

A Specific Macula-Predominant Retinal Phenotype Is Associated With the *CDHR1* Variant c.783G>A, a Silent Mutation Leading to In-Frame Exon Skipping

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PURPOSE. To report the clinical and molecular findings in patients with retinal dystrophy associated with the c.783G>A variant in *CDHR1*.

METHODS. The retinal phenotype of 10 patients with *CDHR1*-related retinopathy was characterized by multimodal imaging including color fundus photography, optical coherence tomography (OCT), and blue- and near-infrared fundus autofluorescence imaging. Functional testing included electroretinography, visual acuity, and visual field testing.

RESULTS. Six patients homozygous for the c.783G>A variant in *CDHR1* showed a retinal phenotype resembling central areolar choroidal dystrophy (CACD) on multimodal imaging. Retinal function outside an area of slowly progressive macular atrophy remained relatively preserved. In contrast, biallelic severe/truncating *CDHR1* mutations result in retina-wide retinal degeneration in addition to macular atrophy, with overall severely reduced retinal function. Patients compound heterozygous for the c.783G>A mutation and a truncating mutation in *CDHR1* showed an intermediate phenotype. All patients except one with biallelic severe *CDHR1* mutations were asymptomatic in the first four decades of life, irrespective of their individual *CDHR1* mutations. Analysis of blood RNA from patients with the c.783G>A variant revealed in-frame skipping of exon 8 in vivo, predicting a partial deletion of *CDHR1* ectodomains 2 and 3.

CONCLUSIONS. Patients with biallelic c.783G>A *CDHR1* mutations demonstrate a retinal phenotype consistent with autosomal recessive CACD. The apparently silent dbSNP-annotated c.783G>A *CDHR1* variant (rs147346345) has a relatively high minor allele frequency (0.31%), with homozygous individuals annotated in the general population, and it may therefore have been disregarded in many next-generation sequencing (NGS)-based studies. The differential diagnosis includes *PRPH2*-associated CACD and age-related macular degeneration.

Keywords: *CDHR1*, autofluorescence, OCT, AMD, phenotyping

Inherited retinal dystrophies are among the most common causes of blindness in children and young adults. Mutations in genes coding for proteins necessary for normal retinal morphology and function, such as proteins involved in photo-transduction, the visual cycle, ciliary structure and physiology, protein transcription, photoreceptor structure, and morphogenesis, have all been described.

The cadherin-related family member 1 gene (*CDHR1*, Online Mendelian Inheritance in Man [OMIM] 609502) codes for a photoreceptor-specific protein with an important role in the morphogenesis and arrangement of photoreceptor outer segment discs.^{1–3} Absence of *cdhr1* in mice results in retinal degeneration,¹ but a first screening of this candidate gene in patients with retinitis pigmentosa (RP) was unsuccessful.⁴ However, later genetic investigations in consanguineous

families identified *CDHR1* mutations in several families with autosomal recessive retinal dystrophies.^{5,6} In reports with sufficiently detailed phenotypic data, a generalized cone-rod dysfunction of variable severity and retinal atrophy mainly in the macular area was described.^{5–13} Other reports also associated *CDHR1* mutations with an RP phenotype, although their phenotypic data are inconclusive as to whether rod involvement is predominant.^{10,14}

In a recent study, mutations in *CDHR1* were disease causing in approximately 5% of our macular and cone-rod dystrophy cohort.¹⁵ The silent c.783G>A variant accounted for 9 of the 12 mutant *CDHR1* alleles (four patients homozygous, one patient compound heterozygous). During the course of our study, this frequent mutation was shown to result in skipping of exon 8 by in vitro splice assays.¹⁰ To date there is limited published



information on the retinal phenotype associated with the c.783G>A mutation in *CDHR1*.^{10,11} Reports on the phenotype in three homozygous patients were inconsistent, with either rather mild and late-onset cone dystrophy and obvious degenerative changes restricted to the macula,¹⁰ or generalized cone and rod dysfunction and more severe panretinal degenerative changes.¹¹

Herein, we report six patients homozygous for the c.783G>A variant in *CDHR1* and two patients with distinct *CDHR1* mutations in trans. Our detailed morphologic and functional clinical observations, the analysis of in vivo mRNA splicing of the mutant allele, and its high minor allele frequency in the general population (with homozygotes among apparently healthy individuals) indicate that c.783G>A is likely to represent a hypomorphic mutation.

METHODS

Patients

This case series was conducted from August 2014 through August 2018. Patients were identified from the databases of two tertiary referral centers for inherited retinal dystrophies: Department of Ophthalmology at the University of Bonn, Bonn, Germany, and the Oxford Eye Hospital, Oxford, United Kingdom. The genotype of six patients (patients 1.3–1.6, 2.1, 3.1) was reported in an earlier publication, however, without detailed phenotypic description.¹⁵ The study was performed in adherence with the Declaration of Helsinki. Institutional Review Board approval and written patient consent were obtained.

All patients and their reported family members underwent a complete ophthalmologic examination including best-corrected visual acuity (BCVA) testing, slit-lamp examination, and indirect ophthalmoscopy through dilated pupils. All patients underwent retinal imaging with a confocal scanning laser ophthalmoscope (Spectralis HRA-OCT; Heidelberg Engineering, Heidelberg, Germany). Fundus autofluorescence (AF) images (covering 30° or 55°) were acquired in the high-resolution mode (1536 × 1536 pixels) with either short-wavelength (SW-AF; 488 nm) or long-wavelength (LW-AF; 790 nm) excitation light. Spectral-domain optical coherence tomography (SD-OCT) images were recorded in the high-speed mode with 768 A-scans per B-scan.

