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Association of Missense Variants in VSX2 With a Peculiar Form of Congenital Stationary Night Blindness Affecting All Bipolar Cells

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1 **Missense variants in VSX2 associated with a peculiar form of congenital**
2 **stationary night blindness affecting all bipolar cells.**

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20 This article contains additional online-only material. The following should appear online-only: eFigure
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23 **Main article:** 2954, Abstract: 282

24 **Key points**

25 **Question** What is the detailed clinical picture and underlying gene defect in patients with a peculiar
26 form of congenital stationary night blindness (CSNB)?

27 **Findings** We report three patients of two families with CSNB, anterior segment abnormalities (ciliary
28 body hypoplasia and lens luxation), and all-bipolar cell dysfunction. We identified two genetic
29 variants in *VSX2*, a major gene important for ocular development and bipolar cell fate in those CSNB
30 families.

31 **Meaning** This genetic defect of an inherited ocular disease had both functional and anatomical eye
32 defects.

33 **Abstract**

34 **Importance** Congenital stationary night blindness (CSNB) is an inherited stationary retinal disorder,
35 which is clinically and genetically heterogeneous. To date, some cases with CSNB and an unusual
36 complex clinical picture are still genetically unsolved.

37 **Objective** To describe an unreported CSNB phenotype and the associated gene defect in three patients
38 from two unrelated families.

39 **Design** Retrospective case-series conducted in 2021-2022.

40 **Setting** Single-institution study in a national referral center for rare ocular diseases.

41 **Participants** Three patients from our cohort of 140 genetically unsolved CSNB cases have been
42 clinically and genetically explored.

43 **Exposures** Complete ocular examination including full-field electroretinography and multimodal
44 fundus imaging (spectral-domain optical coherence tomography, color, infrared reflectance and
45 short-wavelength autofluorescence photographs) were performed. The gene defect was identified by
46 exome sequencing, confirmed by Sanger sequencing and co-segregation analysis in one family.

47 Additionally, screening of our genetically unsolved CSNB cases for *VSX2* variants was performed by
48 direct Sanger sequencing.

49 **Main outcome measures** Ocular and molecular biology findings.

50 **Results** Patients had nystagmus, low stable visual acuity and myopia from birth and complained of
51 night blindness. Two older patients had bilateral lens luxation and underwent lens extraction. Full-
52 field electroretinography revealed an electronegative Schubert-Bornschein appearance, combining
53 characteristics of incomplete and complete CSNB, affecting the function of rod and cone ON- and
54 OFF-bipolar cells. Exome sequencing and co-segregation analysis in a consanguineous family with
55 two affected members identified a homozygous variant in *VSX2*. Subsequently, screening of our
56 CSNB cohort identified another unrelated patient harboring a distinct *VSX2* variant.

57 **Conclusion and Relevance** We reported a peculiar pan-bipolar cell retinopathy with lens luxation
58 associated with variants in *VSX2*. Clinicians should be aware of this peculiar association. We
59 recommend that *VSX2* might be added to CSNB diagnostic gene panels.

60

61 **Main text**

62 **Introduction**

63 Congenital stationary night blindness (CSNB) with largely normal fundus appearance is a vast group
64 of genetically and clinically heterogeneous congenital retinal disorders. The most common form
65 manifests with infantile nystagmus, reduced visual acuity, variable degree of myopia, poor visual
66 behavior in dim lightning and/or photophobia¹. The diagnosis is ascertained by full-field
67 electroretinography (ffERG), displaying characteristic waveform changes.

68 Under dark adaptation, the stimulation with a dim flash (DA 0.01 ERG, dark adapted 0.01 cd/m²)
69 generates a positive going b-wave reflecting the rod ON-bipolar cell depolarization². In the same dark-
70 adapted conditions, the stimulations with brighter flashes (DA 3.0 ERG, DA 10.0 ERG) produce a
71 negative a-wave, reflecting the hyperpolarization of photoreceptors, mainly dominated by rods in this
72 adaptation state^{3,4}, followed by a positive b-wave, which ascending limb is dominated by ON-bipolar
73 cell depolarization⁵. Under light adapted conditions, the stimulations with bright flashes (LA 3.0 ERG,

74 light adapted 3.0 cd/m²) lead to a negative a-wave generated by the hyperpolarization of cones and
75 then of OFF-bipolar cells. The second deflection is a positive b-wave which ascending limb is
76 dominated by cone ON-bipolar cell depolarization and descending limb by OFF-bipolar cell
77 hyperpolarization^{6,7}. Long-duration stimulations allow the separation of responses generated by cone
78 ON- and OFF- bipolar pathway^{6,8}.

