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Multimodal imaging evaluation of occult macular dystrophy associated with a novel RP1L1 variant

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ABSTRACT

Purpose: Occult Macular Dystrophy (OMD) is an autosomal dominant inherited retinal dystrophy caused by mutations in the retinitis pigmentosa 1-like 1 (*RP1L1*) gene. The present study describes a novel *RP1L1* variant, identified for the first time in two Italian sisters diagnosed with OMD, along with multimodal imaging features, including Optical Coherence Tomography (OCT) Angiography.

Methods: We performed multimodal imaging including spectral-domain OCT, blue light autofluorescence (BAF), infrared autofluorescence (IRAF), swept-source OCT Angiography (OCTA), full-field and multifocal electroretinography. Genetic analysis was performed using Next-Generation Sequencing. Pathogenic potential of non-synonymous novel variants was scored with two in silico algorithms.

Results: Proband 1 (P1) and proband 2 (P2) were two Italian sisters of 61 and 56 years old. Both reported a history of progressive visual loss without fundoscopic alterations. P1 reported a 4-year history of rapid visual function worsening, and her best-corrected visual acuity (BCVA) was counting fingers in both eyes. P2 reported a 20-year history of mild but progressive visual acuity loss, and her BCVA was 1/10 and 2/10 respectively in her right and left eye. Structural OCT displayed disorganization of outer retinal bands at the macula and foveal cavitation; loss of foveal photoreceptors was remarkably evident on *en-face* OCT slabs. OCTA quantitative analysis found that vessel density was reduced both at SCP and DCP while choriocapillaris blood flow was relatively spared. Genetic analysis found the same rare dominant c.2873G > C, p.Arg958Pro variant in the *RP1L1* gene. The substitution was regarded as moderately radical according to Grantham score while PolyPhen2 classified the amino acidic substitution as probably damaging.

Conclusions and importance: Our study expands the mutational spectrum of *RP1L1* gene: the rare c.2873G > C, p.Arg958Pro missense variant may be considered a new pathogenic variant for OMD, the first to be identified exclusively in an Italian family. Moreover, our quantitative OCTA data suggest that OMD is characterized by a rarefaction of superficial and deep capillary plexus.

1. Introduction

Occult Macular Dystrophy (OMD; OMIM #613587) is an autosomal dominant inherited retinal dystrophy with age-dependent incomplete penetrance first characterized by Miyake *et al.* in 1989 and caused by dominant mutations in the retinitis pigmentosa 1-like 1 (*RP1L1*) gene,[1,2] which encodes for a photoreceptor-specific protein that participates in the formation of the axoneme.[3] *RP1L1* protein contains two doublecortin, one RP1 and two repeat domains; the most common OMD-associated variant causes the p.Arg45Trp substitution in the first microtubule-binding (doublecortin) domain.[4,5] The unique clinical finding is the progressive decline in visual acuity despite the absence of any fundus finding. However, multifocal electroretinography (ERG) shows a reduction in cone responses and optical coherence tomography (OCT) confirms the anatomical disruption of both the inner and outer segment of photoreceptors.[6,7] Recently, the progression of

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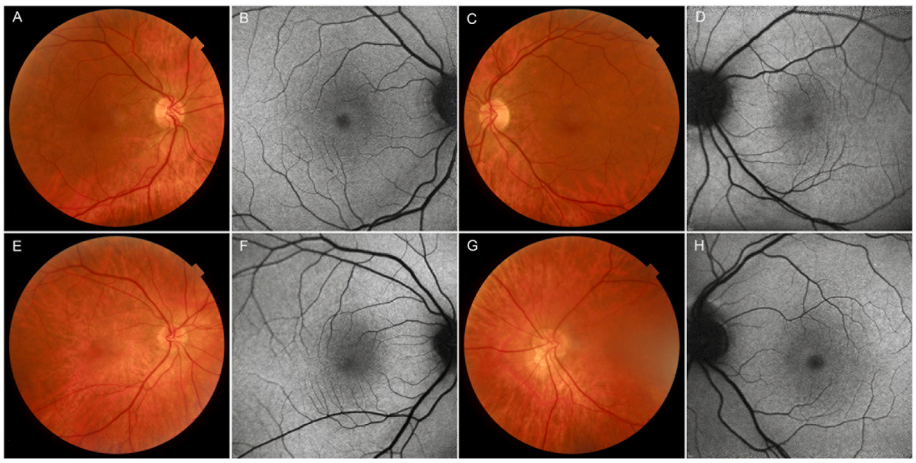

