Research Square

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Myocilin gene mutations: A cause of juvenile open-angle glaucoma in north India

Manoj Yadav
Maharshi Dayanand University
Anupama Deora
Maharshi Dayanand University
Sumit Sachdeva
Pandit Bhagwat Dayal Sharma University of Health Sciences
Manisha Rathi
Pandit Bhagwat Dayal Sharma University of Health Sciences
Jitender Phogat
Pandit Bhagwat Dayal Sharma University of Health Sciences
Chand Singh Dhull
Pandit Bhagwat Dayal Sharma University of Health Sciences
Minakshi Vashist
Maharshi Dayanand University
Mukesh Tanwar (mukeshtanwar@gmail.com)
Maharshi Dayanand University

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Abstract

Purpose

Juvenile-onset open-angle glaucoma (JOAG) is an uncommon type of primary open-angle glaucoma that affects individuals during childhood and early adulthood. Pathogenic variants in the myocilin gene account for varying frequencies of primary open-angle glaucoma and JOAG cases in different populations. This study has screened and identified novel and previously identified myocilin variants in a north Indian cohort of JOAG patients.

Methods

Eighty unrelated JOAG cases and one hundred controls have been screened for MYOC variants by PCR and DNA sequencing of exons.

Results

DNA sequencing revealed seventeen different variants. Out of these variants, five (p.G122A, p.R136I, p.S173T, p.K216I, and p.R200KTer*15) were novel and registered in NCBI. Pathogenic MYOC variants identified in 7.5% of JOAG cases.

Conclusion

Pathogenic myocilin variants account for 7.5% of cases of JOAG in our patient's cohort. This study augments the mutation spectrum of the MYOC gene, provides population-specific information, and aids in better understanding the underlying lesions of the disease.

Introduction

Glaucoma is a neurodegenerative disorder of the human eye responsible for a large number of blindness cases worldwide [1]. It may show a simple Mendelian pattern of inheritance in some familial cases [2]. Juvenile open-angle glaucoma (JOAG) is an uncommon type of glaucoma that affects individuals during childhood and early adulthood between 3–40 years of age [3]. Pathogenic myocilin (MYOC) variants account for 8%-36 % of cases of JOAG worldwide [4, 5]. Quigley and Broman (2006) have postulated that by the year 2020, around 89 million individuals will be affected and, approximately 6 million people bilaterally blind because of glaucoma [6]. A total of sixteen genetic loci have been associated with POAG cases by Genome-wide association studies. Pathogenic variants in *MYOC*, *CYP1B1, OPTN* and, *WDR36* were reported to cause open-angle glaucoma [7–9]. Among these, MYOC was the first gene to be identified and responsible for POAG and JOAG phenotype in different populations [4, 10]. Because the genetic causes of the JOAG vary in genetically distinct populations. This study was initiated to identify pathogenic MYOC variants and their frequency in a north-Indian cohort of JOAG Patients.

Materials And Methods

The study was approved by the Institutional Review Board of Pt. B.D. Sharma University of Health Sciences Rohtak (HR) India, and participating individuals or their guardians gave written informed consent consistent with the Declaration of Helsinki's tenets before the study. A total of eighty unrelated JOAG patients from the Glaucoma OPD facility of Regional Institute of Ophthalmology of the Pt. B.D. Sharma University of Health Sciences Rohtak were enrolled in this study. Patients are diagnosed to have JOAG if they met the following criteria "1) elevated IOP>21 mmHg by Goldmann applanation tonometry at the initial hospital visit, 2) open-angle configuration on gonioscopy, and 3) glaucomatous optic neuropathy (neural rim thinning, focal notching or a vertical cup-to-disc ratio >0.5) and/or glaucomatous visual field defects." The age of onset age of disease in enrolled patients varied from 3 years to 40 years.[3] A total of hundred healthy individuals without any history of ocular and systemic disease enrolled as controls.

Exclusion Criterion: Cases of primary congenital glaucoma, traumatic glaucoma, and glaucoma with syndromes such as Axenfeld-Rieger syndrome/Sturge-Weber syndrome are excluded in this study. Cases of developmental glaucomas with late presentations (patients with Haab's striae) were also excluded.

A five ml sample of venous blood was collected into EDTA vacutainer tubes (VACUETTE Greiner Bio-One) from patients and controls. Blood samples were stored at -20º Celsius until DNA isolation.

