 microsatellite markers distributed with an average spacing of 10 cM throughout the human genome.

Results: 14 members of the study family exhibited clinical features of the disease including decreased central vision and macular abnormalities in the posterior pole of the retina. Analysis of loci known to be associated with macular dystrophy did not show positive linkage. A genomewide linkage scan showed linkage to chromosome 19q, with a two-point maximum LOD score of 5.809 at $\theta = 0$ between the disease and marker locus D19S412. On the basis of recombination events, the disease interval was localised between markers D19S420 and D19S540 on chromosome 19q, at a span of about 3.8 cM, in an area known to contain 120 known genes/transcripts. Eleven of these genes/transcripts were sequenced, and no disease-causing mutation was identified.

Conclusions: This study describes a new locus on 19q associated with autosomal-dominant macular dystrophy, designated as MCDR5. Additional study of other family members will be necessary to further narrow the interval and identify the responsible gene. The study of MCDR5 will aid in elucidation of the underlying pathogenic mechanisms for this and other macular diseases, including age-related macular degeneration.

Macular dystrophies can present with autosomal dominant, autosomal recessive, X-linked recessive and mitochondrial inheritance. The autosomal dominant form of macular dystrophy is a heterogeneous group of disorders that typically present within the first two decades of life with progressive central visual loss and macular atrophy. Ophthalmoscopic findings include bilateral atrophic macular lesions with or without subretinal deposits at the level of the retinal pigment epithelium (*RPE*). The resulting central visual loss is variable and can be severe with visual acuities ranging between 20/20 and 20/400. So far, 12 loci for autosomal dominant forms of macular dystrophy have been localised to particular regions of the human genome.[1 2] They include Stargardt-like macular dystrophy (*STGD3* and *STGD4*).[3 4] Best macular dystrophy,[5] adult vitelliform dystrophy and pattern dystrophy,[1 2 6 7] Doyne honeycomb retinal dystrophy,[8] progressive bifocal chorioretinal atrophy,[9] Sorsby's fundus dystrophy,[10] central areolar choroidal dystrophy,[11 12] dominant cystoid macular dystrophy,[13] North Carolina macular dystrophy (*MCDR1*),[14 15] autosomal dominant ''bull's eye'' macular dystrophy (*MCDR2*),[16] autosomal dominant macular dystrophy resembling *MCDR1* (*MCDR3*)[17] and North Carolina-like macular dystrophy associated with deafness (*MCDR4*).[18] Five genes have been identified.[6–8 11 19–21]

In this report, we describe a large Greek family with autosomal-dominant macular dystrophy that maps to a new disease locus on chromosome 19. Linkage was established with a two-point maximum LOD score of 5.809 at $\theta = 0$ between the disease and marker locus *D19S412*. We designated this locus *MCDR5*.

METHODS

Study subjects

This project was approved by the Institutional Review Board of the Aghia Sophia Children's Hospital, Athens, Greece and the Institutional Review Board of the University of Utah Health Sciences Center, Salt Lake City, Utah, USA. Twenty six members of the study family, 11 females and 15 males, were included. Informed consent was obtained from all members. In all, 14 affected and 12 unaffected members underwent ophthalmic examination including best corrected Snellen visual acuity determination and fundus examination. Members were designated as affected on the basis of decreased visual acuity and the presence of flecks with or without atrophic macular lesions. Fluorescein angiography was carried out on five members.

Genotyping and linkage analysis

Blood was collected by venepuncture and genomic DNA was isolated from the samples with the Puregene genomic DNA purification kit (Gentra Systems, Minneapolis, Massachusetts, USA) according to the manufacturer's instructions. A genomewide linkage scan was carried out using 400 microsatellite markers distributed with an average spacing of 10 cM throughout the human genome (Proligo LLC, Boulder, Colorado, USA). Forward primers were labelled with fluorescent dye D2, D3 or D4 and samples were amplified using the polymerase chain reaction (PCR) and a standard cycling programme of 30 cycles at 94℃ for 30 s, 55℃ for 30 s, and 72℃ for 45 s. A 1 µl sample of PCR product and 0.5 µl internal size standard (Size Standard Kit 400, Beckman-Coulter, Fullerton, California, USA) were combined in a total volume of 40 µl sample loading solution (SLS, Beckman-Coulter) and loaded on to the CEQ 8000 Genetic Analysis System (Beckman-Coulter).

