

A Dominant form of Congenital Stationary Night Blindness (adCSNB) in a Large Chinese Family

X. Liu¹, S. Zhuang^{2,#}, S. Hu^{2,3}, F. Zhang³, B. Lin¹, X. Li¹, D. Xu⁴ and S.-H. Chen^{5,*}

¹Eye Hospital, School of Optometry and Ophthalmology, Wenzhou Medical College, Wenzhou, Zhejiang 325003, P.R. China

²Hangzhou Genomics Institute, Hangzhou, Zhejiang 310008, P.R. China

³Beijing Genomics Institute, Beijing 101300, P. R. China

⁴Department of Medicine, Division of Medical Genetics, 357720, University of Washington, Seattle, WA 98195, USA

⁵Department of Pediatrics, Box 359300, 6P-1, University of Washington and Children's Hospital and Regional Medical Center, Seattle, WA 98195, USA

Summary

A pedigree of congenital stationary night blindness (CSNB) is described in a large Chinese family. The clinical description, pedigree, dark adaptation and electroretinogram (ERG) studies indicate that the patients have an autosomal dominant form (ad) of CSNB. The disorder has been transmitted through at least 12 generations with over 40 affected individuals identified. The ERG data reveal that affected persons have severely diminished b-wave responses to dim light, but normal a-wave and subnormal b-wave responses to maximum light stimuli. The dark adaptation curves of three patients show a monophasic curve, typical for night blindness. We have excluded the five previously known mutations in the three genes (*RHO*, *PDE6B* and *GNAT1*) associated with adCSNB, and linkage studies have excluded tight linkage between the disease locus and markers associated with these three genes. Thus, this family has adCSNB caused by a different gene from the previously identified *RHO*, *PDE6B*, and *GNAT1*.

Keywords: adCSNB, Chinese family

Introduction

Congenital stationary night blindness (CSNB) consists of a group of eye disorders clinically characterized by a non-progressive severe deficiency of vision under dim illumination. Affected families have been identified with autosomal dominant (ad), autosomal recessive (ar) and X-linked inheritance (xl) for CSNB. Historically, several large pedigrees have been reported to have adCSNB (Nettleship, 1907; Rosenberg *et al.* 1991). The most famous family was that of Jean Nougaret, a southern

Frenchman. He was born around 1637 and had 135 affected descendants in eleven generations before 1907 (Bell, 1922). In China, a few pedigrees with adCSNB have been recorded (Chen, 1982; Yan *et al.* 1991; Fei *et al.* 1992).

Patients with adCSNB have always been described as having normal fundi, an abnormal dark adaptation curve and severe attenuation of a-wave and an abnormal b-wave by electroretinograms (ERG). Recent molecular studies of families with adCSNB (Dryja, 2000) have identified three genes with five different missense mutations as being responsible for the clinical phenotype. The genes are rhodopsin (*RHO*), rod cGMP phosphodiesterase beta-subunit (*PDE6B*) and the alpha-subunit of rod transducin (*GNAT1*). In this paper, we report a large Chinese family with adCSNB with an apparently different gene responsible for the condition.

[#]Current address: Department of Chemistry, Zhejiang University, Hangzhou, Zhejiang 310027, P.R. China.

*Corresponding author: Molecular Diagnostic Laboratory, 6P-1, Children's Hospital & Regional Medical Center, 4800 Sand Point Way NE, Seattle, WA 98105. Tel: (206) 987-3872, Fax: (206) 987-3838. E-mail: shi-han.chen@seattlechildrens.org

