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

Lin Zhou[1], Tuo Li[1], Yi-Qiao Xing[2], Yin Li[1], Qing-Song Wu[1], Mao-Ju Zhang[1]

[1]Department of Ophthalmology, the Central Hospital of Enshi Autonomous Prefecture, Enshi Clinical College of Wuhan University, Enshi 445000, Hubei Province, China
[2]Department of Ophthalmology, Remin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China
Correspondence to: Tuo Li. Department of Ophthalmology, the Central Hospital of Enshi Autonomous Prefecture, Enshi Clinical College of Wuhan University, Enshi 445000, Hubei Province, China. 13986840088@139.com

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

INTRODUCTION

Myopia is characterised by the focal point of parallel light rays falling in front of the fovea, resulting in blurred vision [1]. This condition is a common ocular disorder worldwide and is one of the leading causes of legal blindness[2]. Myopia is heterogeneous with respect to ethnicity, with the prevalence of this disease being higher in Asian than in African and Caucasian [3-4], reaching 70% -90% in certain Asian populations[5]. High myopia (<-6.0 dioptres) can lead to blindness resulting from severe complications of glaucoma, macular degeneration and retinal detachment [6]. The development of myopia was the result of both genetic and environmental factors [7-9]. 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
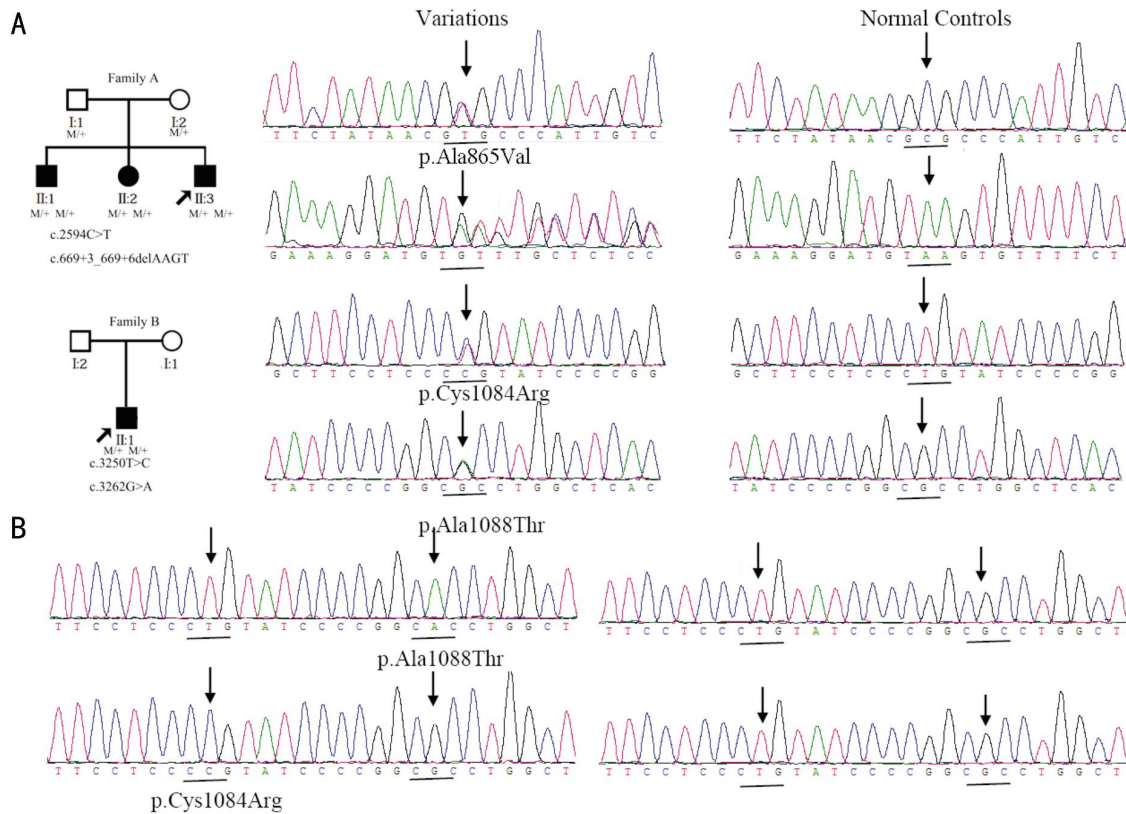
Figure 1 Two compound heterozygous variations in *TRPM1* detected in two families with high myopia A: The family pedigrees and the sequences obtained from the two probands and normal controls; B: The cloned sequences of mutations detected in family B as well as normal controls. Circle: Female; Square: Male; Filled symbols: Patients; Arrow: Proband; M: Mild type; +: Variations.

