

# CYP1B1 variants have low contribution to Pakistani patients with Primary Open Angle Glaucoma Page

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## Research article

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# Abstract

**Background** Glaucoma is a group of complex neurodegenerative ophthalmological disorders and is the second common of cause of irreversible blindness around the globe. Primary open-angle glaucoma (POAG) is the commonest type of all glaucoma and is characterized by elevated intraocular pressure (IOP) leading to optic nerve damage and visual field defects. Inherited mutations in *CYP1B1* are a rare cause of POAG. **Objective** This study was conducted to screen Pakistani population with POAG for *CYP1B1* variants. **Methods** Detailed family history was recorded from all participating families. The disease was confirmed through ophthalmological examinations. Blood samples were collected for genomic DNA extraction. *CYP1B1* exons were directly sequenced in one affected individual from each family. **Results** *CYP1B1* sequencing revealed a novel heterozygous missense variant, c.649G>A (p.Asp217Asn). All affected individuals having the variant had characteristic glaucomatous changes with mean disease onset at 35 years. The unaffected individuals in the family had no signs of POAG. The prevalence of *CYP1B1* associated POAG in study cohort is 4% (1/25). *In silico* predictions showed that p.Asp217Asn, substitution affects the structure and function of the protein. **Conclusion** The study suggests that *CYP1B1* mutations are considered to have less contribution in pathogenesis of POAG in Pakistani population studied. Identification of novel dominantly inherited variant shows allelic heterogeneity and may help in early diagnosis for effective management of the disease through genetic counseling.

## Background

Glaucoma, the second most important cause of irreversible blindness in the entire globe, is characterized by raised intraocular pressure (IOP) and damage to retinal ganglionic cells of the retina which together result in cupping of the optic disc and its atrophy with resultant visual field defects. (Wójcik-Gryciuk et al., 2015) Based on anatomy and structure of irido-corneal angle, glaucoma is into Primary open angle glaucoma (POAG) and Primary angle closure glaucoma (PACG). POAG is divided into Adult onset glaucoma which occurs mostly after 40 years of age and Juvenile onset glaucoma which starts between 3 and 40 years of age (Liu and Allingham, 2017).

POAG is the most common variety of glaucoma in Asia and Europe including Pakistan. (Iqbal et al., 2011) It produces more structural damage than other forms of glaucoma due to its asymptomatic progression and late diagnosis in most of the cases (Kwon et al., 2009, Cheng et al., 2012). It is genetically heterogeneous in nature and is usually inherited as an autosomal dominant trait but in the majority of the cases, the pattern of inheritance is complex and multifactorial. Up till now more than 20 loci have been identified for POAG. (Wirtz et al., 2010, Abu-Amero et al., 2012) Myocilin (MYOC) is the most common gene responsible for POAG, but some cases have also been reported with variants in *CYP1B1*. (Liu and Allingham, 2017, Abu-Amero et al., 2015)

*CYP1B1* (OMIM 601771) encodes an enzyme, cytochrome P450 B1, which is required for the metabolism of endogenous substrates in ciliary epithelium, and has a role in the production of aqueous humour.

(Tang et al., 1996, Stoilov et al., 1998, Civan and Macknight, 2004) The absence or reduced activity of cytochrome P450B1 may result in abnormal flow of aqueous humour and causes increased intraocular pressure and subsequent glaucomatous changes (Micheal et al., 2015). Mutations in *CYP1B1* have been associated with anterior segment disorders, including Primary Congenital Glaucoma (PCG), POAG, Juvenile open angle glaucoma (JOAG), Peter's anomaly and Rieger's anomaly. To date, over 200 pathogenic variants have been identified in *CYP1B1* and the majority of the mutations are substitutions, followed by insertions and deletions (Stenson et al., 2017). It is noteworthy that *CYP1B1* mutations are population specific and have been detected in patients with diverse ethnic backgrounds. (Sheikh et al., 2014, Rauf et al., 2016)

The Pakistani population is genetically heterogeneous with combination of several ethnicities. Previous research indicates presence of population specific variants in numerous disease causing genes. Thus, the present study was aimed to investigate the role of *CYP1B1* in the pathogenesis of POAG in Pakistani population resides in Sindh province. This may help to study the contribution of *CYP1B1* mutation in POAG patients and may also help identify common variants for genetic counseling.