Fig. 1. Colour fundus photograph (CFP) and blue-light autofluorescence (BAF) in Occult Macular Dystrophy. CFPs of proband 1 (A, C) and 2 (E, G) show normal macular morphology. BAF images of proband 1 (B, D) and 2 (F, H) reveal a subtle foveal hyper-autofluorescence. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

photoreceptor layer damage was investigated using ultrahigh-resolution OCT: the cone interdigitation zone is disrupted at the fovea even in early stages while the damage of outer segments and ellipsoid zone is preceded by microstructural abnormalities such as clusters of hyperreflective dots.[8]

The disease was initially described in a Japanese population and only subsequently was identified in Caucasian patients. Currently, about 24 different OMD-associated *RP1L1* disease-causing variants have been reported in literature.[5] Herein we describe a novel *RP1L1* variant, identified for the first time in two Italian sisters diagnosed with OMD, along with an extensive multimodal imaging investigation which includes the first quantitative OCT Angiography (OCTA) analysis of retinal vascular impairment in OMD.

2. Materials and methods

2.1. Study participants

Study participants were two Italian sisters referred in November 2020 to the Retinal Heredodystrophies Unit of San Raffaele Hospital in Milan with a suspect diagnosis of OMD. The research was approved by the local ethical committee (MIRD) and adhered to the tenets of Helsinki declaration.

2.2. Imaging assessment and quantitative analysis

We performed a complete ophthalmologic examination including best-corrected visual acuity (BCVA) measurement, spectral-domain OCT, blue light autofluorescence (BAF) and infrared autofluorescence (IRAF) (Spectralis HRA + OCT; Heidelberg Engeneering, Heidelberg, Germany), swept-source (SS) OCT and OCTA (DRI Triton; Topcon Medical Systems, Tokyo, Japan). Visual evoked potential (PEV), pattern ERG (pERG), full field ERG (ffERG) and multifocal ERG (mfERG) were performed according to standard International Society for Clinical Electrophysiology of Vision (ISCEV) protocols.

To extract quantitative OCTA parameters, we used 3 × 3 mm macular scans and performed manual segmentation of superficial capillary plexus (SCP), deep capillary plexus (DCP) and choriocapillaris (CC). Foveal avascular zone (FAZ) area was measured using a tool provided by Topcon IMAGEnet 6 software. Then, all reconstructions were loaded in Fiji software. SCP and DCP images were binarized using automatic "mean" thresholding to calculate vessel density (VD), or the proportion of white pixels to black ones.[9] CC images were binarized using automatic local "Phansalkar" thresholding method to calculate VD and to measure the number of flow voids using the "analyze particles command".[10] Quantitative data acquired from OMD-affected probands were compared with four eyes of four age-matched healthy subjects. Descriptive data for quantitative variables are expressed as median [interquartile range]. Distribution differences among non-normal variables were assessed using Mann-Whitney test on SPSS Statistics 25 (IBM; Armonk, NY).

2.3. Genetic testing

Genetic analysis was performed as follows: extraction of genomic DNA from the peripheral blood, sample enrichment using the TruSight One sequencing kit (Illumina Inc., San Diego, CA, USA) and Next-Generation Sequencing (NGS) of exons and flanking intronic regions (+20/-20 bases) on a panel of genes linked to inherited retinal dystrophies using Illumina NexSeq500 (Illumina Inc., San Diego, CA, USA). After read alignment with the hg19/GRCh37 reference genome, allele frequency data were obtained through gnomAD. Only variants with a frequency <1% in the general population were evaluated by expert geneticists in relation to the patient's phenotype. Reported variants were confirmed by means of direct Sanger sequencing.

Pathogenic potential of nonsynonymous low-frequency novel variants was scored utilizing two different in silico algorithms. Grantham matrix score predicts the effect of amino acid substitutions on the basis of their polarity and molecular volume; codon replacements can be categorized as conservative (score 0–50), moderately conservative (score 51–100), moderately radical (score 101–150), or radical (score ≥151).[11] PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) predicts the impact of an amino acid substitution on protein structure and function using physical and sequence homology data and determines if the substitution is probably damaging, possibly damaging or benign.[12] American College of Medical Genetics and Genomics (ACMG) criteria have been adopted for clinical interpretation of variants.[13]

3. Results

3.1. Clinical data

Proband 1 (P1) and proband 2 (P2) were two Italian sisters of 61 and 56 years old with a personal history of progressive visual loss starting in adulthood without visible fundus alterations. Familiar history was suggestive because the deceased father and paternal grandmother experienced a similar bilateral impairment in visual function. P1 reported a 4-year history of visual acuity loss and her BCVA on the day of the

