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Novel mutation in *SCO2* of patients with high myopia from Enshi Tujia and Miao Autonomous Prefecture of China

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中国恩施土家族苗族自治州高度近视患者 *SCO2* 基因新突变

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摘要

目的:探讨 *SCO2*(OMIM 604272)基因在恩施土家族苗族自治州高度近视患者中的致病性变异。

方法:共招募 384 例高度近视患者,其中至少一眼球镜度数≤-6.00 D,且眼轴长度≥26.00 mm。应用苯酚-氯仿法从 5 mL 外周静脉血中提取 DNA。通过 Sanger 测序以鉴定 *SCO2* 第 2 外显子的致病性变异。运用计算机预测软件对检测到的变异进行评估。来自同一地区的 288 名健康人群作为正常对照。

结果:共检测出 7 个突变位点,分别为 4 个同义突变(c.201C>T/p.=,c.576C>T/p=,c.633A>C/p.=,c.780T>C/p.=.),2 个错义突变(c.187A>G/p.Ile63Val,c.59G>C/p.Arg20Pro)和 1 个无义突变(c.544C>T/p.Gln182*)。通过 PolyPhen2、SIFT 和 Provean 软件预测,两个错义突变没有致病性。新的无义突变(c.544C>T/p.Gln182*)在 1000G 中未被发现,在 288 个正常对照中也未被发现。Variant Taster 预测该无义突变位点是保守的。

结论:新发现的无义突变可能是我们研究的高度近视患者的致病原因。*SCO2* 与高度近视相关,而这批高度近视人群中 *SCO2* 基因突变的发生率低至 1/384;该无义突变可能是中国恩施土家族苗族自治州高度近视的一种罕见变异。

关键词:高度近视;*SCO2*;Sanger 测序;突变;无义突变

Abstract

• AIM: To evaluate the pathogenic variants of the *SCO2* (OMIM 604272) gene in patients with high myopia from Enshi Tujia and Miao Autonomous Prefecture of China.

• METHODS: A total of 384 patients with high myopia whose spherical refractive error was ≤ -6.00 D and whose axial length was ≥ 26.00 mm in at least one eye were recruited. DNA was extracted by the phenol-chloroform method from 5 mL of peripheral venous blood. Sanger sequencing was performed to identify pathogenic variants in exon 2 of *SCO2*. The detected variants were evaluated *via in silico* prediction software. A total of 288 people from the same district were included as the normal control cohort.

• RESULTS: Seven variants were detected, namely, four synonymous variants (c.201C>T/p.=, c.576C>T/p.=, c.633A>C/p.=, c.780T>C/p.=.), two missense variants (c.187A>G/p.Ile63Val, c.59G>C/p.Arg20Pro) and one nonsense variant (c.544C>T/p.Gln182*). The two missense variants were not damaging, as predicted by PolyPhen2, SIFT and Provean. The novel nonsense variant (c.544C>T/p.Gln182*) cannot be found in the 1000 Genomes Project and was not identified in 288 normal controls. Variant Taster suggested that the nonsense variant site was conserved.

• CONCLUSION: The newly identified nonsense mutation may be responsible for high myopia of the patients in our cohort. *SCO2* is associated with high myopia, while the incidence of *SCO2* variants in high myopia in this cohort was as low as 1/384; the nonsense mutation may be a scarce variant of high myopia in the Enshi Tujia and Miao Autonomous Prefecture of China.

• KEYWORDS: high myopia; *SCO2*; Sanger sequencing; mutation; nonsense mutation

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INTRODUCTION

Myopia is a worldwide disease characterized by refractive error. Parallel light is focused in front of the retina, which leads to blurred vision. Patients with myopia will have their symptoms alleviated or entirely corrected by wearing glasses or undergoing surgeries. However, in some cases, especially when the refractive error is less than -6.00 diopters sphere (DS), complications occur so that vision sharply declines and can never be corrected[1-2]. Patients with high myopia are susceptible to cataracts, glaucoma, maculopathy, fundus haemorrhage, posterior staphyloma, retinal detachment and so on, which negatively affect the quality of life of the patients[3].

