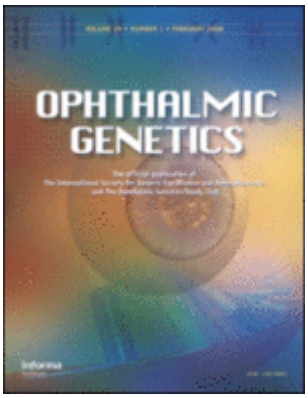

Macula-predominant retinopathy associated with biallelic variants in *RDH12*

Journal:	*Ophthalmic Genetics*
Manuscript ID	NOPG-2020-0103.R1
Manuscript Type:	Case Report
Date Submitted by the Author:	n/a
Complete List of Authors:	Ba-Abbad , Rola; Moorfields Eye Hospital NHS Foundation Trust; University College London; King Khaled Eye Specialist Hospital Arno, Gavin; Moorfields Eye Hospital & UCL Institute of Ophthalmology Robson, Anthony; Moorfields Eye Hospital, Electrophysiology Bouras, Konstantinos; Moorfields Eye Hospital NHS Foundation Trust, Retina Georgiou, Michalis; Moorfields Eye Hospital NHS Foundation Trust Wright, Genevieve; Moorfields Eye Hospital NHS Foundation Trust Webster, Andrew; University College London; Moorfields Eye Hospital NHS Foundation Trust Michaelides, Michel; MOORFIELDS EYE HOSPITAL, INHERITED EYE DISEASE
Keywords:	RDH12-retinopathy, Early-onset retinal dystrophy, Macular dystrophy

Macular ~~dystrophy~~ -predominant retinopathy associated with biallelic variants in *RDH12*

Rola Ba-Abbad, FRCS, PhD[1,2,3§]; Gavin Arno[1,2], PhD[§]; Anthony G. Robson, PhD[1,2]; Konstantinos Bouras, PhD[1]; Michalis Georgiou[1,2]; Genevieve Wright, MSc[1]; Andrew R. Webster, MD, FRCOphth; Michel Michaelides, MD, FRCOphth [1,2]

1. UCL Institute of Ophthalmology, University College London, London, United Kingdom
2. Moorfields Eye Hospital, London, United Kingdom
3. King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia

§ These authors have contributed equally to the manuscript and should be considered as joint first authors

Keywords: *RDH12*-retinopathy; early-onset retinal dystrophy; macular dystrophy

Disclosures: None

Funding: None

Word Count: 1504

Corresponding Authors:

Professor Michel Michaelides, MD, FRCOphth

UCL Institute of Ophthalmology, London EC1V 9EL, United Kingdom

Email: michel.michaelides@ucl.ac.uk

Abstract

Purpose

To describe the clinical, electrophysiological, and molecular features of an unusual ~~macular~~ macula-predominant~~dystrophy~~ retinopathy in two unrelated probands with biallelic variants in *RDH12*.

Methods

Retrospective case series

Results

A 29-year old female presented with visual loss since the age of 14 years. Retinal examination revealed symmetric outer retinal atrophy in the posterior pole with peripapillary sparing. Fundus autofluorescence (AF) showed patchy loss of AF in the posterior pole, with hyper-autofluorescent borders. Optical coherence tomography (OCT) showed loss of the macular outer retinal layers. Pattern electroretinography (PERG) showed macular dysfunction and full-field ERG indicated mild loss of photoreceptor function. Next generation sequencing (NGS) identified two variants in *RDH12*: p.(Arg234His) and c.448+1G>A in *trans*.

The second patient was a 10-year old male with bilateral macular changes and visual loss. Retinal examination showed bilateral macular clover-leaf-like outer retinal changes, with relative foveal sparing. Fundus AF showed bilateral macular hypo-autofluorescent patches with a border of increased signal and preserved foveal AF. OCT showed attenuation of the perifoveal outer retinal layers in the regions of reduced AF signal. PERG showed macular dysfunction, but the full field ERG was normal. NGS and whole genome sequencing identified two variants in *RDH12*: p.(Arg234His) and p.(Cys245_Leu247del) in *trans*.

Conclusions

Disease-causing variants in *RDH12* are typically associated with early-onset severe retinal dystrophy with significant macular involvement. Hypomorphic alleles of this gene cause relatively mild retinopathy with predominant macular involvement. This phenotype demonstrates vulnerability of the macular photoreceptors to certain perturbations of *RDH12*.

