

# Exome sequencing study of 20 patients with high myopia

Ling Wan<sup>1,2</sup>, Boling Deng<sup>2</sup>, Zhengzheng Wu<sup>2</sup> and Xiaoming Chen<sup>1</sup>

<sup>1</sup> Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu, Sichuan, China

<sup>2</sup> Department of Ophthalmology, Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital and Affiliated Hospital of University of Electronic Science and Technology, Chengdu, Sichuan, China

## ABSTRACT

**Background:** High myopia is a common ocular disease worldwide. To expand our current understanding of the genetic basis of high myopia, we carried out a whole exome sequencing (WES) study to identify potential causal gene mutations.

**Methods:** A total of 20 individuals with high myopia were exome sequenced. A novel filtering strategy combining phenotypes and functional impact of variants was applied to identify candidate genes by multi-step bioinformatics analyses. Network and enrichment analysis were employed to examine the biological pathways involved in the candidate genes.

**Results:** In 16 out of 20 patients, we identified 20 potential pathogenic gene variants for high myopia. A total of 18 variants were located in myopia-associated chromosomal regions. In addition to the novel mutations found in five known myopia genes (*ADAMTS18*, *CSMD1*, *P3H2*, *RPGR*, and *SLC39A5*), we also identified pathogenic variants in seven ocular disease genes (*ABCA4*, *CEP290*, *HSPG2*, *PCDH15*, *SAG*, *SEMA4A*, and *USH2A*) as novel candidate genes. The biological processes associated with vision were significantly enriched in our candidate genes, including visual perception, photoreceptor cell maintenance, retinoid metabolic process, and cellular response to zinc ion starvation.

**Discussion:** Systematic mutation analysis of candidate genes was performed using WES data, functional interaction (FI) network, Gene Ontology and pathway enrichment. FI network analysis revealed important network modules and regulator linker genes (*EP300*, *CTNNB1*) potentially related to high myopia development. Our study expanded the list of candidate genes associated with high myopia, which increased the genetic screening performance and provided implications for future studies on the molecular genetics of myopia.

**Subjects** Bioinformatics, Computational Biology, Genetics, Genomics, Translational Medicine

**Keywords** Whole exome sequencing, High myopia, Pathway enrichment, GO and FI network enrichment, QTL, Genomics, Bioinformatics

## INTRODUCTION

Myopia, recognized as the most common ocular disease (*Holden et al., 2016; Shi et al., 2011; Vitale, Sperduto & Ferris, 2009*), has a prevalence in 20–30% of Australian, American, and Western European populations (*Kempen et al., 2004*). One extreme type of

Submitted 17 June 2018  
Accepted 7 August 2018  
Published 17 September 2018

Corresponding author  
Xiaoming Chen,  
chenxm058@163.com

Academic editor  
Yong Wang

Additional Information and  
Declarations can be found on  
page 12

DOI 10.7717/peerj.5552

© Copyright  
2018 Wan et al.

Distributed under  
Creative Commons CC-BY 4.0

OPEN ACCESS

myopia is high myopia, defined as a refractive error of at least  $-6.0$  diopters (D) or axial length  $>26$  mm (Young *et al.*, 1998). It occurred in about 2.7% of the world population in 2000 and has shown significant increase in prevalence over the last two decades (Holden *et al.*, 2016). Although myopia is usually a benign disorder that can be corrected by glasses and contact lenses, patients with high myopia are at increased risk of other complications, such as cataracts, glaucoma, retinal detachment, and posterior staphyloma (Morgan, Ohno-Matsui & Saw, 2012; Saw *et al.*, 2005; Xu *et al.*, 2006).

Genetic factors play a critical role in the development of high myopia based on family aggregation and twin studies (Hammond *et al.*, 2001; Katz, Tielsch & Sommer, 1997), with its heritability estimated to be over 70% in large twin studies (Dirani *et al.*, 2006; Lopes *et al.*, 2009). Family linkage studies have identified 26 Quantitative Trait Loci (QTLs) (OMIM: 160700) so far (Andrew *et al.*, 2008; Li *et al.*, 2009; Nakanishi *et al.*, 2009; Schwartz, Haim & Skarsholm, 1990; Young *et al.*, 1998). However, only seven QTLs are fine-mapped to the causal genes (Table S1), a large proportion of linkage signal found in family studies still remain to be elucidated.

The inheritance mode for familial form of high myopia is complex. It may be inherited in an autosomal recessive, autosomal dominant, or X-linked recessive manner (Ng *et al.*, 2009; Zhang, 2015). Quite a few disease-associated genes have been identified in individual studies, such as dominant genes *SCO2* (Tran-Viet *et al.*, 2013), *ZNF644* (Shi *et al.*, 2011), and *P4HA2* (Guo *et al.*, 2015); recessive genes *LRPAP1* (Aldahmesh *et al.*, 2013) and *LEPREL1* (Mordechai *et al.*, 2011); X-linked gene *ARR3* (Xiao *et al.*, 2016). As the sequencing costs keep dropping, whole exome sequencing (WES) has been gradually adopted in studies of familial or early-onset high myopia. Variants in *LRPAP1*, *CTSH*, *LEPREL1*, *ZNF644*, *SLC39A5*, and *SCO2* genes were comprehensively screened in 298 families with early-onset high myopia (Jiang *et al.*, 2014). However, only a small proportion of patients were found to have deleterious mutations in these six genes. Sun *et al.* (2015) investigated mutations in 234 genes associated with retinal dystrophies in 298 patients with early-onset high myopia. They found that 34 of 234 genes had potential pathogenic mutations including *GNAT1*, *GUCY2D*, *COL2A1*, *COL11A1*, *PRPH2*, *FBN1*, *TSPAN12*, *CACNA1F*, *OPA1*, *PAX2*, and *RPGR*. Recently, Kloss *et al.* (2017) analyzed 14 families of high myopia through WES combining with QTL overlapping analysis and identified 73 rare and 31 novel pathogenic variant candidates. Jin *et al.* (2017) took another approach searching for de novo mutations through WES in 18 family trios with healthy parents and early-onset high myopia children. In addition to the novel pathogenic gene *BSG*, two known high myopia candidate genes (*LEPREL1* and *GRM6*), three oculopathy-related genes (*FAM161A*, *GLA*, and *CACNA1F*), and a further possible gene (*MAOA*) were also identified in this trio study.

The high prevalence of high myopia in East Asian population (Holden *et al.*, 2016; Wu *et al.*, 2015) and previous WES studies suggested the complex genetic mechanisms behind the disease despite its strong heritability in families. In this study, we applied a novel phenotype driven variant filtering approach on the WES data of 20 patients with familial high myopia to search for potential causal mutations. We also explored the functions of the candidate genes through gene expression profiling, network and pathway enrichment analysis.

## MATERIALS AND METHODS

### Human subjects

This study included a total of 20 samples from 19 families (nine males and 11 females) of Han Chinese ancestry with non-syndromic familial high myopia. The human subjects were recruited at the ophthalmic clinic at Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, China. Approval for the study was provided by the Institutional Review Board of Sichuan Provincial People's Hospital. Written informed consent forms were signed by all patients before the collection of peripheral blood and clinical data. The refractive errors of all patients were greater than  $-6.00$  D, as diagnosed by a team of high myopia specialists.

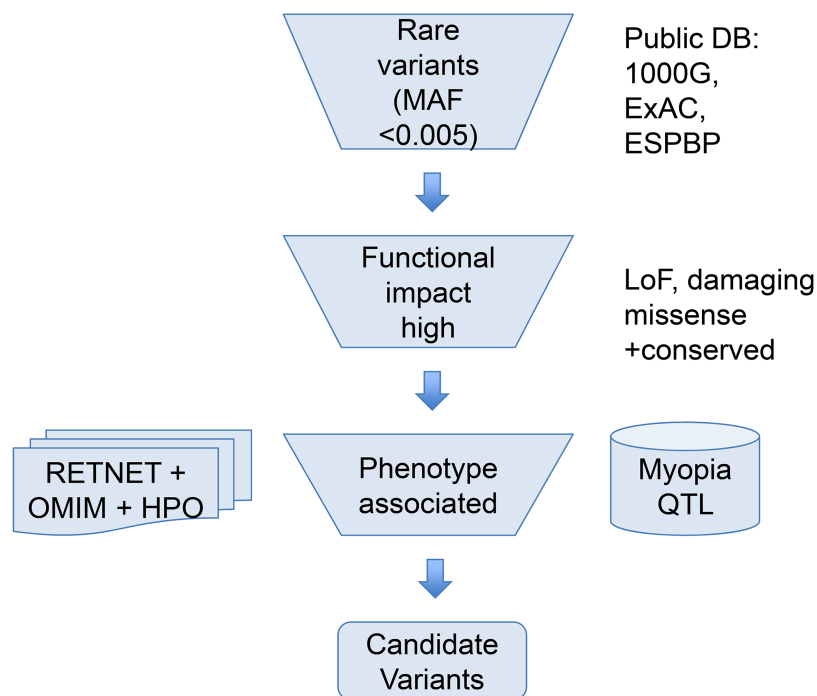
### Exome sequencing and variant calling

Genomic DNA was isolated from peripheral blood samples of patients by using Genomic DNA Extraction Kit (Invitrogen, South San Francisco, CA, USA). The sequencing libraries were prepared and captured using SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's instructions. Paired-end ( $2 \times 150$  bp) NGS was performed using HiSeq X-10 (Illumina, San Diego, CA, USA) according to the manufacturer's protocol.

