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Retinal Phenotype of Patients with CLRN1-Associated Usher 3A Syndrome in French Light4Deaf Cohort

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Purpose. Biallelic variants in CLRN1 are responsible for Usher syndrome 3A and nonsyndromic rod–cone dystrophy (RCD). Retinal findings in Usher syndrome 3A have not been well defined. We report the detailed phenotypic description of RCD associated with CLRN1 variants in a prospective cohort.

Methods. Patients were clinically investigated at the National Reference Center for rare ocular diseases at the Quinze-Vingts Hospital, Paris, France. Best-corrected visual acuity (BCVA) tests, Goldmann perimetry, full-field electroretinography (ffERG), retinal photography, near-infrared reflectance, short-wavelength and near-infrared autofluorescence, and optical coherence tomography (OCT) were performed for all patients.

Results. Four patients from four unrelated families were recruited. Mean follow-up was 11 years for three patients, and only baseline data were available for one subject. Median BCVA at baseline was 0.2 logMAR (range, 0.3–0). ffERG responses were undetectable in all subjects. The III4e isopter of the Goldmann visual field was constricted to 10°. The retinal phenotype was consistent in all patients: small whitish granular areas were organized in a network pattern around the macula and in the midperiphery. OCT showed intraretinal microcysts in all patients. Upon follow-up, all patients experienced a progressive BCVA loss and further visual field constriction. Four distinct pathogenic variants were identified in our patients: two missense (c.144T>G, p.(Asn48Lys) and c.368C>A, p.(Ala123Asp)) and two frameshift variants (c.176del, p.(Gly59Valfs*13) and c.230dup, p.(Ala78Serfs*52)).

Conclusions. RCD in Usher 3A syndrome has some distinctive features. It is a severe photoreceptor dystrophy with whitish granular posterior pole appearance and cystic maculopathy.

Keywords: Usher 3 syndrome, retinal degeneration, natural history
Retinal Phenotype in Usher 3A Syndrome

and RCD. Pathogenic variants in MYO7A (MIM *276903), encoding myosin VIIA, an unconventional myosin linked with intracellular movement, are the most common cause of USH1 and are responsible not only for USH1B (MIM *276900) but also for non-syndromic SNHL.

Usher syndrome type 2 (USH2) is characterized by RCD and variable severity (mild to severe) of congenital SNHL without vestibular dysfunction. Bisallelic variants in USH2A (MIM *608400), encoding usherin, a protein involved in periliminar membrane complex in photoreceptors and in interstereocilia ankle in the inner ear sensory cells, are the leading cause of USH2 (underlying USH2A, MIM *276901) and are also responsible for non-syndromic autosomal recessive RCD (RP39, MIM *613809).

Usher syndrome type 3 (USH3) was defined on the basis of audiological features in the 1970 and 1980s. It is characterized by progressive postlingual SNHL and variable (none to progressive) vestibular dysfunction. Language and motor development are normal in most subjects. Onset of progressive SNHL begins in the first decade of life. About 50% of patients are profoundly deaf by their 40s.

Pathogenic variants in two genes have been associated with USH3: CLRN1 (MIM *606597), which encodes clarin-1, a four transmembrane-domain protein with a possible role in sensory synapses (involved in USH3A, MIM *276902), and HARS1 (MIM *142810), encoding histidyl-tRNA synthetase 1, catalyzing the ligation of histidine to its cognate tRNA (involved in USH3B, MIM *614504). Bisallelic variants in CLRN1 have also been reported in non-syndromic RCD (RP61, MIM *614180). Patients with HARS1 variants are rare, and all present syndromic manifestations including cochleo-vestibular involvement, axonal peripheral neuropathy consistent with Charcot–Marie–Tooth 2W syndrome (MIM *616625), and/or ataxia.

USH3A associated with CLRN1 mutations is uncommon. It represents only 2% of all patients with Usher syndromes in non-Finnish European populations, but it causes 40% to 45% of the Usher cases in Finland. The most common pathogenic variant in the Finnish population is c.528 T>G, p.(Tyr176*), suggesting a founder effect. USH3A is also more common (about 10% of the Usher patients) in Israeli and Palestinian populations. A founder pathogenic variant of CLRN1 is also reported in Ashkenazi Jews (c.144T>G, p.(Asn48Lys)).

