New disease loci

ORIGINAL ARTICLE

SLC39A5 mutations interfering with the BMP/TGF-β pathway in non-syndromic high myopia

Hui Guo,1,2 Xuemin Jin,3 Tengfei Zhu,1 Tianyun Wang,1 Ping Tong,4 Lei Tian,3 Yu Peng,1 Liangdan Sun,5 Anran Wan,1 Jingjing Chen,1 Yanling Liu,1 Ying Li,1 Qi Tian,1 Lu Xia,1 Lusi Zhang,1 Yongcheng Pan,1 Lina Lu,1 Qiong Liu,1 Lu Shen,1 Yunping Li,1,4 Wei Xiong,4 Jiada Li,1,2 Beisha Tang,6 Yong Feng,6 Xuejun Zhang,5 Zhuohua Zhang,1 Qian Pan,1 Zhengmao Hu,1,2 Kun Xia1,2

ABSTRACT

Background High myopia, with the characteristic feature of refractive error, is one of the leading causes of blindness worldwide. It has a high heritability, but only a few causative genes have been identified and the pathogenesis is still unclear.

Methods We used whole genome linkage and exome sequencing to identify the causative mutation in a non-syndromic high myopia family. Direct Sanger sequencing was used to screen the candidate gene in additional sporadic cases or probands. Immunofluorescence was used to evaluate the expression pattern of the candidate gene in the whole process of eye development. Real-time quantitative PCR and immunoblot was used to investigate the functional consequence of the disease-associated mutations.

Results We identified a nonsense mutation (c.141C>G: p.Y47*) in SLC39A5 co-segregating with the phenotype in a non-syndromic severe high myopia family. The same nonsense mutation (c.141C>G: p.Y47*) was detected in a sporadic case and a missense mutation (c.911T>C: p.Y47*) in SLC39A5 was co-segregating and co-segregated in another family.

Conclusions Our study reveals that loss-of-function mutations of SLC39A5 are associated with the autosomal dominant non-syndromic high myopia, and interference with the BMP/TGF-β pathway may be one of the molecular mechanisms for high myopia.

INTRODUCTION

Myopia, which is characterised by refractive error resulting primarily from excess elongation of the eye, is one of the most common eye disorders. The prevalence varies across countries, but is really high (~71–96%) in Asian countries such as China, Singapore, and Japan.1–3 High myopia—clinically defined as a spherical equivalent refractive error ≥6 dioptre—affects 1–10% of the general population.4 It may result in several severe complications including macular degeneration, retinal detachment, cataract and glaucoma, and greatly increases the risk of blindness. There are several syndromic forms of high myopia, with additional findings involving skeleton, heart, and ears such as Marfan, Stickler and the recently defined deafness and myopia syndrome.

Both environmental (close working habits, higher education levels, higher socioeconomic class, etc) and genetic factors are involved in the aetiology of myopia.5–6 Twin and family studies demonstrated that myopia, especially high myopia, has a very high heritability.7 Candidate and whole genome linkage analysis has identified dozens of loci for non-syndromic myopia, but no associated gene has been found in these linkage intervals up to now. Several genome-wide association studies (GWAS) and meta-analyses have found that myopia or refractive error is associated with common variants at GJD2, RASGRF1, GRIA4, KCNQ5, RDH12, BMP25, SIX6, and PRSS56, which may involve eye development by regulating ion transport, neurotransmission, retinoic acid metabolism, and extracellular matrix remodelling.8–12 Several other susceptible genes or loci were also identified, increasing the risk of high myopia by GWAS, such as CTNNND2, 1q41, ZC3H11B, SNTB1, VIPR2, and ZFHX1B.13–17 Recently, by using family based conventional positional cloning or exome sequencing, four genes (ZNF644,18 SC02,19 LEPREL1,20 LRPAR1,21) have been identified in monogenic non-syndromic high myopia families. However, all these genes can only explain a very small proportion of high myopia subjects and the pathogenesis is still unclear.

