Loss of Function of RIMS2 Causes a Syndromic Congenital Cone-Rod Synaptic Disease with Neurodevelopmental and Pancreatic Involvement

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Congenital cone-rod synaptic disorder (CRSD), also known as incomplete congenital stationary night blindness (iCSNB), is a non-progressive inherited retinal disease (IRD) characterized by night blindness, photophobia, and nystagmus, and distinctive electrotetroretinographic features. Here, we report bi-allelic RIMS2 variants in seven CRSD-affected individuals from four unrelated families. Apart from CRSD, neurodevelopmental disease was observed in all affected individuals, and abnormal glucose homeostasis was observed in the eldest affected individual. RIMS2 regulates synaptic membrane exocytosis. Data mining of human adult bulk and single-cell retinal transcriptional datasets revealed predominant expression in rod photoreceptors, and immunostaining demonstrated RIMS2 localization in the human retinal outer plexiform layer, Purkinje cells, and pancreatic islets. Additionally, nonsense variants were shown to result in truncated RIMS2 and decreased insulin secretion in mammalian cells. The identification of a syndromic stationary congenital IRD has a major impact on the differential diagnosis of syndromic congenital IRD, which has previously been exclusively linked with degenerative IRD.

Introduction

Retinal rod and cone cells are photosensitive neurons that possess ribbon synapses to provide rapid and sustained transmission of graded light responses to second-order neuron bipolar and horizontal cells that shape the visual message for the cortex.1 Genetic alterations affecting synaptic transmission from photoreceptors to bipolar cells manifest in congenital stationary night blindness (CSNB [MIM: PS310500]). It is a rare but also overlooked retinal disorder with an estimated prevalence of at least 1:40,000 (according to data drawn from a French cohort that included individuals confirmed to have the disease). This form of CSNB can be subdivided in complete (cCSNB) and incomplete (iCSNB) forms.2 Whereas cCSNB affects mainly proteins located post-synaptically at the dendritic tips of on-center bipolar cells (ON-bipolar cells), which are active when the light is on, iCSNB affects mainly proteins located at the synapse of photoreceptor cells.2 Affected individuals with iCSNB are characterized by the dysfunction of both off-center bipolar cells (OFF-bipolar), which are active when the light is off, and ON-bipolar cells as shown in the electroretinogram (ERG).2 Pathogenic variants in CACNA1F (MIM: 300110),4 CABP4 (MIM: 608965),5 and CACNA2D4 (MIM: 608171)6 were identified in affected individuals with iCSNB.2 Of note, in addition to the fact that phenotypic variability can lead to mildly progressive inherited retinal disorders (IRDs), stable low vision, nystagmus, and more importantly, photophobia might also be major symptoms in affected individuals with variants in CACNA1F, CABP4, and CACNA2D4.2

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and thus, the term iCSNB might be misleading. Therefore, we use the term congenital cone-rod synaptic disorder (CRSD).

The ocular presentation in infants might be reminiscent of Leber congenital amaurosis (LCA [MIM: PS204000]), a degenerative retinal disease that is a leading cause of childhood blindness; it has an estimated prevalence of 1:30,000. Occasionally, LCA can be the earliest manifestation of syndromic disease, such as ciliopathies, neuro-metabolic disorders, or tubulinopathies. ERG is a critical test for early differential diagnosis. ERG responses are undetectable, in keeping with the extremely severe rod and cone dysfunction in LCA. However in CRSD, ERG traces reveal generalized inner retinal dysfunction with both ON- and OFF-bipolar cell dysfunction: normal photoreceptor function manifests with an electronegative waveform in response to a bright flash under dark adaptation and with severely reduced and delayed light-adapted responses. However, ERG recordings might be challenging in young children, and this might contribute to a misdiagnosis of the ultra-rare CRSD as the more prevalent LCA.

Here, a genomic study of individuals with IRD but an unresolved diagnosis revealed bi-allelic loss-of-function variants in regulating synaptic membrane exocytosis 2 (RIMS2 [MIM: 606630]) in seven affected individuals with an initial diagnosis of CSNB or LCA and who were from four unrelated families of Senegalese, French, and Saudi Arabian origin. Consistent with a role of RIMS2 in regulating synaptic membrane exocytosis in the brain, pancreas, and photoreceptors and with its localization in human tissues, we demonstrated syndromic CRSD with neurodevelopmental and possible pancreatic involvement in individuals with bi-allelic mutations in RIMS2.