Additional functional assessment in patients included electroretinography performed in accordance with the standards of the International Society of Electrophysiology of Vision (ISCEV) and retina-tracking mesopic perimetry (MAIA; CenterVue, Padova, Italy) using a test pattern examining 50 loci, mostly placed along the horizontal line through the foveal center covering 30° of the central visual field.

Next-Generation Sequencing

The index patients from the families reported herein underwent next-generation sequencing (NGS) of a panel of 48 macular and cone/cone-rod dystrophy genes (known at the time of panel design, 2015): *ABCA4*, *ACBD5*, *ADAM9*, *AIPL1*, *BEST1*, *C1QTNF5*, *C21orf2*, *C8orf37*, *CABP4*, *CACNA1F*, *CACNA2D4*, *CDH3*, *CDHR1*, *CERKL*, *CNGA3*, *CNGB3*, *CNNM4*, *CRX*, *CTNNA1*, *ELOVL4*, *GUCA1A*, *GUCA1B*, *GUCY2D*, *IFT140*, *KCNV2*, *MFSN8*, *NR2E3*, *PCYT1A*, *PDE6C*, *PITPNM3*, *POC1B*, *PRDM13*, *PROM1*, *PRPH2*, *RAB28*, *RAX2*, *RBP3*, *RDH5*, *RGS9*, *RGS9BP*, *RIMS1*, *RP1L1*, *RPGR*, *RPGRIP1*, *SEMA4A*, *TIMP3*, *TTL5*, *UNC119*). Enrichment was conducted using Roche/NimbleGen (Madison, WI, USA) sequence capture technology, and NGS was carried out on an

Illumina HiSeq 1500 system (San Diego, CA, USA). Bioinformatic evaluation was performed as described previously.¹⁶

Sequence Variant Verification and Segregation Analysis

Verification of identified variants and segregation analyses were carried out by PCR amplification of the corresponding exon, followed by Sanger sequencing. Sequence data for *CDHR1* were compared to the reference sequence NM_033100.3. Asymptomatic relatives of index patients received genetic counseling prior to mutation testing.

Investigation of c.783G>A_{CDHR1}-Associated In Vivo Splicing

To investigate if the c.783G>A variant results in altered splicing, we isolated mRNA from whole blood (PAXgene blood RNA tubes; Qiagen, Hilden, Germany) of two affected (homozygous) brothers (patients 1.3 and 1.4), their healthy brother, and the parents (all heterozygous carriers of c.783G>A). From the resulting cDNA, we PCR amplified and Sanger sequenced fragments with primers binding to sequences of the exon 6/7 (forward primer, 5'-TACTTCCTGCAGAACCTGCACTC-3') and the exon 10/11 junction (reverse primer, 5'-GAGCTGCATGTACAGGTGACTG-3'), with an expected PCR product size of 457 base pairs. Complementary DNA-PCR amplification was carried out with HotStar Taq DNA Polymerase and Q solution (Qiagen) on a Biometra T3000 PCR cycler (Analytik Jena, Jena, Germany) as follows: 1× (95°C, 15 minutes), 40× (95°C, 1 minute/58°C, 1 minute/72°C, 1 minute), 1× 72°C, 5 minutes.

RESULTS

Retinal Phenotype

The genetic findings and clinical features of 10 individuals with *CDHR1*-associated retinopathy are summarized in the Table. Six patients were homozygous for the c.783G>A mutation (group 1; patients 1.3 and 1.4 are brothers, all others are unrelated), and two were compound heterozygous for the c.783G>A mutation and a truncating mutation (group 2). For comparison, one patient with a homozygous truncating mutation and one patient with a truncating and a novel splice site mutation are included (group 3) whose history and phenotype are in line with previously reported characteristics of *CDHR1*-associated retinopathy with biallelic truncations. Of note, all patients except patient 3.2 were asymptomatic in at least the first four decades of life, indicating that *CDHR1*-related retinal dystrophies generally have a late onset. Initial symptoms may be largely explained by macular dysfunction except in patient 3.2, whose initial complaint was nyctalopia. In group 1, symptoms at last follow-up included reduced visual acuity, reading difficulties, glare, poor contrast vision, and metamorphopsia, but no patient of this group had nyctalopia. In contrast, patients of group 2 and group 3 also reported difficulties seeing in dim illumination. None of the patients had extraocular symptoms suggestive of syndromic retinal disease.

