Recessive Retinopathy Consequent on Mutant G-Protein β Subunit 3 (GNB3)

Gavin Arno, PhD; Graham E. Holder, MSc, PhD; Christina Chakarova, PhD; Susanne Kohl, PhD; Nikolas Pontikos, PhD; Alessia Fiorentino, PhD; Vincent Plagnol, MSc, PhD; Michael E. Cheetham, PhD; Alison J. Hardcastle, PhD; Andrew R. Webster, MD (Res), FRCOphth; Michel Michaelides, MD (Res), FRCOphth; for the UK Inherited Retinal Disease Consortium

Many inherited retinal diseases are associated with mutation of genes encoding proteins involved in phototransduction and consequent signaling within the retina (RetNet; http://www.sph.uth.tmc.edu/RetNet/). This report describes a human retinopathy associated with a homozygous null mutation in the G-protein β subunit 3 (GNB3) gene, identified using whole-exome sequencing. The GNB3 gene encodes the G-protein β subunit 3 (Gβ3), involved in the signaling of mammalian cone photoreceptors and ON-bipolar cells. To our knowledge, GNB3 has not previously been implicated in human disease, although a homozygous GNB3 mutation can cause retinal dystrophy in chickens.

Methods

The proband underwent full ophthalmic examination including dilated retinal examination and color fundus photography (Topcon Great Britain Ltd and Optos plc), spectral-domain optical coherence tomography and fundus autofluorescence imaging (Spectralis; Heidelberg Engineering Ltd), and Goldmann visual field testing. Full-field electroretinography (ERG) was performed using gold-foil electrodes to incorporate the International Society for Clinical Electrophysiology of Vision standard responses but pattern ERG was recorded using surface electrodes and a 24 × 30° field owing to nystagmus. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the National Health Service Health Research Authority Committee London, Camden, and Islington. Parental informed written consent was provided.

Supplemental content at jamaophthalmology.com

Results

The proband, a male child with several siblings (GC20578) born to Somali parents, initially presented several years earlier with horizontal nystagmus and intermittent convergent squint. His parents reported difficulties with near vision and no night blindness. Best-corrected visual acuity was 20/40 OU (0.3 LogMAR). A high hypermetropic refractive error was identified (+7.50 DS OU) and was prescribed. Visual acuity did not improve over time despite correction, with a small left/alternating convergent squint noted. At last follow-up several years after his initial presentation, his best-corrected visual acuity wearing +6.25/−0.50 × 180 in both eyes was 20/40 OD and 20/100 OS (0.7 LogMAR), with no change in symptoms.
Dilated retinal examination and color fundus photography findings were unremarkable (Figure 1). Spectral-domain optical coherence tomography and fundus autofluorescence imaging were suggestive of bilateral central macular disturbance given both the smaller size and less reduction in fundus autofluorescence of the macular hypautofluorescent area than would be expected and less foveal cone outer segment lengthening associated with central mild inner segment ellipsoid layer interruption on spectral-domain optical coherence tomography (Figure 1). Goldmann visual field testing revealed relatively intact isopters to the larger, brighter targets, with mild constriction to smaller, dimmer targets to a greater extent in the right eye than the left (Figure 1). Electrophysiological assessment showed a reduced rod-specific (dark adapted, 0.01)

Key Points

**Question** What genes are associated with retinal dystrophies in extensively prescreened patients?

**Findings** A collaborative next-generation sequencing study identified a homozygous nonsense mutation of \( \text{GNB3} \) in a patient with unusual retinal dysfunction.

**Meaning** To our knowledge, this is the first report of human \( \text{GNB3} \) mutation associated with an inherited retinal disease presenting in childhood with a distinct phenotype characterized by nystagmus, a normal ophthalmoscopic examination, mild disturbance on detailed retinal imaging, and distinctive electrophysiographic findings.

Figure 1. Retinal Examinations

A and C, Color fundus imaging showing normal central and peripheral retinal appearance. B, Spectral-domain optical coherence tomography showing mild central inner segment ellipsoid interruption and reduced foveal cone outer segment lengthening. D, Fundus autofluorescence imaging showing less reduction than expected of the central macular hypautofluorescent area. E, Goldmann visual fields reveal relatively intact isopters to the larger, brighter targets, with mild constriction to smaller, dimmer targets to a greater extent in the right eye than the left.
Besubjecttononsense-mediateddecay.However,shouldatruncatedmessengerRNAtranscriptislikelyto
occurinexon0.000009).Theprematureterminationcodonoccursinexon
was noted in the ExAC database (minor allele frequency =
sequenceandisexpectedtobehaveasatruenull;only1allele
Supplement).ThiswasconfirmedbybidirectionalSangerse-
3:c.124C>T;andp.Arg42Ter)(eAppendix2andeTable1inthe
whole-genome sequencing revealed no further mutations in
was noted in the ExAC database (minor allele frequency =

The numbers refer to the stimulus strength in cd.s.m−2 as recommended by the International Society for Clinical Electrophysiology of Vision. See the Results section for full details. DA indicates dark adapted; LA, light adapted.