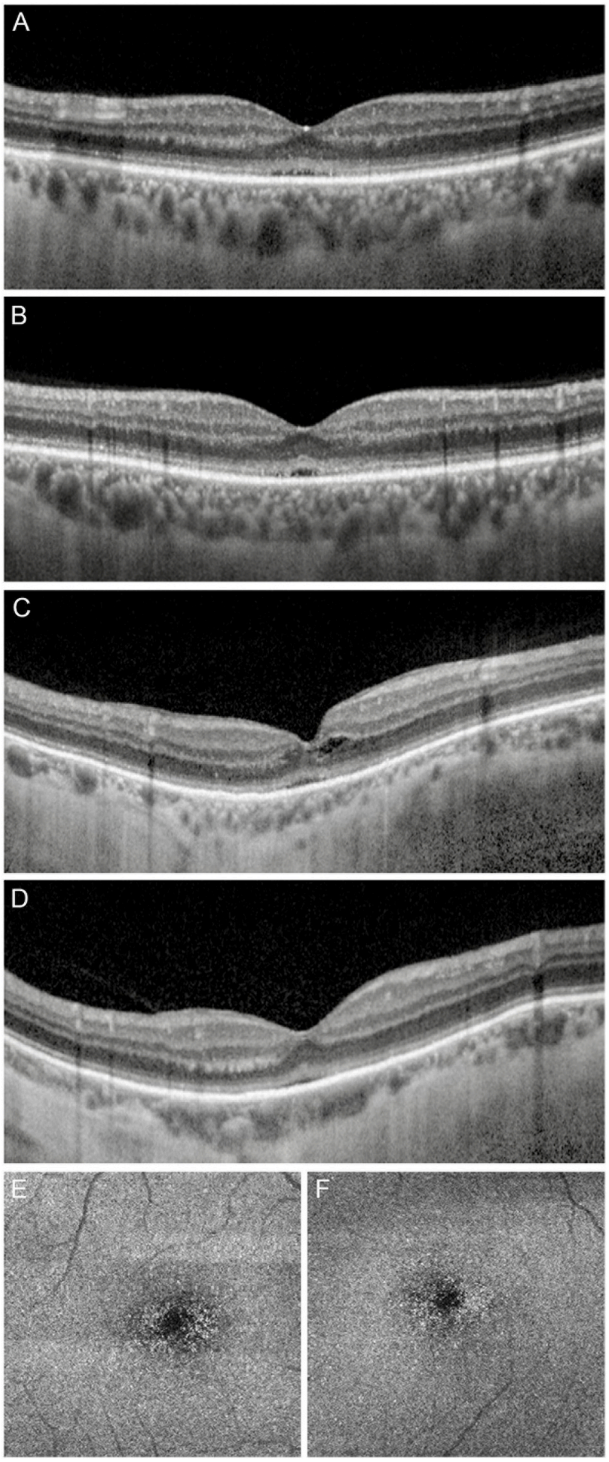

Fig. 2. Optical Coherence Tomography (OCT) in Occult Macular Dystrophy. Vertical radial scans centered on the fovea of proband 1 right (A) and left eye (B) and proband 2 right (C) and left eye (D) demonstrate that the cone interdigitation zone is extinguished and the ellipsoid zone (EZ) is thinner in the entire region, except the fovea. At the foveal region, the EZ appears blurred and dome-shaped with a small cavitation in the region of photoreceptor outer segment. The retinal pigment epithelium (RPE) is well preserved in the entire region. A tractional lamellar macular hole is present in P2 right eye (C). *En-face* OCT slabs at the level of the ellipsoid zone of proband 1 (E, F) confirm the loss of foveal photoreceptors.

examination was counting fingers in both eyes. P2 reported a 20-year history of mild but progressive worsening of visual function and her BCVA was 1/10 in RE and 2/10 in LE. P2 LE was affected by nuclear cataract. Both probands had two children (P1 had two daughters, P2 had one son and one daughter): all had a BCVA of 10/10 in both eyes and a normal macula on fundoscopy and OCT.

3.2. Imaging features

Fundus examination of the posterior pole and retinal periphery was normal in both sisters (Fig. 1A, C, E, G). A faint increased BAF signal at the fovea was noted in all eyes, especially in P1 LE and P2 RE (Fig. 1B, D, F, H); specularly, IRAF imaging demonstrated a foveal hypo-autofluorescence.

