A clinical study of patients with novel CDHR1 genotypes associated with late-onset macular dystrophy

Rola Ba-Abbad1,2 · Anthony G. Robson1,2 · Omar A. Mahroo1,2 · Genevieve Wright1 · Elena Schiff1 · Emma S. Duignan1,3 · Michel Michaelides1,2 · Gavin Arno1,2 · Andrew R. Webster1,2

Abstract

Purpose To describe the clinical and electrophysiological features of adult-onset macular dystrophy, due to novel combinations of CDHR1 alleles, and compare the associated phenotypes with previous reports.

Methods The clinical records of patients with macular dystrophy and biallelic variants in CDHR1 were reviewed. Data analysed included best corrected visual acuity (BCVA), fundus images: autofluorescence (AF) and optical coherence tomography (OCT); full field electroretinography (ERG) and pattern ERG (PERG).

Results Seven patients from six pedigrees were ascertained. One patient was homozygous for a known synonymous variant p.(Pro261=), four were compound heterozygous for the p.(Pro261=) variant and a novel allele of CDHR1: p.(Gly188Ser), p.(Met1?), or p.(Val458Asp); one patient was compound heterozygous for two previously unreported variants: c.297+1G>T in trans with p.(Pro735Thr). The range of BCVA at the last clinic review was (6/5 – 6/60). Autofluorescence showed macular flecks of increased AF in mild cases and patches of reduced AF in severe cases. The OCT showed attenuation of the ellipsoid zone (EZ) in mild cases and loss of the EZ and the outer nuclear layer in severe cases; one patient had subfoveal hyporeflective region between the EZ and the retinal pigment epithelium. The full field ERG was normal or borderline subnormal in all cases, and the PERG was subnormal in mild cases or undetectable in severe cases.

Conclusions This report corroborates previous observations that genotypes distinct from those causing pan-retinal dystrophy can cause a milder phenotype, predominantly affecting the macula, and expands the spectrum of these genotypes. The findings in this cohort suggest a potential macular susceptibility to mild perturbations of the photoreceptor cadherin.

Introduction

Inherited retinal dystrophies (IRDs) are a major cause of visual disability in the young and working age populations. Genetic testing, using next generation sequencing (NGS) clinical panels, whole exome sequencing (WES) and whole genome sequencing (WGS), can reveal unexpected genotype–phenotype associations adding to the complexity of IRDs, an inherently heterogeneous group of disorders. The cadherin-related family, member 1 (encoded by CDHR1—OMIM 609502) is a transmembrane protein, expressed at the base of the rod and cone outer segments, and maintains outer segment structure [1]. Biallelic mutations of CDHR1 have been associated with a severe, rapidly progressive cone–rod dystrophy with early macular involvement [2]. Recently, a milder adult-onset retinopathy predominantly affecting the macula, has been associated with the synonymous variant c.783G>A, p.(Pro261=) in CDHR1 (NM_033100.3) [3–7].

This study expands the spectrum of CDHR1 mutations by reporting novel genotypes associated with this rare form of adult-onset maculopathy.

Methods

This is a retrospective case series including patients reviewed at the inherited retinal disorders clinic at
Moorfields Eye Hospital. All patients with retinal dystrophy predominantly affecting the macula, identified by clinical examination, retinal imaging, and electrophysiological testing, who had biallelic likely pathogenic variants in CDHR1 were included. All patients gave informed consent for genetic testing as part of their clinical care, or clinical research to investigate rare causes of IRDs [8, 9]. Segregation of candidate genetic variants was performed after obtaining consent from available family members. The study was approved by Moorfields Eye Hospital and the Northwest London Research Ethics Committee and conformed to the tenets of the Declaration of Helsinki [8, 9]. Genetic testing was performed using a targeted NGS gene-panel of 176 retinal genes including CDHR1, WES or WGS as previously described [8, 9]. Patients with pathogenic or likely pathogenic variants in other IRD-associated genes were excluded. Clinical data analysed included best corrected visual acuity (BCVA), wide field fundus imaging, fundus autofluorescence (FAF) and spectral-domain optical coherence tomography (OCT). Full field electrophoretography (ERG), pattern ERG (PERG) and multifocal ERG (mfERG; four subjects) were recorded according to the standards of the International Society for Clinical Electrophysiology of Vision [10–12]. ERG was used to assess generalised (peripheral) rod and cone system function, and the PERG P50 component and mfERG were used as measures of macular cone system function. The main ERG components were compared with control (normative) data obtained from healthy subjects (age range 10–79 years), which included validated recordings for dark-adapted (DA 10.0) strong flash ERGs (n = 141 subjects), and light-adapted (LA 3.0) 30 Hz (n = 131 subjects) and single flash cone ERGs (n = 109 subjects).