The prevalence of myopia varies among different areas, among which Asian countries have the highest prevalence of myopia[4]. Epidemiological studies have demonstrated that both environmental and genetic factors play a role in the development of myopia[5]. Environmental factors such as lack of outdoor time, high education level, and too much close-up work have been proven to have negative effects on myopia[6]. Twin studies and pedigree studies have shown that myopia, especially early onset high myopia, is affected more by genetic susceptibility than environmental factors. Linkage analysis and genome-wide association studies have identified more than 30 loci that may be associated with high myopia. Moreover, due to the great help of next-generation sequencing and bioinformatics, variants of several genes have been identified to be the underlying cause of high myopia, such as *ZNF*644 (OMIM 614159), *SCO*2 (OMIM 604272), *SLC*39*A*5 (OMIM 608730), *LRPAP*1 (OMIM 104225), *CTSH* (OMIM 116820), *P3H*2 (OMIM 610341), *CCDC*111 (OMIM 615421) and *P4HA*2 (OMIM 600608)[7]. The *SCO*2 gene was identified as the causative gene of an autosomal dominant high myopia pedigree from Europe. A nonsense variant c.157C>T (p.Gln53*) was found to cosegregate with high myopia within the family[8]. Afterwards, variant screening of *SCO*2 was conducted among a European high myopia cohort (140 cases), a Chinese early-onset high myopia cohort (298 cases)[9] and a Japanese extreme myopia cohort (101 cases)[10]. In this study, we attempted to enlarge the variant spectrum of *SCO*2 by evaluating the variant in a cohort of 384 high myopia patients from the Enshi Tujia and Miao district of Hubei Province in China.

MATERIALS AND METHODS

Study Subjects Subjects were recruited from Enshi Tujia and Miao Autonomous Prefecture, Hubei Province, with spherical refractive errors ≤-6.00 dioptres and axial lengths ≥26.00 mm. A total of 384 patients with high myopia and 288 normal controls were recruited in the present study. Each participant signed an informed consent form, juvenile participants had guardians sign informed consent forms. This study was conducted in compliance with the principles of the Declaration of Helsinki and the Association for Research in Vision and Ophthalmology statement for research involving human subjects. This study was approved by the ethics committee of the Central Hospital of Enshi Tujia and Miao Autonomous Prefecture (No.2023-080-001). After obtaining written consent, 5 mL of peripheral venous blood was collected for the extraction of DNA, a procedure described in our previous study[11]. All of the patients received a comprehensive ophthalmic examination including optometry (Topcon KR-8000, Paramus, Japan), uncorrected visual acuity, best corrected visual acuity (BCVA), axial length (IOL master V5.0, Zeiss, Germany, or A-scan, Souer SW-2100, China), corneal curvature (IOL master V5.0, Zeiss, Germany), and fundus examination (Canon CR-2, Japan). Moreover, population-matched subjects who had a refractive error between +0.5 DS and -0.5 DS and naked vision ≥1.0 were enrolled as normal controls. None of the normal controls had family history of high myopia or had previously undergone any kinds of ophthalmic surgery.

Polymerase Chain Reaction and Resequencing We extracted genomic DNA using the phenol-chloroform method. Polymerase chain reaction (PCR) primers for exon 2 of *SCO*2 were designed by the online software Primer 3 (http://primer3.ut.ee/), as shown in Table 1. For each sample, PCR reaction was performed with 40 ng of genomic DNA, 10 μL of 2X GC-rich buffer I (Shanghai Bioengineering, Shanghai, China), 1.6 μL of a dNTP mixture (10 mM, Shanghai Bioengineering, China), 1.5 units of rTaq DNA polymerase (Shanghai Bioengineering, Shanghai, China), 0.4 μL (10 μmol/L) of each primer and ddH2O to a final volume of 20 μL. The PCR procedure was 95℃ for 30s of denaturation, 65℃ for 30s of annealing, and 72℃ for 40s of extension for 15 cycles (0.5℃ touchdown every cycle). After the PCR reaction, the amplified product is loaded into an agarose gel for electrophoresis to verify whether the PCR reaction is successful. Sequencing was performed on an ABI 3130xl genetic analyser (Applied Biosystem, Foster City, CA) with a BigDye Terminator cycle sequencing kit version 3.1.

