Novel *TRPM1* mutations in two Chinese families with early–onset high myopia, with or without complete congenital stationary night blindness

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**Received:** 2015-03-22 **Accepted:** 2015-05-25

**Abstract**

- **AIM:** To investigate the relationship between high myopia [with or without complete congenital stationary night blindness (CSNB1)] and *TRPM1* and *NYX*.
- **METHODS:** Two unrelated families with early–onset high myopia (eoHM) and 96 normal controls were recruited. Sanger sequencing or clone sequencing were used for mutation screening. Further analyses of the available family members and the 96 normal controls were subsequently conducted to obtain additional evidence of the pathogenicity of these variants. The initial diagnosis of the probands was eoHM. We performed a further comprehensive examination of the available family members after mutations were detected in *TRPM1* or *NYX*.
- **RESULTS:** Two novel compound heterozygous mutations in *TRPM1* were detected in the recruited families. The proband in family A with eoHM carried a c.2594C>T missense mutation in exon 19 and a c.669+3_669+6delAAGT splicing mutation, which was co-segregated with CSNB1 in this family. A patient in family B with a compound heterozygous missense mutation (c.3262G>A and c.3250T>C) was detected. No mutations were found in *NYX*. These two identified compound heterozygous mutations were not found in the 96 normal controls. After further examination of the family members, the patients in family A could be diagnosed as eoHM with CSNB1. However due to the limited clinic data, the patient in family B cloud not clearly diagnosed as CSNB1.
- **CONCLUSION:** This study has expanded the mutation spectrum of *TRPM1* for CSNB1 and additional studies are needed to elucidate the association between isolated high myopia and *TRPM1* and *NYX*.

**KEYWORDS:** *TRPM1*, *NYX*, mutations; high myopia; complete congenital stationary night blindness

**DOI:** 10.18240/ijo.2016.10.05


**INTRODUCTION**

Myopia is characterised by the focal point of parallel light rays falling in front of the fovea, resulting in blurred vision [8]. This condition is a common ocular disorder worldwide and is one of the leading causes of legal blindness [9]. Myopia is heterogeneous with respect to ethnicity, with the prevalence of this disease being higher in Asian than in African and Caucasian [14], reaching 70%-90% in certain Asian populations [9]. High myopia (<-6.0 dioptres) can lead to blindness resulting from severe complications of glaucoma, macular degeneration and retinal detachment [8]. The development of myopia was the result of both genetic and environmental factors [19]. Genetic factors are well known to be closely associated with high myopia [10]. However, early-onset high myopia (eoHM), which occurred before school age, is an ideal model for monogenic studies on high myopia because of the rarity of environmental influence on this condition.

Complete congenital stationary night blindness (CSNB1) is one of the syndromes that may accompany high myopia. Congenital stationary night blindness (CSNB) refers to a group of genetically and clinically heterogeneous retinal disorders accompanied by non-progressive night blindness. CSNB has been reported to be associated with more than 360 mutations in 17 genes [11]. According to the pattern of inheritance, CSNB can be divided into X-linked, autosomal recessive and autosomal dominant patterns. CSNB1 is one of the types of CSNB that are characterised by abnormality of the electroretinogram (ERG). The clinical features may vary between different types of CSNB, with CSNB1 often being associated with high myopia, nystagmus, reduced visual acuity, strabismus and a normal fundus appearance, except...
for myopic changes [12-14]. NYX [15-16], TRPM1 [17-19], GRM6 [20], GPR179 [21], and LR173 [22] are the genes responsible for CSNB1, and the products of these genes localise at the dendritic tips of retinal-bipolar cells. Among these pathogenic genes, NYX and TRPM1 play major roles in CSNB1 [23]. Recently, two studies have reported that NYX might be responsible for the development of isolated high myopia [23-24].