Results

Seven patients, from six pedigrees, were ascertained (referred to as patients 1–6; a GC identifier for each pedigree is given in Table 1 and Fig. 1). Three patients were compound heterozygous for the c.783G>A allele and a novel missense change c.562G>A p.(Gly188Ser) (siblings 1-a, 1-b and patient 2). Patient 3 was homozygous for the c.783G>A p.(Pro261=) allele. Patient 4 had two previously unreported variants in CDHR1: a splice site mutation, c.297+1G>T, in trans with a novel missense change: c.2203C>A, p.(Pro735Thr). Patient 5 was compound heterozygous for the c.783G>A allele and a start-loss mutation c.1A>G, p.(Met1?) patient 6 was compound heterozygous for the c.783G>A allele and a novel missense change: c.1373T>A, p.(Val458Asp).

All patients had adult-onset symptoms, such as difficulty reading, photopsias in the central visual field, photoaversion and/or difficulty seeing under dim illumination, but all had good navigation ability (Table 1). Except for the eldest patient at age 72, all patients retained good visual acuity in the better seeing eye with the youngest patients (age 46 and 41) having normal acuity. Fundus examination showed outer retinal atrophy in the macula with or without foveal involvement, with one patient showing small, yellow flecks at the macula. The peripheral retina was unremarkable in all patients (Fig. S1).

Figure 2 shows the FAF (short and medium wavelength) and OCT images from one eye of each patient in patients with symmetric retinal features on FAF and OCT, or from both eyes if the features were asymmetric. The FAF changes ranged from a ring of perifoveal mottling with increased AF signal to a large patch of loss of the signal in the macula. The OCT showed disruption of the perifoveal ellipsoid zone (EZ) (n = 4), or nearly complete loss of the EZ with marked thinning of the band representing the outer nuclear layer (n = 1). The macular OCT of patient 1-a showed a hypo-reflective region between the EZ and the retinal pigment epithelium (RPE), which is more prominent in the left eye.

The main full field ERG parameters are summarised for patients 1-a and 1-b, and 2–5 and compared with control values across a range of ages (Fig. 3a–h), the data from patient 6 were obtained using previous ERG protocols and therefore were not compared. The DA10 ERG a-wave and LA ERG amplitudes were within the normal range or were borderline (n = 3; patients 1-b, 3, 6); almost all had amplitudes lower than the mean for the control group. No subject showed delay in any of the main ERG components. There was no evidence of an increased rate of ERG decline with increasing age compared with the control group. PERG P50 was undetectable in six cases and showed delay and reduction in two (cases 3 and 4). mfERGs, performed in cases 1-a, 1-b, 4 and 5 (Fig. 3i–k), showed reduction over large macular areas with relative sparing over localised central locations in three eyes of two subjects (left eye of case 1-a; both eyes of case 4).

Discussion

This study reports new genotype–phenotype combinations in patients with CDHR1 maculopathy and adds a genotype that does not include the reported p.(Pro261=) variant. The findings also corroborate the previous reports associating the p.(Pro261=) variant with autosomal recessive maculopathy.

