Molecular profiling of complete congenital stationary night blindness: a pilot study on an Indian cohort

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Molecular profiling of complete congenital stationary night blindness: A pilot study on an Indian cohort

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Purpose: Congenital stationary night blindness (CSNB) is a non-progressive retinal disorder that shows genetic and clinical heterogeneity. CSNB is inherited as an autosomal recessive, autosomal dominant, or X-linked recessive trait and shows a good genotype–phenotype correlation. Clinically, CSNB is classified as the Riggs type and the Schubert-Bornschein type. The latter form is further sub-classified into complete and incomplete forms based on specific waveforms on the electroretinogram (ERG). There are no molecular genetic data for CSNB in the Indian population. Therefore, we present for the first time molecular profiling of eight families with complete CSNB (cCSNB).
Methods: The index patients and their other affected family members were comprehensively evaluated for the phenotype, including complete ophthalmic evaluation, ERG, fundus autofluorescence, optical coherence tomography, and color vision test. The known gene defects for cCSNB, *LRIT3*, *TRPM1*, *GRM6*, *GPR179*, and *NYX*, were screened by PCR direct sequencing. Bioinformatic analyses were performed using SIFT and PolyPhen for the identified missense mutations.
Results: All eight affected index patients and affected family members were identified as having cCSNB based on their ERG waveforms. Mutations in the *TRPM1* gene were identified in six index patients. The two remaining index patients each carried a *GPR179* and *GRM6* mutation. Seven of the patients revealed homozygous mutations, while one patient showed a compound heterozygous mutation. Six of the eight mutations identified are novel.
Conclusions: This is the first report on molecular profiling of candidate genes in CSNB in an Indian cohort. As shown for other cohorts, *TRPM1* seems to be a major gene defect in patients with cCSNB in India.

Congenital stationary night blindness (CSNB) is a group of clinically and genetically heterogeneous nonprogressive retinal disorders. CSNB is caused by mutations in genes that are involved in the phototransduction cascade or in retinal signaling from photoreceptors to bipolar cells (second-order neurons). Many patients with CSNB experience a strong visual disability during night time when artificial light is limited. CSNB is also associated with other ocular features such as myopia, nystagmus, and strabismus without striking fundus abnormalities [1,2]. Based on full-field electroretinogram (ERG), CSNB can be distinguished into two types, the Riggs type and the Schubert-Bornschein type. In the Riggs type [3], rod adaptation is present, although it is slower, and cone responses are normal. This type may be inherited in an autosomal dominant (AD) or autosomal recessive trait [4-7]. The Schubert-Bornschein type is characterized by a negatively shaped dark-adapted ERG response to a bright flash in which the amplitude of the a-wave is normal but larger than that of the b-wave [8]. This type is divided into two sub-types, complete CSNB (cCSNB or CSNB1), associated with a drastically reduced rod b-wave response and a peculiar square a-wave with relatively preserved amplitudes in response to a standard flash under photopic conditions indicating ON bipolar dysfunction, and incomplete CSNB (icCSNB or CSNB2), which is associated with a reduced rod b-wave and substantially reduced cone responses indicating ON and OFF bipolar dysfunction [9]. The mode of inheritance of the Schubert-Bornschein type is X-linked recessive [10-14], autosomal recessive (AR) [15-26], or autosomal dominant [27].

CSNB does not usually show fundus abnormalities, except for myopic changes; however, there are two other variants of CSNB with distinctive fundus abnormalities, Oguchi disease and fundus albipunctatus. Oguchi disease is characterized by a golden or diffuse gray-white fundus discoloration. After prolonged dark adaptation of 2–3 h, the discoloration of the fundus returns to normal, and normal

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function of rod cells is resumed [28,29]. The fundus of patients with fundus albipunctatus shows numerous small white or yellow flecks spread throughout the retina, and these patients show recovery of scotopic responses after prolonged dark adaptation [30]. Oguchi disease and fundus albipunctatus are inherited in an autosomal recessive trait [28-30].