In this study, we recruited a three-generation Chinese high myopia family with autosomal dominant inheritance. By using whole genome linkage and exome sequencing, we identified a segregating nonsense mutation of SLC39A5 in this family. Candidate screening in other high myopia families and sporadic samples identified another family with a missense mutation and a sporadic case with the same nonsense mutation. Immunofluorescence revealed that SLC39A5 involves the whole process of eye development and expresses mainly in sclera and retina. Real-time quantitative PCR (qPCR) and immunoblot showed that both disease-associated


© 2014 BMJ Publishing Group Ltd

mutations are loss-of-function and interfere with the BMP/ TGF-β (bone morphogenic protein/transforming growth factor-β) pathway.

METHODS

Study subjects and clinical characterisation

A Chinese Henan non-syndromic high myopia family (HM-FR3) with autosomal dominant inheritance participated in this study (figure 1A). All recruited members underwent clinical examination and blood collection after providing informed consent. All of the affected cases have a history of myopia onset before 10 years of age. A comprehensive ophthalmic examination was performed and the refractive error and axial length were measured and recorded. All of the affected individuals have no known ocular disease or insult that could predispose to myopia, such as retinopathy of prematurity or early age media opacification, and no known genetic diseases associated with myopia, such as Stickler or Marfan syndrome. The study was approved by the Institutional Review Board of The State Key Laboratory of Medical Genetics and adhered to the tenets of the Declaration of Helsinki.

Whole genome genotyping and linkage analysis

Genomic DNA was extracted from leucocytes of all individuals by the standard proteinase K digestion and phenol-chloroform method. Whole genome genotyping was performed using Illumina HumanLinkage-12 Bead Chip on all recruited members of pedigree HM-FR3, strictly following the instructions of Illumina’s protocol. Genotype calling and quality control were performed using the Illumina GenomeStudio Genotyping Module (V6.0). Single nucleotide polymorphisms (SNPs) were excluded if the call rate was lower than 95%, departed from Hardy-Weinberg equilibrium (p<0.001) or had Mendelian inconsistencies. Finally, the call rates for all individuals were above 98% and a total of 5440 autosomal SNPs were left for linkage analyses. Parametric two-point and multi-point linkage analysis using the genotypes after quality control were carried out with MERLIN based on an autosomal

Figure 1 Identification of SLC39A5 mutations in high myopia patients. (A) The pedigree plot for HM-FR3: solid symbols represent affected individuals. M numbers denote individuals whose DNA samples were analysed. The nonsense mutation (Y47X) identified by whole genome linkage and exome sequencing segregated with the phenotype exactly. (B) Fundus photograph for the proband of HM-FR3 (M16346) appeared tigroid and had focal atrophy of the choroid. OD represents right eye and OS represents left eye. (C) Multi-point parametric linkage analysis shows four regions with LOD score more than 1 on chromosomes 2, 10, and 12, respectively, and other eight low peaks with maximum LOD score no more than 0.5. (D) The pedigree plot for family HM-ZZ19 with the missense mutation (M304T) of SLC39A5. (E) Schematic of human SLC39A5 protein structure and mutational locations: the nonsense mutation occurred at the 47th amino acid and is located in the amino-terminal domain; the missense mutation is located at the terminal of the third transmembrane domain.
dominant (AD) model with 0.9 penetrance and an allele frequency of 0.01. The affection statuses were defined strictly according to the clinical diagnosis.

**Targeted region capturing and exome sequencing**

For each patient selected for exome sequencing, 1 μm of genomic DNA was fragmented and selected, aiming for a 350–400 base pair product and PCR amplified. Exome capturing was performed to collect the protein coding regions of human genome DNA using Illumina TruSeq array as described in the manufacturer’s instructions. The exon-enriched DNA libraries were sequenced using the Illumina HiSeq 2000 platform, following the manufacturer’s instructions (Illumina, San Diego, USA).

