

Exons sequencing identifies a novel mutation in GPR157 in a high myopia family

Tao Tang

North Sichuan Medical University

Mohan Liu

North Sichuan Medical College

Ting Wei

North Sichuan Medical College

Lin Deng

North Sichuan Medical College

Yueyang Zhang

North Sichuan Medical College

Yu Pu

North Sichuan Medical College

Yan Cai

Affiliated Hospital of North Sichuan Medical College

Qiling Song

Affiliated Hospital of North Sichuan Medical College

Ting Zhang

Affiliated Hospital of North Sichuan Medical College

qingsong liu (✉ liuqingsong00@aliyun.com)

North Sichuan Medical University <https://orcid.org/0000-0001-5261-2162>

Research article

Keywords: high myopia, WES, GPR157

Posted Date: May 18th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-21983/v1>

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Abstract

Background Next-generation sequencing (NGS) and whole exome sequencing (WES) have identified many potential disease-causing loci and genetic mutations of high myopia(HM). However, these known genes can only explain the heritability of a small proportion of HM patients. A large proportion of variants have yet to be discovered. Herein we aimed to investigate the genetic characteristics of HM through a Chinese HM family(the inheritance pattern unknown) .

Methods We performed WES on the parent-offspring trio and identified mutations by Sanger sequencing. All the members in this family were sequenced to validate phenotype co-segregated with candidate genes via Sanger sequencing as well. Besides, mutations detected were further evaluated in a cohort of 110 sporadic high myopia controls and 200 unrelated ethnically-matched controls. And reverse transcription PCR(RT-PCR) was applied to measure the mRNA expression levels of GPR157 in the 4-week-old KM mice.

Results A novel heterozygous nonsense mutation, c.859C>T (p.Arg287*) of GPR157 gene, was detected in the proband and her father by WES. And this disease-associated mutation was not found in 310 control individuals. For the family under study, HM was classified as autosomal dominant inheritance with reduced penetrance. And RT-PCR results showed GPR157 was abundantly expressed in the eye.

Conclusion The hybrid nonsense mutation of the GPR157 gene identified in this study may constitute a novel genetic cause of HM. **Keywords** :high myopia, WES, GPR157

Background

Myopia (short-sightedness or near-sightedness), is a common eye disorder worldwide, in which the image of distant objects focus in front of the retina^[1]. Reports noted that 80%-90% of teenagers and young adults in urban areas of East and Southeast Asia are myopic eyes^[1-3]. The prevalence rate of HM is 0.5-5% worldwide and higher in China. It reaches nearly 10% according to a population-based epidemiological investigation in 2018, in which school-aged children students aged 10-15 years old or older in the western of China involved^[4]. The incidence of refractive error, particularly myopia, is increasing worldwide. Wojciechowski R^[5] estimated there are 2.5 billion people with myopia by the year 2020. When ocular accommodation is relaxed, myopia can be divided into low myopia (the spherical equivalent refractive error is ≤ -0.5 and ≥ -6 D) and HM (the spherical equivalent refractive error is ≤ -6.00 D)^[6]. HM is the focus of research in the field of ophthalmology since it gives rise to elevated incidences of its associated complications, such as macular degeneration, cataract, glaucoma^[7-9] and so on. Note that not all highly myopic eyes have fundus lesions—the most critical difference between HM and pathological myopia (PM, high myopia accompanied by the presence of diffuse atrophy in the fundus)^[10].

Myopia can sometimes be associated with symptoms in other diseases. Thus, HM can be divided into non-syndromic myopia (myopia occurs alone) and syndromic myopia (myopia couples with other ocular diseases or systemic diseases). And most cases of HM are isolated forms^[11]. Myopia can be inherited in an autosomal dominant, autosomal recessive and X-linked recessive manner. The exact pathogenesis of myopia remains unclear, but it's believed to encompass environmental factors and genetic factors. Many studies indicate that genetic variation contributes to the development of myopia, extremely HM. To date, based on pedigree studies with WES and bioinformatic works, many disease-causing genes have been uncovered, such as BSG, SCO2, CCDC111, P4HA2, ZNF644, and so on^[12-16]. Nevertheless, these known genes were detected in a limited number of cases-hence they are still regarded as yet unidentified causative genes.