Raw sequencing reads were filtered using the Trimmomatic program (<http://www.usadellab.org/cms/index.php?page=trimmomatic>) to remove reads with low sequencing quality at both ends. The cleaned reads were mapped to human reference genome (GRCH37) by the BWA-MEM software (*Li & Durbin, 2009*), and Polymerase Chain Reaction (PCR) duplications were removed by Picards (<http://broadinstitute.github.io/picard>). Variant calling was performed by a consensus call method to reach a good balance of high sensitivity and low false positive rate. Only variants detected by at least two methods out of four haplotype-based calling algorithms (Platypus, samtools, freebayes, and GATK haplotype caller) were kept in the final variant file. Variant annotation including gene functional consequence (gene context, amino acid change, splicing effect etc.), pathogenicity predictions by multiple computational methods (SIFT, Polyphen2, MutationTaster, CADD etc.), as well as healthy population allele frequencies from 1000G (*Sudmant et al., 2015*), ExAC (<http://exac.broadinstitute.org/>), and ESP (<http://evs.gs.washington.edu/>) database (*Lek et al., 2016*) were performed using the VEP software (*McLaren et al., 2016*).

### Variant filtering

To help narrow down the potential candidate variants for high myopia, we took a phenotype driven strategy focusing on rare variants in ocular diseases and high myopia phenotype related genes as illustrated in [Fig. 1](#). First, we used a maximum allele frequency of 0.005 in any of the three public population databases: 1000G (<http://browser.1000genomes.org/index.html>), ExAC, and ESP dataset to filter out common variants. Then, only variants with high functional impact (loss-of-function, or computationally predicted pathogenic missense variants) were considered. Pathogenicity of missense mutations was assumed if predicted pathogenic by at least six out of eight computational methods (SIFT, PolyPhen2, LRT, MutationTaster,



**Figure 1** Overview of the variant filtering strategy and analysis of WES data. We applied a phenotype driven strategy to filter the potential candidate variants for high myopia. Variant frequency in populations, mutation functional impact, and phenotype associations from RetNet, HPO, and OMIM are combined to narrow down the candidate variants. [Full-size !\[\]\(5f471a71b78d7676bc356df190b88ab4\_img.jpg\) DOI: 10.7717/peerj.5552/fig-1](https://doi.org/10.7717/peerj.5552/fig-1)

MutationAssessor, FATHMM, CADD, DANN). Lastly, we focused on phenotype relevant genes. We considered only genes meeting both of the following two criteria. Firstly, genes must be associated with eye-related diseases. The ocular disease genes were assembled from the following three databases: genes associated with the myopia phenotype term (HP:0000545) in Human Phenotype Ontology (HPO: <http://human-phenotype-ontology.github.io>), or ocular disease genes from RetNet database (<http://www.sph.uth.tmc.edu/retnet/>) and OMIM (<https://www.omim.org/>). Secondly, genes must be known high myopia genes from previous publications (*Aldahmesh et al., 2013*; *Chen et al., 2013*; *Jiang et al., 2014*; *Jin et al., 2017*; *Kloss et al., 2017*; *Shi et al., 2011*; *Sun et al., 2015*; *Tran-Viet et al., 2013*) (complete gene list of known genes and ocular disease genes in [Table S2](#)) or within the myopia-associated (MYP) QTL regions ([Table S1](#)). Because not all patients have syndromic severe ocular diseases, a few variants in genes causing dominant OMIM ocular diseases not satisfying clinical phenotypes were excluded.

### Functional enrichment and network analysis

We chose a recent RNA-seq expression profile [GSE94437](#) and a gene microarray expression profile [GSE41102](#) to explore the expression pattern of the compiled complete list of ocular disease genes. For the [GSE94437](#), RPKM was transformed by  $\log_2(\text{RPKM} + 0.001)$ . To test the expression differences of the 709 ocular disease genes versus other genes, one-sided *t*-test was applied to obtain the statistical significance. The Gene Ontology (GO) is a hierarchically organized, controlled vocabulary to

consistently describe and annotate gene products (Ashburner et al., 2000). Joint terms may give insight on the shared biological processes, and enrichment analysis can make use of term-term relationships (Huang, Sherman & Lempicki, 2009; Kuleshov et al., 2016). The ReactomeFIViz app (Wu et al., 2014) was designed to find GO, pathways and network patterns. Thus we used the ReactomeFIViz app and Cytoscape 3.5.1 to perform GO enrichment analysis of candidate genes. Using all the protein-coding genes in the genome as background, we carried out the GO enrichment for both the 12 candidate gene set and the 709 eye disease gene set. ReactomeFIViz was also used to explore the network features of candidate genes in the manually curated pathway-based protein functional interaction (FI) network covering over 60% of human proteins (Wu, Feng & Stein, 2010).

## RESULTS

In this study, WES from 20 patients with high myopia were performed to find disease-associated mutations, followed by pathway, GO enrichment and network analysis (Materials and Methods). From WES, we generated an average of 10.7 Gb of sequence with  $121\times$  mean target coverage for each individual as paired-end, 150 bp reads. All samples had more than 95% of target bases covered by at least 10 reads. There are an average of 49,904 Single Nucleotide Polymorphisms (SNPs) and small indels called per sample. After multiple steps of variant filtering considering Minor Allele Frequency (MAF), functional impact, phenotype associations and QTL overlapping information, a total of 20 potential pathogenic (three splicing, one frame-shift, and 16 deleterious missense) heterozygous variants in 12 genes were identified in 16 patients (Table 1).

There were eight pathogenic variants identified from five known high myopia genes, including *ADAMTS18*, *CSMD1*, *P3H2*, *RPGR* and *SLC39A5*. Patient R0020 had a clear molecular diagnosis with one missense variant rs756666376 in gene *SLC39A5* (MYP24, autosomal dominant inheritance). Rs756666376 (p.Gly293Arg) is extremely rare in healthy populations, absent in 1000G data, with only one heterozygote in 4,325 East Asians (MAF = 0.00012) and two heterozygotes in 33,359 Non-Finnish Europeans (MAF = 0.00003) in the ExAC database. The SNP is highly conserved and predicted to be deleterious by all of the eight computational methods in our variant annotation. The *RPGR* gene is located on the Xp21.1 and plays a role in ciliogenesis and photoreceptor integrity. In gene *RPGR*, sample R0015 had a heterozygous loss-of-function mutation in the second intron (c.154+4A>G) potentially affecting splicing as predicted by the adaptive boosting method in the splicing consensus region (Jian, Boerwinkle & Liu, 2014) and sample R0016 had a rare heterozygous pathogenic missense mutation (rs774982456, c.1832A>G).

Other than the known high myopia genes, we also discovered 12 rare potentially pathogenic heterozygous variants located in other MYP QTL regions, suggesting potential new candidate genes explaining these QTLs. Among these ocular disease related or direct high myopia related genes, five genes (*CSMD1*, *HSPG2*, *RPGR*, *SEMA4A* and *USH2A*) have pathogenic variants in multiple patients.