Thus far, 17 genes have been identified as associated with CSNB, Oguchi disease, and fundus albipunctatus and show a good genotype–phenotype correlation. Mutations in *NYX* (MIM: 300278) lead to X-linked recessive cCSNB whereas mutations in *CACNA1F* (MIM: 300110) cause X-linked recessive icCSNB [10-14]. *GRM6* (MIM: 604096), *TRPM1* (MIM: 603576), *GPR179* (MIM: 614515), and *LRIT3* (MIM: 615004) are implicated in autosomal recessive cCSNB [15,16,19-26], while *CABP4* (MIM: 608965) mutations cause autosomal recessive icCSNB [17]. Of note, *CACNA2D4* (MIM: 608171) mutations were identified in a patient with icCSNB, which showed upon reinvestigation as autosomal recessive cone dystrophy [18]. However, *SLC24A1* (MIM: 603617) mutations have been reported in autosomal recessive CSNB with the Riggs type [7]. Autosomal dominant CSNB with the Riggs type are due to mutations in *RHO* (MIM: 180380), *GNAT1* (MIM: 139330), and *PDE6B* (MIM: 180072) [4-6]. Recently, homozygous mutations in *GNAT1* in a consanguineous Pakistani family were reported, indicating involvement of *GNAT1* in autosomal recessive CSNB as well [31]. Oguchi disease is caused by mutations in *GRK1* (MIM: 180381) and *SAG* (MIM: 181031) [28,29] whereas the gene thus far predominantly identified in fundus albipunctatus is *RDH5* (MIM: 601617) [30], although two other genes *RPE65* (MIM: 180069) and *RLBP1* (MIM: 180090) have also been associated with fundus albipunctatus [32-34]. There are no data on the mutation prevalence of patients with CSNB in the Indian population. Here we report molecular genetic data from a preliminary study of eight families with cCSNB from India.

METHODS

Research procedures were conducted in accordance with Institutional Review Board guidelines of Vision Research Foundation and the Declaration of Helsinki. Thirteen patients from eight unrelated Indian families were recruited and twelve patients underwent complete ophthalmic examination, which included refraction, full-field ERG, optical coherence tomography (OCT), fundus autofluorescence (FAF), color fundus photography, and color vision test (D15 test).

Full-field ERG was recorded using VERIS Science 5.1 (Electro-Diagnostic Imaging, EDI; San Mateo, CA) following the guidelines by the International Society for Clinical Electrophysiology of Vision (ISCEV) after pupil dilation with the use of a bipolar Burian Allen electrode with a gold cup electrode attached to the earlobe as a ground electrode [35]. In four of the eight families, more than one member were affected (men and women; Family 2, Family 4, Family 6, Family 7), in one family a single index patient (female; Family 3) was affected, while in three families, only the index male patient was affected (Family 1, Family 5 and Family 8).

After informed consent was obtained, 10 ml of heparin blood samples of the index patients along with their affected and unaffected sibs and parents were collected. The samples were stored at 4 ºC prior to extraction. The DNA was extracted using the NucleoSpin Blood XL kit (Macherey-Nagel, Duren, Germany) according to manufacturer's protocol. The index patients were screened for mutations in the coding regions of the genes underlying autosomal recessive cCSNB, namely, *LRIT3*, *TRPM1*, *GPR179*, *GRM6*, and *NYX*, underlying X-linked recessive by Sanger sequencing. Primer sequences for these genes were obtained from published articles [16,23,24,26]. Primers for *NYX* were designed by us using the Primer3 online tool [36]. PCR was performed using 50 ng genomic DNA in a 12.5 µl reaction consisting of 5 µM primers (Shrimpex Biotech, Chennai, India), 250 µM dNTPs (Applied Biosystems, Foster City, CA), and 0.1 U of Taq DNA polymerase (GeNei, Merck, Mumbai, India) as described below. PCR for genes *LRIT3*, *TRPM1*, *GPR179*, and *GRM6* was performed as following, an initial denaturation of 5 min at 94 °C, 35 cycles of denaturation at 94 °C for 20 s, annealing for 20 s and extension at 72 °C for 45 s followed by a final extension at 72 °C for 7 min. The annealing temperature for different amplicons varied and the conditions were as described in the publications [16,23,24,26]. PCR for *NYX* was done using an initial denaturation of 5 min at 94 °C, 35 cycles of denaturation at 94 °C for 20 s, annealing at 58 °C for 20 s and extension at 72 °C for 45 s followed by a final extension at 72 °C for 7 min.