for myopic changes [12-14]. *NYX* [15-16], *TRPM1* [17-19], *GRM6* [20], *GPR179* [21], and *LRIT3* [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 [12]. 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, nyctalopin, 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 abnormities 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 (Opthalmoscope, YZ6F, 6 6 Vision Tech Co., LI, 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.

Table 1 Primers used in Sanger sequencing

Exon	Forward primer	Reverse primer	Product size (bp)
TRPM1-1a	CCAGACGCCCAAATCCTTCCCATTT	GCCCCATGCCCACCCAGCACAGTTT	584
TRPM1-1	CTCAATTATGCAAACCCTGCTGACATTT	GCACCTGAGTTTGTCCACGCTTGAGTTT	466
TRPM1-2	GGGCAGACATATTGTTTTATAAAGGTAT	CCTTCTGGACTCCTCTTTCTGCCTCTTA	279
TRPM1-3	GGGAAAGGGGGAGATTGTTGTAGAAA	GCCACAGCCATGAAGAAGACCAGGTAT	352
TRPM1-4	GCCTCACTCCCCTTTGACACGAGAA	CACAGTGAGTTCTGGGTGGTACATTGATTA	339
TRPM1-5	GGGAATGTAGGGACTCAGGGCAAGT	GGCTGCAAGGGAGCTCTCTTATTATTCTTA	335
TRPM1-6	GGCAGGGTAGAGATGTGAGAATAAGTTTTA	CCCAGGAGGACTGCGTGCCTTT	332
TRPM1-7	CCAGATAGAACATCCCCCAAGTCGTAAT	GGAGATCTAGCAAATCTTCCTGATTTAA	423
TRPM1-8	CCTCCCACAGCAAAGTCTCAAATCAAA	CCGGACTAAAATATGAATAACCCTGTCAT	334
TRPM1-9	CCCCATATCTCCTTCTTGTTTTCAACTT	CCAACATCAGAGGGACAGGAGTCACCAT	174
TRPM1-10	CCAGGCGTGGAGTGAAAAATCATT	GGGAGAACATACTAATAGGTCACTTGTACAAA	217
TRPM1-11	GCACATACAGAGGTGGGAGGGTCAAT	GGTGGGACTGAAGCAAGGACAAGAA	352
TRPM1-12	GGAATCTGGCGTTCGGAATACCCTTT	GCACAATATGCACCAGTGACAAACACATT	313
TRPM1-13	GGCCTCAAACTACTGACCTCAAGTGAT	CCTGTGCCTGGAATAATAATACATTTTGAAA	283
TRPM1-14	CCACTTACCCCCTAGACGTGTGATGAA	CTCTGAACCGGCCAAGTTGTTGAAAA	309
TRPM1-15	GACAGAAGAAGAGTTTCTATATTAAAGCACTT	GGGCTATGTATATTTGACCAGGATATTAT	267
TRPM1-16	GGAGTGGAAATGTAAGATGTACTTCAGAA	CCACCCCTCCCTGCAGAGACAAGTA	504
TRPM1-17	GTCCTTGGTGGATATGCCTGTCTAAGAAA	CCTCAGGGGGTTGCTAGAAGGAATAAAT	426
TRPM1-18-19	GGGATAACATTCCAGGGCTCCTAGGTT	CCAACATATCAAAGCATTCAAAATATACTGAAT	640
TRPM1-20	CCCAAACACGGCTAACAGCTTGTCTTT	CGCCTGGCCCAACATGCATAATTT	272
TRPM1-21	GAATGTTCCATCAGTCTTATTGTTCCAA	CCCAAGGCCTGTTCTTATGTCCTAACTA	414
TRPM1-22	CCCACAAGCCAAGACAGTGAGAGACAAA	GCCTCAGAAGCAGGGAAGAGAATCTTA	265
TRPM1-23	CCTCCCCTCCTGTCAAAACAAAACAAA	CTGGCACCAAAAAACAAGAGAAGTATTT	201
TRPM1-24	GCTGGGGCTACAGAGTTTATTTTTT	CTGCAAAAATTGGATCTACTTAAAAACCCTAAT	446
TRPM1-25	GGCCATCTCATTGAGTATTTTCACTTAAAT	CACACGAGCAAGTAGTTGAGTGAGATTT	409
TRPM1-26	GGAGTGGCGGAGAAGAGAGCTTAATTAAA	CAGGAGAACTCGGAGTGCATGTTTAAAT	356
TRPM1-27	GGGGGATTGTGAAGCTTGTAAATATACTCAAA	CCTGTCCATGTATCACATCTGTTTTAT	751
TRPM1-27-1a	GGAAGATGATGAAAGACAGACAGACTCTAA	GACACCCATTAGTGGTTCTGACTGTTAAA	782
NYX-1	TGGGGAGCTTCTGATTTTCTGTTG	ATCCCCACCACCTGCTGTTTTCTT	443
NYX-2A	GCGGGTGTCTTAGGTGGATA	GCGTGATGAAGGACAGGTTG	472
NYX-2B	GACCTTTGGCTGACGGTTG	TTGTCGTTGAGCAGCAGATG	756
NYX-2C	CTTCGACAACCTGTTCCGC	CTCCATCCAGTCCCTCAGC	559
NYX-2D	CTCTACCTGGACCGCAACA	TTTCACCTCTGCCCTCCATT	689