All patients were phakic and had lens opacities in keeping with their age. Fundoscopic findings are illustrated in Figure 1 (first column) and Supplementary Figure S1. In group 1 patients, the earliest disease manifestation was a bull's-eye macular appearance with foveal sparing, some paracentral yellowish-orange flecks/dots, and mild pigmentary changes (patients 1.1 and 1.2). Those with later disease stages showed a sharply demarcated atrophy of the retinal pigment epithelium (RPE) within the macular area. The peripheral retina and the

TABLE. Clinical Features of 10 Patients With *CDHR1*-Associated Retinopathy

Patient, Age at Last Examination (y)	First Symptoms, Age at First Symptoms (y)	Current Symptoms	Best- Corrected Visual Acuity	Refraction (Spheric/ Cylinder)	Fundus	OCT	Autofluorescence	Visual Field	ERG
Group 1: c.783G>A—Homozygous									
1.1 (46)	Metamorphopsia (45)	Metamorphopsia	OD 20/20 OS 20/20	OD $-4.0/-1.25$ OS $-3.0/-1.0$	Bull's-eye maculopathy, paracentral yellowish- brownish flecks	Paracentral irregularity in the ellipsoid and RPE bands, slight thinning of the photoreceptor layer	Paracentral slightly decreased AF, some flecks of decreased and increased AF	Humphrey 30°: relative paracentral scotoma	Borderline low photopic responses, scotopic responses within normal limits
1.2 (45)	Reading difficulties (40)	Mild patchy vision OD	OD 20/20 OS 20/20	Not assessed	Bull's-eye maculopathy, paracentral yellowish- brownish flecks	Paracentral irregularity in the ellipsoid and RPE bands, slight thinning of the photoreceptor layer	Paracentral slightly decreased AF, some flecks of decreased and increased AF	NP	Photopic and scotopic responses within normal limits
1.3 (49)	Glare (42)	Glare, reading difficulties	OD 20/25 OS 20/25	OD $-6.25/-1.0$ OS $-6.0/-1.5$	Round-oval atrophy of the RPE/outer retina	Central atrophy of the outer retina, sharp border toward relatively preserved retinal areas	Hypo fluorescent round-oval central area; few spots of increased AF adjacent to the border of the atrophy	Goldmann III/4 normal peripheral thresholds Microperimetry: sensitivity outside atrophy normal	Photopic > scotopic responses mildly reduced NP
1.4 (51)	Metamorphopsia, reading difficulties (41)	Reduced visual acuity, glare	OD 20/200 OS 20/200	OD $-4.0/-1.0$ OS $-2.25/-0.75$	Oval atrophy of the RPE/ outer retina, few small yellowish flecks adjacent to the border of the atrophy				
1.5 (63)	Metamorphopsia (43)	Reduced visual acuity, glare, poor contrast vision	OD CF OS 20/1000	OD $-6.25/-1.0$ OS $-5.75/-0.75$	Roundish atrophy between the large arcades; parapapillary atrophy			NP	Photopic and scotopic responses within normal limits
1.6 (76)	? (55)	Reduced visual acuity, glare	OD 20/800 OS 20/50	OD $+1.75/-0.75$ OS $+2.5/-0.25$	Round-oval atrophy of the RPE/outer retina OD>OS; minor parapapillary atrophy		Hypo fluorescent round-oval central area; OS: mottled decreased AF surrounding the small atrophic area	Goldmann III/4 normal peripheral thresholds	Photopic and scotopic responses within normal limits

TABLE. Continued

Patient, Age at Last Examination (y)	First Symptoms, Age at First Symptoms (y)	Current Symptoms	Best- Corrected Visual Acuity	Refraction (Spheric/ Cylinder)	Fundus	OCT	Autofluorescence	Visual Field	ERG
Group 2: c.783G>A—Compound Heterozygous With a Severe/Truncating Second <i>CDHRI</i> Mutation*									
2.1 (52)	Glare (47)	Reduced visual acuity, glare, night vision problems	OD 20/200 OS 20/200	OD −0.75 OS −0.5/−0.25	Macular pigment mottling, paracentral atrophy of the RPE/outer retina OS, slightly narrowed vessels; midperipheral hypopigmented fundus; minor parapapillary atrophy	Widespread severe thinning of the outer nuclear layer	Central mottled decreased AF, surrounding diffuse increase of AF in the area between the large vascular arcades	Microperimetry: sensitivity outside atrophy reduced	Photopic ~ scotopic responses reduced
2.2 (54)	Glare (45)	Reduced visual acuity, glare, poor reading ability, reduced color vision	OD 20/50 OS 20/80	OD −2.0/−0.25 OS −2.5/−1.5	Macula with paracentral atrophy of the RPE/outer retina	Paracentral atrophy of the outer nuclear layer, foveal sparing	Low AF within areas of atrophy, surrounding ring- like increase of AF	NP	Photopic responses reduced, scotopic responses within normal limits
Group 3: Two Severe <i>CDHRI</i> Mutations*									
3.1 (49)	Glare, reduced visual acuity (43)	Reduced visual acuity, glare, night vision problems	OD 20/800 OS 20/800	OD +2.5/−4.0 OS +2.5/−4.75	Oval atrophy of the RPE/outer retina; narrowed vessels; hypopigmented fundus; minor parapapillary atrophy	Central atrophy of the outer retina; surrounding retina shows severe thinning of the outer nuclear layer	Low AF within central area of atrophy, midperipheral areas of mottled decreased AF	Microperimetry: sensitivity outside atrophy severely reduced	Photopic and scotopic responses extinguished
3.2 (39)	Nyctalopia (~25)	Nyctalopia, glare, reduced visual acuity	OD 20/600 OS CF	NP (bilateral corneal graft for keratoconus)	Macular chorioretinal atrophy, narrowed vessels, midperipheral and peripheral outer retinal atrophy with bone spicule pigmentations	Central atrophy of the outer retina; surrounding retina shows severe thinning of the outer nuclear layer	Low AF within central area of atrophy, surrounding ring- like of AF, periphery with diffuse decrease of AF	Goldmann III/4 concentric restriction	Photopic and scotopic responses extinguished

CF, counting fingers; NP, not performed.