There are only a few reports describing the ophthalmological features of CLRN1-related USH3A due to the rarity of this disorder. A large study cohort that included 90 Finnish patients demonstrated that the clinical course of CLRN1-related syndromic RCD due to the founder variant c.528 T>G, p.(Tyr176*) was similar to that for USH1 and USH2 individuals with non-specific retinal imaging findings. In a series of 10 non-Finnish USH3A patients, RCD was more severe and rapidly progressive compared to patients harboring USH2A gene defects. The cone mosaic assessed by adaptive optics was normal within the four central retinal degrees with spared ellipsoid zone (EZ) in three USH3A patients. In Ashkenazi Jewish patients harboring the c.144T>G, p.(Asn48Lys) founder pathogenic CLRN1 variant, night blindness and visual field constriction appeared early in life (early childhood to late teens) in one third of patients prior to the auditory symptoms. One third of patients were legally blind (i.e., below 1.3 logMAR) by their 50s.

The aim of our study was to report the retinal phenotype of a series of French patients with CLRN1-related USH3A and to compare it with previously reported European and Israeli/Palestinian cohorts.

**Patients and Methods**

**Clinical Investigation**

Patients with a molecular diagnosis of USH were included in a large prospective cohort study of USH syndromes (Light4Deaf, NCT04665726, www.clinicaltrials.gov). Patients were clinically investigated at the National Reference Center for Rare Retinal Diseases of Quinze-Vingts Hospital, Paris, France. Ophthalmic examination was performed as previously described. Prior to testing, written informed consent, approved by the Comité de Protection des Personnes, was obtained from each study participant or their parents. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local ethics committee. The study was performed in compliance with Oviedo Convention and Treaty of Lisbon.

**Genetic Analysis**

For most patients, a multiplex amplicon panel (Fluidigm Access Array; Fluidigm Corporate, San Francisco, CA, USA) was applied as previously described to analyze all coding and noncoding exons of the 10 USH genes: MYO7A, myosin VIIA (MIM *276903); USH1C, harmonin (MIM *605242); CDH23, cadherin 23 (MIM *605516); PCDH15, protocadherin 15 (MIM *605514); USH1G, sans (MIM *607696); CIB2, calcium- and integrin-binding 2 (MIM *605564); USH2A, usherin (MIM *608400); ADGRV1, adhesion G protein-coupled receptor V1 (MIM *602851); WHRN, whirlin (MIM *607928); CLRN1, clarin-1 (MIM *606397); and the USH2 modifier gene PDZD7, PDZ domain-containing 7 (MIM *612971). For some individuals, direct Sanger sequencing of coding sequences and flanking intronic regions of CLRN1 were performed.

Family segregation of the identified variants was performed when possible. All patients harboring biallelic pathogenic variants in CLRN1 were subsequently included in this study.

**Clinical Data Collection**

Clinical data were retrospectively collected from medical records. These included sex, age at time of diagnosis and examination, personal or family history, symptoms, best-corrected visual acuity (BCVA) assessed by the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, refractive errors, slit-lamp biomicroscopy, Lanthony D-15 panel, Goldmann kinetic visual fields (VFVs; Haag-Streit, Koeniz, Switzerland), full-field electroretinogram (ffERG; ColorDome, Diagnosys, Cambridge, UK), spectral-domain optical coherence tomography (SD-OCT), fundus photography, and short-wavelength fundus autofluorescence (SWAF) and near-infrared fundus autofluorescence (NIRAF) imaging (SPECTRALIS HRA-OCT; Heidelberg Engineering, Heidelberg, Germany). Structural changes were evaluated using Heidelberg Eye Explorer, version 1.9.10.0 (Heidelberg Engineering). We measured the horizontal and vertical diameters of the remaining central retina on three retinal imaging modalities: first, the preserved ellipsoid zone on SD-OCT scans passing through the fovea; second, the ring of increased autofluorescence on SWAF (SWAF-RIA); and, third, the area of preserved autofluorescence on NIRAF (NIRAF-
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APA). For SD-OCT, the follow-up setting for horizontal and vertical images was used to ensure consistent measurements in serial images.