Figure 2. Full-Field Electroretinography (ERG) and Pattern ERG (PERG)

A \hspace{1cm} \text{Right eye}

B \hspace{1cm} \text{Left eye}

C \hspace{1cm} \text{Normal}

response; an electronegative bright-flash dark-adapted ERG
(dark adapted, 10.0) with normal amplitude but delayed a-
wave; a profoundly delayed 30-Hz flicker ERG of subnormal
amplitude; and a markedly delayed and reduced single-flash photopic ERG (light adapted, 3.0) of markedly altered waveform (Figure 2). Pattern ERG was bilaterally profoundly subnormal.

Whole-exome sequencing revealed a likely homozygous nonsense mutation in GNB3 (Chr12: 6952161C>T; NM_002075.3: c.124C>T; and p.Arg42Ter) (eAppendix 2 and eTable 1 in the Supplement). This was confirmed by bidirectional Sanger sequencing and is expected to behave as a true null; only 1 allele was noted in the ExAC database (minor allele frequency = 0.000009). The premature termination codon occurs in exon 4 of 11 and the truncated messenger RNA transcript is likely to be subject to nonsense-mediated decay. However, should a transcript survive, the protein is terminated before the first of 7 WD40 consensus sequences and would be rendered nonfunctional.

Subsequent Sanger sequencing of GNB3 coding exons or whole-genome sequencing revealed no further mutations in 13 patients exhibiting electrophysiological similarity to enhanced 5-cone syndrome, 16 patients with congenital stationary night blindness (CSNB), and 213 patients with other retinopathy (189 complete and incomplete achromatopsia, 9 stationary cone dysfunction syndromes, and 15 cone dystrophy). Polymorphisms and rare sequence variants observed are summarized in eTable 2 in the Supplement.

Discussion

G-protein β subunit 3 is expressed in cones and ON-bipolar cells in the mammalian retina, where it forms the heterotrimeric G-protein second messenger of the metabotropic receptor coneopsin and mGlu6R in cones and ON-bipolar cells, respectively. Knockout of Gnb3 (Gnb3−/−) in mice results in ERG abnormalities exhibiting as a partial loss of ON-bipolar cell sensitivity and downregulation of signal cascade protein expression including mGluR6, Goo, Gy13, and Trpml. Furthermore, the absence of Gβ3 in mouse cones leads to reduced expression of the Gαt2 and Gγt2 subunits and corresponding lossof cone
terference and downregulation of signal cascade protein expression including mGluR6, Goo, Gy13, and Trpml. Furthermore, the absence of Gβ3 in mouse cones leads to reduced expression of the Gαt2 and Gγt2 subunits and corresponding loss of cone response sensitivity. The murine knockout photopic ERGs appear not to show the profound delay present in this patient, and thus do not faithfully model the human disease, but the retinal structure and photopic ERGs in the mouse differ markedly from those in humans and extrapolation from a mouse knockout model to human disease should always be made with caution.

A naturally occurring homozygous chicken mutation of Gβ3, p.D153del, abolishes protein function and leads to a retinopathy with a globe enlargement phenotype and complete visual loss. However, Gβ3 in chickens is expressed in both rods and cones in addition to ON-bipolar cells, thereby differing from the mammalian retina.
The delayed ERG bright-flash a-wave is compatible with loss of rod photoreceptor sensitivity, with the electronegative waveform suggesting additional dysfunction occurring after phototransduction. However, although there is a negative ERG waveform, the marked a-wave delay is not a feature of CSNB and the patient also denies night blindness.

Electroretinographic data in the cone system are more challenging. Selective loss of the ON-bipolar cell pathway but preservation of OFF-bipolar cell pathway function associated with CSNB gives pathognomonic findings, including that the light-adapted 3.0 a-wave commences normally but has a broadened trough with sharply rising b-wave showing marginal delay and reduced b to a ratio. The b-wave is of higher amplitude than the a-wave. The flicker ERG shows minimal peak-time shift and amplitude change, but with some broadening of the trough. When there is additional OFF-pathway involvement, the findings are far more abnormal. Both photopic a- and b-waves are profoundly reduced and are of equivalent amplitude, and the flicker ERG shows a characteristic triphasic waveform with profound delay and amplitude reduction.

The ERG data in our patient differed from the aforementioned findings associated with CSNB, including the profound photopic a-wave delay, possibly indicating cone photoreceptor sensitivity loss, but with additional waveform simplification and no evidence of the features expected in pure ON-bipolar cell pathway dysfunction (Figure 2). OFF-bipolar cell pathway involvement is not entirely excluded electrophysiologically but would not be anticipated based on GJB3 expression data. The peak times and waveforms resemble those usually associated with S-cone function, but the flicker ERG of higher amplitude than the light-adapted 3.0 a-wave suggests this response cannot exclusively be arising in S-cones; however, the initial ERG impression could be that of an atypical enhanced S-cone syndrome.

Conclusions
This report describes a distinct inherited retinal disease presenting in childhood, with a phenotype characterized by nystagmus, normal retinal examination, mild disturbance on detailed retinal imaging, and previously undescribed ERG findings associated with recessive null GNB3 mutations. While there has been no progression to date in this patient, longer follow-up would be needed to have greater insight regarding progression. Moreover, the identification of further affected patients may allow description of the phenotypic and genotypic spectrum of disease associated with GNB3 retinopathy.