**Read mapping, variant analysis, and mutation validation**

Generated reads by exome sequencing were aligned to human reference genome hg19 (UCSC version) using BWA (Burrows-Wheeler Aligner) 0.5.6. 25 Read qualities were recalibrated using GATK Table Recalibration 1.0.2905. 24 Picard 1.14 was used to flag duplicate reads (http://picard.sourceforge.net/). GATK IndelRealigner 1.0.2905 was used to realign reads around insertion/deletion (InDel) sites. Single nucleotide variants (SNVs) were generated with GATK Unified Genotyper 24 and in parallel with the SAM tools pipeline. 23 The small InDels were also called with the GATK Unified Genotyper and SAMtools. The called SNVs and InDels are annotated with manufacturer sequenced using the Illumina HiSeq 2000 platform, following

**Immunoblot analyses**

Cells were seeded in 24-well plates transfected with empty SLC39A5-FLAG, SLC39A5-M304T-FLAG or SLC39A5-Y46X-FLAG plasmid using Lipofectamine 2000 (Invitrogen). Cells were harvested 48 h after transfection for protein extraction. Cells were lysed and homogenised with SDS (sodium dodecyl sulfate) sample buffer (63 mM Tris-HCl, 10% glycerol, 2% SDS) and Protease Inhibitor Cocktail (Sigma). Twenty micrograms of total protein per lane were separated on 10% SDS-PAGE (polyacrylamide gel electrophoresis) gels and followed by transfer to PVDF membranes (Millipore). Membranes were blocked with 5% non-fat milk for 1 h at room temperature followed by incubation overnight with primary antibody at 4 °C. The antibody used was rabbit-anti Smad1 (Cell Signaling), M2 (Sigma), mouse-anti SLC39A5 (sigma), mouse-anti β-actin (Sigma).

**SLC39A5 shRNA plasmid construction**

The short hairpin RNA targeting SLC39A5 cDNA was designed with Block-iT RNAi Designer (Invitrogen). SLC39A5 sh1 (5′-GGAATCTCGAACCAGCCACACT-3′), SLC39A5 sh2 (5′-GACCACTGGAATGAGGTGGT-3′), SLC39A5 sh3 (5′-GCCCTGCTTTATCATGAGCTGC-3′), and a control shRNA (5′-GACCACTGGCATGAACTGA-3′), which was not homologous with any known mammal genes, were used in the experiment. Oligonucleotides were annealed following by insertion into pSUPER vector (OligoEngine).

**Immunofluorescence for subcellular localisation of SLC39A5**

Cells were cultured on glass coverslips. Wide type and mutant vectors were transfected with lipo2000 (Invitrogen). The cells were then fixed in 4% paraformaldehyde/sucrose for 10 min. Cell permeabilisation was performed with 0.1% Triton X-100/ PBS (phosphate buffered saline) for 15 min followed by blocking with BSA (bovine serum albumin) for 30 min. The cells were then probed by primary antibody and stained by Alexa Fluor 488-conjugated or cy3-conjugated secondary antibody. DAPI was used for nuclear stain. Images were acquired with an Eclipse TCS-SP5 inverted confocal microscope (Leica).

**RESULTS**

**Clinical description**

Nine members (five affected, figure 1A) of HM-FR3 were recruited into the study. The refractive errors for the five affected subjects range from −5.00 to −25.00 dioptre sphere (DS) for the left eye (OS) and from −7.00 to −20.00 DS for the right eye (OD). Eye globe axial length ranges from 26.14 to 33.32 mm for OS and 26.29 to 32.09 mm for OD (table 1). The proband (M16346) and two elderly patients showed typical fundus features of high myopia, tigroid and focal atrophy of choroid (figure 1B). All of the affected individuals have a history of myopia onset before the age of 10 years old (table 1)
and have no known ocular disease or insult that could predispose to myopia.

Whole genome linkage and exome sequencing identified segregating mutation in SLC39A5

To narrow the chromosome intervals and increase the possibility of identifying the causative mutation, we firstly performed whole genome linkage analysis using Illumina Human Linkage-12 BeadChip. Multi-point parametric linkage analysis identified four regions on chromosome 2, 10, and 12 with maximum multi-point parametric LOD (logarithm of odds) scores from 1 to 1.42 (figure 1C, see online supplementary table S1). We also identified eight other signals distributed in seven chromosomes, with maximum parametric and non-parametric LOD scores of no more than 0.5 (figure 1C). Nevertheless, we also included these eight signals in the following filtering procedures as linkage intervals.

