Chapter 48
Congenital Stationary Night Blindness: Mutation Update and Clinical Variability

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48.1 Introduction

Congenital stationary night blindness (CSNB) represents a group of low vision disorders in which patients exhibit a negative ERG, reduced visual acuity, impaired night vision, myopia, nystagmus, and strabismus (Miyake et al. 1986; Héon and Musarella 1994; Miyake 2006; Lodha et al. 2009) with abnormal retinal neurotransmission (Tremblay et al. 1995) and different modes of genetic inheritance (Bech-Hansen et al. 1998a, b; Strom et al. 1998; Bech-Hansen et al. 2000; Zeitz et al. 2005a, b; Zeitz 2007; Bellone et al. 2008; Audo et al. 2009; Li et al. 2009; Nakamura et al. 2010). Analysis of individuals with CSNB diagnosed based on a negative ERG has revealed *CACNA1F* mutations in the patients with incomplete X-linked CSNB (CSNB2A), *NYX* mutations in patients with complete X-linked CSNB (CSNB1A), and *GRM6* and *TRMP1* mutations in patients with complete autosomal recessive CSNB (CSNB1B and CSNB1C). Several reports have revealed phenotypic variability among genetically defined CSNB1 and CSNB2 patients (Boycott et al. 2000; Jacobi et al. 2002; Allen et al. 2003), though limited information is available on the genotype–phenotype correlation in patients with CSNB.

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In this report we describe the type and distribution of mutations in *CACNA1F*, *NYX*, *GRM6*, *TRMP1*, *and CAPB4* genes in a cohort of 199 patients with CSNB together with the worldwide experience. In addition, we have analyzed the degree of clinical variability of 182 genetically defined patients with X-linked CSNB in an effort to refine the phenotype of patients with CSNB.

48.2 Methods

48.2.1 Subjects

A total of 199 patients were contributed by the CSNB Study Group for genetic analysis from Canada (Alberta, British Columbia, Nova Scotia, Ontario, Quebec), Denmark, Finland, Netherlands, United Kingdom, and USA (California, Georgia, Illinois, Missouri, New York, Oregon, Pennsylvania, Texas, Wisconsin) (manuscript in preparation). Furthermore, a retrospective chart review of 182 patients (males, except for 1 female; from 52 different families) with genetically defined X-linked CSNB (CSNB1A and CSNB2A) was undertaken. Clinical data collected included visual acuity (VA), refractive error (ref), color vision (CV), where available electrophysiological (ERG), dark adaptation (DA), nystagmus, strabismus, and complaint of impaired night vision.

48.2.2 Analyses

48.2.2.1 Genetic Analysis

Genomic DNA was PCR-amplified using a series of primers designed to amplify the coding and flanking intronic regions of the *CACNA1F*, *NYX*, *GRM6*, *TRMP1*, and *CAPB4* genes (see Boycott et al. 2001). PCR fragments were then sequenced and sequence variants were evaluated for their consequence on the encoded protein (e.g., nonsense, frameshift, or missense that involved a change in a highly conserved residue; analysis by PolyPhen).

48.2.2.2 Statistical Analysis

Within-group variability for individual clinical measures was calculated and variability between groups was compared using the variability ratio test. Binary variables (nystagmus, strabismus, impaired night vision) were compared using χ-square analysis (O'Brien 1981).

48.3 Results

48.3.1 Genetic Analysis

In a total of 199 patients diagnosed clinically with CSNB, 112 for X-linked iCSNB, 73 for X-linked cCSNB, and 14 with ar-cCSNB, we have identified a total of 116 unique mutations (65 unpublished; manuscript in preparation) in 159 (80%) of these families. Of the mutations, 69 were in *CACNA1F* (Table 48.1, Fig. 48.1), 32 in *NYX* (Table 48.2), seven in *GRM6* (Table 48.3), and eight in *TRMP1* (Table 48.4). No mutations were identified in *CAPB4*. *CACNA1F* mutations were distributed across the coding region from exon 2 to 47 (Fig. 48.1), while *NYX* mutations predominated in conserved or immediately adjacent residues across the leucine-rich repeats of NYX. The *CACNA1F* mutations were identified among patients originally diagnosed

