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Case Report

GNB1-Related Rod-Cone Dystrophy: A Case Report

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Keywords

GNB1 · Rod-cone dystrophy · Retinitis pigmentosa · Inherited retinal disease · Case report

Abstract

Introduction: The *GNB1* (guanine nucleotide-binding protein, β1) gene encodes for the ubiquitous β1 subunit of heterotrimeric G proteins, which are associated with G-protein-coupled receptors (GPCRs). *GNB1* mutations cause a neurodevelopmental disorder characterized by a broad clinical spectrum. A novel variant has recently been confirmed in a case of rod-cone dystrophy. ***Case Presentation:*** We describe the second confirmed case of a classical rod-cone dystrophy associated with a mutation located in exon 6 of *GNB1* [NM_002074.5:c.217G>C, p.(Ala73Pro)] in a 56-year-old patient also presenting mild intellectual disability, attention deficit/hyperactivity disorder, and truncal obesity. ***Conclusion:*** This paper confirms the role of *GNB1* in the pathogenesis of a classic rod-cone dystrophy and highlights the importance of including this gene in the genetic analysis panel for inherited retinal diseases.

Giovanni Marco Conti and Francesca Cancellieri contributed equally to this work.

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Introduction

The *GNB1* gene encodes for the ubiquitous β1 subunit of heterotrimeric G proteins, which are associated with G-protein-coupled receptors. It forms a functional complex with the α and γ subunits and is involved in a variety of cellular functions [1]. G-protein-coupled receptor binding to its own ligand promotes dissociation between the subunits and enables the β1 subunit to activate the signal cascade and its various effectors [1]. Despite *GNB1* being ubiquitous, it is highly expressed in the outer segments of rod photoreceptors and in the brain [2–4]. In rods, together with $Gt\alpha_1$ (G protein subunit alpha transducin 1) and $Gt\delta_1$ (G protein subunit gamma transducin 1), the GNB1 protein forms transducin and is therefore crucial for phototransduction and for the general physiology of this cell type [5].

GNB1 germline mutations have been associated with an autosomal dominant neurodevelopmental disorder characterized by a large clinical spectrum [6–10]. Neurological impairments include global developmental delay, impaired intellectual development, seizures, ataxia, delayed myelination, cerebellar hypoplasia, hypotonia, and limb hypertonia. Patients with *GNB1* mutations often present also with failure to thrive and with craniofacial, genito-urinary, and skeletal anomalies. Finally, it is not uncommon for some individuals to suffer from ocular conditions such as strabismus, nystagmus, impaired smooth pursuit, cortical visual impairment, and ophthalmoplegia (OMIM: # 616973) [6, 7, 11]. It is important to note that the majority of patients with *GNB1* mutations were reported to display signs and symptoms at a young age [6–8, 11]. In addition, somatic heterozygous *GNB1* mutations have been associated with hematologic malignancies, including acute lymphoblastic leukemia (OMIM: # 613065) and myelodysplastic syndrome (OMIM: # 614286) [12].

GNB1 is considered to be a good candidate gene for retinal dystrophies in humans. Recently, a novel missense heterozygous variant in exon 8 of *GNB1* [NM_002074.5:c.437T>G, p.(Leu146Arg)] was identified in association with a classic phenotype of retinitis pigmentosa in a 45-year-old woman also suffering from developmental disorders, apraxia, and moderate intellectual disability [13]. Previously, Hemati et al. [11] reported the case of a patient with neurodevelopment delay, lateral nystagmus, and abnormal full-field electroretinography (ffERG) suggestive of rod-cone dystrophy that has not been confirmed, in which genetic analysis identified a de novo NM_002074.5:c.239T>C, p.(Ile80Thr) variant. Furthermore, it has been shown that a murine strain with dominant retinal degeneration carried a large inversion on chromosome 4 causing the inactivation of *Gnb1* [14]. We report here the second case ever described of a person with rod-cone dystrophy, who also presented mild intellectual disability, ADHD, truncal obesity and a heterozygous mutation in *GNB1*, confirming the involvement of this gene in hereditary retinal diseases.

Case Report

The patient was managed by ophthalmologists of the Jules-Gonin Eye Hospital (JGEH) genetics service and, after taking a comprehensive past and family medical history, underwent a full functional and anatomical investigation. Best-corrected visual acuity and uncorrected visual acuity were assessed according to the logMAR chart and slit-lamp biomicroscopy and intraocular pressure measurements were performed. Color fundus photographs, short wavelength fundus autofluorescence, enhanced depth imaging spectral-domain optical coherence tomography, functional examinations such as visual field test and ffERG were carried out. The investigations were mostly performed in accordance with the ERG standards of the International Society of Clinical Electrophysiology of Vision (ISCEV) [14]. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000537997).