Electrophoresis was carried out using the following programme: (a) 0.5 µl sample injection; (b) 5 min DNA strand denaturation at 93℃; (c) separation at 6000 V at 50℃; (d) signal detection with calibrated D2, D3 or D4 emission spectra. Fragment size determinations were carried out using the default fragment analysis parameters of the CEQ 8000

Abbreviations: CORD, cone-rod dystrophy; PCR, polymerase chain reaction; RPE, retinal pigment epithelium

Table 1 Two-point LOD score between macular dystrophy and STR markers on 19q

	LOD scores at different recombination fractions (θ)				
Markers	**0**	**0.1**	**0.2**	**0.3**	**0.4**
D19S245	−5.5743	0.49704	0.82841	0.7649	0.46702
D19S211	−4.2199	2.0171	1.6731	1.103	0.4586
D19S420	−6.8152	2.3554	2.0798	1.4075	0.6123
D19S408	2.8386	4.6271	3.6037	2.3543	1.0499
D19S559	5.4475	4.4733	3.4061	2.2386	1.0182
D19S219	4.4364	3.6128	2.7689	1.8829	0.9381
D19S412	5.8086	4.7384	3.5628	2.2856	0.9876
D19S540	−1.6092	3.9727	3.2824	2.2841	1.1271
D19S606	−3.1395	4.8823	3.8078	2.5003	1.1291
D19S246	−5.3555	3.3266	2.8207	1.9723	0.9725

software. Two-point LOD scores were calculated using the subroutine MLINK of the LINKAGE program (V.5.1; http://www.hgmp.mrc.ac.uk/; Human Genome Mapping Project Resources Center, Cambridge, UK).[22 23] An autosomal-dominant mode of inheritance with full penetrance and a disease allele frequency of 0.0001 were assumed in the computations. Additional microsatellite markers for fine mapping on chromosome 19 were chosen from the Marshfield database, amplified using PCR (30 cycles at 94℃ for 30 s, 55℃ for 30 s and 72℃ for 45 s), labelled with α[32P]-2′-deoxycytidine 5′-triphosphate, and separated by electrophoresis on a 6.6% denaturing polyacrylamide gel.[24 25]

DNA sequence analysis

Direct sequencing of *GNG8*, *SIX5*, *ZNF224*, *XTP7*, *GPR4*, *FKRP*, *ZNF45*, *ZNF342*, *PLAUR*, *CCDC8*, *RTN2, RDS, ELOVL4* and *CRX* was carried out using the Taq Dyedeoxy Terminator Cycle Sequencing Kit (Beckman-Coulter). For *RDS*, *ELOVL4* and *CRX*, the primers used were described previously.[19 26 27] For the other genes, primers were designed to amplify the