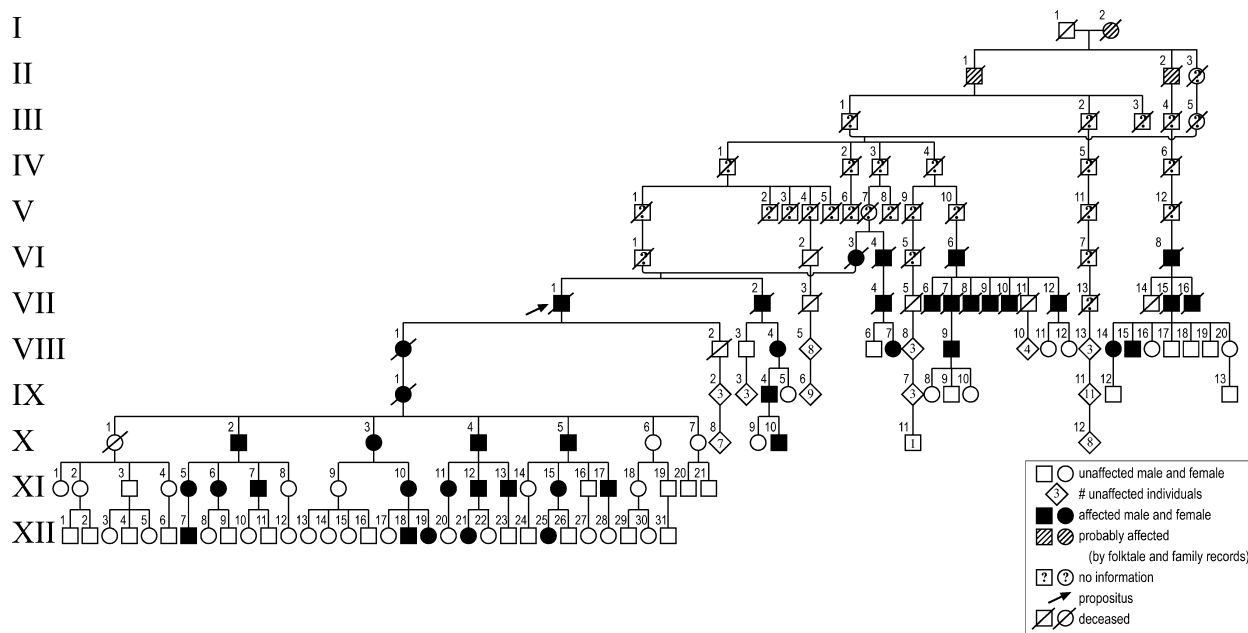


Figure 1 Pedigree of the Chinese family with adCSNB.

Methods

Propositus (Figure 1, VII-1) and his Family

The propositus and his ancestors originally resided in a small village in Pengyang county in the southeastern part of Zhejiang province, China, for at least three hundred years. He was considered to be in good health, with normal intelligence and normal day vision until his death in 1962 at the age of 80. The night blindness was stationary all through his life with no known associated eye anomalies such as myopia, cataract or pigmentary changes of the retina. Under ordinary night illumination he had no visual difficulty. When he walked in an unlit place, his vision was considerably impaired and did not improve even if he remained in the dark for a long time. Several individuals with the same condition were identified in the family: his mother (VI-3), her brother (VI-4), his brother (VII-2), their daughters (VIII-1, 4), his granddaughter (IX-1) and four of her seven children (X-2, 3, 4 and 5). Recently, several individuals in the XI and XII generations have been identified as having the same condition. Questionnaires were sent to over 50 individuals in the family and those individuals identified with the condition were further questioned regarding the age of onset and progression of problems related to night vision.

Dark Adaptation

Testing was performed on a modified version of the Goldmann-Weekers Adaptometer (Roland Consult Co, LIZENZ program, Germany) in Wenzhou Medical College. Bleaching and target light are generated by an array of 139 two-colour LEDs, built into the right-eye viewing field and extending to 60 degrees off the central visual axis. Each LED contains a super-red (635 nm) and a green (565 nm) element, so that either red or green stimuli can be presented. Prior to an experimental test, the eye was dilated and then light adapted for 7 min. The size of pupils was recorded before and after testing to make sure the pupils remained constant. The wavelength stimuli was 565 nm (green), stimulus length 98 to 500 ms, reaction time 300 to 2000 ms. After 3 min of bleaching, the absolute threshold to light was tested for 30 min. Their curves were compared with normal controls.