Until recently, the phenotypes associated with CDHR1 mutations were autosomal recessive cone–rod dystrophy and retinitis pigmentosa, both leading to severe visual impairment in adulthood [2, 13].
Table 1 A summary of the clinical and molecular data for all the patients from six unrelated families (GC numbers).

| Patient (family number) | Age at onset of symptoms (age at ERG-years) | Allele 1                     | Allele 2                     | Presenting symptoms                                      | Visual acuity at last clinic visit & age | PERG (P50) | ERG | miERG | Fundus appearance                                      |
|-------------------------|---------------------------------------------|------------------------------|------------------------------|---------------------------------------------------------|---------------------------------------|------------|-----|-------|-------------------------------------------------------|
| 1-a (GC17748)           | 41 (60)                                     | c.783G>G, p.(Pro261=)       | c.562G>A, p.(Gly188Ser)      | Difficulty with night vision, blind spots in the central field | RE 6/12; LE 6/9, Age 63             | Undetectable | Normal | Bilaterally subnormal with relative sparing of the central response on the left | Outer retinal atrophy, macular hypopigmentation; peripapillary atrophy |
| 1-b (GC17748)           | 40 (61)                                     | c.783G>G, p.(Pro261=)       | c.562G>A, p.(Gly188Ser)      | Reduced central vision, constant photopsias, photoaversion | RE 6/9; LE 6/18, Age 73            | Undetectable | DA10 ERG a-wave & LA ERGs: borderline amplitudes & normal peak time | Bilaterally subnormal over central region | Outer retinal atrophy, macular hypopigmentation; peripapillary atrophy |
| 2 (GC26788)             | 34 (43)                                     | c.783G>G, p.(Pro261=)       | c.562G>A, p.(Gly188Ser)      | Difficulty reading and recognising faces, dyschromatopsia | RE 6/9; LE 6/12, Age 47           | Undetectable | Normal | NP                                             | Outer retinal atrophy, macular hypopigmentation |
| 3 (GC24117)             | 42 (29 & 30)                               | c.783G>G, p.(Pro261=)       | c.783G>G, p.(Pro261=)        | Metamorphopsia, photoaversion                            | RE 6/5; LE 6/5, Age 46            | Delayed & subnormal | DA10 ERG a-wave & LA ERGs: borderline amplitudes & normal peak time | NP                                             | Small, yellow flecks at the macula; blunt foveal reflex |
| 4 (GC26837)             | 40 (41)                                     | c.297+1G>T                  | c.2203C>A, p.(Pro735Thr)     | Difficulty transitioning from light to dark             | RE 6/5; LE 6/6, Age 41            | Delayed & subnormal | Normal | Bilaterally subnormal with relative sparing of the eccentric responses | Sharply demarcated outer retinal atrophic lesions in the macula with foveal sparing |
| 5 (GC20637)             | 58 (72)                                     | c.783G>G, p.(Pro261=)       | c.1A>G, p.(Met17)            | Difficulty reading, could not play ball sports under dusk-like illumination during childhood | RE 6/60; LE 6/60, Age 72          | Undetectable | Normal | Bilaterally subnormal with relative sparing of the eccentric responses | Extensive macular atrophy |
| 6 (GC27924)             | 40 (51)                                     | c.783G>G, p.(Pro261=)       | c.1373T>A, p.(Val458Asp)     | Difficulty with central vision                           | RE 6/36; LE 6/24, Age 64          | Undetectable | Normal | NP                                             | Outer retinal atrophy, macular hypopigmentation; peripapillary atrophy; relative foveal sparing in LE |

All patients had biallelic mutations of the photoreceptor cadherin CDHR1. Patients 1-a and 1-b are siblings. The visual acuity was measured using Snellen chart. PERG pattern electroretinogram, ERG full field electroretinogram, NP not performed.
The synonymous change c.783G>A substitutes the guanine nucleotide at the exon–intron boundary of exon 8 with adenine and may therefore weaken the donor splice site consensus sequence. Although this synonymous variant does not cause a change at the amino acid level, since proline is translated from four different DNA codons, including the canonical (for CDHR1) CCG and the variant CCA, this codon is located within the donor splice site consensus sequence and may impact splicing. RNA analysis of this variant was previously shown to result in aberrant splicing and in-frame skipping of exon 8 of CDHR1 [7]. If translated, the resulting protein would lack 48 amino acid residues [7]. However, it is unknown whether normal splicing would still occur.