Primer design

A total of seven pairs of primers (Table 1) covering all exon and intron-exon junctions of *MYOC* (against reference sequence ENSG00000034971) were designed by using the primer design tool ***Primer3,*** available at https://www.ncbi.nlm.nih.gov/tools/primer-blast/

DNA isolation, Polymerase Chain Reaction (PCR) and, DNA Sequencing

DNA was isolated from all blood samples by using an established method [11]. All three exons and intron-exon junctions of *MYOC* were amplified by PCR using the primers detailed in Table 1, and products visualized on a 1.8% agarose gel. PCR reactions performed in 40µl containing 100-150ng of genomic DNA with 4µl of 10X buffer, 2.0 µl of 15mM MgCl2, 2µl of dNTPs, 2µl of each primer and, 1 µl of Taq DNA Polymerase (Genei Labs). During PCR, temperature profiles included initial denaturation at 94ºC for 3 minutes followed by 35cycles consisting of 30 seconds denaturation step at 94ºC, annealing at 54º-60ºC for 45-60 seconds and, extension at 72ºC for 50-60 seconds. After electrophoresis on a 1.8% agarose gel, amplified PCR products were purified using a PCR purification kit (Favorgen, Biotech Corp.) PurifiedPCR products were used for commercial DNA sequencing by *Eurofins Genomics India Pvt Ltd.* DNA

sequences were compared to the ensemble MYOC reference sequence ENSG00000034971 using the CLUSTALW program. Identified sequence variants were confirmed by bidirectional sequencing and described as per latest HGVS recommendations and follow the universally accepted ACMG (American College of Medical Genetics) guidelines for characterizing a variant [12,13].

Pathogenicity prediction of missense variants

Identified missense variants were searched in *ClinVar* and *gnomAd*. The pathogenicity of identified missense sequence variants was predicted using SIFT (**S**orting **I**ntolerant **F**rom **T**olerant)[14], PROVEAN (**Pro**tein **V**ariation **E**ffect **An**alyzer)[15], PolyPhen2 [13,14,16] and Mutation Taster [3,17].

Results

A total of eleven missense variants, one frameshift variant and, five synonymous/neutral variants were identified in this study (Table 2) *(chromatograms and pedigree included as supplement data)*. Variants identified in intron and untranslated regions (both 5′ and 3′) have not been considered. Details of all identified variants, associated amino acid variants, predictions of their pathogenicity (*only for missense variants*) and, other information is given in Table 3. The variants identified included:

1. p.Gln48His (g.171652468G>T):

This variant is observed as a heterozygous variant in JOAG patient P28 but not observed in controls. This variant is present in *ClinVar* and *gnomADv2.1.1* as a pathogenic variant.

2. p.Arg76Lys (g.171652385G>A):

This variant is observed as a homozygous variant in eleven JOAG patients and heterozygous variant in thirteen JOAG patients (Table 2). This variant is also observed in control samples. This variant is present in *ClinVar* and gnomAD *v2.1.1* database with conflicting interpretations of pathogenicity.

3. p.Gly122Ala (g.171652247G>C):

This variant is observed as a homozygous variant in one JOAG patient (P12) but not observed in the control samples. This is a novel variant and has been registered in GenBank with accession no. MT126741

4. p.Arg136Ile (g.171652205G>T) :

This variant is observed as a heterozygous variant in JOAG patient P13 but not observed in the control samples. This variant was found to be a novel and has been registered in GenBank with accession no. MT126742.

5. p.Ser173Thr (g.171652094G>C):

This variant was observed as a heterozygous variant JOAG patient P45, and not observed in control samples. This variant was also found to be a novel and registered in GenBank with accession no. MT126743.

6. Compound heterozygous substitution/ p.[(Leu215Pro; Lys216Ile)] (g.171607823T>C,g.171607822G>C, g.171607820A>T, g.171607819G>T):

A compound heterozygous complex substitution was present in a JOAG patient P7 with a positive family history. This variant is also novel and has been registered in GenBank with accession no. MT126744. This variant is not observed in controls and databases.

7. p.Ser238Asn (g.171638614G>A):

This is observed as a homozygous variant in two JOAG patients (P24 and P36) but not observed in the control samples. This variant is not present in *ClinVar* and *gnomADv2.1.1*

8. Pro370Leu (g.171605471C>T):

This variant is identified in three JOAG patients (P5, P12, and P42). P5 had a positive history (in supplementary data) of the disease on her paternal side, but P12 and P42 were sporadic cases. This variant is present in *ClinVar* as a pathogenic variant.