Introduction

Early-onset severe retinal dystrophy (EOSRD) manifests in infancy as absent or markedly reduced visually guided behavior, roving eye movements, and undetectable or severely abnormal full-field electroretinogram (ERG)[1]. Early diagnosis and genotyping may identify patients who require screening for renal disease, neurological assessment, or establishing suitability for treatment in the case of *RPE65*-associated retinopathy[2].

Biallelic disease-causing variants in *RDH12* (OMIM 608830), encoding retinol dehydrogenase 12, have been associated with EOSRD (LCA13, MIM 612712), characterized by severe, progressive rod-cone dystrophy with macular atrophy, with an excavated macular lesion in some cases[3]. Recently, the phenotypic spectrum of *RDH12*-associated retinopathy has been expanded to include retinitis pigmentosa, and macular dystrophy[4, 5].

The present report describes the clinical and electrophysiological features of unusual macula-predominant~~r~~ ~~dystrophy~~ retinopathy in two unrelated probands~~,~~: a child and an adult with an age difference of 19 years, who each harbor a known missense change c.701G>A p.(Arg234His) in *RDH12*, *in trans* with a novel in-frame deletion in the young proband and a splice-site variant in the adult.

Case reports

Case 1

A 29-year old woman presented with progressive central visual loss since the age of 14 years. She failed a driving test because of reduced acuity at the age of 19. She did not have a problem with navigation and was otherwise fit and well. The parents had no visual symptoms and were said to be distantly related. The visual acuity (VA) at presentation was 6/60 bilaterally. The ocular media were clear, and both fundi showed bilateral outer retinal changes with intraretinal pigment migration in the posterior pole ~~with~~ and peripapillary sparing (Figure 1-A). Fine refractile crystal-like deposits were noted at the border of the atrophic changes, and there was no retinal vascular attenuation in either eye. Fundus autofluorescence (AF) showed bilateral patches of hypoautofluorescence in the macula, extending over areas superior and nasal to the optic disc with a rugged hyperautofluorescent border giving a leaf-like appearance. Macular optical coherence tomography (OCT) showed the outer nuclear and ellipsoid zone layers to be markedly disrupted. Full-field electroretinography (ERG) was performed according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards and showed moderate bilateral reduction of the rod and cone-mediated ERG responses, without peak time delay (Figure S1). The pattern ERG (PERG) were bilaterally undetectable, in keeping with severe macular dysfunction (Figure S1). Next generation sequencing (NGS) of a panel of 176 retinal genes, performed at the Manchester Centre for Genomic Medicine, showed the patient to harbor a previously reported missense variant in *RDH12* (NM_152443.3): c.701G>A, p.(Arg234His) and a splice-site variant c.448+1G>A[4-6]. Parental testing showed these two variants to be in *trans* (Figure S2).

Case 2

An eight-year old asymptomatic boy was referred with bilateral macular changes noted during routine ocular screening. The child was otherwise fit and well and the family history was non-contributory. ~~The v~~Visual acuity at the time of referral was 6/9 bilaterally. At the age of 10 years, the ~~visual acuity~~VA was 6/36 in both eyes without a significant refractive error. He was able to identify only 2 of the 17 Ishihara color vision plates with the right eye, and 4 of 17 with the left. Clinical examination showed clear ocular media, and both fundi had outer retinal changes in the perifoveal region, but the foveal reflex appeared intact. The optic discs and retinal vasculature had a normal appearance. Fundus AF showed unusual cloverleaf-shaped hypoautofluorescent areas with a border of increased AF, and relative preservation of the fovea (Figure 1-B). OCT identified attenuation of the ellipsoid zone (EZ) nasal to the fovea, with sharp decline of the outer nuclear layer thickness, and preservation of the foveal EZ with a prominent band representing the external limiting membrane. The outer retinal bands temporal to the fovea appeared severely attenuated, with preservation of inner retinal lamination. There was no ERG evidence of generalized (peripheral) retinal dysfunction but PERG P50 reduction indicated macular dysfunction bilaterally (Figure S1). A normal electro-oculogram (EOG) excluded generalized RPE dysfunction.