We subsequently performed exome capturing and sequencing for two patients (M16346 and M16350, figure 1A) from family HM-FR3. In total, we generated an average of 13.09 Gbp of sequence with more than 50× and 90× coverage for two patients (M16346 and M16350, respectively). We identified 21 963 (9083 non-synonymous SNV, splicing SNV and InDels) coding variants in M16346 and M16350, respectively. The called SNVs and InDels were filtered as described in the method (Read mapping, variant analysis and mutation validation). Following the filtering procedures described above, only four variants were left for our further analysis (table 2). We used Sanger sequencing to validate these four variants and analyse the co-segregating status of the variants in all family members. Finally, only one nonsense mutation in SLC39A5 (c.141C>G; p.Y47*) co-segregated with the phenotype in all family members. Considering that the HM-FR3 family is not large enough to detect complete linkage signals (the MAX-LOD score is 1.42), we also assessed all the variants shared between the two affected individuals (left by procedure 5). Only the nonsense mutation in SLC39A5 co-segregated with the phenotype in all family members. This mutation is located in the linkage region in chromosome 12 with the maximum multi-point parametric LOD score of 1.42 (rs774033 and rs10122, see online supplementary table S1). To confirm that the segregating status of the mutation is consistent with the haplotype segregating status, we performed the haplotype analysis using the genotyped SNPs around this mutation. The haplotype plot shows that the mutation segregated within the haplotype exactly (see online supplementary figure S1). This mutation is not identified in 500 population-matched controls using Sanger sequencing, or