Materials and Methods

Subjects

This study involved seven affected subjects (three females and four males) with early-onset IRD and five healthy relatives from four unrelated families. Families 1 and 2 were studied at Imagine-Necker-Enfants Malades University Hospital. Affected individual III-3 of family 1 (F1:III-3 in Figure 1A) was also studied at the Centre Hospitalier National d’Ophthamologie des Quinze-Vingts. Family 3 was clinically studied at the Pediatric Ophthalmology Division at the Dhahran Eye Specialist Hospital in Dhahran, Saudi Arabia, and Family 4 was studied at the Centre Hospitalier National d’Ophthamologie des Quinze-Vingts. Family 1, referred for LCA, has an inbred Senegalese pedigree with several consanguinity loops and three affected individuals over two generations (two adult sisters and the child of one of them; the child was born to a first-cousin marriage). Family 2, referred for LCA, comprises a single affected individual and his two unrelated parents of French origin. Family 3 includes an affected sibling born to a first-cousin marriage and is from Saudi Arabia. Family 4, with a clinical diagnosis of CSNB, consists of a single affected individual originating from first-degree cousins; the mother is Franco-Senegalese, and the father is of Senegalese descent (Figure 1A). All individuals or legal representatives consented with the study, which received approval from the institutional review boards Comité de Protection des Personnes Ile de France II (Necker), Ile de France V (project number 06693, EudraCT number 2006-003474-44, 11 December 2006, Quinze-Vingts), and Ghent University Hospital Ethics Committee (B670201734438). Genomic DNA was extracted from peripheral blood by standard procedures.

Genetic Analysis

The notation of the variants was based on RIMS2 transcript GenBank: NM_00134848.1, corresponding to the variant X5 transcript for GenBank: XM_033203201 (version XM_033203201.1).

Gene-Panel Testing and Exome Sequencing

Families 1 and 2 underwent testing with a small IRD panel followed by exome sequencing (ES). Genomic DNA libraries were generated from DNA (F1:III-3, F1:III-5, F2:1-1, F2:1-2, and F2:II-2) sheared with a Covaris S2 Ultrasonicator via SureSelectXT Library Prep Kit (Agilent). Regions of interest (ROIs) were captured with the SureSelect All Exon V5 kit (Agilent) and sequenced on an Illumina HiSeq2500 HT system (Illumina). Data analysis was performed with a homemade pipeline (POLYWEB) created by the Imagine Institute Bioinformatics core facilities of Paris Descartes University.

Whole-Genome Homozygosity Mapping and ES

DNA from F3:II-1 and F3:II-2 of family 3 were genotyped with the HumanCytoSNP-12 BeadChip platform (Illumina). The genotypes were evaluated for runs of homozygosity (ROH) >1 Mb via PLINK software integrated in-house software ViVar. Resulting ROH were ranked according to their length and number of consecutive homozygous single-nucleotide polymorphisms (SNPs). For ES, exome enrichment and sequencing were performed with the Agilent SureSelect Human All exon V5/V6 kit followed by paired-end sequencing on a HiSeq2000 (2×100 cycles). The CLC Genomics Workbench version 9.0.1 (CLCBio) was used for read-mapping against the human genome reference (NCBI build37/hg19 version), post-mapping duplicate read removal, coverage analysis, and quality-based variant calling via Alamut (visual version 2.7.2; interactive biosoftware).

Targeted Testing of RIMS2

Forty-six affected individuals diagnosed with iCSNB and 133 affected individuals diagnosed with LCA underwent RIMS2 testing either by Sanger sequencing (Big Dye Terminator v3.1 Kit, Applied Biosystems) or targeted next-generation sequencing (Nextera XT DNA Library Prep kit, MiSeq, Illumina) (Table S1A and S1B). We confirmed RIMS2 variants identified by ES, and we performed segregation analysis by Sanger sequencing in all available family members (Figure 1A).

RIMS2 Expression Studies in Human Tissues

Data Mining in Human Adult Single-Cell Retinal Transcriptional Datasets

Data was processed for evaluating RIMS2 expression at the single-cell (sc) level. The expression matrix derived from pooling three donor neural retina samples was retrieved and imported into R (v.3.6.2) with the Seurat sc analysis package (v3.1.4). We conducted pre-processing and quality control to remove outlier cells. We filtered out cells that had unique (gene) feature counts less than 200 or greater than 2,500 and that expressed >5% mitochondrial counts. The dataset was subsequently normalized via the built-in global-scaling normalization method “LogNormalize.”
Prior to dimensional reduction, the data was subjected to scaling, and heterogeneity associated with the number of unique molecular identifiers (UMIs) per cell and mitochondrial contamination was regressed out. After quality control pre-processing, a total of 15,635 cells were kept and clustered via the K-nearest neighbor graph method implemented in Seurat after PCA-reduction. We applied the non-linear dimensional reduction technique UMAP (uniform manifold approximation and projection)19 to visualize and explore the dataset. As input, the same principal components from the clustering analysis were used. We used markers associated with major neural retina cell populations to assess RIMS2 expression at the sc level.

\[\text{Data Mining in Human Adult Bulk Retinal Transcriptional Datasets}\]

Expression levels in terms of transcripts per million (TPM) were retrieved from postmortem retina samples characterized in Ratnapriya et al.20 A total of 453 samples that passed quality control were considered first. In order to avoid introducing confounding variables in the downstream analysis, we selected only donor retinas without age-related macular degeneration, resulting in 105 individual samples. For gene set selection, TPM values were further filtered for a set of candidate genes, which included all genes reported to cause IRD (RetNet) and genes identified in synaptic vesicle pools and/or with pre- and post-synaptic-curated annotations (SynGO).21 A total of 379 genes were eventually considered. For normalization, stabilization, and statistics, prior to examining correlations in the expression of the candidate genes, the set was further filtered by both mean expression and variance. To remove potential noise, we first filtered out the 25% of genes with the lowest mean expression across all samples. We then subjected the set to a variance-stabilizing transformation to correct for

\[\text{Figure 1. Bi-allelic RIMS2a Variants in the Four Families and Location in RIMS2a, RIMS2b, and RIMS2γ Isoforms and Domain Structure}\]

(A) Pedigrees of families and segregation analysis of the variants. Positions of c.3126G>A (p.Trp1042*), c.2884C>T (p.Arg962*), c.4363+1G>A (p.?), c.3508C>T (p.Arg1170*), and c.1595C>G (p.Ser532*) substitutions corresponding to RIMS2a. The WT allele is represented by an equal sign.