The apparently normal fundus contrasted with the alterations on structural OCT, which consisted mainly in a disorganization and thinning of the photoreceptor layer in the entire macular region. In more detail, outer retinal structures displayed the typical features of OMD in all four eyes: the cone interdigitation zone (IZ) extinguished and the ellipsoid zone (EZ) thinned in the entire region; at the foveal region, the EZ appeared blurred and dome-shaped with a small cavitation in the region of photoreceptor outer segments, a typical feature of cone dysfunction syndromes. The RPE-Bruch's membrane complex was intact in the entire region (Fig. 2A, B, C, D). All eyes may be defined as Stage IIb according to the clinical classification proposed by Nakamura.[14] Moreover, P2 RE had a concomitant lamellar macular hole characterized by a "schisis-like" appearance in the outer nuclear layer, which was categorized as tractional due to the presence of an epiretinal membrane (Fig. 2C). E*n-face* SS-OCT slabs remarkably enhanced the loss of foveal photoreceptors and the alterations of the EZ parafoveally (Fig. 2E and F).

3.3. Electrophysiological tests

The ffERG was normal for rod and cone responses in both eyes of both probands. On the other hand, mfERG displayed severely altered cone responses in the macular area in both eyes of P2 and in LE of P1; cone responses resulted normal in RE of P1. Surprisingly, PEV and pERG demonstrated reduced amplitude and increased latency of P100 and P50 in both eyes of P1.

3.4. OCT Angiography

Quantitative SS-OCTA analysis demonstrated a rarefaction of retinal capillary plexuses in the two probands affected by OMD (Fig. 3): vessel density at SCP and DCP were significantly lower when compared to healthy eyes (43% [42,2–44,4] vs 45,6% [44,9–45,8] and 41,7% [40,8–42,1] vs 42,8% [42,5–45,8]; all P-values $< .05$); moreover, FAZ area at DCP was larger than in controls (0,76 mm^2 [0,69–0,91] vs 0,35 mm^2 [0,33–0,42]; P-value $< .05$) while the difference was not significant at superficial capillary plexus (0,31 mm^2 [0,25–0,36] vs 0,25 mm^2 [0,22–0,26]; P-value $> .05$). On the other hand, choriocapillaris vessel density (61% [60,6–62,3] vs 62,3% [60,8–62]; P-value $> .05$) and flow voids number (2216 [2111–2397] vs 2196 [2045–2266]; P-value $> .05$) did not differ significantly from control eyes.

3.5. Genetic testing

Genetic analysis in P1 and P2 found the heterozygous missense c.2873G > C, p.Arg958Pro variant in the *RP1L1* gene. The *RP1L1* c.2873G > C variant has an allelic frequency of 0,000004% (gnomAD general population database) and had never been described in association with OMD. The variant co-segregated with the disease phenotype in the two affected sisters and a different missense at the same codon c.2873G > A, p.Arg958His in a case of OMD was reported by Davidson et al.[15] Given that functional studies on *RP1L1* protein are lacking, we used two in silico algorithms to assess the pathogenic potential of the

