Science & Technology Development Journal 2024, 26(SI):45-49

Open Access Full Text Article

Special issue: Vietnam International Conference On Genome Biology 2023 Proceedings

Identification of COL11A1 single-gene polymorphisms in patients with primary angle-closure glaucoma

Vo Thi Bich Thuy[1,*], Hoang Quang Vinh[2], Bui Thuy Linh[1], Nghiem Ngoc Minh[1], Nguyen Dinh Ngan[3], Nguyen Linh Toan[2]

ABSTRACT

Introduction: According to a survey of the Rapid Assessment of Avoidable Blindness (RAAB) in Vietnam in 2015, glaucoma is the third most common cause of blindness after cataracts and fundus diseases, with a rate of 4%. Worldwide, studies on primary angle-closure glaucoma (PACG) have revealed approximately 50 genes and many related gene variants. In primary angle-closure glaucoma, *COL11A1* was identified as one of the eight genes with the most important role related to the disease. There have been many studies analyzing gene mutations associated with eye diseases in Vietnam, but few studies have been conducted on PACG. Therefore, we focused on detecting some gene variants of *COL11A1* in patients with PACG in several military hospitals. **Methods:** Blood samples from 30 patients with primary angle-closure glaucoma were clinically evaluated. Total DNA was extracted from the samples using a mixture of phenol, chloroform, and isoamyl alcohol (PCI). Primer design to multiply *COL11A1* gene segments based on reference gene sequences published on NCBI. The designed gene fragment was amplified by PCR on a thermocycler. After electrophoresis on a 1% agarose gel, the PCR products were purified using GenJET PCR purification kits according to the manufacturer's instructions. Gene sequencing was performed by Sanger sequencing. The obtained sequences were processed and compared with published sequences in the International Gene Data Bank using BioEdit software. **Results:** In 12/30 patients, the rs12138977 (C>T) variant of the *COL11A1* gene, which is a mutation in the intron region, was detected. The rs12138977 variant is thought to be related to disease severity. In the exon region of the *COL11A1* gene, the rs1676486 (A>G) variant was detected in 21/30 patient samples. At the mutation site, the Ser residue at position 1535 changes to a Pro. **Conclusion:** Although we have identified some alterations in *COL11A1* genes, further investigation is needed to understand the underlying mechanisms and patient-specific and clinical manifestations associated with these variants. These findings contribute to the understanding of the genetic basis of primary angle-closure glaucoma, with the hope of supporting the diagnosis and treatment of this disease in Vietnam.

Key words: COL11A1 gene, primary angle-closure glaucoma, sequencing, clinical samples

[1]*Institute of Genome Research, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha noi*

[2]*Vietnam Military Medical University, 160 Phung Hung, Ha Dong, Hanoi*

[3]*103 Military Hospital, 261 Phung Hung Ha Dong, Hanoi*

Correspondence

Vo Thi Bich Thuy, Institute of Genome Research, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Ha noi

Email: thuytbvo.igr@gmail.com

History

- Received: 2023-10-26
- Accepted: 2024-02-18
- Published Online: 2024-6-30

DOI :

https://doi.org/10.32508/stdj.v26iSI.4205

Check for updates

INTRODUCTION

Primary angle-closure glaucoma is one of the most common types of glaucoma affecting more than 15 million individuals worldwide[1], including Vietnam. In Vietnam, glaucoma is the third most common cause of blindness, with a disease incidence of 4%. The rate of people with glaucoma is 2.1% of the population over 40 years old. Because acute angle-closure glaucoma is very common for unknown reasons, this disease is a dangerous threat to public health. A previous review on PACG revealed approximately 50 genes and genetic mechanisms associated with this disease[2]. Although the mutations that cause severe genetic disease have not been clearly identified, several recent association studies have implicated gene/genetic factors that may contribute to a person's risk of developing PACG. Additionally, genetic factors that alter disease endophenotypes have been identified[1].