**Statistical Analysis**

Statistical analyses were performed using SPSS Statistics 21.0 (IBM, Chicago, IL, USA), with \( P \leq 0.05 \) considered statistically significant. We applied the Wilcoxon signed-rank test for BCVA, NIRAF-APA, and EZ diameters to evaluate the agreement between eyes. We then averaged the two values for further analyses. The rate of annual progression for NIRAF-APA and EZ diameters was calculated as follows: (value on first visit – value on last visit)/(years of follow-up).

**RESULTS**

We identified four patients (two males and two females) with \( CLRN1 \)-related USH3A in our Usher database comprised of 289 index patients in total, leading to a prevalence of 1.4% in our USH cohort. Three families were of African ancestry: F65 (CIC00088) from Algeria, F196 (CIC03005), and F2792 (CIC05477) from Ivory Coast. F937 (CIC02644) was of Ashkenazi Jewish descent.

The clinical data at first assessment are summarized in Table 1. Night blindness was the first symptom of disease, appearing at 10 to 12 years of age in all patients. The mean age at the initial examination was 18.5 years. The mean BCVA was 0.2 \( \pm \) 0.08 logMAR. The Goldmann visual field at the III4e/V4e target was severely constricted in all patients. Subjects CIC03005 and CIC05477 had a residual temporal peripheral crescent of visual field at V4e target. Static perimetry showed a ring-shaped scotoma with variable preservation of foveal threshold. Color vision tests showed normal results (CIC02644), multiple without-axis errors (CIC00088 and CIC05477), or tritan axis dyschromatopsia (CIC03005). Full-field ERG was undetectable in all patients.

At slit-lamp examination, patients CIC00088 and CIC02644 had a posterior subcapsular cataract in each eye. Multimodal retinal imaging is presented in Figure 1. Fundus findings were typical of severe RP: waxy pallor of the optic disc, retinal vasculature narrowing, and pigmentary changes (Figs. 1A–1D). However, all patients presented an unusual whitish granularity of the midperipheral retina as a distinctive feature (Figs. 1A–1D, Supplementary Fig. S1). SWAF, NIRAF, and SD-OCT findings were typical of RP.

Long-term follow-up data were available for three patients (Table 2, Figs. 1A–1D, Figs. 2A–2C). BCVA was stable until 25 years of age and then gradually decreased (Fig. 2A). The peripheral visual field rapidly became constricted with relative preservation of central vision (Table 2). EZ and NIRAF-APA diameters decreased altogether but not completely in parallel (Figs. 2B, 2C). Cystic macular changes were observed in all patients on initial examination. Patient CIC05477 was assessed only once at 19 years of age. Unfortunately, the patient did not return for follow-up visits.

We identified four \( CLRN1 \) variants (Fig. 3A, Supplementary Table S2): one duplication (c.230dup, p.(Ala78Serfs*52)); one deletion (c.176del, p.(Gly59Valfs*13)); and two missense (c.368C>A, p.(Ala123Asp)) and c.144T>G, p.(Asn48Lys)). All variants had been previously reported and were classified as pathogenic according to the American College of Medical Genetics and Genomics guidelines. Patient CIC02644 (Ashkenazi Jewish) was a compound heterozygous for c.176del, p.(Gly59Valfs*13) and c.144T>G, p.(Asn48Lys), the latter being a founder. The remaining three patients carried homozygous changes: patient CIC00088 carried the homozygous duplication (c.230dup, p.(Ala78Serfs*52)), whereas the other two patients (CIC03005 and CIC05477) carried the homozygous missense variant (c.368C>A, p.(Ala123Asp)). Two patients reported a history of parental consanguinity (CIC00088 and CIC05477). Parents of CIC03005 were born in the same village from Ivory Coast, but there was no identifiable parental consanguinity (Fig. 3B).

**DISCUSSION**

\( CLRN1 \)-related USH3A is relatively rare in non-Finnish European populations. It accounts for 1.4% of our cohort of Usher patients. At present, there are only a few reports of audiological and retinal phenotypes in USH3A.