79 Most of the patients with CSNB have a distinct Schubert-Bornschein electronegative waveform (b/a
80 amplitude ratio < 1) under dark-adapted conditions⁹, in relation with a signaling defect from
81 photoreceptors to adjacent bipolar cells. Two subtypes of Schubert-Bornschein CSNB can be
82 distinguished on the basis of additional fERG features: the incomplete (icCSNB) and complete
83 (cCSNB)¹⁰. In icCSNB, there is a preserved but reduced and delayed b-wave to the DA0.01 ERG¹⁰.
84 Light-adapted responses are markedly reduced and delayed, reflecting a cone to cone ON- and OFF-
85 bipolar cell transmission defect which can be documented using long duration stimulations^{10,11}.
86 Patients have variable degrees of night blindness, strabismus, nystagmus, a wide range of refractive
87 errors¹² and a normal fundus, apart from myopic changes. Photophobia may be the main complaint for
88 some patients who also experience difficulties in dimly lighted environment. Pathogenic variants in
89 *CACNA1F* (MIM #300110)^{13(p),14} and *CABP4* (MIM# 608965)¹⁵ lead to icCSNB. Variants were also
90 identified in *CACNA2D4* (MIM# 608171)¹⁶ in patients initially diagnosed with icCSNB but later
91 associated with a cone dystrophy¹⁷. Proteins encoded by these genes are localized at the presynaptic
92 membrane of both rods and cones. They play a role in glutamate release into the synaptic cleft.
93 icCSNB represents thus a photoreceptor to ON- and OFF-bipolar cell transmission defect¹. Light
94 sensitivity present in icCSNB patients may be explained by the worse dysfunction in cone bipolar
95 circuitry unlike in other forms of CSNB¹². In cCSNB, the b-wave is typically absent on the DA 0.01
96 ERG. The a-wave has typically a square shape with a sharply arising b-wave and a reduced b/a
97 amplitude ratio at the LA 3.0 ERG¹⁰, with absence of ON-bipolar responses but preserved OFF-
98 responses to long duration stimulations¹⁸. Patients with cCSNB present consistently with high myopia,
99 nystagmus, night blindness and low visual acuity¹². Variants in *NYX* (MIM# 300278)^{13,19}, *GRM6*
100 (MIM# 604096)^{20,21}, *TRPM1* (MIM# 603576)²²⁻²⁴, *GPR179* (MIM# 614515)²⁵ and *LRIT3* (MIM#

101 615004)²⁶ lead to cCSNB. Genes listed above code for proteins localized mainly in the dendritic tips
102 of the ON-bipolar cells. They are important for glutamate-induced signaling. cCSNB represents thus a
103 photoreceptor to ON-bipolar cell transmission defect¹.

104 Other rare forms of CSNB with a different ERG and clinical phenotype have been associated with
105 *SAG* (MIM# 181031), *GNAT1* (MIM# 139330), *RHO* (MIM# 180380), *PDE6B* (MIM# 180072),
106 *GRK1* (MIM# 180381) and *SLC24A1* (MIM# 603617)¹.

107 The goal of this study was to report an unusual CSNB phenotype and associated gene defects in three
108 patients from two unrelated Turkish families.

109 **Material and Methods**

110 Research procedures adhered to the tenets of the Declaration of Helsinki and were approved by the
111 local Ethics Committee (CPP, Ile de France V, Project number 06693, N°EUDRACT 2006-A00347-
112 44, 11 December 2006 and CPP, Ile de France II). Prior to testing, written informed consent was
113 obtained from adult participants and the parents of the subject IPP 1889196 who was under 18. No
114 compensation or incentive was offered to patients to participate in the study. Relevant reporting
115 guideline has been followed.

116 *Clinical studies.* The cases were clinically investigated at the national reference center for rare ocular
117 diseases REFERET of the Centre Hospitalier National d’Ophtalmologie (CHNO) des Quinze-Vingts
118 as previously described²⁷. Patient IPP1889196 was also assessed at the national reference center for
119 rare ocular diseases OPHTARA of Necker-Enfants Malades Hospital at the age of 5 months.

120 *Genetic analysis.* Blood samples from cases and all available family members were collected for
121 genetic research and genomic DNA was extracted as previously reported²⁸. These DNA samples were
122 collected within the NeuroSensCol DNA bank, for research in neuroscience (PI: JA Sahel, co-PI I
123 Audo, partner with CHNO des Quinze-Vingts, Inserm and CNRS).

124 Direct Sanger sequencing was performed for patient CIC00257 for all exons and exon-intron
125 boundaries of the following genes: *CACNA1F*, *NYX*, *TRPM1*, *LRIT3*, *GRM6*, *GPR179*, *CABP4*,

126 *CACNA2D4* and for some other genes associated with inner retinal dysfunctions: *RIMS2*, *GNB3* and
127 *GNB5*. The amplification and sequencing conditions can be obtained on request.