In the present study, we detected a novel heterozygous nonsense mutation of GPR157 via WES.

Methods

Participants and clinical data

All procedures used in this study adhered to the tenets of the Declaration Helsinki. The Institutional Review Board and the Ethics Committee of Affiliated of North Sichuan Medical College approved the present study. Written informed consent was obtained from the patients in this study.

For this study, we recruited a three-generation family with HM included seven members, 2/7 presented with HM. Further, 110 sporadic high myopia controls and 200 unrelated ethically-matched controls were involved.

Mutation analysis

Genomic DNA was extracted from peripheral venous leukocyte using the Relax Gene

Blood DNA Extraction kit according to the manufacturer's instructions (Tiangen Beijing, China). Twenty ugs of genomic DNA from the trio were sheared into about 500bp DNA fragments randomly. Exome capture was conducted to collect the exons of human genome DNA by a Nimble Gen 2.1M HD array as described in the protocol (Roche NimbleGen, Inc, Madison, WI, USA). Then the exon-enriched DNA libraries were subject to a second library construction and were sequenced via the Illumina Hiseq 2000 Sequencing System (Illumina, SanDiego, USA). The sequencing results were analyzed utilizing a series of bioinformatic work to screen the potentially disease-causing variants. Moreover, we performed Sanger sequencing on all members of this family via ABL 3730XL Genetic Analyzer, using the following primers: GPR157-F, 5'-CAA CAG CGC GTG TGT TTC TG-3'; GPR157-R, 5'-GCA GGG ACT CAC AGA AGT GC-3'. Confirmed variants were further validated in all 310 controls using Sanger sequencing as well.

GPR157 expression

Total RNA in various tissues (heart, kidney, spleen, eye, testis, liver, lung and brain)

from the 4-week-old KM mice were extracted by Trizol. CDNA was synthesized utilizing the Hiscript® III Reverse Transcriptase (Vazyme). The obtained cDNAs were subjected to PCR (Applied Biosystems) by using Prime STAR® Max DNA Polymerase (TaKaRa). The relative mRNA expression levels of GPR157 were normalized to GAPDH. Primers used were as follows: GPR157-F, 5'-CGC CAA CAC CAG CTC CTT CTT C-3'; GPR157-R, 5'-GAC ACG GTC CTC AGC CTC CAG-3'. The KM mice were purchased from the Chengdu Dossy Experimental Animals Company, China. The animal care guidelines are comparable with those published by the Institute for Laboratory Animal Research (NIH Publications No 8023, revised 1978). All of the animal studies were approved by the Animal Care and Ethics Committee at North Sichuan Medical College, China.

Statistical analysis

Graphpad Prism 8.0 (San Diego, CA) was used for statistical analysis. Data are shown as mean±SD (SD: standard error of the mean). Comparisons between groups were performed using one-way ANOVA with post hoc comparison by Dunnett test. A two-sided $P < 0.05$ was considered statistically significant.

Results

Clinical findings

This HM pedigree (Figure 1) is a three-generation family, and HM just occurred in the III generation. The proband (III:1), HM was observed at 2-year-old, is a 22-year-old female with bilateral HM of -12.00D now. And optical coherence tomography (OCT) examination of the index showed no abnormalities (Figure 2). Her parents are shortsighted but not met the criteria of HM. And the cousin of the index case (III:2) was diagnosed as HM from her middle-school years. There was no other diseases in all members of this family.

Mutation analysis

By exome sequencing of the patient (III:1), we identified a heterozygous nonsense mutation in the GPR157 gene (c.859C>T, p.Arg287*) (Figure3). The dbSNP has recorded its' SNP feature (rs762095374), but not revealed its' incidence rate in different populations. But the identified mutation is absent in either the Exome Variant Server (ESP6500) or 1000 Genomes Project. Furthermore, this variant was found in the proband's father, but not in the remaining family members and the 310 control individuals. In conclusion, our data show that the novel mutation in GPR157 is probably responsible for HM in this studied family.