On initial examination, the RCD was severe in our patients. Despite a relatively preserved visual acuity, all patients had abnormal color vision with tritan or anarchic color vision defects. Visual field was severely constricted from the teen years; if initially present, the remaining temporal crescent disappeared within a decade on Goldmann perimetry. None of the studied patients had detectable fERG responses at presentation in their teen years.

The functional data of our patients were similar to those of previously reported series. On initial examination, the RCD was severe in our patients. Despite a relatively preserved visual acuity, all patients had abnormal color vision with tritan or anarchic color vision defects. Visual field was severely constricted from the teen years; if initially present, the remaining temporal crescent disappeared within a decade on Goldmann perimetry. None of the studied patients had detectable fERG responses at presentation in their teen years.

The severity of retinal dysfunction in our cohort was mirrored by structural alterations. Only a small foveal island of outer retina was preserved. SWAF-RIA in the posterior pole was present at the initial visit in two of the four subjects (CIC00088 and CIC05477). It was faint and rapidly replaced by patches of hypo-autofluorescence due to rapid outer retinalatrophy. Poor outer retinal preservation was also apparent on the NIRAF imaging, with only a small remaining area of normal autofluorescence that rapidly diminished (e.g., – 107 \( \mu \)m/y on average for CIC02644). NIRAF alterations have been reported to precede SWAF alterations and have been found to better correlate with SD-OCT findings in RP. Indeed, the borders of NIRAF-APA usually coincide with the relative preservation of the EZ on SD-OCT. We recog-
## Table 1. Initial Clinical Features of Patients with CLRN1-Related USH3A

<table>
<thead>
<tr>
<th>Patient, Sex, Age at First Examination (y)</th>
<th>Symptoms</th>
<th>BCVA (logMAR) and Refraction</th>
<th>Anterior Segment</th>
<th>Fundus</th>
<th>SWAF</th>
<th>NIRAF</th>
<th>OCT</th>
<th>SP</th>
<th>MD/FT (RE/LE)</th>
<th>Color Vision</th>
<th>fERG</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIC00088, M, Algerian 22</td>
<td>Hearing difficulties at 20 y; night blindness from 10 y</td>
<td>RE: 0.5; –1.50 (-1.25); 20° LE: 0.2; +1.50 (-1.25); 150°</td>
<td>Anterior polar cataract surgery at 54 y</td>
<td>Waxy disc pallor, vessel narrowing</td>
<td>Whitish retina at posterior pole and whitish granularity of the midperipheral retina</td>
<td>Bone spicules-like, paravascular pigmentary cuffs, coarse pigment clumps in midperiphery</td>
<td>Small central RIA, patchy paravascular and midperipheral hypoAF</td>
<td>Small foveolar area of preserved EZ, outer retinal layers disorganization outside</td>
<td>III4e constricted to 5° OU</td>
<td>17.9/15 16.7/22</td>
<td>Severe defect without axis (RE) Normal (LE)</td>
<td>Undetectable</td>
</tr>
<tr>
<td>CIC02644, F, Ashkenazi Jewish 16</td>
<td>Mild hearing loss at 5 y; night blindness at 12 y</td>
<td>RE: 0; +1.75 (-2.0); 45° LE: 0.1; +1.75 (-2.0); 135°</td>
<td>Posterior subcapsular opacities OU</td>
<td>Waxy disc pallor, vessel narrowing</td>
<td>Normal aspect of the macula Midperipheral whitish granularity, spotted marble appearance Bone spicules, paravascular pigmentary cuffs, sparse pigmentary clumps in midperiphery</td>
<td>Narrow peripheral RIA, patchy paravascular and midperipheral hypoAF, some punched-out AF loss in midperiphery</td>
<td>Foveal area of preserved EZ, disorganization of the outer retinal layers replaced by hyper-reflective dots outside the fovea Microcystic maculopathy (in INL and OPL)</td>
<td>KP: III4e constricted to 10°</td>
<td>17.3/29 16.8/32</td>
<td>Normal (both eyes)</td>
<td>Undetectable</td>
<td>—</td>
</tr>
</tbody>
</table>
### Table 1. Continued