128 IPP1889196 was analyzed on a next generation sequencing (NGS) molecular diagnostic panel
129 including CSNB- and pediatric retinal degeneration-associated genes (eTable 1 in Supplements).
130 Subsequently, exome sequencing was performed in the patient and both parents. Genomic DNA
131 libraries were generated from DNA sheared with a Covaris S2 Ultrasonicator via SureSelectXT
132 Library Prep Kit (Agilent). Regions of interest (ROIs) were captured with the SureSelect All Exon V5
133 kit (Agilent) and sequenced on an Illumina HiSeq2500 HT system (Illumina). Data analysis was
134 performed with a homemade pipeline (POLYWEB)²⁹ created by the Imagine Institute Bioinformatics
135 core facilities of Paris University. Considering that pathogenic variants are uncommon, we searched
136 for recessive variants absent in the dbSNP, 1000Genomes, EVS, ExAC, GnomAD and all in-house
137 databases and for variants with minor allele frequency up to 0.01. We searched in priority for
138 homozygous consensus splice-site changes, nonsense variants, insertions and deletions in coding
139 regions. After this stringent filtering, variants in four candidate genes (*ITIH2*, *PRDM10*, *VSX2* and
140 *BCR*) were identified. All variants were classified using Human Genome Variation Society
141 nomenclature (<http://www.hgvs.org/>) and their pathogenicity was assessed in accordance with the
142 American College of Medical Genetics and Genomics recommendations (ACMG)³⁰. Validation of
143 putative pathogenic variants and co-segregation study were performed by direct Sanger sequencing of
144 exons of interest (conditions and primer sequence will be delivered on request) in all available family
145 members.

146 In a second step, a cohort of 140 unsolved CSNB cases was screened for *VSX2* variants. One
147 additional patient (CIC12548) was identified. He previously tested negative on a NGS panel³¹
148 including all known-to-date CSNB-associated genes.

149

150

151 **Results**

152 All three patients clinically investigated at 30, 4 and 45 years of age (CIC00257, IPP1889196 and
153 CIC12548, respectively), presented infantile nystagmus, low visual acuity, myopia and night blindness
154 complaints from birth, but did not report changes in their symptoms or vision since childhood. Clinical
155 data of patients are summarized in Table 1.

156 Their ffERG recordings (Fig.1) under dark adapted (DA) conditions showed no detectable responses
157 to a dim 0.01 cd.s.m⁻² flash (DA 0.01 ERG), a normal a-wave but a severely reduced b-wave leading
158 to an electronegative waveform to bright flashes (DA 3.0 and DA 10.0 ERG). These alterations
159 resembled ON-bipolar dysfunction in cCSNB. However, light-adapted responses to a 3 cd.s.m⁻² single
160 flash and to a 30 Hz 3 cd.s.m⁻² flicker (LA 3.0 ERG and LA 3.0 flicker) were reduced and delayed,
161 unlike patients with cCSNB but resembling functional alterations in icCSNB¹.

162 The female patient CIC00257, was referred to our clinic at 30 years of age. Best corrected visual
163 acuity (BCVA) was 20/200 both eyes (OU). Color vision was normal. Goldmann visual field was
164 flattened in the superior part on all target sizes. Static visual field found a diffuse reduction of retinal
165 sensitivity and reduction of foveal threshold. Both lenses were subluxated superiorly and showed
166 cataract. Ophthalmoscopic evaluation revealed typical myopic changes including oval tilted optic discs
167 surrounded with peripapillary chorio-retinal atrophy, thin retinas with increased visibility of choroidal
168 vasculature and narrowed retinal vessels (Fig.2, A). Numerous glistening white crystals and atrophic
169 chorio-retinal patches were visible in the peripheral retina (Fig.2, B). Infrared reflectance (IRR)
170 (eFig.1, B in the Supplement) and short-wave length fundus autofluorescence (SW-FAF) (eFig.1, C in
171 the Supplement) were unremarkable. Spectral domain optical coherence tomography (SD-OCT) was
172 not performed at the first visit. The patient underwent intracapsular cataract extraction without
173 intraocular lens implantation. BCVA improved to 20/63 both eyes.

174 The male patient IPP1889196, a nephew of CIC00257, was referred to the clinic at 4 years old.
175 Convergent squint and infantile nystagmus syndrome with an initial intermittent and transient upbeat
176 component were present at the age of 5 months. Slit lamp examination was unremarkable. Fundus
177 examination revealed similar myopic changes as reported in the aunt (Fig.2, C). In the peripheral
178 retina there was a 360° white-without-pressure. SD-OCT (Fig.2, D) showed preserved outer retina and

179 thinned inner retinal layers. Multimodal retinal imaging was difficult due to nystagmus, with
180 unremarkable IRR and SW-FAF (eFig.1, E in the Supplement).

181 The male patient CIC12548, from an unrelated family, was referred to the clinic at the age of 45 years.
182 He had infantile nystagmus and poor vision since birth. At the age of 42 years he underwent
183 intracapsular lens extraction for bilateral subluxated cataract. Ultrasound biomicroscopy (UBM)
184 revealed hypoplastic ciliary body and posterior iris bowing (eFig.2 in the Supplement).
185 Ophthalmoscopic examination revealed triangular atrophic chorio-retinal lesions inferior to the macula
186 and white-without-pressure in peripheral retina (Fig.3, A). Atrophic chorio-retinal lesions were
187 hypoautofluorescent with hyperautofluorescent edges on SW-FAF (Fig.3, B), were bright on IRR
188 (Fig.3, C) with outer retinal disruption on SD-OCT. Grade 3 foveal hypoplasia³² as well as a thin
189 epimacular membrane could be seen on SD-OCT (Fig.3, D).

