Children with complete or incomplete congenital stationary night blindness: ophthalmological findings, standard ERGs and ON-OFF ERGs for differentiation between types

Otroci s prirojeno stacionarno nočno slepoto: oftalmološke značilnosti, standardni ERG ter ON-OFF ERG razlikovanje med kompletno in nekompletno obliko

Maja Šuštar, Branka Stirn-Kranjc, Jelka Brecelj

Eye Clinic, University Medical Centre Ljubljana, Ljubljana, Slovenia

Korespondenca/ Correspondence:
Maja Šuštar, e-mail: sustar.majchi@gmail.com

Ključne besede:
kompletna oblika CSNB, elektroretinogram, nekompletna oblika CSNB, nočna slepota, ON-OFF ERG, otroci

Key words:
complete CSNB, electroretinogram, incomplete CSNB, night blindness, ON-OFF ERG, children

Citirajte kot/Cite as:
Zdrav Vestn 2012; 81: I-16–28

Prispelo: 9. jan. 2012,
Sprejeto: 2. feb. 2012

Abstract

Purpose: Congenital stationary night blindness (CSNB) is a group of retinal disorders with diverse clinical characteristics. The most common types are complete and incomplete CSNB. Both can have normal fundus appearance and an electronegative waveform of the electroretinogram (ERG). Our aim was to define ERG differences between complete and incomplete CSNB in a pediatric population.

Subjects and methods: In 12 children (5–18 years old) with clinical signs of stationary night blindness, standard full-field ERGs and ON-OFF ERGs were recorded. These were abnormal if the implicit time was above 95 % of the upper confidence limit and the amplitude below 5 % of the lower confidence limit of the normative data. Comparisons between the data were performed with Mann-Whitney U tests, with $p < 0.01$ considered as significant.

Results: According to the ERG characteristics, complete CSNB was diagnosed in 8 of the children, and incomplete CSNB in the remaining 4 children. Dark-adapted ERGs showed negative waveforms of the combined rod–cone response in all 12 children, with normal a-waves and reduced b-waves, which indicated post-photoreceptor dysfunction. No rod response was detectable in the children with complete CSNB, with reduced rod response in those with incomplete CSNB. Light-adapted ERGs showed normal or subnormal amplitudes of the cone response and the 30-Hz flicker response in complete CSNB, where the cone response a-waves were also distinctly broadened. In the children with incomplete CSNB, the light-adapted ERGs were significantly reduced. In complete CSNB, the ON-OFF ERGs showed alterations of only the ON-response component (ON-bipolar cell dysfunction), while in incomplete CSNB, both the ON- and OFF-responses were reduced (ON- and OFF-bipolar cell dysfunction). Comparisons of the ERG amplitudes between the children with complete and incomplete CSNB demonstrated significant differences in rod responses, cone responses, 30-Hz flicker responses, and OFF-responses.

Conclusion: Distinct electrophysiological characteristics can be used to differentiate between complete and incomplete CSNB. Moreover, ON-OFF ERGs are important for precise localization of the retinal bipolar cell dysfunction, and these can also be reliably recorded in children.

Izvleček

Namen: Prirojena stacionarna nočna slepota ('Congenital stationary night blindness'–CSNB) je mrežnična bolezen, za katero je značilen normalen izgled očesnega ozadja in elektronegativ-

na izoblikovanost elektroretinograma (ERG). Glede na elektrofiziološke značilnosti ter genetsko variabilnost je mogoče prepoznati kompletno in nekompletno obliko nočne slepote. Cilj raziskave je bil preučiti elektrofiziološke razlike med kompletno in nekompletno obliko nočne slepote v slovenski pediatrični populaciji.

Metode in preiskovanci: V študijo je bilo vključenih 12 otrok (5–18 let), katerih klinični znaki so bili v soglasju s stacionarno nočno slepoto. Pri vseh otrocih je bil opravljen skotopični in fotopični ERG po standardu Mednarodne zveze za klinično elektrofiziologijo vida (ISCEV) ter ON--OFF ERG. Rezultati so bili prepoznani kot abnormni, če je čas do vrha posameznega odgovora presegal 95 % zgornje meje zaupanja, amplituda pa je bila nižja od 5 % spodnje meje zaupanja normativnih podatkov. Primerjava med rezultati je bila izvedena s testom Mann-Whitney U in prepoznana kot statistično pomembna pri $p < 0{,}01$.

Rezultati: Glede na vzorec elektrofizioloških abnormnosti je bila kompletna oblika CSNB prepoznana pri 8 otrocih in nekompletna oblika pri 4 otrocih. Skotopični ERG je pri vseh 12 otrocih pokazal elektronegativno izoblikovanost maksimalnega odgovora, z normalnim valom a in znižanim valom b, ki kaže na prizadetost delovanja bipolarnih celic. Odgovor paličnic je bil neizziven pri kompletni obliki CSNB in izziven, a pomembno znižan pri otrocih z nekompletno obliko CSNB. Fotopični ERG je pri kompletni obliki CSNB pokazal normalno ali mejno abnormno amplitudo odgovora čepnic in odgovora 30 Hz, odgovor čepnic pa je kazal značilno razširjeno izoblikovanost vala a. Pri otrocih z nekompletno obliko CSNB je bil fotopični ERG pomembno znižan. ON-OFF ERG je pri kompletni obliki CSNB pokazal znižanje zgolj ON-odgovora (disfunkcija ON-bipolarnih celic), medtem ko sta bila pri nekompletni obliki CSNB znižana tako ON- kot tudi OFF-odgovor (disfunkcija ON- in OFF-bipolarnih celic). Primerjava med kompletno in nekompletno obliko CSNB je pokazala signifikantne razlike v amplitudi odgovora paličnic, odgovora čepnic, 30 Hz odgovora in OFF--odgovora.

Zaključek: Značilne elektrofiziološke spremembe v delovanju mrežnice omogočajo prepoznavo in razlikovanje kompletne in nekompletne oblike prirojene stacionarne nočne slepote. ON-OFF ERG je uporaben za natančno umestitev disfunkcije mrežničnih bipolarnih celic pri nočni slepoti in ga je mogoče zanesljivo zabeležiti tudi pri otrocih.

Introduction

Congenital stationary night blindness (CSNB) is a clinically and genetically heterogeneous retinal disorder that is characterized by vision impairment under dim-light conditions. CSNB can be classified according to ophthalmological findings, mode of inheritance, genetic testing, and specific characteristics of the electroretinogram (ERG).[1] A schematic representation of the different forms of night blindness according to mode of inheritance, genetic diversity and level of dysfunction is shown in Figure 1.