Genetic testing was undertaken,[6] which showed him to harbor two variants in *RDH12*: c.701G>A, p.(Arg234His), and a novel in-frame deletion c.735_743del, p.(Cys245_Leu247del). Due to the unusual phenotype, further genotyping was performed through whole genome sequencing (WGS) of the patient's, as well as parental DNA for phasing, as part of the Genomics England Study as described previously[7]. The same variants were identified in the absence of additional candidates and shown by trio-WGS to be *in trans* (figure S2).

Discussion

This report describes an unusual presentation of ~~maculopathy~~ macula-predominant retinopathy associated with compound heterozygosity for a known missense variant in *RDH12* with one of two variants: an in-frame deletion of three amino acids; and a splice-site variant: c.448+1G>A. Case 1 had fine intra-retinal crystals, akin to those noted in Bietti crystalline dystrophy. The crystals were detected at the border between the atrophic retina in the posterior pole and normal appearing retina. The 176 retinal gene NGS panel included *CYP4V2* and therefore the possibility of comorbidity is unlikely. Another differentiating point between the phenotype of case 1 and *CYP4V2* retinopathy is that while Bietti's crystalline dystrophy presents with retinal pigment epithelial and the choriocapillaris atrophy and scanty intra-retinal pigment migration, *RDH12* retinopathy affects primarily the photoreceptors and intra-retinal pigmentation is a prominent feature. Case 1 had ERG evidence of loss of peripheral photoreceptor function, atypically mild for *RDH12*-retinopathy. The peripapillary sparing in this case confirms previous reports and suggests that peripapillary sparing can be a feature of *RDH12* retinopathy in addition to *ABCA4* and autosomal recessive bestrophinopathy[4,8-10].

The 8-year-old child (case 2) presented with maculopathy, but showed no ERG evidence of peripheral retinal dysfunction, although monitoring will be required to establish whether progression to generalized retinal dysfunction will ensue as reported in other cases[11]. The apparent foveal sparing on OCT, despite visual deterioration, suggests that the foveal cones are particularly vulnerable to reduction of the dehydrogenase function and this manifests at an early stage as foveal cone dysfunction, possibly preceding structural changes. The presentation of subnormal VA with preservation of the foveal structure, noted in our case 2, corroborates the findings of Zou et al in a 3-year old child with biallelic missense changes in *RDH12*[11]. This could represent a window of opportunity for therapeutic intervention in children where the foveal cones are dysfunctional but could be still rescued. The preservation of the foveal EZ contrasts with the early central macular involvement reported by Aleman et al[9]. In their series, the foveal EZ and outer nuclear layer were undetectable in children as young as 2 years of age with central macular excavation noted in a 9-year old patient[9]. This suggests that nullizygousity for *RDH12* impacts the fovea before it is completely developed in infancy.

The variant c.448+1G>A has been reported in compound heterozygous state with a frameshifting variant in *RDH12* in a proband with EOSRD[10,12]. It is predicted to abolish the canonical donor splice site of intron 6, and therefore may lead to aberrant splicing of this intron. One possible outcome would be skipping of exon 6 leading to an in-frame loss of the encoded 35 amino acid residues encompassing a highly conserved short chain dehydrogenase motif. Indeed, it is possible that alternate splice donor sites up/downstream of the abolished site may be used leading to frameshift truncations. The EOSRD phenotype reported in association with this allele and the current case suggest that this variant is likely to be a loss of function allele.

The p.(Arg234His) allele has a maximum population allele frequency of 0.00065 in the gnomAD dataset and has been shown experimentally to reduce retinol dehydrogenase activity by approximately 56% compared to the wild type protein[11]protein[13]. Previous reports showed that when paired with acompound heterozygosity for this allele, null allelelikely results in a relatively milder *RDH12* retinopathy , it presents clinically as macular dystrophy with outer retinal changes that expand nasal to the optic disc with characteristic peripapillary sparing[4,5,13].