Ophthalmologic Examination and Electroretinogram (ERG)

General eye examinations were performed in the Wenzhou Medical College. Eye examinations included pupillary responses, (cycloplegic) refraction, acuity measurement, visual fields by automated perimetry (Octopus,

Interzeag, Switzerland), and dilated fundus examination and photography.

Full field ERG was recorded and analyzed by a Roland Consult Electrophysiological Diagnostic System (Roland Consult Co, Germany). The instrument measured the retinal response to a brief flash of light from a Ganzfeld Q 400 sphere. For scotopic ERG, eyes were first dark adapted for 30 min and pupils were fully dilated. An active jet electrode was placed on the cornea, the ground electrode was attached to the forehead and a reference electrode was placed near each orbital rim temporally for the corresponding eye (ISCEF standards). The scotopic ERGs were recorded and averaged to a series of single light flashing of low intensity setting (0.01 cd/m², fix on, plot time 150 ms and backlight off), and the ERG responses to maximum white light flashing (3.0 cd/m², fix on, plot time 150 ms and backlight off) were then recorded and averaged under the same condition. For photopic ERG, the eyes were light adapted to the background illumination for 10 min, single flash (3.0 cd/m², fix on, backlight on). The flicker (3.0 cd/m², fix on, backlight on, 30 Hz and plot 300 ms) cone responses were recorded.

Mutation and Family Linkage Analysis

Genomic DNA was isolated in Hangzhou from peripheral blood samples from 16 affected and 14 unaffected family members (mainly offspring of IX-1) by Animal Tissue Genomic DNA Mini-prep Kit (VITAGENE biochemical technique Co.Ltd). Primers were synthesized based on the sequences flanking each of the following exons and their exon/intron junctions. They included all 5 exons of *RHO*, exon 4 of *PDE6B* and exon 2 of *GNAT1*. The DNA was amplified by polymerase chain reaction (PCR) and the amplified fragments were then sequenced by ABI PRISM[®] 377 DNA Sequencer (PE Applied Biosystems).

For linkage studies, seven sets of variable number dinucleotide tandem repeat (VNTR) primers were synthesized. The primer sequences and locations of the markers are shown in Table 1. Two of the markers flank the *RHO* locus, two flank *GNAT1* and three flank *PDE6B*. The method for genotyping was PCR amplification followed by genotyping. PCR conditions were optimized over a range of annealing temperatures (55–59°C)

Table 1 Sequences and locations of the primers used for linkage analysis

Primer name	Forward primer sequences*	Reverse primer sequences	Product length	Distance to the gene	Heterozygosity#
VNTR 1	5'-CATTAGGATGCATCTTCTG	5'-GTCAGGATTGAAGTGGGAAC	118 bps	Intron 1 of <i>RHO</i>	0.68
VNTR 2	5'-AGGGCTTTGAGGTTATCCAG	5'-GATGTGGAGTCCGAGGTT	340 bps	60 kb downstream of <i>RHO</i>	0.84
VNTR 3	5'-CCCTGTGGGAAGGCATAA	5'-AAACTGAGGCCCTAGAGAGG	142 bps	139 kb upstream of <i>GNAT1</i>	0.78
VNTR 4	5'-CAGGACCCAGAAAAGCCC	5'-TGATGATCTTGGCGGAGG	336 bps	3.4 kb upstream of <i>GNAT1</i>	0.72
VNTR 5	5'-CGCCTGGCCAACTAAATG	5'-GGGTGAATGAAGGAAGAATGTG	168 bps	78 kb upstream of <i>PDE6B</i>	0.70
VNTR 6	5'-TGTAATCCCAGTACTCAGG	5'-TCATTTAACCATCGCCGGAG	192 bps	28 kb upstream of <i>PDE6B</i>	0.61
VNTR 7	5'-AGACTCCTGTCCGACGTTG	5'-GTTAGAAAACCAGCCCAGC	263 bps	102 kb downstream of <i>PDE6B</i>	0.64

*: Forward primer 5'-FAM labeled.