The most common forms of CSNB are those associated with mutations in genes subsequent to the phototransduction cascade, which can be inherited as either X--linked (*NYX* or *CACNA1F* gene mutations) or autosomal recessive (*GRM6*, *TRPM1* or *CAPB4* gene mutations). These forms have been previously described as the Schubert–Bornschein type of CSNB,[4] and they have a nonspecific fundus appearance and are currently classified into complete (cCSNB) or incomplete (icCSNB) types.[5] CSNB with autosomal dominant inheritance has a defect at the level of rod phototransduction. This type is associated with mutations in the *GNAT1*, *PDE6B* or *RHO* genes, and its clinical presentation can include a normal fundus or bone-spicule pigmentation in the peripheral retina for the specific locus of the *RHO* mutation (Gly90Asp).[1] Another form of night blindness with photoreceptor dysfunction is known as Oguchi disease, an autosomal recessive night blindness disorder with a diffuse grayish fundus appearance that normalizes after a long period of dark adaptation. Oguchi disease is associated with mutations in the *SAG* or *GRK1* genes, which are involved in the phototransduction cascade.[6,7] A similar autosomal recessive disorder of night vision that is caused by impairment of rodopsin regeneration is known as fundus albipunctatus. This disorder is as-

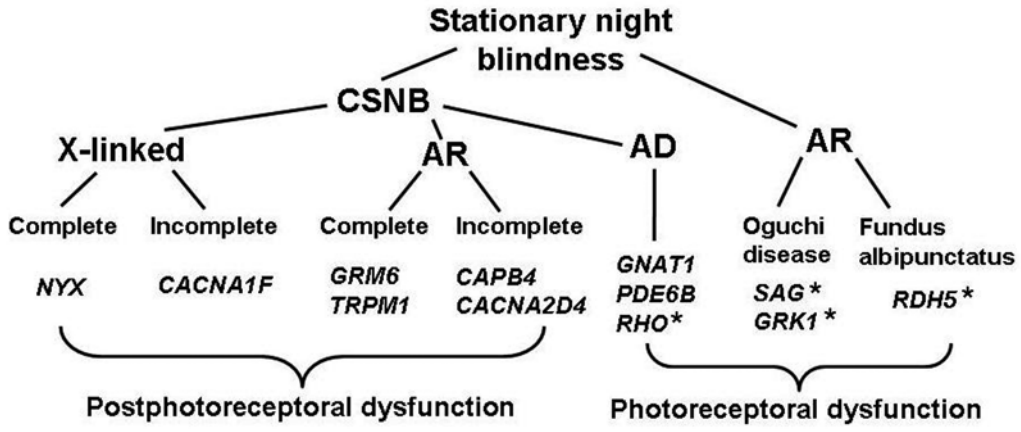

Figure 1: Classification of stationary night blindness disorders according to mode of inheritance [2–3]. CSNB, congenital stationary night blindness; AR, autosomal recessive; AD, autosomal dominant; *, night blindness with abnormal fundus appearance.

sociated with mutations in the *RDH5* gene, and fundoscopy reveals distinct multiple yellow-white dots that are located at the level of the retinal pigment epithelium in the mid-periphery.[8]

ERGs are relevant for confirmation of these disorders, as they can indicate the level of dysfunction and reveal specific ERG abnormalities in CSNB. The negative waveform of ERGs is a highly characteristic sign in both cCSNB and icCSNB, where dysfunction occurs at the post-photoreceptor level. This can be seen as larger amplitudes of the a-wave compared with those of the b-wave in the combined rod–cone response of dark-adapted ERGs.[4,5] These ERG features indicate that photoreceptor function is preserved, and thus that there is a block in the signal transmission between the photoreceptors and the bipolar cells.[9,10] ERG classification into cCSNB and icCSNB is based on the complete absence or the residual presence of rod function, respectively. Moreover, cCSNB has been shown to be associated with a defect in the post--photoreceptor retinal ON-pathway, which can be seen in abnormal ON- and normal OFF-components of the ON-OFF ERGs. In contrast, in icCSNB, there is a partial defect of both the ON- and OFF-pathways, which

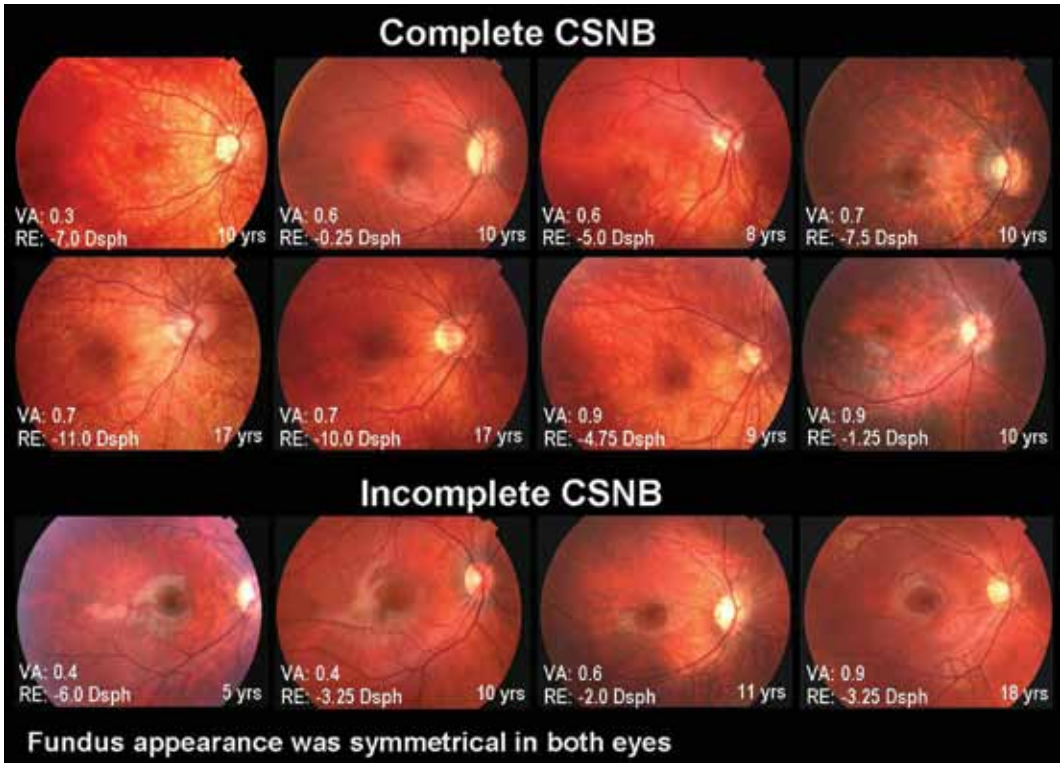

Figure 2: The fundus appearance in the 8 children with cCSNB and the 4 children with icCSNB. The findings were symmetrical across both eyes, with the fundus of the right eye shown here for all of the children.

Table 1: Summary of the clinical findings in the 8 children with cCSNB and the 4 children with icCSNB.