The in-frame deletion p.(Cys245_Leu247del) removes a cysteine and two leucine amino acid residues carboxyl to the catalytic site of RDH12 (Conserved Domain Database: https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) from a region that is conserved in mammalian species (Figure 2). The functional effect of removing these amino acids is not readily predicted without *in vitro* transcript analysis, but the following reasons suggest that this variant can reduce or abolish the function of RDH12: first, case 2 had precipitous loss of central vision during a 2-year follow up period suggesting that the in-frame deletion may have a significant effect on the protein function. Second, *in silico* analysis of this allele predicted it to involve an exonic splicing enhancer motif within exon 8 (www.umd.be/HSF3/) and may therefore impact splicing of this exon. Finally, gGiven that the

p.(Arg234His) can confer about 44% of the dehydrogenase function, the severity of the phenotype in case 2, suggests that the p.(Cys245_Leu247del) allele does not provide sufficiently functional protein to rescue the phenotype.

In conclusion, this report expands the phenotypic spectrum of the macula-predominant *RDH12* retinopathy associated with the missense change: p.(Arg234His) *in trans* with likely loss of function alleles, and reports a novel in-frame deletion in exon 8 of the gene. The detailed retinal imaging highlighted the early features of *RDH12* maculopathy in the pediatric age group. There is increasing importance of early detection of macular abnormalities in asymptomatic children since future clinical trials for this retinopathy may rescue the dysfunctional, yet probably surviving foveal cones in these patients.

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Figures and legends

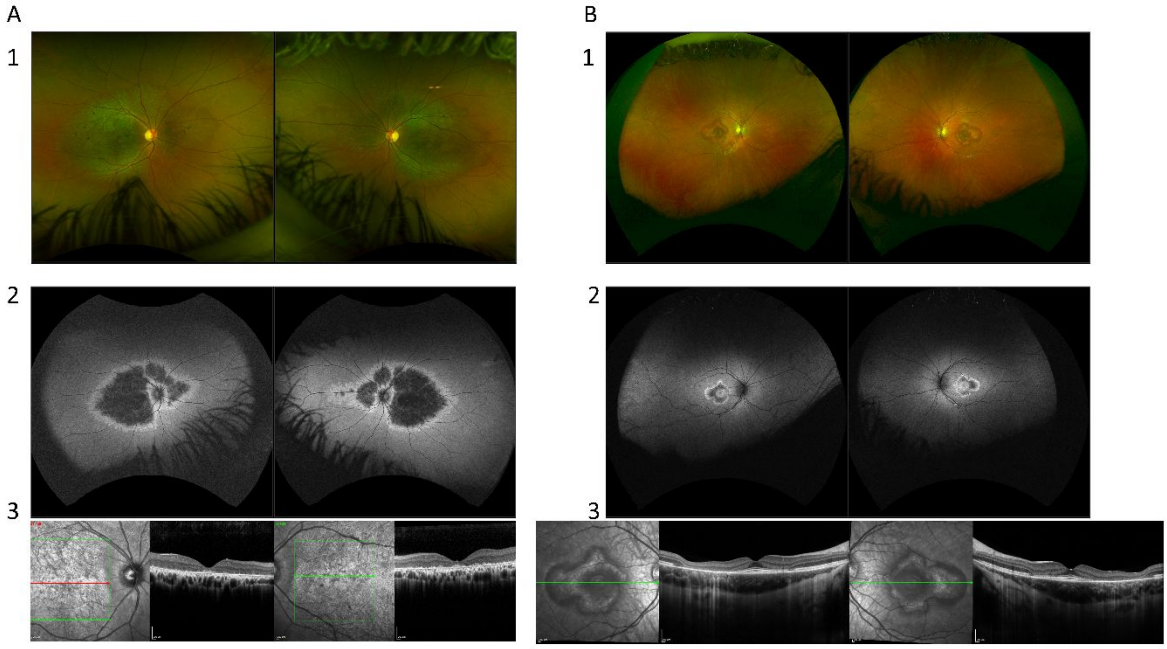


Figure 1. (*A*): Retinal images of case 1. *Top(1)*: widefield pseudo-color images showing bilateral, symmetric outer retinal atrophy and intraretinal pigment migration in the

posterior pole. *Middle(2)*: Fundus autofluorescence (AF), note the hypoautofluorescence in the posterior pole and the feathery hyperautofluorescent border and distinct sparing of peripapillary AF. The peripheral retina AF has a normal appearance. *Bottom(3)*: Optical coherence tomography (OCT) through the fovea showing absence of the outer nuclear layer and severe disruption at the ellipsoid-retinal pigment epithelial complex. (*B*): Retinal images of case 2. *Top(1)*: both fundi showing an unusual macular reflex with a dark cloverleaf-like reflex at the border of hypopigmented patches in the posterior pole. *Middle(2)*: AF showing a hyperautofluorescent border resembling the shape of the dark reflex on pseudo-color images surrounding an area of reduced AF, with mild increase of the AF in the fovea. *Bottom(3)*: OCT through the fovea showing a hyperreflective external limiting membrane in the fovea with thickened foveal ellipsoid zone-interdigitation zone bands. Note the severe attenuation of the outer retinal bands in the perifoveal region.