190 *Genetic assessment.* Patients CIC00257 and IPP1889196 were members of a family (F178) with
191 North Turkish ancestry and were born from consanguineous unions (Fig.4A). Patient CIC12548
192 (F7248) was also of Turkish ancestry (Fig.4A). He did not report parental consanguinity.

193 Sanger sequencing and targeted NGS revealed the absence of disease-causing variants in CIC00257
194 and IPP1889196, respectively. Exome sequencing (ES) performed in IPP1889196 and his parents
195 revealed homozygous putative disease-causing variants in the following genes, *ITIH2* (MIM
196 #146640), *PRDM10* (MIM #618319), *BCR* (MIM #151410) and *VSX2* (MIM #142993), consistent
197 with autosomal-recessive inheritance (eTable 2 in Supplement). However only the missense variant in
198 *VSX2* (NM_182894.3): c.595C>T p.(Arg199Cys) (Fig. 4A and B) co-segregated with the disease. This
199 variant, is rare in population databases (minor allele frequency 8×10^{-6} in gnomAD; not present in
200 ExAC database) is predicted to be damaging by 25 out of 40 prediction algorithms available at
201 <https://varsome.com>. Thus this variant is considered likely pathogenic (PM1, PM2, PP1-M, PP3) by
202 ACMG standards³⁰. *VSX2* Sanger sequencing in CIC12548 revealed another missense homozygous
203 variant: c.698C>T p.(Pro233Leu) (Fig. 4A and B). This variant is absent in population databases and
204 predicted damaging by all prediction algorithms available at <https://varsome.com>. It is considered as

205 variant of unknown significance (PM2, PM1, PP3) by ACMG standards. Unfortunately, other
206 members of the family were not available for co-segregation analyses.

207 **Discussion.**

208 The clinical picture of the patients presented herein that included infantile nystagmus, low stable
209 BCVA and myopia was suggestive of CSNB. The upbeat component of nystagmus, present in patient
210 IPP1889196, has also been reported as an early and transient feature of infantile nystagmus in
211 CSNB³³. Regarding functional alteration, the ffERG revealed an electronegative dark-adapted
212 response in keeping with a Schubert-Bornschein type of CSNB. However, both dark- and light adapted
213 response alterations were different than in cCSNB or icCSNB¹⁰. On one hand, dark-adapted responses
214 were in line with cCSNB with absent b-wave and on the other hand, severely reduced light-adapted
215 responses were suggestive for icCSNB. These ffERG alterations would reflect generalized both rod
216 and cone post-phototransduction disorder. Since - in contrast to c and icCSNB- the herein presented
217 patients had absent rod and cone ON- and OFF-bipolar cell responses, we propose to call this finding a
218 “pan-bipolar” cell dysfunction.

219 In general, *VSX2* (Visual System Homeobox 2, 14q24.3, formerly described as *HOX10/CHX10*)
220 recessive gene defects have been reported in association with isolated microphthalmia/anophthalmia
221 (MIM #610093)³⁴, microphthalmia with coloboma (MIM #610092)³⁵, microphthalmia with cataracts
222 and iris abnormalities³⁶⁻⁴⁰ (Fig.4, B). The adult patients, CIC00257 and CIC12548, presented lens
223 subluxations and cataracts. All patients assessed in this study had no microphthalmia. One report
224 mentions lens ectopia without microphthalmia in a patient with a c.456-6C>G r.(?) change in *VSX2*
225 but detailed data are lacking⁴¹.

226 Our patients presented a peculiar retinal dysfunction, between cCSNB and icCSNB, in keeping with
227 generalized rod and cone post-phototransduction disorder. Only few data on retinal dysfunction in
228 both heterozygous and homozygous pathogenic variants in *VSX2* are available^{42,43} as discussed in the
229 Supplementary Materials.

230

231 *VSX2* is a five-exon gene (Fig.4, B) located on chromosome 14q24.3⁴⁴ with a conserved sequence
232 across vertebrates³⁴. It is abundantly expressed in retinal progenitor cells during embryonic and foetal
233 eye development^{45,46}. This abundant expression is transient: in adult vertebrate retina the protein is
234 found only in the nuclei of bipolar cells^{45,47,48,34,49}. According to available retinal gene expression
235 databases, *VSX2* is present in all types of bipolar cells and thus it is considered as a pan-bipolar cell
236 marker⁵⁰⁻⁵³. *VSX2* encodes a 361 amino-acid residue protein (Fig.4, B) with transcription factor
237 activity. *VSX2* is composed of several domains: residues 149-205 constitute the homeobox domain
238 (HOX), residues 208 to 261 - CVC domain (standing for CHX10, VSX1 and CEH10) and residues
239 300 to 318 - OAR domain (OTP, aristaless and RAX). HOX or homeodomain has a helix-turn-helix
240 structure binding directly regulatory DNA sequences of target genes⁵⁴ (Fig.4,B). Disease causing
241 truncating or missense *VSX2* variants were identified all over the gene (Fig.4,B). *VSX2* presence
242 defines bipolar cell fate of retinal precursor cells⁵⁵. Several retinal targets of *VSX2* have been
243 identified. *VSX2* acts as a transcriptional repressor of *RHO*, cone opsin locus control region and *SAG*
244 (S-antigen visual arrestin), thus suppressing photoreceptor gene expression in bipolar cell
245 precursors^{56,57}.