One study revealed that single nucleotide polymorphisms (SNPs) in the *PLEKHA* and *COL11A1* genes may be associated with PACG disease and disease severity[3]. In particular, the *COL11A1* gene was identified as one of the most important genes related to PACG disease[4,5]. It is associated with congenital conditions, including nearsightedness and blindness due to retinal detachment due to Stickler and Marshall syndrome type II[6,7]. Recent relevant studies have shown that genetics may contribute to the risk of developing PACG. Among them, the gene polymorphisms rs1676486 and rs12138977 in *COL11A1* may be associated with an increased risk of PACG[3]. Most genome-wide association studies in the Asian population[5] have identified single-nucleotide polymorphisms (SNPs) of rs3753841 (*COL11A1*; 1p21.1) and rs1015213 (8q11.23) that are strongly associated with

Cite this article : Thuy V T B, Vinh H Q, Linh B T, Minh N N, Ngan N D, Toan N L. **Identification of COL11A1 single-gene polymorphisms in patients with primary angle-closure glaucoma**. *Sci. Tech. Dev. J.* 2024; 26(SI):45-49.

the PACG phenotype. Here, we selected the *COL11A1* gene variants rs1676486 and rs12138977 to survey and evaluate their relevance to patients with primary angle-closure glaucoma in Vietnam.

MATERIALS AND METHODS

Patient selection

Blood samples from thirty patients with PACG were screened and collected at the Military Central Hospital and several hospitals in Hanoi.

Total DNA extraction and PCR method

Whole blood samples obtained from patients were extracted using a mixture of phenol, chloroform, and isoamyl alcohol (PCI). PCR was performed using Taq DNA polymerase and primers (Table 1) under the following conditions.

The PCR was optimized and performed in an Eppendorf Mastercycler Pro S. The cycling conditions consisted of initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturing at 95°C for 30 seconds, primer annealing at 64°C for 30 seconds, extension at 72°C for 30 seconds, and finally at 72°C for 5 minutes. PCR products were subjected to 1% agarose gel electrophoresis, and the results were read with a gel documentation system (Bio-Rad).

Gene sequencing and data analysis

The *COL11A1* gene sequence [NC_000001.11:c103108522-102876473][5] in GenBank/NCBI was used as the reference sequence. The BioEdit bioinformatics tool was used to identify nucleotide and amino acid mutations.

RESULTS AND DISCUSSION

As a result, we identified the rs1676486 variant in 21 out of 30 patients at the position where nucleotide A became G. This 1535 position in the *COL1A1* gene causes the amino acid serine to change to proline. Our 30 patients had a median age of 65 years, suggesting a likely association between the variant and glaucoma. It is hypothesized that different amino acid residues at this site may affect the configuration and function of collagen, and even the extracellular matrix leading to normal aqueous humor circulation pathways may be altered[8].

We also identified the rs12138977 variant in 12 out of 30 patients at the position where the C to T nucleotide was changed. This variant is located in the intron region, without changes in amino acids. Although there have not been many detailed studies on this variant, it is likely that this variant, along with other variants, affects the severity of glaucoma.

Patients with glaucoma associated with *COL11A1* gene mutations may have an increased risk of primary angle-closure glaucoma. Wan Y et al. discovered an association between the *COL11A1* SNP and primary angle-closure glaucoma. Two SNPs, rs1676486 and rs12138977, in the *COL11A1* gene increased the risk of primary angle-closure glaucoma in the Han Chinese population. The study also revealed an association between *COL11A1* expression and the severity of primary angle-closure glaucoma[3]. In this study, the patients had an average age of 65 years, and the majority were aged 60 - ≥70 years. The acute glaucoma form accounted for the majority, with a rate of 64.28%. The axial length of the 30 patients was within the normal range of approximately 22-24 mm. The anterior chamber angle of the diseased eye of 17 patients was at closure level 4, accounting for 56.67%, and the remaining patients were at closure levels 3 and 2. The intraocular pressure of patients with SNPs was > 23 mmHg, and headache was one of the signs of glaucoma. COL11A1 gene expression was increased in the ethmoids of glaucoma patients, further suggesting the involvement of the expression and regulation of the extracellular reticulum gene in the pathogenesis of this disease. It is hypothesized that the different amino acid residues of the two SNPs and their combination may influence the configuration and function of collagen, and even the extracellular matrix leading to normal aqueous humor circulation pathways can often be altered[8]. The *COL11A1* gene is a member of the collagen family and a major component of the trabecular extracellular network. Extracellular reticulum substances play a role in creating resistance to the flow of aqueous humor, increasing intraocular pressure[9]. There are several possible mechanisms for these altered mutations in glaucoma patients, such as changes in mRNA stability, secondary structure, and transcriptional activity or alterations in protein synthesis. Additionally, unknown genetic pathways and differences in environmental factors as well as patient age may contribute to phenotypic variation. Thus, additional studies are needed to understand the exact role of genetic polymorphisms in the pathogenesis of glaucoma.