## Methods

### 2.1. Recruitment of subjects and Clinical Investigations

The study approval was obtained from Ethical Review Committee of Liaquat University of Medical and Health Sciences, Jamshoro. Total 25 families with two or more than two members affected with POAG were recruited for the study. All the families were visited to record detailed medical history and pedigrees were drawn. Informed written consent was obtained from all members of enrolled families for the study. All affected members of the enrolled families were subjected to extensive ophthalmological examination to confirm the diagnosis of POAG. Intra ocular pressure (IOP) was measured by Goldmann applanation tonometry, irido-corneal angles (anterior chamber angles) were evaluated by the Goldmann gonioscope. The fundus was examined using slit lamp biomicroscopy with +90 Diopter lens. Venous blood was taken in EDTA containing tubes from all affected and normal members of enrolled families and genomic DNA was extracted by using standard non-organic protocol (Grimberg et al., 1989).

### 2.2. Mutation Screening and Bioinformatics Analysis:

Both coding exons of *CYP1B1* gene were amplified and directly sequenced as described previously (Sheikh et al., 2014). Chromatograms were analyzed by using Chromas v 1.45 software. The Pathogenic role of the novel variant was assessed by using mutation taster, Sorting Intolerant from Tolerant (SIFT) and HOPE web tools. The Custal Omega was used to find the conservation status of substituted amino acid in seven species. Biochemical properties of wild type and mutated amino acids and their importance in normal structure and function of protein was assessed using HOPE web tool.

## Results

Total 25 consanguineous families with two or more than two affected members with POAG were enrolled for this study. Pedigree analysis and medical history showed that all families had dominantly inherited disease with onset between 35 to 55 years. Ophthalmological examination confirmed the presence of POAG. Both coding exons of *CYP1B1* were sequenced in single affected from each family.

The sequencing data revealed a novel heterozygous transition, c.649 G>A, substituting Aspartic acid into Asparagine at codon 217 (p.Asp217Asn) of *CYP1B1* in one family, POAG-02, (4%, 1/25) (Fig 1.B). No *CYP1B1* variant was found in any other family. Pedigree analysis of POAG-02 revealed an autosomal dominant trait segregating in 8 affected individuals in 2 generations (Fig. 1.A). Mutation analysis showed that all 8 affected were heterozygous carriers of the variant, whereas, phenotypically normal individuals had wildtype genotype. The mean age of affected patients of POAG-02 family was 45 years, ranging from 40 to 88 years. The findings of the clinical examination showed severe characteristic glaucoma features (Table 1). The c.649 G>A, p.Asp217Asn is a novel variant and has not been reported earlier in association with POAG. Bioinformatics studies predicted Aspartic acid at 217<sup>th</sup> codon of *CYP1B1* as a conserved residue across the six species and its substitution with Asparagine is deleterious with pathogenic effects on normal protein. In addition, this variant was not found in 1000 genome database and Exome Aggregation Consortium (ExAC) database. This variant was also examined in

60 ethnically matched controls and was not detected.