	Child	Sex	Age	VA	Refr. E	Initial complaint	Nys.	Dark adapt.	Fundus appearance	Inherit.
Complete CSNB	1	M	8	0.6 0.6	-5.0 -5.5	Low visual acuity	+		Mild optic disc pallor, peripapillary atrophy	AR,X?
	2	M	9	0.9 0.8	-4.75 -6	Family history of compromised night vision	+		Myopic	X
	3	M	10	0.9 0.8	-1.25 -3.25	Low visual acuity, problems with night vision	+		Mild optic disc pallor	AR,X?
	4	M	10	0.3 0.1	-7 -8	Problems with night vision	+	Abnormal	Optic disc pallor	AR,X?
	5	F	10	0.6 0.6	-0.25 -0.25	Low visual acuity	+	Abnormal	Mild optic disc pallor	AR?
	6	M	10	0.7 0.5	-7.5 -6.5	Low visual acuity, problems with night vision		Abnormal	Temporal optic disc pallor, peripap. atrophy	AR,X?
	7	M	17	0.7 0.8	-11 -11	Family history of compromised night vision	+		Optic disc pallor, peripapillary atrophy	X
	8	M	17	0.7 0.8	-10 -8	Family history of compromised night vision			Myopic	X
Incomplete CSNB	9	M	5	0.4 0.6	-6.0–7.0	Family history of low visual acuity			Normal	X
	10	M	10	0.4 0.4	-3.25–1.75	Low visual acuity			Mild attenuation of the vessels	AR,X?
	11	M	11	0.6 0.6	-2.0–2.0	Family history of low visual acuity			Temporal optic disc pallor, tilted optic disc	X
	12	M	18	0.9 0.8	-3.25–6.0	Low visual acuity		Abnormal	Optic disc pallor	AR,X?

VA – visual acuity; Refr.E – refractive error; Nys. – presence of nystagmus; Dark adapt. – dark adaptometry; Inherit. – pattern of inheritance; M – male; F – female.

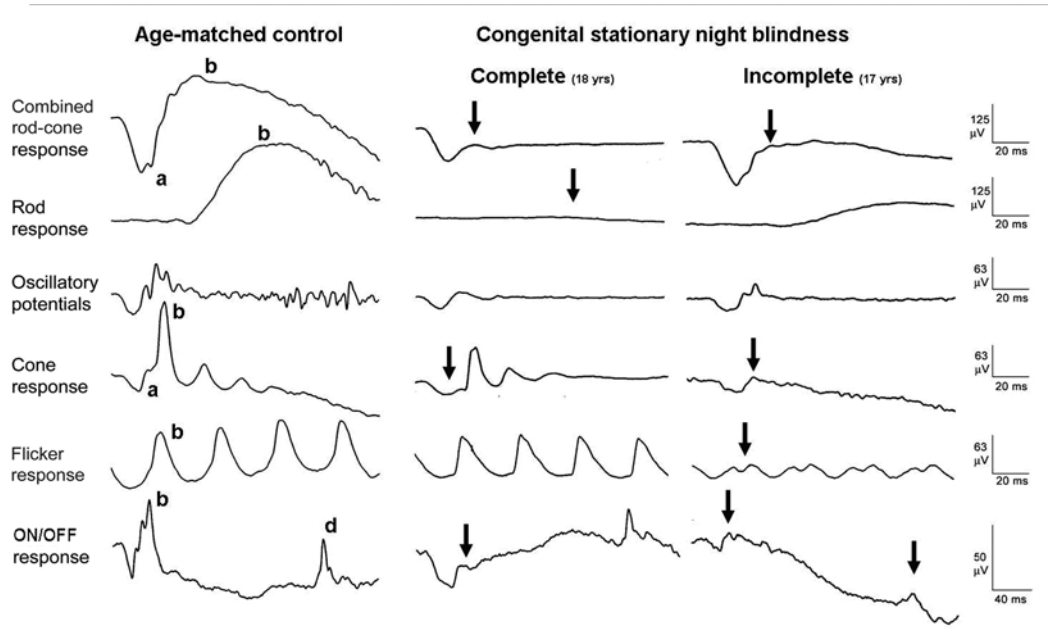

Figure 3: Standard full-field ERGs and ON- and OFF-responses, as a comparison of an age-matched control child (left) with a representative child (Child 8) with cCSNB (middle) and a representative child (Child 12) with icCSNB (right). The same patterns of changes were seen in the other children with cCSNB and icCSNB.

is seen as reductions in both the ON- and OFF-components of the ON-OFF ERGs.[5] This classification, which was first defined according to these electrophysiological findings, was subsequently confirmed by genetic analysis. In cCSNB with an X-linked mutation of the *NYX* gene, which encodes a proteoglycan protein known as nyctalopin, promotion of the retinal ON-pathway interconnections is most probably disturbed.[11] In cCSNB with an autosomal-recessive mutation of the *GRM6* gene[12] or the recently discovered mutation of the *TRPM1* gene,[13] there is a defect in glutamate transmission between the photoreceptors and the ON-bipolar cells. IcCSNB is associated with either an X-linked mutation of the *CACNA1F* gene[14] or, more rarely, an autosomal-recessive mutation of the *CAPB4*[2] and *CACNA2D4*[15] genes. All three of these mutations lead to disruption of glutamate release from the photoreceptors to the ON- and OFF-bipolar cells.[7]

However, impaired night vision is not always the major complaint for those with CSNB, and the fundus appearance can be indistinctive in some forms of CSNB. Therefore, diagnosis of CSNB can be challenging, especially in children. The aim of the present study was to screen the Slovene population of children with CSNB to distinguish between cCSNB and icCSNB using standard ERGs *versus* recently developed ON-OFF ERGs. Furthermore, the purpose was also to determine whether ON-OFF ERGs are significant for the differentiation of children with CSNB.

Subjects and methods

Subjects

This study was performed according to the tenets of the Declaration of Helsinki, and it was approved by the National Ethics Committee. All of the children and their parents were informed about the investigation protocol. The study included 8 children with a diagnosis of cCSNB (7 ♂, 1 ♀), which were 8–17 years old (mean age: 10.6 years), and 4 children with icCSNB (4 ♂), aged from 5–18 years (mean age: 11.0 years). Three of the patients with CSNB were adolescents at the time of inclusion in this study (17–18 years old), although they were followed from a younger age, and for the sake of convenience they were considered here as children.

The main opthalmological findings of these children with CSNB are summarized in Table 1, and these include: variable visual loss (cCSNB: 0.1–0.9, mean: 0.6; icCSNB: 0.4–0.9, mean: 0.6; visual acuitiy Snellen equiv.), refractive error ranged from moderate to high myopia (spherical equivalent range, cCSNB: -0.25 to -11.00 D; icCSNB: -1.75 to -7.00 D), abnormal dark adaptation,

nystagmus (six of these 12 children), normal anterior eye segment, clear optic media, normal fundi (Figure 2) or temporal optic disc pallor or attenuated vessels, normal color vision, and variable constriction of the visual field. No clinical progression was identified by follow-up over at least 3 years. According to their family histories, X-linked inheritance was recognized in five of these 12 children, while with the others, none of their family members had any symptoms of night blindness. All of the children were included in the molecular genetic procedures, although to date these results have been obtained for only one of these children (Table 1, child 10).