GPR157 expression

RT-PCR results showed GPR157 is ubiquitously expressed in mice tissues examined and the mRNA level of GPR157 in the eye significantly increased compared with other mice tissues (Figure4.A, B). From this result, GPR157 probably has potential impacts on the development and function of the eye.

Discussion

G protein-coupled receptors (GPCRs) are the largest class of membrane protein receptors families in the human genome. GPCRs have a typical structure including a single polypeptide with an extracellular N-terminus, an intracellular C-terminus and seven membrane-spanning regions linked by three extracellular loops (ECL1-ECL3) and three intracellular loops (ICL1-ICL3)^[17]. GPCRs play a vital role in physiological processes such as embryonic growth, gonadal development, learning and organismal homeostasis. Most GPCRs transmit extracellular signals into the cell, for example, many hormones, neurotransmitters, chemokines, autocrine and paracrine factors^[18]. However, there are still some orphan GPCRs lacking endogenous ligands^[19].

GPR157 gene is located on chromosome 1p36, contains four exons, and encodes a protein of 366 amino acids. GPR157 is an orphan GPCR and has no subfamily and splice variant yet. GPCRs transmit environmental information into the cell via activating cognate heterotrimeric G-protein. Generally speaking, heterotrimeric G-protein can be classified as Gs, Gi, Gq and G12/G13 families according to their G-sequence similarity^[18,21]. There are only two pieces of literature about GPR157 both at home and abroad up to now. One in 2005 reported 2 SNPs of GPR157 (c.795C>T, p.Arg218 Leu; c.811C>T, p.Ala223Va) were identified in the patients with Schnyder crystalline corneal dystrophy (SCCD) as well as 102 healthy control individuals. Thus, these two SNPs might have no association with SCCD^[20]. The other in 2016 revealed that GPR157 is mainly expressed in the developing neocortex and coupled with Gq-class G-proteins in both neuronal differentiation and Ca²⁺ release through the GPR157-Gq-IP3 signaling^[21]. Our experiment result (Figure4) shows that GPR157 may involve in the development of eye. However, there is no article reported regarding whether GPR157 participates in the development of myopia so far.

Enormous efforts have been made in identifying the mechanism underlying the development of myopia and HM. With the advent of NGS, lots of causative genes association with HM were reported. But these disease-causing genes often lead to controversial results for they mostly can't be replicated from each other because HM has typical genetic heterogeneity. It remains unknown whether the novel heterozygous nonsense mutation in GPR157 (c.859C>T, p.Arg287*) induces inactivation of the GPR157 protein. Furthermore, the variant we detected in this study existed in the proband's father as well, who presented with only low refractive error.

Given that patient(III:1), the proband, do not reach the clinical manifestations of SCCD (subepithelial crystalline deposits, central discoid or annular corneal stromal opacification and so on), we assume that the hybrid nonsense mutation may not result in SCCD. Patient(III:2), the cousin of the proband, not detected the same variation as the index case, together with HM developed during her school age. We presume her HM phenotype is mostly caused by environmental factors^[3, 7]. Our findings show this family recruited descends as an autosomal dominant inheritance with reduced penetrance, as mentioned above.

The heterozygous nonsense mutation in the current study, c.859C>T (p.Arg287*) of GPR157, is present in the dbSNP data (frequency unknown). And it is absent in the 110 sporadic HM controls and 200 healthy controls. In conclusion, these results suggest that the novel hybrid nonsense variant associated with the occurrence of early-onset high myopia in the proband with doubt whether it is just a SNP or Indels in Chinese population because of the limited number of controls. Hence future functional study about this locus of GPR157 is essential and further studies are expected to exclude the presence of other variants, such as mutations in the non-coding region and large deletion in the coding region.