## Discussion

Glaucoma is a chronic and heterogeneous group of ocular disorders characterized by raised intraocular pressure (IOP) causing degeneration and atrophy of optic nerve fibers and manifesting as visual field defects and irreversible blindness if remain untreated. Many studies have suggested the role of elevated IOP in the pathogenesis of POAG indicating its close association in its development but the etiology of glaucoma is still unclear. Genetic and molecular studies have indicated an important role of genes in the development of POAG like many other ocular disorders. Identification of disease causing genes may provide insight into the pathogenesis of inherited disorders. (Fingert, 2011)

This study was aimed to evaluate the role of *CYP1B1* variants in families affected with dominantly inherited POAG in Sindh Province. Twenty five families affected with POAG were screened for variants in *CYP1B1*; a heterozygous *CYP1B1* variant, segregating with the disease phenotype was found in all 8 affected individuals of family POAG2. The prevalence of the *CYP1B1* was 4% (1/25) in our group of POAG patients; whereas, the previous study on 40 Pakistani families affected with POAG described *CYP1B1* disease causing variants in 10% (4/40) of the patients. In addition, the prevalence of *CYP1B1* in non-familial cases (3%, 6/190) was not significantly different from our findings (Micheal, Ayub et al. 2015). The variation of affected individuals in familial cases may be due to different ethnic backgrounds of the patients within Pakistani population. Moreover, results of this study in familial POAG are comparable with the findings of an Indian study, where *CYP1B1* variants were found in 4% of the POAG patients (4/200) (Melki et al., 2004).

The patients included in this study had the onset of POAG from 38-50 years (average= 43.3 years). All patients had normal or near-normal IOP as they underwent early trabeculectomy soon after diagnosis with POAG except one (patient IV: 12). There was complete cupping in the patient (IV:12) who had not undergone trabeculectomy, whereas all other affected individuals exhibited significant cupping corresponding to respective reduced visual acuity.

c.649 G>A, p.Asp217Asn is predicted to be pathogenic by mutation taster as disease causing and damaging. Whereas SIFT web tool indicted it as pathogenic. According to HOPE tool, the mutated amino acid (Asparagine) is neutral whereas the wild-type amino acid (Aspartic Acid) has a negative charge. The wild type amino acid forms linkages with Arginine at 122<sup>nd</sup> and 183<sup>rd</sup> positions of CYP1B1 protein; however incorporation of mutated amino acid causes alterations in the polarity and affects ionic interactions of the protein (Fig 2 A). In addition, the mutated amino acid is located in a highly important domain that has the vital role in interactions between different protein domains and it also affects binding capabilities of the protein with other molecules (Fig 2 A). The mutated amino acid thus affects the overall activity of the protein thus results in the manifestation of glaucomatous symptoms. Aspartic acid at 217<sup>th</sup> position is not fully conserved in all seven species studied (Fig 2 B), but it is still important for normal function of the protein in humans.

Previously, only one study was conducted on the role of *CYP1B1* in Pakistani patients with family history of POAG and revealed one novel heterozygous mutation (p.D316V) and a previously reported p.E229K mutation. Moreover, three novel heterozygous variants (p.T234K, p.A287P & p.Q362\*) and three previously reported heterozygous variants (p.G61E, p.E229K & p.R368H) were detected in 190 sporadic POAG patients (Michael S, Ayub 2015). It is significant that, heterozygous *CYP1B1* variants cause POAG, whereas in case of familial PCG, heterozygous are phenotypically normal (Sheikh et al., 2014).

*CYP1B1* mutations have shown interfamilial clinical variations; some patients may have congenital glaucoma, whereas other carrying same mutation may have POAG (Melki et al., 2004). In our study, all the affected have onset from 35 to 50 years and family history did not reveal incidence of congenital glaucoma cases. Interestingly, homozygous *CYP1B1* mutations have also been associated with POAG, where heterozygous individuals with *CYP1B1* variants were phenotypically normal (Acharya et al., 2006). Similarly, in Iranian patients with POAG; homozygous *CYP1B1* variants are associated with POAG and JOAG (Suri et al., 2008). It is hypothesized that there are other genetic or epigenetic factors which influence the expression and pathogenicity of *CYP1B1* variants. Mutation in *CYP1B1* gene may be an important risk factor for early-onset POAG (Vincent et al., 2002). Screening of *CYP1B1* mutations in affected persons and relatives of POAG patients is clinically important for early diagnosis and prognosis. It may help to monitor vision and detect early symptoms of POAG in such individuals. *CYP1B1* screening in these individuals may help to prevent visual impairment caused by glaucoma.

