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Congenital Stationary Night Blindness with Hypoplastic Discs, Negative Electroretinogram and Thinning of the Inner Nuclear Layer

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ABSTRACT

Purpose: To describe congenital stationary night blindness (CSNB) with a negative electroretinogram, hypoplastic discs, nystagmus and thinning of the inner nuclear layer (INL).

Methods: Retinal structure was analyzed qualitatively with spectral domain optical coherence tomography and wide field imaging. Retinal function was evaluated with full-field electroretinography (ffERG). Molecular genetic testing included next-generation sequencing (NGS) of the known genes involved in CSNB.

Results: Patients presented with CSNB presented with nystagmus, high myopia, hypoplastic discs and negative ffERG with no measurable rod response. The retinas appeared normal and automated segmentation of retinal layers demonstrated a relative reduction of thickness of the INL. There was no significant change in the ffERG after prolonged 2 hour dark adaptation compared to standard 30 minute dark adaptation. Affected family members harboured the homozygous 1-bp deletion c.2394delC in exon 18 of the *TRPM1* gene, whereas their unaffected parents were heterozygous carriers.

Conclusions: This data expands the genotype and phenotype spectrum of CSNB. The lack of improvement of rod responses after prolonged dark adaptation, together with thinning of the INL, is compatible with postreceptoral transmission dysfunction in the bipolar cells. Such knowledge may prove useful in future development of treatment for outer retinal dystrophies, using opsin genes to restore light responses in survivor neurons in the inner retina.

Key words: Congenital stationary night blindness, optical coherence tomography, optic disc hypoplasia, full-field electroretinography.

INTRODUCTION

Congenital stationary night blindness (CSNB) refers to a group of mainly non-progressive retinal disorders featuring night blindness due to mutations in genes affecting either retinoid metabolism in the retinal pigment epithelium, photoreceptor transduction or signal transmission through the retinal bipolar cells [1]. CSNB is clinically and genetically heterogeneous. Some forms are associated with poor visual acuity, myopia, nystagmus, strabismus and fundus abnormalities [2-4]. CSNB is referred to as "complete" (MIM#613216), when there is no full-field electroretinography (full-field ERG) rod response combined with an electronegative rod-cone ERG with a normal a-wave (emanating from the photoreceptors) and severely reduced b-wave [1]. Such findings are consistent with an ON-bipolar cell dysfunction.

The rods connect with the dendritic tips of ON-bipolar cells (rod bipolar cells). An increase in light intensity induces a depolarization in the ON-bipolar cells via a reduction of glutamate release from presynaptic rods. The signal transduction cascade in the dendritic tips of ON-bipolar cells involves the metabotropic Glutamate Receptor, 6 (mGluR6) glutamate receptors signalling to Transient Receptor Potential cation channel, subfamily M, member 1 (TRPM1) proteins that form part of the transduction channel. Mutations in *TRPM1* are thought to be the leading cause for the complete form of autosomal recessive CSNB, also called CSNB1 [1]. Like *TRPM1*, the other mutations causing autosomal recessive complete CSNB occur in genes expressed in ON-bipolar cells, whose protein products are thought to participate in the rod pathway signal transduction [1].

Clinically autosomal recessive complete CSNB is typically associated with high myopia and nystagmus [5]. Here, we describe a consanguineous family with autosomal recessive complete CSNB and high myopia, nystagmus and in addition optic disc hypoplasia, associated with the novel homozygous frameshift mutation c.2394delC in the *TRPM1* gene. We present data that indicate that there is a specific postreceptoral transmission dysfunction in the bipolar cells in this form of CSNB. Such knowledge may prove useful in future development of treatment for outer retinal dystrophies, using opsin genes to restore light responses in survivor neurons in the inner retina (Optogenetics).

METHODS

This is a retrospective study of a family with 2 affected twin 20-year old brothers (II:1 and II:2) with CSNB and their unaffected parents. Informed consent was obtained. Institutional Review Board (IRB)/Ethics Committee approval was obtained. The research adhered to the tenets of the Declaration of Helsinki.