<table>
<thead>
<tr>
<th>Patient, Sex, Ancestry</th>
<th>Age at First Examination (y)</th>
<th>Symptoms</th>
<th>BCVA (logMAR) and Refraction</th>
<th>Anterior Segment Fundus</th>
<th>SWAF</th>
<th>NIRAF</th>
<th>OCT</th>
<th>KP</th>
<th>SP MD/FT (RE/LE)</th>
<th>Color Vision</th>
<th>fERG</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIC03005, F, Ivory Coast</td>
<td>17</td>
<td>Night blindness from 12 y</td>
<td>RE: 0.4; +3.0 (-0.75); 135° LE: 0.6; +2.0 (-0.75); 55°</td>
<td>Normal</td>
<td>Arteriolar narrowing Yellowish macula, microcystic maculopathy Whitish granularity of midperipheral retina, spotted marble appearance Bone spicule-like and paravascular pigmentary cuffs</td>
<td>SWAF: posterior pole globally hypoAF with foveolar hyperAF Patchy midperipheral hypoAF</td>
<td>Small central APA</td>
<td>Small foveolar area of preserved EZ, disorganized outer retinal layers outside the fovea Microcystic maculopathy ILM thickening and irregularity</td>
<td>V4e constricted to 60° + temporal crescent OU</td>
<td>18.8/15 24.3/12</td>
<td>Tritan defect (both eyes)</td>
<td>Undetectable Seizures, valproate treatment</td>
</tr>
<tr>
<td>CIC05477, M, Ivory Coast</td>
<td>20</td>
<td>Progressive hearing loss starting at 7 y; night blindness at 10 y</td>
<td>RE: 0.1; +1.75 (-1.75); 15° LE: 0.2; +1.75 (-175); 170°</td>
<td>Normal</td>
<td>Arteriolar narrowing Normal aspect of the macula, midperipheral whitish granularity, spotted marble appearance Few bone spicule-like pigments in midperiphery</td>
<td>SWAF: well-circumscribed perifoveal RIA, patchy midperipheral hypoAF</td>
<td>Foveal APA</td>
<td>Foveal area of preserved EZ, disorganization of the outer retinal layers replaced by hyper-reflective dots outside the foveal region Microcystic maculopathy (in INL and OPL)</td>
<td>KP: V4e constricted to 20° + temporal crescent OU</td>
<td>21.2/22 21.6/21</td>
<td>Multiple errors without axis (both eyes)</td>
<td>Undetectable Audiogram: severe middle and high-frequency loss MD REar: −100 dB MD LEar: −75 dB Normal speech Hearing aids from 7 y, cochlear implant at 21 y</td>
</tr>
</tbody>
</table>
Retinal Phenotype in Usher 3A Syndrome

**FIGURE 1.** Multimodal retinal imaging in patients with CLRN1 USH3A at first assessment and follow-up. Color fundus photography, SWAF, NIRAF, near-infrared reflectance (NIRR), and SD-OCT horizontal and vertical cross-section images. (A) Patient CIC00088, (B) Patient CIC2644, (C) Patient CIC03005, and (D) Patient CIC05477. All patients had classical signs of RP on fundus photography: optic disc pallor, arterial narrowing, and pigmentary changes. The distinctive feature was a granularity and whitishness of retina, present in all patients and prominent in CIC2644 and CIC03005. Only patient CIC05477 had a typical ring of increased autofluorescence on SWAF. The remaining cases show ill-defined perifoveal changes with hyper-autofluorescence (CIC03005) or iso-autofluorescence (CIC00088). On follow-up, hypo-autofluorescent zones increased. NIRAF showed a progressive narrowing of the area of preserved autofluorescence (APA) in the posterior pole, seen in CIC05477, where it was no longer present in patient CIC00088 at the last visit. Patient CIC03005 had some black dots in APA suggestive of foveal outer retinal disruption. SD-OCT revealed various degrees of outer nuclear layer, EZ, and RPE preservation. There were multiple hyperreflective dots in the subretinal space, especially in patients with prominent whitishness of the retina (CIC2644 and CIC03005). All patients had microcystic macular changes. Patient CIC00088 developed an epimacular membrane. CF, counting fingers.