DNA samples of the proband and family members were obtained from whole-blood or saliva samples. Whole-exome sequencing was performed on the proband's DNA at CeGaT GmbH (Tübingen, Germany), where sequencing libraries were generated using the Twist Human Core Exome Plus kit (Twist Bioscience), following the manufacturer's protocols. Libraries underwent paired-end sequencing on a Novaseq 6000 (Novogene, CeGaT) resulting in sequences of 100 bases. The average coverage was higher than 150X in targeted regions and resulting in ~90% of targeted regions with a coverage higher than 20X.

Mapping, variant calling, and variant annotation were performed using standard tools and in-house scripts as previously described [15]. The variant was classified following the recommendations of the American College of Medical Genetics (ACMG) [16]. Homozygosity mapping was done using AutoMap [17].

The *GNB1* variant was confirmed by Sanger sequencing and co-segregation analysis was performed in all available family members of the proband. Specifically, Primer3Plus [18] was used to design primers for polymerase chain reactions, performed using the GoTaq polymerase (Promega) and approximately 2 ng of template DNA, according to the manufacturers' protocol, and the following primer pair: 5′-tctgggttttagtctgttgctc-3′ (CR-7973); 5′-gctgccttccct atcctgta-3′ (CR-7974), enabling the amplification of a 250bp fragment. Polymerase chain reaction products were treated with ExoSAP-IT (ThermoFisher) and Sanger sequencing was performed by Microsynth (Balgach, Switzerland). Sequences were visualized with the CLC Genomics Workbench 12 software (QIAGEN).

Clinically, our patient is a Caucasian woman affected by mild intellectual disability, ADHD and truncal obesity. The clinical diagnosis of terminal rod-cone dystrophy has been made at the age of 56 years, but the patient had been complaining of symptoms for some years before.

Family history was negative for cases of binocular visual impairment or genetic syndromes. The father suffered from amblyopia. The parents were deceased at the time of diagnosis, and the patient's only sister is healthy. LogMAR best-corrected visual acuity was 0.2 in the right eye and 0.3 in the left. Slit-lamp examination revealed bilateral posterior cortical cataract, the ocular fundus characterized by bilateral reduced caliber of arterial vessels, bilateral "salt and pepper" retinopathy, and decreased foveolar reflex in the left eye caused by cystoid macular edema (shown in Fig. 1a, b).

Enhanced depth imaging spectral-domain optical coherence tomography confirmed left cystoid macular edema and loss of parafoveolar photoreceptors in both eyes. The thickness of the choroid is slightly greater in the eye with cystoid macular edema (left eye) than in the right eye but otherwise still within normal range upon age [19] (shown in Fig. 1e, f). Macular fundus autofluorescence was normal in the central 10° but was encircled by a hypoautofluorescent ring surrounded by a hyperautofluorescent zone delimited by vascular arcades; outside the latter, the retina was characterized by confluent hypofluorescent spots (shown in Fig. 1c, d). The visual field was restricted to the central 10°; the scotopic ffERG and the flicker 30 Hz were not recordable while the photopic ffERG showed a severely decreased amplitude of the b wave with a significant delay of culmination time.

The negative family history and absence of consanguinity, after the analysis of regions of homozygosity, guided our analysis toward an autosomal recessive or a de novo dominant pattern of inheritance. First, we analyzed all variants in genes that were already clearly associated with IRDs but found no pathogenic DNA change or good candidate gene. We therefore extended the analysis to all coding genes of the human genome and identified a heterozygous variant in *GNB1*: NM_002074.5:c.217G>C, p.(Ala73Pro) (chr1:1737964C>G, hg19). This variant was the only one detected to have a predicted impact at the protein level, to be absent from databases of healthy individuals (https://gnomad.broadinstitute.org/), to have a very high DOMINO score (0.999, out of a max of 1.000, indicative of autosomal dominant inheritance) [20] and a very high MutScore (0.991, out of a max of 1.000, indicative of high deleteriousness of the missense change) [21]. The