The missense change p.(Gly188Ser) exchanges a conserved non-polar glycine with a polar serine residue. This missense change is classified as probably damaging (Polyphen), and deleterious (SIFT); the valine is conserved in primates and some mammalian species, and is substituted by isoleucine, a similarly non-polar amino acid with hydrophobic side chain in other species, conserving the main characteristics of the amino acid in the 458 position (Fig. 1d). Unlike valine and isoleucine, aspartic acid is a polar amino acid that may alter the hydrophobic region of the protein and impact the protein function if the protein is expressed in the photoreceptor cell.

The effect of the start-loss mutation c.1A>G, p.(Met1?), which replaces the adenine of the ATG codon at the canonical start site for translation with guanine, remains inconclusive and RNA was not available from this patient. However, the phenotypic similarity to previously reported patients with homozygosity for the c.783G>A allele suggests that this genotype may be functionally similar. The c.297+1G>T variant alters the canonical donor splice site at intron 3 of CDHR1 and is likely to represent a loss of function allele.

The CDHR1-related maculopathy resembles that seen in patients with dominant mutations of PRPH2, PROM1, and recessive ABCA4 maculopathy. Although central macular atrophy is a common denominator of the end stage of these disorders, examining the early images may give an insight
into the possible causative gene. The resemblance between the CDHR1-related maculopathy and the maculopathy associated with the dominantly inherited missense change p.(Arg373Cys) of PROM1 may reflect the close interaction between the photoreceptor cadherin and prominin 1 at the base of the photoreceptor outer segments as previously suggested [14].

The submacular hypo-reflective region noted on the OCT of subject 1-a is unusual and may represent RPE dysfunction that persisted over the 4-year follow-up period. The preservation of the visual acuity in the left eye at the level of 6/9 suggests the presence of functional foveal cones. This feature could be part of the spectrum of CDHR1 maculopathy, but as it is detected in one patient, it could result from a process similar to central serous chorioretinopathy.

Previously, classification of the genotype–phenotype associations in CDHR1 retinopathy suggested that patients homozygous for the p.(Pro261=) variant have the mildest phenotype, with central macular involvement and preservation of the retinal function adjacent to the atrophic lesion (classified by Charbel Issa et al. group 1) [7]. The second group consisted of patients with the p(Pro261=) variant in trans with a loss of function allele, and microperimetry showed reduced retinal sensitivity at the edge of the atrophic lesion [7]. Due to the retrospective nature of this study, none of our patients underwent microperimetry. However, some
insights can be gained from electrophysiology. Full-field ERGs tended to be towards the lower end of the normal range or of borderline amplitude; and longitudinal data would help establish ERG stability. However, there is no evidence of significant peripheral retinal involvement of rod or cone systems and there is no evidence of accelerated ERG decline compared with the control group, suggesting that none of the patients in the present cohort have group 3 retinopathy according to the proposed classification [7]. The PERG was detectable but abnormal in cases 3 and 4, with additional mfERG evidence of spared foveal function in cases 1a and 4 (Fig. 3i, j). Patient 3 was homozygous for the p.[Pro261=] variant and patient 4 had a novel genotype, suggesting that the combination of c.2203C>A, p.(Pro735Thr) and c.297+1G>T have similarly mild impact on the macular photoreceptors with foveal sparing, possibly fitting the description of group 1 [7]. Contrary to the classification that suggest good genotype–phenotype correlation, patient 1b had an undetectable PERG around the same age as the sibling and therefore, unlike patient 1-a, may not fit into group 1 despite having the same genotype. Except for patient 5 who had severe reduction of visual acuity at the age of 72, the patients in this study retained relatively good acuity in their sixth and seventh decades in spite of undetectable PERG recordings; likely reflecting the lower spatial resolution of the PERG compared with mfERG and psychophysical measures of macular function.