In addition, in the 30 patients described above, we detected several other variations (Table 2). The additional detected variants are all located in the intron region and have not been studied in depth, nor are they related to primary angle-closure glaucoma.

Table 1: Sequences of Primers used in the study

Primer		Sequences (5' -> 3')	Size (bp)	Tm (oC)
COL.86	FW	TGGCAGAATGTGCTTTTTGT	408	54.3^{o}C
	RV	GGTCCTCCAGGCTTACCAGT		62.5^{o}C
COL.77	FW	GGAACATGTTGTTTAAGAGTCCAA	519	60.1^{o}C
	RV	TGACGAGTTAGTGGGTGCAG		60.5^{o}C

(Tm: Melting temperature)

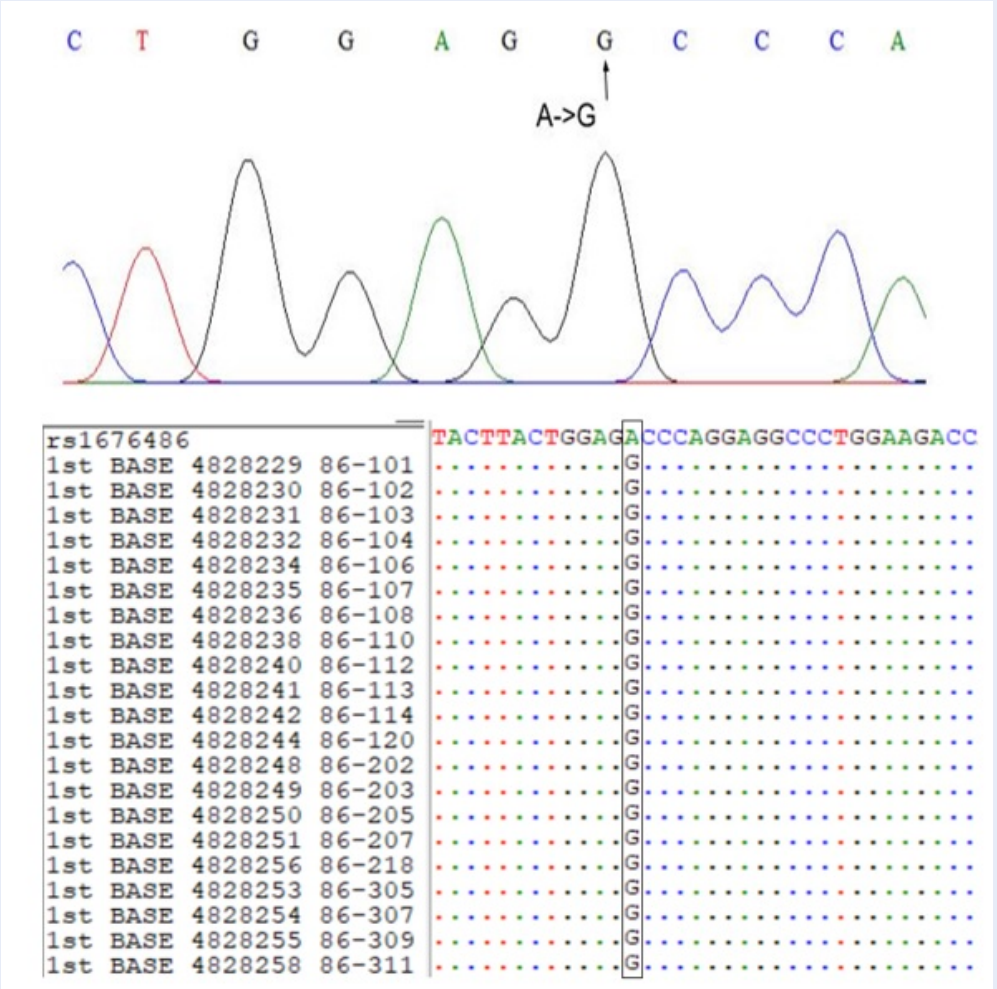

Figure 1: Sequence of the location of the rs1676486 variant in 21 patients.

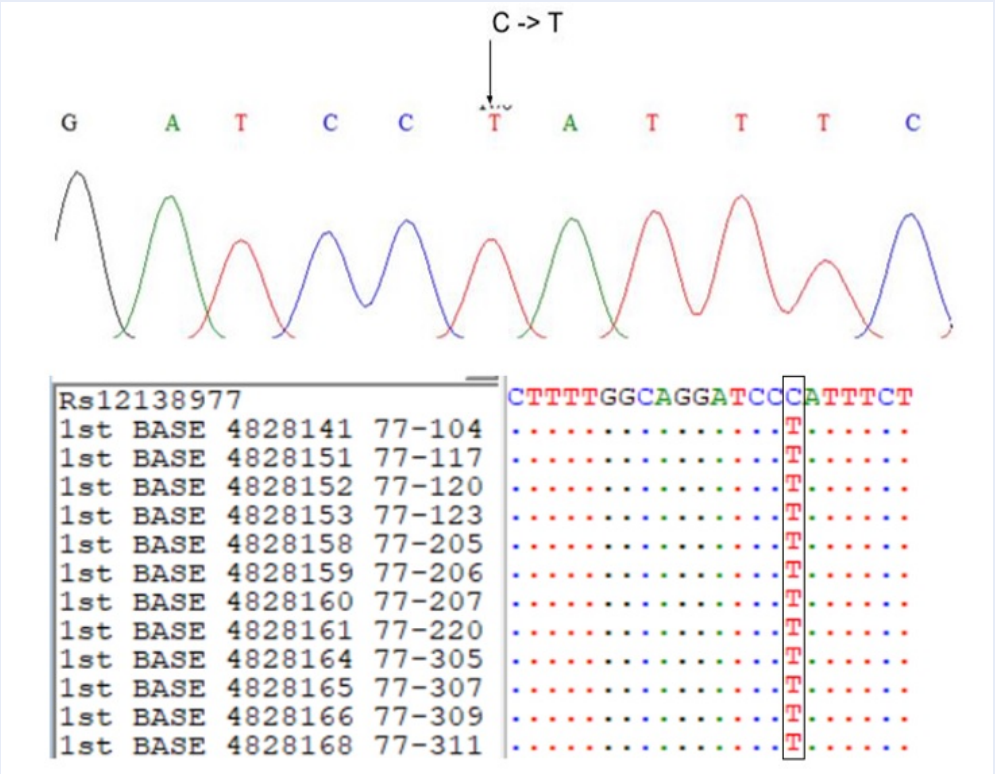

Figure 2: Sequence of the location of the rs12138977 variant in 12 patients.

Table 2: Other variations were detected in the sequences of 30 samples

Number of mutations	ID Sample	SNP	Location	Clinical significance and condition
3	126, 120, 206	rs1307453598	Intron 1p21.1	Not Reported
1	206	rs1557831528	Intron 1p21.1	Not Reported
1	123	rs1656834761	Intron 1p21.1	Not Reported
2	113, 303	rs568394367	Intron 1p21.1	Not Reported
1	123	rs758253549	Intron 1p21.1	Not Reported
1	111	rs17127203	Intron 1p21.1	Benign: not specified. not provided
1	108	rs756869175	Intron 1p21.1	Not Reported
1	107	rs1348053414	Intron 1p21.1	Not Reported
1	107	rs1223813323	Intron 1p21.1	Not Reported
2	101, 104	rs778700431	Intron 1p21.1	Not Reported

CONCLUSIONS

This study revealed two changes in the *COL11A1* gene that are linked to PACG disease. These changes are variants rs1676486 and rs12138977, and they are linked to PACG symptoms in Vietnamese patients. A number of other SNPs were also found; however, it seems that these variants have not been mentioned much in previous studies. This initial research has scientific value and is aimed at disease diagnosis applications. However, future studies require more extensive research on a larger number of patients.