(B) Diagram of the human RIMS2 showing positions of the c.1595C>G, c.2884C>T, c.3126G>A, c.3508C>T, and c.4363+1G>A variants. RIMS2α, GenBank: NM_00134848.1 (GRCh38). RIMS2β, GenBank: NM_00134849.4 (GRCh38). RIMS2γ, GenBank: NM_001282882.1 (GRCh38). The positions of specific RIMS2α, β, and γ promoters are located above the diagram.

(C) Diagram showing predicted protein domains in the three isoforms RIMS2a, RIMS2b, and RIMS2γ; the positions of p.Ser532*, p.Arg962*, p.Trp1042*, p.Arg1170*, and p.δ variants correspond to the RIMS2a isoform. Abbreviations used: Zn 2+, N-terminal zinc finger domain; PDZ, central PDZ domain; C2A and C2B, central and C-terminal C2 domains, respectively; PxxP, proline-rich sequence; asterisk, SH3 domain-binding motif.
mean-variance dependency; this resulted in a total of 289 genes being retained. We then examined the expression of RIMS2 and several synaptic genes (CACNA1F, CACBP4, CACNA2D4, and SV2B). For each gene pair, Spearman’s correlations were computed along with pairwise p values adjusted for multiple comparisons (Holm’s method). A matrix correlation plot was then generated for visualization.

Expression Analysis
RT-qPCR was performed on the total RNA of fetal brain (22 weeks), retina (16 weeks), and head of the pancreas (25 weeks) (RNasey Mini Kit, QIAGEN), and for cDNA synthesis, random hexamer anchored oligo(dT) primers were used (Verso cDNA Kit, Life Technologies Thermo Fisher Scientific). We measured RIMS2 expression by amplifying 138, 78, and 89 bp fragments (Table S1C). Real-time PCR amplification and normalization were performed as described. Data were analyzed with Realplex software (Eppendorf).

Immunohistochemistry
Human retinal, brain, and pancreatic tissues used for immunohistochemistry were fixed in 10% neutral buffered formaldehyde and embedded in paraffin. Staining for RIMS2 was performed on 3-µm-thick sections via an automatic immunostainer (BenchMark Ultra, Ventana Medical Systems). The rabbit polyclonal antibody anti-RIMS2 (1:25; Antibodies-Online: ABIN1003091) was used, and visualization was achieved with the OptiView Amplification Kit (Ventana Medical Systems). Heat-induced epitope retrieval was performed with Cell Conditioning 2 (Ventana Medical Systems).

Functional Analysis of RIMS2 Nonsense Variants
Site-Directed Mutagenesis
Constructs were made for the following variants: c.1595C>G, c.2884C>T, c.3126G>A, and c.3508C>T. We did this by using mutagenesis via inverse PCR with Phusion polymerase and by using vector pcDNA3.1(+)-(K)-DYK-RIMS2x (RIMS2a, NM: 00134848.1; 10,277 bp) (Genscript) as a template. Primers can be found in Table S1D. We digested the amplified product with DpnI to avoid re-ligation of the original non-mutated DNA. We amplified constructs in TOP10 Chemically Competent Escherichia coli cells, and we sequenced inserts to assess the mutagenesis and the rest of the sequence.

Immunoblot Analysis in HEK293 Cells
5 × 10^5 cells/well in 6-well plates were co-transfected with wild-type (WT) or mutant FLAG-tagged RIMS2 plasmids (1.6 µg; Genscript) and the pCAGGS-GFP plasmid (400 ng; Clontech) with the FuGene HD transfection reagent according to the manufacturer’s protocol (Promega). GFP-expressing cells from cell pools were sorted on a BD FACSAria II (BD Biosciences) with special order research products (SORP) program. GFP-MIN6 B1 cells were seeded at 5 × 10^5 cells/well in 96-well plates. After 1 h of DMEM Glutamax I medium (5 mM glucose), the medium was replaced by DMEM Glutamax I medium (25 mM glucose). Concentration of insulin in the medium was determined with the Insulin Mouse ELISA Kit (Invitrogen Thermo Fisher Scientific) after 25 min of incubation. Absorbance was measured immediately at 450 nm and 550 nm by a VICTOR X4 2030 multilabel plate reader (PerkinElmer). Using Prism6 software, we determined the significance of variations among samples via a one-way ANOVA with a post hoc Tukey’s test.