* Individual severe mutations of patients in groups 2 and 3 are shown in Figure 4.

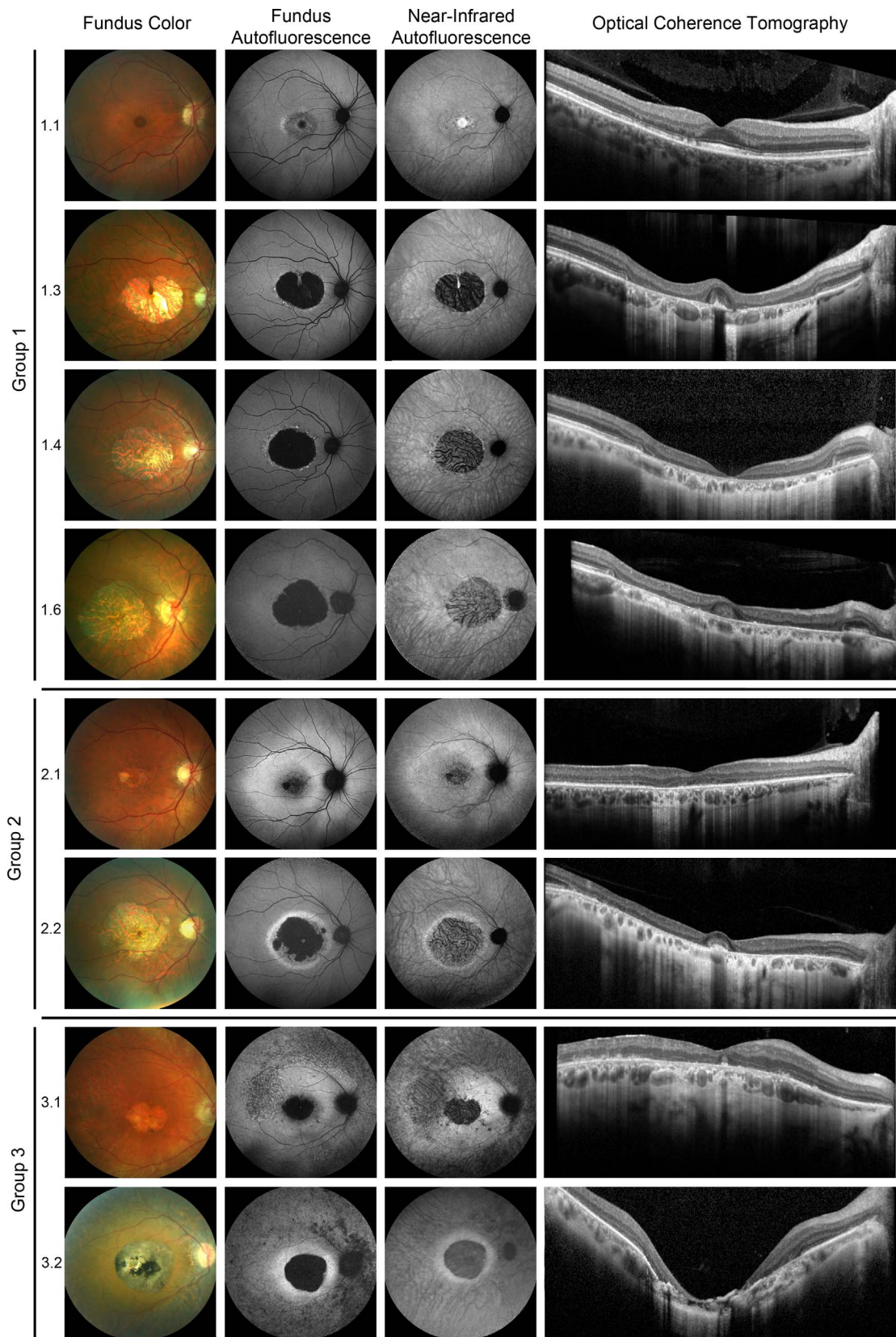


FIGURE 1. Retinal phenotype in patients with *CDHR1*-associated retinopathy. Fundus color (*left column*), fundus autofluorescence with blue (*second column*) and near-infrared (*third column*) excitation light, and horizontal SD-OCT (*right column*) scans centered on the fovea. Group 1: patients homozygous for the c.783G>A variant. Group 2: patients compound heterozygous for the c.783G>A mutation and a truncating mutation. Group 3: patients with biallelic severe *CDHR1* mutations. Findings in patients 1.2 and 1.5 are illustrated in Supplementary Figure S1. Due to the overall high symmetry (see Supplementary Fig. S2), only one eye is shown for each patient.

