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Mutations in *GRM6* identified in consanguineous Pakistani families with congenital stationary night blindness

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Purpose: This study was undertaken to investigate the causal mutations responsible for autosomal recessive congenital stationary night blindness (CSNB) in consanguineous Pakistani families.
Methods: Two consanguineous families with multiple individuals manifesting symptoms of stationary night blindness were recruited. Affected individuals underwent a detailed ophthalmological examination, including fundus examination and electroretinography. Blood samples were collected and genomic DNA was extracted. Exclusion analyses were completed by genotyping closely spaced microsatellite markers, and two-point logarithm of odds (LOD) scores were calculated. All coding exons, along with the exon–intron boundaries of *GRM6*, were sequenced bidirectionally.
Results: According to the medical history available to us, affected individuals in both families had experienced night blindness from the early years of their lives. Fundus photographs of affected individuals in both the families appeared normal, with no signs of attenuated arteries or bone spicule pigmentation. The scotopic electroretinogram (ERG) response were absent in all of the affected individuals, while the photopic measurements show reduced b-waves. During exclusion analyses, both families localized to a region on chromosome 5q that harbors *GRM6*, a gene previously associated with autosomal recessive CSNB. Bidirectional sequencing of *GRM6* identified homozygous single base pair changes, specifically c.1336C>T (p.R446X) and c.2267G>A (p.G756D) in families PKRP170 and PKRP172, respectively.
Conclusions: We identified a novel nonsense and a previously reported missense mutation in *GRM6* that were responsible for autosomal recessive CSNB in patients of Pakistani decent.

Congenital stationary night blindness (CSNB) refers to a group of diseases involving impaired night vision with stationary rod dysfunction [1]. It is associated with decreased visual acuity, nystagmus, myopia, and strabismus or retinal changes [2]. CSNB has been reported to have autosomal dominant, autosomal recessive, and X-linked inheritance. Mutations in *RHO* (Gene ID: 6010; OMIM: 180380), *PDE6B* (Gene ID 5158; OMIM: 180072), and *GNAT1* (Gene ID 2779; OMIM: 139330) have been associated with autosomal dominant CSNB [3–5], while mutations in *GRM6* (Gene ID 2916; OMIM: 604096), *CABP4* (Gene ID 57,010; OMIM: 608965), *TRPM1* (Gene ID 4308; OMIM: 603576), *GPR179* (Gene ID 440,435; OMIM: 614515), and *LRIT3* (Gene ID 345,193; OMIM: 615004) have been identified in patients with autosomal recessive CSNB [6–13]. Likewise, mutations in *NYX* (Gene ID 60,506; OMIM: 300278), and *CACNA1F* (Gene ID 778; OMIM: 300110) have been linked to X-linked CSNB [14–16]. Causal mutations in *SLC24A1* (Gene ID 9187; OMIM: 603617) and *GNAT1* have been identified in patients of Pakistani origin with autosomal recessive CSNB [17,18].

Previously, Hashimoto et al. (1997) mapped *GRM6* to chromosome 5q and demonstrated that the gene contains 10 exons that span approximately 17 kb and encode for an 877 amino acid protein [19]. The authors further demonstrated that GRM6 is a G protein-coupled receptor that contains a signal peptide, a large extracellular domain, and seven transmembrane segments [19]. Subsequently, it was discovered that GRM6 is used by ON bipolar cells for light-activated depolarization [20,21].

Here, we report two consanguineous Pakistani families with multiple affected individuals manifesting cardinal symptoms of CSNB. Exclusion linkage analysis localized the