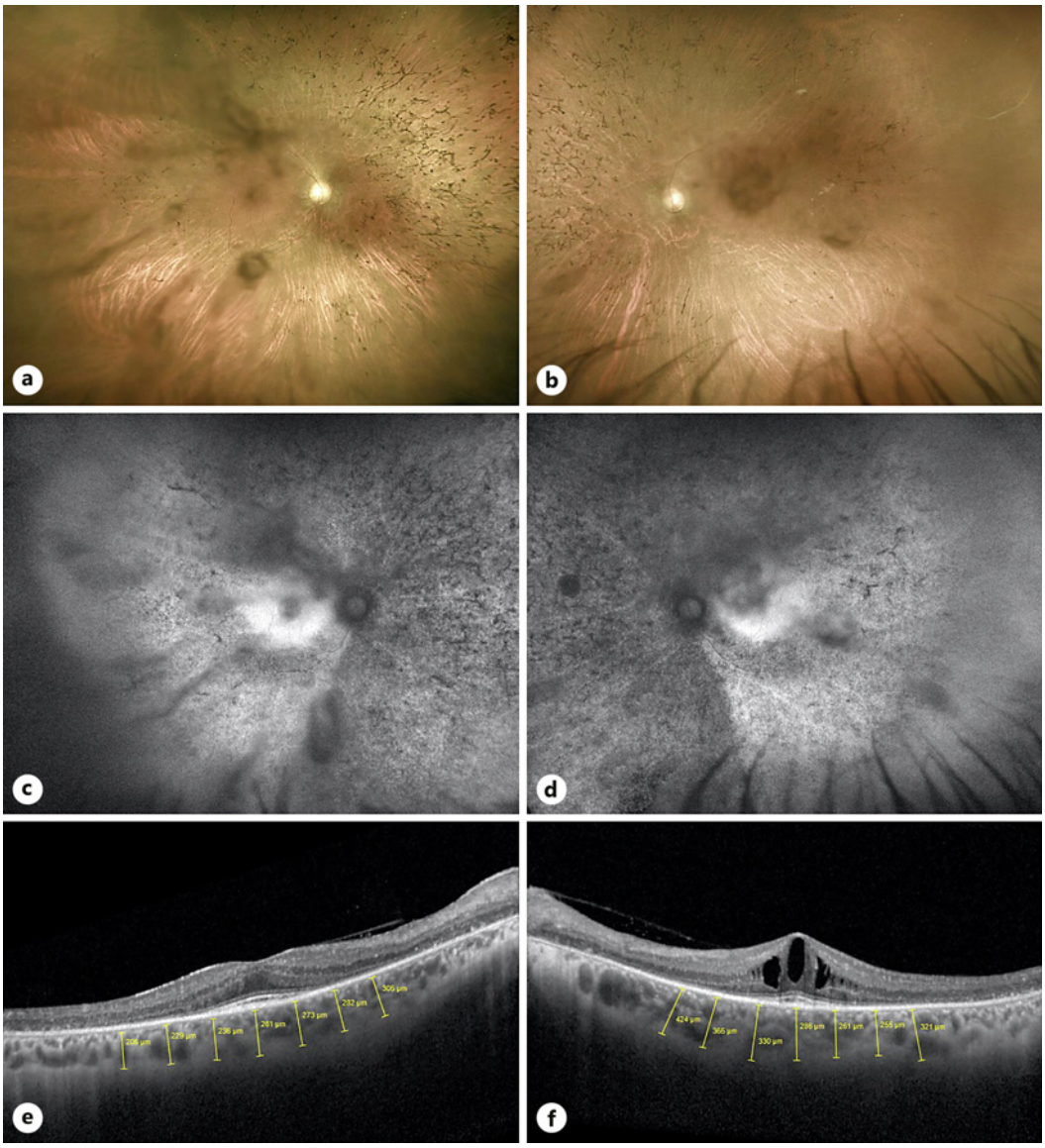
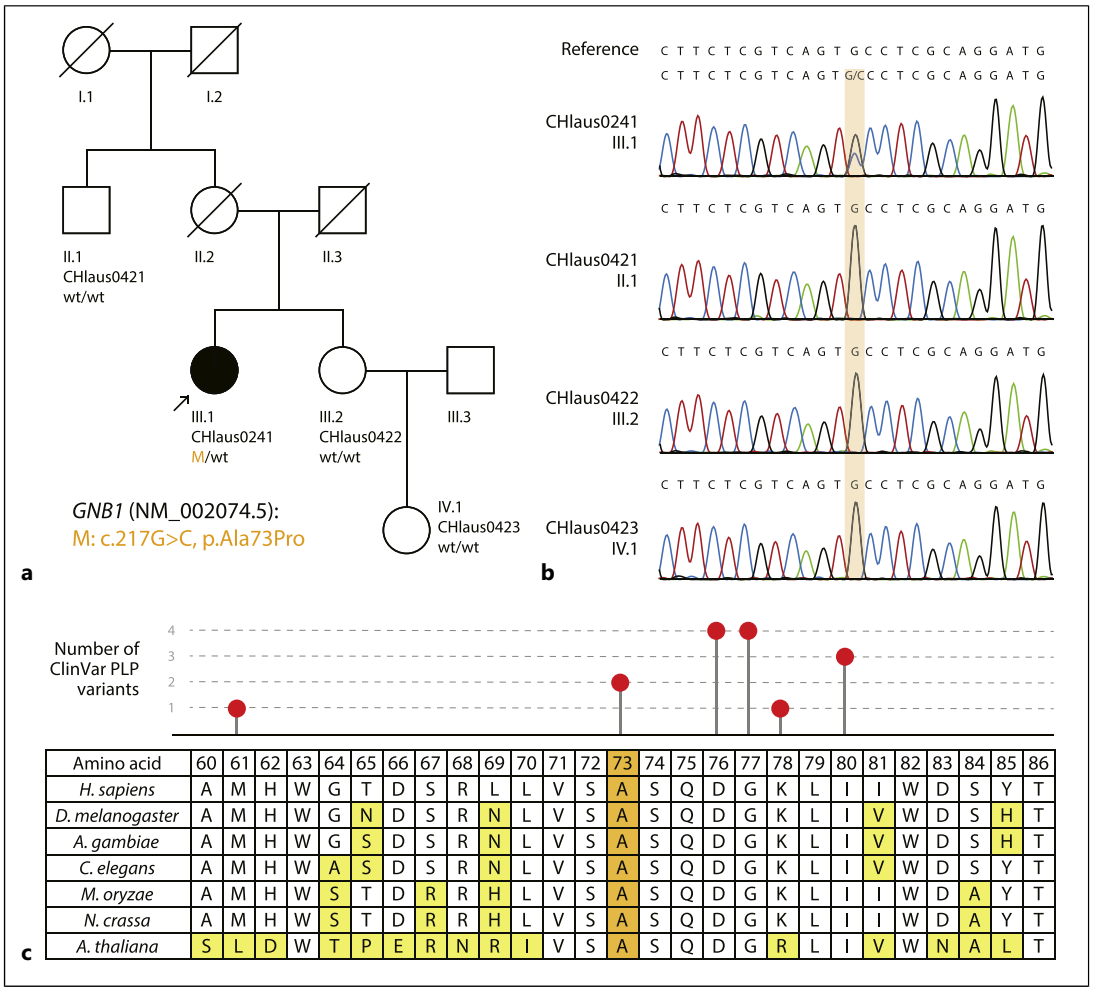