RDH12,	*H.sapiens*	NP_689656.2	206	ANVLFTRELAKRL--QGTGVTTYAVHPG-VVRSELVRH-SSLLCLLW---	245
	P.troglodytes	XP_003314454.1	206	ANVLFTRELAKRL--QGTGVTTYAVHPG-VVRSELVRH-SSLLCLLW---	245
	M.mulatta	XP_002805156.1	206	ANILFTRELAKRL--QGTGVTTYAVHPG-VVRSELVRH-SSLLCLLW---	245
	C.lupus	XP_547866.3	206	ANMLFTRELAKRL--QGTGVTTYAVHPG-VVSSELVRH-SFLLCLLW---	245
	B.taurus	NP_899207.1	206	ANVLFTRELAKRL--KGTGVTTYAVHPG-IVRSKLVRH-SFLLCLLW---	245
	M.musculus	NP_084293.1	206	ANLLFTRELAKRL--QGTGVTAYAVHPG-VVLSEITRN-SYLLCLLW---	245
	R.norvegicus	NP_001101507.1	206	ANVLFTRELAKRL--QGTGVTAYVVHPG-CVLSEITRH-SFLMCLLW---	245
	G.gallus	XP_421193.1	216	ANVLFTRELARRL--QGTKVTANSLHPG-SVHSELVRH-SFVMTWLW---	255
	D.rerio	NP_001002325.1	209	ANVLFTRELARRL--QGSNVTVNSVHPG-TVRSELVRH-STLMSLLF---	248

Figure 2. Multiple sequence alignment of the CLL amino acids of RDH12, showing conservation of these residues in mammals.

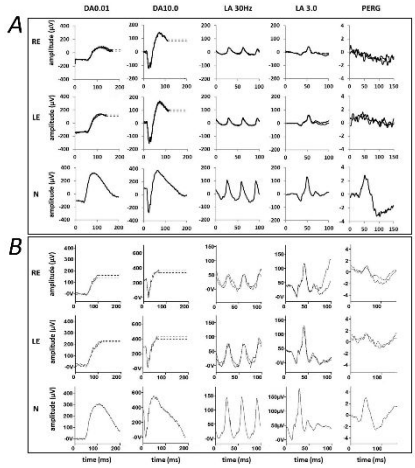


Figure S1. Electroretinograms (ERG) of cases 1 and 2; testing was performed on a different recording system for each patient, representative normal traces are shown for each dataset for reference. ***A***. Full-field ERG and PERG findings in case 1, shown for right (RE) and left (LE) eyes and compared with a representative control subject (N). Full-field ERGs including the DA10 ERG and LA3 ERG a-waves are reduced with preservation of the b:a amplitude ratio, consistent with a loss of rod and cone photoreceptor function. Pattern ERG P50 is undetectable, in keeping with severe macular dysfunction. Patient traces are superimposed to demonstrate reproducibility. Broken lines replace blink artefacts for clarity. ***B***. Full-field ERG and PERG findings in case 2, shown for right (RE) and left (LE) eyes and compared with a

representative control subject (N). Dark-adapted full-field ERGs are normal and reveal no evidence of generalized rod system dysfunction. Light adapted (LA) 30Hz flicker ERG show mild reduction without delay, but due to marked eye closure during testing. The LA3.0 ERGs are normal and consistent with preserved peripheral retinal cone system function. PERG P50 shows reduction, in keeping with significant macular dysfunction. Patient traces are superimposed to demonstrate reproducibility. Broken lines replace blink artefacts for clarity.

