Pseudodominant inheritance of autosomal recessive congenital stationary night blindness in one family with three co-segregating deleterious GRM6 variants identified by next-generation sequencing

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Abstract

**Background:** The congenital stationary night blindness (CSNB) affects the patients' dim light vision or dark adaptation by impairing the normal function of retina. It is a clinically and genetically heterogeneous disorder and can be inherited in an X-linked, autosomal dominant or autosomal recessive pattern. Several genetic alterations to the genes involved in visual signal transduction of photoreceptors and/or bipolar cells underlie its pathogenesis.

**Methods:** In this study, we used Sanger sequencing and next-generation sequencing (NGS)-based gene panel screening to investigate a family of three patients with CSNB inherited in an apparent autosomal dominant pattern. We expected to find out the disease-causing gene defects carried by this family.

**Results:** We found that the patients in this family did not carry the RHO, GNAT1, or PDE6B mutation, but carried compound heterozygotes mutations of GRM6. Three deleterious GRM6 variants, p.Arg621Ter, p.Gly51Val, and p.Gly464Arg, were found to be co-segregating with the disease, causing a pseudodominant inheritance of GRM6-related autosomal recessive complete CSNB.

**Conclusion:** This study presents a rare case of autosomal recessive CSNB (arCSNB) pseudodominant inheritance, which potentially leads us to expand our gene candidate list in future genetic testing for apparent dominant pedigrees. The discovery of the two novel likely pathogenic variants p.Gly51Val and p.Gly464Arg could broaden our knowledge about the genetics of CSNB and provide insights into the structure and function of the GRM6 protein.

**KEYWORDS**

congenital stationary night blindness, GRM6, next-generation sequencing, pseudodominant inheritance

1 INTRODUCTION

Congenital stationary night blindness (CSNB) is a group of nonprogressive retinal disorders with clinical and genetic heterogeneity (Zeitz, Robson, & Audo, 2015). Patients with this disease have impaired night vision or poor adaption to darkness. Poor visual acuity, myopia, photophobia, nystagmus, strabismus, and fundus abnormalities are other possible
manifestations associated with some forms of CSNB (Zeitz et al., 2015). To date, 17 genes have been implicated in the pathogenesis of this disease, most of which are crucial for the normal function of photoreceptor or bipolar cells (Zeitz et al., 2015).

CSNB with normal fundi is divided into Riggs, (1954) and Schubert-Bornschein (Schubert & Bornschein, 1952) subtypes according to electroretinography (ERG) patterns. The latter further diverges into two forms, the complete (cCSNB) and the incomplete (icCSNB) (Miyake, Yagasaki, Horiguchi, & Kawase, 1987; Miyake, Yagasaki, Horiguchi, Kawase, & Kanda, 1986). Riggs-type CSNB presents a reduced a-wave and reduced b-wave, while Schubert-Bornschein-type has a normal a-wave in addition to a severely reduced b-wave under scotopic conditions at a bright flash. In contrast to icCSNB, cCSNB has no detectable ERG to a dim flash. These CSNB subtypes possess its own specific inheritance patterns and distinct gene drivers. Today, autosomal dominant CSNB (adCSNB) is only associated with Riggs phenotype driven by RHO (OMIM: 180380; Dryja, Berson, Rao, & Oprian, 1993), GNAT1 (OMIM: 139330; Dryja, Hahn, Reboul, & Arnaud, 1996), or PDE6B (OMIM: 180072; Gal, Orth, Baehr, Schwinger, & Rosenberg, 1994); X-linked CSNB (xlCSNB) is associated with NYX (OMIM: 300278; Bech-Hansen et al., 2000; Pusch et al., 2000) and CACNA1F (OMIM: 300110; Bech-Hansen et al., 1998; Strom et al., 1998); autosomal recessive CSNB (arCSNB) could be complete, incomplete or Riggs-type, which may be caused by GRM6 (OMIM: 604096; Dryja et al., 2005; Zeitz et al., 2005), TRPM1 (OMIM: 603576; Audo et al., 2009; Li et al., 2009; van Genderen et al., 2009), GPR179 (OMIM: 614515; Audo et al., 2012; Peachey et al., 2012), LRIT3 (OMIM: 615004; Zeitz et al., 2013), C4BP4 (OMIM: 608965; Zeitz et al., 2006), CACNA2D4 (OMIM: 608171; Wycisk, Budde, et al., 2006; Wycisk, Zeitz, et al., 2006), SLC24A1 (OMIM: 603617; Riazuddin et al., 2010), or GNAT1 (OMIM: 139330) (Naeem et al., 2012).