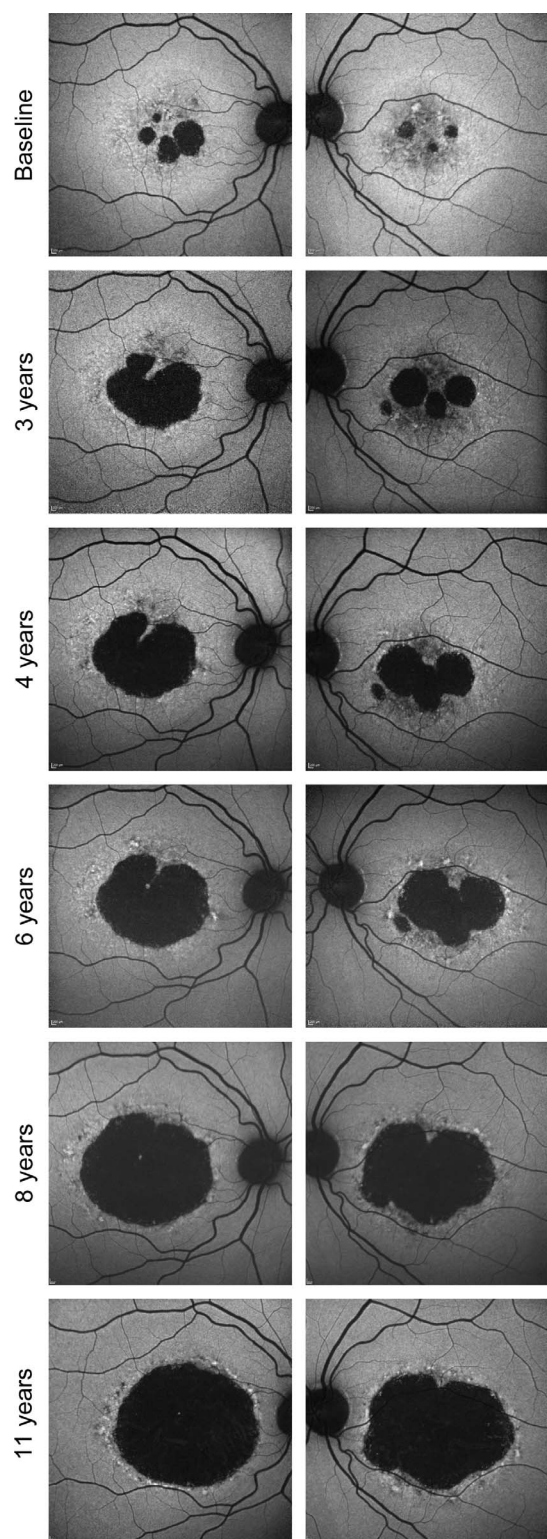


FIGURE 2. Longitudinal AF imaging in patient 1.4 over 11 years.

retinal vessels consistently appeared normal. In contrast, group 2 and 3 patients of similar age revealed more widespread fundus changes including hypopigmentation and narrowed retinal vessels and—in the most severe case (3.2)—peripheral bone spicule pigmentation.

Short-wavelength fundus AF imaging (Fig. 1; Supplementary Fig. S1, second column) in group 1 patients with an early

disease stage revealed a slightly reduced signal in the paracentral area, surrounded by some spots of increased AF (patients 1.1 and 1.2). In later disease stages, there was a markedly reduced AF signal within areas of RPE atrophy, which initially may spare the foveal center (patient 1.3). There were spots of increased and decreased AF at the border of the atrophy, but no obvious changes could be detected outside the macular area. By contrast, patients of groups 2 and 3 also showed AF changes outside the central atrophy. Individual pattern and extent of retinal pathology was clearly visible on AF imaging and showed an overall high degree of symmetry between eyes (Supplementary Fig. S2). Longitudinal AF imaging with a minimum follow-up time of 5 years was available for four patients, showing progressive enlargement and—if initially multifocal—coalescence of the atrophic patches (Fig. 2; Supplementary Fig. S3).

Long-wavelength fundus AF imaging (Fig. 1; Supplementary Fig. S1, third column) revealed an increased foveal signal in the earliest observed disease stage (patient 1.1). In all other patients, the pattern as well as the retinal area with degenerative changes was very similar to that observed on SW-AF imaging (this imaging modality was not available for patient 1.2).

Examination of the cross-sectional retinal structure using SD-OCT imaging (Fig. 1; Supplementary Fig. S1, fourth column) in group 1 patients with early disease revealed mild paracentral thinning of the photoreceptor layer, disruption of the ellipsoid band, and small hyperreflective lesions protruding from the RPE layer. In eyes with later disease stages, there was increased signal transmission below Bruch's membrane within areas of RPE atrophy. The junctional zone typically showed a sharp break of the RPE and ellipsoid band, a curved external limiting membrane, disappearance of the outer nuclear layer, and hence approximation of the outer plexiform layer/inner nuclear layer toward Bruch's membrane within the area of atrophy. The perilesional zone seemed to show mild photoreceptor layer thinning and irregularities of the ellipsoid band. In patients of group 2 and 3, thinning or loss of the photoreceptor layer was much more pronounced also in areas outside the macular atrophy.

All patients tested with retina-tracking mesopic perimetry showed loss of function within areas of RPE atrophy; and in patients of group 2 and 3, severely reduced retinal function was even observed in central areas with partially preserved outer nuclear layer (yellow layer on OCT scans). Differences depending on genotype were best observed in slightly eccentric areas (Fig. 3): In group 1 patients, the retina directly adjacent to the border of atrophy showed apparent normal retinal function. However, group 2 and 3 patients revealed severe functional loss, which was more pronounced in the tested group 3 patient with biallelic truncating mutations.

Retina-wide functional testing using full-field electroretinogram (ERG) showed more severe functional decline of the photopic compared to the scotopic responses. Group 1 patients showed no or only mild functional decline, whereas patients of group 2 had more severe functional loss (Table). ERG responses were nonrecordable in group 3 patients.