246 HOX is a common peptide found in transcription factors⁵⁸. Within the HOX peptide, residue 50 and its
247 surrounding amino acids are important for DNA binding⁵⁹. Residue 50 binds the DNA backbone,
248 while the surrounding residues bind to neighbor DNA bases. These important residues would
249 correspond to residue 200 and its surrounding amino acids in *VSX2* that would explain the pathogenic
250 mechanism associated with Arg200 missense variants leading to severe ocular malformations (micro-
251 and anophthalmia)^{34,60}. Computational structure-based evaluation of p.(Arg199Cys) variant predicts a
252 modification of *VSX2* binding to a neighbor base of targeted DNA sequence⁶¹. We hypothesize that
253 Arg199 residue is probably less crucial for protein function as a regulator of ocular morphogenesis but
254 is still required for correct bipolar cell differentiation and function. Indeed, in patients from family
255 F178 we are reporting that the p.(Arg199Cys) substitution leads to the CSNB phenotype without
256 microphthalmia. The CVC domain and OAR domain, both less commonly found in the structure of
257 transcription factors and found in proteins involved in neural tube and ocular development, are

258 supposed to be involved in the specificity of DNA recognition, protein-protein interactions and
259 stability⁶²⁻⁶⁴. The p.(Pro233Leu) variant found in CIC12548 is in the CVC domain. The pathogenic
260 variant described by Khan A. et al. c.773delA p.(Lys258Serfs*44)⁴³ is also located in the CVC domain
261 and leads to an ocular phenotype close to the phenotype of the subjects presented herein. Missense
262 variants in CVC domain could change the pattern of gene interactions due to impairment of specific
263 DNA-binding activity and lead to severe ocular phenotypes in mice⁶⁵ and humans³⁷⁻³⁹. It is unclear,
264 why mutations in this region lead either to microphthalmia/anophthalmia or the CSNB-like phenotype.
265 The 3D-structures of different VSX2 mutants were similar (data not shown). Different variants may
266 influence gene interactions differently leading to the variability of the VSX-associated phenotypes.

267 A naturally occurring mouse model carrying a nonsense variant in VSX2, p.Tyr176* (in the HOX
268 domain) is known under the term of ocular retardation phenotype (*Or*). *Or* mice are blind with
269 microphthalmia, congenital cataracts, and underdeveloped iris, retina and optic nerve⁶⁶⁻⁶⁸. They have
270 abnormal retinal development with reduced retinal progenitor proliferations and a complete absence of
271 bipolar cells⁶⁸. Dependent on the genetic background of the mice, the ocular phenotype could be
272 milder. In *modified Or* mice⁶⁹ with only mild microphthalmia and better visual behavior there was
273 evidence of underdeveloped ciliary body⁶⁹. Lenses became cataractous by 6 months of age. The retina
274 extended less in the periphery and did not reach the ciliary body root. Bipolar cells were present but
275 their number was severely reduced compared to wild-type mice. Bipolar cell connections were
276 abnormal: their dendrites extended into the outer nuclear layer, whereas the axons were not found in
277 the inner plexiform layer. ERG recordings of *modified Or* mice showed an electronegative response to
278 a bright flash (DA 5.0) in dark-adapted animals, resembling the ffERGs alteration of the patients
279 presented herein. We hypothesize that the underdeveloped ciliary body might be a cause for lens
280 subluxation. Peripheral retinal underdevelopment might be a cause of peripheral retinal alterations in
281 our patients. The depletion of the bipolar cell population and abnormal bipolar cell wiring might be
282 linked to a peculiar ffERG waveform, which combines features of incomplete and complete CSNB.
283 Future studies need to be performed to better understand the different phenotypes associated with

284 VSX2 variants. This study is limited to only two families, making it difficult to determine the
285 frequency or clinical relevance of this genetic defect.

286 **Conclusion.**

287 While only identified in three patients of two distinct families, the clinical phenotype of our patients
288 harboring missense variants in VSX2 is in accordance with retinal VSX2 expression/localization data
289 and resembles the phenotype reported in a *Vsx2* knock-out animal model. Here we describe a “pan-
290 bipolar cell” CSNB phenotype which might be due to the global lack and/or dysfunction of bipolar
291 cells

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298 **Author Contributions:** IA and CZ had full access to all the data in the study and takes responsibility
299 for the integrity of the data and the accuracy of the data analysis. IA and CZ contributed equally to the
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317 JAS has received personal fees from Pixium Vision, GenSight Biologics, SparingVision, Prophesee,
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321 **References.**

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512

Table 1. Clinical characteristics of patients.