Results
Identification of Bi-allelic RIMS2 Variants in Seven Cases from Four Unrelated Families
Exome datasets were generated for affected siblings F1:III-3 and F1:III-5 and F3:II-1 and F3:II-2 and for affected individual F2:II-2 and his unaffected parents, F2:I-1 and F2:I-2 (Figure 1A). By applying stringent filtering, we found a total of seven and two candidate genes in family 1 and family 2, respectively (Table S2). This strategy revealed bi-allelic RIMS2 variants (GenBank: XM_033203201) in the two families: c.3126G>A (p.Trp1042*) in F1:III-3 and F1:III-5 (homozygous) and c.2884C>T (p.Arg962*) in trans with c.4363+1G>A (p.? ) in F2:II-2. Similarly, homozygosity mapping combined with ES in family 3 identified a homozygous RIMS2 variant, c.3508C>T (p.Arg1170*), in the largest 25 Mb autozygous region in F3:II-1 and F3:II-2 as the most plausible candidate variant (Table S2). Of note, no LOD score could be calculated as a result of the small size of the family. Subsequent targeted RIMS2 testing of pre-screened affected individuals with iCSNB identified two affected individuals with a homozygous RIMS2 variant: c.1595C>G (p.Ser532*) in F4:II-1 and c.3126G>A (p.Trp1042*) in an affected individual that retrospectively appeared to be related to family 1 (F1:III-3) (Figures 1B and 1C). Co-segregation of the RIMS2 variants with the disease was confirmed (Figure 1A).

Clinical Re-evaluation of Affected Individuals
The complete lack of Rims2 in the mouse has been reported to cause behavioral anomalies, defective synaptic visual signal transmission from photoreceptors to second-order retinal neurons consistent with iCSNB, and insulin resistance. In family 1, the affected individual F1:III-5 and her elder, affected sister F1:III-3 were born to consanguineous parents from Senegal and were reported to have manifested nystagmus, poor visual function, and altered ERGs around birth. Upon ophthalmological evaluation, the
sisters, currently aged 23 and 31 years and having had myopic correction, displayed features of inner retinal dysfunction: in response to a bright flash under dark-adapted conditions such features included a preserved a-wave (originating from photoreceptor hyperpolarization) but a severely reduced b-wave (originating from ON-bipolar depolarization) and severely reduced and delayed light-adapted responses, in keeping with cone ON- and OFF-bipolar cell dysfunction (Figure 2A). Funduscopy showed optic disc pallor, moderate vascular attenuation, normal macular area, and a normal mid-peripheral and peripheral aspect with no pigmentary migration (Figure 2B). Spectral-domain optical coherence tomography (SD-OCT) of the macular region revealed inner retinal thinning throughout the posterior pole and a disruption of the foveal ellipsoid line in F1:III-5 (Figure 2C), and SD-OCT of the retinal nerve fiber layer (RNFL) revealed a temporal RNFL loss (Figure 2D). These data are consistent with a cone-rod synaptic disorder with additional foveal changes. Disc pallor, inner retinal changes, and thinned retinal vessels have to be interpreted in the context of high myopia. The eldest sister (F1:III-3) had a known history of persistent elevated blood glucose since the age of 29 years, and fasting blood glucose testing confirmed the diagnosis of insulin-dependent diabetes mellitus (Table 1). Clinical neurologic assessment and neuroimaging of the youngest affected sister, F1:III-5, demonstrated autistic features with unremarkable cerebral anomalies with the exception of temporal optic-nerve atrophy. She displayed marked lipatrophy with facial sparing, but the metabolic workup was unremarkable; in particular, there was no resistance to insulin and no alteration of blood-glucose homeostasis.
| Individual | F1:III-3 | F1:III-5 | F1:IV-4 | F2:II-2 | F3:II-1 | F3:II-2 | F4:II-1 |
|------------|---------|---------|---------|---------|---------|---------|---------|
| Age (years) | 33      | 25      | 2       | 7       | 7       | 6       | 9       |
| Origin     | Senegal | Senegal | Senegal | France  | Saudi Arabia | Saudi Arabia | France/Senegal |

**Ophthalmologic Features**

|                  | F1:III-3 | F1:III-5 | F1:IV-4 | F2:II-2 | F3:II-1 | F3:II-2 | F4:II-1 |
|------------------|----------|----------|---------|---------|---------|---------|---------|
| Nystagmus        | +        | +        | erratic ocular movement | erratic ocular movement | +        | +        | +        |
| Photophobia      | +        | +        | +        | +        | +        | +        | +        |
| Night blindness  | –        | –        | –        | –        | –        | –        | +        |
| Visual acuity (RE) | 20/250  | 20/3200  | NP      | 20/320  | LP      | LP      | around 20/200 |
| Visual acuity (LE) | 20/320  | 20/3200  | NP      | 20/320  | LP      | LP      | around 20/200 |
| Refractive error (RE) | –2 (1.75) 90° | –0.75 (1.25) 20° | +2.25 | +6 | NA | NA | –3 (~1) 175° |
| Refractive error (LE) | –1 | –2.50 (~2.25) 170° | +3 | +6 | NA | NA | –4.50 (~2) 170° |
| ERG electronegativity | present | present | NP | present | NP | NP | present |
| Fundus           | optic disc pallor; no peripheral pigmentary migration, greyish retina | temporal optic disc pallor; no peripheral pigmentary migration | temporal optic disc pallor; no peripheral pigmentary migration | optic disc pallor; no peripheral pigmentary migration | temporal optic disc pallor; no peripheral pigmentary migration | temporal optic-disc pallor; no peripheral pigmentary migration | temporal optic-disc pallor; no peripheral pigmentary migration |
| Macular SD-OCT  | retinal thinning at the expense of inner retina | retinal thinning at the expense of inner retina; retinal thinning at the expense of inner retina | NA | NA | normal |
| RNFL-SD-OCT     | NA (optic nerve dysversion) | temporal RNFL loss | NP | NP | NA | NA | NA |
| Autofluorescence| normal | normal | NP | normal | NA | NA | normal |