Genetic Analysis

Figure 4 shows the pedigrees of all patients included in this study and their mutations (Figs. 4A, 4B). The synonymous *CDHR1* variant c.783G>A affects the last nucleotide of exon 8 (Fig. 4C). We hypothesized that this nucleotide change may interfere with normal splicing at the donor splice site because the last exonic nucleotide is part of an "extended splice site consensus" and represented by a guanine residue in approximately 80% of cases.^{17,18} Computer-assisted analysis yielded

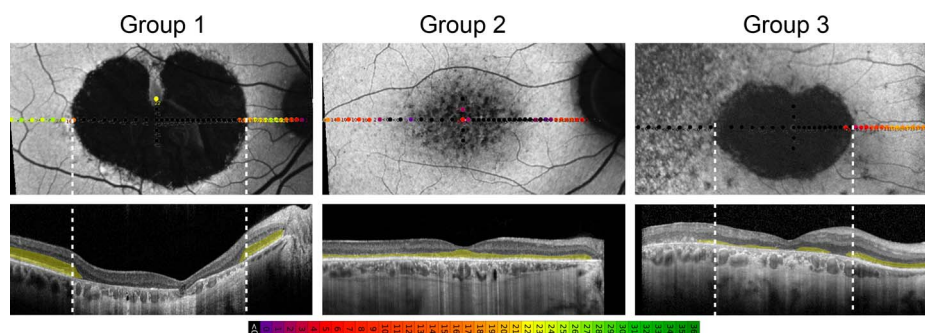


FIGURE 3. Structure-function correlation in patients with *CDHR1*-associated retinopathy. Fundus AF imaging (*top row*), OCT (*bottom row*), and retina-tracking perimetry (overlay in *top row*) in patients 1.3 (*left*; group 1), 2-1 (*middle*; group 2) and 3-1 (*right*; group 3) with *CDHR1*-associated retinopathy. All patients are in their fifth decade of life. There was severe loss of retinal sensitivity over the central area of reduced AF. Adjacent areas show different sensitivity, with almost normal retinal function in the group 1 patient and most severe function loss in the group 3 patient. There is corresponding variable thinning of the photoreceptor layer on OCT scans (marked in *yellow*). The color-coded perimetry scale shows preserved function in *green*, while *yellow*, *orange*, *red*, and *black* illustrate increasing functional loss.

inconsistent results, with only moderate changes predicted by SpliceView (<http://bioinfo.itb.cnr.it/oriel/splice-view.html>; score reduced from 86 to 80), but loss of the donor splice site according to NetGene2 (<http://www.cbs.dtu.dk/services/NetGene2/>). Sequencing of cDNA PCR amplicons generated from whole blood RNA from patients confirmed aberrant splicing with in-frame skipping of exon 8 (Figs. 4D, 4E). The *CDHR1* protein derived from the c.783G>A mutation would lack 48 amino acid residues and thus significant parts of cadherin ectodomains 2 and 3 (provided that the c.783G>A allele produces stable *CDHR1* protein; Fig. 4F).

The c.783G>A_{*CDHR1*} variant is annotated as rs147346345 in dbSNP (National Center for Biotechnology Information, Bethesda, MD, USA). Its minor allele frequency (MAF) in the general population is surprisingly high (0.31%, corresponding to 850 out of 277,164 alleles in the Genome Aggregation Database, gnomAD).¹⁹ Of note, gnomAD reports five homozygous individuals from its assumed-to-be-healthy reference cohort.

Apart from the c.783G>A variant (M1) and two previously reported truncating mutations (M2: c.2522_2528del, M4: c.1503_1507del), three of the mutations described herein are novel (M3, M5, M6). The c.1570_1592del (M3; frameshift) and the c.18G>A (M5) both lead to a premature stop codon, and subsequent mRNA degradation (nonsense-mediated decay) or truncation of the protein. Based on *in silico* predictions, the c.438+1G>A variant (M6) leads to significant alteration of the mRNA splicing due to an altered donor splice site of exon 5. Details of the *in silico* analysis for those variants are provided in Supplementary Table S1.

DISCUSSION

The CACD Phenotype of *CDHR1*-Associated Retinopathy

In this cohort, patients with a homozygous c.783G>A mutation in *CDHR1* consistently presented with a phenotype similar to central areolar choroidal dystrophy (CACD). CACD has previously been associated with specific mutations in *PRPH2* (OMIM 613105, CACD2; reviewed in Ref. 20). Mutations in *GUCY2D* (OMIM 215500, CACD1) and *GUCA1A* (OMIM 602093, CACD4) have also been reported to cause CACD (Supplementary Table S2),^{21,22} although previously reported cases do not indicate similarity in all disease stages. Mutations in *PRPH2*, *GUCY2D*, and *GUCA1A* have an autosomal dominant mode of inheritance, with extensive clinical variability, and nonpenetrance has been observed in

up to 20% of *PRPH2* CACD mutation carriers.²³ Autosomal recessive²⁴ or inconclusive (OMIM 613144, CACD3) pedigrees have been reported, but no link to a genetic cause had been established. The novel genotype-phenotype correlation of *CDHR1* with CACD indicates that sporadic cases not only may be due to nonpenetrance or *de novo* occurrence of dominant mutations, but may also represent autosomal recessively inherited group 1 *CDHR1*-associated retinopathy (suggested terminology: CACD5; Supplementary Table S1).

