Recessive Retinopathy Consequent On Mutant G Protein β Subunit 3

(\textit{GNB3})

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\textbf{Abstract}

Importance: Mutations in phototransduction and retinal signaling genes are implicated in many retinopathies. To our knowledge, GNB3 encoding the G protein beta subunit 3 (Gβ3) has not previously been implicated in human disease.

Observations: Whole exome sequencing on a patient with distinct inherited retinal disease presenting in childhood, with a phenotype characterized by nystagmus, normal retinal examination, mild disturbance of the central macula on detailed retinal imaging, and previously undescribed ERG findings revealed a homozygous GNB3 nonsense mutation (c.124C>T ; p.Arg42Ter).

Conclusions and relevance: Gβ3, expressed in cone photoreceptors and ON-bipolar cells, is essential in phototransduction and ON-bipolar cell signaling. Knockout of Gnb3 in mice results in dysfunction of cone photoreceptors and ON-bipolar cells and a naturally occurring chicken mutant leads to retinal degeneration. Identification of further affected patients may allow description of the phenotypic and genotypic spectrum of disease associated with GNB3-retinopathy.

Introduction
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 GNB3 gene, identified using whole exome sequencing (WES). GNB3 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\(^2\).

**Methods**

The study protocol adhered to the tenets of the Declaration of Helsinki and received local ethics committee approval. Parental, informed written consent was provided. The proband underwent full ophthalmic examination including dilated retinal examination and color fundus photography (Topcon Great Britain Ltd, Berkshire, UK; Optos plc, Dunfermline, UK), spectral-domain optical coherence tomography (SD-OCT) and fundus autofluorescence (FAF) imaging (Spectralis, Heidelberg Engineering Ltd, Heidelberg, Germany), and Goldmann visual field testing. Full field electroretinography (ERG) was performed using gold foil electrodes to incorporate the ISCEV standard responses but pattern electroretinography (PERG) was recorded using surface electrodes and a 24x30 degree field due to nystagmus.

Full details of molecular investigations are included in the online supplementary data (eMethods). The proband underwent WES as part of a collaborative study (UK Inherited Retinal Disease Consortium) of 95 probands in whom previous extensive genetic screening proved negative.

**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. Parents reported difficulties with near
vision, and no night blindness. Best corrected visual acuity (BCVA) was 20/40 (0.3LogMAR) in both eyes. A high hypermetropic refractive error was identified (+7.50DS OU) and was prescribed. Vision 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 BCVA wearing +6.25/-0.50x180 in both eyes was 20/40 OD, 20/100 (0.7LogMAR) OS, with no change in symptoms.

Dilated retinal examination and color fundus photography was unremarkable (Figure 1). SD-OCT and FAF imaging were suggestive of bilateral central macular disturbance given both the smaller size and less reduction in FAF of the macular hypoautofluorescent area than would be expected, and less foveal cone outer segment lengthening associated with mild inner segment ellipsoid (ISE) layer interruption centrally on SD-OCT (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 left (Figure 1). ERGs showed a reduced rod specific (DA 0.01) response; an electronegative bright flash dark-adapted ERG (DA 10.0) with normal amplitude but delayed a-wave; a profoundly delayed 30Hz flicker ERG of subnormal amplitude; and a markedly delayed and reduced single flash photopic ERG (LA 3.0) of markedly altered waveform (Figure 2). PERG was bilaterally profoundly subnormal.

WES revealed a likely homozygous nonsense mutation in GNB3 (Chr12: 6952161C>T; NM_002075.3: c.124C>T; p.Arg42Ter) (see eResults and
Table 1). This was confirmed by bi-directional Sanger sequencing and is expected to behave as a true null; only one allele was noted in the ExAC database (MAF=0.000009). The premature termination codon occurs in exon 4 of 11 and the truncated mRNA transcript is likely to be subject to nonsense mediated decay. However should a transcript survive, the protein is terminated before the first of seven WD40 consensus sequences and would be rendered non-functional.

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

Discussion

Gβ3 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 cone-opsin 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, Gαo-Gγ13 and Trpm1\textsuperscript{1}.

Furthermore, absence of Gβ3 in mouse cones leads to reduced expression of the Gαt2- and Gγt2 subunits and corresponding loss of cone response and sensitivity\textsuperscript{6}. 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 human 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 (RGE) phenotype and complete visual loss\textsuperscript{2,5,7,8}. However Gβ3 in chicken is expressed in both rods and cones in addition to ON-bipolar cells, thereby differing from the mammalian retina\textsuperscript{5}.

The delayed ERG bright flash a-wave is compatible with loss of rod photoreceptor sensitivity, with the electronegative waveform suggesting additional dysfunction occurring post-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.

ERG data in the cone system are more challenging. Selective loss of the ON-pathway but preservation of OFF-pathway function associated with CSNB gives pathognomonic findings\textsuperscript{9}, including that the LA 3.0 ERG a-wave commences normally, but has a broadened trough with sharply rising b-wave
showing marginal delay and reduced b: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-wave 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 differ 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-pathway dysfunction (Figure 2). OFF-pathway involvement is not entirely excluded electrophysiologically, but would not be anticipated based on Gβ3 expression data. The peak times and waveforms resemble those usually associated with S-cone function, but the flicker ERG of higher amplitude than the LA 3.0 a-wave suggests this response cannot exclusively be arising in S-cones; however, the initial ERGs impression could be that of an atypical ESCS.

In conclusion, 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. Whilst 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.

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Group information

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### References


Figure 1: Fundus imaging, SD-OCT, FAF and Goldmann visual fields. CP: Colour fundus photography, OCT: SD-OCT showing mild central ISe interruption, WF: Wide field imaging, FAF: showing less reduction than expected of the central macular hypoautofluorescent area, GVF: Goldmann Visual Fields

Figure 2: Full field ERGs and pattern ERGs (PERG). DA: dark-adapted; LA: light-adapted; the numbers refer to the stimulus strength in cd.s.m-2 as recommended by the International Society for Clinical Electrophysiology of Vision. See text for full details.