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Sex</th>
<th>Affection status</th>
<th>Age at onset (years)</th>
<th>Age at exam (years)</th>
<th>Corneal power OD</th>
<th>Corneal power OS</th>
<th>Refractive error (DS) OD</th>
<th>Refractive error (DS) OS</th>
<th>Axial length (mm) OD</th>
<th>Axial length (mm) OS</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>HM-FR3 M16344</td>
<td>M</td>
<td>U</td>
<td>NA</td>
<td>70</td>
<td>42.75/43.01</td>
<td>42.50/44.62</td>
<td>−0.34</td>
<td>−0.50</td>
<td>24.2</td>
<td>23.8</td>
<td>NO</td>
</tr>
<tr>
<td>M16345</td>
<td>F</td>
<td>A</td>
<td>10</td>
<td>68</td>
<td>43.50/44.75</td>
<td>43.01/44.25</td>
<td>−5.00</td>
<td>−7.00</td>
<td>26.14</td>
<td>27.65</td>
<td>c.C141G:p.Y47X</td>
</tr>
<tr>
<td>M16346</td>
<td>F</td>
<td>A</td>
<td>3</td>
<td>47</td>
<td>41.57/42.75</td>
<td>42.01/43.50</td>
<td>−25.00</td>
<td>−20.00</td>
<td>33.32</td>
<td>32.09</td>
<td>c.C141G:p.Y47X</td>
</tr>
<tr>
<td>M16348</td>
<td>M</td>
<td>U</td>
<td>NA</td>
<td>44</td>
<td>40.65/41.34</td>
<td>40.50/42.45</td>
<td>+1.00</td>
<td>+0.50</td>
<td>23.5</td>
<td>23.9</td>
<td>NO</td>
</tr>
<tr>
<td>M16347</td>
<td>F</td>
<td>A</td>
<td>5</td>
<td>42</td>
<td>41.75/42.23</td>
<td>41.50/42.35</td>
<td>−9.00</td>
<td>−13.00</td>
<td>28.13</td>
<td>29.41</td>
<td>c.C141G:p.Y47X</td>
</tr>
<tr>
<td>M19118</td>
<td>F</td>
<td>U</td>
<td>NA</td>
<td>28</td>
<td>41.21/43.89</td>
<td>41.50/43.35</td>
<td>−0.30</td>
<td>−0.25</td>
<td>24.0</td>
<td>24.5</td>
<td>NO</td>
</tr>
<tr>
<td>M16349</td>
<td>M</td>
<td>A</td>
<td>8</td>
<td>35</td>
<td>42.35/44.62</td>
<td>42.75/44.25</td>
<td>−5.00</td>
<td>−7.00</td>
<td>27.87</td>
<td>28.52</td>
<td>c.C141G:p.Y47X</td>
</tr>
<tr>
<td>M16350</td>
<td>M</td>
<td>A</td>
<td>8</td>
<td>15</td>
<td>43.25/44.00</td>
<td>43.75/44.50</td>
<td>−7.50</td>
<td>−7.00</td>
<td>26.35</td>
<td>26.29</td>
<td>c.C141G:p.Y47X</td>
</tr>
<tr>
<td>M21932</td>
<td>M</td>
<td>U</td>
<td>NA</td>
<td>17</td>
<td>42.75/43.50</td>
<td>42.02/43.65</td>
<td>−0.22</td>
<td>−0.45</td>
<td>23.8</td>
<td>24.1</td>
<td>NO</td>
</tr>
<tr>
<td>HM-ZZ19 M20111</td>
<td>M</td>
<td>U</td>
<td>NA</td>
<td>50</td>
<td>40.87/41.50</td>
<td>40.25/42.50</td>
<td>−1.00</td>
<td>−1.00</td>
<td>23.9</td>
<td>24.0</td>
<td>NO</td>
</tr>
<tr>
<td>M20112</td>
<td>F</td>
<td>A</td>
<td>10</td>
<td>48</td>
<td>43.19/44.25</td>
<td>43.94/45.25</td>
<td>−8.00</td>
<td>−8.50</td>
<td>27.50</td>
<td>28.35</td>
<td>c.T911C:p.M304T</td>
</tr>
<tr>
<td>M20113</td>
<td>M</td>
<td>A</td>
<td>5</td>
<td>23</td>
<td>43.75/44.12</td>
<td>44.00/45.25</td>
<td>−6.50</td>
<td>−5.25</td>
<td>26.54</td>
<td>26.52</td>
<td>c.T911C:p.M304T</td>
</tr>
<tr>
<td>Sporadic M21789</td>
<td>M</td>
<td>A</td>
<td>9</td>
<td>45</td>
<td>41.26/42.79</td>
<td>41.38/42.21</td>
<td>−20.00</td>
<td>−18.50</td>
<td>31.34</td>
<td>29.58</td>
<td>c.C141G:p.Y47X</td>
</tr>
</tbody>
</table>

OD represents right eye and OS represents left eye.
A, affected; DS, diopter sphere; F, female; M, male; U, unaffected.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>NS/SS/InDel</th>
<th>LowFreq (MAF&lt;0.01)</th>
<th>Dominant (Heterozygous)</th>
<th>Absent in our 50 exomeSeq data</th>
<th>Segregating in exomeSeq subjects</th>
<th>In linkage regions</th>
<th>Segregating in all family members</th>
</tr>
</thead>
<tbody>
<tr>
<td>M16346</td>
<td>8692/77313</td>
<td>630/2639/0993</td>
<td>262/21/202</td>
<td>437/8/46</td>
<td>72/26</td>
<td>3/1/0</td>
<td>1/00 (SLC39A5:c.C141G;p.Y47X)</td>
</tr>
<tr>
<td>M16350</td>
<td>8300/693/0993</td>
<td>549/19/293</td>
<td>540/14/295</td>
<td>385/2/39</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

InDel, insertions and deletions; LowFreq represents low allele frequency (MAF<0.01) both in 1000g2012Ari and ESP6500NS, non-synonymous variants; SNV, single nucleotide variant; SS, splicing variants.
in another cohort of 776 population-matched controls with exome sequencing data. High myopia and other related syndromes were excluded for all of the 1276 controls.