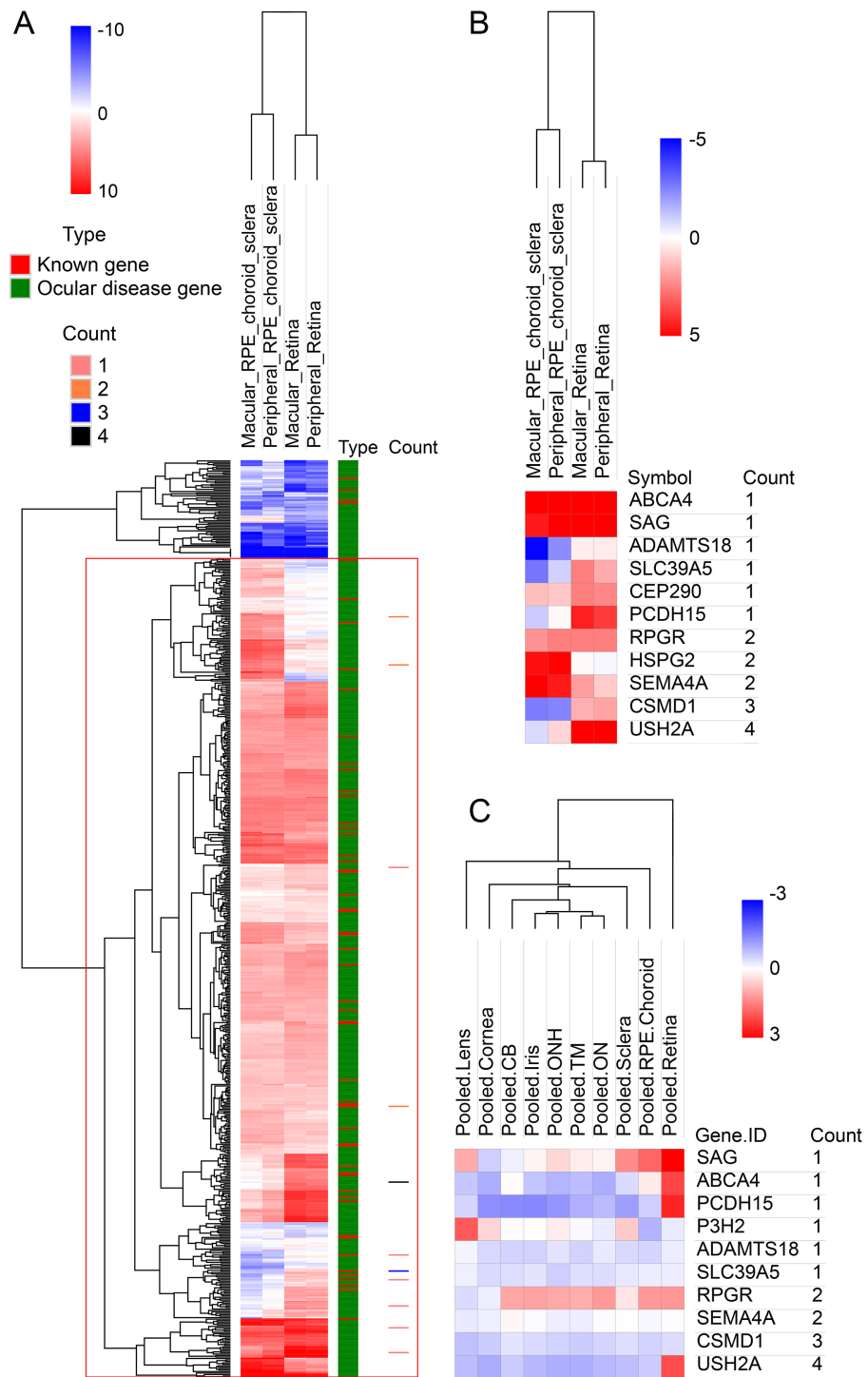
To look deep into the genes with pathogenic variants associated with high myopia, we assembled a list of 709 genes associated with eye diseases (details in variant filtering

Table 1 Variants identified in patients with high myopia.

Gene	Inheritance	Type	Patient ID	Position	Variations		dbSNP	QTL
					Nucleotide	Amino acid		
ABCA4	AR	Ocular disease gene	R0025	1:94490591	c.4553G>A	p.Ser1518Asn	-	MYP14
ADAMTS18	AR	Known HM gene	R0017	16:77334288	c.2546G>A	p.Gly849Asp	-	-
CEP290	AR	Ocular disease gene	R0031	12:88519100	c.1112T>C	p.Val371Ala	-	MYP3
CSMD1	AR	Known HM gene	R0026	8:2975943	c.6408C>G	p.Asn2136Lys	rs778164827	MYP10
			R0027	8:3165341	c.3826G>A	p.Glu1276Lys	rs534926586	MYP10
HSPG2	AR	Ocular disease gene	R0029	8:3165341	c.3826G>A	p.Glu1276Lys	rs534926586	MYP10
			R0023	1:22154919	c.12238G>A	p.Val4080Met	-	MYP14
P3H2	AR	Known HM gene	R0011	1:22207207	c.1940G>A	p.Arg647Gln	-	MYP14
			R0031	3:189692463	c.1336C>T	p.Leu446Phe	-	-
PCDH15	AR	Ocular disease gene	R0018	10:55973726	c.1081_1082delGA	p.Asp361LeufsTer6	-	MYP15
RPGR	X-linked	Known HM gene	R0016	X:38147035	c.1832A>G	p.Asn611Ser	rs774982456	MYP13
			R0015	X:38182648	c.154+4A>G	Splicing	rs764483977	MYP13
SAG	AR	Ocular disease gene	R0021	2:234217902	c.72_75+15delATC GGTGAGTGGT GCACAA	Splicing	rs771810575	MYP12
			R0019	1:156146474	c.1972C>T	p.Arg658Trp	rs756847201	MYP14
SEMA4A	AR/AD	Ocular disease gene	R0012	1:156126310	c.245A>G	p.Glu82Gly	-	MYP14
			R0020	12:56629416	c.877G>A	p.Gly293Arg	rs756666376	MYP24
USH2A	AR	Ocular disease gene	R0018	1:216419965	c.2771C>T	p.Pro924Leu	-	MYP14
			R0019	1:216372968	c.3811+1G>A	Splicing	-	MYP14
USH2A	AR	Ocular disease gene	R0014	1:215821999	c.14453C>T	p.Pro4818Leu	rs143344549	MYP14
			R0015	1:216498726	c.1064T>C	p.Val355Ala	rs746683099	MYP14

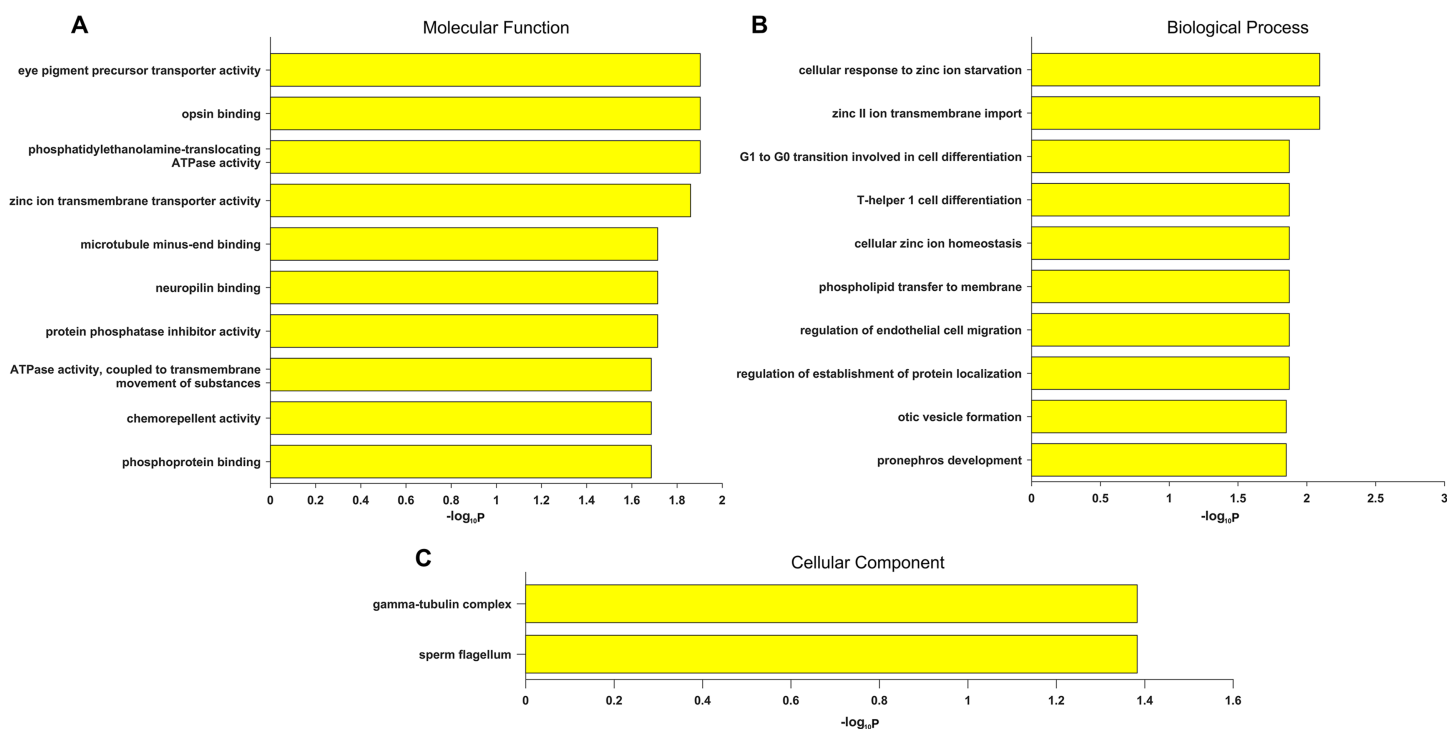
**Notes:**

Chromosome position in accordance with GRCh37/hg19 assembly.  
The HM is abbreviation of the high myopia.