Patient, age at 1 st assessment	BCVA, Snellen	Refraction	Axial lengt h, mm	Colour vision (15 hue Lantho ny)	Visual field	ffERG	Anterior segment	Fundus	SW-FAF	OCT
Family F178, VSX2: c.595C>T, p.(Arg199Cys)										
CIC00257, 30 y.o.	RE : 20/200 LE : 20/200 *** RE : 20/63 LE : 20/63af ter cataract surgery	RE: -15.0 LE: -15.0	RE: 28.76 LE: 28.23	Normal	GP: superior flattening at all target sizes SP: diffuse reduction of retinal sensitivity, MD : RE - 6.6dB, LE - 10.2dB FT : OU 25dB	DA 0.01: undetectable DA 3.0, DA 10.0: electronegative Schubert-Bornschein configuration LA 3.0, LA 3.0 flicker: severely	Superior lens subluxation OU Cataract OU Phaco- and iridodonesis	Oval pale tilted discs, peripapillary chorio- retinal atrophy, vascular narrowing, increased visibility of choroidal vasculature White glistening crystals, atrophic patches and retinal tears in peripheral retina	Normal	NA

IPP1889196, 4 y.o.	20/200 OU	RE:-10.50 (-1.50) 175° LE: -8.75 (-1.50) 155° cycloplegi c	RE: 24.77 LE: 24.43	NA	NA	DA 0.01: undetectable DA 3.0, DA 10.0: electronegative Schubert-Bornschein configuration LA 3.0, LA 3.0 flicker: reduced and delayed	Normal	Oval pale tilted discs, peripapillary chorio- retinal atrophy, vascular narrowing, increased visibility of choroidal vasculature White-without- pressure in peripheral retina	Normal	Preserved outer retina Thinned inner retinal layers
Family F7248, VSX2: c.698C>T, p.(Pro233Leu)										
CIC12548, 45 y.o.	RE: 20/50 LE: 20/100 after cataract surgery; *** RE: -2.75 (-3.0)0° LE: -3.0 (-	RE: +2.0 LE: +3.50 (-0.50) 90° after cataract surgery; *** RE: -2.75 (-3.0)0° LE: -3.0 (-	RE: 22.3 LE: 21.94	NA	SP: diffuse reduction of retinal sensitivity	DA 0.01: undetectable DA 3.0, DA 10.0: electronegative Schubert-Bornschein configuration LA 3.0, LA 3.0 flicker: reduced	Pseudophakic OU *** UBM: hypoplastic ciliary body *** Surgery for lens subluxation	Normal discs, mild vascular narrowing, vessels crossing fovea, increased visibility of choroidal vasculature, triangular zone of chorio- retinal atrophy pointing macula inferiorly	Tringular hypoAF lesion with hyperAF edges	Grade 3 foveal hypoplasia Disappeara nce of outer reflective layers (ONL, EZ, RPE)

		3.5) 25° before surgery ■■■■■■■■					and cataracts at 42 y.o. OU			correspond ing to atrophic fundus lesion
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BCVA – best corrected visual acuity, OU – *oculi utriusque*, both eyes, RE – right eye, LE – left eye, GP – Goldman perimetry, SP – automated static perimetry, MD – mean deficit, FT – foveal threshold, NA – not available, UBM – ultrasound biomicroscopy

Figure 1. Full field ISCEV-standard ERG. A, adult patients CIC00257 and CIC12548, B, patient IPP1889196. Undetectable responses to a dim flash under dark adapted condition (DA 0.01).

Electronegative waveform (b/a amplitude ratio < 1) to bright flashes under dark-adapted conditions (DA 3.0 and DA10.0). Severely reduced light adapted responses (LA 3.0 and LA 3.0 flicker).

Figure 2. Retinal findings in patients from family F178. Top, patient CIC00257. A-B, fundus photos. Myopic fundus abnormalities with oval tilted optic discs, temporal pallor and peripapillary chorio-retinal atrophy, narrowed retinal vessels and thinned retina with increased choroidal visibility. Peripheral snowflake degeneration and patches of chorio-retinal atrophy. . Bottom, patient IPP1889196. C, fundus photo. Myopic fundus abnormalities with oval titled optic disc, temporal pallor, narrowed retinal vessels, retinal thinning. D. SD-OCT, horizontal scans passing through fovea. Preserved outer retina with inner retinal thinning.