For both affected twin brothers, retinal structure was analyzed qualitatively with transfoveal horizontal spectral domain optical coherence tomography scans (OCT, Heidelberg Engineering, Inc., Heidelberg, Germany) and wide field imaging (Optos PLC, Dunfermline, UK). Five myopic age-matched controls, were recruited to analyze differences in retinal structure between the patients and controls. For this analysis, automated retinal sublayer segmentation using the Heidelberg software was done, and the following 5 retinal sublayers were isolated and enlarged and compared qualitatively for differences in thickness, while preserving the proportions within each layer, and between the patient II:2 and each control: A. Photoreceptor and retinal pigment epithelium (RPE) layer: Between the external limiting membrane and Bruchs membrane B. Outer nuclear layer: Between the outer plexiform layer line and the external limiting membrane C. Inner nuclear layer: Between the inner nuclear layer line and the inner plexiform layer line D. Ganglion cell layer: Between the ganglion cell layer line and the retinal nerve fiber layer line. E. Total retinal and RPE thickness.

In both affected twin brothers retinal function was evaluated with full-field electroretinography (ffERG, Nicolet Biomedical Instruments, Madison, Wisconsin, USA), in dark adapted and light adapted state according to ISCEV standards [6], with a few modifications as follows. Retinal function was evaluated with full-field electroretinography (ffERG, Nicolet Biomedical Instruments, Madison, Wisconsin, USA), in dark adapted and light adapted state according to ISCEV standards, with a few modifications as follows [6].Full-field electroretinograms were recorded in a Nicolet analysis system(Nicolet Biomedical Instruments, Madison, Wisconsin, USA), after dark adaptation of subjects for 40 min, dilatation of the pupils with topical cyclopentolate 1% and metaoxedrine 2,5% and topical anaesthesia, with a Burian Allen bipolar contact lens and a ground electrode applied to the forehead. Responses were obtained stimulating with single full-field flash (30 ms) with blue light light (0.81 cd-s/m2: rod response) and with white light (10.02 cd-s/m2: combined rod-cone response). Photopic responses (not shown) were obtained with a background illumination of 3.4 cd-s/m2 in order to saturate the rods.

Molecular genetic testing in both affected twin brothers included next-generation sequencing (NGS, Illumina HiSeq 1500, performed by Center for Human Genetics Bioscientia, Ingelheim, Germany) of the known genes involved in CSNB: *CABP4, CACNAIF, GNAT1, GPR179, GRK, GRM6, LRIT3, NYX, PDE6B, RHD, SLC24A1, TRPM1*. Genomic DNA was fragmented and the coding exons of the analyzed genes as well as the corresponding exon-intron

boundaries were enriched using the Roche/NimbleGen sequence capture approach, amplified and sequenced using NGS. The target regions were sequenced with an average coverage of 506-fold. For more than 99% of the regions of interest a 20-fold coverage was obtained. NGS data analysis was performed using bioinformatic analysis tools as well as JSI Medical Systems software (version 4.1.2). Identified variants and indels were filtered against external and internal databases and filtered depending on their allele frequency focusing on rare variants with a minor allele frequency (MAF) of 1% or less. Nonsense, frameshift and canonical splice site variants were primarily considered likely pathogenic. Variants that have been annotated as common polymorphisms in databases or in the literature were not considered further.

Putatively pathogenic differences between the wildtype sequence (human reference genome according to UCSC Genome Browser: hg19, GRCh37) and the patients sequence mentioned and interpreted in this report were validated using polymerase chain reaction (PCR) amplification followed by conventional Sanger sequencing. The resulting sequence data for the TRPM1 gene (OMIM: #603576; locus: chromosome 15q13.3) were compared to the reference sequence NM_001252020.1.

For the unaffected parents, exon 18 of *TRPM1* was apmlified by PCR and directly sequenced. In addition, wide field fundus photography and autofluorescence imaging was obtained.

We did not perform mutation screening in genes that may be associated with optic nerve hypoplasia, such as *HESX1*, *SOX2*, or *ATOH7*.