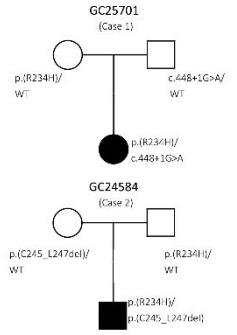
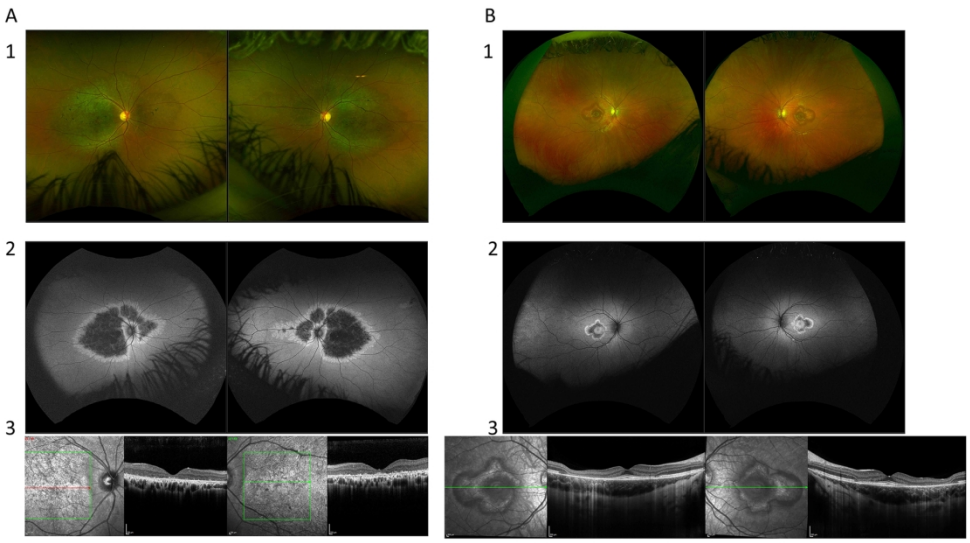


Figure S2: Pedigrees for cases 1 (GC25701) and 2 (GC24584), showing biallelic variants in *RDH12* to be *in trans* in each proband.



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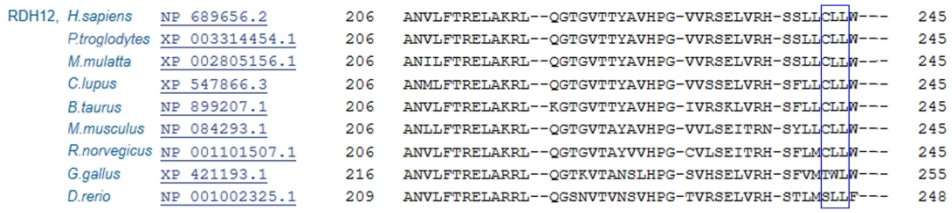