**Neurologic Features**

|                  | F1:III-3 | F1:III-5 | F1:IV-4 | F2:II-2 | F3:II-1 | F3:II-2 | F4:II-1 |
|------------------|----------|----------|---------|---------|---------|---------|---------|
| Neurological examination | NP | autistic behavior neuro developmental delay; general movement disorganization; ataxia manifestations; poor language | autistic features: stereotypies/OCD | severe autistic behavior; aggressivity; anxiety; no language | autistic behavior; poor language | autistic behavior; no language | autistic behavior; poor language |
| MRI              | normal | normal | dysmorphic corpus callosum | normal | NA | NA | normal |

**Metabolic Features**

|                  | F1:III-3 | F1:III-5 | F1:IV-4 | F2:II-2 | F3:II-1 | F3:II-2 | F4:II-1 |
|------------------|----------|----------|---------|---------|---------|---------|---------|
| Fasting blood glucose | insulin-dependent diabetes mellitus | normal: 0.94 g/L | NP | normal: 0.85 g/L | NP | NP | NP |
| Blood glucose    | hyperglycemia: 1.26 g/L | hyperglycemia: 1.13 g/L | hyperglycemia: 1.53 g/L | NP | NP | NP | normal |

Ophthalmologic, neurologic, and metabolic investigations in all affected individuals. Abbreviations used: CF, count fingers; LP, light perception; ERG, electroretinogram; RE, right eye; LE, left eye; BE, both eyes; SD-OCT, spectral-domain optical-coherence tomography; RNFL-SD-OCT, spectral-domain optical-coherence tomography measuring the retinal nerve fiber layer thickness; MRI, magnetic resonance imaging; OCD, obsessive-compulsive disorders; NA, not available; NP, not performed.
Lipid homeostasis and liver function were also not altered. Her affected son, F1:IV-4, was born to a first-cousin marriage and presented at 13 months of age with severe visual dysfunction and marked neurodevelopmental delay (poor language, mild ataxia, general movement disorganization) with a dysmorphic corpus callosum at brain magnetic resonance imaging (MRI; Figure 2E). The metabolic workup was unremarkable (Table 1). The young individual IV-4 in family 1 was born to a double-consanguineous marriage. Because the familial RIMS2 pathogenic variant in IV-4 was found by targeted RIMS2 testing and not by whole-exome sequencing (WES), we cannot rule out the possibility that dysmorphology of the corpus callosum is attributed to a homozygous pathogenic variant in another gene because of this parental consanguinity.

Affected individual F2:II-2 was born at term after an uneventful pregnancy. He was first seen in ophthalmology at 2.5 months for pendular nystagmus, strabismus, and photophobia. Upon examination, he presented with hyperopia, the inability to follow lights or objects, and a normal fundus. At the age of 7 months, the poor quality of ERG recording led to a presumed diagnosis of LCA. At 2 years of age, the child manifested behavioral problems and an inability to walk without assistance. In addition to nystagmus and photophobia, the ophthalmologist noted some albinoid characteristics, including fair ashy hairs, peripheral iris transillumination, and pale fundus. Furthermore, bilateral active pupillary reflexes were present, calling the initial ERG results and diagnosis of LCA into question. However, ERG recordings could not be repeated at that time because of high agitation. Genotype-directed clinical reexamination was conducted at the age of 5.5–6 years. ERG recordings under general anesthesia revealed an electronegative waveform under limited dark adaptation and barely detectable photopic responses, consistent with iCSNB (Figure 2A). Funduscopy showed optic nerve pallor and a normal mid-peripheral and peripheral aspect (Figure 2B). Neuropediatric assessment showed autistic features, including stereotypies and obsessive-compulsive disorder (OCD). Neuroimaging with MRI displayed no brain anomalies. The blood-glucose homeostasis dosage was in the normal range (Table 1).

The two affected sibling of family 3 (F3:II-1 and F3:II-2) were born to consanguineous Saudi Arabian parents and displayed poor visual acuity reaching light perception in both eyes, photophobia, pendular nystagmus, the absence of pupillary response, and oculo-digital signs. Retinal imaging of both siblings showed a waxy pallor of the optic discs and attenuated retinal vessels (Figure 2B). Both siblings displayed autistic behavior with a variable degree of severity: whereas the female younger sibling, F3:II-2 (3 years), could produce simple speech, the male sibling, F3:II-1 (4 years), was not capable of speech and showed aggressive and anxious behavior (Table 1), thus not allowing for ERG recordings.