9. Arg422His (g.171636175G>A):

This variant is observed as a heterozygous variant in one JOAG patient (P36) but not present in controls. This variant is present in *gnomADv2.1.1* but absent in *ClinVar.*

10. Frameshift mutation:

One frameshift mutation **p.Arg200Lysfs*15** also identified in the patient group. This mutation is predicted to produce a truncated myocilin protein of 214 amino acids with altered amino acids from position 200 to 214 due to the frameshift. This variant is identified as a homozygous change in JOAG patient P45 but is absent in controls. This variant is a novel mutation and has been registered in GenBank with accession no. MT126745.

Following five neutral sequence variants are also identified:

1. p.Glu14= (g.171652570G>A)

This variant is present as a heterozygote in three JOAG patients (P37, P38, and P39) but absent in controls. This variant is present in *gnomADv2.1.1* as a synonymous variant.

2. p.Lys156= (g.171652144G>A)

This variant is present as a heterozygote in one JOAG patient P39 but absent in controls. This variant is not present in *gnomADv2.1.1*

3. p.Tyr347= (g.171636399T>C)

This variant is present as a heterozygote in one JOAG patient P67 but absent in controls. This variant is present in *gnomADv2.1.1* as a benign synonymous variant.

4 p.Phe430= (g.171636150C>T)

This variant is present as a heterozygote in one JOAG patient P61 but absent in controls. This variant is present in *gnomADv2.1.1* as a synonymous variant.

4. p.Lys484= (g.171635988G>A)

This is present as a homozygous variant in four JOAG patients (P26, P43, P47, P48) and a heterozygous variant in twenty-three JOAG patients (Table 2). This variant was also present in controls. This variant is present in *gnomADv2.1.1* as a synonymous variant.

A total of 80 JOAG patients and 100 controls individuals have been enrolled in the current study. Of the 80 patients, 27 were females, 53 were males, and the onset age of glaucoma varied from 5 years to 38 years. The most common presenting complaints were heaviness of the forehead and vision problems. DNA sequencing analysis identified pathogenic MYOC variants in six out of eighty (7.5%) JOAG patients. Three patients of these six patients are positive for more than one sequence variant. These are as follows:

Patient P7 was a male with the onset of glaucoma at the age of 25 years. He had four heterozygous complex substitutions in which (g.171607823T>C (Heterozygous), g.171607822G>C (Heterozygous), g.171607820A>T (Heterozygous), g.171607819G>T (Heterozygous) substituted by other bases. This variant produced codon CTG>CCC and AAG>ATT, which resulted in the amino acid changes p.[(L215P; K216I)]. His IOP at diagnosis was 22mmHg in both eyes (Table 2). He was presented with a headache and diminished vision, having a cup/disc ratio of 0.9:1 in both eyes. He underwent glaucoma surgery for both eyes in 2013, 2014, and 2016. He currently has vision 3/60and, 1/60 in the right and left eyes, respectively, and his current visual field is VF<10^0. One additional sibling of the proband has bilateral glaucoma, and his mother (now deceased) was also having glaucoma in both eyes. After multiple attempts, we could not get a blood sample from proband's brother and unable to confirm that he might be harbouring the same variants.

Patient P12 had compound heterozygous p.[(G122A; P370L)] mutations. Patient P12 was presented at 21 years of age with the heaviness of the forehead, vision problems, and redness of his eyes. He had IOP of 22 and 25 mmHg in the right and left eyes in conjunction with a cup disc ratio of 0.9:1 in the right eye and 0.8:1 in the left eye, respectively. He has no vision in the right eye, and the vision in his left eye is 6/18.

Patient P45 was a female who first presented in the ocular clinic at the age of 26 years. The patient had symptoms of vision loss, watering, and redness of her eyes. Her latest cup/disc ratio was 0.7:1 in both eyes with a vision of 6/36 in the right and 6/18 in the left eye, respectively. She had a deletion of guanine at genomic position g.171652013, predicted to result in ***p.R200Kfs*15,*** resulting in an abbreviated protein of 214 amino acids (Figure 1). Since this patient also harbours p.S173T variant, a truncated protein of 215 amino acids produced with threonine at position 173 and 14 novel amino acids (from 200-214) will be different from wild type protein as a result of frameshift (*shown in red color in Figure 1*). This truncated protein might act as a mutated myocilin protein forming protein aggregates responsible for JOAG.