Given the predilection of CDHR1 to affect the macular photoreceptors, it is plausible that the central macula is vulnerable to minor perturbations of the cadherin function, while the foveal cones are relatively resilient in the early course of the maculopathy. Examining patients with scotopic and photopic microperimetry may give insight into the differential effect of these CDHR1 mutations on the macular rods and cones or an earlier effect on the DA cone function. This could explain the reason that some patients had difficulty adjusting to dim lights in the presence of normal scotopic function on full field ERG.

In summary, we have identified new genotypes for predominantly macular disease in CDHR1-associated retinopathy.
In addition, we confirm previous reports showing that homozygosity for the c.783G>A variant gives rise to predominantly macular disease. As we enter a phase of widespread feasibility of genetic testing for IRDs, distinguishing specific effects of different variants, and precise correlation of phenotype to genotype is increasingly relevant, in enabling a decision as to whether a clinical case has been molecularly solved, and in yielding insight into potential mechanisms of disease.

Summary

What was known before

- Mutations of CDHR1 cause severe and progressive cone–rod dystrophy.
- Recently, a synonymous change of CDHR1: p.(Pro261=) has been associated with adult-onset macular dystrophy.

What this study adds

- The present study corroborates the association between the p.(Pro261=) variant and macular dystrophy and presents novel alleles associated with the macular dystrophy phenotype.
- Our study adds CDHR1 to the list of candidate genes to screen in patients with likely autosomal recessive macular dystrophy.

Table 2  CDHR1 mutations reported in trans with the synonymous change p.(Pro261=), and the new genotype c.297+1G>T in trans with c.2203C>A, p.(Pro735Thr) presenting with retinopathy predominantly affecting the macula.

| Variant | Allele frequency (gnomAD) | Genomic coordinate (GRCh37) | Reference |
|---------|---------------------------|----------------------------|-----------|
| c.783G>A, p.(Pro261=) | 0.3% (rs147346345) | Chr10: 85962879G>A | Glücke et al. (2014) [3]; Stingl et al. (2017) [4]; Besette et al. (2018) [5]; Jespersgaard et al. (2019) [6]; Charbel Issa et al. (2019) [7]. |
| c.562G>A, p.(Gly188Ser) | 0.007% (rs748412274) | Chr10: 85961599G>A | This study |
| c.2203C>A, p.(Pro735Thr) | 0.0008% (rs1464226905) | Chr10: 85954070G>T | This study |
| c.1A>G, p.(Met1) | 0.002% (rs94726954) | Chr10: 8594517A>G | This study |
| c.152G>A, p.(Val51Glu) | 0.002% (rs120620318) | Chr10: 8594534G>A | This study |
| c.434+1G>A | Not available | Chr10: 85956280G>A | This study |
| c.1311_1316del, p.(Leu437_Thr438del) | 0.0004% (rs1257781536) | Chr10: 85956628_8596633del | Glücke et al. (2014) [3]; Stingl et al. (2017) [4]. |
| c.1503_1507del, p.(Gly502Lysfs*32) | 0.001% (rs126698628) | Chr10: 85971421_85971425del | Birtel et al. (2018); Charbel Issa et al. (2019) [7]. |
| c.1570_1592del, p.(Ser524Lysfs*4) | 0.004% (rs751597954) | Chr10: 85971951_85971973del | Charbel Issa et al. (2019) [7]. |
| c.252_2528del, p.(Ile841Serfs*119) | 0.003% (rs124953310) | Chr10: 85971439_85971432del | Stingl et al. (2017) [4]; Birtel et al. (2018); Charbel Issa et al. (2019) [7]. |
| c.152_2A>G | Not available | Chr10: 85956259A>G | Besette et al. (2018) [5]. |
| c.1373T>A, p.(Val458Asp) | 0.0004% (rs760942217) | Chr10: 85970809T>A | This study |

The allele frequency data from gnomAD (https://gnomad.broadinstitute.org/) were rounded to the nearest 10.