**Figure 2 Hierarchical cluster analysis of gene expression.** (A) The heatmap of 709 known genes associated with eye diseases in RNA-seq expression profile. The 709 genes were divided into known high myopia genes and ocular disease genes, as shown in different colors in type column. Count column represents the sample count of pathogenic variants for our candidate genes. (B) The heatmap of candidate genes in RNA-seq expression profile. (C) The heatmap of candidate genes in microarray expression profile.

Full-size DOI: [10.7717/peerj.5552/fig-2](https://doi.org/10.7717/peerj.5552/fig-2)

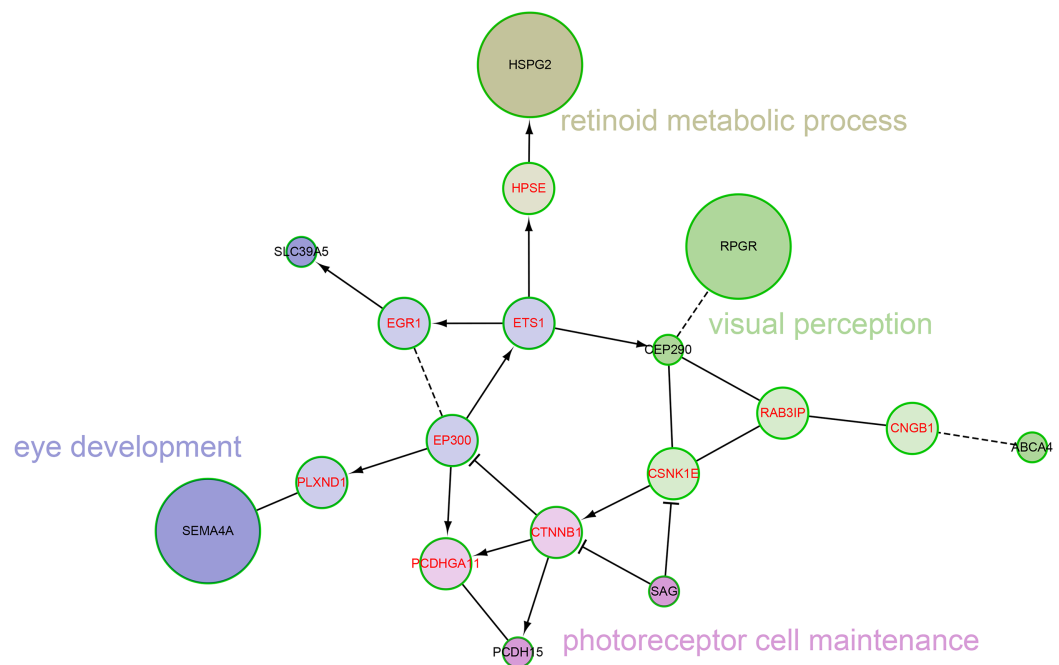


**Figure 3** Top 10 significantly enriched GO terms only in candidate genes and not in the 709 eye disease genes. (A) Molecular function. (B) Biological process. (C) Cellular component.  $x$ -axis is the  $-\log_{10}$  (BH adjusted  $P$ -value) from hypergeometric test of enrichment analysis. Complete list of enriched GO terms in [Table S3](#). [Full-size](#) DOI: [10.7717/peerj.5552/fig-3](https://doi.org/10.7717/peerj.5552/fig-3)

section of Materials and Methods), including 58 known high myopia genes and 651 ocular disease genes ([Table S2](#)). Clinical phenotype related studies have shown that retinal, choroidal, and scleral lesions are high-frequency phenotypes in high myopia patients ([Brussee et al., 2014](#); [Gupta et al., 2015, 2017](#)). It would be natural to assume that myopia related genes would be highly expressed in retina and sclera tissues. To test this hypothesis, we first explored the expression pattern of all the 709 eye disease genes. The results showed that in the RNA-seq dataset, the expression of 709 genes was significantly higher than that of other genes in retina, retinal pigment epithelium, choroid and sclera ( $p < 0.001$ ; [Fig. S1](#)). All candidate genes in our own high myopia cohort were located in the positive expression class when using the 709 eye disease genes as background ([Fig. 2A](#)). Looking further into the candidate gene expression differences among the three tissues, we found that five genes (*PCDH15*, *CEP290*, *SLC39A5*, *CSMD1* and *USH2A*) have much higher expression in retinas (macular retina and peripheral retina), while *ABCA4*, *SAG*, *RPGR*, and *SEMA4A* are highly expressed in both sclera and retina ([Fig. 2B](#)). Microarray dataset confirmed the higher expression in retina for genes *PCDH15* and *USH2A* ([Fig. 2C](#)).

Gene Ontology term enrichment was carried out for a comprehensive functional analysis of the 12 genes found in this study. Because our bioinformatics pipeline has a filtering step to include only eye disease related genes, this could lead to potential bias in the enrichment signal. Thus we did two GO enrichment analyses using either our 12 genes,





**Figure 4** The network modules associated with high myopia candidate genes and linker genes in the functional interaction (FI) network. This network can be divided into four network modules. The background colors of genes represent different modules. Principal biological process of each module was described. The candidate genes are black in text color, and the linker genes are red in text color. The circle size of each candidate gene is proportional to the number of samples with pathogenic variants for the gene. [Full-size](#) DOI: 10.7717/peerj.5552/fig-4

or the 709 eye disease genes (complete list of enriched GO terms from both analyses are in [Table S3](#)). Other than the enriched GO terms like visual perception, photoreceptor cell maintenance, retinoid metabolic process shared with the 709 genes, significant enrichment was uniquely observed in our candidate genes in eye pigment precursor transporter activity, opsin binding, zinc ion transmembrane related processes, microtubule binding etc. ([Fig. 3](#)). Interestingly, the visual phototransduction pathway was enriched by pathway analysis. Visual phototransduction is the photochemical reaction that takes place when light is converted to an electric signal in the retina ([Smith, Sivaprasad & Chong, 2016](#)). It's not surprising the malfunction of genes in this pathway will potentially lead to myopia. This pathway was also found highly enriched in differentially expressed genes during myopia development in a mouse study ([Metlapally et al., 2016](#)), which corroborated these genes' potential causal roles in high myopia.

We also explored the network characteristics in a FI network ([Wu, Feng & Stein, 2010](#)). Focused on the 12 genes from our own cohort, except for the direct interaction between *RPGR* and *CEP290*, other genes are scattered, but all genes can be linked to form a large network by some linker genes, such as *EP300*, *CTNNB1* ([Fig. 4](#)). A network clustering algorithm ([Newman, 2006](#)) divided this network into four network modules. The principal biological process of each module was eye development, retinoid metabolic process, visual perception and photoreceptor cell maintenance, respectively. There were some differences between modules in biological processes, but they were all related to

vision. When using the 709 genes as background, only GO term photoreceptor cell cilium was found enriched in the modules (Fig. S2). *EP300* encodes protein p300, which is a transcriptional co-activator regulating hundreds of genes' transcription via chromatin remodeling, and is important in the processes of cell proliferation and differentiation. Mutations in *EP300* are known to cause Rubinstein–Taybi syndrome (RTS). Interestingly, RTS patients with *EP300* mutations were frequently documented with severe myopia phenotype (Bartholdi et al., 2007; Fergelot et al., 2016). Various visual defects (including familial exudative vitreoretinopathy, retina detachment and myopia etc.) have also been reported in recent WES studies of people with mutations in *CTNNB1* (Li et al., 2017; Panagiotou et al., 2017).

## DISCUSSION

Whole genome sequencing and WES have now entered medical practice (Biesecker & Green, 2014). Whole genome sequencing is the most powerful method of disease gene identification, because this method targets both coding and regulatory non-coding variants, and can identify disease genes caused by SNVs, large indels, and other large structure variants as well. However, its cost is still daunting. Some previous studies have estimated that ~85% of Mendelian disease mutations are located within the coding region, canonical splice acceptor and donor sites (Stenson et al., 2017). Thus the WES becomes the sweet spot rather than Whole Genome Sequencing (WGS) (Teer & Mullikin, 2010). We used WES to identify causal gene mutations in 20 patients with high myopia. Potential pathogenic mutations were identified in 16 patients (80.0%, 16/20). A total of 20 mutations in 12 genes were potentially pathogenic variants for high myopia. Compared to the discovery rate in previous studies (Chen et al., 2013; Kloss et al., 2017; Sun et al., 2015), our phenotype driven filtering method yielded higher diagnostic rate.