Patients P5 and P42 are heterozygous for already reported pathogenic p.P370L (g.171605471C>T) variant (Table 2). Patient P5 was a female, who presented at the ocular clinic with complaints of the heaviness of her forehead and vision loss at the age of 30. She had an IOP of 26 and 34 mmHg in the right and left eyes and optic cupping of 0.8:1 and 0.9:1 in the right and left eye, respectively. She had a vision of 6/18 in both eyes. She also underwent glaucoma surgery. Patient P42 was a male who presented at the ocular clinic at the age of 32 years with complaints of halos around light and scattered vision. He had IOPs of 28 and 22 mmHg in the right and left eye, respectively with a cup disc ratio of 0.8:1 in both eyes. He had a vision of 6/36 in both eyes in his most recent clinical examination.

Patient P36 is harbouring p.R422H (g.171636175G>A) variant. She is presented to the ocular clinic at the age of 24 years with the complaints of the heaviness of her forehead, watering in both eyes, with vision problems. She had an IOP of 28 mmHg in both eyes and visual acuities of 6/24 and 6/60 in the right and left eyes, respectively. The pathogenicity of p.R422H is uncertain because it shows an ambiguous pathogenicity prediction in which PolyPhen2 and Mutation taster predict it to be probably damaging and disease-causing. But it is predicted to be tolerated by SIFT and neutral by PROVEAN. Functional analysis of p.R422H mutation essentially required for a final verdict on its pathogenicity.

The presence of the p.Q48H (g.171652468G>T) mutation had already been observed in the JOAG/POAG/PCG cases in earlier glaucoma studies but never reported in controls [19-23].This variant lies in the N-terminal domain of the myocilin protein. A Triton-X-100 assay done using recombinant myocilin containing a histidine residue at the 48th position showed protein aggregation, confirming the deleterious effect of the p.R48H variant [24]. The p.Arg76Lys reported as a non-pathogenic variant in earlier glaucoma studies [19,25,26]. The same message conveyed by its presence in both patients and controls in this study as well [19,25,26].

Myocilin variants predicted to be deleterious/pathogenic by all four programs are p.[(Leu215Pro;Lys216Ile)] and p.Pro370Leu. The position of p.P370L lies in the C-terminal domain of myocilin. It has been reported as a disease-causing variant in several glaucoma studies.[30-32] Transfection of human trabecular meshwork (TM) cells with the p.P370L myocilin mutant resulted in increased levels of endogenous reactive oxygen species (ROS and reduced ATP production, and increased cell death [18]. Sakai et al. (2007) reported that the myocilin protein enters into the mitochondria with a high molecular weight translocator complex in the outer and inner mitochondrial membranes and that myocilin mutations alter the protein structure and its confirmation [31,32]. The p.P370L mutation in the myocilin protein increases the mitochondria's sensitivity to various cellular injuries in the TM cells. It also disables normal TM cell functions and eventually contributes to the TM's failure to control IOP and glaucoma pathogenesis [18].

Discussion

GLC1A was the first locus to connect with primary open-angle glaucoma [33, 34]. In 1997, pathogenic MYOC variants were identified as a cause of glaucoma, and these variants account for 8%-36 % of cases of JOAG worldwide [35, 4, 5]. The myocilin gene encodes a 55kDa olfactomedin-related secretory protein with 504 amino acids [42]. In the human myocilin protein, the initial thirty-three amino acids form a signal peptide. Amino acids from 111 to 184 form an alpha-helical coiled-coil region identical to the myosin tail fibre accommodating a leucine zipper pattern, which is involved in myocilin-myocilin interplay [43]. This leucine zipper section shows similarity to other known leucine zipper proteins. Myocilin shows homology to olfactomedin of bullfrogs, the Z sector of a neuron-specific olfactomedin-related protein derived from rat brain and an express sequence tag (EST). Its olfactomedin-like motif in amino acids 246 to 504 contains beta-sheets with a disulfide bond connecting Cys245-Cys433 [43–45]. Cysteine is a well-preserved residue among all the myocilin proteins among different species and, it is well-maintained in olfactomedin and olfactomedin-like proteins throughout evolution [46]. It is a belief to be involved in protein oligomerization by disulfide-linkage in other olfactomedins. The linker section between 185 to 245 amino acids is pliable.