#: Heterozygosity in 100 Chinese controls.

and magnesium concentrations (1.0–1.5 mM MgCl₂). Amplifications were performed in 96-well PCR plates on an Applied Biosystems 9700 PCR machine. Each 10 μ l PCR reaction contained 30 ng DNA, 0.5 U *Taq* polymerase, 5 pmol of each primer, 1X buffer. Amplified DNA fragments were electrophoresed on 7% acrylamide gels, which were run for 1h using a model 3730XL automated sequencer (Applied Biosystems). Semi-automated DNA fragment sizing was performed using GENEMAPPER (version 3.5) software (Applied Biosystems). One hundred normal individuals were typed for each marker as controls. Twenty-nine individuals of the family were also genotyped, including 16 affected and 13 at risk. Pair-wise LOD scores (*Z*) between adCSNB and marker loci were calculated by use of FASTLINK v.3.0. (Lathrop *et al.* 1984) and LINKAGE versuib 5.1 (Cottingham *et al.* 1993) under a model of autosomal dominant inheritance of the disease. At risk individuals under four year of age were typed “unknown” for the eye condition.

Results

Inheritance

Figure 1 shows the twelve-generation pedigree with 42 affected individuals and another 3 individuals probably affected by CSNB. The affected individuals were identified by an eye physician examination or by self-recognition (orally and written). They all indicated that they had night vision difficulties that did not deteriorate over time. There was no apparent difference between the eyes of a single individual. The information of the likely “affected” individuals was obtained via oral history and the family pedigree book (Chen’s Jia-Pu in Chinese).

Dark Adaptation Thresholds

Three patients (VIII-4, IX-4 and XI-7) were tested for dark adaptation thresholds. All three showed a monophasic curve, which is different from the bipartite curve of normal controls. Figure 2 shows the dark adaptation curves of a patient’s (XI-7) left eye and a normal control. The curve of the patient’s eye fell rapidly at first, paralleling with the normal curve and then (in about 10 minutes) reached a threshold. The level of sensitivity

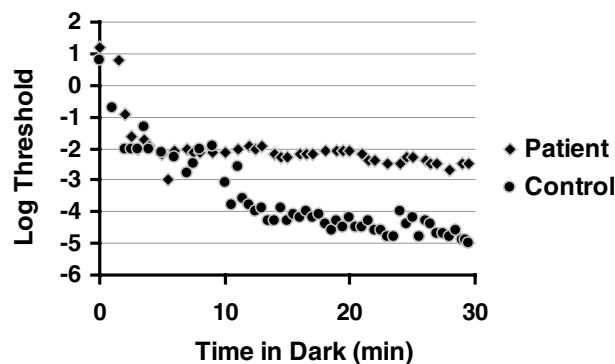


Figure 2 Dark adaptation curves and thresholds of the left eye of patient XI-7 and a normal eye.

was about 3 log₁₀ units improvement and then maintained at about that level throughout a subsequent testing period of 30 minutes. For the normal control, when the curve reached the first threshold, a normal bipartite curve was seen. The sensitivity of the control eye continued to improve and reached another threshold (about 2 log₁₀ units) in about another 15 to 20 minutes. This represented the cone/rod break in the normal eye that was lacking in the patient’s eyes.

Ophthalmologic and ERG Findings

Three patients had thorough eye examinations and were also tested by ERG. They were X-3 (female, age 61), X-4 (male, 57) and XI-17 (male, 25). They all had normal day-time vision, normal pupils, no pigment changes in the retina and no narrowing of the retinal vessels. X-3 had a visual acuity of 0.5 (20/40) and refraction of 1.0/–0.75/110; X-4 had a visual acuity of 1.0 (20/20); and XI-17 had a visual acuity of 1.0 (20/20) and refraction of 0.5/–/–.