Table 48.1 Unique *CACNA1F* mutations in patients with CSNB2A

Mutation type	Calgary experience (%)	World experience (%)
Missense point mutation	25 (36)	34 (37)
Nonsense point mutation	14 (20)	19 (21)
Splicing	9 (13)	15 (16)
Frameshift deletion	11 (16)	13 (14)
In-frame deletion	4 (6)	4 (4)
In-frame deletion/insertion	2 (3)	2 (2)
Frameshift duplication	3 (4)	3 (3)
Frameshift insertion	1 (1)	2 (2)
Total	69 (100)	92 (100)

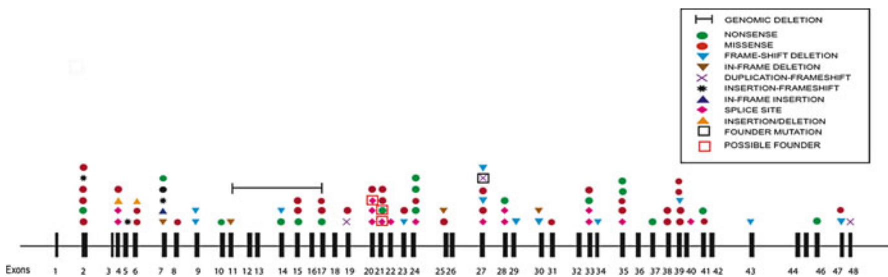

Fig. 48.1 Distribution of *CACNA1F* mutations in patients with CSNB2A. Genomic organization of *CACNA1F* on the X-chromosome between nucleotides 48,087,757 and 48,116,066 showing the distribution of 92 unique mutations (65 from our studies plus published mutations from other labs). The *CACNA1F* gene codes for the α_1-subunit of the voltage-gated calcium channel $Ca_v1.4$. ☐: founder mutation in *CACNA1F* c.3166dupC (exon 27, frameshift mutation in patients with Dutch-German Mennonite ancestry). The following *CACNA1F* mutations show multiple occurrences: c.2576+1G>A (Exon 20 splice mutation) in 8 families from Canada and United States. c.2683C>T (Exon 21 nonsense mutation) in 7 European families

Table 48.2 Unique *NYX* mutations in patients with CSNB1A

Mutation type	Calgary experience (%)	World experience (%)
Missense point mutation	17 (53)	22 (46)
Nonsense point mutation	4 (13)	6 (13)
Splicing	2 (6)	3 (6)
Frameshift deletion	3 (9)	6 (13)
In-frame deletion	1 (3)	5 (10)
In-frame deletion/insertion	1 (3)	2 (4)
In-frame duplication	4 (13)	4 (8)
Total	32 (100)	48 (100)

Table 48.3 Unique *GRM6* mutations in patients with CSNB1B

Mutation type	Calgary experience	World experience
Missense point mutation	4	10
Nonsense point mutation	0	4
Splicing	1	1
Frameshift deletion	2	2
Frameshift duplication	0	2
Total	7	19

Table 48.4 Unique *TRMP1* mutations in patients with CSNB1C

Mutation type	Calgary experience	World experience (%)
Missense point mutation	3	20 (44)
Nonsense point mutation	2	8 (18)
Splicing	2	10 (22)
Frameshift deletion	1	5 (11)
Regulatory	0	1 (2)
Silent	0	1 (2)
Total	8	45 (100)

with incomplete X-linked CSNB (iCSNB or CSNB2A), Åland Island eye disease (AIED and AIED-like), and Åland eye disease (AED) (Strom et al. 1998; Boycott et al. 2001; Jalkanen et al. 2007) suggesting that these conditions are the same genetic condition (Rosenberg, Lodha, Bech-Hansen, manuscripts in preparation). We consistently observed the *CACNA1F* founder mutation (c.3166dupC, exon 27) among individuals from Canada with a Dutch-German Mennonite ancestry (Orton et al. 2008). A 24-bp deletion mutation in *NYX*, which results in the loss of the RACPAACA amino acids in the N-terminal Cys-rich region of nyctalopin, was detected in 12 North American CSNB families. Of 164 CSNB mutations known worldwide (Bech-Hansen, manuscript in preparation), we note that 18 of 48 unique *NYX* mutations, 24 of 93 unique *CACNA1F* mutations, 7 of 17 unique *GRM6* mutations, and 3 of 45 unique *TRMP1* mutations have been detected in more than one family. No mutations were identified in 40 of 199 CSNB patients (20%); the clinical picture and the family histories of these patients were not inconsistent with CSNB and autosomal inheritance.