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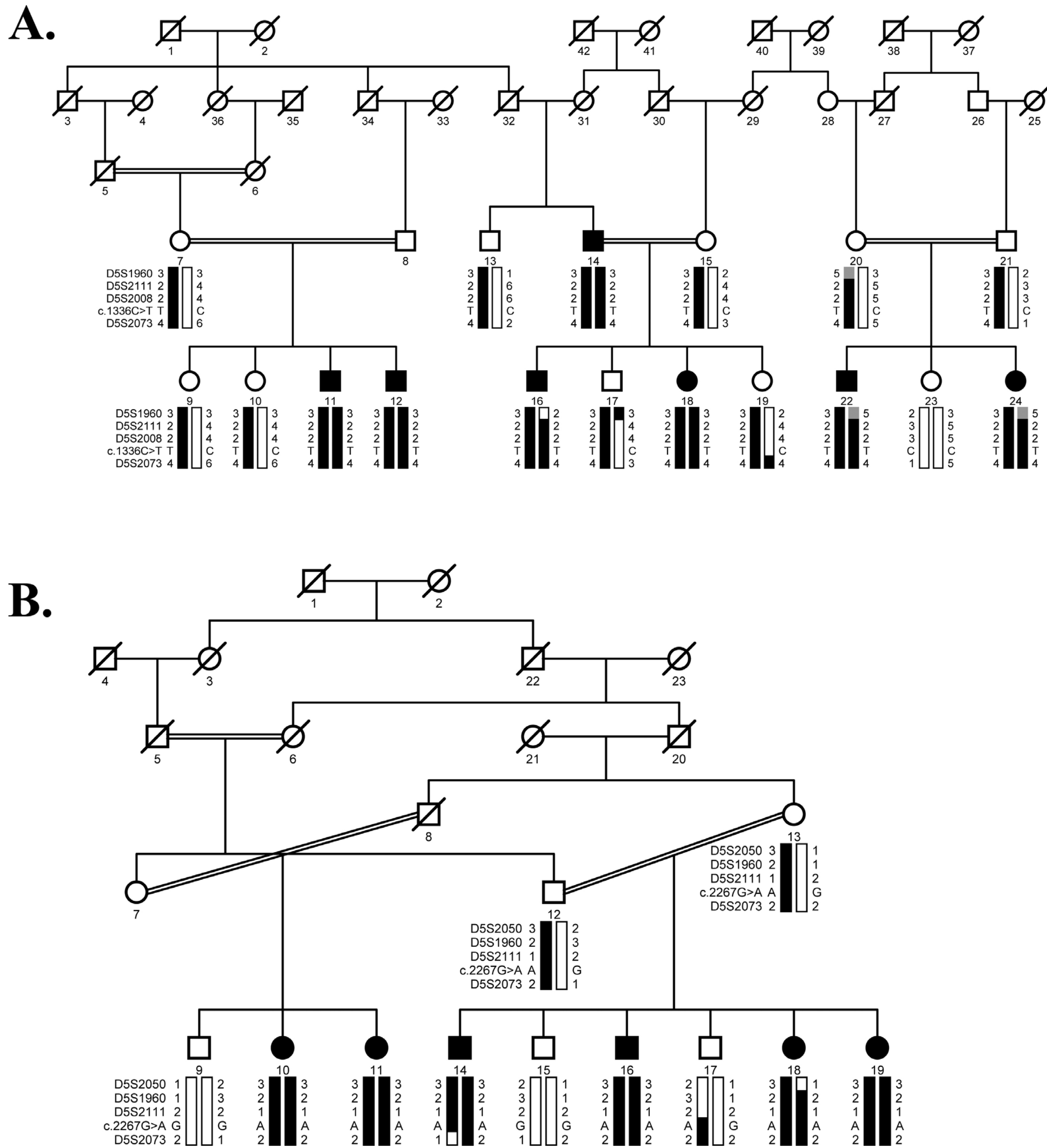

Figure 1. Pedigree drawing of families **A**: PKRP170 and **B**: PKRP172 with haplotypes of chromosome 5q microsatellite markers. Squares represent male, circles represent female, filled symbols are affected individuals, a double line between individuals indicates consanguinity, and a diagonal line through a symbol is a deceased family member. Alleles forming the risk haplotype (black), and alleles not cosegregating with arCSNB are shown white.

Table 1. Clinical characteristics of families PKRP170 and PKRP172.

Family	ID	Sex	Age (Years)	First Symptoms	Color Vision	Visual acuity		auto refraction
						OD	OS	
PKRP170	14	M	65	N.B.	Nor.	6/60	6/60	N.A.
PKRP170	16	M	35	N.B.	Nor.	6/6	6/6	astigmatism/ hyperopia
PKRP172	14	M	40	N.B.	Nor.	6/9	6/12	astigmatism/ hyperopia
PKRP172	18	F	25	N.B.	Nor.	CF	CF	N.A.
PKRP172	19	F	22	N.B.	Nor.	CF	CF	N.A.
PKRP172	10	F	32	N.B.	Nor.	6/36	CF	N.A.
PKRP172	15	M	23	Normal	Nor.	6/6	6/6	hyperopia

M: male; F: female; CF: counting fingers.

disease phenotype to chromosome 5q, whereas bidirectional sequencing of *GRM6* identified causal mutations that segregated with the disease phenotype in the respective families.

METHODS

Patient ascertainment: We recruited two large consanguineous Pakistani families comprising multiple affected individuals with a history of night blindness to participate in a study investigating autosomal recessive CSNB. The institutional review boards (IRBs) of the National Centre of Excellence in Molecular Biology (Lahore, Pakistan), National Eye Institute (Bethesda, MD), and Johns Hopkins University (Baltimore, MD), approved for the study. All participating family members provided an informed written consent form that had been endorsed by the respective IRBs and was consistent with the tenets of the Declaration of Helsinki.