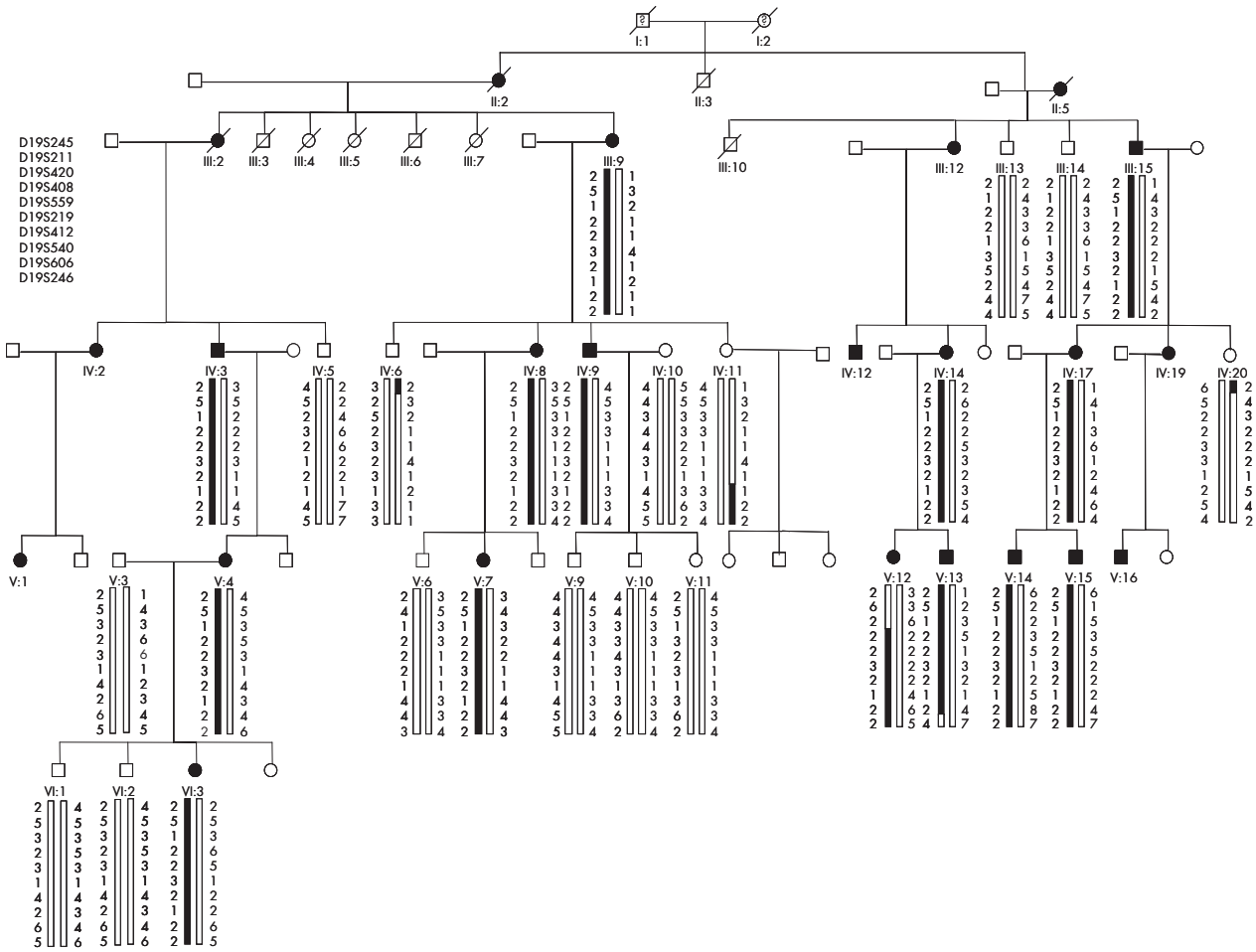

Figure 1 Pedigree of a large Greek family with autosomal-dominant macular dystrophy. Haplotypes of microsatellite markers spanning the linked region on 19q13.2–q13.3 are shown. Affected members are identified by solid squares (males) or solid circles (women). Unaffected members are identified by open symbols; deceased members are indicated by a slash (/). The clinical status of deceased grandparents (I-1 and I-2) is not known. Solid bars denote the haplotype that segregates with the disease phenotype.

complete coding regions and intron splice sites. Amplified products were purified using the QIAquik Gel Extraction Kit (Qiagen, Valencia, California, USA) and sequenced with forward and reverse primers using the Taq Dyedeoxy Terminator Cycle Sequencing Kit (Beckman-Coulter) according to the manufacturer's instructions.

RESULTS

Twenty six members of a large Greek family spanning four generations were included in this study. In all, 14 members were affected and 12 were unaffected (fig 1). All 26 family members underwent clinical examination and ophthalmoscopy. Ages of examined members ranged from 14 to 88 years. Of the 26 individuals examined, 11 were females and 15 were males. Affected patients experienced decreased central vision in the second decade of life and exhibited fundus changes including central macular atrophy with or without subretinal deposit surrounding the macula (fig 2). No dark choroid phenomena were seen on fluorescein angiography (fig 2). Colour vision and night vision were not affected.

Polymorphic STR markers surrounding loci previously known to be associated with autosomal dominant macular dystrophy, cone dystrophy (COD), central areolar choroidal dystrophy (CACD) and cone-rod dystrophy (CORD) were examined first. These included *RDS/Peripherin* on 6p21.1-cen, *MCDR1* on 6q14-q16.2, *MCDR2* and *STGD4* on 4p, *MCDR3* on 5p15.33-p13.1, *MCDR4* on 14p, *EFEMP1* on 2p16, autosomal dominant butterfly-shaped macular dystrophy on 5q21.2-q33, *CORD7* on 6cen-q14, *STGD3* on 6q14, *VMD2* on 11q13, *CACD* on 17p, *CORD5* on 17p13-p12 and *TIMP3* on 22p12.1-q13.[1–4 11–15 19 21 27–30] No marked linkage was found to any of these loci. Additionally, direct sequencing of the coding regions of *RDS/Peripherin*, *ELOVL4* and *CRX* for affected member III:9 did not show any mutations. We then carried out a whole genome scan using 400 microsatellite markers spaced at 10 cM intervals throughout the human genome.

Results of the genome scan showed that the disease phenotype was linked to marker D19S559. Negative or non-significant two-point LOD scores were obtained for all other test loci. This locus was refined using the following markers: D19S245, D19S211, D19S420, D19S408, D19S559, D19S219, D19S412, D19S540, D19S606 and D19S246. Table 1 shows the LOD scores. A maximum two-point LOD score of 5.809 was obtained at $\theta = 0$ with marker D19S412. The LOD scores for loci D19S559, D19S219 and D19S412 were all >4.

Extended haplotypes were constructed using markers in the following order: D19S420–D19S408–D19S559–D19S219–D19S412–D19S540–D19S606–D19S246. The disease-associated haplotype was determined as a common extended haplotype for all affected members. On the basis of recombination events, the disease interval was localised between markers D19S420 and D19S540 on chromosome 19q, at a span of about 3.8 cM. No recombination was detected at loci D19S408, D19S559, D19S219 or D19S412 (fig 1). There are about 120 known genes/transcripts within this interval. Among these, 11 (*GNG8*, *SIX5*, *ZNF224*, *XTP7*, *GPR4*, *FKRP*, *ZNF45*, *ZNF342*, *PLAUR*, *CCDC8*, *RTN2*) were sequenced, and no disease-causing mutation was identified.