Electroretinography

Full-field ERGs were recorded simultaneously from both eyes following the standards of the International Society of Clinical Electrophysiology of Vision (ISCEV).[16] The recording electrode was a HK-loop, placed in the fornix of the lower eyelid.[17] The silver-chloride reference electrode was placed on the ipsilateral temple, and the ground electrode was positioned on the forehead. The pupils were dilated with 1 % tropicamide (Mydriacyl®). Full-field ERGs were recorded using a Ganzfeld stimulator of the RETIport unit (Roland Consult, Wiesbaden, Germany). The dark-adapted ERG responses were recorded after 20 min of dark adaptation, and the light-adapted responses after 10 min of light adaptation (background luminance, 22 cd/m^2). The stimulus intensities were consistent with the ISCEV standard values,[16] the intensity of the standard flash (as combined rod–cone response, oscillatory potentials, cone response and 30-Hz flicker) was 2.4 cd s/m^2, and the intensity of the attenuated dark-adapted flash (rod response) was 0.03 cd s/m^2. ON-OFF ERGs were recorded from one eye, as described previously,[18] with a full-field hand-held Espion ColorBurst stimulator (Diagnosys LLC, Littleton, MA, USA), which was powered by white LEDs. The ON-OFF ERG responses were elicited with 200 ms stimuli of 1.9 log cd s/m^2 intensity, which were presented on a photopic background of 50 cd/m^2 luminance. The flashes were delivered at 2 Hz and the means were taken over 20 to 30 responses. At least two measurements were

Table 2: Summary of the ERG data and statistical comparisons between cCSNB and icCSNB (Mann-Whitney U tests).

	Comb. rod-cone resp. – a-wave		Comb. rod-cone resp. – b-wave		Rod resp. – b-wave	
	Amplitude [mV]	Impl. time [ms]	Amplitude [mV]	Impl. time [ms]	Amplitude [mV]	Impl. time [ms]
Complete CSNB	133 ±23	18.4 ±1.3	69.7 ±21	31.6 ±1.7	0	-
Incomplete CSNB	125 ±17	18.5 ±1.2	92.1 ±24	33.9 ±4.3	51.4 ±20	107 ±7
Difference	NS	NS	NS	NS	P < 0.001	P < 0.001
	Cone resp. – a-wave		Cone resp. – b-wave		30 Hz – b-wave	
Complete CSNB	23.8 ±6.8	16.7 ±0.9	98.9 ±34	32.1 ±1.1	87.2 ±22	28.4±1.5
Incomplete CSNB	14.6 ±3.4	16.3 ±1.2	16.9 ±4.3	28.4 ±1.6	17.4 ±4.7	25.8±1.0
Difference	P = 0.003	NS	P < 0.001	P < 0.001	P < 0.001	P< 0.001
	ON resp. – b-wave		OFF resp. – d-wave			
Complete CSNB	12.6 ±5.6	37.5 ±2.7	26.7 ±6.9	216 ±1.6		
Incomplete CSNB	6.9 ±5.1	34.0 ±6.7	8.7 ±4.2	225 ±1.2		
Difference	NS	NS	P < 0.001	P < 0.001		

NS: No significant difference between the groups

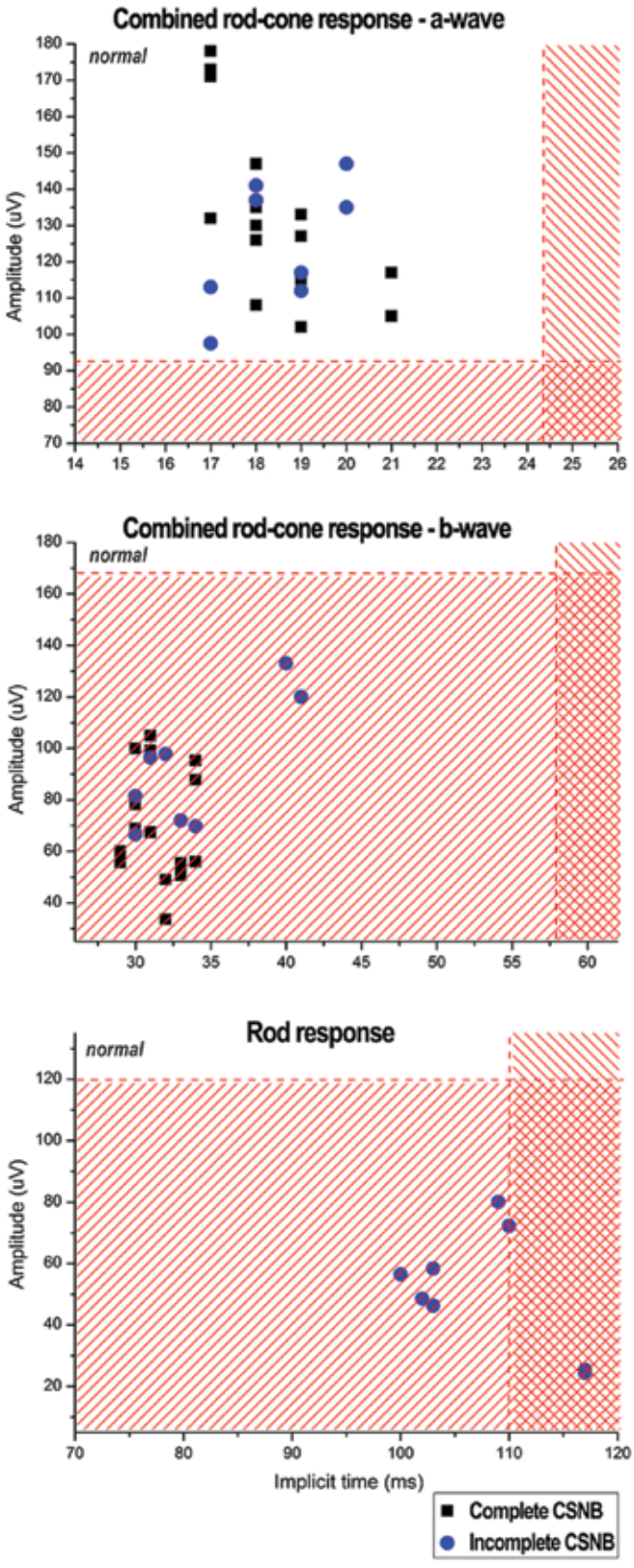

Figure 4: Individual data of dark-adapted ERGs (rod–cone responses and rod responses, as indicated) where the amplitudes from both eyes were plotted against the implicit time; normative limits indicate abnormality of the values when located in the area marked with red. cCSNB – black ■ symbols; icCSNB – blue ● symbols.

repeated and averaged for each of the full-field and ON-OFF ERG responses. All of the responses were differentially amplified and stored on the hard disc of a computer. The flash intensity and background luminance were calibrated with a photometer/ radiometer (IL-1700; International Light INC, Newburyport, USA) using a photopic light detector.