A detailed clinical and medical history was obtained from the individual families. Funduscopy was performed at the Layton Rehmatulla Benevolent Trust (LRBT) Hospital (Lahore, Pakistan). Electroretinogram (ERG) responses were recorded using equipment manufactured by LKC (Gaithersburg, MD). Dark-adapted rod responses were determined through incident flash attenuated by −25 dB, whereas rod–cone responses were measured at 0 dB. The 30 Hz flicker responses were recorded at 0 dB to a background illumination of 17 to 34 cd/m^2.

All participating members voluntarily provided a blood sample of approximately 10 ml that was stored in 50 ml Sterilin® falcon tubes containing 400 µl of 0.5 M EDTA. Blood samples were stored at −20 °C for long-term storage.

Genomic DNA extraction: Genomic DNA was extracted from white blood cells using a modified procedure, as described previously [22,23]. Approximately, 10 ml blood samples were mixed with 35 ml of TE buffer (10 mM Tris-HCl, 2 mM EDTA, pH 8.0) and the TE-blood mixture was centrifuged at 2,000 × *g* for 20 min. The red blood cells were discarded and the pellet was re-suspended in 35 ml of TE buffer. The TE washing was repeated for 2–3 times and the washed pellet was re-suspended in 2 ml of TE buffer. Next, 6.25 ml of protein digestion cocktail (50 µl [10 mg/ml] of proteinase K, 6 ml TNE buffer [10 mM Tris HCl, 2 mM EDTA, 400 mM NaCl] and 200 µl of 10% sodium dodecyl sulfate) was added to the re-suspended pellets and incubated overnight in a shaker (250 rpm) at 37 °C. The digested proteins were precipitated by adding 1 ml of 5 M NaCl, followed by vigorous shaking and chilling on ice for 15 min. The precipitated proteins were pelleted by centrifugation at 2,000 × *g* for 20 min and removed. The supernatant was mixed with equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) and the aqueous layer containing the genomic DNA was carefully collected. The DNA was precipitated with isopropanol and pelleted by centrifugation at 3,500 × *g* for 15 min. The DNA pellets were washed with 70% ethanol and dissolved in TE buffer. The concentration of the extracted genomic DNA was estimated with a SmartSpec plus Bio-Rad Spectrophotometer (Bio-Rad, Hercules, CA).

Exclusion analysis: Exclusion analyses were performed for reported regions of autosomal recessive CSNB with fully informative polymorphic short tandem repeat (STR) markers flanking the CSNB locus or gene. PCR products were mixed with a loading cocktail containing HD-400 size standards (Applied Biosystems, Foster City, CA) and resolved in an Applied Biosystems 3100 DNA Analyzer. Genotypes were assigned using the Gene Mapper software from Applied Biosystems.

Linkage analysis: Linkage analysis was performed with alleles of PKRP170 and PKRP172 obtained through exclusion analysis using the FASTLINK version of MLINK from the LINKAGE Program Package [24,25]. Maximum logarithm