DISCUSSION

Here we describe a new locus, MCDR5, on 19q associated with autosomal dominant macular dystrophy. This locus shows variable expressivity evidenced by variable fundus appearance and visual acuities in affected patients. In addition, there is no dark choroid on fluorescein angiography. Several loci and genes have been identified for an autosomal dominant form of macular dystrophy. We now add to this list a new locus, on the basis of the description of a large Greek family with autosomal-dominant macular dystrophy that links to a locus on chromosome 19q13.2–13.3. Recombination and haplotype analyses have narrowed the interval containing the gene responsible for this disease to a region spanning 3.8 cM between markers D19S420 and

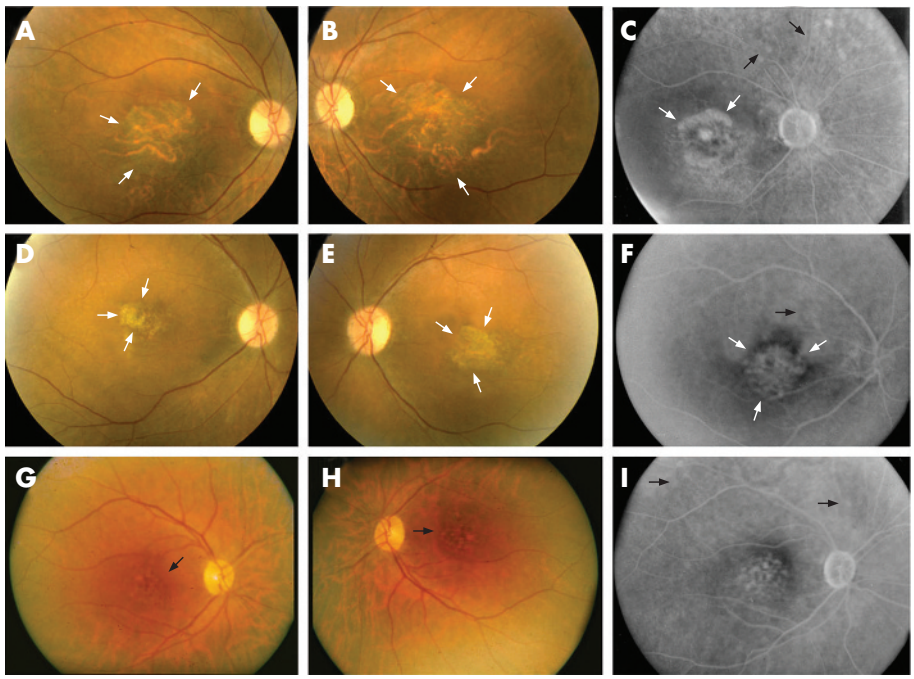

Figure 2 Fundus photographs and fluorescein angiograms of three affected members: IV:9 (A–C), 49 years old, visual acuity (VA): 20/800 OD, 20/800 OS; IV:8 (D–F), 53 years old, VA: 20/400 OD, 20/400 OS; V:4 (G–I), 49 years old, VA: 20/50 OD, 20/70 OS. Fundus photographs and angiograms exhibit central macular atrophy (white arrows) and subretinal deposits (black arrows).

D19S540. Additional study of other family members will be necessary to further narrow the interval.

The identification of this locus will hopefully lead to further understanding and treatment of this disabling disease. The study of MCDR5 and other similar hereditary macular disorders will also potentially help elucidate the complex mechanisms behind age-related macular degeneration, the most common cause of irreversible blindness in older people in the developed world. Through investigation of early-onset macular dystrophy associated with single-gene mutations that share clinical and histopathological features with age-related macular degeneration, it is possible to identify pathways that are common to these diseases. In this manner, a better understanding of the pathogenesis of retinal degeneration and macular dystrophies can be attained. This knowledge will hopefully lead to earlier diagnosis and new strategies for prevention and treatment.

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Funding: K Zhang was supported by grants from the U.S. National Institutes of Health (NIH) NIH RO1 EY14428, NIH RO1 EY14448; the American Health Assistance Foundation; the Karl Kirchgessner Foundation; the Ruth and Milton Steinbach Fund; Ronald McDonald House Charities; Val and Edith Green Foundation and an unrestricted grant to the Department of Ophthalmology and Visual Sciences from Research to Prevent Blindness, New York, NY: Z Yang was supported by grants from the Department of Science and Technology of Sichuan Province, Sichuan, People's Republic of China.

Competing interests: None declared.

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Received 11 December 2005
Revised 17 May 2006
Accepted 20 May 2006

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