In this study, we used Sanger sequencing and NGS-based gene panel screening to investigate a family with three patients affected by CSNB inherited in an apparent autosomal dominant pattern. We aimed to identify potential gene defects underlying the case.

2 | METHODS AND MATERIALS

2.1 | Ethical compliance

Four members of the pedigree (II3, II4, III3, and III4 in Figure 1) and a control (Figure S1) were included in this study, which was carried out in accordance with the tenets of the Declaration of Helsinki and was approved by the Internal Review Board of Henan Provincial People's Hospital, People's Hospital of Zhengzhou University. Informed consent was obtained from the participants for the study.

2.2 | ERG examination

ERG was performed according to ISCEV standard for full-field clinical ERG (McCulloch et al., 2015). For scotopic condition, 0.01/3.0/10.0 cd.s.m−2 and oscillatory potentials ERG were performed. For photopic condition, 3.0 cd.s.m−2 and 30 Hz Flicker ERG were performed.

2.3 | Gene panel screening

The screening of the proband for genetic variants was performed with Illumina TruSight™ One Sequencing Panel following the manufacturer's instructions. 150-bp paired-end reads were generated with an Illumina MiSeq platform. Sequencing data were analyzed with MiSeq Reporter (Illumina) and variant annotation was performed with VariantStudio (Illumina).

2.4 | In silico prediction

The function of genetic variants was predicted in silico with PolyPhen-2 (Adzhubei et al., 2010), PROVEAN (Choi, Sims, Murphy, Miller, & Chan, 2012), and MutationTaster (Schwarz, Cooper, Schuelke, & Seelow, 2014) following the instructions on their online interfaces and using default parameters.

2.5 | Database accessibility

Clinical significance of variants was obtained from ClinVar (Landrum et al., 2016) (May 13, 2019; https://www.ncbi.nlm.nih.gov/clinvar/). Allele frequency was obtained from ExAC (Lek et al., 2016) (release 1.0 updated February 27, 2017; http://exac.broadinstitute.org/) and gnomAD (Lek et al., 2016) (version 2.0.2 updated October 3, 2017; http://gnomad-old.broadinstitute.org/).

2.6 | Sanger sequencing of GRM6

The following primers were used to screen GRM6. GRM6-E2-3F: TGTTCAGGACACAGCTTGTACC. GRM6-E2-3R: CTATTCAGTCTGGGCTTGTGGC. GRM6-E4F: CCTCTGACCCTCTGACACTG. GRM6-E4R: CAATTCCTCCCCGGGCAAGG. GRM6-E5-6F: GTTCACCTGGCCACTCCTAG. GRM6-E5-6R: TAGACCACTCAGCCTCACCG. GRM6-E7-8F: CGGCTTGGATTTGCACGTCC. GRM6-E7-8R: CACATTTTTGACACGCT. GRM6-E9F: AGAGCCTAAGGGGATCCTG. GRM6-E9R: AACAAGCAGCCAGTACGGG. GRM6-E10F: GTGCTCATTCCCAGTTCCCC. GRM6-E10R: TGGTCTTGGCAAAACCTCCTG.
RESULTS

As shown in Figure 1, three members in a pedigree were affected by CSNB, the proband (II3) and his two children (III3 and III4). All the three patients had mild myopia (−1.0 to −2.0 D) with mild astigmatism; the uncorrected visual acuity of them was between 0.3 and 0.5 whereas the corrected visual acuity could reach 1.0. The proband had nystagmus and strabismus. Both of his children had amblyopia. His partner (II4) had no symptoms of CSNB. The children were diagnosed with night blindness when 4–5 years old. The age of the proband at diagnosis was not available. When the pedigree was received by Genetic Counseling Clinic, Henan Provincial People’s Hospital, no properly performed ERG was available.