Variant Analysis Sequences were analysed by Lasergene DNAStar 7.1 software. The reference sequences were downloaded from NCBI (NCBI: http://www.ncbi.nlm.nih.gov/) (NM_005138.2). Variants were re-sequenced with both forward and reverse primers. All the variants detected were submitted to functional prediction by the online software

Table 1 Polymerase chain reaction and sequencing primers for exon 2 of *SCO*2

Name	Forward	Reverse	Product size (base pairs)
*SCO*2-1	CAGGTGTGGATGTTTGGTGG	ATGTCAGGGCAGTGAGTGAA	599
*SCO*2-2	ATGTCAGGGCAGTGAGTGAA	TGGTCCACGATGTAGTCCTG	410
*SCO*2-3	GCAGCCTGTCTTCATCACTG	CCCGGCCTAATAAAGCAGTG	491

PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2/), SIFT (http://sift.jcvi.org) and PROVEAN (http://provean.jcvi.org/index.php). We obtained the allele frequency of the variants from the 1000 Genome Project (1000G: http://browser.1000genomes.org/), Exome Variant Server (EVS: http://evs.gs.washington.edu/EVS/), Exome Aggregation Consortium (ExAC: http://exac.broadinstitute.org/) and Genomic Evolutionary Rate Profiling score (GERP++, https://bio.tools/gerp).

Structure of Proteins The 3D models of wild-type (Swiss-Prot accession O43819) and mutant protein of *SCO2* were generated by using the SWISS-MODEL online server (https://swissmodel.expasy.org)[12]. The PyMOL software (https://pymol.org/2/) was used to visualize proteins.

RESULTS

In total, 384 subjects were recruited in this study. All of them originated from Enshi Tujia and Miao Autonomous Prefecture. The basic information of the subjects is shown in Table 2. A total of seven heterozygous variants were identified (Figure 1), namely, four synonymous variants (c.201C>T, c.576C>T, c.633A>C, and c.780T>C), two missense variants (c.187A>G and c.59G>C) and one nonsense variant (c.544C>T), among which the nonsense variant (c.544C>T, p.Gln182*) and one missense variant (c.187A>G, p.Ile63Val) were novel and were not found in the 288 normal controls. The missense variant, c.187A>G, was predicted to be benign by PolyPhen2, SIFT or PROVEAN (Table 3). GERP++ (NR score 4.84, RS score 2.6) suggested that the nonsense variant, c.544C>T, was relatively conserved among species. The patient who harboured the nonsense variant was a 44-year-old man suffering retinal detachment in his left eye that had occurred several years prior. His right eye had a refractive error of -35.00 DS, and his axial length was as long as 34.34 mm. The right fundus of the patient is shown in Figure 2. The BCVA was 0.1 in the right eye, and there was no light perception in the left eye. The patient did not complain of other medical conditions, such as night blindness, deafness, skeletal abnormalities and developmental abnormalities. He has negative family history, and additional details about his parents and siblings were not available.

Table 2 Basic information of patients with high myopia

Category	Age (years)	Refractive error (D)		Axial length (mm)	
		R	L	R	L
Min	3.00	-6.25	-6.00	26.28	26.41
Max	67.00	-30.00	-30.00	34.34	35.25
Mid	38.27	-12.00	-12.75	28.09	28.77
Average	35.30	-10.50	-11.00	28.37	28.41

R: Right eye; L: Left eye; D: Dioptre.