Purified (Exo-SAP *E. coli* exonuclease I and Fast Alkaline phosphatase, Thermo Scientific, Vilnius, Lithuania) PCR products were bidirectionally sequenced using a reaction kit (Big Dye Terminator v3.0 Ready, Applied Biosystems) and passed on a sequencer (ABIPRISM 3100- or 3730 *Avant* Genetic Analyzer, Applied Biosystems). The sequences were compared with the reference (*LRIT3* - ENSG00000183423, *TRPM1* - ENSG00000134160, *GPR179* - ENSG00000260825, *GRM6* - ENSG00000113262, and *NYX* - ENSG00000188937). Cosegregation analysis was performed on the DNA of available family members. Control screening was done using PCR-based direct sequencing or allele-specific PCR (ASPCR). SIFT [37] and PolyPhen [38] analyses were performed to predict the possible impact of amino acid substitution on the

structure and function of the protein and the conservation of a particular amino acid across closely related species. One hundred and twenty controls were taken from samples collected as part of epidemiological studies conducted at our hospital (Medical Research Foundation), underwent complete ophthalmic examination, and had no retinal abnormalities [39,40].

RESULTS

Clinical characteristics: The clinical data for each affected index patient and affected siblings from the eight families were analyzed. Based on full-field ERG waveforms, the patients were all classified as having cCSNB showing electronegative waveforms. The full-field ERG traces of patients with *TRPM1*, *GRM6*, and *GPR179* mutations along with a normal ERG trace are shown in Figure 1.

All patients carrying mutations showed typical post-photoreceptoral dysfunction on full-field ERG: The b-wave was absent in response to a dim flash under dark-adapted conditions; the response to a bright flash under the same conditions had the typical electronegative waveform. The oscillatory potentials were undetected. Clinical details of the affected family members are given in Table 1. Comparison of the ERG amplitude and implicit time values between the *TRPM1*, *GRM6*, and *GPR179* mutation-positive patients and the normal controls are given in Table 2 and Table 3, respectively. The autofluorescence and OCT examinations did not reveal any obvious abnormality. The color vision test was normal in all.

Candidate gene screening: First, the recently identified *LRIT3* gene in autosomal recessive cCSNB was screened in all eight patients. No pathogenic variation was found in this gene in any of the patients. This was followed by screening the most frequently mutated gene underlying autosomal recessive cCSNB, *TRPM1* [19]. We identified six mutations: c.1870C>T p.(R624C), c.3326_3327dupC p.(P1110Tfs*39), c.416G>T p.(G139V), c.398C>A p.(A133D), c.2783G>A p.(R928Q), and c. 857C>T p.(S286L) in *TRPM1* in six families. Five of the mutations identified are novel. The mutation, predicted change in protein, consanguinity, details of segregation analysis, and results of the SIFT and PolyPhen analyses are given in Table 4. Five of the six families showed homozygous mutation, while the sixth family had compound heterozygous mutations (Figure 2A–F).

The two index patients with no mutation in the *TRPM1* gene were screened for *GPR179,* which underlies another form of autosomal recessive cCSNB. We identified a novel missense variation, in patient 3 of family 3, a homozygous c.1811C>T leading to p.(P604L) change in exon 9. The parents and unaffected sister were heterozygous carriers of the mutation (Figure 2G).