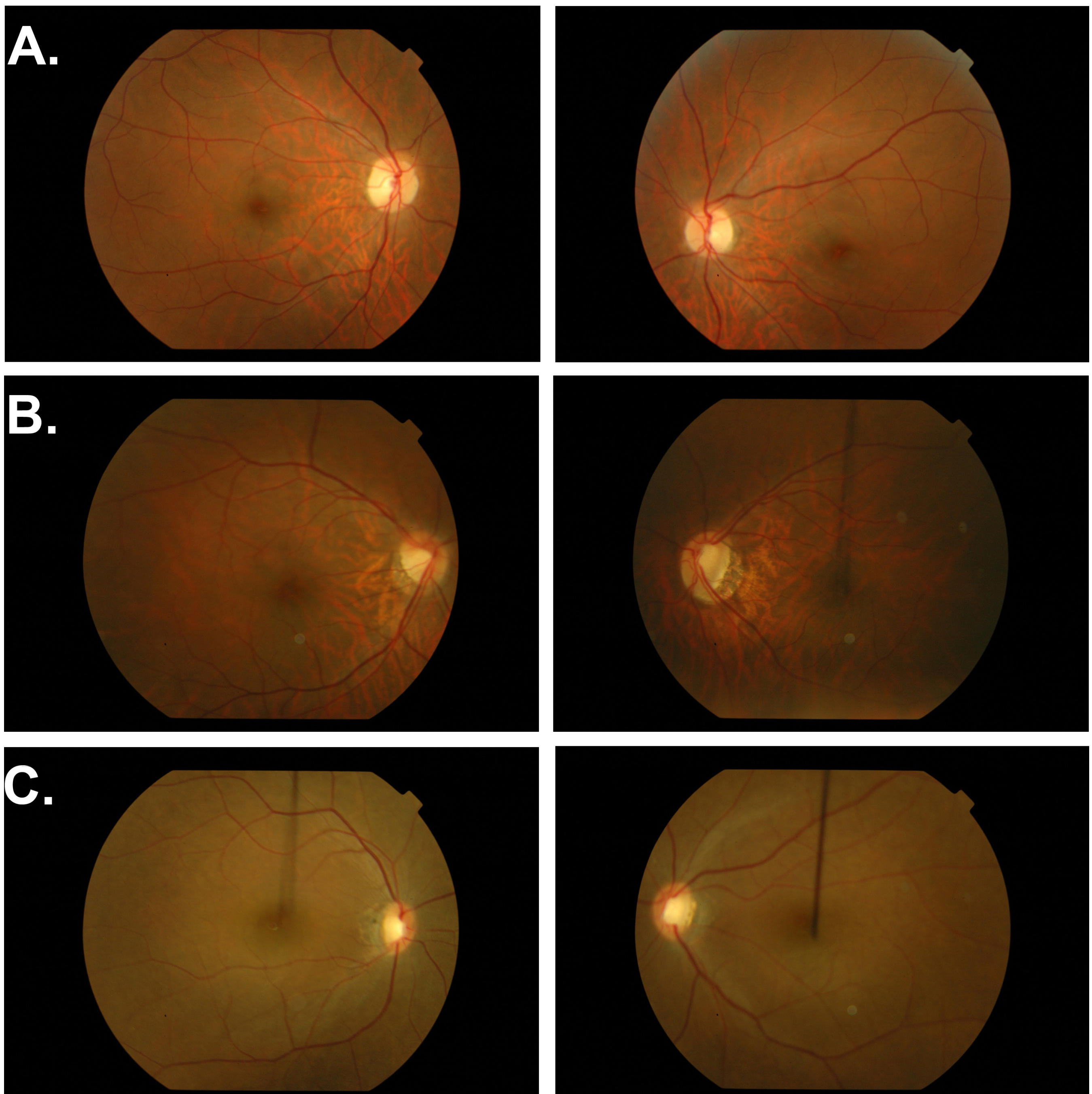

Figure 2. Fundus photographs of PKRP170 and PKRP172 family members. OD and OS of (**A**) affected individual 16 of PKRP170; (**B**) affected individual 14 of PKRP172; and (**C**) unaffected individual 15 of PKRP172. Fundus photographs of affected individuals in both the families show signs of tilted optic disc, while the macula and vasculature are normal. There are diffuse chorio-retinal atrophic changes involving the macula, equator and periphery. In contrast, the unaffected individual (normal night vision) exhibits mild chorio-retinal atrophic changes outside the arcades and the posterior pole. OD (oculus dextrus: right eye) and OS (oculus sinister: left eye).