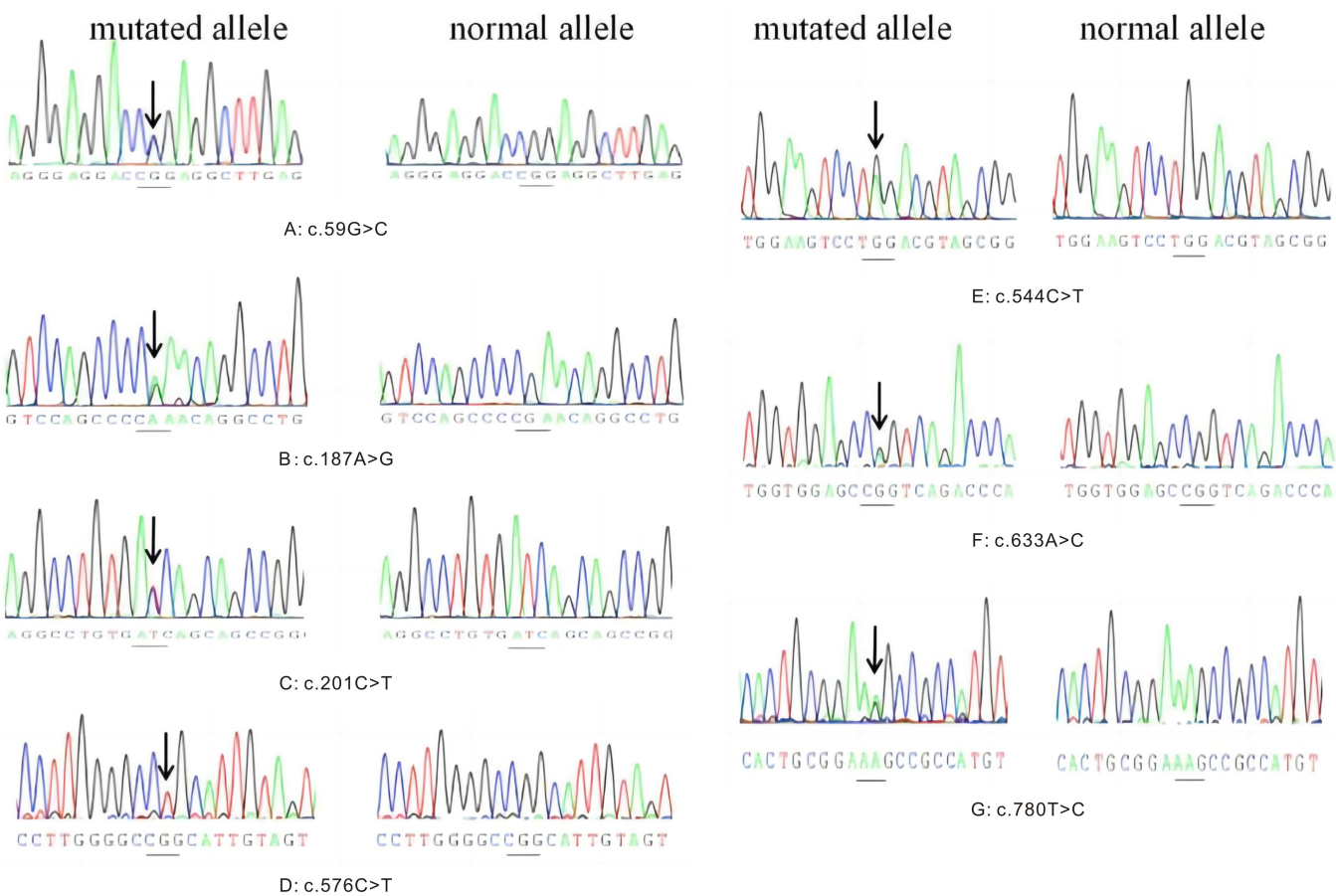

Figure 1 Seven heterozygous variants screened in this study. A and B were missense variants; C, D, F, and G were synonymous variants; E was a novel nonsense variant. The left side of each figure indicates the mutated allele, and the right side indicates the normal allele. The black arrows point to the variant alleles.