Analysis

The amplitude of the a-wave was measured from the stimulus onset to the middle of the first negative trough. The amplitude of the b-wave was measured from the trough of the a-wave to the first positive peak. The amplitude of the d-wave was measured from the stimulus offset to the following positive peak. The results were considered as abnormal if the implicit time was above 95 % of the upper confidence limits and the amplitude was below 5 % of the lower confidence limits of the normative data.[19] Comparisons between the data were performed with Mann-Whitney U tests, and were considered significant for $p < 0.01$. The data were analyzed using Origin 7.0 (OriginLab Corp., Northampton, USA) and SPSS, version 12.0 (SPSS Inc., Chicago, USA).

Results

Representative ERGs of a healthy control child and children with cCSNB and icCSNB are shown in Figure 3. Both types of CSNB were characterized by an electronegative combined rod–cone response of the dark-adapted ERG, which can be recognized as the smaller b-wave amplitude compared with the a-wave amplitude. Rod responses to dim stimuli were not detectable with cCSNB, although residual responses remained with icCSNB. There were no peaks in the oscillatory potentials with cCSNB, with some seen with icCSNB.

Abnormalities were also present in the light-adapted ERGs, which were more prominent with icCSNB, where both the cone response and the 30-Hz flicker response were severely reduced. On the contrary, these two responses appeared nearly normal

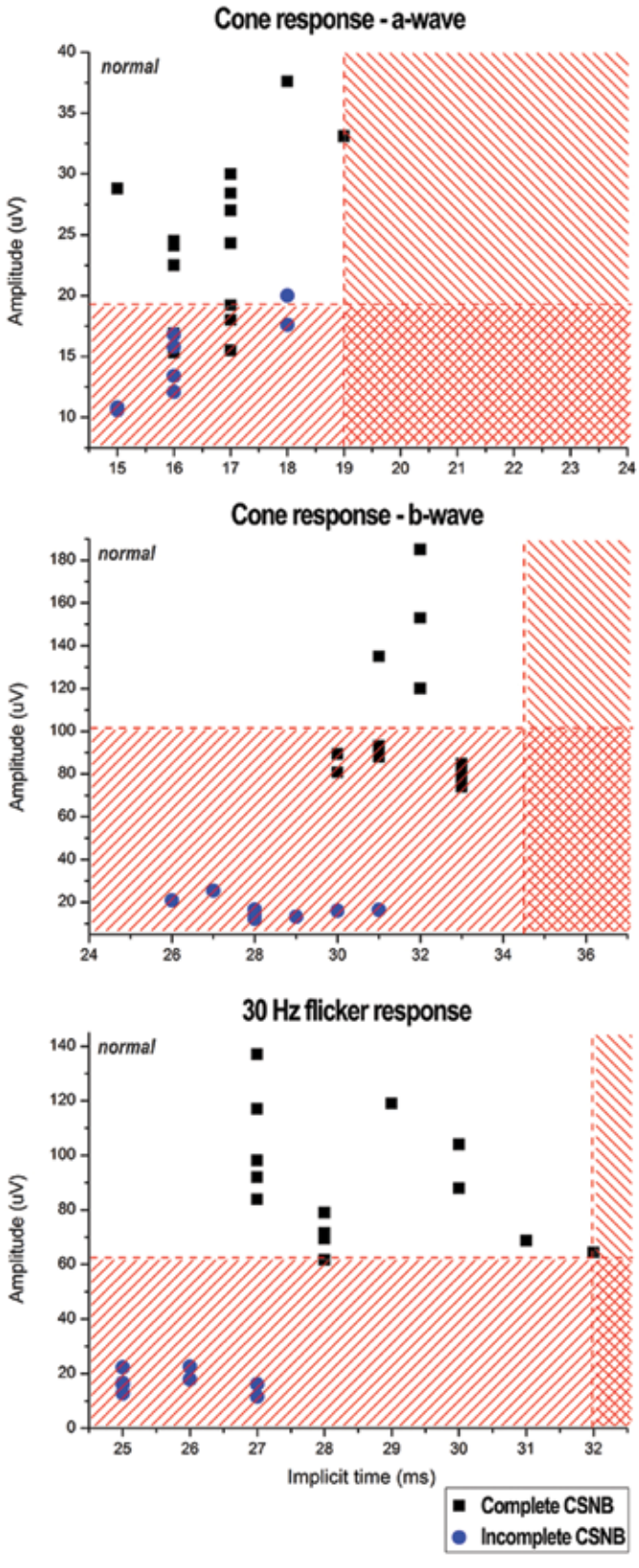

Figure 5: Individual data of light-adapted ERGs (cone responses and 30-Hz flicker responses, as indicated) where the amplitudes from both eyes were plotted against the implicit time; normative limits indicate abnormality of the values when located in the area marked with red. cCSNB – black ■ symbols; icCSNB – blue ● symbols.

with cCSNB, except that the a-wave of the cone response was broadened. The ON- and OFF-responses elicited with long duration flashes under light-adapted conditions were also changed in both types of CSNB. The ON-response (b-wave) was severely reduced in both types of CSNB, while the OFF--response (d-wave) was abnormal only in icCSNB.

Figures 4, 5 and 6 show the response data of all of these children with CSNB, where the amplitudes measured from both eyes were plotted against the implicit time. The limits were also plotted, which indicate the normal and abnormal ranges of the data. Figure 4 shows the plots of the full-field ERGs recorded under dark-adapted conditions. The a-wave of the combined rod–cone response had a normal amplitude and implicit time in all of these children with cCSNB and icCSNB. The b-wave of the combined rod–cone response was significantly reduced in both CSNB types, while the time to peak of the b-wave remained within the normal range. The rod response was not detectable with cCSNB (the values are not plotted), while the rod response amplitude was reduced in the children with icCSNB; one child also showed a delayed implicit time of the rod response.

The plots summarizing the data of the light-adapted ERG responses in children with cCSNB and icCSNB are shown in Figure 5. The amplitude of the cone response a-wave was normal to borderline subnormal in cCSNB, while it was borderline subnormal to reduced in the children with icCSNB. The a-wave implicit time was normal in the majority of these children with both CSNB types; only one child with cCSNB showed borderline prolongation of the implicit time. The b-wave of the cone response had normal to borderline subnormal amplitudes with cCSNB, while a reduction in the amplitude was distinct in the children with icCSNB. The b-wave implicit time was within normal limits with both CSNB forms. The 30-Hz flicker response had normal to borderline amplitudes and implicit times in the children with cCSNB, while a severe reduction of its amplitude was seen in the children with icCSNB.