Patients who were compound heterozygous for c.783G>A and a truncating *CDHR1* mutation (group 2) showed thinning of the photoreceptor layer, reduced retinal function adjacent to the central area of atrophy, and a considerably poorer global retinal function on full-field ERG testing. In the patient with two truncating mutations (group 3), the retinal morphologic degeneration and functional decline was clearly the most severe. Thus, there appears to be a continuum of retinal disease severity with homozygous c.783G>A mutations at the mild end and biallelic truncating mutations at the severe end of the spectrum.

To our knowledge, the retinal phenotype associated with a homozygous c.783G>A mutation in *CDHR1* has previously been reported for only three patients, one of whom matches the consistent phenotype-genotype correlation in our cohort.¹⁰ The different phenotype (RP) in two previously reported patients of ethnic background comparable to our patients¹¹ indicates an influence of genetic modifiers, but the actual reason for this considerable phenotypic difference currently remains unclear.

Because of its relatively late onset, *CDHR1*-associated maculopathy in sporadic (simplex) patients may be misdiagnosed as age-related macular degeneration (AMD),²⁵ and indeed, one of our patients with group 1 disease had previously participated in a clinical trial for AMD patients in a different hospital. Careful ophthalmologic investigation may aid in distinguishing the phenotypes of CACD and AMD,²³ and it seems reasonable to keep *CDHR1*-related retinopathy in mind as a differential diagnosis, especially in cases of “atypical” AMD and familial occurrence compatible with recessive inheritance. Consequently, genomic (based on whole exome or whole genome) data of patients with AMD or geographic atrophy could be searched for individuals with homozygosity for c.783G>A_{*CDHR1*} to verify the assumption that some of these patients may be misdiagnosed as AMD.

Genetic Analysis

Although *CDHR1* has probably been included in most targeted NGS panels covering the known retinal disease genes, *CDHR1*-