Abbreviations

GPR157: G protein-coupled receptor 157; Next-generation sequencing (NGS); WES: whole exome sequencing; HM: high myopia; RT-PCR: reverse transcription polymerase chain reaction; mRNA: message ribonucleic acid; KM mice: Kunming mice; D: dioptre; PM: pathological myopia; BSG: basigin; SCO2: synthesis of cytochrome C oxidase 2; CCDC111: coiled-coil domain-containing 111; P4HA2: Prolyl 4-Hydroxylase Subunit Alpha 2; ZNF644: zinc finger protein 644, DNA: deoxyribonucleic acid; RNA: ribonucleic acid; cDNA: complementary deoxyribonucleic acid; PCR: polymerase chain reaction; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; SD: standard error of the mean; ANOVA: analysis of variance; OCT: optical coherence tomography; OS: the left eye; OD: the right eye; dbSNP: The Single Nucleotide Polymorphism Database; ESP6500: exome sequencing project 6500; GPCR: G protein-coupled receptor; ECL: extracellular loop; ICL: intracellular loop; SNP: single nucleotide polymorphism; SCCD: Schnyder crystalline corneal dystrophy; GPR157-Gq-IP3: GPR157 couples with Gq-class of the heterotrimeric G-proteins and inositol triphosphate; NGS: next-generation sequencing.

Declarations

Authors' information

Affiliations

Department of Laboratory Medicine, North Sichuan Medical College, Nanchong, China

Tao Tang, Mohan Liu, Ting Wei, Lin Deng, Yueyang Zhang, Yu Pu, Yan Cai, Qiling Song & Qingsong Liu

Prenatal Diagnosis Center, Affiliated Hospital of North Sichuan Medical College, Nanchong, China

Yan Cai, Qiling Song, Ting Zhang & Qingsong Liu

Corresponding author

Correspondence to Qingsong Liu.

Ethics approval and consent to participate

The present study was approved by the ethics committee of the Affiliated Hospital of North Sichuan Medical College(No.2020ER002-1). Written informed consent was obtained from each participant(all the subjects are adults).

Consent for publication

Written and verbal informed consent was obtained from participants (none of the participants are children). The images and genetic results in this study are not identifiable to participants.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by The Education Department Of Sichuan Province (No.16ZA0233)and Bureau of Science&Technology Nanchong City (No. NSMC20170438). The funding had no role in study design, data collection and analysis, or preparation of this manuscript.

Authors' contributions

Conceived and designed the experiments: ML, QL. Performed the experiments: TT, YC, QS. Analyzed the data: TW, LD, YZ, TZ. Wrote the paper: TT, YP. All authors have read and approved the final manuscript.

Acknowledgements

We thank all subjects enrolled in this study.