Previous studies have shown that transient receptor potential cation channel, subfamily M, member 1 (TRPM1) shows the same localisation and function as mGluR6 and NYX. Additionally, nytalopin, which is encoded by NYX, has been reported to be interacted with TRPM1 [17,25], thus confirming the hypothesis that these proteins form a functional cascade [26]. Based on these results, we hypothesise that TRPM1 might also be associated with high myopia.

The relationship of high myopia with or without CSNB1 and the NYX or TRPM1 gene has been investigated in this study. Two families with eoHM with or without CSNB1 were recruited, and two compound heterozygous mutations in TRPM1 were identified. Further comprehensive examinations and analyses of the available family members were then performed.

SUBJECTS AND METHODS

Subjects Two unrelated families with eoHM (with or without CSNB1) and 96 normal controls were collected from the Department of Ophthalmology at the Central Hospital of Enshi Autonomous Prefecture, Enshi Clinical College, Wuhan University. The criteria for the probands with eoHM were as follows: 1) axial length greater than 26 mm or the refractive error less than -6.0 dioptres; 2) myopia detected before school age; and 3) no other detectable abnormalities in patients other than high myopia via the best visual acuity test, slit-lamp examination and direct ophthalmoscopy. We enrolled the probands from two families; family A, with three patients, and family B, with one patient. The visual acuity test (Topcon KR 8900, Japan), slit-lamp examination (SL-1E, Japan), and direct ophthalmoscopy (Ophthalmoscope, YZ6F, 6 Vision Tech Co., Ltd, China) were conducted in every proband. After mutations in NYX and TRPM1 were detected, a comprehensive ophthalmic examination were conducted the same as previous study [27]. Written informed consent was collected from individuals or family members before the study were collected, which was consistent with the tenets of the Declaration of Helsinki.

Mutation Screening Genomic DNA of the individuals was prepared from peripheral leukocytes. The primers for NYX were designed using Primer3 (Table 1) (http://bioinfo.ut.ee/primer3-0.4.0/), and the primers for TRPM1 were based on the 27 published exons of this gene [18]. Sanger sequencing was used for mutation screening of NYX and TRPM1 as previous reported [27] (Figure 1A). The detected variations were confirmed through a segregation analysis of the available family members and the evaluation of 96 normal controls.

Figure 1 Two compound heterozygous variations in TRPM1 detected in two families with high myopia A: The family pedigrees and the sequences obtained from the two probands and normal controls; B: The cloned sequences of mutations detected in family B as well as normal controls. Circle: Female; Square: Male; Filled symbols: Patients; Arrow: Proband; M: Mild type; +: Variations.
Table 1 Primers used in Sanger sequencing

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size (bp)</th>
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<tr>
<td>TRPM1-1a</td>
<td>CCAGACGCCTCATAATTCCTCATT</td>
<td>GCCCCATGCCCACCCGCAAGAAT</td>
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<tr>
<td>TRPM1-1</td>
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<td>GCACCTCTAGTTTGGTACCCGCTGATTT</td>
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<tr>
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</tr>
<tr>
<td>TRPM1-3</td>
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<td>GCCACAGCCATAGAAGAACAGGACGTTT</td>
<td>352</td>
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<tr>
<td>TRPM1-4</td>
<td>GCCTACTCCCTTCCCTGACAGGAGA</td>
<td>CACAGTGATTTCTCGGGGTGATACAGGATT</td>
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</tr>
<tr>
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<td>GGCTGCAAGGGGAGCTCTGTATCTTTT</td>
<td>335</td>
</tr>
<tr>
<td>TRPM1-6</td>
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<td>CCCCATACGGACGAGGACGATTCCTTTT</td>
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<td>GGCTGCAAGGGGAGCTCTGTATCTTTT</td>
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<tr>
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<td>TRPM1-12</td>
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<td>TRPM1-13</td>
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<td>TRPM1-14</td>
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<td>TRPM1-15</td>
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<td>TRPM1-16</td>
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<td>TRPM1-17</td>
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<tr>
<td>TRPM1-18</td>
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<td>TRPM1-19</td>
<td>GGAGGTGACATATTGTTTATAAAGGTAT</td>
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</table>

The primer sequences used for amplification and the sizes of the PCR products are listed.