Fig. 1. a, **b** Fundus photographs demonstrating bilateral reduced caliber of arterial vessels, bilateral "salt and pepper" retinopathy. **c**, **d** Fundus autofluorescence (AF) photographs characterized by a hypoautofluorescent ring encircling the normal central 10° surrounded by a hyperautofluorescent zone delimited by vascular arcades and confluent hypofluorescent spots outside the latter. **e**, **f** EDI SD-OCT confirmed left cystoid macular edema and loss of parafoveolar photoreceptors in both eyes. The thickness of the choroid is slightly greater in the eye with cystoid macular edema (left eye) than in the right eye but otherwise still within normal range upon age.

amino acid affected by the genetic change, alanine 43, was conserved in 46 vertebrates and was located in an α-helix secondary structure, in which its substitution by a proline would be likely disruptive. Co-segregation analysis in the family revealed that the variant was absent from all available unaffected family members of the proband (shown in Fig. 2a, b), suggesting potential de novo inheritance, as it was the case for the two *GNB1*-associated ocular cases reported previously [13]. However, in our case de novo inheritance could not be proven experimentally since both parents of the probands are deceased. The variant was classified as likely pathogenic using the ACMG classification for the neurological phenotype, according to criteria PM1, PM2,



Amino acid	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
H. sapiens	A	M	H	W	G	T	D	S	R	L	L	V	S	A	S	Q	D	G	K	L	I	I	W	D	S	Y	T
D. melanogaster	A	M	H	W	G	N	D	S	R	N	L	V	S	A	S	Q	D	G	K	L	I	V	W	D	S	H	T
A. gambiae	A	M	H	W	G	S	D	S	R	N	L	V	S	A	S	Q	D	G	K	L	I	V	W	D	S	H	T
C. elegans	A	M	H	W	A	S	D	S	R	N	L	V	S	A	S	Q	D	G	K	L	I	V	W	D	S	Y	T
M. oryzae	A	M	H	W	S	T	D	R	R	H	L	V	S	A	S	Q	D	G	K	L	I	I	W	D	A	Y	T
N. crassa	A	M	H	W	S	T	D	R	R	H	L	V	S	A	S	Q	D	G	K	L	I	I	W	D	A	Y	T
A. thaliana	S	L	D	W	T	P	E	R	N	R	I	V	S	A	S	Q	D	G	R	L	I	V	W	N	A	L	T

Fig. 2. Characteristics of the *GNB1* missense variant identified. **a** Family pedigree showing co-segregation of this variant in the family and respective electropherograms (**b**). **c** Location and number of ClinVar pathogenic or likely pathogenic (PLP) missense in proximity of alanine 73, as well as protein alignment of this region of GNB1 in selected non-vertebrates, showing in yellow the non-conserved amino acids among the species.