Affected individual F4:II-1 of family 4 was born to consanguineous Senegalese parents. He was first referred to the electrophysiology unit when he was 2.5 years of age for photophobia, nystagmus, and poor vision. He was a premature (born at 33 weeks after amenorrhea) twin baby, and his fraternal twin brother was exempt of any relevant medical and ophthalmic history besides the prematurity and a mild ataxia that developed while he was starting to walk at 14 months of age. F4:II-1 had been diagnosed with developmental delay with delayed speech and walking; these delays were resolving upon rehabilitation. After thorough psychomotor examination, a diagnosis of attention disorder with hyperactivity within the spectrum of autistic behavior was diagnosed; he had no intellectual disability. His metabolic work-up was otherwise normal. F3:II-1 was myopic and had a visual acuity around 20/200 for both eyes at age 7 years (Table 1). Fundus examination revealed a mild optic-disc pallor and some vessel attenuation that could be attributed to the myopia (Figure 2B). SD-OCT of the macula was normal (Figure 2C). Fundus autofluorescence was normal. ERG recordings revealed an electronegative waveform to a bright flash in dark-adapted conditions as well as delayed and reduced responses in light adaptation, leading to a diagnosis of iCSNB. The child manifested neurodevelopmental delay with speech difficulties but no learning disability in elementary school, an ataxic gait, and a certain degree of hyperactive autistic behavior (Table 1).

RIMS2 Is Present in the Human Retina, Brain, and Pancreas

Consistent with the neuro-ophthalmometabolic phenotype of the knock-out model, Rims2 is detected in mouse retinal, brain, and pancreatic tissues (see BioGPS in Web Resources). According to the human retinal transcriptome dataset of the Ocular Genomics Institute, the Human Protein Atlas, and BioGPS, human RIMS2 is equally detected in these tissues. Data mining of sc transcriptional datasets of human adult neural retina showed that RIMS2 was predominantly expressed in rod photoreceptor clusters (Figure S1A) and has an expression pattern similar to that of CARP4 (Figure S1B). Next, we assessed the expression patterns of RIMS2, CACNA1F, CABP4, CACNA2D4, and SV2B in human adult bulk retinal transcriptional datasets for coordinated correlation, reasoning that this could provide insight into potential regulatory interactions. A matrix correlation plot (Figure S1C) showed RIMS2 expression to be anti-correlated with CACNA1F (rho = −0.3435, p = 0.002) and to be correlated with SV2B (rho = 0.6565, p < 0.0001). The latter is known to be localized in synaptic vesicles, where it might function in the regulation of vesicle trafficking and exocytosis.

In addition to a promoter located in the 5′ UTR region driving the longest RIMS2α transcript, the gene has two internal alternative promoters yielding shorter transcripts (RIMS2β and RIMS2γ) (Figure 1B). RT-qPCR analysis with primers specific for each of the three isoforms applied to fetal human tissues showed that all transcripts were expressed far more highly in the brain than in other tissues.
In the retina, the three transcripts seemed equally expressed. In the pancreas, RIMS2γ had the highest expression, followed by RIMS2α then RIMS2β, whereas RIMS2α expression was undetectable in fibroblasts; these non-neuronal, non-secretory cells showed predominant RIMS2γ expression (Figure 3).

We assessed RIMS2 localization in adult human retina, brain, and pancreas by immunostaining using an antibody recognizing both RIMS2α and RIMS2β isoforms but not RIMS2γ. Strong and specific RIMS2 immunostaining was observed in the outer plexiform retinal layer (Figure 4A), cerebellar cortical neurons (more specifically in Purkinje cells; Figure 4B), and pancreatic Langerhans islets (Figure 4C). This localization is in agreement with the spectrum of oculo-cerebro-pancreatic anomalies in individuals with bi-allelic mutant RIMS2 variants.

**In Silico Predictions and In Vitro Functional Characterization of RIMS2 Variants**

**Effect of a Splice Variant**

The c.4363+1G>A splice variant found in family 2 is the only one out of five unique RIMS2 variants that is expected to affect the three transcripts. *In silico* predictions suggest the presence of an abolished consensus donor splice-site leading to the skipping of the adjacent exon, a frameshift, and the introduction of a premature termination codon (c.4251_4363del [p.Asn1417Lysfs*2]). The insertion of a stop codon in the shortest RIMS2γ transcript most likely results in nonsense-mediated decay (NMD) and in the absence of this an isoform. The mutant RIMS2α and RIMS2β transcripts, however, might escape NMD and lead to truncated isoforms (Figure S2).

**Protein Abundance of Nonsense Variants**

The four RIMS2 nonsense variants, however, are predicted to produce normal RIMS2γ and truncated RIMS2α and RIMS2β. Consistent with this, immunoblot analysis using FLAG antibody of lysates from HEK293 cells overexpressing individually the WT, p.Ser532*, p.Arg962*, p.Trp1042*, and p.Arg1170* FLAG-tagged RIMS2α cDNAs revealed an ~210 kDa protein and truncated isoforms of expected ~60, ~120, ~130, and ~150 kDa sizes, respectively (Figure 5A). The WT and truncated FLAG-tagged proteins were produced with the same intensity (Figure 5B). When using the primary antibodies polyclonal rabbit IgG anti-RIMS2 (1:1000; Synaptic Systems Cat. No. 140 303) and monoclonal mouse IgG anti-β-actin (1:4000; Abcam) and the secondary antibodies goat anti-rabbit IgG-HRP (1:2000; Abcam) and goat anti-mouse IgG-HRP (1:2000; Abcam), we detected no endogenous RIMS2α or RIMS2β in lysates from untransfected HEK293 cells (Figure S3).