Figure 3, Retinal findings in patient from family F7248. Patient CIC12548. A, fundus photo. Normal optic disc, narrowed retinal vessels, a triangular zone of chorio-retinal atrophy inferior to the macula. B, Short-wavelength fundus autofluorescence. Zone of extinguished autofluorescence with hyperautofluorescent edges inferior to the macula. C, Infrared reflectance. Increased reflectance inferior to the macula. Vessels crossing fovea. D, SD-OCT, vertical scan passing through fovea. Grade 3 foveal hypoplasia. Epirteinal membrane. Loss of outer reflective layers in scan passing through the zone of chorioretinal atrophy.

Figure 4. A, pedigrees. Top, family F178. The c.595C>T variant in VSX2 co-segregated with disease in this consanguineous Turkish family. Bottom, family F7248. The c.698C>T variant was identified in one affected member for whom other family members were unavailable for co-segregation analyses.

B, schematic VSX2 gene (top) and protein (bottom) structure. Microphthalmia/anophthalmia-associated variants are listed above and retinal dysfunction associated variants are listed below the schematic gene structure. In addition, the consequences on protein levels are depicted and the schematic protein structure given. Variants identified by us are highlighted in red. The different

domains of VSX2 are indicated as following: HOX – homeobox domain, CVC - CHX10, VSX1 and CEH10 domain, OAR - OTP, aristaless and RAX domain. Amino acid residue positions of each domain are depicted in green, light-blue and red, respectively.