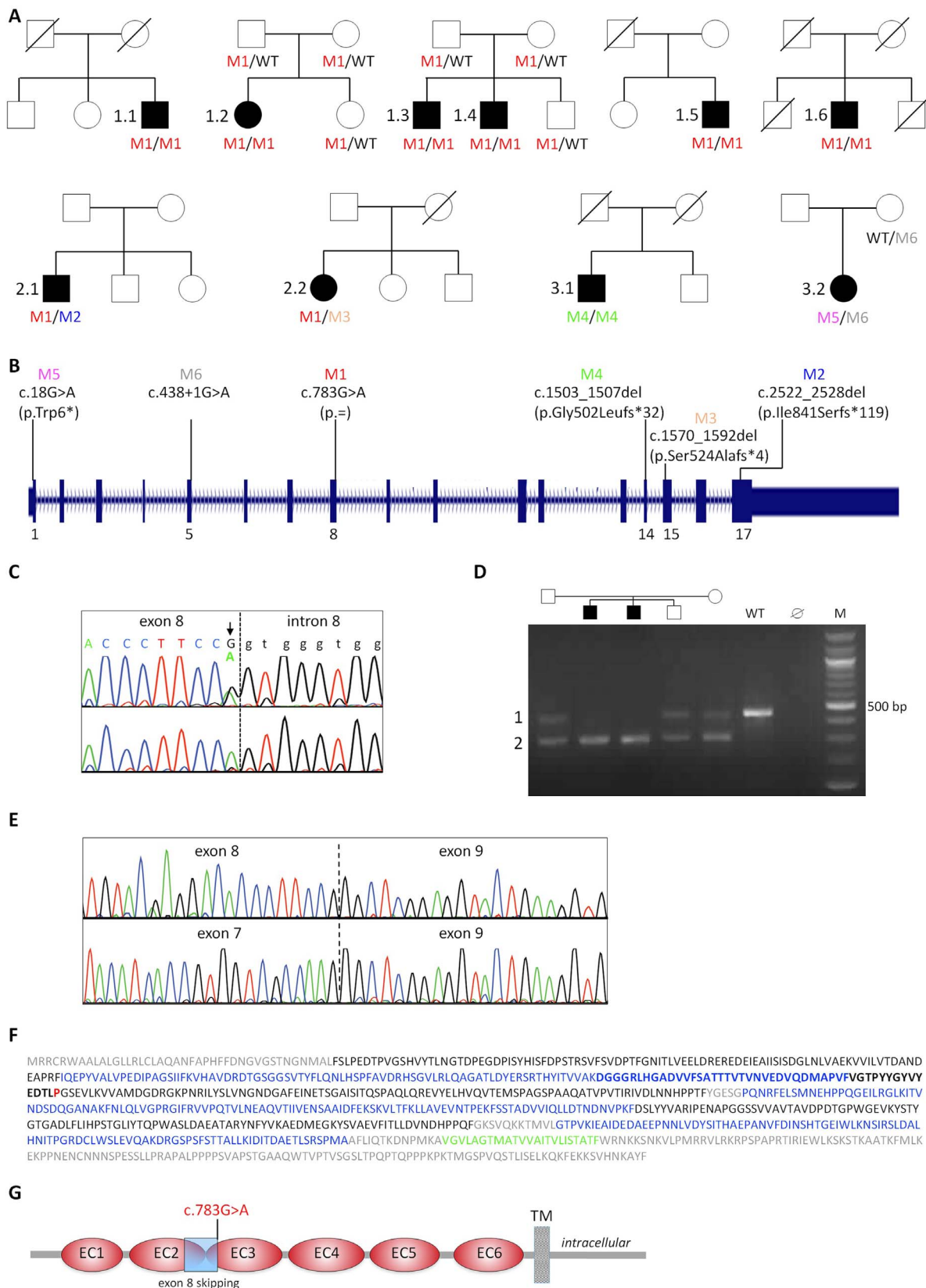


FIGURE 4. Genetic analyses. **(A)** Pedigrees. Compound heterozygosity in patient 2.1 was inferred from the genotype of one son who was a heterozygous carrier of M1 (WT/M1). **(B)** *CDHR1* gene with exons (vertical bars) and identified mutations. **(C)** c.783G>A, a silent mutation affecting the last nucleotide of exon 8, in a heterozygous carrier (upper) and homozygous patient (lower). **(D)** PCR products from blood-derived cDNA of patients 1.3 and 1.4. WT, wild-type (unrelated control individual), Ø, water control; M, size marker. Normal splicing (1), skipping of exon 8 due to 783G>A (2). **(E)** cDNA PCR products with normal splicing (upper) compared to skipping of exon 8 in homozygous patients. **(F)** CDHR1 protein sequence (1-letter code). Green, transmembrane domain. Cadherin motifs (ectodomains, EC) are in alternating color (black/blue). In case of stable c.783G>A-derived protein, 48 protein residues would be deleted from EC2 and EC3 (bold). **(G)** CDHR1 protein scheme with indicated c.783G>A-derived deletion.

associated retinopathy may often have been missed (with the exception of Glöckle et al.,²⁶ who had considered the mutation as causative in an earlier study) because only very recently, c.783G>A was classified as pathogenic. The c.783G>A mutation represents an example of pathogenic variants that are highly prone to misclassification, for the following reasons. First, although the cutoff for the MAF of autosomal recessive mutations in filtering NGS data is usually 1%, most recessive disease-causing mutations are either absent from databases such as ExAC or gnomAD or documented with very low MAF based on a low number of heterozygous carriers. Secondly, of note, five apparently healthy individuals listed in the Genome Aggregation Database (gnomAD) carry c.783G>A in the homozygous state—generally a strong argument against a variant's pathogenicity—and many bioinformatics pipelines consulting ExAC and/or gnomAD may discard the variant or consider it nonpathogenic. The relatively mild and late-onset phenotype associated with homozygosity of the mutation may have meant that these five individuals were in the presymptomatic stage of disease before the fifth decade of life and were consequently included in the “healthy” control cohort. Alternative explanations include incomplete penetrance of c.783G>A_{CDHR1} homozygosity or the contribution of modifier(s).

The c.783G>A mutation is a paradigm for the challenges of high-throughput data interpretation. Even sound bioinformatics analysis and the availability of large-scale databases such as ExAC and gnomAD obviously cannot replace the critical evaluation by clinical and genetic experts.

The effect of silent (and other) variants on splicing is probably underestimated, and the evaluation of RNA transcripts may yield important information on the likely effect of such variants. We have shown in vivo that c.783G>A leads to skipping of *CDHR1* exon 8, thereby supporting the recent data from Stingl et al.,¹⁰ which were based on an artificial minigene assay. The large deletion on the RNA level, albeit in frame, predicts loss of 48 residues from the protein's ectodomain and likely loss of CDHR1 protein function (as can be assumed for out-of-frame truncations). It is thus surprising that CDHR1 protein function is apparently not completely lost and that the mutation is probably only hypomorphic.

CDHR1 is a membrane-bound protein composed of six extracellular cadherin domains that are presumed to form contacts with the inner segment.³ However, the precise mechanism of degeneration resulting from skipping of exon 8 in patients biallelic for c.783G>A is difficult to predict. Additionally, the mechanism underlying the apparent predominant cone degeneration in this patient group, despite CDHR1 expression in both rods and cones, is currently unknown.

Therapeutic Implications

Deciphering the genetic basis of retinal dystrophies is a prerequisite step in developing gene therapy to stop or slow disease progression.²⁷ Recent technological advances have led to a number of clinical studies delivering gene therapy to patients with specific retinal diseases, and recently, gene therapy for choroideremia has been the first clinical trial targeting foveal photoreceptors.²⁸ However, factors limiting the wider applicability of retinal gene therapy include the restricted cargo capacity of certain viral vectors, the need for a therapeutic window between diagnosis and function loss, and the restricted number of patients with individual genetic disease causes.

CDHR1 cDNA has a size of 2.7 kb and thus can be packaged into adeno-associated viral vectors, which have been established as a safe and efficient vector to deliver genes to retinal cells. Moreover, a *cdhr*^{-/-} mouse is available for preclinical assessment of *CDHR1*-related retinal degeneration.¹ There also appears to be a considerable time interval between first

symptoms and substantial vision loss, resulting in an adequate therapeutic window. Patients with *CDHR1*-related retinal degeneration due to severe mutations in trans to c.783G>A should account for a significant fraction of this genetic subtype and might represent ideal candidates for proof-of-concept *CDHR1* gene augmentation therapy.

CONCLUSIONS

The prevalent, hypomorphic c.783G>A mutation in *CDHR1* induces in-frame skipping of exon 8 in vivo. The retinal phenotype of *CDHR1*-associated retinopathy depends on the underlying mutations' severity: Homozygosity for the mild c.783G>A mutation results in disease essentially restricted to the macula, whereas biallelic truncating mutations lead to retina-wide cone-rod dystrophy with severe function loss.

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