

STUDY OF GRM 6 METABOTROPIC GLUTAMATE RECEPTOR 6 GENE IN  
NIGHT BLIND FAMILIES

Muhammad Fayyaz<sup>1\*</sup>, Masroor Elahi Babar<sup>2</sup>, Khawar Ali<sup>2</sup>, Anam Shahzadi<sup>3</sup>, Ali Raza<sup>4</sup>, Muhammad Masood Ahmed<sup>4</sup>, Mehreen Arshad<sup>5</sup>, Dr Jawwad azeem khan

<sup>1\*</sup>Department of Biochemistry and Biotechnology University of Gujrat Pakistan.

<sup>2</sup>Department of Molecular Biology Virtual University of Pakistan.

<sup>3</sup>Department of Biochemistry and Biotechnology University of Gujrat Pakistan.

<sup>4</sup>Department of Management Sciences University of Engineering & Technology Lahore.

<sup>4</sup>College of Pharmacy, Nishtar Medical University, Multan.

<sup>5</sup>Ziauddin University Karachi, Pakistan.

\*Corresponding Author: Muhammad Fayyaz, Khawar Ali.

\*Corresponding Author Email [fayyazahmad198@gmail.com](mailto:fayyazahmad198@gmail.com) , [khawarali1202@gmail.com](mailto:khawarali1202@gmail.com)

**Abstract**

Night blindness is characterized by poor vision in low light and is frequently related to retinal rod cell degeneration. While it can be congenital and classified as an Autosomal recessive ambiguity, it is regarded as rare around the world and is usually distinguished from X chromosome. The study focused on families that had the history of night blindness, and entire blood samples were taken for DNA extraction. Exon 4 of the GRM6 gene was targeted using specific primers to amplify the targeted DNA fragments. Gel electrophoresis and sequencing techniques were used to examine the amplified fragments. However, there were no significant alterations in the GRM6 Exon 4 area in our findings, indicating that this specific gene may have no role in night blindness in the examined families. The work emphasizes the significance of comprehending the numerous genetic mechanisms that contribute to congenital fixed night blindness, as well as the involvement of the GRM6 gene in retinal function. The study focused on the GRM6 gene in connection to congenital stationary night blindness (CSNB). The GRM6 gene was premeditated in seven families having night blindness symptoms. Blood samples were taken, DNA was extracted by organic method and specific primers for Exon 4 of the GRM6 gene were designed. Gel electrophoresis was performed that indicated the successful PCR amplification and subsequent DNA sequencing revealed no substantial changes in Exon 4 as compared to the NCBI reference sequence.

**Keywords:** GRM6, PCR, CSNB, DNA, Exon 4, DNA Sequencing, NCBI, Visual Acuity.

## INTRODUCTION

Mutations in the glutamate receptor Metabotropic 6 (GRM6) gene cause congenital stationary night blindness. (Xu 2009). CSNB is a collection of non-progressive retinal illnesses characterized by reduced night vision that can be inherited as an Autosomal dominant, Autosomal recessive, or X-linked trait. (Zeitz *et al.*, 2005, Khan *et al.*, 2005).

Clinically Significant Non progressive Retinal Disorders are clinically and genetically varied group of non-progressive retinal diseases. Congenital stationary night blindness (CSNB) (Nakamura *et al.*, 2010) is caused by mutations in genes involved in the photo-transduction cascade or retinal signaling from photoreceptors to bipolar cells (second-order neurons). Many people with CSNB have severe night vision impairment since artificial light is restricted. In the absence of evident fundus abnormalities, CSNB is associated with other ocular features such as Myopia, Nystagmus, and strabismus. (Malaichamy *et al.*, 2014)