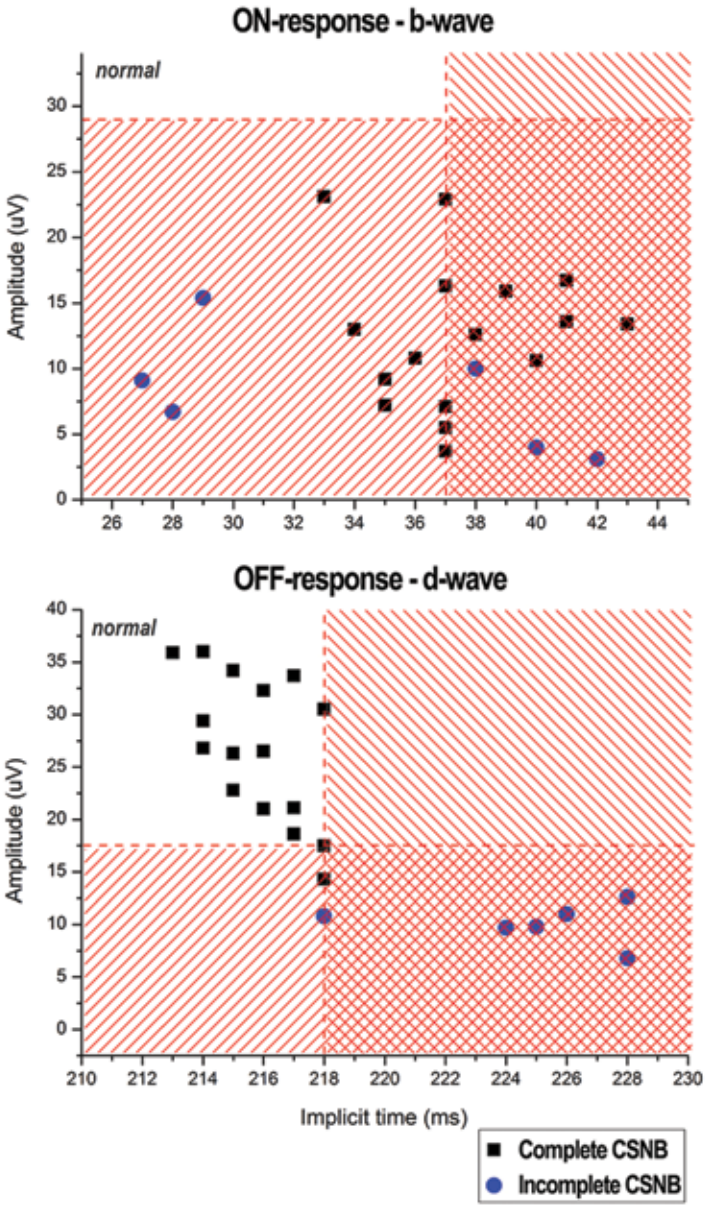

Figure 6: Individual data of the ON-OFF ERGs (ON- and OFF-responses, as indiacted) where the amplitudes from both eyes were plotted against the implicit time; normative limits indicate abnormality of the values when located in the area marked with red. cCSNB – bblack ■ symbols; icCSNB – blue ● symbols. In icCSNB, data from only 6 eyes are plotted, because in one child ON-OFF ERG was contaminated with blinking artifacts.

The alterations in the ON-OFF ERG data with cCSNB and icCSNB are summarized in Figure 5. The ON-response (b-wave) was markedly abnormal in both types of CSNB, as it was reduced in all of these children. Prolongation of the implicit time was seen in some of these children with cCSNB and icCSNB. The OFF-response (d-wave) amplitude and implicit time were normal or borderline subnormal in the children with cCSNB, while they were severely reduced and prolonged with icCSNB.

The statistical comparisons between these data for cCSNB and icCSNB are summarized in Table 2. The differences in the values of the responses between these two types of CSNB were the most significant for the rod response, the b-wave of the cone response, the 30-Hz flicker response, and the d-wave or OFF-response, which was elicited with long-duration stimuli. The combined rod–cone responses were similar with both types of CSNB.

Discussion

The present study reveals distinct ERG features that can be used to differentiate between children with cCSNB and icCSNB, inherited as either X-linked or autosomal recessive trait. Standard full-field ERGs show an electronegative waveform of the combined rod–cone responses with both types of CSNB. cCSNB was characterized by complete rod dysfunction and borderline cone dysfunction, while with icCSNB, there was preservation of rod function and severe cone dysfunction seen. ON-OFF ERGs revealed ON-bipolar cell dysfunction with cCSNB, while with icCSNB, there was both ON- and OFF-bipolar cell dysfunction.

According to Boycott et al.,[20] clinical diagnosis of CSNB can be made with the following findings: a history of night blindness, reduced visual acuity, characteristic findings on ERGs, myopia or hyperopia, nystagmus and strabismus in 50 % to 70 % of cases, and in general, normal color vision and normal fundus appearance. A positive family history is seen in cases of X-linked inheritance. All of the clinical findings of the children included in the present study were consistent with these criteria. With the exception of the ERGs, all of the other clinical findings were of very similar appearance with both cCSNB and icCSNB. All of these children were myopic, and they all manifested a similar loss of visual acuity, which appeared to be the major complaint in both of these CSNB groups. Both CSNB types also had nonspecific fundus changes, which varied from a normal appearance to myopic changes and mild optic disc pallor. Similar descriptions have also been reported in other studies.[1,10,20,21] Nystagmus, squint, tilted optic discs and paradoxical pupil responses have been reported in association with both conditions.[22] In the group of children in the present study,

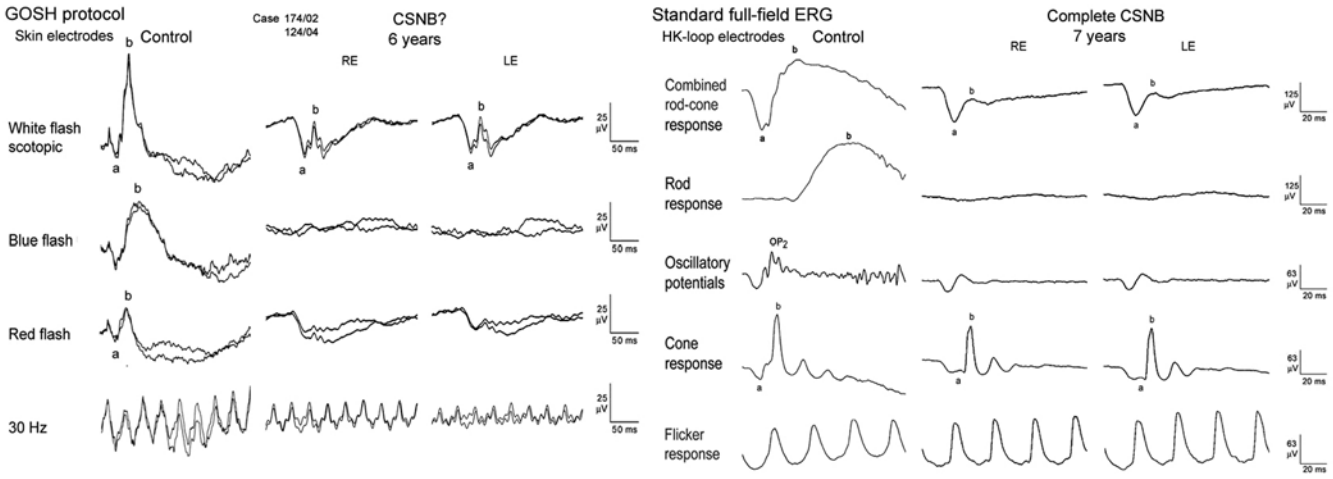

Figure 7: ERGs according to the GOSH protocol at the age of 6 years and the standard full-field ERGs at the age of 7 years in a child (Child 6) with cCSNB.

there was evident nystagmus in six of these 12 children.