The night blindness transmission from the proband to his son ruled out the possibility of X‐linked inheritance (Figure 1). Initially, we assumed the disease was inherited autosomal dominantly. Three genes underlying adCSNB, GNAT1, RHO, and PDE6B, were screened on the proband with Sanger sequencing, but no variant was found.

Next, TruSight™ gene panel sequencing was performed on the proband. Two GRM6 variants were identified and subsequently validated via Sanger sequencing (Figure 1). One is p.Arg621Ter (c.1861C>T, CGA to TGA), p.Gly51Val (c.152G>T, GGC to GTC), and p.Gly464Arg (c.1390G>A, GGA to AGA). Another single nucleotide variant c.1392A>G can be also observed in the top right. It is at the wobble position of codon 464, synonymous and benign. (GRM6: NM_000843.3) Bottom: the CSNB family’s pedigree with genotype. Arrow marks the proband and asterisk marks the clinically evaluated member.

Sanger sequencing targeting GRM6 was then carried out for the proband’s children. It was found that the son and the daughter were both compound heterozygous for p.Arg621Ter and p.Gly51Val and possibly affected by autosomal recessively inherited GRM6-related CSNB.

Together with the proband’s genotype, in this family three deleterious GRM6 variants, p.Arg621Ter, p.Gly51Val, and p.Gly464Arg, were identified, which could explain the apparent autosomal dominant inheritance pattern of GRM6‐related arCSNB in the pedigree. By the criteria of ACMG (Richards et al., 2015), both of the missense variants were classified into Likely Pathogenic category.
did not alter amino acid sequence of the protein or locate in a splice site. Its frequency in ExAC and gnomAD were 71592/119380 and 164878/275666, respectively. ClinVar documented it as a benign variant.

GRM6-related CSNB was the complete form of this disease. To subtype the CSNB of the pedigree, the daughter and a control were recruited to take ERG examination. From the result (Figure S1 and Table S1), we could see that the daughter showed a dark-adapted ERG of negative waveforms with a normal a-wave and a severely reduced b-wave, which matched the characteristic of cCSNB. The ERG results indicated that the function of bipolar cells and amacrine cells in both eyes of the daughter decreased, while other functions were basically normal.

4 | DISCUSSION

In this study, we reported a rare case of pseudodominantly inherited arCSNB in a family with three co-segregating deleterious variants of GRM6. To our knowledge, this is the first report of CSNB pseudodominant inheritance, and the first report of likely pathogenic variants p.Gly51Val and p.Gly464Arg in GRM6-related CSNB.

Pseudodominant inheritance of autosomal recessive diseases happens when a homozygote (or compound heterozygote) has a partner with a heterozygous mutation. Higher carrier frequency in a population brings higher incidence of this phenomenon. Similar cases also have been reported in DUOX2-caused nonautoimmune hypothyroidism and GDAP1-caused Charcot-Marie-Tooth type 2 (Abe, Narumi, Suwanai, Hamajima, & Hasegawa, 2015; van Paassen et al., 2017). Although adCSNB is usually caused by RHO, GNAT1, or PDE6B defects, this study reminds us that some apparent autosomal dominant CSNB can also be caused by some other autosomal recessive genes. Considering the possibility of this situation, unbiased methods like next-generation sequencing simultaneously targeting multiple genes are more helpful when patients’ clinical manifestations are unclear.