In reaction to a bright white flash in the dark-adapted eye, CSNB is typically coupled with an electronegative or 'negative' (ERG). As a result, the b/a ratio has decreased. CSNB is frequently associated with an electronegative or 'negative' in response to a bright white flash in the dark-adapted eye (ERG). The b/a ratio have dropped as a result. GRM6 encodes the Metabotropic glutamate receptor 6 (mGluR6) which is found on the dendrites of rod and cone ON bipolar cells. (Sergouniotis *et al.*, 2012)

ERGs obtained in response to single brief flashes of light revealed visible a-waves generated by photoreceptors and dramatically reduced b-waves generated by second-order inner retinal neurons. ERG responses to saw-tooth flickering light demonstrated a drastically reduced ON response and a nearly normal OFF response. (Dryja *et al.*, 2005)

Patients with CSNB may have impaired night vision, as well as decreased visual acuity, Nystagmus, and refractive error. Except for a few known examples involving GRM6 (the gene encoding Metabotropic glutamate receptor 6), mGluR6 (Koike .,2010) , which is found at the dendritic ends of the ON bipolar cells) mutations, which frequently but not always result in myopia, Emmetropia, and Myperopia, full CSNB usually invariably has extreme myopia. (Almutairi *et al.*, 2021)

The researchers investigated the potential of a link between GRM6 gene alterations and congenital stationary night blindness (CSNB). CSNB is a group of retinal diseases characterized by impaired night vision that can manifest in some inherited forms. Mutations in genes involved in the Photo transduction cascade or retinal signaling from photoreceptors to bipolar cells can result in CSNB. The researchers collected blood samples from families with night blindness and carefully saved the genetic information for future research.

L-glutamate is the central nervous system's principal excitatory neurotransmitter, activating both Ionotropic and Metabotropic glutamate receptors. Glutamatergic neurotransmission is involved in almost every aspect of normal brain function and can be disrupted in a variety of Neuropathologic disorders. Metabotropic glutamate receptors are a G protein-coupled receptor family that has been split into three categories based on sequence homology, proposed signal transduction processes, and structure, as well as pharmacologic characteristics. Group III comprises GRM4, GRM6, GRM7, and GRM8. [Source: Ref Seq, May 2018] The researchers used PCR amplification with particular primers designed for the GRM6 Exon 4 region and confirmed efficient amplification by gel electrophoresis, which exhibited distinct and visible bands. Further DNA sequencing of this region across seven samples identified no mutations or polymorphisms when compared to the reference sequence. This means that genetic alterations in the GRM6 Exon 4 area did not produce night blindness in the samples studied. (Naem *et al.*,2015)

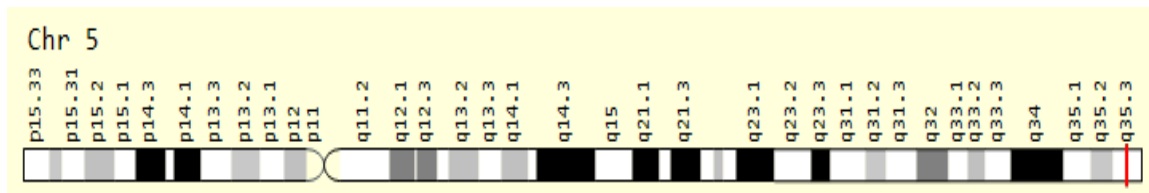


Figure 1 Genomic View for GRM6 Gene

The findings of the study are consistent with previous research establishing a relationship between GRM6 gene alterations and CSNB. It emphasizes the role of the GRM6 gene, which encodes the Metabotropic glutamate receptor, in visual information transfer from photoreceptors to ON bipolar cells in the retina (Kew JN.,2005) People with CSNB usually exhibit minimally reduced or normal visual acuity under certain lighting circumstances, despite synaptic abnormalities in the transmission route. Electrophysiological investigations, such as Electroretinography (ERG), reveal significant abnormalities in the responses of patients, especially a reduction in inner second-order retinal neurons generated by ON pathways.