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

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

Sample ID	Nucleotide change	Amino acid change	State	Computational prediction		Frequency in controls
				Polyphen	SIFT	
Family A	c.2594C>T	p.Ala865Val	Hetero	PrD	D	0/192chromosomes
	c.669+3_669+6delAAGT	-	Hetero	-	-	0/192chromosomes
Family B	c.3262G>A	p.Ala1088Thr	Hetero	PrD	T	0/192chromosomes
	c.3250T>C	p.Cys1084Arg	Hetero	PrD	D	0/192chromosomes

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

Table 3 Clinical data for individuals with mutations in *TRPM1*

Patient ID		Variations	Gender	Age (a) at		BCVA		Spherical refraction (D)		Axial length (mm)		ERG	
				Exam	Onset	OD	OS	OD	OS	OD	OS	Rod	Cone
Family A	I:1	c.[2594C>T];[0]	M	40	-	1.0	1.0	-0.50	0.25	NA	NA	Normal	Normal
	I:2	c.[669+3_669+6delAAGT];[0]	F	38	-	1.2	1.0	0.25	0.25	NA	NA	NA	NA
	II:1	c.[669+3_669+6delAAGT];[2594C>T]	M	17	EC	0.7	0.6	-7.00	-6.25	NA	NA	Extinguished	Normal
	II:2	c.[669+3_669+6delAAGT];[2595C>T]	F	37	EC	1.0	1.0	-7.00	-7.25	27.49	27.67	Extinguished	Normal
	II:3	c.[669+3_669+6delAAGT];[2596C>T]	M	39	NA	1.0	1.0	-9.25	-9.25	28.37	28.28	Extinguished	Normal
Family B	II:1	c.[3250T>C];[3262G>A]	M	3	EC	0.3	0.3	-5.75	-5.50	NA	NA	NA	NA