Additionally, for the proband of family B, whose family members were not available, clone sequencing was conducted. The polymerase chain reaction products with the compound heterozygous mutations were subcloned into the pMD19-T vector (TaKaRa BIO Japan). Sanger sequencing was then used to detect the variants present in the cloned fragments (Figure 1B).

The variations detected in this study were described according to the currently accepted nomenclature (HGVS: http://www.hgvs.org/). Splice site mutations were predicted using Splice Site Prediction by Neural Network (http://www.fruitfly.org/seq_tools/splice.html), and the potential functional effect of an amino acid substitution resulting from a missense mutation was predicted using the Sorting Intolerant From Tolerant Program (SIFT: http://sift.jcvi.org/) and the Polymorphism Phenotyping algorithm (Polyphen-2: http://genetics.bwh.harvard.edu/pph2/) (Table 2). In addition, the MegAlign programme was adopted to analyse the degree of evolutionary conservation at amino acid positions altered through mutations (Figure 2).

RESULTS
Two eoHM families with or without CSNB1 were recruited. Two novel and compound heterozygous mutations in TRPM1 were detected: one missense mutation
Figure 2 Protein sequence alignment for the nine TRPM1 orthologues showing the variations detected in this study.

Table 2 Variations in TRPM1 identified in two highly myopic families, with or without CSNB1

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Nucleotide change</th>
<th>Amino acid change</th>
<th>State</th>
<th>Computational prediction</th>
<th>Frequency in controls</th>
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<tbody>
<tr>
<td>Family A</td>
<td>c.2594C&gt;T</td>
<td>p.Ala865Val</td>
<td>Hetero</td>
<td>PrD</td>
<td>0/192chromosomes</td>
</tr>
<tr>
<td></td>
<td>c.669+3_669+6delAAGT</td>
<td></td>
<td>Hetero</td>
<td>-</td>
<td>0/192chromosomes</td>
</tr>
<tr>
<td>Family B</td>
<td>c.3262G&gt;A</td>
<td>p.Ala1088Thr</td>
<td>Hetero</td>
<td>PrD</td>
<td>0/192chromosomes</td>
</tr>
<tr>
<td></td>
<td>c.3250T&gt;C</td>
<td>p.Cys1084Arg</td>
<td>Hetero</td>
<td>PrD</td>
<td>0/192chromosomes</td>
</tr>
</tbody>
</table>

Hetero: Hemizygous; PrD: Probably damaging; D: Damaging; -: Not applicable; T: Tolerated; B: Benign.

Table 3 Clinical data for individuals with mutations in TRPM1

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Variations</th>
<th>Gender</th>
<th>Age (a) at BCVA</th>
<th>Spherical refraction (D)</th>
<th>Axial length (mm)</th>
<th>ERG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>OS</td>
<td>OD</td>
</tr>
<tr>
<td>I:1</td>
<td>c.[2594C&gt;T];[0]</td>
<td>M</td>
<td>40</td>
<td>-</td>
<td>1.0</td>
<td>0.50</td>
</tr>
<tr>
<td>I:2</td>
<td>c.[669+3_669+6delAAGT];[0]</td>
<td>F</td>
<td>38</td>
<td>-</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>II:1</td>
<td>c.[669+3_669+6delAAGT];[2594C&gt;T]</td>
<td>M</td>
<td>17</td>
<td>EC</td>
<td>0.7</td>
<td>0.6</td>
</tr>
<tr>
<td>II:2</td>
<td>c.[669+3_669+6delAAGT];[2595C&gt;T]</td>
<td>F</td>
<td>37</td>
<td>EC</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Family B</td>
<td>II:1</td>
<td>c.[3250T&gt;C];[3262G&gt;A]</td>
<td>M</td>
<td>3</td>
<td>EC</td>
<td>0.3</td>
</tr>
</tbody>
</table>

BCVA: Best corrected visual acuity; D: Diopter; OD: Right eye; ERG: Electroretinography; OS: Left eye; M: Male; F: Female; -: No; EC: Early childhood; NA: Not available.