RESULTS

The affected brother (II:1) presented with a corrected visual acuity (CVA) of 20/30 OU (Refraction OD -7_1.75x175, OS-8.75-2.25x105) and his brother (II:2) with 20/40 OD and 20/50 OS (Refraction OD -8_-1x60, OS -8_-0.5x150). Both presented with was a small amplitude pendular horizontal nystagmus OU. Fundi were normal except for myopic changes (such as apparently thin retinas with visible choroidal vessels) and hypoplastic tilted discs (Fig. 1A). Fundus autofluorescence demonstrated a normal distribution of autofluorescence (Fig. 1B) Optical coherence tomography showed normal retinal layers that could be automatically segmented using the Heidelberg software in brother II:2 (Fig. 1C right column). Full field electroretinography demonstrated a non-recordable rod response and an electronegative pattern, with a reduced b wave amplitude compared to a wave amplitude in the combined rod-cone

response (Fig. 2A), and light adapted responses were normal to mildly reduced including a mildly reduced b/a ratio in the single flash cone response (Fig. 2B) There was no significant change in the full field electroretinogram after prolonged 2 hour dark adaptation compared to after standard 30 minute dark adaptation (Fig. 2B).

Total retinal thickness did not seem to be reduced compared to controls, however there seemed to be a relative thinning of the inner nuclear layer compared to other retinal layers (Fig. 3A-E).

In both, MRI had been done because of hypoplastic discs, to rule out septo-optic dysplasia, with normal findings, including normal midline structure and normal appearing optic nerves. Visual fields were normal. The mother and father had normal fundi, normal fundus autofluorescence imaging (not shown) and were asymptomatic.

In both brothers, next-generation sequencing (NGS, Illumina HiSeq 1500) revealed the homozygous 1-bp deletion c.2394delC in exon 18 of the *TRPM1* gene, which leads to a frameshift and subsequent formation of a premature stop codon (p.Thr799Profs*110). This frameshift mutation probably results either in mRNA degradation by nonsense-mediated mRNA decay (NMD) or in the expression of a truncated TRPM1 protein, and can be regarded pathogenic. The result was confirmed by Sanger sequencing. To the best of our knowledge, this mutation has neither been annotated in databases nor been described in the literature so far. Specifically, it is not mentioned in the ExAc browser (http://exac.broadinstitute.org/, access date March 29, 2016). The detection of the homozygous frameshift deletion in TRPM1 is in accordance with the CSNB phenotype of these patients [7]. Parental consanguinity indicates that it is highly likely that the alteration is homozygous. In principle, high coverage of NGS data generated by Illumina HiSeq sequencing enables copy number variation (CNV) analysis. Here, we found no indication for a large deletion or duplication comprising exon 18 on the other TRPM1 allele. Both parents were heterozygous carriers for the mutation.

DISCUSSION

TRPM1 (MIM #603576) is a member of the transient receptor potential (TRP) channel family of proteins which permit Ca^{2+} entry into hyperpolarized cells, leading to depolarization. The mechanism may involve phosphatidylinositol and protein kinase C signaling [1,8]. TRPM1 is believed to be the cation channel in the ON bipolar cells that is responsible for the depolarization of these cells during light stimulation as follows. In the dark, glutamate is released from photoreceptors, binds to the metabotropic gluatamate receptor mGluR6 (encoded by *GRM6*) on the rod bipolar cells [9], which in turn leads, by an unidentified mechanism which probably involves nyctalopin (NYX) [10], G-Protein coupled Receptor 179 (GPR179) [11], and Leucine-rich Repeat, Immunoglobulin-like, and Transmembrane

domains-containing protein 3 (LRIT3) [12], to the closure of the cation channel TRPM1 [1]. Upon light exposure, the cessation of glutamate release from the photoreceptors leads to the opening of TRPM1, in turn leading to ON bipolar cell depolarization, giving rise to the b-wave. Mutations in *GRM6* lead to the loss of mGluR6 at the cell surface of the ON bipolar cells, resulting in the failure of depolarization of these cells and thus a severely reduced b-wave and complete CSNB [2]. Likewise, mutations in NYX, GPR179, and LRIT3, may cause complete CSNB [1]. These proteins, including TRPM1, are all localized to the dendritic tips of ON-bipolar cells, and are all implicated in autosomal recessive complete CSNB with negative electroretinogram [9-13].