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.
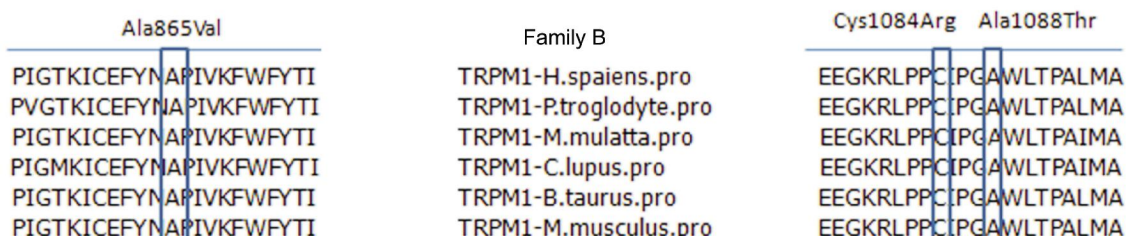

Figure 2 Protein sequence alignment for the nine *TRPM1* orthologues showing the variations detected in this study.

c.2594C>T (p.Ala865Val) and one splicing mutation (c.669+3_669+6delAAGT) in family A; and c3262G>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.

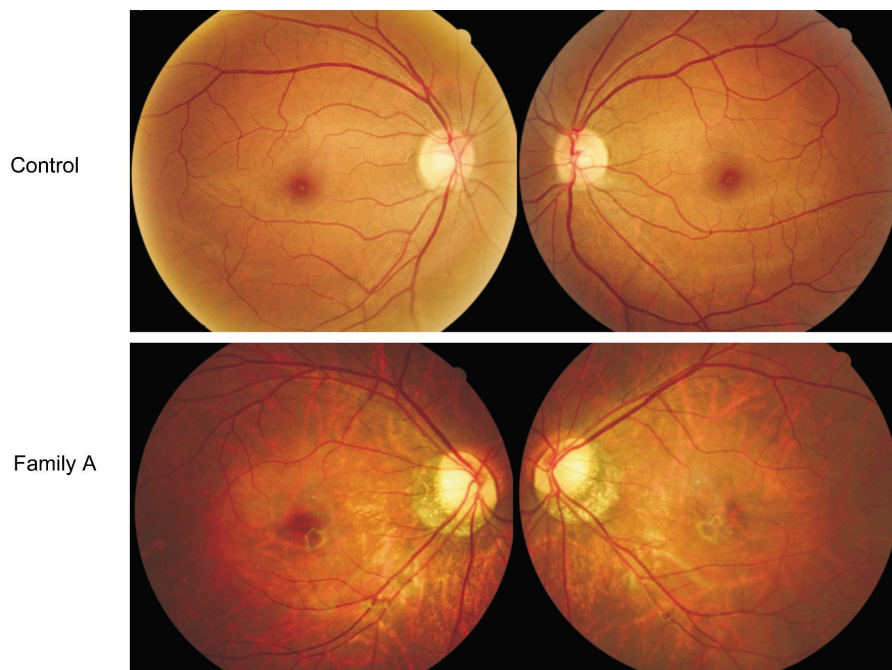Affected proband in family B harboured two deleterious

Figure 3 Fundus photographs form the proband of family A and normal controls The "tigroid" appearance of the posterior retina and the optic nerve head crescent, which typical of high myopia were observable in patients in family A and B.

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 TRPM 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 [17-18,30]. 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 [26]. 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 [22,35-37], Stickler syndrome[38-40], Marfan syndrome [41-42],

Knobloch syndrome[43], 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 [45-47]. 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 *NYX* 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 *NYX* and *TRPM1* in isolated high myopia.

ACKNOWLEDGEMENTS

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

REFERENCES

1 Kempen JH, Mitchell P, Lee KE, Tielsch JM, Broman AT, Taylor HR, Ikram MK, Congdon NG, O'Colmain BJ; Eye Diseases Prevalence Research Group. The prevalence of refractive errors among adults in the United States, Western Europe, and Australia. *Arch Ophthalmol* 2004;122 (4): 495-505.

2 Pan CW, Ramamurthy D, Saw SM. Worldwide prevalence and risk factors for myopia. *Ophthalmic Physiol Opt* 2012;32(1):3-16.