## Conclusions

This is the first report of *CYP1B1*-linked Primary open angle glaucoma from Sindh province of Pakistan. Identification of novel diseased allele in *CYP1B1* gene and its segregation with primary congenital glaucoma indicates genetic heterogeneity of our population. The study suggests that Aspartic acid residue at 217<sup>th</sup> codon of *CYP1B1* is functionally and structurally important for normal functioning of *CYP1B1* protein and its substitution with Asparagine is pathogenic. The results may help to better understand the pathophysiology of *CYP1B1* induced Primary open angle glaucoma and may be helpful in genetic counselling of affected family for early screening and better management of the disease.

## Abbreviations

POAG: Primary open angle glaucoma

CYP1B1: Cytochrome P-450 1B1

IOP: Intraocular pressure

EDTA: Ethylenediaminetetraacetic acid

DNA: Deoxyribonucleic acid

## Declarations

### Ethics approval and consent to participate

Study was approved by Research ethics committee of Liaquat University of Medical & Health Sciences, Pakistan, and written consent was taken from study participants

### Consent for Publication

Not applicable

### Availability of data and material

Available

### Competing interests

The authors declare that they have no competing interests

### Funding

Study was funded by Liaquat University of Medical & health Sciences

### Authors' contributions

- 1.MYS: study design, data collection, interpretation
2. SM: study design, study writing, interpretation of results

3. AMW: genetic study analysis
4. AN: provision of patients
5. UB,SS,SB data collection, physical examination, sample collection, laboratory analysis