Family A c.2594C>T (p.Ala865Val) and one splicing mutation (c.669+3_669+6delAAGT) in family A; and c.3262G>A (p.Ala1088Thr) and c.3250T>C (p.Cys1084Arg) in family B (Table 2). The mutations identified in family A co-segregated with CSNB1 with high myopia, and these two compound heterozygous variations were not observed in the 96 normal control individuals. Furthermore, all of the detected variants were predicted to be pathogenic and located in conserved region according to the bioinformatics analysis. No variants were found in the NYX gene.

In fact, the two recruited patients were initially diagnosed with eoHM by the same ophthalmologists, and we did not observe any other abnormalities according to the best visual acuity test, slit-lamp examination and direct ophthalmoscopy. After the mutations in TRPM1 were detected, we invited the available family members to return for comprehensive ocular examinations.

A missense mutation, c.2594C>T, that causes a p.Ala865Val substitution and a c.669+3_669+6delAAGT splicing mutation were detected in family A. The proband of family A was a 19-year-old girl exhibiting a spherical equivalent of -7.0 D (OD) and -7.25 D (OS) (Table 3), who stated that she had detected myopia before school age. Her best-corrected visual acuity was 20/32 bilaterally. Fundus changes typical of high myopia can be found in the proband with mutation (Figure 3). But nystagmus, strabismus, and night blindness were not observed in the examination. Initially, the girl was diagnosed with isolated high myopia. After the compound heterozygous mutation was detected, we performed a further comprehensive examination. In the full field ERG of this patient, we detected an extinguished dark-adapted, rod-mediated b-wave response, a deficient electronegative configuration of the combined rod response, absent scotopic oscillatory potentials and a normal response of the cones, typical of CSNB1. Based on the previous clinic data, she can be clearly diagnosed as CSNB1 accompanied with high myopia. The girl's affected brothers exhibited the same signs as the girl according to the same ophthalmologist, but the parents had normal sight. The compound heterozygous missense mutation detected in this family was predicted to affect the function of the encoded protein. Although the c.669+3_669+6delAAGT mutation was located in an intron, this mutation is predicted to affect the splicing of TRPM1 according to the Berkeley Drosophila Genome Project (BDGP: http://www.fruitfly.org/seq_tools/splice.html).

Furthermore, this novel compound heterozygous mutation segregated with CSNB1 with high myopia. Affected proband in family B harbour two deleterious...
mutations in exon 24, leading to a p.Ala1088Thr substitution, and c.3250T>C, causing a p.Cys1084Arg substitution. The bilateral sphere refraction of the 3-year-old proband was -5.75 D (OD) and -5.50 D (OS). Strabismus was detected in this individual, but nystagmus was not found by the ophthalmologist. According to his parents, the proband was afraid of playing alone in dim environments. Unfortunately, this patient's ERG was unavailable. Therefore, we could not clearly diagnose whether he had high myopia with or without CSNB1. Because DNA samples from his family members were unavailable, the compound heterozygous mutations detected in this boy were confirmed via clone sequencing.