3 Lin LL, Chen CJ, Hung PT, Ko LS. Nation-wide survey of myopia among schoolchildren in Taiwan, 1986. *Acta Ophthalmologica Suppl* 1988;185: 29-33.

4 Wilson A, Woo G. A review of the prevalence and causes of myopia. *Singapore Med J* 1989;30(5):479-484.

5 Ahmed I, Rasool S, Jan T, Qureshi T, Naykoo NA, Andrabi KI. TGIF1 is a potential candidate gene for high myopia in ethnic Kashmiri population. *Curr Eye Res* 2014;39(3):282-290.

6 Fredrick DR. Myopia. *BMJ* 2002;324(7347):1195-1199.

7 Stambolian D. Genetic susceptibility and mechanisms for refractive error. *Clin Genet* 2013;84(2):102-108.

8 Wojciechowski R. Nature and nurture: the complex genetics of myopia and refractive error. *Clin Genet* 2011;79(4):301-320.

9 Young TL, Metlapally R, Shay AE. Complex trait genetics of refractive error. *Arch Ophthalmol* 2007;125(1):38-48.

10 Sun W, Huang L, Xu Y, Xiao X, Li S, Jia X, Gao B, Wang P, Guo X, Zhang Q. Exome sequencing on 298 probands with early-onset high myopia: approximately one-fourth show potential pathogenic mutations in RetNet genes. *Invest Ophthalmol Vis Sci* 2015;56(13):8365-8372.

11 Zetiz C, Robson AG, Audo I. Congenital stationary night blindness: an analysis and update of genotype-phenotype correlations and pathogenic mechanisms. *Prog Retin Eye Res* 2015;45:58-110.

12 Bijveld MM, Florijn RJ, Bergen AA, van den Born LI, Kamermans M, Prick L, Riemslag FC, van Schooneveld MJ, Kappers AM, van Genderen MM. Genotype and phenotype of 101 Dutch patients with congenital stationary night blindness. *Ophthalmology* 2013;120(10):2072-2081.

13 Pieh C, Simonsz-Toth B, Gottlob I. Nystagmus characteristics in congenital stationary night blindness (CSNB). *Br J Ophthalmol* 2008;92(2): 236-240.

14 Sergouniotis PI, Robson AG, Li Z, Devery S, Holder GE, Moore AT, Webster AR. A phenotypic study of congenital stationary night blindness (CSNB) associated with mutations in the GRM6 gene. *Acta Ophthalmol* 2012;90(3):e192-e197.

15 Bech-Hansen NT, Naylor MJ, Maybaum TA, Sparkes RL, Koop B, Birch DG, Bergen AA, Prinsen CF, Polomeno RC, Gal A, Drack AV, Musarella MA, Jacobson SG, Young RS, Weleber RG. Mutations in NYX, encoding the leucine-rich proteoglycan nyctalopin, cause X-linked complete congenital stationary night blindness. *Nat Genet* 2000;26 (3): 319-323.

16 Pusch CM, Zeitz C, Brandau O, Pesch K, Achatz H, Feil S, Scharfe C, Maurer J, Jacobi FK, Pinckers A, Andreasson S, Hardcastle A, Wissinger B, Berger W, Meindl A. The complete form of X-linked congenital stationary night blindness is caused by mutations in a gene encoding a leucine-rich repeat protein. *Nat Genet* 2000;26(3):324-327.

17 Audo I, Kohl S, Leroy BP, *et al.* TRPM1 is mutated in patients with autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet* 2009;85(5):720-729.

18 Li Z, Sergouniotis PI, Michaelides M, Mackay DS, Wright GA, Devery S, Moore AT, Holder GE, Robson AG, Webster AR. Recessive mutations of the gene TRPM1 abrogate ON bipolar cell function and cause complete congenital stationary night blindness in humans. *Am J Hum Genet* 2009;85 (5):711-719.

