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Retinitis pigmentosa (RP), a clinically and genetically heterogeneous retinal disease, entails abnormalities of the rod and cone photoreceptors or the retinal pigment epithelium, leading to progressive visual loss (1). Since RP predominantly affects the rods, the visual impairment is usually manifested as night blindness and progressive loss of the visual field. The cones are usually affected at a later stage, allowing preservation of the patient's daytime vision for several years or even decades (1). More than 50 different genes or loci are involved in cases of non-syndromic RP (2). One of these, *CNGB1*, a gene encoding the beta-subunit of the cGMP-gated channel of the rods, is involved in an autosomal recessive form of non-syndromic RP (RP45, MIM 613767). Here, we present the first case of RP45 caused by an unequivocal homozygous nonsense variant of *CNGB1* identified by run of homozygosity (ROH) analysis. In addition, we review the pathogenic variants of *CNGB1* as reported to date (Table 1) (3-13).

Table 1. Pathogenic variants of the *CNGB1* gene reported in the literature according to NCBI Reference Sequence: NM_001297.4.

Reference	Affected individuals	Age of onset	Clinical course	*CNGB1* variants [hg19] ADN	p
Bareil et al.[3]	3 siblings	Childhood	Visual field severely reduced at age 30 y	c.2978G>T; homozygous	p.Gly993Val
Kondo et al.[4]	1 male	Childhood	At age 67 y: Slowly progressive	c.3463+1G>A; homozygous	p.?
Simpson et al.[5]	1 individual	NA	NA	c.2957A>T; homozygous	p.Asn986Ile
Azam et al.[6]	2 families with several affected individuals	1st or 2nd decade	NA	c.2284C>T; homozygous c.412+1G>A homozygous	p.Arg762Cys p.?
Schorderet et al.[7]	1 individual	NA	NA	c.2293C>T homozygous	p.Arg765Cys
Bocquet et al.[8]	2 male siblings	10 y	-1st individual at age 44 y: OD: visual acuity 0.3; OS NLP (ocular phtisis) -2nd individual at age 57 y: OD/OS: LP/LP	c.2284C>T; homozygous	p.Arg762Cys
Fu et al.[9]	1 male	NA	At age 40y: OD/OS BCVA: 0.1/LP	c.1589C>G; homozygous	p.Pro530Arg
Nishiguchi et al.[10]	2 female siblings	NA	NA	c.1896C>A; c.3150del;	p.Cys632*p. Phe1051Leufs*12
Xu et al.[11]	1 male	NA	At age 34 y: OD/OS BCVA: 0.5/0.4	c.2361C>A; c.2888_2889del;	p.Tyr787* p.Phe963Serfs*4
Maria et al.[12]	1 female	NA	NA	c.2493-2A>G	p.?
Saqib et al.[13]	1 male and 1 female siblings	1st decade	The male individual at age 24 y: NLP	c.413-1G>A	p.?
Present study	1 female	Childhood	At age 51 y: BCVA (ETDRS charts) 20/25 both eyes	c.939G>A; homozygous	p.Trp313*

BCVA, best corrected visual acuity (on the Snellen decimal scale if not otherwise specified); OD, right eye; OS, left eye; LP, light perception; NLP, no light perception; y, year; NA, not available

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The pedigree of the family of French origin is shown in Figure 1. The family history revealed the consanguineous marriage of the parents (f = 1/32) but there was no history of RP.

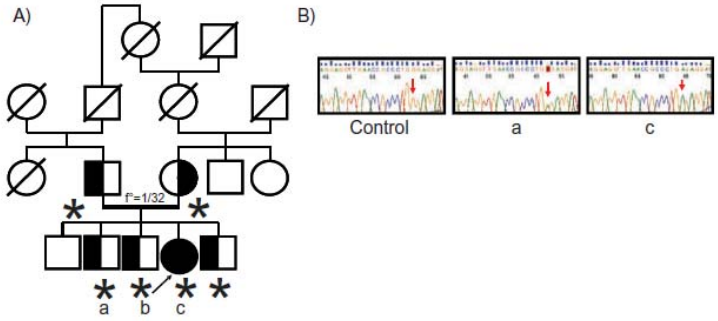

Figure 1. (A) Pedigree of the family showing the consanguinity loop. The arrow indicates the proband, and the asterisks indicate the individuals for whom DNA was available. Half-filled circles and squares represent heterozygous individuals, filled circle the homozygous proband. (B) DNA sequence of *CNGB1* (GenBank NM_001297.4, NC_000016.9) from an unaffected brother (a) and from the affected index case (c).

The affected individual was a 51-year-old female who had complained of disturbances in night vision since early childhood. RP, diagnosed at age 23, had led to severe constriction of the visual field by age 45. She had no other medical history and, in particular, no olfactory impairment. She had undergone cataract surgery at age 50 with significant visual improvement. The best corrected visual acuity, assessed using the ETDRS chart, was 20/25 in both eyes, with optical correction of plano (−50)30° in the right eye and +0.25(−1.50)175° in the left eye. Fundus examination revealed a waxy pallor of the optic discs, slightly narrowed retinal vessels, and pigmentary changes in the fundus periphery, which relatively spared the macular region (Figure 2A). Fundus autofluorescence showed a high density perimacular ring characteristic of RP (Figure 2B). The macular function was preserved as confirmed by spectral domain optical coherence tomography, which showed normal macular structure (Figure 2C). The visual field was constricted in both eyes and the binocular kinetic visual field, using the III4e stimulus, was reduced to the central 10°. The full-field electroretinogram, performed according to the ISCEV standard, did not reveal any detectable response.