Figure 3 shows the scotopic ERG patterns of the left eye of XI-17. Patient XI-17 is a great grandson of the proband. Following dark adaptation, his scotopic ERG response (Fig. 3A) to the dim light flash was 76 μ V (left eye), which is 24% of a normal value of 309 μ V. Under the high intensity white light flashing, the patient’s ERG response (Fig. 3B) showed relatively normal a-waves of 213 μ V and grossly abnormal b-waves of 197 μ V. The latter is 43% of the normal value of 449 μ V. The b/a ratio is about 0.9, which is low compared to the normal ratio of 2. Following 10 min. of light

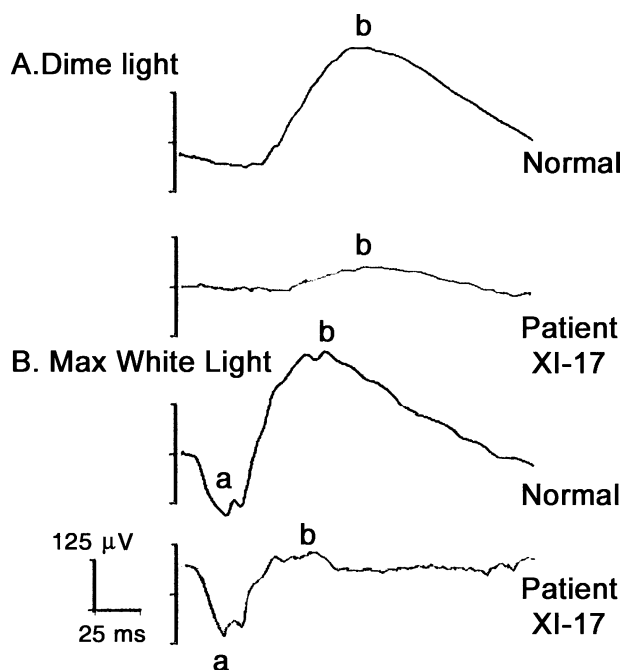


Figure 3 Scotopic ERGs of the left eye of patient X-17, compared to a normal eye. The time scale is 25 ms and the amplitude scale is 125 μ V. (A) Dark adapted rod ERG response to dim light stimulus (0.01 cd/m^2 , fix on and backlight off). (B) Dark adapted mix ERG response to maximum light stimulus (3.0 cd/m^2 , fix on and backlight off).

adaptation, the photopic ERGs and 30 Hz flicker were in the normal range (results not shown).

The calculated ERG data from eight normal eyes and the eyes of three patients are summarized in Table 2. The data indicate that each patient had diminished rod b-wave responses, that were 20–30% of normal, to scotopic dim flashes, and a decrease (\sim 50% of normal) of b-wave, but with normal a-wave response to bright white light flashes. For the patients, the b/a ratio was 0.9–1.5 and the shapes of the b-waves were grossly ab-

normal but with normal implicit time. Cone responses to a series of single bright flashes under light adaptation were in the normal range. The data were consistent with the diagnosis of adCSNB.

Mutation Search

We amplified and sequenced the DNA of all 5 exons of the rhodopsin gene (*RHO*), exon 4 of *PDE6B* and exon 2 of *GNAT1* in 9 affected and 12 unaffected individuals in the family (results not shown). No mutation was found in all the sequences, including codons 90, 94 and 292 of *RHO*; codon 258 of *PDE6B* and codon 38 of *GNAT1*. These mutations were identified in other families with adCSNB.

Linkage Analysis

Genotyping of 100 Chinese DNAs showed that the seven markers all had multiple alleles with heterozygosities of 0.61 \sim 0.89 (Table 1).

Linkage analyses (Table 3) did not support a close linkage between the markers and previous identified genes (*RHO*, *GANT1* and *PDE6B*) that cause the clinical phenotype of adCSNB. Assuming a dominant form of inheritance for the disorder, the LOD scores (*Z*) of the seven markers all had negative values at a recombination fraction (θ) of zero. Thus, linkage analyses excluded the three known genes associated with adCSNB.

