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# Mutations in *MFSD8*, Encoding a Lysosomal Membrane Protein, Are Associated with Nonsyndromic Autosomal Recessive Macular Dystrophy

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***Purpose:*** This study aimed to identify the genetic defects in 2 families with autosomal recessive macular dystrophy with central cone involvement.

***Design:*** Case series.

***Participants:*** Two families and a cohort of 244 individuals with various inherited maculopathies and cone disorders.

***Methods:*** Genome-wide linkage analysis and exome sequencing were performed in 1 large family with 5 affected individuals. In addition, exome sequencing was performed in the proband of a second family. Subsequent analysis of the identified mutations in 244 patients was performed by Sanger sequencing or restriction enzyme digestion. The medical history of individuals carrying the *MFSD8* variants was reviewed and additional ophthalmic examinations were performed, including electroretinography (ERG), multifocal ERG (mfERG), perimetry, optical coherence tomography (OCT), fundus autofluorescence, and fundus photography.

***Main Outcome Measures:*** *MFSD8* variants, age at diagnosis, visual acuity, fundus appearance, color vision defects, visual field, ERG, mfERG, fundus autofluorescence, and OCT findings.

***Results:*** Compound heterozygous variants in *MFSD8*, a gene encoding a lysosomal transmembrane protein, were identified in 2 families with macular dystrophy with a normal or subnormal ERG, but reduced mfERG. In both families, a heterozygous missense variant p.Glu336Gln was identified, which was predicted to have a mild effect on the protein. In the first family, a protein-truncating variant (p.Glu381*) was identified on the other allele, and in the second family, a variant (c.1102G>C) was identified that results in a splicing defect leading to skipping of exon 11 (p.Lys333Lysfs*3). The p.Glu336Gln allele was found to be significantly enriched in patients with maculopathies and cone disorders (6/488) compared with ethnically matched controls (35/18 682; $P < 0.0001$), suggesting that it may act as a genetic modifier.

***Conclusions:*** In this study, we identified variants in *MFSD8* as a novel cause of nonsyndromic autosomal recessive macular dystrophy with central cone involvement. Affected individuals showed no neurologic features typical for variant late-infantile neuronal ceroid lipofuscinosis (vLINCL), a severe and devastating multisystem lysosomal storage disease previously associated with mutations in *MFSD8*. We propose a genotype–phenotype model in which a combination of a severe and a mild variant cause nonsyndromic macular dystrophy with central cone involvement, and 2 severe mutations cause vLINCL. *Ophthalmology 2014;■:1–10 © 2014 by the American Academy of Ophthalmology.*

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Inherited macular and cone diseases, such as achromatopsia (ACHM), cone dystrophy (COD), and cone-rod dystrophy (CRD), have an estimated worldwide prevalence of 1:30.000 to 1:40.000.[1–3] Individuals with ACHM typically present shortly after birth with significantly reduced visual acuity, severe photophobia, a congenital pendular nystagmus, and color vision defects in all color axes. Absent or residual cone responses with normal rod responses are measured with full-field electroretinography (ERG).[4,5] Optical coherence tomography (OCT) shows initial loss of inner and outer cone segments with disruption of the ciliary layer, followed by the appearance of a bubble with cell loss in the cone photoreceptor layer. The end stage of ACHM is characterized by atrophy of the retinal pigment epithelium



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(RPE). This degradation takes place in the second decade of life and progresses with age.[6] Individuals with COD present with a normal cone function at birth, but develop progressive loss of cones and central vision during the first or second decade of life. Most individuals develop severe visual acuity loss before the fourth decade.[7] Clinical features of COD are poor visual acuity, disturbances in color vision, and a fundus appearance varying from normal, to a bull's eye maculopathy, or total atrophy of the macular region, with a variable degree of temporal pallor of the optic nerve. The visual fields of these individuals show a central scotoma, and ERG demonstrates progressive deterioration of the cone-derived photopic amplitude responses. Both diseases are characterized by loss of cone photoreceptors and a progressive visual decline, but CRD can be distinguished from COD by early involvement of rod photoreceptors. In addition to the symptoms seen in COD, patients with CRD also may experience nyctalopia caused by rod dysfunction.[8] Both disorders have a high genetic heterogeneity and display all Mendelian inheritance forms. The most prevalent mode of inheritance is autosomal recessive (ar). Eight genes have been implicated in ar COD (*ABCA4*,[9] *CACNA2D4*,[10] *CNGA3*,[11] *CNGB3*,[12,13] *KCNV2*,[14] *PDE6C*,[3] *PDE6H*,[15] and *TULP1*[16]). Three of these genes initially have been associated with ACHM, a form of cone dysfunction that can develop into COD. Eighteen genes have been associated with ar CRD (*ABCA4*,[17] *ADAM9*,[18] *C8orf37*,[19] *CDHR1*,[20,21] *CERKL*,[22] *CNGB3*,[23] *CRB1*,[24] *CRX*,[24] *EYS*,[25] *FSCN2*,[26] *GUCY2D*,[27] *KCNV2*,[14] *PDE6C*,[23] *POC1B*,[28,29] *PROM1*,[30] *RAB28*,[31] *RPE65*,[24] *RPGRIP1*,[23] and *TULP1*[16]). In both disorders, mutations in these genes explain disease in an estimated 21% (COD) and 25% (CRD) of patients.[1,2,14,32,33]