19 van Genderen MM, Bijveld MM, Claassen YB, Florijn RJ, Pearring JN, Meire FM, McCall MA, Riemslag FC, Gregg RG, Bergen AA, Kamermans M. Mutations in TRPM1 are a common cause of complete congenital stationary night blindness. *Am J Hum Genet* 2009;85(5):730-736.

20 Dryja TP, McGee TL, Berson EL, Fishman GA, Sandberg MA, Alexander KR, Derlacki DJ, Rajagopalan AS. Night blindness and abnormal cone electroretinogram ON responses in patients with mutations in the GRM6 gene encoding mGluR6. *Proc Natl Acad Sci U S A* 2005;102 (13):4884-4889.

21 Audo I, Bujakowska K, Orhan E, *et al.* Whole-exome sequencing identifies mutations in GPR179 leading to autosomal-recessive complete congenital stationary night blindness. *Am J Hum Genet* 2012;90 (2): 321-330.

22 Zeitz C, Jacobson SG, Hamel CP, *et al.* Whole-exome sequencing identifies LRIT3 mutations as a cause of autosomal-recessive complete

congenital stationary night blindness. *Am J Hum Genet* 2013;92(1):67–75.

23 Zhang Q, Xiao X, Li S, Jia X, Yang Z, Huang S, Caruso RC, Guan T, Sergeev Y, Guo X, Hejtmancik JF. Mutations in NYX of individuals with high myopia, but without night blindness. *Mol Vis* 2007;13:330–336.

24 Yip SP, Li CC, Yiu WC, Hung WH, Lam WW, Lai MC, Ng PW, Fung WY, Chu PH, Jiang B, Chan HH, Yap MK. A novel missense mutation in the NYX gene associated with high myopia. *Ophthalmic Physiol Opt* 2013; 33(3):346–353.

25 Morgans CW, Ren G, Akileswaran L. Localization of nyctalopin in the mammalian retina. *Eur J Neurosci* 2006;23(5):1163–1171.

26 Klooster J, Blokker J, Ten Brink JB, Unmehopa U, Fluiter K, Bergen AA, Kamermans M. Ultrastructural localization and expression of TRPM1 in the human retina. *Invest Ophthalmol Vis Sci* 2011;52(11):8356–8362.

27 Zhou L, Li T, Song X, Li Y, Li H, Dan H. NYX mutations in four families with high myopia with or without CSNB1. *Mol Vis* 2015;5 (21): 213–223.

28 Morgans CW, Brown RL, Duvoisin RM. TRPM1: the endpoint of the mGluR6 signal transduction cascade in retinal ON–bipolar cells. *BioEssays* 2010;32(7):609–614.

29 Malaichamy S, Sen P, Sachidanandam R, Arokiasamy T, Lancelot ME, Audo I, Zeitz C, Soumittra N. Molecular profiling of complete congenital stationary night blindness: a pilot study on an Indian cohort. *Mol Vis* 2014; 20:341–351.

30 Nakanmura M, Sanuki R, Yasuma TR, Onishi A, Nishiguchi KM, Koike C, Kadowaki M, Kondo M, Miyake Y, Furukawa T. TRPM1 mutations are associated with the complete form of congenital stationary night blindness. *Mol Vis* 2010;16:425–437.

31 Flaxel CJ, Jay M, Thiselton DL, Nayudu M, Hardcastle AJ, Wright A, Bird AC. Difference between RP2 and RP3 phenotypes in X linked retinitis pigmentosa. *Br J Ophthalmol* 1999;83(10):1144–1148.

32 Jayasundera T, Branham KE, Othman M, Rhoades WR, Karoukis AJ, Khanna H, Swaroop A, Heckenlively JR. RP2 phenotype and pathogenetic correlations in X–linked retinitis pigmentosa. *Arch Ophthalmol* 2010;128 (7):915–923.

33 Yokoyama A, Maruiwa F, Hayakawa M, Kanai A, Vervoort R, Wright AF, Yamada K, Niikawa N, Na ō i N. Three novel mutations of the RPGR gene exon ORF15 in three Japanese families with X–linked retinitis pigmentosa. *Am J Med Genet* 2001;104(3):232–238.