<table>
<thead>
<tr>
<th>Fundus</th>
<th>SWAF</th>
<th>NIRAF</th>
<th>NIRR</th>
<th>SD-OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>22 y.o.</td>
<td>CIC00088 Baseline, LogMAR 0.5</td>
<td>NA</td>
<td>Follow-up, CF</td>
<td></td>
</tr>
<tr>
<td>27 y.o.</td>
<td>CIC2644 Baseline, LogMAR 0.1</td>
<td>Follow-up, LogMAR 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34 y.o.</td>
<td>CIC03005 Baseline, LogMAR 0.4</td>
<td>Follow-up, LogMAR 0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19 y.o.</td>
<td>CIC05477 Baseline only, LogMAR 0.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>Follow-Up Period (y)</td>
<td>BCVA at Last Visit (logMAR), RE/LE; Age</td>
<td>Visual Field Progression</td>
<td>Color Vision at Last Visit</td>
</tr>
<tr>
<td>----------</td>
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<td>--------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>CIC00088</td>
<td>12</td>
<td>CF/0.6; 34 y</td>
<td>Temporal crescents dimished and finally disappeared at the age 25. The central V4e isopter reduction was only of 5° over the 12 y.</td>
<td>Multiple without-axis errors appeared in RE, whereas the LE color vision remained unchanged.</td>
</tr>
<tr>
<td>CIC02644</td>
<td>11</td>
<td>0.2/0.3; 27 y</td>
<td>Progressive loss and finally disappearance (at 24 y) of the temporal peripheral crescents. The extent of the central V4e isopter did not change. Mean defect was slowly worsening, with a loss of 3 dB in the RE (~18%) and 2 dB in the LE (~12%) compared with the first assessment.</td>
<td>Color vision was first normal but a tritan defect became obvious.</td>
</tr>
<tr>
<td>CIC03005</td>
<td>9</td>
<td>0.5/0.5; 30 y</td>
<td>Kinetic visual field was normal under the age of 20 y. Temporal peripheral crescents disappeared at 24 y, and the isopter V4e became constricted to 10° at 25 y.</td>
<td>A tritan color vision defect was present at the first assessment and remained unchanged.</td>
</tr>
</tbody>
</table>
Changes, however, the difficulty of obtaining reliable NIRAF-APA measurements in late stages of RCD when NIRAF-APA limits become ill defined (e.g., in patient CIC03005) (Fig. 1C). This may explain some discrepancies between observed EZ and NIRAF-APA parameters. Previously published OCT data in CLRN1–USH3A patients documented the progression of retinal thinning with age. Our study takes into account the EZ diameter, as it probably correlated better with the functional parameters.35,36 The loss of outer retinal layers on SD-OCT was in line with SWAF and NIRAF findings (e.g., ~76 μm/y on average for CIC02644). All of our patients had cystic macular changes in the course of our monitoring.

Of note, we found an unusual phenotype of CLRN1 RCD consisting of a whitish granularity of the midperipheral retina giving a spotted marbled appearance that was present in all four patients. This finding was correlated with the presence of numerous hyperreflective dots in the subretinal space on SD-OCT, especially in patients with prominent whitishness of the retina (Figs. 1B, 1C; Supplementary Fig. 1). We hypothesize that it might be debris of degenerating photoreceptors and/or retinal pigment epithelium. Alternatively, we cannot exclude the possibility that the whitishness could be a general feature of RCD at this time point. Indeed, our study involved patients of nearly the same age. This finding was not reported in the description of the Finnish or Ashkenazi Jewish cohorts or on evident on published fundus photographs.

Patients CIC03005 and CIC05477, both from Ivory Coast ancestry and harboring the same homozygous variant c.368C>A, p.(Ala123Asp), were initially assessed by the end of their 20s. Foveal EZ and RPE were already disorganized in CIC03005 at 17 years of age, whereas there was a well-preserved foveal island of outer retina in CIC05477 at 19 years of age. These data suggest some phenotypic variability in CLRN1-related RCD, also common for other inherited retinal disorders. This pathogenic variant was first reported in a French Canadian patient.33 The retinal phenotype of this patient has not been published. Patients CIC03005 and CIC05477 did not report any common ancestor and were born in different Ivory Coast districts; however, a founder effect cannot be excluded considering an enrichment of this allele in the French Light4Deaf cohort.