LIST OF ABBREVIATIONS

PACG: Primary Angle-Closure Glaucoma
SNP: Single Nucleotide Polymorphism
RAAB: Rapid assessment of avoidable blindness
PCR: Polymerase chain reaction
PCI: Phenol, Chloroform, Isoamyl alcohol

COMPETING INTERESTS

The author(s) declare that they have no competing interests.

ACKNOWLEDGEMENTS

This work was supported by the No 41/QĐ-NCHG (27/02/2023) and ĐTCS2023 of the Institute of Genome Research, Vietnam Academy of Science and Technology.

REFERENCES

1. Ahram DF, Alward WL and Kuehn MH. The genetic mechanisms of primary angle closure glaucoma. Eye (Lond). 2015; 29(10):1251-9;Available from: https://www.ncbi.nlm.nih.gov/pubmed/26206529.10.1038/eye.2015.124.
2. Kondkar AA. Updates on Genes and Genetic Mechanisms Implicated in Primary Angle-Closure Glaucoma. Appl Clin Genet. 2021; 14(89-112;Available from: https://www.ncbi.nlm.nih.gov/pubmed/33727852.10.2147/TACG.S274884.
3. Wan Y, Li S, Gao Y, Tang L, Cao W and Sun X. COL11A1 Polymorphisms Are Associated with Primary Angle-Closure Glaucoma Severity. J Ophthalmol. 2019; 2019(2604386;Available from: https://www.ncbi.nlm.nih.gov/pubmed/30809385.10.1155/2019/2604386.
4. Khor CC, Do T, Jia H, et al. Genome-wide association study identifies five new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2016; 48(5):556-62;Available from: https://www.ncbi.nlm.nih.gov/pubmed/27064256.10.1038/ng.3540.
5. Vithana EN, Khor CC, Qiao C, et al. Genome-wide association analyses identify three new susceptibility loci for primary angle closure glaucoma. Nat Genet. 2012; 44(10):1142-6;Available from: https://www.ncbi.nlm.nih.gov/pubmed/22922875.10.1038/ng.2390.
6. Annunen S, Korkko J, Czarny M, et al. Splicing mutations of 54-bp exons in the COL11A1 gene cause Marshall syndrome, but other mutations cause overlapping Marshall/Stickler phenotypes. Am J Hum Genet. 1999; 65(4):974-83;Available from: https://www.ncbi.nlm.nih.gov/pubmed/10486316.10.1086/302585.
7. Richards AJ, McNinch A, Whittaker J, et al. Splicing analysis of unclassified variants in COL2A1 and COL11A1 identifies deep intronic pathogenic mutations. Eur J Hum Genet. 2012; 20(5):552-8;Available from: https://www.ncbi.nlm.nih.gov/pubmed/22189268.10.1038/ejhg.2011.223.
8. Last JA, Pan T, Ding Y, et al. Elastic modulus determination of normal and glaucomatous human trabecular meshwork. Invest Ophthalmol Vis Sci. 2011; 52(5):2147-52;Available from: https://www.ncbi.nlm.nih.gov/pubmed/21220561.10.1167/iovs.10-6342.
9. Huang W, Fan Q, Wang W, Zhou M, Laties AM and Zhang X. Collagen: a potential factor involved in the pathogenesis of glaucoma. Med Sci Monit Basic Res. 2013; 19(237-40;Available from: https://www.ncbi.nlm.nih.gov/pubmed/24002298.10.12659/MSMBR.889061.