Discussion

The clinical description, mode of inheritance, dark adaptation thresholds and ERG data described are

Table 2 Summary Results of three patients' ERGs

Subject		Skotopic to dim light		Skotopic to bright light		30 Hz flicker and photopic ERGs
		b-wave, μ V	a-wave, μ V	b-wave, μ V	b/a ratio	
Normal	8 eyes	309 \pm 62 * (% of Normal)	223 \pm 61	449 \pm 113 * (% of Normal)	1.8–2.3*	normal
XI-17	left'	76 (24%)	213	197 (43%)	0.9	normal
	right	56 (18%)	176	234 (52%)	1.3	normal
X-4	left'	92 (30%)	185	222 (49%)	1.2	normal
	right	97 (31%)	186	235 (52%)	1.3	normal
X-3	left'	68 (22%)	205	283 (63%)	1.4	normal
	right	66 (21%)	174	259 (58%)	1.5	normal

* Indicates values of the patients' eyes in the column are significantly deviated from the normal controls.

Table 3 LOD score of VNTR markers at three chromosomal regions

Markers	LOD Score at $\theta =$					
	0	0.1	0.2	0.3	0.4	0.5
VNTR 1	-6.95	-0.95	-0.22	-0.01	0.00	0.00
VNTR 2	-0.91	1.52	1.53	1.09	0.44	0.00
VNTR 3	-12.9	-3.60	-1.89	-0.94	-0.35	0.00
VNTR 4	-7.39	-1.63	-0.59	-0.11	0.06	0.00
VNTR 5	-3.47	-0.12	0.22	0.19	0.04	0.00
VNTR 6	-7.06	-0.80	-0.06	0.13	0.11	0.00
VNTR 7	-10.4	-2.00	-0.91	-0.37	-0.01	0.00

consistent with the diagnosis of adCSNB for this family.

Men as well as women were affected with a ratio of 28 to 16 respectively. The higher ratio of male/female affected might be due to unreported affected females in the early generations. There were 13 cases of father to son transmission, excluding X-linkage of the disorder. All offspring of normal individuals were not affected. The ratio of normal to affected offspring born to affected parents was 35 to 39, close to the expected ratio of 1:1.

Although there was no direct evidence to show that the CSNB was present beyond generation V, the fact that there were two branches of affected individuals in the pedigree points to a common ancestor, I-2, who passed the mutation to her two sons.

In the dark adaptation studies, the absence of cone-rod break in 3 patients established that the entire adaptation process is due to the cone system, with a possible defect in rods, rod/bipolar cell junctions, or the bipolar cells themselves.

The scotopic ERGs of three patients showed an abnormal attenuated b-wave response (20–30% of the normal) to dim light; normal a-wave, reduced b-wave amplitudes (43–63% of the normal) and abnormal b-wave shapes to the maximum white light flashing (Figure 3 and table 1). The patterns were nearly consistent with the Schubert-Bornschein type of CSNB for the family. These deviated from previous reports of absent or undetectable scotopic b-wave responses to dim light, and no or severe reduction of both a-wave and b-wave responses to the maximum white light stimuli in most patients with adCSNB (Dryja, 2000), the Riggs type of CSNB. It was apparent that the ERGs of our patients were dif-

ferent from most patients with adCSNB reported in the literature. For the few previously reported Chinese adCSNB patients with ERG data (Fei *et al.* 1991; Gan *et al.* 1997), the results have been mixed. Some of the patients had no rod b-wave and others had normal or reduced a-wave and reduced b-wave. No comparison studies could be performed between the families.

We found no mutation in the three known genes previously documented as a cause of adCSNB in this extensive family. The entire coding and splice junction regions of *RHO*, exon 4 of *PDE6B* and exon 2 of *GNAT1* were all normal. No evidence for linkage was found in analyses of the family, indicating the responsible gene was neither at 3q21, the approximate site of *RHO*, at 3p21 for *GNAT1* or at 4p16.3, the location of *PDE6B*. Thus, the gene responsible for adCSNB in this family is different from those three genes previously identified as being responsible for adCSNB.

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