The gene coding for clarin-1, CLRN1, is a four exon gene located on chromosome 3q25.1. Multiple transcripts have been reported for this gene, but the major mRNA transcript variant, comprised of exons 0, 2, and 3 (RefSeq NM_174878.3), is expressed in the retina and encodes a protein of 232 amino acid residues (clarin-1 isoform a). A minor mRNA transcript, comprised of exons 1, 2, 3a, and 3b (RefSeq NM_052995.2), encodes a protein of 120 amino acid residues (clarin-1 isoform c). Clarin-1 is a small transmembrane protein belonging to the tetraspanin and claudin families. A major retinal isoform a is predicted to be comprised of four transmembrane domains and is glycosylated at Asn48 residue. Multiple sequence alignment algorithms have depicted a close similarity of clarin-1 with the voltage-dependent calcium channel gamma-2 subunit, CACNG2 (MIM #602911), also known as stargazin. The putative function of clarin-1 in cells was deduced from this resemblance. Adato and colleagues speculated that clarin-1 may have a role in the structure and function of the ribbon synapse and in protein–protein bridging in the synaptic cleft. The protein is localized ubiquitously with higher levels in the spiral ganglion cells of the inner ear, in the olfactory epithelium, in the testis and in the retina. RNA in situ hybridization and single-cell RNA sequencing demonstrated the localization of mRNA transcripts in the inner nuclear layer and more precisely in the Müller cells for both mouse and human retinas.

The mechanism of CLRN1-related RCD remains unknown because there is no mouse model of clarin-1–associated RCD: knock-in and knock-out mice for Clrn1 do not produce any detectable retinal disease. Plausible hypotheses for the discrepancy between human and mouse phenotypes have been formulated. First, it is possible that CLRN1 expression does not follow the same spatial and temporal pattern across mammalian species. For example, there is a developmental downregulation of Clrn1 expression in mouse retina and not in human. The absence of well-developed, actin-filled ciliary processes at the apical region of inner segments of mouse photoreceptors represents a second possibility. As the Usher syndromes are a part of the ciliopathy spectrum, clarin-1 could take part in the structure or and function of the photoreceptor connecting cilium; unfortunately, limited experimental data are available to support this hypothesis. A better understanding of the retinal localization and function of clarin-1 would be crucial for...
translational research, such as developing gene replacement protocols, as CLRN1 is a small gene, suitable for viral delivery, unlike other USH-related genes.45

In contrast, the inner ear dysfunction phenotype could be obtained and rescued in mutant mice. Clarin-1 is essential for the morphogenesis and maintenance of the stereocilia hair bundle in auditory hair cells.46 At the molecular level, the protein is supposed to be necessary for the regulation of actin cytoskeleton and of the F-actin core of the stereocilia hair cell.47,48 The stereocilia in Clrn1 knock-out mice are poorly developed and disorganized.43,49 Viral-based gene augmentation therapy prevents early-onset deafness and limits the progression of SNHL in this model.48

Two founder pathogenic variants have been reported and account for most cases: c.528T>G, p.(Tyr176*) in the Finnish population52 and c.144T>G, p.(Asn48Lys) in Ashkenazi Jews.17 Thirty-eight other disease-causing variants in CLRN1 (Fig. 3A) have been reported to date (http://www.hgmd.cf.ac.uk/; https://databases.lovd.nl/shared/variants/CLRN1/unique), with missense and nonsense pathogenic variants being the most frequent.44

Some genotype–phenotype associations related to CLRN1 variants have been described. Amino acid changes found in the intra- and extracellular loops are thought to be more damaging than non-polar amino acid substitutions within the transmembrane domains that could give rise to non-syndromic RP61.53 As our patients were selected on the basis of their clinical phenotype and their number was small, we cannot come to conclusions. Larger cohorts of patients in collaborative projects would be necessary to explore genotype–phenotype correlations.

In summary, we have described relatively severe and rapidly progressive CLRN1-associated RCD with a distinctive retinal granularity and whiteness not previously reported (Fig. 1, Supplementary Fig. S1). It accounts for 1.4% of the cases in our large French USH cohort. Although the variants in CLRN1 harbored by our patients have already been reported, the associated ocular phenotype has not.

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