Table 3 Detailed variant information of patients with high myopia

Site	Change	Effect	1000G	EVS	ExAC	Provean	SIFT	PolyPhen2	dbSNP
50962297, G>A	c.544C>T	p.(Gln182*)	none	NA	8.254×10−6	NA	NA	NA	rs200354211
50962782, C>G	c.59G>C	p.(Arg20Pro)	0.348	36.931	0.639	N	T	NO	rs140523
50962654, T>C	c.187A>G	p.(Ile63Val)	none	NA	8.683×10−6	N	T	NO	NA
50962640, G>A	c.201C>T	synonymous	0.001	0.318	2.340×10−3	N	T	NO	rs61748568
50962265, G>A	c.576C>T	synonymous	0.001	NA	5.032×10−4	N	T	NO	rs201909075
50962208, T>G	c.633A>C	synonymous	0.344	36.491	0.6383	N	T	NO	rs12148
50962061, A>G	c.780T>C	synonymous	none	NA	NA	N	T	NO	NA

NA: Not available; N: Neutral; T: Tolerant; NO: No effect.

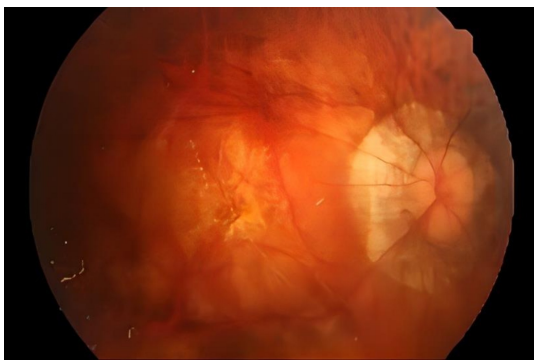

Figure 2 Right fundus photograph of the patient with the nonsense mutation. The figure showed tessellated fundus changes, diffuse chorioretinal atrophy, and peripapillary crescent.

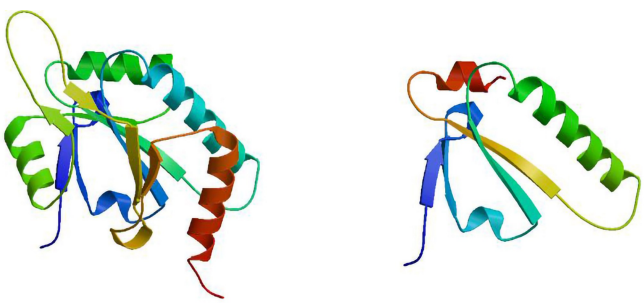

Figure 3 Wild-type and mutant protein conformation of *SCO2*.

SWISS-MODEL software modelling in our research showed that the substitution of c.544C>T caused a premature stop codon on base 182 (p.Gln182*), which may detrimentally impact the structure of the translated protein, as shown in Figure 3. The length of the coding product is 181 amino acids, which is much shorter than the normal length of 266 amino acids. The protein conformation is obviously changed, which is suspected to have a major impact on protein function.

DISCUSSION

After variant screening of *SCO2* in 384 high myopia patients from Enshi Tujia and Miao Autonomous Prefecture of China, a total of seven variants were detected: four synonymous variants, two missense variants, and one novel nonsense variant (c.544C>T). The novel nonsense variant was predicted to be damaging by PolyPhen2 and SIFT and was not found in 288 population-matched normal controls. Our findings expanded the variant spectrum of *SCO2* in high myopia. These results suggest that c.544C>T is possibly a high myopia-causing variant.

SCO2 consists of two exons, and only exon 2 encodes a functional protein that is essential for adenosine triphosphate (ATP) metabolism. Mutations in *SCO2* lead to a deficient protein function and lower ATP production. The retina is an organ with a high level of metabolism. Pathological changes may occur in the retina due to mutation in *SCO2*, which may explain the development of high myopia[13-14].