Furthermore, the study suggests a relationship between myopia and CSNB, particularly in cases with mutations in the NYX and GRM6 genes. This emphasizes the significance of these genes in CSNB and its many manifestations. Overall, the work adds to our understanding of the GRM6 gene's function in the development of CSNB and the potential hereditary variables linked with this disorder. More study is needed to confirm these findings and acquire a better understanding of the genetic basis of night blindness and associated illnesses.

## METHODOLOGY:

With the Consent, blood samples were collected from families affected by night blindness and processed to extract genomic DNA organically. To avoid blood clotting, these samples were collected in 0.5M EDTA anticoagulant vacutainers. Each participant supplied roughly 5 ml of whole blood, which was immediately mixed with the anticoagulant. Following that, the samples were carefully preserved in an icebox before being sent to the laboratory for further processing, to extract DNA.

Blood samples were stored at -20°C in EDTA vacutainers. This meticulous sample collection approach ensured the integrity of the genetic material needed for subsequent genomic DNA extraction and analysis, which was a critical step in the study's methodology. The GRM6 Exon 4 region was targeted using specific primers (table1), and PCR was performed to amplify the DNA segment. The PCR results were checked on gel electrophoresis, and possible alterations in GRM6 Exon 4 were investigated using DNA sequencing technique. Bioinformatics techniques were also used to compare the sequenced samples to reference sequences, demonstrating that no SNPs related to night blindness were found in the tested samples. The primers were developed for human GRM6 Exon 4 using primer3 software, as shown in Table1, and they include the product size of 410 bp of human GRM6 Exon 4. The human GRM6 Exon 4 was amplified using these primers, which had a forward primer length of 20 bp and a reverse primer length of 20 bp. The oligonucleotide characteristics calculator was used to determine complementarity and hairpin formation, and the findings showed no hairpin formation, indicating that the complementarity was zero. GRM6 Ex4 F Hum To optimize the annealing temperature of GRM6 Ex4 primers, the reaction mixture in Table 2 was employed, and PCR conditions were used to achieve the required target sequence amplification. In a 0.2ml PCR tube, DNA Taq polymerase, dNTPs, MgCl<sub>2</sub>, taq buffer, primer forward, primer reverse, and ddH<sub>2</sub>O were added.

The study comprised nine patients from seven families who showed Autosomal recessive inheritance and clinical and electrophysiological findings consistent with complete CSNB. Informed consent was obtained, and blood samples were collected for DNA extraction and subsequent GRM6 mutation screening. The study was approved by the local research ethics committee and adhered to the Helsinki Declaration standards.

Gene	Primer	Primer sequence	Length	Tm	GC%	nm
Hum GRM6	GRM6 Ex4 F	AGGCCTAGGGTCAGAGGAAA	20	57.5	51%	42.5
Hum GRM6	GRM6 Ex4 R	GAAAATGAGCATCCCCAAGA	20	53.4	49%	50.4

*Table 1 Primers set for amplification of Hum GRM6 Gene*

Step	Temperature	Duration	No of cycles
Initial denaturation	95°C	5 minutes	1X
Denaturation	95°C	45 seconds	
Annealing	60°C (-0.5°C/ cycle)	60 seconds	11X
Extension	72°C	45 seconds	
Denaturation	95°C	45 seconds	25X
Annealing	52°C	60 seconds	
Extension	72°C	45 seconds	
Final Extension	72°C	10 minutes	1X
Infinite hold	4°C	∞	

*Table 2 Thermocycler conditions for characterization and amplification of Hum GRM6 Gene*

Table 3 shows Thermocycler was used to amplify a specific DNA sequence of human GRM6 Exon 4. The annealing temperature of human GRM6 Exon 4 primers was set at 60oC to 52oC for -0.5oC/cycle using the touch-down method. The PCR product band was then confirmed on a 2 percent Agarose gel with 5ul of DNA and 2ul of loading dye, and a 50 base pair ladder was used to assess the quality and quantity of the DNA. After that, the gel was placed in the gel documentation system to view the DNA bands on the gel.