The present study compared children with cCSNB and icCSNB from the age of 5 years, when they were old enough to cooperate with the recordings with ocular contact electrodes. The ERG features have been previously reported in adults and mixed populations, including children.[5,9,23] One study investigated a pediatric population but did not specify the ages of the patients.[24] In our study, the ERG characteristics in the children with cCSNB and icCSNB were consistent with previous descriptions.[21] As previously shown, the electronegative waveform of the dark-adapted combined rod–cone response indicates post-photoreceptor dysfunction.[5] In the present study, the combined rod–cone responses were reduced with cCSNB and icCSNB to similar extents, and therefore the electronegative waveform did not provide information for differential diagnosis between cCSNB and icCSNB. The most significant differentiation between the children with cCSNB and icCSNB in the present study was seen in the amplitude and implicit time of the rod response, cone response, 30-Hz flicker response, and OFF--response. The cone response a-wave was distinctly broadened in children with cCSNB, as also indicated previously.[21] This broadening is a consequence of a missing positive component of the cone ON-bipolar cells. However, this functional anomaly can be more easily detected with ON-OFF ERGs.

ON-OFF ERGs do not represent a standardized electrophysiological procedure, although the ISCEV currently aims to include these in the standards, together with some other extended ERG protocols. Cones synapse onto ON-bipolar and OFF-bipolar cells. With short duration flashes, such as light-adapted cone responses, the activities of both bipolar cell types merge into a single positive b-wave. With long duration stimuli, as used for the recording of the ON-OFF responses, two positive waves occur, the b--waves and the d-waves.[18,25] The first one of these, the ON-response b-wave, indicates the activity of the ON-bipolar cells, while the second one, the OFF-response d-wave, measures the contributions from the OFF--bipolar cells.[26,27] ON-OFF ERGs have previously been shown to be useful for the selective evaluation of ON- and OFF-pathway abnormalities of the retinal cone system.[26] More recent studies have shown that this response is highly indicative for detection of the preferential ON-pathway abnormality in retinal pathologies, such as cCSNB or melanoma-associated retinopathy.[24,26,28] On the other hand, in icCSNB, where both the ON- and OFF-responses are reduced, this functional abnormality indicates dysfunction of both the ON- and OFF-bipolar cells. This difference between cCSNB and icCSNB was seen in all of our pediatric patients here, and it appears to be highly characteristic for differential diagnosis between those two clinical entities, as previously described.[5,10,21] However, the ON-OFF ERG abnormalities alone do not provide information for the diagnosis of cCSNB and icCSNB, as the defective night vision has to be confirmed by

abnormal dark-adapted ERGs, as well as for the light-adapted ERG characteristics.

The ISCEV does not currently have a standard for ERG recording in younger children (less than seven years old), as these children do not always cooperate in the recordings with ocular contact electrodes and Ganzfield stimuli. Indeed, some laboratories sedate younger children for their ERG recordings.[29] Other laboratories use a noninvasive Great Ormond Street Hospital (GOSH) protocol that uses skin electrodes, while the children are alert and sit in a parent's lap.[30,31] According to our experience, the noninvasive GOSH protocol is already sensitive enough for the detection of CSNB features in babies and young children from the age of a few months. An example of the sensitivity of detection of CSNB abnormalities is shown in Figure 7, where the ERG abnormalities that were detected following the GOSH protocol at a younger age (child of 6 years) were subsequently confirmed using the standard full-field ERG protocol one year later.

In recent years, genetic testing has become fundamental for the diagnosis of patients with CSNB, as ophthalmological and ERG findings are similar for all the genetic forms of cCSNB or icCSNB. To our knowledge, the only phenotypic differences have been detected using dark-adapted 15-Hz-flicker ERGs in cCSNB patients with different gene mutations.[32] Patients with *NYX* and *TRPM1* gene mutations showed similar 15-Hz-flicker ERG responses, while differences were found for those with *GRM6* gene mutations, which suggested some differences in the rod pathways.[33] For all of the children included in the present study, the blood samples were sent for genetic screening, although to date we have received the result of only one of the children with icCSNB, in whom the *CACNA1F* mutation was confirmed. In five of these children, X-linked inheritance is suspected, according to their family histories. In the other children, none of the family members have shown signs of CSNB, and therefore the manner of inheritance and the mutation cannot be revealed without genetic testing. In obligate carriers of X-linked icCSNB that do not show any other clinical signs for night blindness, ERG changes might be observed. These can be seen as significant reductions in the sum of the oscillatory potential amplitude associated with the rod activity or with a reduced light-adapted b-wave and 30-Hz flicker amplitude.[34] Consistently with this description, we found reduced light-adapted ERG amplitudes in the mother of a boy with icCSNB (child 12), who was a possible carrier, but this finding has not yet been confirmed by genetic analysis. However, even when genetic testing is accessible, ERGs still have an important role in the diagnosis of cCSNB from icCSNB, even though they cannot discriminate between X-linked and autosomal recessive mutations that cause these types of retinal disorder.

To conclude, cCSNB and icCSNB are genetically heterogeneous and their clinical features are not specific enough to establish diagnosis. However, children can be diagnosed and differentiated by the use of standard full-field ERGs. ON-OFF ERG recordings are also relevant in children, because they define ON- *versus* OFF-bipolar cell dysfunction, and therefore these also differ between cCSNB and icCSNB.

Acknowledgement

The authors wish to thank Mrs. Marija Jesenšek and Mrs. Ana Jeršin, who were involved in the clinical ERG recording, and to Mrs. Barbara Klemenc, who prepared some of the figures. This study was supported by the Slovene Research Agency, Grant No. J3–6167 and P3–0333, and was in part presented at the anual meeting of the European Paediatric Ophthalmological Society (EPOS), in Portorož, Slovenia, in 2007.