* Birtel J, Eisenberger T, Gliem M, Müller PL, Hermann P, Betz C, et al. Clinical and genetic characteristics of 251 consecutive patients with macular and cone/rod dystrophy. Sci Rep 2018;8:4824.
A clinical study of patients with novel CDHR1 genotypes associated with late-onset macular dystrophy

References

1. Rattner A, Smallwood PM, Williams J, Cooke C, Savchenko A, Lyubarsky A, et al. A photoreceptor-specific cadherin is essential for the structural integrity of the outer segment and for photoreceptor survival. Neuron 2001;32:775–86.

2. Henderson RH, Li Z, Abd El Aziz MM, Mackay DS, Eljinini MA, Zeidan M, et al. Biallelic mutation of protocadherin-21 (PCDH21) causes retinal degeneration in humans. Mol Vis 2010;16:46–52.

3. Glöckle N, Kohl S, Mohr J, Scheurenbrand T, Sprecher A, Weisschuh N, et al. Panel-based next generation sequencing as a reliable and efficient technique to detect mutations in unselected patients with retinal dystrophies. Eur J Hum Genet. 2014;22:99–104.

4. Stingl K, Mayer AK, Llavona P, Mulahasanovic L, Rudolph G, Jacobson SG, et al. CDHR1 mutations in retinal dystrophies. Sci Rep. 2017;7:6992.

5. Jespersgaard C, Fang M, Bertelsen M, Dang X, Jensen H, Chen Y, et al. Molecular genetic analysis using targeted NGS analysis of 677 individuals with retinal dystrophy. Sci Rep. 2019;9:1219.

6. Charbel Issa P, Gliem M, Yusuf IH, Birtel J, Müller PL, Mangold E, et al. A specific Macula-predominant retinal phenotype is associated with the CDHR1 variant c.783G>A, a silent mutation leading to in-frame exon skipping. Invest Ophthalmol Vis Sci. 2019;60:3388–97.

7. Fiorentino A, Fujinami K, Arno G, Robson AG, Pontikos N, Arasanz Armengol M, et al. Missense variants in the X-linked gene PRPS1 cause retinal degeneration in females. Hum Mutat 2018;39:80–91.

8. Carss KJ, Arno G, Erwood M, Stephens J, Sanchis-Juan A, Hull S, et al. Comprehensive rare variant analysis via whole-genome sequencing to determine the molecular pathology of inherited retinal disease. Am J Hum Genet. 2017;100:75–90.

9. Bach M, Brigell MG, Hawlina M, Holder GE, Johnson MA, McCulloch DL, et al. ISCEV standard for clinical pattern electroretinography (PERG): 2012 update. Doc Ophthalmol 2013;126:1–7.

10. McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R, et al. ISCEV standard for full-field clinical electroretinography (2015 update). Doc Ophthalmol 2015;131:81–3.

11. Hood DC, Bach M, Brigell M, Keating D, Kondo M, Lyons JS, et al. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition). Doc Ophthalmol. 2012;124:1–13.

12. Ostergaard E, Batbayli M, Duno M, Vilhelmsen K, Rosenberg T. Mutations in PCDH21 cause autosomal recessive cone–rod dystrophy. J Med Genet. 2010;47:665–9.

13. Yang Z, Chen Y, Lillo C, Chien J, Yu Z, Michaelides M, et al. Mutant prominin 1 found in patients with macular degeneration disrupts photoreceptor disk morphogenesis in mice. J Clin Invest. 2008;118:2908–16.