NYX was screened in patient 8. He was the only male patient affected with cCSNB with no mutation in *LRIT3*, *TRPM1*, and *GRP179*. No pathogenic variation was found. Thus, this index patient was screened for *GRM6*. A reported missense mutation [41], homozygous c.2267G>A, was identified leading to a p.(G756D) change in exon 10 (Figure 2H). None of the eight mutations were seen in the more than 110 controls screened. In addition, none of the identified missense

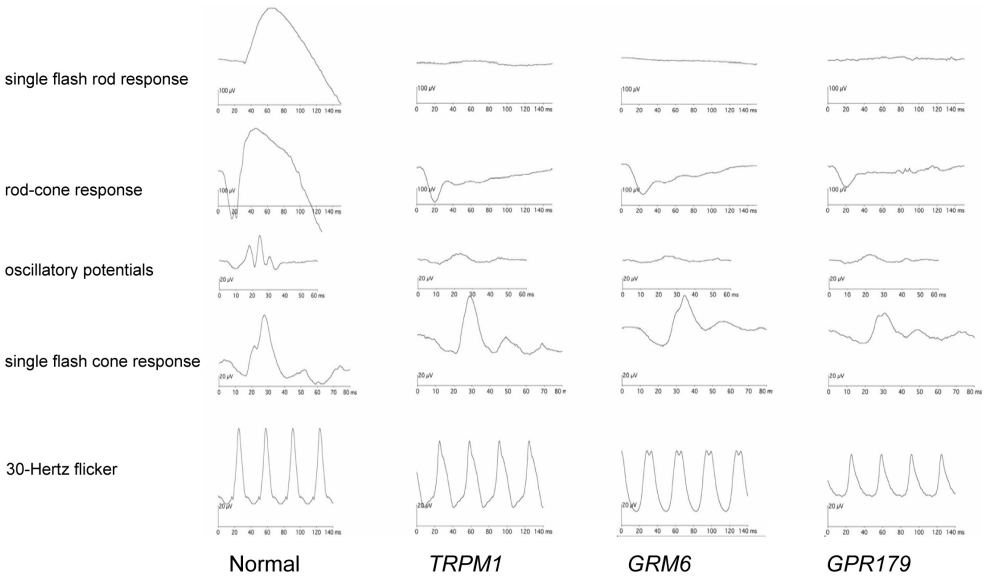

Figure 1. Electroretinogram traces. Full-field electroretinogram (ERG) trace of a normal, *TRPM1*, *GRM6*, and *GRP179* mutation-positive patient, respectively. The waveform of single flash rod response, combined rod-cone response, oscillatory potentials, single flash cone response, and response to 30-Hz flicker are represented.

Table 1. CSNB Clinical parameters

Family/Patient	Age/Sex	Gene	Spherical equivalent (Diopter)	BCVA in logMAR	Fundus Findings	
					Myopic tessellated fundus	Disc
Family 1/ Patient 1	31/M	*TRPM1*	OD: −8.38 OS: −7.88	OD: 0.2 OS: 0.2	YES	Tilted disc
Family 2/ Patient 2	46/F	*TRPM1*	OD: −3.75 OS: −7.50	OD: 0.8 OS: 0.5	YES	Titled disc
Affected daughter of patient 2 from Family 2	25/F	*TRPM1*	OD: −2.00 OS: −2.25	OD: 0.5 OS: 1.3	YES	Tilted disc
Family 4/ Patient 4	32/M	*TRPM1*	OD: −6.50 OS: −6.13	OD: 0.5 OS: 0.5	YES	Myopic disc
Family 5/ Patient 5	30/M	*TRPM1*	OD: −2.50 OS: −1.25	OD: 0.2 OS: 0.4	YES	Tilted disc
Family 6/ Patient 6	20/F	*TRPM1*	OD: −8.50 OS: −7.50	OD: 1.0 OS: 0.6	YES	Tilted disc
Affected sib of patient 6 from Family 6	24/M	*TRPM1*	OD: −5.00 OS: −9.50	OD: 1.3 OS: 0.8	YES	OD: Hypotonic Disc and Macula, 0.4 CDR, Post Trabeculectomy OS: Tilted Disc with 0.7 CDR
Family 7/ Patient 7	34/F	*TRPM1*	OD: −6.00 OS: −6.25	OD: 0.6 OS: 0.6	YES	Tilted disc
Affected daughter of patient 7 from Family 7	14/F	*TRPM1*	OD: −5.50 OS: −5.75	OD: 0.6 OS: 0.5	YES	Tilted disc
Affected son of patient 7 from Family 7	9/M	*TRPM1*	OD: −5.50 OS: −5.50	OD: 0.5 OS: 0.5	YES	Myopic disc
Family 8/ Patient 8	49/F	*GRM6*	OD: −5.00 OS: −4.50	OD: 0.3 OS: 0.5	YES	Tilted disc
Family 3/ Patient 3	17/F	*GPR179*	OD: −6.25 OS: −6.63	OD: 0.3 OS: 0.2	YES	Tilted disc

