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Supplemental Data

Biallelic Mutations in *GNB3* Cause a Unique Form of Autosomal-Recessive Congenital Stationary Night Blindness

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Supplemental Data:

Supplemental Methods:

Whole exome sequencing and analysis pipeline:

Briefly, 2 × 100bp paired end sequencing was done using the Illumina Hi-Seq 2500 platform after target enrichment of 3µg of genomic DNA using the Agilent SureSelect Human Exome Library V5 kit followed by an in house standardized pipeline analysis. Overall mean exon coverage in trio-1 was 87-111X with >20X target base coverage of 96-97%. Overall mean exon coverage in trio-2 was 68-131X with >20X base coverage of 92-97%. Sequence reads were trimmed, and subsequently aligned to the reference human genome (hg 19/GRCh 37) using Burrows-Wheelchair aligner. [1; 2] PCR duplicate reads were removed (MarkDuplicates, Picard tools v.1.112) and local realignment was performed (GATK 3.2.2). GATK haplotype caller3.2.2 was used to annotate single nucleotide variants (SNVs) and insertions/deletions (indels). [3; 4] Only variants with minor allele frequency ≤ 0.01 reported in external and internal databases [NHLBI Exome Sequencing Project (ESP) Exome Variant Server; 1,000 genomes; dbSNP; Exome Aggregation Consortium (ExAC); and Complete Genomics (CG) control database (54 exomes)] were retained. Pathogenic prediction was performed using six different tools that included Polyphen-2, SIFT, mutation taster, combined annotation-dependent depletion Phred, and conservation values amongst PhyloP placental mammals and PhyloP 100-vertebrates.[5-9]

Supplemental Results:

Full-field electroretinogram (ERG):

The ERG assessments of families A and B were done at separate sites using comparable basic International Society for Clinical electrophysiology of Vision (ISCEV) protocols. The amplitude

and implicit time data of standard ERG responses obtained from affected members are detailed in supplemental table (Table S3). The DA 0.01 ERG b-wave showed moderate (n=2; sib-ship from Family A) to severe (n=2; II-5 from Family A and proband from family B) amplitude reduction. The DA3.0 ERG showed normal a-wave and reduced b-wave in all four cases. The bright-flash DA10.0 ERG was electronegative (b/a <1) in all three tested individuals. These findings suggest partial (sib-ship; p.Lys57del/p.Trp339*) or severe rod ON-bipolar dysfunction (2 cases; p.Trp339*/p.Trp339*; p.Ser67Phe/ p.Ser67Phe). Flicker ERG (LA3.0 30Hz) showed normal amplitude and implicit-time in the sib-ship; delayed implicit-timing with near normal amplitudes were noted in two subjects (II-5, Family A, p.Trp339*/p.Trp339* and IV-3, Family B, p.Ser67Phe/p.Ser67Phe). The LA 3.0 single flash ERG results are detailed in the main text.

Cone phototransduction sensitivity (S_c) and Maximal amplitude (Rm_{p3}) measurement:

After pupil dilatation (right eye) and ten minute light adaptation, Case-II-5 (Family A) was tested using a series of four bright white flashes (25, 63, 157 and 241 cd.s.m^{-2}) under a rod saturating (30.0 cd.m^{-2}) background. Burian-Allen electrodes were used for the recording; at each step, five or more reproducible traces were averaged. Pupils measured 9mm after the testing. The cone sensitivity (S_c) and maximal cone photoreceptor amplitude (Rm_{p3}) were calculated as described.[10; 11] In brief, equation 1 was fitted to the initial 12 ms of the waveforms obtained from the 4 flash intensities using a grid search algorithm.

$$P3(i,t) = \left\{\frac{i \cdot S_c \cdot (t-t_d)^3}{i \cdot S_c \cdot (t-t_d)^3+1}\right\} \cdot Rm_{p3} \qquad \text{(Equation 1)}$$

Where i = intensity (log scotopic trolands), t = time (ms), S_c = Sensitivity (td·s^{-1}·sec^{-3}), t_d = time delay (ms) and Rm_{p3} = maximal cone photoreceptor amplitude (µV). To determine $t_{d,}$ waveforms from the control subjects were fitted allowing all 3 parameters (S_c, Rm_{p3} and t_d) to vary; modal

value of t_d (2.2 ms) was then used to fit all control and patient data. Case II-5 had Rm_{p3} of -97µV (Normal Range: ¯74 - ¯103 µV); S_c was reduced [$\log(S_c)$] = 0.83 (Normal Range: 1.59 – 2.17).