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## Tables

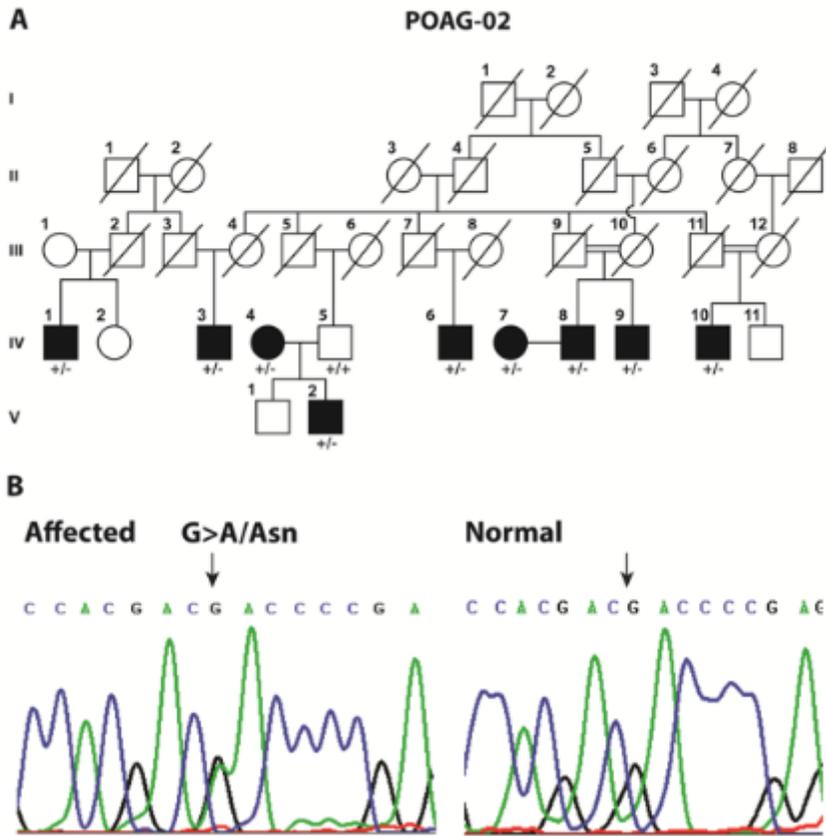
Table 1

ID	Sex/Age	Age of onset	BCVA	IOP mmHg	C/D ratio	Gonioscopy	Surgery
			OD/OS	OD/OS	OD/OS		
IV: 1	M/65	39	6/60,6/36	20/16	0.8/0.7	OA	Left Trabe
IV: 3	M/70	42	6/24,PL+VE	14/26	0.7/0.9	OA	Right Trabe
IV: 6	M/40	38	6/9,6/9P	12/14	0.4/0.5	OA	Bilateral Trabe
IV: 7	F/60	45	6/12,6/60	18/22	0.6/0.7	OA	Right Trabe
IV: 8	M/88	49	NPL,NPL	25/28	1.0/1.0	OA	NIL
IV: 9	M/60	43	CF,6/60	20/18	0.9/0.8	OA	Left Trabe
V: 2	M/49	40	6/18, 6/12	14/16	0.7/0.6	OA	Bilateral Trabe

**Table 1: Clinical data of patients of family POAG02**

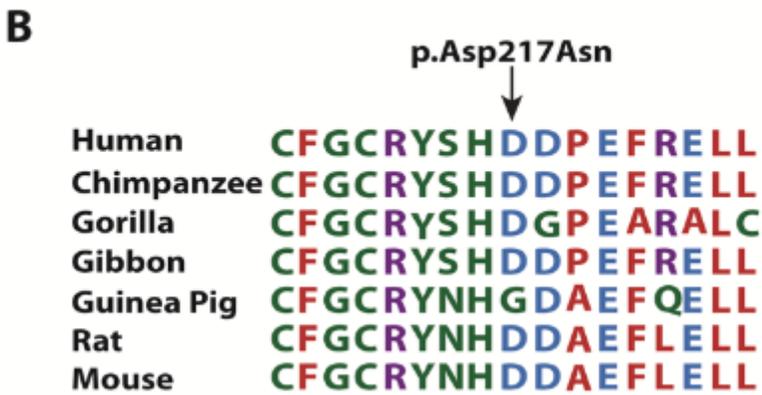
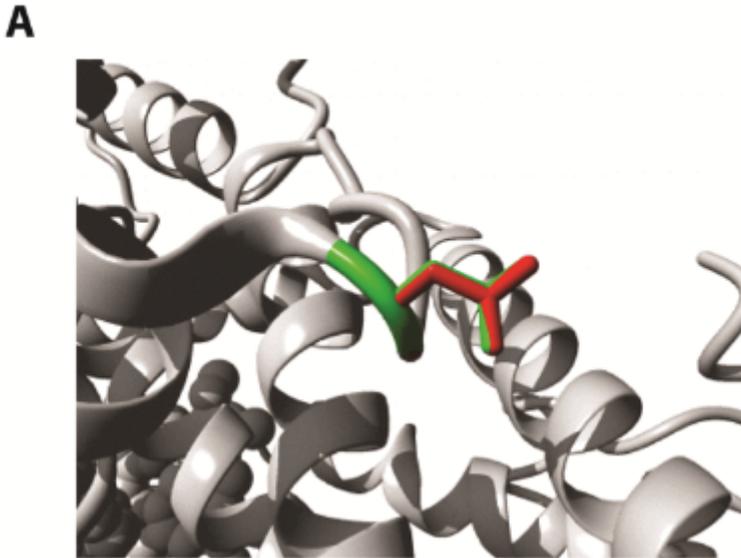
BCVA: Best Corrected Visual Acuity. IOP: Intraocular pressure. C/D ratio: Cup/Disc ratio. NPL: No Perception of Light. Trabe: Trabeculectomy. OD: right eye, OS: left eye. OA: Open Angle.

## Figures



**Figure 1**

Pedigree analysis of POAG-02 shows an autosomal dominant trait segregating in 8 affected individuals in 2 generations



**Figure 2**

Showing that the mutated amino acid (Asparagine) is neutral whereas the wild-type amino acid (Aspartic Acid) has a negative charge according to HOPE tool

## Supplementary Files

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