In our study, *SEMA4A*, *RPGR*, and *HSPG2* each had two pathogenic mutations detected in our probands. Gene *SEMA4A* encodes a member of the semaphorin family of soluble and transmembrane proteins. This gene maps to chromosome 1p36, which is located within the MYP14 locus (Abid et al., 2006; Wojciechowski et al., 2006). Mutation in codon 345 from G to C and in codon 350 from T to G in exon 10 of the *SEMA4A* gene resulted in an asp345-to-his (D345H) and a phe350-to-cys (F350C) substitution, respectively. A link between those two mutations and retinitis pigmentosa and retinal degeneration has been demonstrated (Abid et al., 2006; Berger, Kloeckener-Gruissem & Neidhardt, 2010). Retinitis pigmentosa GTPase regulator (*RPGR*) is one of the main genes causing X-linked retinitis pigmentosa (XLRP). It was suggested that over 70% of XLRP cases were caused by *RPGR* mutations (Breuer et al., 2002; Sharon et al., 2003). Multiple studies have shown that female heterozygous *RPGR* mutation carriers had early-onset high myopia in one of two eyes, especially those with protein truncating variants (Jin et al., 2006; Yokoyama et al., 2001). Some families showed even full penetrance of high myopia in heterozygous carriers (Parmeggiani et al., 2016). Both patients with a *RPGR* mutation are female carriers in our study. The incomplete penetrance of XLRP phenotype of other *RPGR* carriers suggested that annual follow up examinations checking symptoms of Retinitis Pigmentosa (RP) is warranted. Gene *HSPG2*

encodes a protein called perlecan (Farach-Carson & Carson, 2007; Warren et al., 2015). Perlecan is a heparan sulfate proteoglycan, which interacts with many other proteins and has a variety of functions, such that mutation of the gene has pleiotropic effects. Schwartz–Jampel syndrome is a rare autosomal recessive skeletal dysplasia with myotonia, short stature, and low-set ears and myopia (Kubrey, Solanki & Agrawal, 2015). The two damaging missense mutations (c.12238G>A and c.1940G>A) in *HSPG2* each occurred in one patient. In our current cohort, four heterozygote variants in gene *USH2A* were identified, which fall within QTL MYP14. *USH2A* is a large gene with 73 exons and encodes at least two different isoforms. The mutations in the *USH2A* genes are responsible for 5~10% of the cases with retinitis pigmentosa and 60~90% of the cases with Usher syndrome type II (Baux et al., 2014; Pennings et al., 2004; Slijkerman et al., 2016).

Gene *ABCA4* is specifically expressed in cone and rod photoreceptor outer segments (Molday, Rabin & Molday, 2000). The *ABCA4* protein is active following phototransduction. *ABCA4* mutations can result in multiple vision related phenotypes (Cideciyan et al., 2009; Klevering et al., 2004; Shroyer et al., 1999; Valverde et al., 2007) including Retinitis pigmentosa, Fundus flavimaculatus, Cone-rod dystrophy, and Stargardt disease (Lin et al., 2016), which is characterized by juvenile macular degeneration. In addition, a missense variant c.1268A>G in *ABCA4* was recently found to be responsible for myopia (D'Angelo et al., 2017). A novel missense rare variant (not observed in 1000G, ESP, or ExAC database) c.4553G>A (p.Ser1518Asn) was found in our patient R0025. Yzer et al. (2012) made a mutation analysis for gene *CEP290* and found that patients with a different mutation c.5587–1G>C showed myopia. Based on linkage and haplotype analysis, Nallasamy et al. (2007) identified a presumptive myopia locus in gene *PCDH15*. A novel frameshift mutation (c.1081\_1082delGA) was found in one patient R0018. These functional evidences suggested that carriers of high impact variants in these severe recessive inheritance ocular disease genes might be at higher risk for high myopia. When we relaxed the computational prediction filtering criteria for missense variants to at least five or four out of eight methods, we obtained one or two extra candidate variants (Table S4). This suggested that our pipeline is relatively robust to the computational prediction cut-offs. Despite published evidences of the connections with myopia for the genes found in our cohort, none of the candidate variants were found in previous published sequencing studies of myopia (Aldahmesh et al., 2013; Chen et al., 2013; Jiang et al., 2014; Jin et al., 2017; Kloss et al., 2017; Shi et al., 2011; Sun et al., 2015; Tran-Viet et al., 2013), which highlighted the strong heterogeneity even in familial high myopia. The molecular functional impact of these mutations on the penetrance and severity of various ocular disease phenotypes would need further functional studies.

There are some limitations to our current study due to the small sample size and availability of family genetic data. Although our phenotype driven analysis approach has boosted the probabilities of the final variants associated with high myopia, the statistical significance of any genuine associated genes would need a large sample size gene-level burden test (Lee et al., 2014). Alternatively, extra genetic validation of the causal relationship of these variants with high myopia would require family segregation

analysis with extended family phenotyping and genotyping. In our study, R0027 (daughter) and R0029 (father) are the only two samples from the same family. After our bioinformatics filtering process, there are two candidate variants left in both patients and both variants are shared and consistent with Mendelian inheritance. One is the heterozygote c.3826G>A SNP in *CSMD1* listed in [Table 1](#), the other is a missense variant c.2338C>G (p.Pro780Ala) in *COL4A5* on chromosome X with the daughter's genotype being a heterozygote and the father's genotype being a hemizygote. Deletions or pathogenic missense variants in *COL4A5* are known to cause X-linked dominant Alport syndrome ([Knebelmann et al., 1996](#)), however, neither patient exhibits the clinical phenotypes of Alport syndrome. Therefore, this *COL4A5* variant was excluded from our final candidate list.

## CONCLUSIONS

There is a large proportion of genetic heritability of high myopia still unexplained by known myopia genes. WES enables thorough and unbiased genetic analysis of candidate genes as well as novel gene discoveries. In this study, we took a novel bioinformatics screening approach combining ocular disease gene annotation, myopia phenotype to gene association, and rare variant functional effect filtering narrowing down potential causal variants. Systematic mutation analysis of high myopia genes was further analyzed by FI network, GO and pathway enrichment, which expanded our current understanding of high myopia. These variants, especially those on novel high myopia genes, expanded the mutation spectrum of myopia genes and provided clues for further genetic screening targets.

## ACKNOWLEDGEMENTS

We would like to thank all the patients for participating in this study, and Dr. Yongcheng Dong and Xin Ma for feedback on bioinformatics analysis.

## ADDITIONAL INFORMATION AND DECLARATIONS

### Funding

This work was supported by Specialized Research Fund for the Doctoral Program of Higher Education (Grant No. 20130181110079) and the National Major Scientific Equipment program (Grant No. 2012YQ12008005). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Grant Disclosures

The following grant information was disclosed by the authors:

Specialized Research Fund for the Doctoral Program of Higher Education:  
20130181110079.

National Major Scientific Equipment program: 2012YQ12008005.

### Competing Interests

The authors declare that they have no competing interests.

## Author Contributions

- Ling Wan conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Boling Deng performed the experiments, approved the final draft.
- Zhengzheng Wu performed the experiments, approved the final draft.
- Xiaoming Chen conceived and designed the experiments, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.

## Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Approval for the study was provided by the Institutional Review Board of Sichuan Provincial People's Hospital.

## Data Availability

The following information was supplied regarding data availability:

The raw sequence data reported in this paper are deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2017), Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession numbers [CRA000764](#) and [CRA000769](#), publicly accessible at <http://bigd.big.ac.cn/gsa>.

## Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.5552#supplemental-information>.

## REFERENCES

- Abid A, Ismail M, Mehdi SQ, Khaliq S. 2006. Identification of novel mutations in the SEMA4A gene associated with retinal degenerative diseases. *Journal of Medical Genetics* 43(4):378–381 DOI 10.1136/jmg.2005.035055.
- Aldahmesh MA, Khan AO, Alkuraya H, Adly N, Anazi S, Al-Saleh AA, Mohamed JY, Hijazi H, Prabakaran S, Tacke M, Al-Khrashi A, Hashem M, Reinheckel T, Assiri A, Alkuraya FS. 2013. Mutations in LRPAP1 are associated with severe myopia in humans. *American Journal of Human Genetics* 93(2):313–320 DOI 10.1016/j.ajhg.2013.06.002.
- Andrew T, Maniatis N, Carbonaro F, Liew SH, Lau W, Spector TD, Hammond CJ. 2008. Identification and replication of three novel myopia common susceptibility gene loci on chromosome 3q26 using linkage and linkage disequilibrium mapping. *PLOS Genetics* 4(10):e1000220 DOI 10.1371/journal.pgen.1000220.
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G, The Gene Ontology Consortium. 2000. Gene ontology: tool for the unification of biology. *Nature Genetics* 25(1):25–29 DOI 10.1038/75556.
- Bartholdi D, Roelfsema JH, Papadia F, Breuning MH, Niedrist D, Hennekam RC, Schinzel A, Peters DJM. 2007. Genetic heterogeneity in Rubinstein–Taybi syndrome: delineation of the

phenotype of the first patients carrying mutations in EP300. *Journal of Medical Genetics* 44(5):327–333 DOI 10.1136/jmg.2006.046698.