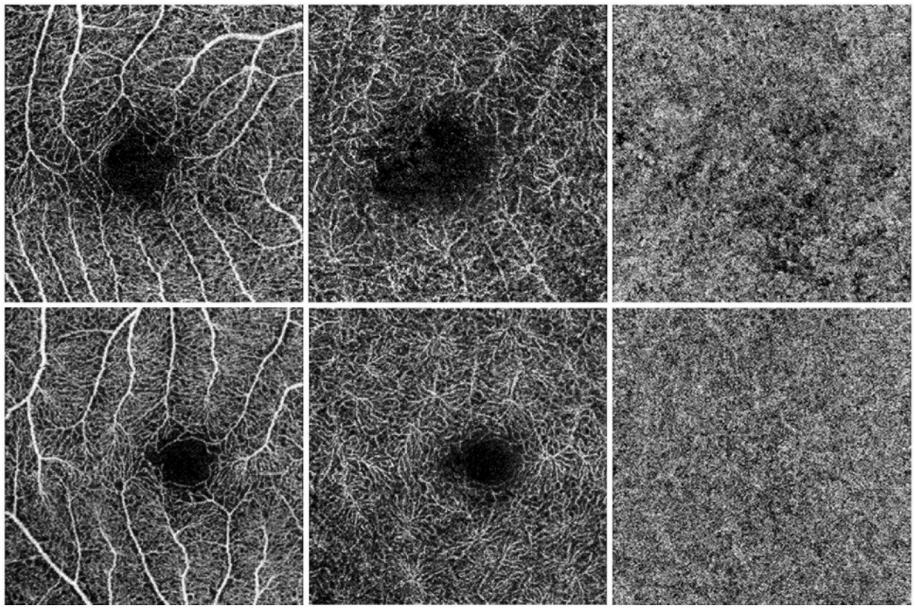

Fig. 3. Optical Coherence Tomography Angiography (OCTA) in Occult Macular Dystrophy. Reconstructions of superficial capillary plexus (first column), deep capillary plexus (middle column) and choriocapillaris (last column) of proband 2 (upper row) in comparison with a healthy subject (bottom row).

identified variant. The substitution of arginine with proline in position 958 of *RP1L1* protein was regarded as moderately radical according to Grantham score (103) while PolyPhen2 classified the amino acidic substitution as probably damaging. Based on ACMG criteria, the p. Arg958Pro substitution was therefore classified as a variant of unknown significance (VUS).

4. Discussion

In this report we describe two Italian sisters affected by OMD and harboring a novel missense c.2873G > C, p.Arg958Pro *RP1L1* variant. Both probands reported a history of progressive loss of central vision without relevant fundoscopic findings but with OCT alterations compatible with middle stages of OMD. However, while P2 had 20 years of disease progression and a BCVA of 1/10 in RE and 2/10 in LE, P1 had counting fingers BCVA in both eyes with only 4 years of disease progression and a mild disruption of outer retinal structures on OCT. This presentation contrasts with previous reports, which highlight that in OMD the severity of visual acuity worsening correlates with the duration of disease and that in most cases the decline stops when BCVA reaches 1/10[2,7,14]. In our opinion, the altered PEV and pERG in both eyes of P1 may explain its disproportionately decreased visual acuity, due to a damage to ganglion cells and central optic pathways. However, the reason for these findings (which were not present in the sister) remains unclear: she had no systemic diseases (apart from arterial hypertension treated with oral medications) or other ocular conditions (her intraocular pressure was normal) and her brain magnetic resonance was completely normal.

Although the identified substitution does not alter any domain of the protein and is classified as a VUS according to ACMG criteria, we hypothesize that it may be considered the causative variant on the basis of co-segregation, in silico prediction of the substitution damaging potential (moderately radical on the structure and probably damaging the function) and a previous report of a different substitution at codon 958 in association with OMD.[15] Moreover, in 2016 Fujinami et al. demonstrated that among patients presenting with occult macular dysfunction syndrome, those with a microstructural phenotype of photoreceptor damage featuring both EZ blurring and IZ absence generally have hereditary OMD caused by pathogenic variants, such in our two probands.[16] Assessment of the clinical significance of the identified novel c.2873G > C variant would require additional data, such as genetic testing of additional family members. Offspring was not tested because asymptomatic and devoid of OCT alterations while the identified variant was classified as of unknown significance and the disease has incomplete penetrance; on the other hand, the affected grandmother and father were already deceased on the day of the examination. However, future studies should consider that the age at onset of OMD spans from 6 to 60 years old[17] and genetic testing in asymptomatic individuals at risk would allow the validation of subclinical disease biomarkers, as it happens for other inherited retinal dystrophies.[18]

We also used SS-OCT and SS-OCTA to investigate structural and vascular alterations occurring in OMD. *En-face* SS-OCT revealed a loss of foveal photoreceptors, in accordance with previous studies[19] and with OMD pathogenesis, since the axoneme is responsible for the homeostasis of the outer segments. On the other hand, our first report on quantitative OCTA data suggests that OMD is characterized by an impairment of intraretinal capillary plexuses while choriocapillaris blood flow is relatively spared and warrants further research on larger cohorts to investigate this lack of knowledge.

In conclusion, the rare c.2873G > C, p.Arg958Pro missense in the *RP1L1* gene may actually be the causative variant explaining the OMD phenotype observed in two Italian sisters. Moreover, our quantitative OCTA data show a rarefaction of superficial and deep capillary plexus in OMD.

Patient consent

Signed consent to use medical data for research purposes was obtained from all participants. The research was approved by the local ethical committee (MIRD) and adhered to tenets of Helsinki declaration.

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Conflicts of interest

FB is consultant for: Alcon (Fort Worth, Texas, USA), Alimera Sciences (Alpharetta, Georgia, USA), Allergan Inc (Irvine, California, USA), Farmila-Thea (Clermont-Ferrand, France), Bayer Shering-Pharma

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

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