References

1. Dryja TP. Molecular genetics of Oguchi disease, fundus albipunctatus, and other forms of stationary night blindness: LVII Edward Jackson Memorial Lecture. Am J Ophthalmol 2000; 130(5): 547–63.
2. Zeitz C, Kloeckener-Gruissem B, Forster U, Kohl S, Magyar I, Wissinger B, e al. Mutations in CABP4, the gene encoding the Ca2+-binding protein 4, cause autosomal recessive night blindness. Am J Hum Genet 2006; 79(4): 657–67.
3. Holder GE, Robson A. Paediatric Electrophysiology: A Practical Approach. In: Lorenz B, Moore AT, eds. Pediatric ophthalmology, neuro-ophthalmology, genetics. Essentials in Ophthalmology Vol 7. Berlin: Springer; 2006. p. 133–55.
4. Schubert G, Bornschein H. [Analysis of the human electroretinogram]. Ophthalmologica 1952; 123(6): 396–413.
5. Miyake Y, Yagasaki K, Horiguchi M, Kawase Y, Kanda T. Congenital stationary night blindness with negative electroretinogram. A new classification. Arch Ophthalmol 1986; 104(7): 1013–20.
6. Yamamoto S, Sippel KC, Berson EL, Dryja TP. Defects in the rhodopsin kinase gene in patients with the Oguchi form of stationary night blindness. Nature Genet 1997; 15: 175–8.
7. Zeitz C, Labs S, Lorenz B, Forster U, Uksti J, Kroes HY, et al. Genotyping microarray for CSNB-associated genes. Invest Ophthalmol Vis Sci 2009; 50(12): 5919–26.
8. Sergouniotis PI, Sohn EH, Li Z, McBain VA, Wright GA, Moore AT, Robson AG, et al. Phenotypic variability in RDH5 retinopathy (Fundus Albipunctatus). Ophthalmology 2011; 118(8): 1661–70.
9. Miyake Y, Yagasaki K, Horiguchi M, Kawase Y. On- and off responses in photopic electroretinogram in complete and incomplete types of congenital stationary night blindness. Jpn J Ophthalmol 1987; 31: 81–7.
10. Miyake Y. (Establishment of the concept of new clinical entities – complete and incomplete form of congenital stationary night blindness). Nippon Ganka Gakkai Zasshi 2002; 106(12): 737–55.
11. Bech-Hansen NT, Naylor MJ, Maybaum TA, Sparkes RL, Koop B, Birch DG, et al. Mutations in NYX encoding the leucine-rich proteoglycan nyctalopin cause X-linked complete congenital stationary night blindness. Nat Genet 2000; 26(3): 319–23.
12. Dryja TP, McGee TL, Berson EL, Fishman GA, Sandberg MA, Alexander KR, et al. Night blindness and abnormal cone electroretinogram ON responses in patients with mutations in the GRM6 gene encoding mGluR6. Proc Nat Acad Sci USA 2005; 102(13): 4884–9.
13. Audo I, Kohl S, Leroy BP, Munier FL, Guillonneau X, Mohand-Saïd S, et al. TRPM1 is mutated in patients with autosomal-recessive complete congenital stationary night blindness. Am J Hum Genet 2009; 85(5): 720–9.
14. Bech-Hansen NT, Naylor MJ, Maybaum TA, Pearce WG, Koop B, Fishman GA, et al. Loss-of-function mutations in a calcium-channel alpha-1-subunit gene in Xp11.23 cause incomplete X-linked congenital stationary night blindness. Nature Genet 1998; 19: 264–7.
15. Wycisk KA, Zeitz C, Feil S, Wittmer M, Forster U, Neidhardt J, et al. Mutation in the auxiliary calcium-channel subunit CACNA2D4 causes autosomal recessive cone dystrophy. Am J Hum Genet 2006; 79(5): 973–7.
16. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M. Standard for clinical electroretinography (2008 update). Doc Ophthalmol 2009; 118: 69–77.
17. Hawlina M, Konec B. New noncorneal HK-loop electrode for clinical electroretinography. Doc Ophthalmol 1992; 81(2): 253–9.
18. Sustar M, Hawlina M, Brecelj J. ON- and OFF-response of the photopic electroretinogram in relation to stimulus characteristics. Doc Ophthalmol 2006; 113(1): 43–52.
19. Skačej H, Friedrich T. Elektrofiziološka ocena delovanja čepnic pri bolnikih s pigmentno retinopatijo (Prešernove naloge). Univerza v Ljubljani, Medicinska fakulteta, 2003.
20. Boycott KM, Bech-Hansen NT, Sauvé Y, MacDonald IM. X-Linked Congenital Stationary Night Blindness. In: Pagon RA, Bird TD, Dolan CR, Stephens K, eds. GeneReviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2008.
21. Miyake Y. Complete and incomplete types of CSNB. In: Miyake Y. Electrodiagnosis of retinal disease. Tokyo: Springer; 2006. p. 90–113.
22. Taylor D, Moore A. Inherited retinal dystrophies. In: Taylor D ed. Paediatric ophthalmology. Cambridge: Blackwell Scientific; 1990. p. 376–403.
23. Langrova H, Gamer D, Friedburg C, Besch D, Zrenner E, Apfelstedt-Sylla E. Abnormalities of the long flash ERG in congenital stationary night blindness of the Schubert-Bornschein type. Vision Res 2002; 42(11): 1475–83.
24. Cibis GW, Fitzgerald KM. The negative ERG is not synonymous with nightblindness. Trans Am Ophthalmol Soc 2001; 99: 171–6.
25. Sustar M, Stirn-Kranjc B, Hawlina M, Brecelj J. Photopic ON- and OFF-responses in complete type of congenital stationary night blindness in relation to stimulus intensity. Doc Ophthalmol 2008; 117(1): 37–46.
26. Sieving PA. Photopic ON- and OFF-pathway abnormalities in retinal dystrophies. Trans Am Ophthalmol Soc 1993; 91: 701–73.
27. Quigley M, Roy MS, Barsoum-Homsy M, Chevrette L, Jacob JL, Milot J. On- and off-responses in the photopic electroretinogram in complete--type congenital stationary night blindness. Doc Ophthalmol 1996; 92(3): 159–65.
28. Alexander KR, Fishman GA, Peachey NS, Marchese AL, Tso MOM. 'On' response defect in paraneoplastic night blindness with cutaneous malignant melanoma. Invest Ophthalmol Vis Sci 1992; 33: 477–83.
29. Fulton AB, Brecelj J, Lorenz B, Moskowitz A, Thompson D, Westall CA, ISCEV Committee for Pediatric Clinical Electrophysiology Guidelines. Pediatric clinical visual electrophysiology: a survey of actual practice. Doc Ophthalmol 2006; 113(3): 193–204.
30. Kriss A, Thompson D. Visual electrophysiology. In: Taylor D ed. Paediatric ophthalmology. Oxford: Blackwell Science Limited; 1997. p. 93–121.

31. Kriss A. Skin ERGs: their effectiveness in paediatric visual assessment, confounding factors, and comparison with ERG srecorded using various types of corneal electrode. Int J Psychophysiol 1994; 16(2–3): 137–46.
32. Scholl HP, Langrová H, Pusch CM, Wissinger B, Zrenner E, Apfelstedt-Sylla E. Slow and fast rod ERG pathways in patients with X-linked complete stationary night blindness carrying mutations in the NYX gene. Invest Ophthalmol Vis Sci 2001; 42(11): 2728–36.
33. Nakamura M, Sanuki R, Yasuma TR, Onishi A, Nishiguchi KM, Koike C, et al. TRPM1 mutations are associated with the complete form of congenital stationary night blindness. Mol Vis 2010; 16: 425–37.
34. Rigaudière F, Roux C, Lachapelle P, Rosolen SG, Bitoun P, Gay-Duval A, Le Gargasson JF. ERGs in female carriers of incomplete congenital stationary night blindness (I-CSNB). A family report. Doc Ophthalmol 2003; 107(2): 203–12.