- Baux D, Blanchet C, Hamel C, Meunier I, Larrieu L, Faugere V, Vache C, Castorina P, Puech B, Bonneau D, Malcolm S, Claustres M, Roux AF. 2014.** Enrichment of LOVD-USHbases with 152 *USH2A* genotypes defines an extensive mutational spectrum and highlights missense hotspots. *Human Mutation* 35(10):1179–1186 DOI 10.1002/humu.22608.
- Berger W, Kloeckener-Gruissem B, Neidhardt J. 2010.** The molecular basis of human retinal and vitreoretinal diseases. *Progress in Retinal and Eye Research* 29(5):335–375 DOI 10.1016/j.preteyeres.2010.03.004.
- Biesecker LG, Green RC. 2014.** Diagnostic clinical genome and exome sequencing. *New England Journal of Medicine* 370(25):2418–2425 DOI 10.1056/NEJMra1312543.
- Breuer DK, Yashar BM, Filippova E, Hiriyan S, Lyons RH, Mears AJ, Asaye B, Acar C, Vervoort R, Wright AF, Musarella MA, Wheeler P, MacDonald I, Iannaccone A, Birch D, Hoffman DR, Fishman GA, Heckenlively JR, Jacobson SG, Sieving PA, Swaroop A. 2002.** A comprehensive mutation analysis of RP2 and RPGR in a North American cohort of families with X-linked retinitis pigmentosa. *American Journal of Human Genetics* 70(6):1545–1554 DOI 10.1086/340848.
- Brussee C, Buitendijk GHS, Springelkamp H, Snabel M, Luyten GPM, Van Rijn GA, Boon CJF, Geerards A, Verhoeven VJM, Klaver CCW. 2014.** Frequency of retinal pathology in high myopia. The MYST study. *Investigative Ophthalmology & Visual Science* 55:3617.
- Chen Y, Zhang Q, Shen T, Xiao X, Li S, Guan L, Zhang J, Zhu Z, Yin Y, Wang P, Guo X, Wang J, Zhang Q. 2013.** Comprehensive mutation analysis by whole-exome sequencing in 41 Chinese families with Leber congenital amaurosis. *Investigative Ophthalmology & Visual Science* 54(6):4351–4357 DOI 10.1167/iovs.13-11606.
- Cideciyan AV, Swider M, Aleman TS, Tsybovsky Y, Schwartz SB, Windsor EA, Roman AJ, Sumaroka A, Steinberg JD, Jacobson SG, Stone EM, Palczewski K. 2009.** ABCA4 disease progression and a proposed strategy for gene therapy. *Human Molecular Genetics* 18(5):931–941 DOI 10.1093/hmg/ddn421.
- D'Angelo R, Donato L, Venza I, Scimone C, Aragona P, Sidoti A. 2017.** Possible protective role of the ABCA4 gene c.1268A>G missense variant in Stargardt disease and syndromic retinitis pigmentosa in a Sicilian family: preliminary data. *International Journal of Molecular Medicine* 39(4):1011–1020 DOI 10.3892/ijmm.2017.2917.
- Dirani M, Chamberlain M, Shekar SN, Islam AF, Garoufalos P, Chen CY, Guymer RH, Baird PN. 2006.** Heritability of refractive error and ocular biometrics: the Genes in Myopia (GEM) twin study. *Investigative Ophthalmology & Visual Science* 47(11):4756–4761 DOI 10.1167/iovs.06-0270.
- Farach-Carson MC, Carson DD. 2007.** Perlecan—a multifunctional extracellular proteoglycan scaffold. *Glycobiology* 17(9):897–905 DOI 10.1093/glycob/cwm043.
- Fergelot P, Van Belzen M, Van Gils J, Afenjar A, Armour CM, Arveiler B, Beets L, Burglen L, Busa T, Collet M, Deforges J, De Vries BB, Dominguez Garrido E, Dorison N, Dupont J, Francannet C, Garcia-Minaur S, Gabau Vila E, Gebre-Medhin S, Gener Querol B, Genevieve D, Gerard M, Gervasini CG, Goldenberg A, Josifova D, Lachlan K, Maas S, Maranda B, Moilanen JS, Nordgren A, Parent P, Rankin J, Reardon W, Rio M, Roume J, Shaw A, Smigiel R, Sojo A, Solomon B, Stembalska A, Stumpel C, Suarez F, Terhal P, Thomas S, Touraine R, Verloes A, Vincent-Delorme C, Wincent J, Peters DJ, Bartsch O, Larizza L, Lacombe D, Hennekam RC. 2016.** Phenotype and genotype in 52 patients with

- Rubinstein-Taybi syndrome caused by EP300 mutations. *American Journal of Medical Genetics Part A* **170**(12):3069–3082 DOI [10.1002/ajmg.a.37940](https://doi.org/10.1002/ajmg.a.37940).
- Guo H, Tong P, Liu Y, Xia L, Wang T, Tian Q, Li Y, Hu Y, Zheng Y, Jin X, Li Y, Xiong W, Tang B, Feng Y, Li J, Pan Q, Hu Z, Xia K. 2015.** Mutations of P4HA2 encoding prolyl 4-hydroxylase 2 are associated with nonsyndromic high myopia. *Genetics in Medicine* **17**(4):300–306 DOI [10.1038/gim.2015.28](https://doi.org/10.1038/gim.2015.28).
- Gupta P, Saw SM, Cheung CY, Girard MJA, Mari JM, Bhargava M, Tan C, Tan M, Yang A, Tey F, Nah G, Zhao P, Yin Wong T, Cheng C-Y. 2015.** Choroidal thickness and high myopia: a case–control study of young Chinese men in Singapore. *Acta Ophthalmologica* **93**(7):e585–e592 DOI [10.1111/aos.12631](https://doi.org/10.1111/aos.12631).
- Gupta P, Thakku SG, Saw SM, Tan M, Lim E, Tan M, Cheung CMG, Wong TY, Cheng CY. 2017.** Characterization of choroidal morphologic and vascular features in young men with high myopia using spectral-domain optical coherence tomography. *American Journal of Ophthalmology* **177**:27–33 DOI [10.1016/j.ajo.2017.02.001](https://doi.org/10.1016/j.ajo.2017.02.001).
- Hammond CJ, Snieder H, Gilbert CE, Spector TD. 2001.** Genes and environment in refractive error: the twin eye study. *Investigative Ophthalmology & Visual Science* **42**(6):1232–1236.
- Holden BA, Fricke TR, Wilson DA, Jong M, Naidoo KS, Sankaridurg P, Wong TY, Naduvilath TJ, Resnikoff S. 2016.** Global prevalence of myopia and high myopia and temporal trends from 2000 through 2050. *Ophthalmology* **123**(5):1036–1042 DOI [10.1016/j.ophtha.2016.01.006](https://doi.org/10.1016/j.ophtha.2016.01.006).
- Huang DW, Sherman BT, Lempicki RA. 2009.** Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Research* **37**(1):1–13 DOI [10.1093/nar/gkn923](https://doi.org/10.1093/nar/gkn923).
- Jian X, Boerwinkle E, Liu X. 2014.** In silico prediction of splice-altering single nucleotide variants in the human genome. *Nucleic Acids Research* **42**(22):13534–13544 DOI [10.1093/nar/gku1206](https://doi.org/10.1093/nar/gku1206).
- Jiang D, Li J, Xiao X, Li S, Jia X, Sun W, Guo X, Zhang Q. 2014.** Detection of mutations in LRPAP1, CTSB, LEPREL1, ZNF644, SLC39A5, and SCO2 in 298 families with early-onset high myopia by exome sequencing. *Investigative Ophthalmology & Visual Science* **56**(1):339–345 DOI [10.1167/iovs.14-14850](https://doi.org/10.1167/iovs.14-14850).
- Jin ZB, Liu XQ, Hayakawa M, Murakami A, Nao-i N. 2006.** Mutational analysis of RPGR and RP2 genes in Japanese patients with retinitis pigmentosa: identification of four mutations. *Molecular Vision* **12**:1167–1174.
- Jin ZB, Wu J, Huang XF, Feng CY, Cai XB, Mao JY, Xiang L, Wu KC, Xiao X, Kloss BA, Li Z, Liu Z, Huang S, Shen M, Cheng FF, Cheng XW, Zheng ZL, Chen X, Zhuang W, Zhang Q, Young TL, Xie T, Lu F, Qu J. 2017.** Trio-based exome sequencing arrests de novo mutations in early-onset high myopia. *Proceedings of the National Academy of Sciences of the United States of America* **114**(16):4219–4224 DOI [10.1073/pnas.1615970114](https://doi.org/10.1073/pnas.1615970114).
- Katz J, Tielsch JM, Sommer A. 1997.** Prevalence and risk factors for refractive errors in an adult inner city population. *Investigative Ophthalmology & Visual Science* **38**:334–340.
- Kempner JH, Mitchell P, Lee KE, Tielsch JM, Bromam AT, Taylor HR, Ikram MK, Congdon NG, O’Colmain BJ. 2004.** The prevalence of refractive errors among adults in the United States, Western Europe, and Australia. *Archives of Ophthalmology* **122**(4):495–505 DOI [10.1001/archophth.122.4.495](https://doi.org/10.1001/archophth.122.4.495).
- Klevering BJ, Mauergeri A, Wagner A, Go SL, Vink C, Cremers FP, Hoyng CB. 2004.** Three families displaying the combination of Stargardt’s disease with cone–rod dystrophy or retinitis pigmentosa. *Ophthalmology* **111**(3):546–553 DOI [10.1016/j.ophtha.2003.06.010](https://doi.org/10.1016/j.ophtha.2003.06.010).