Our report adds to the complexity of CSNB genotypes and phenotypes. We demonstrated preservation of all retinal layers, to a degree that enabled automated layer segmentation, in spite of nystagmus, in 1 of the 2 affected twins. Godara et al. examined 3 patients with GRM6 mutations with spectral domain optical coherence tomography, reporting retinal thinning outside the foveal region due to changes in the inner retina, including the ganglion cell layer, with preservation of the outer retina [14]. These data were compared to 93 controls which however were probably not matched for myopia, which may have a significant impact on retinal thickness measurements. Here, we compared qualitatively the thickness of several automatically segmented retinal sublayers in the right eye of 1 of the 2 affected twins with those from 5 healthy myopic control eyes, indicating that there may be a selective thinning of the inner nuclear layer in this form of CSNB. However, we also note that among our myopic normal controls, there was variability in the thickness of the various retinal sublayers, including total retinal thickness.

A lack of improvement of rod responses after prolonged dark adaptation and a negative electroretinogram indicates a postreceptoral transmission dysfunction in the bipolar cells in this form of CSNB. This was also supported by the finding of a novel homozygous mutation 1-bp deletion c.2394delC in exon 18 of the *TRPM1* gene, known to encode a calcium channel in the ON-bipolar cells, with a prominent role in the propagation of the light driven neural signaling in the retina. The associated myopia and nystagmus have been reported previously,[5] but optic disc hypoplasia have not been described as far as we know.

The reason why autosomal recessive CSNB may be associated with high myopia, nystagmus and (as in this study) optic disc hypoplasia is not known. One possibility is that TRPM1 may act as a trophic factor during embryonic development, considering its role in synaptic activity and the fact that proper synaptic activity is required for synapse formation and development. The associated optic disc hypoplasia could possibly be due failure of proper optic nerve axon formation due to disturbed synaptogenesis, for example between the bipolar cells and the ganglion cells. The latter

is the last synaptic element during embryogenesis to link photoreceptors in the outer retina and RGCs in the inner retina [15,16]. This could possibly also account for the observed reduction in thickness of the inner nuclear layer.

In summary, we describe clinical and molecular genetic data that indicate a specific involvement of the ON bipolar cells in this form of CSNB. Such knowledge may prove useful in future development of treatment for outer retinal dystrophies, using opsin genes to restore light responses in survivor neurons in the inner retina (Optogenetics). For example, a successful *TRPM1* gene transfer using an appropriate viral vector to target the bipolar cells in this form of CSNB, could be assessed by demonstrating a reversal of the negative electroretinogram. Such a viral vector could subsequently be manipulated to carry an appropriate opsin gene, instead of *TRPM1*,to treat outer retinal dystrophies.

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Conflict of Interest: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Figure legends

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Figure 1: Fundus appearance (A. Top row), fundus autofluorescence (B. Middle row) and Spectral domain optical coherence tomography (C. Lower row) in 2 affected family members with Congenital Stationary Night Blindness due to the homozygous 1-bp deletion c.2394delC in exon 18 of the *TRPM1* gene. Left column represent II:1 and right column represent II:2.

A. Wide field imaging of the right eyes shows myopic fundi with mild macular pigment abnormalities and with hypoplastic tilted discs.

B. Wide field fundus autofluorescence imaging of the right eyes demonstrated normal distribution of fundus autofluorescence. C.Spectral domain optical coherence tomography of the right eyes shows normal retinal structure and layers and automated layer segmentation was possible in II:2 (shown for the left column), BM=Bruchs membrane, RPE= Retinal pigment epithelium, PR1 and PR2=photoreceptor layers, ELM=External limiting membrane, ONL=Outer nuclear layer, OPL=Outer plexiform layer, INL=inner luclear layer, IPL=inner plexiform layer, GCL=Ganglion cell layer and inner nuclear layer. RNFL=Retinal nerve fiber layer.

Figure 2. Full field electroretinography in 2 affected family members (II:1 and II:2) with Congenital Stationary Night Blindness due to the homozygous 1-bp deletion c.2394delC in exon 18 of the *TRPM1* gene. A normal age-matched (age 25 years) myopic (8 diopters) patient was examined with electroretinography as reference. Examinations of the right eyes of patients and control are shown.

A. Dark adapted responses. There are recordable rod responses, and an electronegative configuration in the combined rod-cone response, with the a-wave being larger than the b-wave, in both patients. There is no significant change after prolonged dark adaptation.