Supplemental Table 1: The filtering steps used in the analysis of WES data from Family A

Filtering Steps	Total Variants /Genes		Shared Genes
	Case III -2	Case II-5	
Total Variants	95,306	93,262	
Coding sequence and splicing Variants	21,496	20,201	
Non-synonymous Coding Variants and splicing Variants	9,721	9,632	
Variants with Allele frequency ≤ 0.01 in public databases	515	461	
Genes with ≥ 2 variants	42 genes (73 variants)	30 genes (48 variants)	13 genes (18 variants)
Genes remaining after removal of homozygous variants in affected, if present in homozygous state in unaffected parents	33 genes (64 variants)	22 genes (40 variants)	6 genes (11 variants)
Genes remaining after removal of heterozygous variants in affected, if present in homozygous state in unaffected parents	32 genes (62 variants)	21 genes (38 variants)	6 genes (11 variants)
Genes remaining after removal of homozygous or compound heterozygous variants shared between Case II-5 (affected) and Case II-4 (unaffected sibling of II-5)	32 genes (62 variants)	10 genes (18 variants)	1 gene (1 variant) ***GNB3***

Supplemental Table 2: Summary of the clinical phenotype in the two families

Family	Case (Sex, Age) Mutation	BCVA RE: LE	Refractive Error RE:LE	Color Vision	CS RE:LE	Visual Field	Comment
A	III-2 (M, 7yr) p.Lysdel57/p.Trp339*	20/25: 20/30	+2.25/-0.75 x 30°: +2.00/-0.75 x 20°	Normal RG & BY (HRR)	1.50: 1.50	I4e target – 105° BE III4e target – 130° BE	Night-blindness since childhood
	III-1 (M, 13yr) p.Lysdel57/p.Trp339*	20/25: 20/25	Plano: Plano	Normal RG & BY (HRR)	1.65: 1.80	I4e target – 100° BE III4e target – 125° BE	Asymptomatic
	II-5 (F, 48yr) p.Trp339*/p.Trp339*	20/30: 20/25	-1.50/-1.00 x 70°: -1.50/-1.50 x 90°	Mild RG deficit, Normal BY (HRR)	1.35: 1:35	I4e target – 100° BE III4e target – 130° BE	Night-blindness since childhood, Photophobia – recent
B	IV-3 (F, 65yr) p.Ser67Phe/p.Ser67Phe	20/25: 20/25	+2.50/-4.50 x 95°: +2.50/-5.00 x 75°	Normal (saturated D15); Moderate BY deficit (unsaturated D15	NA	IIIc target – 120° RE IIIc target – 115° LE	Night blindness since childhood; photophobia since thirties; Late onset (55 years) cerebellar ataxia and peripheral neuropathy of undetermined etiology

Abbreviations: RE – right eye; LE – left eye; BE – both eyes; BCVA – best-corrected visual acuity; CS – contrast sensitivity; HRR – Hardy Rand Rittler; RG – red-green; BY – blue-yellow; NA – not available; GVF – Goldmann visual fields.

Supplemental Table 3: Detailed Electroretinogram phenotype in the two families

Family	Case (Age/ Age Range)	Eye	DA 0.01 b-wave Amp	DA 3.0 a-wave Amp	DA 3.0 b-wave Amp	DA 3.0 b:a	DA 10.0 b:a	LA 3.0 a-wave IT	LA 3.0 a-wave Amp	LA 3.0 b-wave IT	LA 3.0 b-wave Amp	LA 30 Hz IT	LA 30 Hz Amp
A	III-2 (11yr)	RE	73	183	214	1.17	0.94	17	48	31	179	29	237
		LE	58	186	228	1.23	0.89	16	42	31	180	NA	NA
	III-1 (13yr)	RE	68	165	208	1.26	0.98	17	56	31	165	29	210
		LE	59	181	233	1.28	NA	NA	NA	NA	NA	29	149
	II-5 (48yr)	RE	0	252	179	0.71	0.73	24	36	36	86	35	86
B	IV-3 (65yr)	RE	0	173	139	0.80	NA	31	100	49	96	33	88
		LE	0	158	126	0.80	NA	34	93	49	79	NA	NA
Control (Normal Range)		EI	161 - 430	172 - 440	296 - 652	1.36 - 2.98	1.33 - 2.07	14 - 17	25 - 62	27 - 30	119 - 232	25 - 29	89 - 184

Abbreviations: RE – right eye; LE – left eye; EI – either eye (only one eye data was included); DA – dark adapted; LA – light adapted; Amp – amplitude (μV); IT – implicit time (ms); NA – not available.

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