Table 48.5 Clinical features of X-linked CSNB

Measures	CSNB1A (*NYX*) $n=43$	CSNB2A (*CACNA1F*) $n=139$
Visual acuity[a]	$m=0.35\pm0.25$, $r=0.05–1$	$m=0.50\pm0.34$, $r=0.00–1.78$
Refraction[b]	$m=-9.19\pm4.61$, $r=-26$ to -0.25	$m=-3.78\pm5.76$, $r=-21$ to 12.25
Impaired night vision	97.2% ($n=36$)	39% ($n=128$)
Nystagmus	40% ($n=10$)	52.5% ($n=137$)
Strabismus	29.4% ($n=17$)	24% ($n=131$)
Dark adaptation	NA	95% ($n=40$)
−ve ERG (b/a ratio)	100% ($n=64$ eyes)	75% ($n=52$ eyes)

−ve ERG negative electroretinogram; *NA* not applicable; *m* mean; *r* range
[a]Vision measured in log MAR
[b]Refraction measured in diopters

Table 48.6 Clinical variability observed in patients with X-linked CSNB

Clinical features	Variability[a] CSNB1A	Variability[a] CSNB2A	*F* value	*p* value
Visual acuity	0.06 ($n=43$)	0.12 ($n=149$)	1.85	<0.05
Refractive error	21.25 ($n=43$)	33.18 ($n=149$)	1.57	<0.05
b/a ratio	0.02 ($n=64$ eyes)	0.11 ($n=52$ eyes)	8.15	<0.001
Impaired night vision	$n=36$	$n=128$		0.41
Nystagmus	$n=10$	$n=137$		0.57
Strabismus	$n=17$	$n=131$		1

[a]Variability: a measure of data distribution reflects how far the data points are from the mean (O'Brien, 1981)

48.3.2 Clinical Variability

Clinical and ERG data were collected from 182 genetically defined patients with CSNB (43 CSNB1A from 14 families and 139 CSNB2A from 38 families). Summary ERG reports were available on all patients, though detailed reports have so far only been available on 79 patients.

Almost all patients (181 out of 182) with X-linked CSNB, CSNB1A and CSNB2A, had abnormal visual acuity (Table 48.5). Patients with CSNB1A had more severely impaired night vision compared to patients with CSNB2A (Table 48.5), and both groups of patients had abnormal ERGs (summary ERG of scotopic and photopic ERG information). All patients with CSNB1A had an abnormal rod-isolated response; negative bright-flash ERGs, normal to square cone a-waves, normal cone b-waves, and normal 30 Hz flicker responses, while patients with CSNB2A had reduced to normal isolated rod responses, abnormal cone ERGs and 30-Hz flicker. However, only 75% of the CSNB2A patients had negative bright-flash ERGs. Nystagmus and strabismus were present in some but not all patients (CSNB1A: 40%, 29.4%; CSNB2A: 52.5%, 24%, respectively) suggesting that these are secondary diagnostic feature of both CSNB1A and CSNB2A. Our statistical analysis of the variability of the clinical and ERG presentations revealed that patients with CSNB2A had more variable phenotype with respect to the visual acuity, refractive error, and the b/a wave ratio than those with CSNB1A (Table 48.6).

The analysis of clinical data in our cohort shows that all of the patients with CSNB1A exhibit the diagnostic characteristics of negative ERG, abnormal visual acuity, impaired night vision, and myopia (Table 48.5). Most of the patients with CSNB2A (99%) have abnormal vision, though only 75% of these patients had the characteristic negative ERG and only 39% of these patients had impaired night vision. The statistical analysis of visual acuity, refractive error, and b/a ratio of ERG waves confirms that the clinical phenotype of CSNB1A is less variable than the clinical phenotype of CSNB2A (Table 48.6).