PM5, PP2, and PP3, the PM1 criterion being applicable since the variant lies within a mutational hotspot (shown in Fig. 2c). The variant also fulfilled the PM5 criterion since ClinVar included two entries for likely pathogenic variants affecting the same amino acid (Fig. 2c). With respect to the retinal phenotype, the variant was considered as a variant of uncertain significance, with criteria PM2, PP2, and PP3 being used.

Discussion

The *GNB1* gene encodes for the β_1 subunit of transducin ($G t \alpha_1 \beta_1 \delta_1$), involved in the phototransduction process that takes place in the outer segments of photoreceptors. In rods, this process starts when light hits the 11-cis-retinal rhodopsin ligand, which isomerizes to form all-trans retinal. In turn, this conformational change activates the rhodopsin molecule, which stimulates the exchange of guanosine diphosphate with guanosine triphosphate in the

$Gt\alpha_1$ subunit. Then, $Gt\alpha_1$-guanosine triphosphate activates the phosphodiesterase PDE6, the effector enzyme, which hydrolyzes cyclic guanosine monophosphate. This results in the closure of the cyclic guanosine monophosphate-gated channels of the plasma membrane of the photoreceptor outer segment with a consequent hyperpolarization of the cell and thus the production of a neuronal response to light [22].

We report the second confirmed case of rod-cone dystrophy associated with a *GNB1* mutation. Staging of the disease is classic as retinitis pigmentosa patients suffer from severe visual impairment at approximately 50 years of age.

Our patient presented some similarities with the first reported case [13]. Both patients are middle-aged women with mild intellectual disability without seizures, had a classic phenotype of rod-cone dystrophy with advanced loss of peripheral vision, subcapsular cataracts, and bilateral parafoveolar photoreceptor loss. In contrast to the previously published case, our patient presented macular involvement with cystoid edema. In addition, the diagnosis of retinal dystrophy was made later in life, while the other patient had already been diagnosed at the age of 5 years, although the disease was stable for several decades before and started to show signs of progression around the age of 40 years. The mutation we report is located in exon 6 (c.217G>C, p.Ala73Pro), while the previously described patient had a mutation in exon 8 (c.437T>G, p.Leu146Arg).

Interestingly, our subject's mutation localizes to the mutational hotspot in exons 6 and 7, where 88% of all mutations linked to neurodevelopmental disorder lie [23]. In particular, the mutation identified in this work (c.217G>C, p.Ala73Pro) affects the same codon found to be mutated in a 15-year-old female reported to be suffering from behavioral disorder (ADHD), mild to moderate intellectual disability, and mixed language disorders, i.e., c.217G>A, p.Ala73Thr [23]. No visual symptoms were described in this subject, and neither she nor our patient had major neurological disorders such as seizures [23].

Finally, and most importantly, all reported *GNB1* cases who presented with no visual symptoms were younger than 20 years, whereas the 2 patients with IRD were in their 40s and 50s. Therefore, it is still unclear whether some mutations in *GNB1* are linked to IRDs while other mutations in *GNB1* are not, or if all individuals with *GNB1* mutations would develop retinal dystrophy later in life. Certainly, it is important to report such cases to increase the awareness that *GNB1* could further cause retinal phenotypes.

Acknowledgment

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Statement of Ethics

This case report was designed in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of "Commission cantonale d'éthique de la recherche sur l'être humain (CER-VD)" (Authorization CER-VD n° 340/15) and by the Ethikkommission Nordwest-und Zentralschweiz. Written informed consent was obtained from the patient for publication of the details of their medical case and any accompanying images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Substantial contributions to the conception and design of the work: H.V.T. and C.R. Analysis and interpretation: G.M.C., F.C., C.R., M.Q., K.K., and H.V.T. Data collection: V.V., C.R., and H.V.T. Drafting of the work: G.M.C., F.C., and H.V.T. Review of the work and final approval of the version: G.M.C., F.C., C.R., V.V., M.Q., K.K., and H.V.T.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author (H.V.T.). The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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