- Kloss BA, Tompson SW, Whisenhunt KN, Quow KL, Huang SJ, Pavelec DM, Rosenberg T, Young TL. 2017. Exome sequence analysis of 14 families with high myopia. *Investigative Ophthalmology & Visual Science* 58(4):1982–1990 DOI 10.1167/iovs.16-20883.
- Knebelmann B, Breillat C, Forestier L, Arrondel C, Jacassier D, Giatras I, Drouot L, Deschenes G, Grunfeld JP, Broyer M, Gubler MC, Antignac C. 1996. Spectrum of mutations in the COL4A5 collagen gene in X-linked Alport syndrome. *American Journal of Human Genetics* 59(6):1221–1232.
- Kubrey S, Solanki D, Agrawal S. 2015. Schwartz-Jampel Syndrome (SJS) a rare entity: case report. *Journal of Evolution of Medical and Dental Sciences* 4(43):7538–7546 DOI 10.14260/jemds/2015/1093.
- Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen GW, Ma'ayan A. 2016. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research* 44(W1):W90–W97 DOI 10.1093/nar/gkw377.
- Lee S, Abecasis GR, Boehnke M, Lin X. 2014. Rare-variant association analysis: study designs and statistical tests. *American Journal of Human Genetics* 95(1):5–23 DOI 10.1016/j.ajhg.2014.06.009.
- Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation Consortium. 2016. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 536(7616):285–291 DOI 10.1038/nature19057.
- Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25(14):1754–1760 DOI 10.1093/bioinformatics/btp324.
- Li YJ, Guggenheim JA, Bulusu A, Metlapally R, Abbott D, Malecaze F, Calvas P, Rosenberg T, Paget S, Creer RC, Kirov G, Owen MJ, Zhao B, White T, Mackey DA, Young TL. 2009. An international collaborative family-based whole-genome linkage scan for high-grade myopia. *Investigative Ophthalmology & Visual Science* 50(7):3116–3127 DOI 10.1167/iovs.08-2781.
- Li N, Xu Y, Li G, Yu T, Yao RE, Wang X, Wang J. 2017. Exome sequencing identifies a de novo mutation of CTNNB1 gene in a patient mainly presented with retinal detachment, lens and vitreous opacities, microcephaly, and developmental delay: case report and literature review. *Medicine* 96(20):e6914 DOI 10.1097/md.0000000000006914.
- Lin B, Cai XB, Zheng ZL, Huang XF, Liu XL, Qu J, Jin ZB. 2016. Clinical and genetic analyses reveal novel pathogenic ABCA4 mutations in Stargardt disease families. *Scientific Reports* 6(1):35414 DOI 10.1038/srep35414.
- Lopes MC, Andrew T, Carbonaro F, Spector TD, Hammond CJ. 2009. Estimating heritability and shared environmental effects for refractive error in twin and family studies. *Investigative Ophthalmology & Visual Science* 50(1):126–131 DOI 10.1167/iovs.08-2385.



- McLaren W, Gil L, Hunt SE, Riat HS, Ritchie GR, Thormann A, Flicek P, Cunningham F. 2016. The ensembl variant effect predictor. *Genome Biology* 17(1):122 DOI 10.1186/s13059-016-0974-4.
- Metlapally R, Park HN, Chakraborty R, Wang KK, Tan CC, Light JG, Pardue MT, Wildsoet CF. 2016. Genome-wide scleral micro- and messenger-RNA regulation during myopia development in the mouse. *Investigative Ophthalmology & Visual Science* 57(14):6089–6097 DOI 10.1167/iovs.16-19563.
- Molday LL, Rabin AR, Molday RS. 2000. ABCR expression in foveal cone photoreceptors and its role in Stargardt macular dystrophy. *Nature Genetics* 25(3):257–258 DOI 10.1038/77004.
- Mordechai S, Gradstein L, Pasanen A, Ofir R, El Amour K, Levy J, Belfair N, Lifshitz T, Joshua S, Narkis G, Elbedour K, Myllyharju J, Birk OS. 2011. High myopia caused by a mutation in LEPREL1, encoding prolyl 3-hydroxylase 2. *American Journal of Human Genetics* 89(3):438–445 DOI 10.1016/j.ajhg.2011.08.003.
- Morgan IG, Ohno-Matsui K, Saw S-M. 2012. Myopia. *Lancet* 379(9827):1739–1748 DOI 10.1016/s0140-6736(12)60272-4.
- Nakanishi H, Yamada R, Gotoh N, Hayashi H, Yamashiro K, Shimada N, Ohno-Matsui K, Mochizuki M, Saito M, Iida T, Matsuo K, Tajima K, Yoshimura N, Matsuda F. 2009. A genome-wide association analysis identified a novel susceptible locus for pathological myopia at 11q24.1. *PLOS Genetics* 5(9):e1000660 DOI 10.1371/journal.pgen.1000660.
- Nallasamy S, Paluru PC, Devoto M, Wasserman NF, Zhou J, Young TL. 2007. Genetic linkage study of high-grade myopia in a Hutterite population from South Dakota. *Molecular Vision* 13(26):229–236.
- Newman MEJ. 2006. Modularity and community structure in networks. *Proceedings of the National Academy of Sciences of the United States of America* 103(23):8577–8582 DOI 10.1073/pnas.0601602103.
- Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, Shendure J. 2009. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 461(7261):272–276 DOI 10.1038/nature08250.
- Panagiotou ES, Sanjurjo Soriano C, Poulter JA, Lord EC, Dzulova D, Kondo H, Hiyoshi A, Chung BH, Chu YW, Lai CHY, Tafoya ME, Karjosukarso D, Collin RWJ, Topping J, Downey LM, Ali M, Inglehearn CF, Toomes C. 2017. Defects in the cell signaling mediator  $\beta$ -catenin cause the retinal vascular condition FEVR. *American Journal of Human Genetics* 100(6):960–968 DOI 10.1016/j.ajhg.2017.05.001.
- Parmeggiani F, Barbaro V, De Nadai K, Lavezzo E, Toppo S, Chizzolini M, Palu G, Parolin C, Di Iorio E. 2016. Identification of novel X-linked gain-of-function RPGR-ORF15 mutation in Italian family with retinitis pigmentosa and pathologic myopia. *Scientific Reports* 6(1):39179 DOI 10.1038/srep39179.
- Pennings RJ, Te Brinke H, Weston MD, Claassen A, Orten DJ, Weekamp H, Van Aarem A, Huygen PL, Deutman AF, Hoefsloot LH, Cremers FP, Cremers CW, Kimberling WJ, Kremer H. 2004. USH2A mutation analysis in 70 Dutch families with Usher syndrome type II. *Human Mutation* 24(2):185 DOI 10.1002/humu.9259.
- Saw SM, Gazzard G, Shih-Yen EC, Chua WH. 2005. Myopia and associated pathological complications. *Ophthalmic and Physiological Optics* 25(5):381–391 DOI 10.1111/j.1475-1313.2005.00298.x.