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Figures

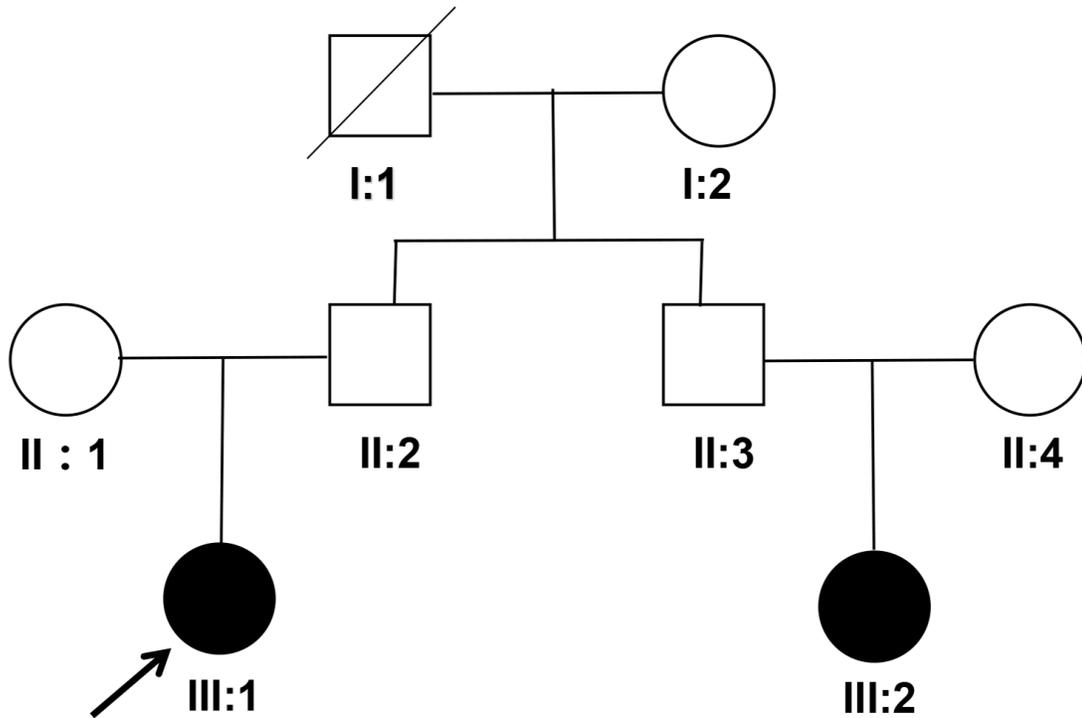


Figure 1

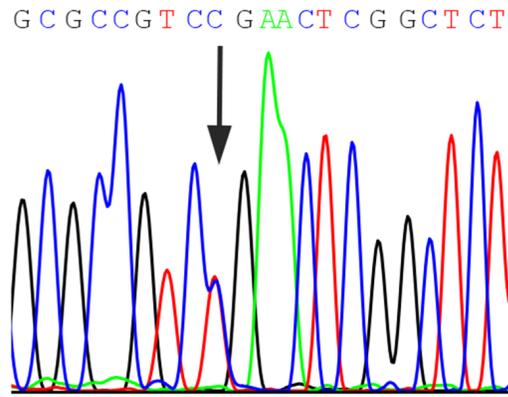
Pedigree of the HM family. Filled circles stand for affected individuals and unfilled symbols stand for unaffected individuals (circle, female; square, male). Proband is indicated by arrow. Deceased individuals are indicated by a slash (/).



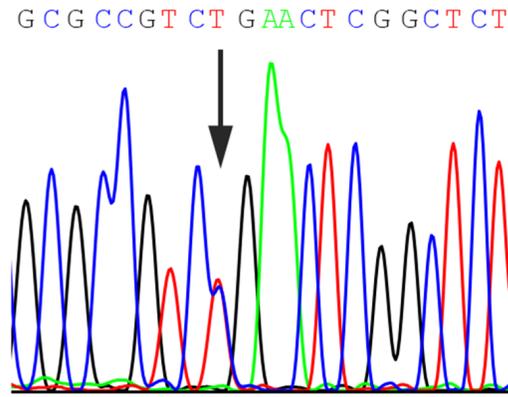
Figure 2

Normal optical coherence tomography (OCT) of the index. OS: left eye; OD: right eye.

the proband
GPR157(c.859C>T, Arg287*)



the proband's father
GPR157(c.859C>T, Arg287*)



the proband's mother
GPR157(normal)

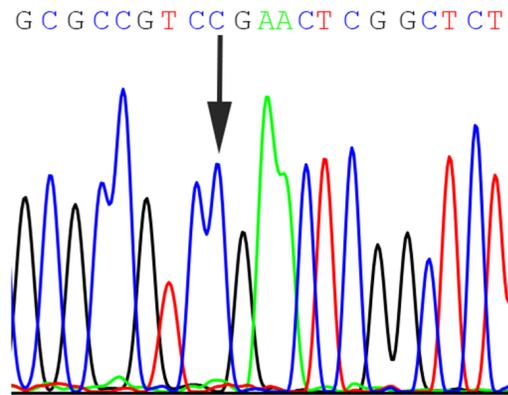


Figure 3

A novel mutation identified in GPR157. The top and middle graphs represent the novel mutation (GPR157, c.859C>T, p.Arg287*) sequence chromatogram. The bottom graph represents a normal sequence chromatogram.