RXT – Right exotropia, LXT – Left exotropia, AXT – Alternate exotropia, CDR- Cup disc ratio, OD – Oculus dexter (right eye), OS – Oculus sinister (left eye)

Table 2. ERG amplitudes.

Amplitude (μv)	CASES			CONTROLS (n=20)
	TRPM1 Gene (n=10)	*GPR179*	*GRM6*	
Rod b-wave	10.2±5.9*	8.8	4.6	296.0±72.6
Combined a-wave	198.5±38.8	133.8	193.6	219.8±54.9
Combined b-wave	79.2±26.6*	84.5	74.5	480.6±94.2
Cone a-wave	30.0±10.8	24.7	34.4	29.4±6.5
Cone b-wave	89.4±29.4	57.4	96.9	106.7±27.7
Flicker b-wave	57.6±16.3	40.4	58.2	67.7±17.9

Comparison of ERG amplitudes of each wave among the *TRPM1*, *GRP179* and *GRM6* mutation positive patients with that of normal controls. *p<0.05 (Independent *t* test) was noted in b-wave of single flash rod and combined responses in *TRPM1* gene group. Statistical analysis was not done for *GPR179* and *GRM6* genes as only one patient was present in those groups.

Table 3. ERG implicit times.

Implicit time (ms)	CASES			CONTROLS (n=20)
	TRPM1 Gene (n=10)	*GPR179*	*GRM6*	
Rod b-wave	68.2±5.9*	67.5	80.0	**64.6** ± 3.7
Combined a-wave	20.0±1.7*	20.0	23.0	17.3±0.6
Combined b-wave	39.6±1.5*	40.0	47.0	**44.8** ± 1.8
Cone a-wave	20.1±1.9*	19.0	22.5	16.3±0.8
Cone b-wave	29.3±1.0*	30.5	34.5	27.4±1.1
Flicker b-wave	25.6±0.8 (0.604)	26.0	28.5	25.3±1.2

Comparison of ERG implicit times of each wave among the *TRPM1*, *GRP179* and *GRM6* mutation positive patients with each other and with that of normal controls. *p<0.05 (Independent *t* test) was noted for all parameters except b-wave of 30 Hz flicker response in *TRPM1* gene group. Statistical analysis was not done for *GPR179* and *GRM6* genes as only one patient was present in those groups.

mutations are reported either in the 1000 Genomes database or the Exome Variant Server database.

DISCUSSION

This is the first report on the mutation profile of CSNB from India. In this pilot study, we screened eight families with cCSNB. Patients with CSNB exhibit a defect in the genes that code for proteins involved in signal transmission from photoreceptors to adjacent bipolar cells or in the phototransduction pathway. Since all eight patients recruited showed ERG waveforms typical of cCSNB, known genes for this form of CSNB, *TRPM1*, *GPR179*, *GRM6*, *LRIT3*, and *NYX*, were screened.

We identified eight mutations in the eight families; six are novel. The missense variations were predicted to be "not tolerated" and "probably pathogenic" by SIFT and PolyPhen analyses, and the amino acid residues were conserved across species. Cosegregation analysis and control screening confirmed the pathogenicity of the mutations as they were segregated among the family members and absent in more than 110 healthy control samples (i.e., 220 chromosomes).