To validate the association of SLC39A5 with HM, we sequenced all the exons and splice sites of this gene in a cohort of 180 patients with high myopia (63 family probands and 117 sporadic cases). The 180 patients had refractive errors ranging from −6.4 to −30.0 DS and an axial eye globe length from 26.00 to 44.38 mm for both eyes (see online supplementary table S3). Two mutations were identified. One of them (c.141C>G:p.Y47*), identified in a sporadic case (M21789, table 1), is the same as that we identified in pedigree HM-FR3. The other one (c.911T>C:p.M304T) was identified and co-segregated in a pedigree with three members (HM-ZZ19, figure 1D, table 1). The c.911T>C mutation was not identified in 1276 population-matched controls.

SLC39A5 is expressed in the sclera and retina across different stages of eye development

SLC39A5 encodes solute carrier family 39, member 5 (SLC39A5 or ZIP5), a member of the ZIP family of transporters for metal ion, specific for zinc.25 It is reported that SLC39A5 is situated in the Golgi membrane27 or cell membrane.28 However, our data showed that it is localised in the membrane of endoplasmic reticulum in HEK293 cells (see online supplementary figure S2–3). Although SLC39A5 has been shown to express in several tissues important for zinc homeostasis, including the intestine, pancreas, liver and kidney,27 no data revealed its expression in ocular tissues. In light of further association between SLC39A5 and high myopia and the substructure localisation of SLC39A5 in the eye, we investigated the expression of Slc39a5 in the mouse eyes with a rabbit polyclonal antiserum against Slc39a5. As shown in figure 2A, B, Slc39a5 was observed in all stages of eye development and mainly expressed in the sclera and several layers of the retina, such as the inner segment (IS), outer plexiform layer (OPL), and ganglion cell layer (GCL).

Disease-associated mutations are loss-of-function and interfere with the BMP/TGF-β pathway

Human SLC39A5 contains eight transmembrane domains, and the N-terminus and C-terminus are extra-cytosolic. There are four potential sites of N-glycosylation: three of them (Asn-49, Asn-91, and Asn-158) are found in the amino-terminal domain, and the other one (Asn-390) is predicted to lie within transmembrane domain 4. The Y47* mutation introduced a stop codon at position 47 (figure 1E). The protein level in the patients’ lymphocyte cell lines was decreased compared to the normal controls as demonstrated by immunoblot, but the mRNA expression level was not different between cases and controls (see online supplementary figure S4). This indicates that the Y47* mutation may produce a truncated protein with 46 amino acids. The missense mutation was located in the third transmembrane domain (figure 1E); however, it did not influence the localisation of SLC39A5 in the membrane of endoplasmic reticulum (see online supplementary figure S3).

SLC39A13, a homologue of SLC39A5, encoding another zinc transporter, has been identified as causing Ehlers-Danlos syndrome (EDS).29 EDS patients also presented with incomplete penetrance of myopia and under-hydroxylated lysyl and prolyl residues of collagens. Interestingly, Slc39a13-deficient mouse showed a disrupted BMP/TGF-β transduction pathway.30 To see if SLC39A5 is also involved in the BMP/TGF-β pathway, the expression of Smad1, a key downstream transcription factor, was examined by real-time qPCR and immunoblot. As shown in figure 3A, B, we observed pronounced upregulation of Smad1 in both mRNA (figure 3A, see online supplementary figure S5)
and protein (figure 3B) expression levels in the patients’ lymphocyte cell lines as compared to controls. We also demonstrated that wild type, but not disease-associated SLC39A5, suppressed the expression of Smad1 in HEK293 cells (figure 3C). To further study the effect of SLC39A5 on the Smad1 expression, we transduced HEK293 cells with three SLC39A5 shRNA vector (SH1, SH2, SH3). As shown in figure 3D,E, silence of SLC39A5 significantly attenuated the suppressive effect of wild-type SLC39A5 on Smad1 expression, implying the disease-associated mutations are loss-of-function. As the BMP/TGF-β pathway has been recurrently implicated in myopia development, the mechanism is still unclear. Our data suggest that zinc may be involved in eye development by regulating the extracellular matrix (ECM) of the sclera,39 which is mainly responsible for the high myopia phenotype. Indeed, BMP/TGF-β pathway has been implicated in high myopia patients,30 there might be some kind of relationship between collagen or collagen hydroxylase disruption of this may cause the refractive error and finally result in the high myopia phenotype.