**Insulin Secretion in MIN6B1 Cells**

We investigated the ability of these truncated proteins that lack all or most of the domain that interacts with RIM-binding proteins 1 and 2 (RIMBP1 and RIMBP2) to promote insulin secretion in MIN6B1 cells. We overexpressed the pCAGGS-GFP plasmid alone or in combination with the WT or mutant FLAG-tagged RIMS2 constructs and measured the accumulation of insulin in the culture medium upon glucose stimulation. The amount of insulin in the culture medium from cells transfected with pCAGGS-GFP alone was in the same range as that in medium from cells transfected with pCAGGS-GFP in combination with the WT FLAG-tagged RIMS2α constructs, suggesting that overexpression of WT RIMS2α does not affect insulin secretion. In contrast, we observed a reproducible reduction of insulin accumulation in cells transfected with each of the three mutant constructs, supporting that truncation of all or most of the RIMBP1- and RIMBP2-binding domain alters insulin secretion in this cellular system (Figure 6).

**Discussion**

The human visual system continues to develop after birth, mainly in the first years of life. The differential diagnosis of early-onset IRD, either stationary (CSNB) or degenerative (LCA), might be challenging in the first months of life, mainly as a result of individuals’ inability to respond verbally to visual testing, the absence of apparent fundus changes, the difficulties in cooperation for testing, especially in individuals with behavioral problems that might lead to unreliable ERG recordings, and the presence of a nystagmus, all of which, without general anesthesia, hamper high-resolution retinal imaging. The identification of the underlying genetic defect can accelerate an early differential diagnosis between a stationary and degenerative retinal disease because there is no genetic overlap.

Here, we demonstrated the bi-allelic loss of function of RIMS2 in a phenotype characterized by syndromic CRS and with neurodevelopmental and pancreatic involvement, consistent with a role of RIMS2 in regulating synaptic...
membrane exocytosis in the brain, pancreas, and photoreceptors. In regard to the retinal phenotype, visual acuity seems low in our individuals with RIMS2-associated CRSD and would be on the lower functional spectrum of the high phenotypic variability spectrum of iCSNB, which is characterized by a post-photoreceptor defect affecting both the cone and rod signaling pathways. Indeed, in a series of 60 affected individuals carrying mutation in CACNA1F, Boycott et al. report a high variability in visual acuity in correlation with the presence of a nystagmus. In addition, for some of the affected individuals in our study, behavioral disturbances made it difficult for us to accurately measure visual acuity, which as a result, might have been underestimated. Additionally, one of the affected individuals presented here had foveal changes that might have also hampered visual acuity. Similar changes have been reported in association with CAPP4 mutations. These changes were only present once in our report, and further studies are needed to document the frequency of this occurrence in RIMS2-associated CRSD. Furthermore, one instance of inner retinal thinning was also found in our series, and such thinning might also contribute to poor visual acuity. This finding has also been reported in association with iCSNB.

RIMS2 is one of the Rab3-interacting molecules, or RIMs, which are multi-domain scaffolding proteins that were first described as putative presynaptic active-zone proteins that play an essential role in neurotransmitter release and are located in the retinal photoreceptors. In vertebrates, RIMs are encoded by four regulating synaptic membrane exocytosis genes (RIMS1–RIMS4). Although Rims1a<sup>−/−</sup> and Rims2a<sup>−/−</sup> mice are viable, the ablation of both genes causes postnatal mortality as a result of a defective neurotransmitter release despite preserved synaptic structure and exocytosis ability. Synapses of double mutants contain active zones and release neurotransmitters, but they cannot mediate normal Ca<sup>2+</sup>-triggered release. The RIMS2-associated phenotype in affected human individuals is consistent with the known function of the mouse ortholog in the photoreceptor ribbon synapse, conventional chemical synapse, and synaptic-vesicle exocytosis and with its localization in the outer retinal plexiform layer, brain synapses, and pancreatic β cells.