Based on the published putative transmembrane regions of the TRPM1 protein [19], the missense mutations p.(A133D), p.(G139V), p.(S286L), and p.(R624C) observed in this study are predicted to localize in the intracellular N-terminal region of the protein. Generally, changes in the N- and C-terminal regions may lead to mislocalization or misfolding of the protein or can lead to altered function [42-46]. A previous study showed R624C causes a failure in localization of the mutant protein to the dendritic tips of ON bipolar cells, thus affecting the channel function of TRPM1 [23]. The observed missense mutation p.(R928Q) is present in transmembrane domain 3. Similarly, we speculate that these mutations contribute to altered or nonfunctional TRPM1 channel activity. The duplication, c.3326_3327dupC, observed was found to code for the extracellular loop connecting the transmembrane domains 5 and 6 of the protein. This duplication is predicted to cause a frameshift leading to a truncated protein p.(P1110Tfs*39) devoid of transmembrane 6 and the intracellular C-terminal region, possibly affecting its structure and function. Alternatively, nonsense-mediated mRNA decay may take place, leading to cCSNB.

GPR179 belongs to the G-protein coupled receptor subfamily of proteins and is reported to be localized in ON bipolar cells. The p.(P604L) missense mutation in *GPR179* is predicted to be in the extracellular loop connecting the sixth and seventh transmembrane domains of the protein [24,25]. The GRP179 protein localizes to the dendritic tip of ON bipolar cells. Wild-type GRP179 has been shown to localize on the cell surface as well as intracellularly in the endoplasmic reticulum and Golgi apparatus. However, localization studies of the previously reported mutant variants, p.(Y220C), p.(G455D), and p.(H603Y), revealed the absence of cell surface staining and showed only intracellular staining. Interestingly, the novel mutation identified in this study, p.(P604L), is adjacent to the reported p.H603Y mutation and may thus also lead to mislocalization of the mutant GRP179 protein [47].

GRM6 is a member of the group III metabotropic glutamate receptor (mGluR) family and is a transmembrane protein in ON bipolar dendrites. The p.(G756D) mutation identified in one of our patients is predicted to be located in the transmembrane 5 of the protein. To date, many cCSNB mutations in *GRM6* and more specifically one in the transmembrane domain have been shown to cause mislocalization of the mutant, which is retained in the endoplasmic reticulum and not trafficked to the cell surface as the wild-type protein

Table 4. Genotype data of the eight cCSNB families.

S.No	Patient/ Family No.	Consanguinity	Gene and Mutations identified	Bioinformatics analysis	Segregation analysis	Number of controls screened	Population frequency as reported in 1000 genome and Exom-eVariant Server database
1	Patient 1/ Family 1	No	*TRPM1* c.[1870C>T];[1870C>T] p.(R624C) (rs3879 06862) Reported [23]	♦SIFT – not tolerated ♦Polyphen – probably damaging ♦Conservation across species – conserved across vertebrates	Heterozygous in parents and homozygous wild-type in unaffected sibling	114	Not reported
2	Patient 2/ Family 2	Yes	*TRPM1* c.[3326_3327dupC]; [3326_3327dupC] p. (P1110Tfs*39) **Novel**	_	Homozygous mutant in the index patient's affected daughter and as heterozygous in the unaffected husband.	_	Not reported
3	Patient 3/ Family 3	Yes	*GPR179* c. [1811C>T];[1811C>T] p.(P604L) **Novel**	♦SIFT – not tolerated ♦Polyphen – probably damaging ♦Conservation across species – conserved across vertebrates	Heterozygous in parents	111	Not reported
4	Patient 4/ Family4	Yes	*TRPM1* c. [416G>T];[416G>T] p. (G139V) **Novel**	♦SIFT – not tolerated ♦Polyphen – probably damaging ♦Conservation across species – conserved across vertebrates	Homozygous mutant in the index patient's affected sister and as heterozygous carrier in unaffected sister	117	Not reported
5	Patient 5/ Family 5	Yes	*TRPM1* c.[398C>A];[398C>A] p.(A133D) **Novel**	♦SIFT – not tolerated ♦Polyphen – probably damaging ♦Conservation across species – conserved across vertebrates	Heterozygous carrier parents and in unaf-fected sib	117	Not reported