Figure 1

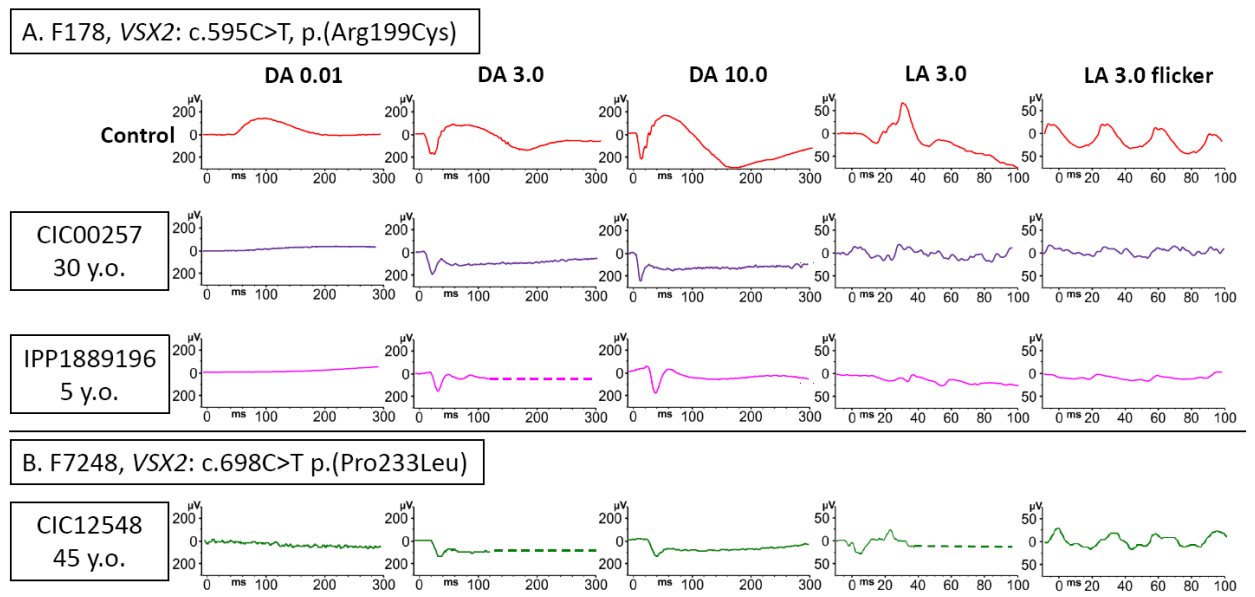


Figure 2

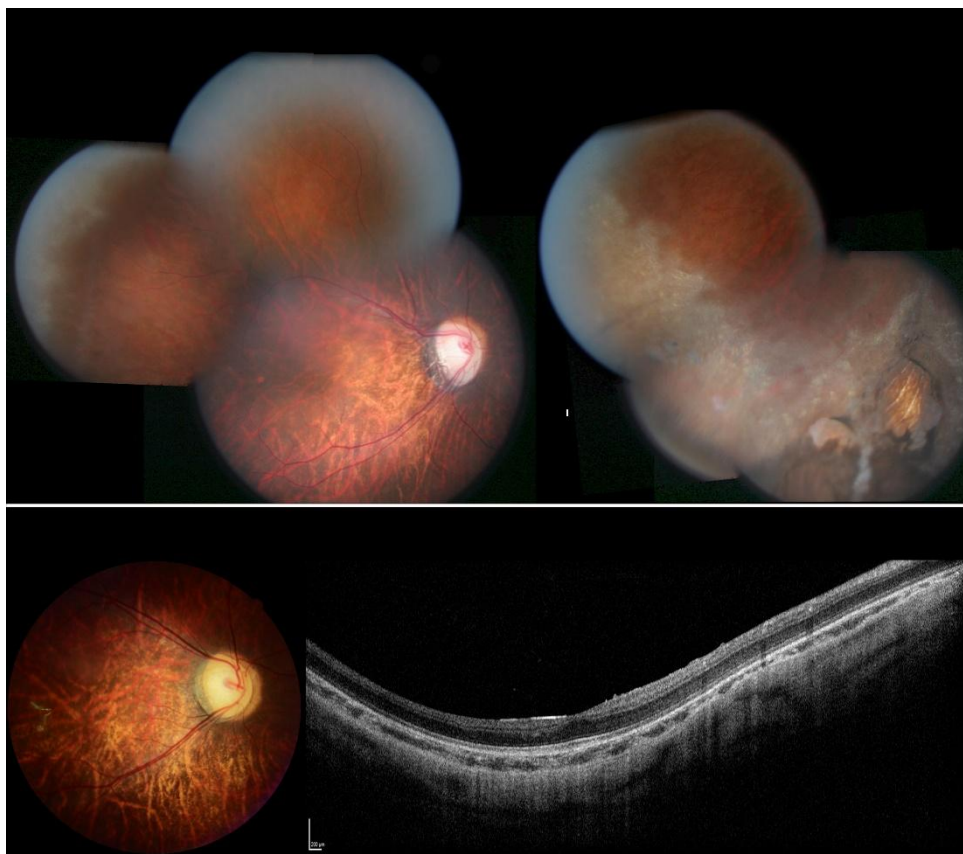


Figure 3

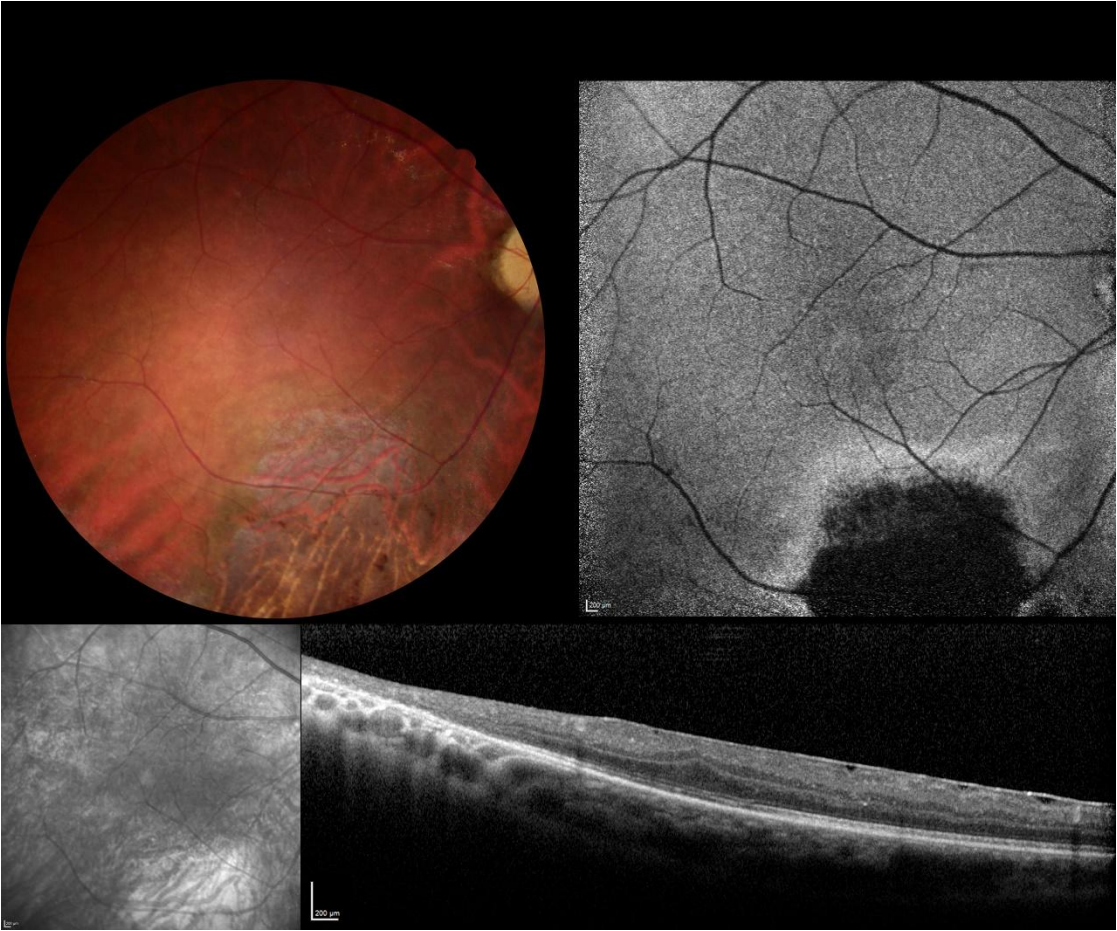


Figure 4