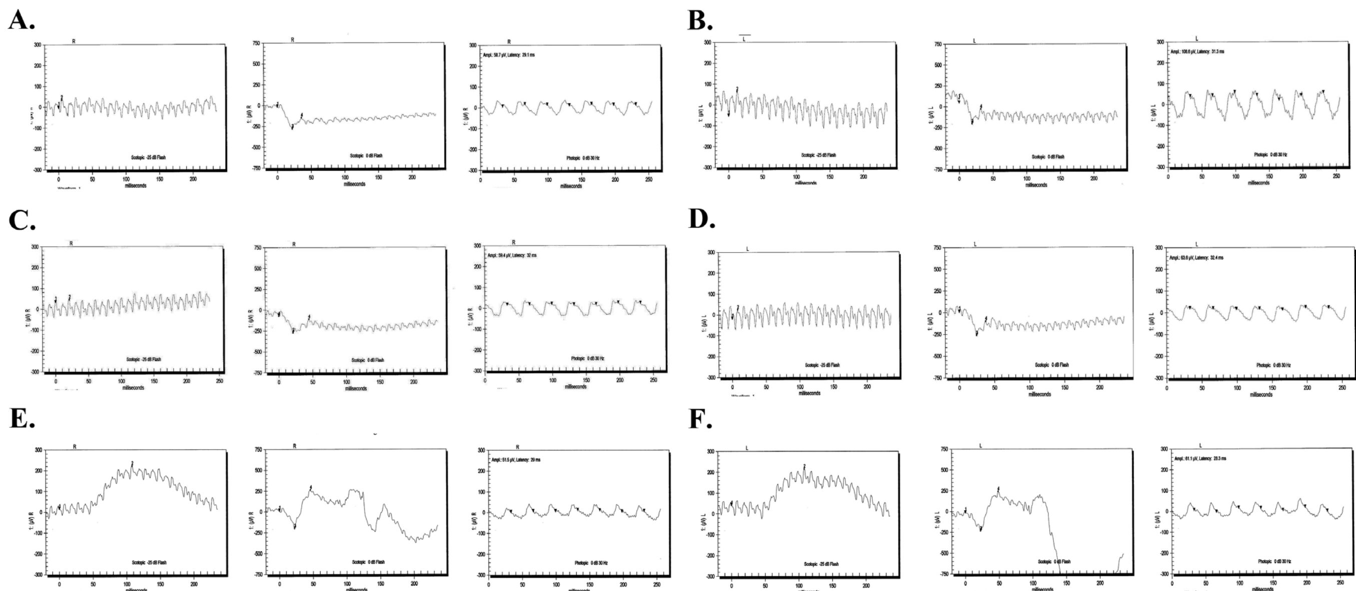

Figure 3. Electroretinogram responses of PKRP170 and PKRP172 family members. Scotopic −25 dB response, scotopic 0 dB response, and photopic 0 dB 30 Hz flicker response, of **A**: oculus dextrus (OD) and **B**: oculus sinister (OS) of affected individual 16 of PKRP170; **C** and **D**: OD and OS, respectively, of affected individual 14 of PKRP172; **E** and **F**: OD and OS, respectively of unaffected individual 15 of PKRP172. These recordings illustrate the absence of response measured at −25 dB and reduced b-waves in all affected individuals, while the 30 Hz flicker responses are indistinguishable when compared with the unaffected individual.

of odds (LOD) scores were calculated using ILINK from the LINKAGE Program Package. Autosomal recessive CSNB was investigated as a fully penetrant disorder with an affected allele frequency of 0.0005.

Mutation screening: Primer pairs to amplify the coding exons along with exon-intron boundaries of *GRM6* were designed using primer3 program and are available upon request. Primer sequences and annealing conditions are available upon request. PCR reactions were completed in a 10 µl volume containing 20 ng of genomic DNA. PCR amplification consisted of a denaturation step at 95 °C for 5 min followed by a two-step touchdown procedure. The first step of 10 cycles consisted of denaturation at 95 °C for 30 s, followed by primer set– pair specific (annealing temperature available upon request) annealing for 30 s (annealing temperature decreases by 1 °C per cycle) and elongation at 72 °C for 45 s. The second step of 30 cycles consisted of denaturation at 95 °C for 30 s followed by annealing (annealing 10 °C below the primer pair specific annealing temperature) for 30 s and elongation at 72 °C for 45 s, followed by a final elongation at 72 °C for 5 min. The PCR primers for each exon were used for bidirectional sequencing using BigDye Terminator Ready reaction mix according to the manufacturer's instructions. The sequencing products were resolved on an ABI PRISM 3100 DNA analyzer (Applied Biosystems), and results were analyzed using Applied Biosystems SeqScape software.

Table 2. Tabulated representation of electroretinogram responses of members of PKRP170 and PKRP172. OD and OS as described in Figure 2.

Family	ID	Eye	Rod response		Rod and cone response				30 Hz flicker response	
			b-wave	Implicit time	a-wave	Implicit time	b-wave	Implicit time	Amplitude	Implicit time
PKRP170	16	OD	31	4.5	−267.9	22	133.1	13.5	58.7	29.1
		OS	112.8	12.5	−301.5	19	176.4	13	108.6	31.3
PKRP172	14	OD	5.6	21	−197.1	21	162.4	23.5	59.4	32
		OS	40.7	8	−281.4	24.5	164.2	13	63.6	32.4
PKRP172	15	OD	179.2	89	−210.6	21	530.4	21	97.6	26.9
		OS	212.3	94.5	−239.3	23.5	546.3	24	71.8	28.1

Table 3. Two-point LOD scores of A) PKRP170 and B) PKRP172 for alleles of chromosome 5q short tandem repeat (STR) markers.