GRM6 is a gene localized on chromosome 5q35.3. It encodes a 7-transmembrane protein of 877 amino acid residues, which belongs to human metabotropic glutamate receptor family and is specifically expressed in ON bipolar cells. GRM6 protein is important for the signal transmission at the postsynaptic site upon light stimulation. In 2005, two research groups reported that several GRM6 variants, including p.Leu26fs, p.Pro46Leu, p.Gly58Arg, p.Gly150Ser, p.Val243fs, p.Cys522Tyr, p.Arg621Ter, p.Glu708Ter, and p.Glu781Lys, could lead to cCSNB in an autosomal recessive manner (Dryja et al., 2005; Zeitz et al., 2005). The variants discovered in this study are both located in the extracellular region of GRM6 spanning from Gly25 to Trp585. Multiple sequence alignment by Clustal Omega suggested Gly51 of GRM6 to be conserved in its family members GRM2 (Gly44), GRM3 (Gly51), GRM4 (Gly61), GRM7 (Gly61), and GRM8 (Gly58), whereas Gly464 is conserved in all eight members of the family (data not shown). This conservation indicates the functional importance of both residues.

Findings in this report remind us to consider the possibility of pseudodominance when facing an apparent autosomal dominant pedigree of CSNB. This potentially leads us to expand gene candidate list in genetic testing. Besides, the discovery of p.Gly51Val and p.Gly464Arg may apply similar mechanism to cause CSNB.

Table 1 The documented allele frequency and the predicted pathogenicity of p.Gly51Val, p.Gly464Arg, and p.Arg621Ter

| Variant      | p.Gly51Val | p.Gly464Arg | p.Arg621Ter |
|--------------|------------|-------------|-------------|
| ClinVar      | N.D.       | N.D.        | Pathogenic  |
| Clinical     |            |             |             |
| significance |            |             |             |
| ExAC         |            |             |             |
| Allele       |            |             |             |
| frequency    | N.D.       | 1/115892    | 21/118580   |
| gnomAD       |            |             |             |
| Allele       |            |             |             |
| frequency    | 1/27876    | 2/245570    | 44/276352   |
| PolyPhen-2   |            |             |             |
| Prediction   |            |             |             |
| Score,       | 0.999      | 1.000       | N.P.        |
| HumDiv       |            |             |             |
| Score,       | 0.993      | 0.990       | N.P.        |
| HumVar       |            |             |             |

GRM6: NM_000843.3.

Abbreviations: ND, not documented; NP, not performed.

In 2007, Zeitz et al. investigated the impact of missense variants in GRM6 and found CSNB-associated variants p.Pro46Leu, p.Gly58Arg, p.Gly150Ser, p.Val243fs, p.Cys522Tyr, p.Arg621Ter, p.Glu708Ter, and p.Glu781Lys, could lead to cCSNB in an autosomal recessive manner (Dryja et al., 2005; Zeitz et al., 2005). The variants discovered in this study are both located in the extracellular region of GRM6 spanning from Gly25 to Trp585. Multiple sequence alignment by Clustal Omega suggested Gly51 of GRM6 to be conserved in its family members GRM2 (Gly44), GRM3 (Gly51), GRM4 (Gly61), GRM7 (Gly61), and GRM8 (Gly58), whereas Gly464 is conserved in all eight members of the family (data not shown). This conservation indicates the functional importance of both residues.

The structure of human GRM6 has not been resolved. From determined structures of its family members, we could infer that Gly51 and Gly464 both reside in the unstructured regions out of flanking alpha helices and/or beta strands, and not involved in the direct contact with the ligand glutamate. In 2007, Zeitz et al. investigated the impact of missense variants in GRM6 and found CSNB-associated variants p.Pro46Leu, p.Gly58Arg, p.Gly150Ser, p.Ile405Thr, p.Cys522Tyr, and p.Arg621Ter, p.Glu708Ter, and p.Glu781Lys, could lead to cCSNB in an autosomal recessive manner (Dryja et al., 2005; Zeitz et al., 2005). The variants discovered in this study are both located in the extracellular region of GRM6 spanning from Gly25 to Trp585. Multiple sequence alignment by Clustal Omega suggested Gly51 of GRM6 to be conserved in its family members GRM2 (Gly44), GRM3 (Gly51), GRM4 (Gly61), GRM7 (Gly61), and GRM8 (Gly58), whereas Gly464 is conserved in all eight members of the family (data not shown). This conservation indicates the functional importance of both residues.

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GRM6 since the early stage of diagnosis will benefit future patients.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

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