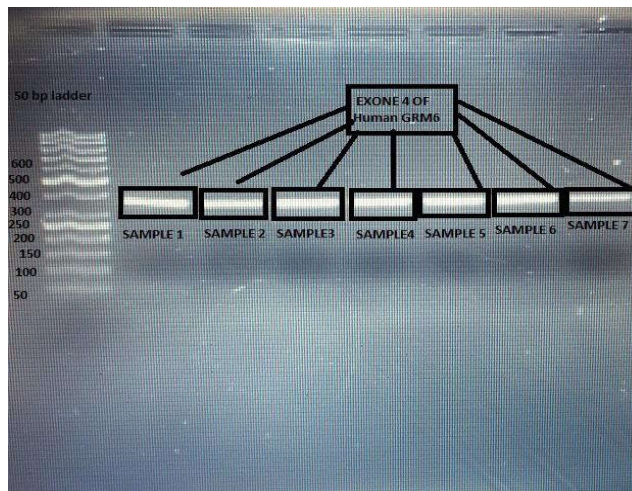


Figure 2. PCR primer gel electrophoresis

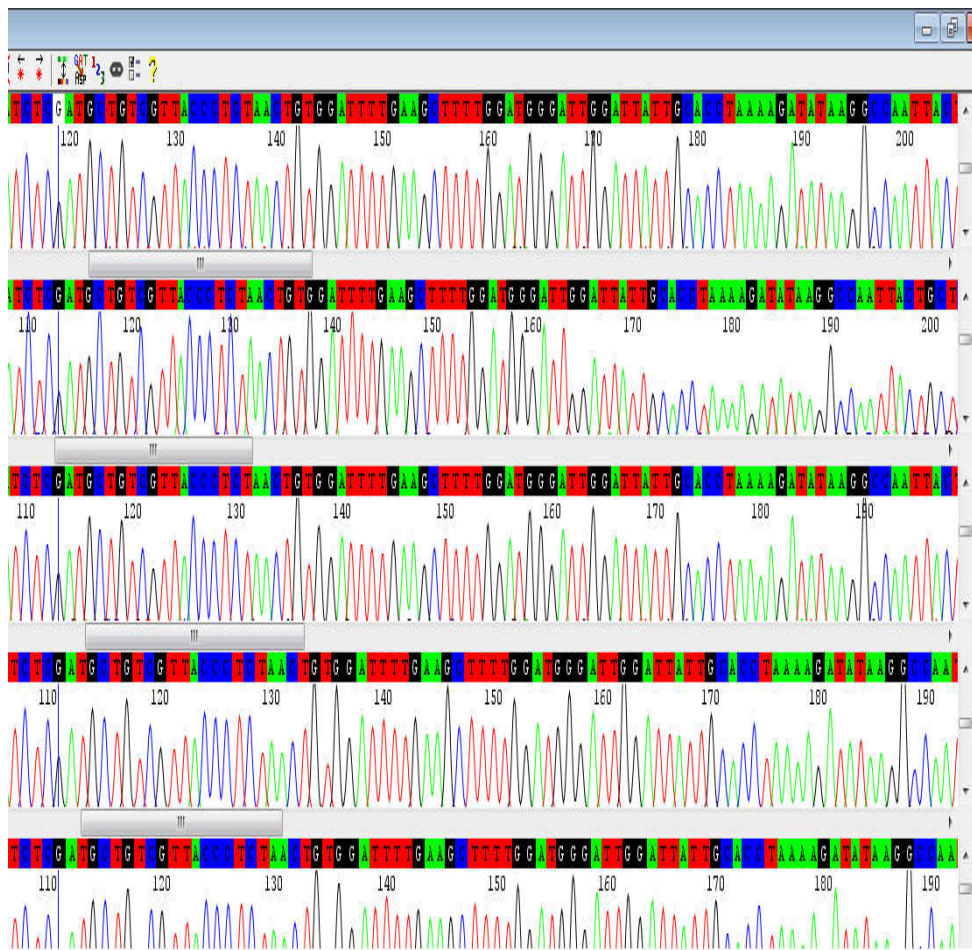
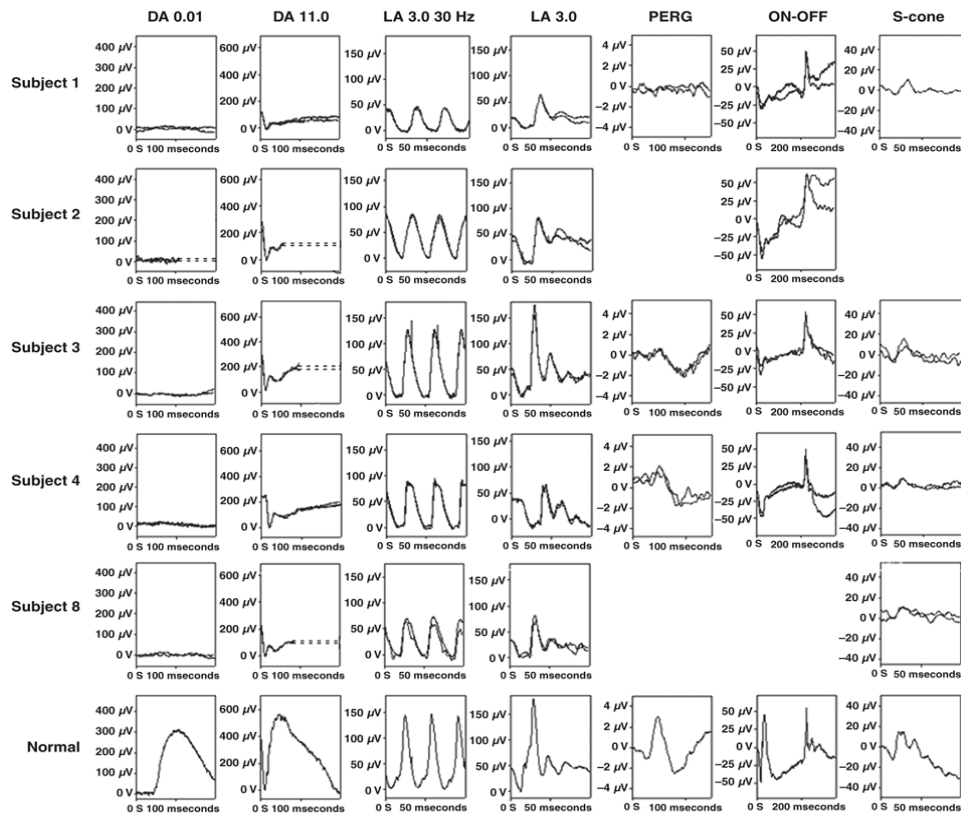


Figure 3. Human GRM6 Exon 4 sequencing data





**Figure 4 Full-field and pattern electroretinograms (ERGs) from patients with GRM6-related complete CSNB**

## Results

This study focuses on the GRM6 gene and its potential link with congenital stationary night blindness (CSNB). The gene GRM6 Exon4, which is found on chromosome 5, was studied in seven families with night blindness symptoms. Myopic astigmatism was the most prevalent refractive error, with a median visual acuity of 0.2 log-MAR. Color vision was normal in the patients who were tested. Most fundus examinations yielded normal results, however, three patients' eyes exhibited severe myopic Maculopathy. The two evaluated patients had normal fundus auto fluorescence imaging. Because no significant changes were found, the absence of genetic variation within the studied area suggests that night blindness in the examined samples may not be associated with genetic abnormalities in the GRM6 Exon 4 region.