Marker	cM	Mb	0	0.01	0.03	0.05	0.07	0.09	0.1	0.2	0.3	Z_{max}	$Ø_{max}$
A.													
D5S1960	179.1	171.5	-∞	−2.4	−1	−0.5	−0.2	0.1	0.1	0.4	0.3	0.4	0.2
D5S2111	187.8	176	5.2	5.1	5	4.7	4.5	4.1	4	2.7	1.5	5.2	0
D5S2008	190.2	177.5	5.3	5.2	5	4.8	4.6	4.2	4.1	2.8	1.6	5.3	0
D5S2073	194.9	179.1	-∞	0.9	1.6	1.9	2	2	2	1.5	0.8	2	0.1
B.													
D5S2050	171.1	166.5	-∞	2.6	3	3.1	3	3	2.9	2.4	1.6	3.1	0.05
D5S1960	179.1	171.5	4.7	4.6	4.5	4.3	4.1	4	3.9	3	2	4.7	0
D5S2111	187.8	176	4.5	4.4	4.3	4.1	3.9	3.7	3.7	2.7	1.7	4.5	0
D5S2073	194.9	179.1	-∞	0.7	1.1	1.2	1.3	1.3	1.2	1	0.6	1.3	0.09

Linkage analysis was performed with alleles of PKRP170 and PKRP172 using the FASTLINK version of MLINK while maximum LOD scores were calculated using ILINK from the LINKAGE Program Package.

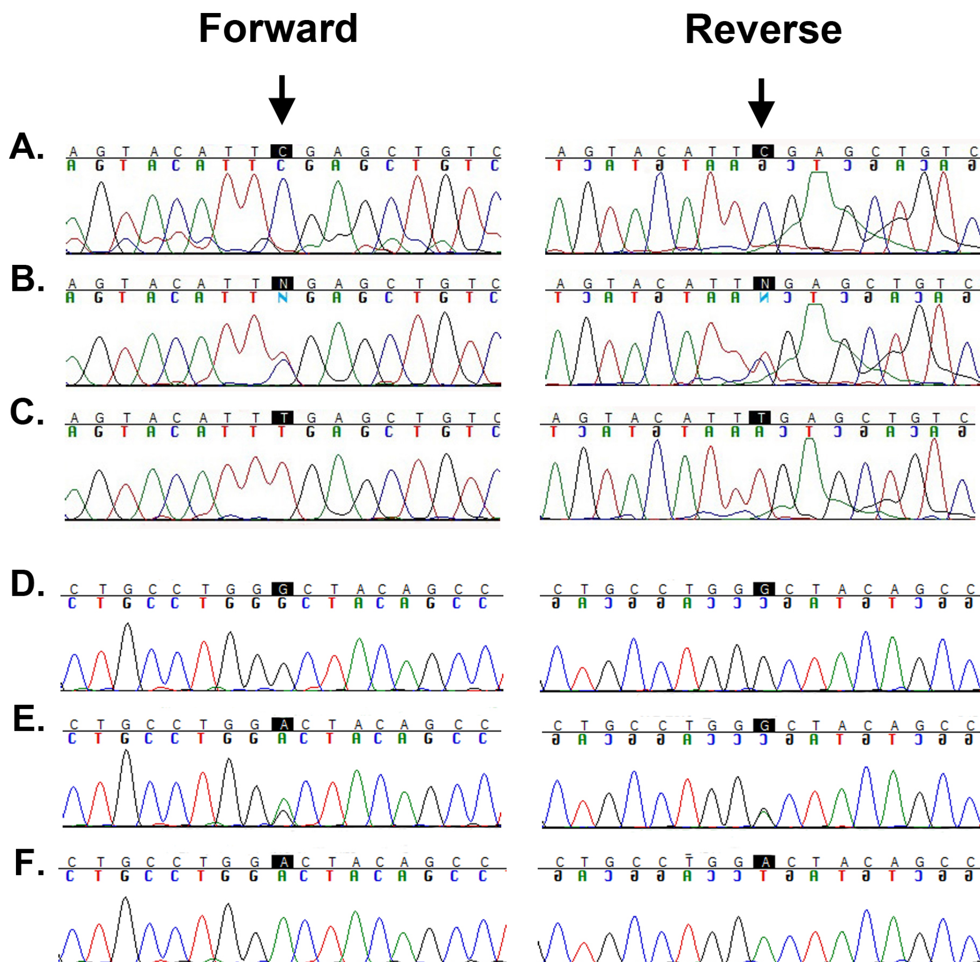

Figure 4. Sequence chromatograms of *GRM6* gene variations identified in families PKRP170 and PKRP172. **A**: Unaffected individual 23 of PKRP170 homozygous for the wild-type; **B**: unaffected individual 21 of PKRP170 heterozygous carriers; and **C**: affected individual 22 of PKRP170 homozygous for the C to T termination in exon 6; c.1336C>T. **D**: Unaffected individual 15 of PKRP172 homozygous wild-type; **E**: unaffected individual 12 of the PKRP172 heterozygous carrier; and **F**: affected individual 16 of PKRP172 homozygous for a G to A transition in exon 9: c.2267G>A.