S.No	Patient/ Family No.	Consanguinity	Gene and Mutations identified	Bioinformatics analysis	Segregation analysis	Number of controls screened	Population frequency as reported in 1000 genome and Exom-eVariant Server database
6	Patient 6/ Family 6	No	*TRPM1* c.[1870C>T]; [(2783G>A)] p. ([R624C];[R928Q]) c.1870C>T-(rs387906862) Reported [23] c.2783G>A - **Novel** (compound heterozygote)	♦SIFT – not tolerated ♦Polyphen – probably damaging ♦Conservation across species – conserved across vertebrates	Father is heterozygous for c.1870C>T. The affected brother is also compound heterozygous for c.1870C>T and c.2783G>A.	114 111	Not reported Not reported
7	Patient 7/ Family 7	Yes	*TRPM1* c. [857C>T];[857C>T] p. (S286L) **Novel**	♦SIFT – not tolerated ♦Polyphen – probably damaging ♦Conservation across species – conserved across vertebrates	The three affected are homozygous for the mutant while the unaf-fected are heterozygous carriers	110	Not reported
8	Patient 8/ Family 8	Yes	*GRM6* c. [2267G>A];[2267G>A] p. (G756D) Reported [41]	♦Provean – deleterious ♦SIFT – tolerated ♦Polyphen –benign Conservation across species – conserved across 78% of vertebrates	Blood samples of the family members are not available.	114	Not reported

Identified mutation, predicted amino acid change, results of bioinformatics, segregation and control screening analyses for the eight families.