B. Light adapted cone responses demonstrate subnormal amplitudes in both patients, with a tendency towards electronegative waveform in the single flash cone response. There is a biphasic peak in the 30 Hz flicker response. µV=microvolts, ms=milliseconds, a=a-wave, b=b-wave.

Figure 3: Retinal sublayer thickness profiles in patient II:2 with Congenital Stationary Night Blindness due to the homozygous 1-bp deletion c.2394delC in exon 18 of the *TRPM1* gene. These were obtained from transfoveal horizontal spectral domain optical coherence tomography line scans. Qualitative comparison with 5 age matched (range 20-30, median 22 years) eyes (C1-C5) from 5 unrelated healthy myopic (myopia range 6-9, median 7.5 diopters) controls. These layers were obtained by automated segmentation with the Heidelberg software with subsequent isolation of retinal sublayers. The scaling within each layer is preserved and equal between patient and controls. There seemed to be a relative and selective reduction of thickness of the inner nuclear layer in II:2, however some degree of generalized retinal thinning, including the inner nuclear layer, seems to be present in controls 3-5.

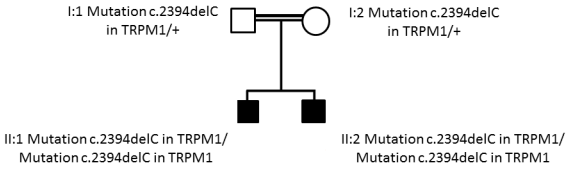
A. Photoreceptor and retinal pigment epithelium (RPE) layer: Between the external limiting membrane and Bruchs membrane.

B. Outer nuclear layer (ONL): Between the outer plexiform layer line and the external limiting membrane.

C. Inner nuclear layer (INL): Between the inner nuclear layer line and the inner plexiform layer line.

D. Ganglion cell layer (GCL): Between the ganglion cell layer line and the retinal nerve fiber layer line.

E. Total retinal and RPE thickness profile.


I:1 Mutation c.2394delC in TRPM1/+
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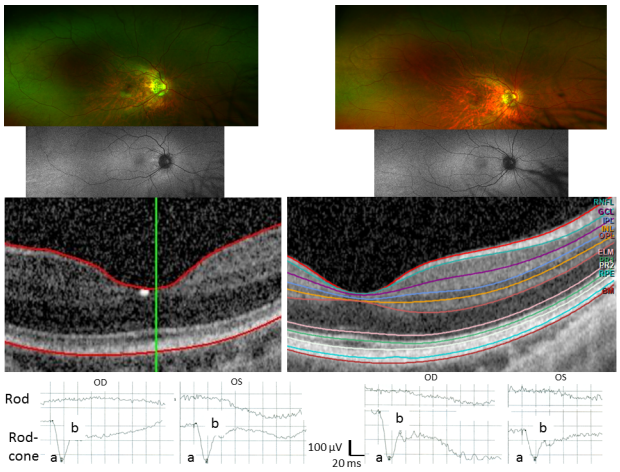
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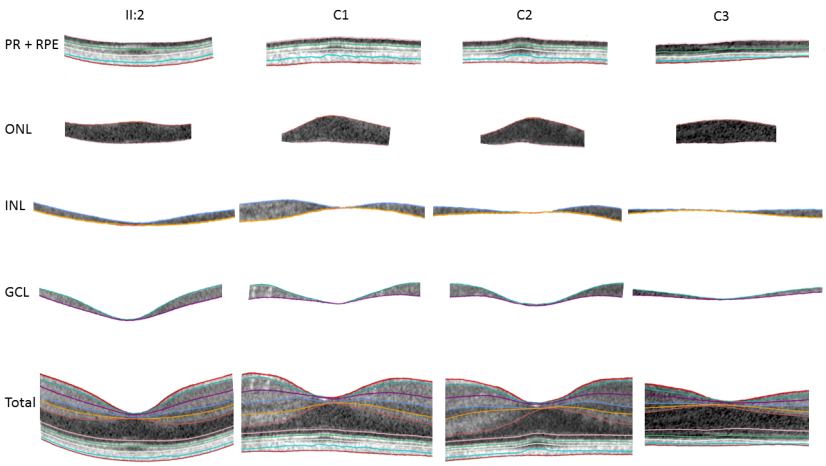
II:2
C1
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PR + RPE
ONL
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Total