48.4 Discussion

Mutation analyses in clinically recognized CSNB patients have provided useful diagnostic information and revealed the spectrum and distribution of sequence changes in *CACNA1F*, *NYX*, *GRM6*, *TRMP1*, and *CAPB4* genes (Tables 48.1–48.4). Definitive DNA diagnosis of CSNB was established in 80% of cases in a cohort of 199 unrelated patients analyzed in our laboratory. Combining our mutation information with that of published reports, a total of 209 mutations are known in *CACNA1F*, *NYX*, *GRM6*, *TRMP1*, and *CABP4*. Within this cumulative data set, we note that the majority of mutations were identified in a single family with only 27% of mutations being detected in more than one family. This finding varied between genes with *TRMP1* having the fewest mutations (6%) detected in more than one family and *GRM6* having the highest rate (41%) with *NYX* and *CACNA1F* having recurrence rates of 37 and 25%. Moreover, mutations in *NYX* and *CACNA1F* represent the most common causes of cCSNB and iCSNB, respectively, which support the notion that X-linked CSNB is the more common form of CSNB. *GRM6* and *TRPM1* mutations were equally common (10% combined) in cCSNB patients. These results have implications for diagnostic labs. The absence of *CAPB4* mutation in the Calgary CSNB cohort might be due to a low incidence together with a bias of ascertainment of ar-iCSNB. From published reports, four of five patients in whom *CAPB4* mutations were identified did not complain of impaired night vision and had ERGs with severely reduced cone function and only negligibly reduced rod function together with abnormal vision, color vision, and photophobia, characteristics similar to cone–rod dystrophy (Zeitz et al. 2006; Zeitz 2007; Littink et al. 2009). For CSNB patients in whom the genetic causes are still to be discovered, mutations are likely to be found among genes for other proteins that function in photoreceptor pre- and postsynaptic processes that affect retinal neurotransmission.

The analysis of clinical data from our cohort shows that all of the patients with CSNB1A exhibit the diagnostic characteristics of negative ERG, abnormal visual acuity, impaired night vision, and myopia (Table 48.5). Almost all (99%) the patients with CSNB2A have abnormal vision, though only 75% of these patients had the characteristic negative ERG and a minority of these patients had impaired night

vision (Table 48.5). Nystagmus and strabismus are present only in 50% or less of patients with CSNB1A and CSNB2A (Table 48.5), suggesting that both of these diagnostic features are not primary, as originally suggested (Miyake et al. 1986).

The clinical phenotype (reduced visual acuity and refractive error) and ERG features were found to be more variable among patients with CSBN2A than among patients with CSNB1A (Table 48.6). Such variability may be explained by various factors, including the presence of other genetic factors modifying the phenotype (Boycott et al. 2000; Allen et al. 2003; Zeitz et al. 2007) and environmental factors acting before or after birth.

The "negative bright"-flash ERG has been considered diagnostic for CSNB and a requisite feature of a defect in the retinal neurotransmission. However, our findings revealed that the negative ERG is *not* invariably present in patients with mutations in the *CACNA1F* gene, as 13 of 52 patients with CSNB2A in whom detailed ERG analysis was available did not have a *negative* bright-flash ERG. Patients with a presynaptic defect due to *CACNA1F* mutations show that both the cone and rod pathways are affected in patients with CSNB2A (Miyake et al. 1986; Tremblay et al. 1995). These findings show that CSNB2A is a cone–rod disorder rather than strictly a night blindness disorder with a predominant rod defect. The cases that present with nonnegative bright-flash ERGs could possibly be explained by a milder retinal neurotransmission deficit. This observation would suggest that the presence of a negative ERG, though normally present, should not be taken as pathopneumonic of this subtype of CSNB, as patients with an abnormal, but nonnegative bright-flash ERG could be misdiagnosed, and potentially lead to unnecessary investigations and patient mismanagement.

A comprehensive clinical assessment, detailed family history, and robust ERG data will aid in the diagnosis of CSNB. The constant clinical findings in patients with X-linked CSNB are abnormal vision and abnormal ERGs. However, the absence of a negative bright-flash scotopic ERG or complaint of impaired night vision does not exclude a diagnosis of CSNB.

48.5 Conclusions

Mutations in the *CACNA1F*, *NYX*, *GRM6*, and *TRPM1* genes established the genetic cause of CSNB in 80% of patients in a cohort of 199. Some patients with mutations in *CACNA1F* (CSNB2A) presented with an abnormal but nonnegative ERG. Furthermore, X-linked CSNB patients with CSNB2A showed greater variability in the key phenotypic features (reduced visual acuity, refractive error, and b/a wave ratio) than those patients with CSNB1A. Furthermore, CSNB1A patients invariantly complained of night blindness, while those with CSNB2A infrequently did consistent with the notion that the later is more correctly a cone–rod disorder.

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