- Schwartz M, Haim M, Skarsholm D. 1990. X-linked myopia: Bornholm eye disease. Linkage to DNA markers on the distal part of Xq. *Clinical Genetics* 38(4):281–286 DOI 10.1111/j.1399-0004.1990.tb03582.x.
- Sharon D, Sandberg MA, Rabe VW, Stillberger M, Dryja TP, Berson EL. 2003. RP2 and RPGR mutations and clinical correlations in patients with X-linked retinitis pigmentosa. *American Journal of Human Genetics* 73(5):1131–1146 DOI 10.1086/379379.
- Shi Y, Li Y, Zhang D, Zhang H, Li Y, Lu F, Liu X, He F, Gong B, Cai L, Li R, Liao S, Ma S, Lin H, Cheng J, Zheng H, Shan Y, Chen B, Hu J, Jin X, Zhao P, Chen Y, Zhang Y, Lin Y, Li X, Fan Y, Yang H, Wang J, Yang Z. 2011. Exome sequencing identifies ZNF644 mutations in high myopia. *PLOS Genetics* 7(6):e1002084 DOI 10.1371/journal.pgen.1002084.
- Shroyer NF, Lewis RA, Allikmets R, Singh N, Dean M, Leppert M, Lupski JR. 1999. The rod photoreceptor ATP-binding cassette transporter gene, ABCR, and retinal disease: from monogenic to multifactorial. *Vision Research* 39(15):2537–2544 DOI 10.1016/s0042-6989(99)00037-1.
- Slijkerman RW, Vache C, Dona M, Garcia-Garcia G, Claustres M, Heterschijt L, Peters TA, Hartel BP, Pennings RJ, Millan JM, Aller E, Garanto A, Collin RW, Kremer H, Roux AF, Van Wijk E. 2016. Antisense oligonucleotide-based splice correction for USH2A-associated retinal degeneration caused by a frequent deep-intronic mutation. *Molecular Therapy—Nucleic Acids* 5:e381 DOI 10.1038/mtna.2016.89.
- Smith RL, Sivaprasad S, Chong V. 2016. Retinal biochemistry, physiology and cell biology. *Developments in Ophthalmology* 55:18–27 DOI 10.1159/000431118.
- Stenson PD, Mort M, Ball EV, Evans K, Hayden M, Heywood S, Hussain M, Phillips AD, Cooper DN. 2017. The human gene mutation database: towards a comprehensive repository of inherited mutation data for medical research, genetic diagnosis and next-generation sequencing studies. *Human Genetics* 136(6):665–677 DOI 10.1007/s00439-017-1779-6.
- Sudmant PH, Rausch T, Gardner EJ, Handsaker RE, Abyzov A, Huddleston J, Zhang Y, Ye K, Jun G, Fritz MH, Konkel MK, Malhotra A, Stutz AM, Shi X, Casale FP, Chen J, Hormozdiari F, Dayama G, Chen K, Malig M, Chaisson MJP, Walter K, Meiers S, Kashin S, Garrison E, Auton A, Lam HYK, Mu XJ, Alkan C, Antaki D, Bae T, Cerveira E, Chines P, Chong Z, Clarke L, Dal E, Ding L, Emery S, Fan X, Gujral M, Kahveci F, Kidd JM, Kong Y, Lameijer EW, McCarthy S, Flicek P, Gibbs RA, Marth G, Mason CE, Menelaou A, Muzny DM, Nelson BJ, Noor A, Parrish NF, Pendleton M, Quitadamo A, Raeder B, Schadt EE, Romanovitch M, Schlattl A, Sebra R, Shabalina AA, Untergasser A, Walker JA, Wang M, Yu F, Zhang C, Zhang J, Zheng-Bradley X, Zhou W, Zichner T, Sebati J, Batzer MA, McCarroll SA, Mills RE, Gerstein MB, Bashir A, Stegle O, Devine SE, Lee C, Eichler EE, Korbelt JO, The 1000 Genomes Project Consortium. 2015. An integrated map of structural variation in 2,504 human genomes. *Nature* 526(7571):75–81 DOI 10.1038/nature15394.
- Sun W, Huang L, Xu Y, Xiao X, Li S, Jia X, Gao B, Wang P, Guo X, Zhang Q. 2015. Exome sequencing on 298 probands with early-onset high myopia: approximately one-fourth show potential pathogenic mutations in RetNet genes. *Investigative Ophthalmology & Visual Science* 56(13):8365–8372 DOI 10.1167/iovs.15-17555.
- Teer JK, Mullikin JC. 2010. Exome sequencing: the sweet spot before whole genomes. *Human Molecular Genetics* 19(R2):R145–R151 DOI 10.1093/hmg/ddq333.
- Tran-Viet KN, Powell C, Barathi VA, Klemm T, Maurer-Stroh S, Limviphuvadh V, Soler V, Ho C, Yanovitch T, Schneider G, Li YJ, Nading E, Metlapally R, Saw SM, Goh L, Rozen S, Young TL. 2013. Mutations in SCO2 are associated with autosomal-dominant high-grade myopia. *American Journal of Human Genetics* 92(5):820–826 DOI 10.1016/j.ajhg.2013.04.005.

- Valverde D, Riveiro-Alvarez R, Aguirre-Lamban J, Baiget M, Carballo M, Antinolo G, Millan JM, Garcia Sandoval B, Ayuso C. 2007. Spectrum of the ABCA4 gene mutations implicated in severe retinopathies in Spanish patients. *Investigative Ophthalmology & Visual Science* **48**(3):985–990 DOI [10.1167/iops.06-0307](https://doi.org/10.1167/iops.06-0307).
- Vitale S, Sperduto RD, Ferris FL III. 2009. Increased prevalence of myopia in the United States between 1971–1972 and 1999–2004. *Archives of Ophthalmology* **127**(12):1632–1639 DOI [10.1001/archophthalmol.2009.303](https://doi.org/10.1001/archophthalmol.2009.303).
- Warren CR, Kassir E, Spurlin J, Martinez J, Putnam NH, Farach-Carson MC. 2015. Evolution of the perlecan/HSPG2 gene and its activation in regenerating *Nematostella vectensis*. *PLOS ONE* **10**(4):e0124578 DOI [10.1371/journal.pone.0124578](https://doi.org/10.1371/journal.pone.0124578).
- Wojciechowski R, Moy C, Ciner E, Ibay G, Reider L, Bailey-Wilson JE, Stambolian D. 2006. Genomewide scan in Ashkenazi Jewish families demonstrates evidence of linkage of ocular refraction to a QTL on chromosome 1p36. *Human Genetics* **119**(4):389–399 DOI [10.1007/s00439-006-0153-x](https://doi.org/10.1007/s00439-006-0153-x).
- Wu G, Dawson E, Duong A, Haw R, Stein L. 2014. ReactomeFIViz: a Cytoscape app for pathway and network-based data analysis. *F1000Research* **3**:146 DOI [10.12688/f1000research.4431.2](https://doi.org/10.12688/f1000research.4431.2).
- Wu G, Feng X, Stein L. 2010. A human functional protein interaction network and its application to cancer data analysis. *Genome Biology* **11**(5):R53 DOI [10.1186/gb-2010-11-5-r53](https://doi.org/10.1186/gb-2010-11-5-r53).
- Wu LJ, You QS, Duan JL, Luo YX, Liu LJ, Li X, Gao Q, Zhu HP, He Y, Xu L, Jonas JB, Wang W, Guo XH. 2015. Prevalence and associated factors of myopia in high-school students in Beijing. *PLOS ONE* **10**(3):e0120764 DOI [10.1371/journal.pone.0120764](https://doi.org/10.1371/journal.pone.0120764).
- Xiao X, Li S, Jia X, Guo X, Zhang Q. 2016. X-linked heterozygous mutations in ARR3 cause female-limited early onset high myopia. *Molecular Vision* **22**:1257–1266.
- Xu L, Wang Y, Li Y, Wang Y, Cui T, Li J, Jonas JB. 2006. Causes of blindness and visual impairment in urban and rural areas in Beijing: the Beijing eye study. *Ophthalmology* **113**(7):1134.e1–1134.e11 DOI [10.1016/j.ophtha.2006.01.035](https://doi.org/10.1016/j.ophtha.2006.01.035).
- Yokoyama A, Maruiwa F, Hayakawa M, Kanai A, Vervoort R, Wright AF, Yamada K, Niikawa N, Naoi N. 2001. Three novel mutations of the RPGR gene exon ORF15 in three Japanese families with X-linked retinitis pigmentosa. *American Journal of Medical Genetics* **104**(3):232–238 DOI [10.1002/ajmg.10035](https://doi.org/10.1002/ajmg.10035).
- Young TL, Ronan SM, Drahozal LA, Wildenberg SC, Alvear AB, Oetting WS, Atwood LD, Wilkin DJ, King RA. 1998. Evidence that a locus for familial high myopia maps to chromosome 18p. *American Journal of Human Genetics* **63**(1):109–119 DOI [10.1086/301907](https://doi.org/10.1086/301907).
- Yzer S, Hollander AI, Lopez I, Pott JW, De Faber JT, Cremers FP, Koenekoop RK, Van Den Born LI. 2012. Ocular and extra-ocular features of patients with Leber congenital amaurosis and mutations in *CEP290*. *Molecular Vision* **18**:412–425.
- Zhang Q. 2015. Genetics of refraction and myopia. *Progress in Molecular Biology and Translational Science* **134**:269–279 DOI [10.1016/bs.pmbts.2015.05.007](https://doi.org/10.1016/bs.pmbts.2015.05.007).