34 Jin ZB, Liu XQ, Hayakawa M, Murakami A, Nao-i N. Mutational analysis of RPGR and RP2 genes in Japanese patients with retinitis pigmentosa: identification of four mutations. *Mol Vis* 2006;12:1167–1174.

35 Xiao X, Jia X, Guo X, Li S, Yang Z, Zhang Q. CSNB1 in Chinese families associated with novel mutations in NYX. *J Hum Genet* 2006;51(7): 634–640.

36 Hemara–Wahanui A, Berjukow S, Hope CI, Dearden PK, Wu SB, Wilson–Wheeler J, Sharp DM, Lundon–Treweek P, Clover GM, Hoda JC, Striessnig J, Marksteiner R, Hering S, Maw MA. A CACNA1F mutation identified in an X–linked retinal disorder shifts the voltage dependence of Cav1.4 channel activation. *Proc Natl Acad Sci U S A* 2005;102 (21): 7553–7558.

37 Xu X, Li S, Xiao X, Wang P, Guo X, Zhang Q. Sequence variations of GRM6 in patients with high myopia. *Mol Vis* 2009;15:2094–2100.

38 Ahmad NN, McDonald–McGinn DM, Zackai EH, Knowlton RG, LaRossa D, DiMascio J, Prockop DJ. A second mutation in the type II procollagen gene (COL2AI) causing stickler syndrome (arthro–ophthalmopathy) is also a premature termination codon. *Am J Hum Genet* 1993;52(1):39–45.

39 Annunen S, Körkkö J, Czarny M, *et al* Splicing mutations of 54–bp exons in the COL11A1 gene cause Marshall syndrome, but other mutations cause overlapping Marshall/stickler phenotypes. *Am J Hum Genet* 1999;65 (4):974–983.

40 van Camp G, Snoeckx RL, Hilgert N, van den Ende J, Fukuoka H, Wagatsuma M, Suzuki H, Smets RM, Vanhoenacker F, Declau F, Van de Heyning P, Usami S. A new autosomal recessive form of stickler syndrome is caused by a mutation in the COL9A1 gene. *Am J Hum Genet* 2006;79 (3):449–457.

41 Dietz HC, McIntosh I, Sakai LY, Corson GM, Chalberg SC, Pyeritz RE, Francomano CA. Four novel FBN1 mutations: significance for mutant transcript level and EGF–like domain calcium binding in the pathogenesis of Marfan syndrome. *Genomics* 1993;17(2):468–475.

42 Montgomery RA, Geraghty MT, Bull E, Gelb BD, Johnson M, McIntosh I, Francomano CA, Dietz HC. Multiple molecular mechanisms underlying subdiagnostic variants of Marfan syndrome. *Am J Hum Genet* 1998;63(6): 1703–1711.

43 Suzuki OT, Sertié AL, Der Kaloustian VM, Kok F, Carpenter M, Murray J, Czeizel AE, Kliemann SE, Rosemberg S, Monteiro M, Olsen BR, Passos–Bueno MR. Molecular analysis of collagen XVIII reveals novel mutations, presence of a third isoform, and possible genetic heterogeneity in Knobloch syndrome. *Am J Hum Genet* 2002;71(6):1320–1329.

44 Douzgou S, Petersen MB. Clinical variability of genetic isolates of Cohen syndrome. *Clin Genet* 2011;79(6):501–506.

45 Marr JE, Halliwell–Ewen J, Fisher B, Soler L, Ainsworth JR. Associations of high myopia in childhood. *Eye* 2001;15(Pt 1):70–74.

46 Logan NS, Gilmartin B, Marr JE, Stevenson MR, Ainsworth JR. Community–based study of the association of high myopia in children with ocular and systemic disease. *Optom Vis Sci* 2004;81(1):11–13.

47 Bansal AS, Hubbard GB, 3rd. Peripheral retinal findings in highly myopic children < or =10 years of age. *Retina (Philadelphia, Pa)* 2010;30(4 Suppl):S15–S19.