## Discussion

The GRM6 gene encodes a protein with two domains that depolarize ON bipolar cells when exposed to light. Night blindness is connected with mutations in genes such as RHO, CABP4, NYX, and GRM6 (Xiao *et al.*, 2007), with probable ties to myopia. The findings imply that the GRM6 gene may be susceptible to night blindness and underline its involvement in visual information transmission. Mutations in the GRM6 gene were examined due to their importance in retinal transmission from photoreceptors to bipolar cells. A cohort research was conducted on a group of patients ranging in age from 7 to 75 years old, with an average age of 24. Except for one case, all symptoms appeared within the first decade of life, with the most prevalent difficulties being diminished visual acuity, even with myopic correction, and Nystagmus. Only two patients were affected by night blindness. Two of the nine patients had strabismus and five had Nystagmus, with one adopting a head position for better eyesight. Two sets of siblings were included, and four patients had

It was distinguished by a distinct and visible band. Furthermore, when the DNA was matched to the NCBI reference sequence, sequencing data from seven human GRM6 Exon 4 samples revealed no mutations or polymorphisms. Because no significant changes were found, the absence of genetic variation within the studied area suggests that night blindness in the examined samples may not be associated with genetic abnormalities in the GRM6 Exon 4 region. DNA extraction and sequencing were performed, findings revealed no substantial alterations in Exon 4 of the GRM6 gene.

Full-field and pattern (ERGs) from GRM6-related full CSNB patients (Frishman *et al.*, 2011). Figure 3 shows a comparison of normal traces. The dark-adapted responses for flash intensities of 0.01 and 11.0 cd.s/m<sup>2</sup> are shown, as are Photopic ERGs for flash intensities of 3.0 cd.s/m<sup>2</sup> and ON-OFF and S-cone system stimulation.

The macular function is assessed using pattern ERGs. All responses are one-sided and show a high degree of Interocular symmetry. A 10-ms Prestimulus delay was used in subject 4. Due to Nystagmus or eye movement artifact, subjects 2 and 8 exhibited un-recordable missing traces. Despite the existence of synaptic defects, people with CSNB often exhibit modestly reduced or normal visual acuity under certain lighting conditions. Notably, electrophysiological tests in

affected. Patients revealed distinct rod signal circuit abnormalities. More study is needed to confirm these findings and acquire a better understanding of the role of GRM6 mutations in night blindness and associated illnesses. The ON-OFF ERGs revealed significant ON b-wave decrease while sparing both the a-wave and the OFF response. Four of the four patients had abnormal S-cone ERGs, indicating ON-pathway malfunction.

Waveforms in four younger individuals assessed using a shorter methodology was qualitatively comparable to those previously described. The research focuses on the GRM6 gene, which encodes the Metabotropic glutamate receptor, and its role in visual information transfer from photoreceptors to ON bipolar cells in the retina. Individuals with CSNB frequently exhibit moderately impaired or normal visual acuity in dim or normal light circumstances sensed by the cone system, despite synaptic abnormalities in the transmission pathway.

Electrophysiological testing such as Electroretinography (ERG) indicated Distinctive anomalies in the ERG responses of afflicted patients, specifically a decrease in inner second-order retinal neurons generated from ON pathways. The report also covers the discovery of unique signal pathway patterns in CSNB patients, underscoring the disease's complexity. The occurrence of homozygous or compound heterozygous GRM6 mutations in afflicted patients emphasizes the gene's significance in the development of CSNB. Furthermore, the study demonstrates a relationship between myopia and CSNB, particularly in individuals with NYX and GRM6 gene mutations (Koike *et al.*, 2010). This conclusion is consistent with the unusual incidence of Autosomal recessive CSNB and is consistent with prior research that has connected GRM6 gene alterations to CSNB.

This shows that these genes have a specific function in CSNB, resulting in distinct phenotypes. (Pearing *et al.*, 2011).

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