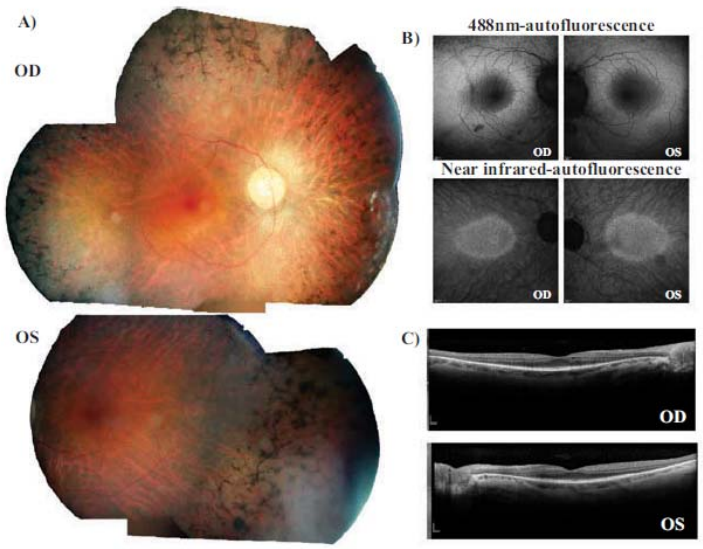

Figure 2. Fundus examination of the proband. (A) Fundus of the right (Ocula Dextra, OD) and left (Ocula Sinistra, OS) eyes showing typical features of rod-cone dystrophy including pigmentary changes in the peripheral retina with preservation of the macular area, narrowing of retinal blood vessels and waxy disc pallor; (B) Fundus autofluorescence at 488nm and in near infrared revealing patchy loss of autofluorescence outside the vascular arcade and a perifoveal ring of hyper-autofluorescence with macular preservation; and (C) Spectral Domain Optical Coherence tomography horizontal scan showing the normal foveal lamination.

DNA samples were obtained from the proband and two of her unaffected brothers. Informed consent was obtained from all participants prior to their participation in this study.

ROH analysis was performed using an Infinium HumanCytoSNP-12v2H array (Illumina, San Diego, CA, USA) containing 220,000 single nucleotide polymorphisms (SNPs) with an average spacing of 30kb. The results were interpreted using the Illumina GenomeStudio software v2011.1 and the Copy Number Variation partition v3.1.6. The *CNGB1* gene sequence was analyzed using PCR and subsequent direct bidirectional sequencing of the coding regions (GenBank NM_001297.4, NC_000016.9) and the flanking intronic regions.

Analysis of the SNP array revealed that 10 ROHs (43Mb) were present in the affected individual but absent in both unaffected brothers. The largest ROH was a 24Mb interval in chromosome 16q12.1q23 (52,325,676-76,532,392). This ROH contained 595 genes including 176 OMIM genes among which only *CNGB1* was known to be involved in RP. Subsequent Sanger sequencing of *CNGB1* showed that the proband was homozygous for the nonsense variant c.939G>A; p.Trp313*. Her healthy parents as well as two of her healthy brothers were heterozygous for this variant, which was not found in 120 ethnically matched control chromosomes (Figure 1). Although variant softwares are inappropriate for analyzing the consequences of nonsense mutations, an evaluation using the Alamut software predicts that the RNA containing c.939G>A encodes for a truncated protein deprived of all its functional domains. Moreover, this variant is referenced neither in the Single Nucleotide Polymorphism database nor in the Exome Aggregation Consortium database. Nonetheless, it is of note that a fairly similar variant of *CNGB1*, namely c.952C>T; p.Gln318*, has been referenced as pathogenic in the ClinVar database (www.ncbi.nlm.nih.gov/clinvar/).

The involvement of *CNGB1* in RP was first reported in 2001 using homozygosity mapping to identify a homozygous variant (c.2978G>T) in three siblings affected with non-syndromic RP (3). To date, only 13 pathogenic *CNGB1* variants have been reported in 12 families (Table 1) (3-13). Although clinical details are lacking in some reports and the methods used for evaluating visual impairment differ from one publication to another, it appears that RP45 has

an early onset with a rather slow progression (4), but no genotype-phenotype correlation has been established thus far.

RPs are highly heterogeneous genetic conditions with mutations in more than 50 different genes or loci, making the delineation of the underlying molecular defect challenging for both patient and clinician. Indeed, establishing a molecular diagnosis is a prerequisite to preventive measures such as genetic counseling, prenatal diagnosis, and preimplantation diagnosis. Moreover, in the near future, the precise determination of the molecular defect responsible for RP will be of major importance in determining the eligibility of patients for clinical gene therapy trials relevant to their specific mutations.

Finally, this study shows that ROH analysis is a clinically relevant tool for performing a genotype-first identification of recessive genes responsible for RP.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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