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Human	D_{748}	L_{749}	S_{750}	L_{751}	I_{752}	G_{753}	C_{754}	L_{755}	G_{756}	Y_{757}	S_{758}	L_{759}	L_{760}	L_{761}	M_{762}	V_{763}
Chimp	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Gorrila	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Orangutan	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Gibbon	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Rhesus	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Crab-eating macaque	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Baboon	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Green monkey	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Marmoset	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Squirrel monkey	D	L	S	L	I	G	C	L	G	Y	S	F	L	L	M	V
Bushbaby	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Chinese tree shrew	D	L	S	L	I	G	C	L	G	Y	S	F	L	L	M	V
Squirrel	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Lesser Egyptian jerboa	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Praire vole	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Chinese hamster	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Golden hamster	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Mouse	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Rat	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Naked mole rat	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Guniea pig	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Chinchilla	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Brush tailed rat	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Rabbit	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Pika	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Pig	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Alpaca	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Bactrian camel	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Dolphin	D	L	S	L	V	G	C	L	G	Y	S	L	L	L	M	V
Killer whale	D	L	S	L	V	G	C	L	G	Y	S	L	L	L	M	V
Tibetan antelope	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Cow	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Sheep	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Domestic goat	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Horse	D	L	S	L	I	G	C	L	G	Y	S	F	L	L	M	V
White rhinoceros	D	L	S	L	I	G	C	L	G	Y	S	F	L	L	M	V
Cat	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Dog	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Ferret	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Panda	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Pacific walrus	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Weddell seal	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Black flying fox	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Megabat	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
David's myotis (bat)	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Big brown bat	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Hedgehog	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Shrew	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Star nosed mole	D	L	S	L	I	G	C	L	C	Y	S	L	L	L	M	V
Elephant	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Cape elephant shrew	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Manatee	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	F
Cape golden mole	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Tenrec	D	L	S	L	I	G	C	L	C	Y	S	L	V	L	M	V
Aardvark	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Armadillo	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Opossum	D	L	S	L	I	G	C	L	G	Y	S	L	L	L	M	V
Tasmanian devil	D	L	S	L	I	G	C	L	G	Y	S	L	V	L	M	V
Platypus	D	L	S	L	I	C	C	L	S	Y	S	I	V	L	M	V

Figure 5. Sequence alignment of amino acids illustrating the conservation of glycine 756 in GRM6 orthlogs. Primates, Euarchontoglires, Laurasiatheria, and Afrotheria are colored brown, green, purple, and orange, respectively. G756 mutated in family PKRP172.

In silico analysis: The degree of evolutionary conservation of positions at which mutations exist in other GRM6 orthologs was examined using the UCSC genome browser. The degree of evolutionary conservation of amino acid positions and the possible effect of amino acid substitution on the structure of GRM6 protein was examined with SIFT and PolyPhen2, respectively.

RESULTS

The consanguineous families PKRP170 and PKRP172 were recruited from the Punjab province of Pakistan (Figure 1). The available medical records of these families suggested that affected individuals in both families had reported night blindness from their early childhood (Table 1). Fundus photographs

of affected individuals in both the families show signs of tilted optic disc, while the macula and vasculature are normal. There are diffuse chorio-retinal atrophic changes involving the macula, equator and periphery (Figure 2A-B). In contrast, the unaffected individual (normal night vision) exhibits mild chorio-retinal atrophic changes outside the arcades and the posterior pole (Figure 2C). The auto refraction data suggests that both affected individuals have astigmatism while the unaffected individual has hyperopia (or far-sightedness) but no astigmatism (Table 1). In the scotopic ERG recordings, rod responses measured at −25 dB were absent in all of the affected individuals when compared with the response of the unaffected individuals (Figure 3 and Table 2). The rod–cone response measured at 0 dB illustrated reduced b-waves in affected individuals, while 30 Hz flicker responses of the affected individuals were indistinguishable from those of unaffected family members (Figure 3 and Table 2). Taken together, these clinical data are suggestive of a complete form of stationary night blindness in both families.