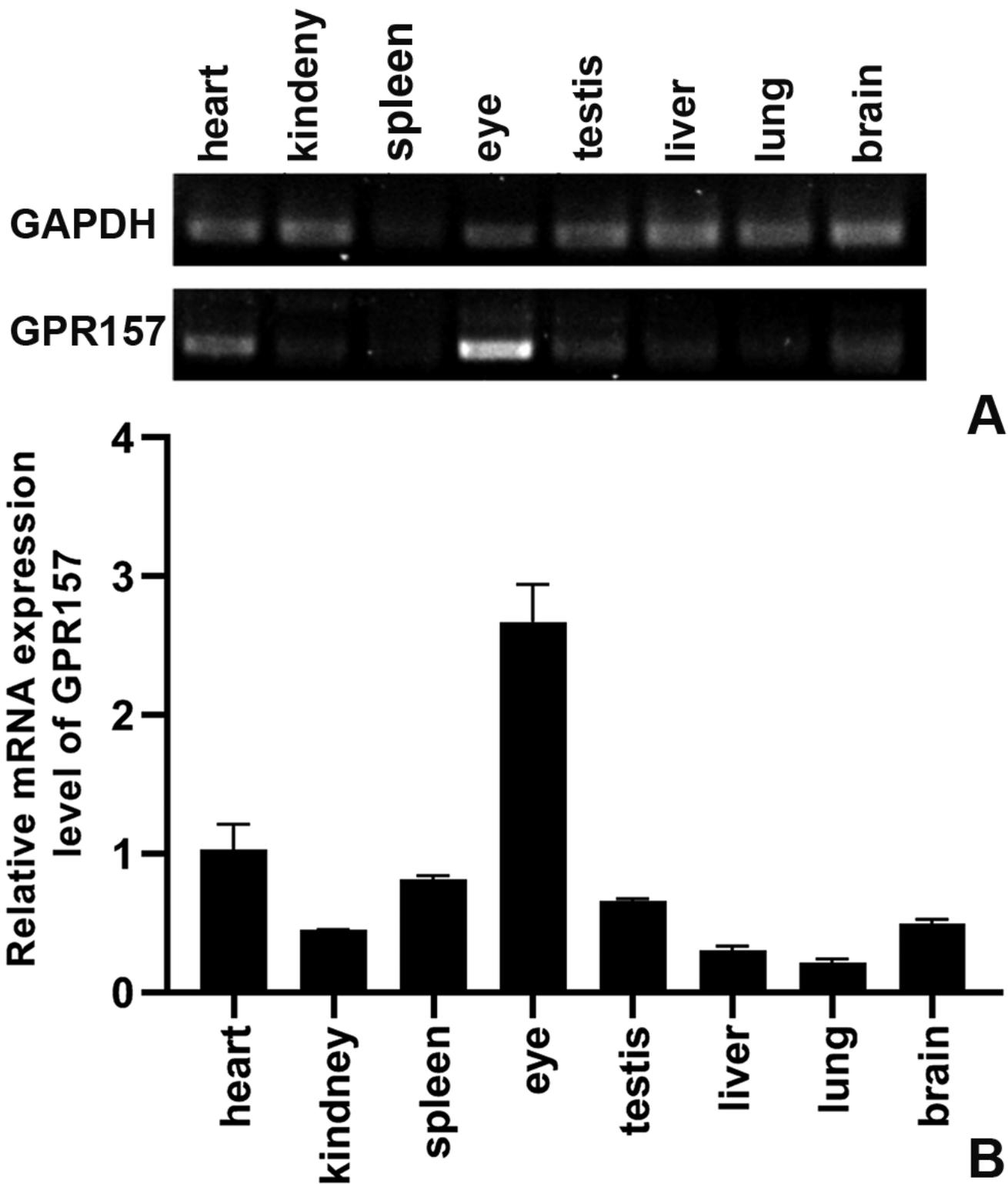


Figure 4

Relative mRNA expression of GPR157 in different mouse tissues. The relative mRNA expression levels of GPR157 in the eye compared to those of other ones were statistically increased. A: RT-PCR analyses of GPR157 expression in the mouse heart, kidney, spleen, eye, testis, liver, lung and brain with 238 bp of products. GAPDH served as the inner reference gene. B: Data are mean \pm SD. ****, $P < 0.0001$, one-way ANOVA, followed by Dunnett's post hoc intergroup comparisons.