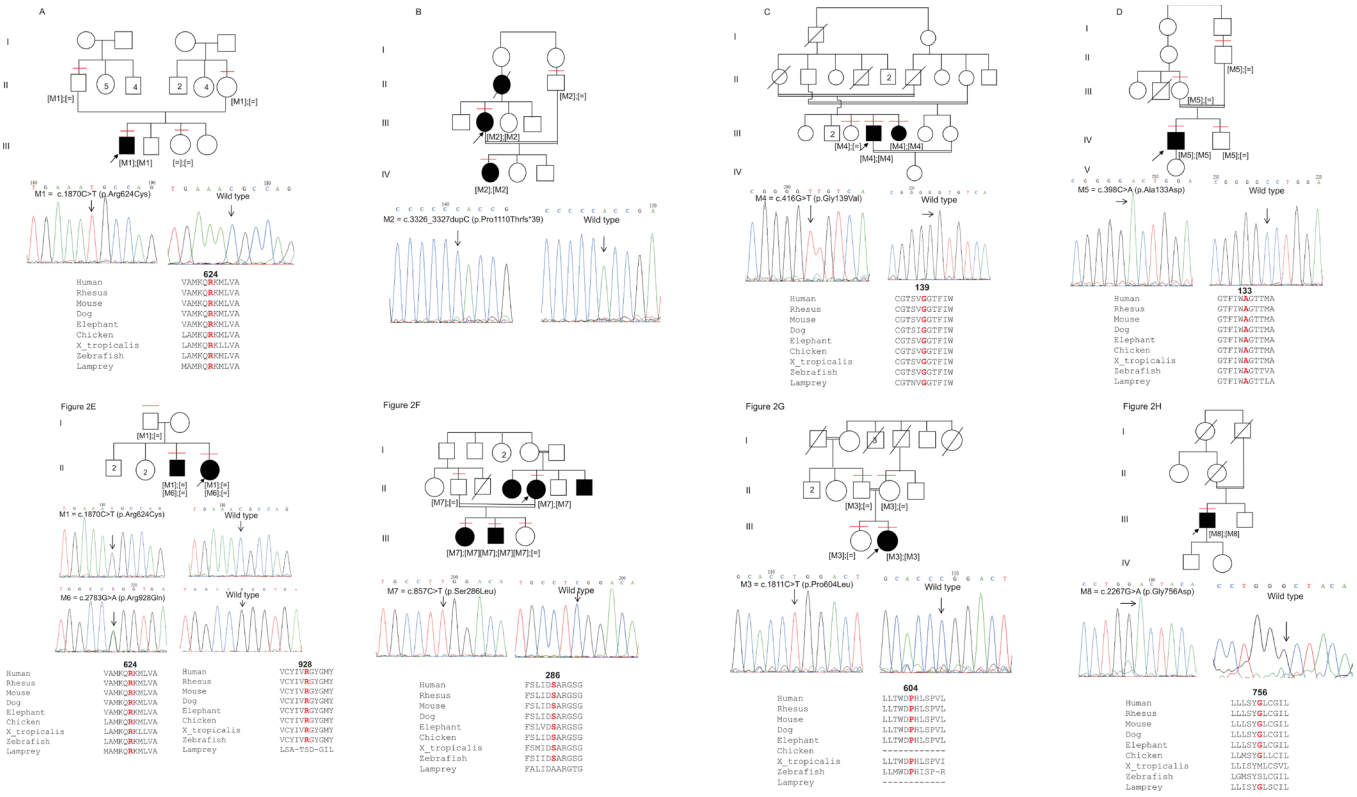

Figure 2. Pedigree, sequence chromatogram traces and conserved amino acid residues of the eight cCSNB families. The squares and the circles in the pedigree represent men and women, respectively, and the filled-in squares and circles represent affected men and women, respectively. The red line above the individuals in the pedigree indicates genotype data are available. The sequence chromatogram trace shows the region of the mutation as seen in the affected individuals. M1–M8 represent the eight mutations identified in the eight families. **A**–**F**: Families with mutations in the *TRPM1* gene. **G**: The pedigree and the sequence chromatogram of the index patient with a mutation in *GRP179* are shown. **H**: The pedigree and the mutation identified in the index patient with *GRM6* are shown. The wild-type sequence chromatogram trace is shown adjacent to the mutant chromatogram trace. The conservation of the wild-type amino acid codon across the vertebrates is also shown for the missense mutations.

[48]. Thus, we hypothesize that the mutation identified in this study, which is also present in the transmembrane domain, results in mislocalization of the protein.

Transient receptor potential cation channel, subfamily M, member 1 (also known as melastatin) (*TRPMI*), G protein coupled receptor 179 (*GPR179*), and metabotropic glutamate receptor 6 (mGluR6) (*GRM6*), and *NYX* all play a key role in ON bipolar cell depolarization that leads to the rise of b-wave after light stimulation. Loss of function of these genes results in impairment of signal mediation between photoreceptors and ON bipolar cells, presenting as a loss of the scotopic b-wave in ERG and night vision disturbance [15,16,19-25,49,50].

Furthermore, immunohistological studies have shown the localization of the respective proteins specific to retinal ON bipolar cells suggesting their role in retinal signaling from photoreceptors to bipolar cells [22,25,49-53]. Studies on animal knockout or mutant models of these genes have revealed a similar absence of the b-wave under dark-adapted conditions and no abnormalities in the retinal structure, differentiation, and synapse formation similar to presentation in patients with CSNB [25,53,54].

Six families (patients) had mutations in *TRPM1* while mutations in *GRP179* and *GRM6* were seen in one family each. Molecular profiling has confirmed or identified that the disease is autosomal recessive in all the families studied. The confirmation or identification of the pattern of inheritance also helps in genetic counseling for patients and their families.

In summary, in our study, we identified mutations in *TRPM1*, *GPR179*, and *GRM6*. All patients showed typical genotype–phenotype correlation, in which the ERG data identified them as cCSNB and the molecular profiling confirmed. Although our analyzed group is small, we

confirm that *TRPM1* as suggested by other cohorts [19-23] is also a major gene defect in India. However, a larger Indian cohort must be screened to deliver a more accurate prevalence of the mutations in various forms of CSNB.

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