DISCUSSION

In the present study, we recruited two eoHM families (with or without CSNB1) to find out the relationship between TRPM1, NYX and eoHM. Two novel compound heterozygous variations were detected in the TRPM1 gene, and no variants were found in the NYX gene. According to the applied online tools, these two compound heterozygous variants in TRPM1 were predicted to be damaging. Furthermore, these two changes were highly conserved in nine spaces. One of the two compound heterozygous variants was detected in a highly myopic Chinese family with CSNB1. The family with the other mutation cannot be clearly diagnosed as CSNB1 due to the limited clinical data because of the limited data. To our knowledge, this is the first study to reveal a pathogenic role of TRPM1 in CSNB1 in Chinese patients. However, the evidence of an association between isolated high myopia and the TRPM1 and NYX genes were not illustrated, and further studies are needed to address this issue. TRPM1 is a member of the TRPML subfamily of TRP channels. The TRPM1 gene is located at chr15q13-q14 and contains 27 exons. This protein was initially identified as a potential suppressor of tumour metastasis \[28\]. TRPM1 immunoreactivity (IR) was observed on the dendrites of ON-bipolar cells invaginating the synaptic terminals of rods and cones near the synaptic ribbon. Defects in the TRPM1 gene, which encodes proteins involved in signal transmission from photoreceptors to adjacent bipolar cells and in the photo transduction pathway, could induce CSNB1 \[29\]. Many studies have reported that mutations in TRPM1 account for half of the autosomal recessive CSNB1 cases in Caucasian and Japanese population \[17-18,20\]. However, the importance of TRPM1 in CSNB1 has not been demonstrated for the Chinese population. TRPM1 localises to and functions in the same intracellular location as mGluR6 and NYX, indicating that these proteins form a functional cascade \[20\]. Mutations in NYX have been associated with isolated high myopia in previous studies \[23-24\], and we hypothesised that mutations in TRPM1 might also be responsible for high myopia. We recruited patients with eoHM (with or without CSNB1) to find out the relationship between high myopia (with or without CSNB1) and the NYX and TRPM1 genes. In this study, the initial diagnosis of all probands was isolated eoHM, and we conducted a further comprehensive examination in these patients after the compound heterozygous mutations in TRPM1 were detected. According to the FF-ERG results and other combined signs, we were able to diagnose individuals in family A with high myopia with CSNB1. EoHM is commonly accompanied by other ocular or systemic diseases, such as retinitis pigmentosa \[31-34\], CSNB1 \[22,31-37\], Stickler syndrome \[34-40\], Marfan syndrome \[41-42\],...
Knobloch syndrome\(^{[30]}\), and Cohen syndrome\(^{[44]}\). Prior to the present study, a number of previous studies indicated that eoHM with associated ocular or systemic conditions could account for half of all patients with eoHM \(^{[46-47]}\). Recently, according to the whole-exome sequencing of 298 patients with eoHM, it was found that one-fourth of these individuals showed potential pathogenic mutations in RetNet genes \(^{[10]}\). Thus, eoHM might constitute a significant clue for other systemic diseases associated with eoHM, such as CSNB1, indicating the importance of clinicians paying attention to the combined signs of high myopia to distinguish isolated high myopia and syndromic high myopia, such as CSNB1. No full field ERG was available for family B, and we could not definitively diagnose isolated eoHM in this family. The identified compound heterozygous mutations (c.3262G>A and c.3250T>C) in TRPM1 were responsible for isolated high myopia or CSNB1 with high myopia. However, due to the limited number of samples included in our study, the associations between isolated high myopia and \(NFX\) and \(TRPM1\) could not be evaluated in detail, and further studies should be conducted.

In this study, we have expanded the spectrum of \(TRPM1\) for autosomal recessive CSNB1, but powerful evidence of an association of \(TRPM1\) with high myopia was not been found out. Further evidence is needed to reveal the roles of \(NFX\) and \(TRPM1\) in isolated high myopia.

**ACKNOWLEDGEMENTS**

In this study, we are thankful to all of the patients and controls for participation. Qing-Jiong Zhang, Department of Ophthalmic Genetics & Molecular Biology, Zhongshan Ophthalmic Center, Sun Yat-sen University, had given us helpful guidance.

**Foundation:** Supported by the National Nature Science Foundation of China (No.81362138).

**Conflicts of Interest:** Zhou L, None; Li T, None; Xing YQ, None; Li Y, None; Wu QS, None; Zhang MJ, None.

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