Linkage analysis was completed with STR markers flanking the previously reported loci or genes associated with autosomal recessive CSNB, and haplotypes were constructed using the alleles of these markers. Linkage analysis gave two-point LOD scores of 5.2 and 5.3 with markers D5S2111 and D5S2008, respectively, for PKRP170 (Table 3A). Similarly, two-point LOD scores of 4.7 and 4.5 were obtained with markers D5S1960 and D5S2111, respectively, for PKRP172 (Table 3B). Alleles of markers linked to *GRM6* were homozygous in affected individuals in both families (Figure 1A,B). The proximal and distal boundaries observed by haplotype analyses narrowed down the critical interval to 15.77 cM and 23.82 cM in families PKRP170 and PKRP172, respectively. The critical interval encompasses *GRM6*, a gene previously implicated in autosomal recessive CSNB.

To identify the causal variant responsible for the CSNB phenotype in PKRP170 and PKRP172, we sequenced entire coding exons, as well as the exon–intron boundaries of *GRM6*. Bidirectional sequencing identified the homozygous missense variation c.1336C>T in exon 6 of *GRM6* in all affected individuals of PKRP170 (Figure 4A-C), which would result in the premature termination of the open reading frame of protein (p.R446X). This variation segregated with the CSNB phenotype in PKRP170 (Figure 1A) and was not present in ethnically matched controls. Likewise, we identified a homozygous missense variation in c.2267G>A in exon 9 of *GRM6* in affected individuals of PKRP172 (Figure 4D-F). This variation results in a substitution of amino acid glycine at position 756 with aspartic acid (p.G756D). This variation segregated with the disease phenotype in the family (Figure 1B), but was absent in ethnically matched control chromosomes.

To evaluate the effect of aspartic acid substitution in the GRM6 protein, we first evaluated the conservation of glycine (Gly756) in other GRM6 orthologs. As shown in Figure 5, Gly756 is highly conserved in primates, placental mammals, and vertebrate species. We further examined the candidacy of Gly756 using SIFT and PolyPhen2 bioinformatics algorithms. SIFT predictions suggested that G756D substitution would not be tolerated by the native three-dimensional structure of the GRM6 protein. The affect protein function score for G756D was 0.01 (amino acids with probabilities <0.05 are predicted to be deleterious). Position-specific score differences obtained from PolyPhen2 algorithms suggested that the G756D substitution was benign, with a score of 0.166 (sensitivity: 0.92; specificity: 0.87).

DISCUSSION

Here, we report two familial cases of autosomal recessive CSNB recruited from the Punjab province of Pakistan. The clinical evaluations confirmed the CSNB diagnosis and microsatellite marker analyses localized the critical interval to a region on chromosome 5q harboring *GRM6*. Sanger sequencing identified two causal mutations: a novel nonsense variation and a previously reported missense variation. Both of these variations segregated with the CSNB phenotype in their respective families and were absent in ethnically matched control chromosomes. To the best of our knowledge, this is the first report identifying *GRM6* variations associated with CSNB in Pakistani patients.

Causal mutations in *GRM6* have been associated with the complete form of autosomal recessive CSNB [2,6]. Zeitz and colleagues identified mutations in the *GRM6* gene, where all patients displayed a distinctive abnormality of the rod pathway characterized by abnormal phase behavior with several minimum responses [2]. They further demonstrated that pathogenic mutations in three different domains of the GRM6 protein—the ligand-binding, cysteine-rich, and intracellular domains—abolished proper protein trafficking to the cell membrane [26]. Subsequently, Dryja and colleagues reported both nonsense and missense mutations in *GRM6* in individuals exhibiting cardinal symptoms of CSNB [6]. Furthermore, Xu and colleagues identified mutations in the *GRM6* gene in CSNB patients with high myopia [27].

The nonsense mutation (p.R446X) identified in family PKRP170 was present in exon 6 (of a total of 10 exons) and the corresponding mutant RNA would likely be subject to nonsense-mediated decay machinery (NMD), resulting in a null phenotype leading to autosomal recessive CSNB. Even

if the mutant protein escapes NMD, the mutant protein would lack the terminal 432 residues, including the seven transmembrane domains and the intracellular G-protein binding region [28]. Previously, G756D was identified in patients of South Asian and Indian origin, respectively [29,30]. Amino acid Gly756 is predicted to reside in the region constituting the transmembrane domain of the protein [28]. Transmembrane domains usually constitute nonpolar amino acids; therefore, we speculate that substitution of glycine with a charged amino acid affects the three-dimensional structure or the proper folding of GRM6, leading to mislocalization of the protein and thus compromising the physiologic function of GRM6.

Identification of causal mutations reaffirmed the role of *GRM6* in the pathogenesis of CSNB and reiterated the heterogeneity associated with the disease phenotype. Discovery of causal alleles associated with autosomal recessive CSNB in the Pakistani population will help in diagnostic efforts to identify carrier status in inbred familial cases and subsequent genetic counseling to prevent hereditary blindness.

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