SLC39A5 encodes a zinc transporter. Interestingly, zinc is essential for eye development and zinc deficiency has been reported in high myopia patients.35,36 Our data show that zinc transporter Slc39a5 is involved in the whole stage of mouse eye development and is abundantly expressed in the sclera and retina. Although it is still unclear how zinc participates in eye development and the development of myopia, we identified that disease-associated mutations of the zinc transporter encoded the gene dysregulated BMP/TGF-β pathway. Interestingly, TGF-β is one of the most reproducibly dysregulated genes in the study of myopia development,21,31,32,35,38 and is thought to exert an effect through modulating the extracellular matrix (ECM) of the sclera, in which SLC39A5 is abundantly expressed. Although the BMP/TGF-β pathway has been implicated in high myopia pathogenesis, the mechanism is still unclear. Our data suggest that zinc may be involved in eye development by regulating BMP/TGF-β, and disruption of this may cause the refractive error and finally result in the high myopia phenotype.

It is also worth highlighting that SLC39A13 mutations caused under-hydroxylated lysyl and prolyl residues of collagens in EDS patients.29 Since Slc39a13-deficient mouse presented with disruption of the BMP/TGF-β pathway, there might be some kind of relationship between collagen or collagen hydroxylase and the BMP/TGF-β pathway. Indeed, BMP/TGF-β has been implicated in modulating ECM of the sclera,30 which is mainly comprised of collagens. Therefore, it is possible that BMP/TGF-β may regulate the transcription of collagens or
New disease loci

collagen-related protein, such as lysyl and prolyl hydroxylase, and control the expression and normal function of collagens. The failure of any process may contribute to myopia or related phenotypes. Interestingly, one homozygous missense mutation in the prolyl hydroxylase encoded gene, LEPRE1, has been identified as the cause of autosomal recessive high myopia in an Israeli family.20 Furthermore, we also identified a homozygous loss-of-function mutation of this gene in a high myopia family from a Chinese population.40 Further studies should be conducted to explore the relationship between collagen or collagen hydroxylase and the BMP/TGF-β pathway, as well as the potential underlying mechanisms in the development of myopia.

Author affiliations

1The State Key Laboratory of Medical Genetics, Central South University, Changsha, Hunan, China
2School of Life Sciences, Central South University, Changsha, Hunan, China
3Department of Ophthalmology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, China
4Department of Ophthalmology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China
5Department of Dermatology, Institute of Dermatology, No. 1 Hospital, Anhui Medical University, Hefei, Anhui, China
6The Xiangya Hospital, Central South University, Changsha, Hunan, China
7Key Laboratory of Medical Information Research, Changsha, Hunan, China

Acknowledgements

We greatly thank all the patients and their family members who participated in this study.

Contributors

Conceived and designed the experiments: XX, ZH and HG. Performed the experiments: TZ, TW, HG, VP, JC, YL, QT, LX, LZ, YP, LL and QL. Analysed the data: HG, TW and LS. Contributed reagents/materials/analysis tools: XJ, XZ, LS, YL and QL. Obtained the data: HG, TW and LS. Contributed reagents/materials/analysis tools: XJ, XZ, LS, YL and QL. Analysed the data: TZ, TW, HG, YP, AW, JC, TL and XJ. Wrote the paper: HG and KX. Critically reviewed the manuscript: ZH, JL, WX, BT, YF, ZZ and QP. Obtained the funding: KX and ZH.

Funding

The research was supported by the National Basic Research Program of China (2012CB517902), and the National Natural Science Foundation of China (81330027, 8116112054).

Competing interests

None.

Patient consent

Obtained.

Ethics approval

The study was approved by the Institutional Review Board of The State Key Laboratory of Medical Genetics and adhered to the tenets of the Declaration of Helsinki.

Provenance and peer review

Not commissioned; externally peer reviewed.

Open Access

This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc-3.0/

REFERENCES