To date, including our research results, a total of seven pathogenic mutations have been detected in *SCO2* in patients with high myopia. The positions of these seven pathogenic mutations in the *SCO2* gene are shown in Figure 4. Since the *SCO2* gene consists of only two exons and the first exon has no protein-encoding function, the seven mutations are all located in exon 2. *SCO2* encodes a protein containing 266 amino acids. Mutations may lead to changes in protein conformation or premature termination[8]. The inability to form effective functional domains will greatly impair its function and cause highly aerobic and highly metabolic tissues, such as in the retina of the eye. Stunted growth or decreased function results in high myopia[15].

So far, at least 16 genes have been reported to be associated with early onset of high myopia, namely *OPN1LW* (OMIM 300822), *ZNF644*, *CCDC111*, *LRPAP1*, *SLC39A5*, *P4HA2*, *ARR3* (OMIM 301770), *CPSF1* (OMIM 606027), *NYX* (OMIM 300278), *LOXL3* (OMIM 607163), *BSG* (OMIM 109480), *DZIP1* (OMIM 608671), *XYLT1* (OMIM 608124), *NDUFAF7* (OMIM 615898), *TNFRSF21* (OMIM 605732), and *SCO2*[16]. Of these 16 genes, 11 genes (*SCO2*, *ZNF644*, *CCDC111*, *SLC39A5*, *P4HA2*, *CPSF1*, *BSG*, *DZIP1*, *XYLT1*, *NDUFAF7*, and *TNFRSF21*) are responsible for autosomal dominant high myopia, two genes are responsible for autosomal recessive high myopia (*LRPAP1* and *LOXL3*), and three genes are associated with X-linked high myopia (*OPN1LW*, *ARR3*, and *NYX*). In our previous study, we identified seven pathogenic or potential pathogenic variants in four genes (*ARR3*, *SLC39A5*, *NDUFAF7*, and

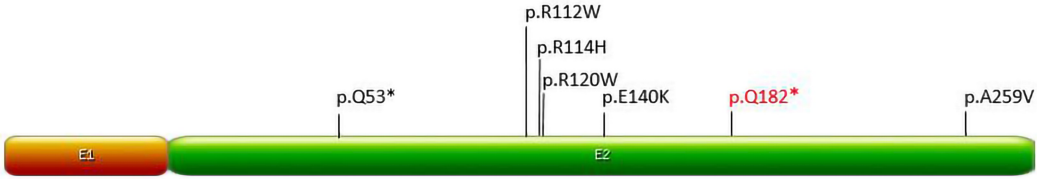

Figure 4 Variants of *SCO2*. E1: exon 1 of *SCO2*; E2: exon 2 of *SCO2*. The nonsense variant identified in our cohort was highlighted in red.

*ZNF*644) in 67 patients with early-onset high myopia[17]. In this study, pathogenic variants of *SCO2* were identified for the first time in our minority population, which expanded the spectrum of pathogenic variants in ethnic minority people with high myopia.

It is worth noting that high myopia has etiological heterogeneity, which is influenced by both environmental and genetic factors. A growing body of research suggests that environmental factors, such as time spent outdoors, play an important role in the development of myopia[18-19]. Meanwhile, the contribution of genetic factors to the development of myopia should not be underestimated, especially for preschool people with high myopia[7]. In this study, although the c.544C>T/p.Gln182* variant of *SCO2* was identified in a 44-year-old patient, the patient had progressed to high myopia by preschool age, suggesting that the patient's high myopia was more influenced by genetic factors and less influenced by environment. However, since pathogenic variants in the *SCO2* gene have only been detected in a singleton, more data are needed to support our finding in future studies.

In summary, a novel nonsense variant of *SCO2* was detected in our minority areas. Our findings support that *SCO2* plays a role in the development of high myopia. Only one pathogenic mutation was detected in 384 patients with high myopia, so the mutation frequency of *SCO2* in this population is approximately 1/384. Our results provide reference value for the clinical interpretation of the *SCO2* gene-related high myopia, in particular, suggesting that there are differences in the pathogenic variation spectrum of high myopia in different populations. Further studies should focus on the molecular mechanism of how variants of *SCO2* contribute to high myopia. We look forward to additional knowledge about the genetic mechanism of high myopia.

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