RIMS2 is the predominant large RIM isofrom present at photoreceptor ribbon synapses. RIMS2 plays an important role in maintaining the normal synaptic connection at the ribbon synapse of the photoreceptors. This can be achieved via several interactions required for synaptic vesicle docking and priming. The majority of photoreceptor RIMS2 lacks the N-terminal zinc finger and part of the RAB3A-binding domain, suggesting that photoreceptor synaptic transmission does not depend on full-length RIMS<sub>a</sub>. The role of RIMS2 at photoreceptor ribbon synapses is different from RIM function in most other types of chemical synapses. At the photoreceptor ribbon active zone, RIMS2 is not essential for vesicle priming or Ca<sup>2+</sup> channel clustering, but it does act as a Ca<sup>2+</sup> channel modulator. We assessed the influence of Rims1 and Rims2 on synaptic processes at rod terminals by generating a conditional double-knockout mouse model in rod photoreceptors at as early as 3 weeks of age. Deletion of Rims1 and Rims2 in mouse rods (called Rims1/2 double knockout) showed a dramatic loss of Ca<sup>2+</sup> influx through Cav1.4 channels and an associated reduction in evoked release. Both Rims isoforms were shown to be expressed in the retina at as early as 3 weeks of age. However, it has been shown that the Rims1-specific antibody supports an absence of Rims1 from photoreceptors and their ribbon synapses in the outer plexiform layer and that Rims1a does not contribute to the regulation of exocytosis at the cone photoreceptor ribbon synapse. Rims2 is also present exclusively in the outer plexiform layer of WT mice, whereas it was absent in the knockout mice. It was concluded that Rims2 potently enhances the influx of Ca<sup>2+</sup>, which is vitally important for the release of vesicles from the rod ribbon possibly through direct or indirect modulation of the Cav1.4 channels. As Rims2 has many partners that have not been linked with any human or animal phenotype so far at the photoreceptor synapse (synthesized in Figure 7), the genes encoding them merit consideration in genomic studies of children with congenital IRD.

In regard to the brain, Rims2, like Rims1, was shown to be expressed at as early as 3 weeks of age in whole brain lysates. Analysis of double knockout mice has demonstrated expression of the two paralogs in overlapping but distinct patterns throughout the brain. Here, we showed localization of RIMS2 in human adult cerebellar cortical neurons, more specifically in Purkinje cells. Of interest,
involvement of RIMS2 in neurological disease, more specifically in autism spectrum disease (ASD), is supported by a genome-wide association study in affected individuals with Asperger syndrome (ASPG [MIM: 608638]), revealing a significant association with the RIMS2-associated SNP rs2080610. A study on alternative splicing in neural tissues showed that one of the RIMS2 transcripts contains a highly-conserved micro-exon that is neuron-specific. RIMS2 has been listed as an ASD-associated gene in a recently developed ASD database.

Interestingly, a RIMS1 variant was found to co-segregate with autosomal-dominant cone-rod dystrophy (CORD7 [MIM: 603649]) in a British family, implicating a gene with a synaptic function in an IRD. In a mouse model carrying the same Rims1 variant as the human CORD7 (p.Arg655His), the mutant was shown to modify Rims1 function in regulating voltage dependent Ca²⁺ channel currents. A Rims1 variant on different presynaptic voltage-dependent calcium channels (VDCCs) might eventually lead to CORD and enhanced cognitive abilities. Moreover, RIMS1 has been associated with ASD by two independent studies. Regarding RIMS2, a copy-number variant (CNV), more specifically a duplication of 277 kb (Chr8: 104901578–105178819), was found in an individual affected with autosomal-dominant retinitis pigmentosa (adRP [MIM: 268000]). Although this CNV was found to be absent in genomic databases, the role of this duplication in the pathogenesis of adRP is still a matter of debate, especially because no segregation analysis could be performed in the rest of the family, including the affected father.

RIMS2 or RIMS1 variants have not been associated with metabolic dysfunction before. Yet, RIMS2 has been identified by a yeast two-hybrid screen performed on pancreatic β cells to determine docking and priming states in insulin granule exocytosis. Consistently, Rims2−/− mice display a phenotype that consists of anomalies of photoreceptor synaptic transmission, deficit in maternal behavior, and insulin resistance. This retino-neuro-metabolic phenotype is reminiscent of the RIMS2-associated disease we report here. Whether abnormal glucose homeostasis is an invariant feature remains to be confirmed. Evidence of insulin resistance in the eldest affected individual of our cohort suggests that this could be an age-related feature, and it will be important to perform a close follow-up of metabolic functions.

In summary, bi-allelic loss-of-function variants of RIMS2 have been shown to cause a previously unreported syndromic iCSNB, manifesting as a CRS with neurodevelopmental disease and occasional anomalies of glucose homeostasis. The identification of a syndromic stationary congenital IRD has a major impact on the differential diagnosis of syndromic congenital IRD, which has previously been exclusively linked with degenerative IRD. Finally, our study implicates a photoreceptor synaptic gene in syndromic disease.
Figure 7. Schematic and Simplified Drawing of Protein Complexes Present at the Photoreceptor Ribbon Synapses
Presynaptic photoreceptor, synaptic cleft, and postsynaptic bipolar areas are shown. The names of proteins are indicated after dashes. The names of protein domains are directly indicated on the protein drawing. Proteins that have already been implicated in CSNB are indicated with an arrow. Abbreviations used: NT, neurotransmitters; iCSNB, incomplete form of congenital stationary night blindness; cCSNB, complete form of congenital stationary night blindness; Ca$^{2+}$, calcium; Na$^+$, sodium.

Supplemental Data
Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2020.04.018.

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Declaration of Interests

The authors declare no competing interests.

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Web Resources

BioGPS, biogps.org/
Ocular Genomics Institute, https://oculargenomics.mei.harvard.edu/
OMIM, https://omim.org/
RetNet, https://sph.uth.edu/retnet/
The Human Protein Atlas, www.proteinatlas.org/

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