In the past few years, exome sequencing has shown its power to elucidate genetic defects in inherited retinal diseases.[19,31,34,35] The aim of this study was to identify novel causes of ar maculopathies and cone disorders using linkage analysis and next-generation sequencing.

## Methods

### Subjects and Clinical Evaluation

A large nonconsanguineous Dutch family (family A) with 5 family members and an isolated Dutch case (family B) affected by macular dystrophy with central cone involvement were included in this study. An additional cohort of individuals with inherited maculopathies and cone disorders (ACHM, n = 22; COD, n = 110; CRD, n = 112), the majority of whom represent isolated cases, were ascertained from various ophthalmic centers in the Netherlands, Belgium, the United Kingdom, and Canada. Individuals were diagnosed with central cone disease if they showed a progressive decline of visual acuity, color vision disturbances, and reduced multifocal electroretinography (mfERG). Individuals were diagnosed with ACHM when they showed shortly after birth a significantly reduced visual acuity, severe photophobia, a congenital pendular nystagmus, and color vision defects in the protan, deutan, and tritan color axes. Persons were diagnosed with COD if they showed a progressive decline of visual acuity, color vision disturbances, and reduced cone amplitude responses on ERG, with normal rod responses for ≥5 years. Inclusion criteria for CRD were a progressive decline of visual acuity, color vision disturbances, and a reduction of both cone and rod ERG responses, with cones equally or more severely reduced.[36]

After identification of the genetic defect, the medical history of the affected individuals of families A and B was reviewed. Ophthalmologic examinations included best-corrected visual acuity (Snellen chart), slit-lamp biomicroscopy, ophthalmoscopy, color vision testing (Hardy−Rand−Rittler color vision test and Lanthony Panel D-15 tests), and visual field testing using Goldmann kinetic perimetry (targets V-4e and I-4e to I-1e). Fundus photographs centered on the macular area and the 4 peripheral quadrants was performed using standard procedures. Electroretinography was performed according to the extended protocol for the full-field ERG and mfERG using 61 hexagons (central 28°) of the International Society for Clinical Electrophysiology of Vision.[37] For individual II-5 of family A and individual II-1 of family B, spectral-domain OCT (Heidelberg Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany) was used to obtain cross-sectional images of the macular region through dilated pupils, one horizontally and one vertically (30° wide, 51 frames per line). For the same individuals, fundus autofluorescence imaging (Heidelberg Spectralis HRA+OCT, Heidelberg Engineering, Germany) of the macula was performed at a 488-nm wavelength using a 30° lens: A mean image of 16 single images was calculated. In addition, we performed a volume scan (20°×15°, 37 lines, 36 frames per line) for all affected individuals. Individual A:II-2 was examined by a neurologist at age 48 years because of a suspected optic neuropathy. Examinations included a cerebral computed tomography scan, measurement of evoked potentials, and an electroencephalogram. This study was approved by the institutional review board and adhered to the tenets of the Declaration of Helsinki. The probands of both families provided written informed consent to perform exome sequencing.

### Molecular Genetic Analysis

Blood samples were obtained of all probands and, when possible, their parents and affected and unaffected siblings. DNA was isolated from peripheral blood lymphocytes by standard procedures. Mutations in various genes previously implicated in inherited maculopathies and cone disorders (i.e., *ABCA4, CNGA3, CNGB3, KCNV2*, *PDE6C,* and *RAB28*) were excluded by Sanger sequencing. By using genomic DNA of 4 affected members of family A (II-1, II-2, II-3, II-4), genome-wide linkage analysis was carried out using 250K single nucleotide polymorphism arrays. Multipoint parametric linkage analysis was performed with Genehunter (deCode Genetics, Reykjavik, Iceland) in the Easylinkage Plus software package (University of Leipzig, Leipzig, Germany; http://nephrologie.uniklinikum-leipzig.de/nephrologie.site,postext,easylinkage,a_id,797.html) using the Marshfield genetic single nucleotide polymorphism map and the Caucasian allele frequencies. An ar mode of inheritance was assumed because neither the parents nor the 10 children of the 5 affected siblings were affected. The disease-allele frequency was estimated at 0.001.

For all 5 affected members of family A and their available unaffected family members, microsatellite analysis was performed for selected markers to analyze the established linkage peaks on