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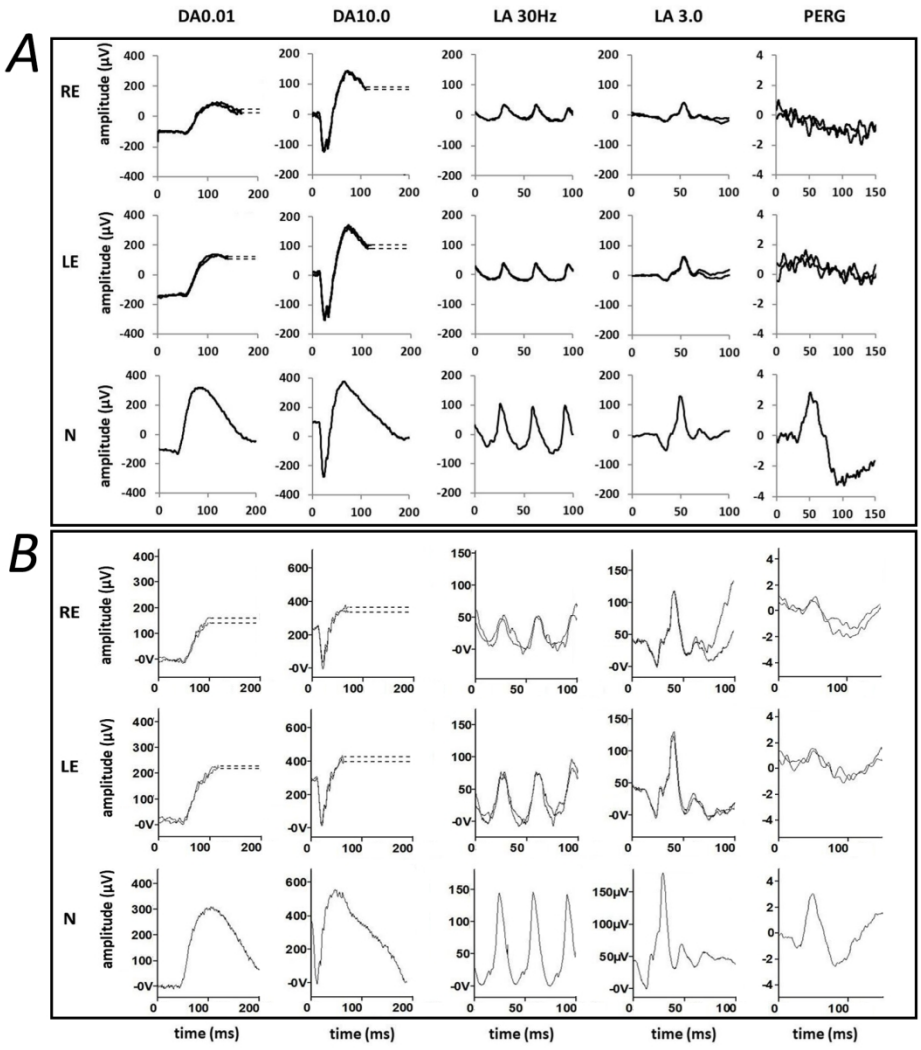

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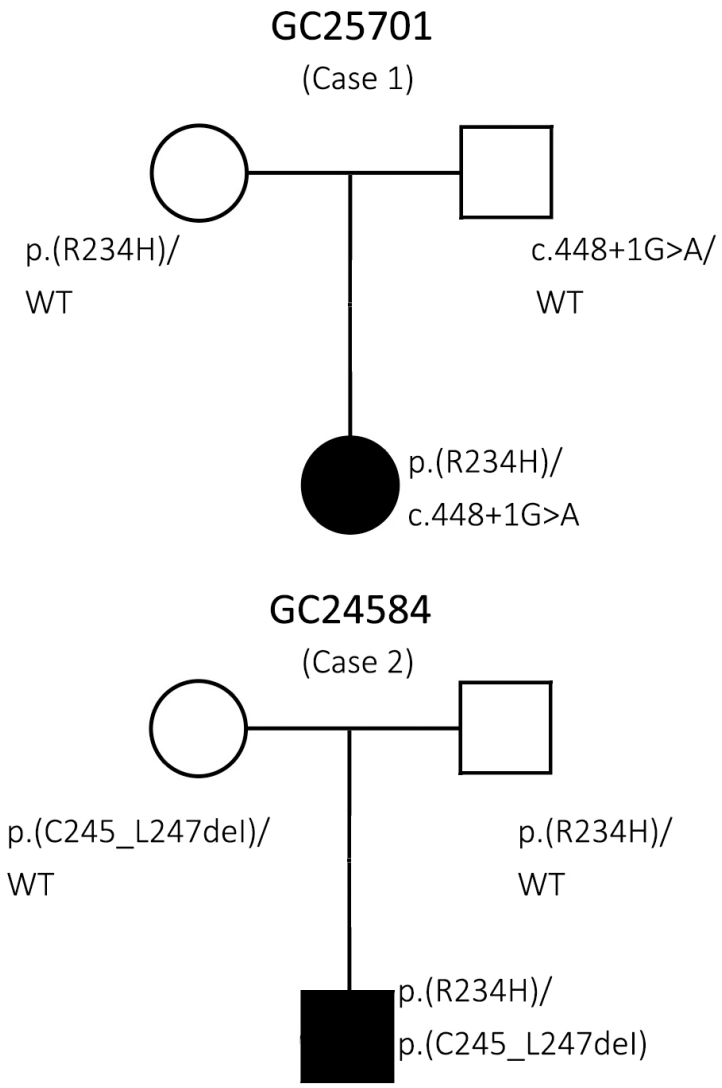

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