Comparative analysis of the amino acid sequences of myocilin protein from fifteen different species (Fig. 2a & 2b) shows conservation of serine at 173, leucine at 215, lysine at 216, proline at 370, and arginine at 422 positions in all analyzed proteins. Glutamic acid at 48 and arginine at 136 also retained in 14/15 species; glycine at 122 retained in 12/15 species studied. Simultaneously, arginine at 76 and serine at 238 was not conserved in most of the proteins analyzed. So p.R76K and p.S238N cannot be regarded as pathogenic variants.

Clinical phenotypes of patients with pathogenic myocilin variants were more severe than most JOAG patients negative for myocilin mutations. In the current study, we have identified pathogenic MYOC variants in 7.5% of cases. Similar findings have been reported by with Sripriya and associates in Indian POAG/JOAG population in 2004 [19].

The mutated myocilin protein is not secreted adequately from trabecular meshwork (TM) cells but retained in the cell bodies [47, 48]. This retained myocilin protein becomes cytotoxic to the cells, causes their death, leading to improper drainage. It causes an elevation in intraocular pressure, which is the most common risk factor for glaucomatous damage [49, 50].

Pathogenic myocilin variants may behave as a gain of function mutations and cause the glaucoma phenotype by hampering other ocular proteins' function [47, 51]. They produce highly misfolded polypeptides and reduce secretion in cultured cells and transgenic mouse models [47, 51–54].

In 2001, Jacobson and associates transduced A549 and TM5 cells with wild-type and mutated MYOC adenovirus, including p.Q368X. In their study, the wild type myocilin protein was detected intracellularly as well as in the medium. But there was no detectable myocilin in the medium of A549 and TM5 cells transduced with p.Q368X adenovirus suggesting the possibility that the truncated myocilin protein may accumulate in intracellular compartments [47]. So, non-secreted mutant myocilin protein could compromise proteasomal function, leading to cell death [47, 51].

In 2008 Aroca-Aguilar and his team studied the heterozygous expression of wild-type and mutant myocilin proteins (p.E323K, p.R346T, p.P370L, p.D380A and p.Q368X) in HEK-293T cells and reported intracellular retention of wild-type myocilin and raised the extracellular concentration of mutant myocilin protein [55]. They observed extracellular mutant myocilin protein accounted for up to 20% of total mutated myocilin. The secreted wild type myocilin significantly decreased by 2.6 fold (for p.E323K) to 36 fold (for p.Q368X). All this might be due to specific structural defects in the protein, which could affect the formation of wild-type/mutant heteroaggregates. They suggested that the extracellular mutant myocilin in patients could be secreted by different tissues such as the ciliary muscle, ciliary epithelium and TM cells [56–58]. So, “heterozygous pathogenic variants in MYOC increase the secretion of the mutant forms and reduce the extracellular processed myocilin” [55]. This could also be due to a dominant-negative effect of pathogenic myocilin variants resulting from hetero-aggregation and might contribute to the pathogenic mechanism leading to IOP rising and glaucoma. Similar findings (i.e. moderate elevation of IOP, loss of approximately 20% of retinal ganglion cells in the peripheral retina, and axonal degeneration in the optic nerve) have reported in the mouse [59].

Identifying new and already identified mutations in known glaucoma genes may enable us to design DNA based diagnostic tests in the near future. Besides, mutations in these genes should help us correctly identify the root cause of this devastating optic neuropathy. This, in turn, may enable us to diagnose the disease before the onset of irreversible vision loss. It also may give us critical information regarding future animal trial/testing with respect to drug response analysis.

Conclusion

Pathogenic myocilin variants identified in 7.5 % of JOAG cases in north India. Five novel myocilin variants identified and registered in NCBI. This study augments the mutation spectrum of the MYOC gene, provides population-specific information, and aids in understanding the underlying lesions of glaucoma pathogenesis.

Abbreviations

JOAG: Juvenile open-angle glaucoma; MYOC: Myocilin; CYP1B1: Cytochrome P450 family 1 subfamily B member 1 ; OPTN: Optineurin ; WDR36: WD repeat domain 36; OPD: Outpatient Department; IOP: Intraocular pressure; NCBI: National Center for Biotechnology Information; TM: Trabecular meshwork; ATP: Adenosine triphosphate; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; PCR: Polymerase chain reaction; ºC: degree Celsius; µl: microlitre; HGVS: Human Genome Variation Society; SIFT: Sorting Intolerant From Tolerant; PROVEAN: Protein Variation Effect Analyzer;

Declarations

Authors Contributions:

Manoj Yadav & Anupama Deora have done the sample collection, benchwork and data analysis. Sumit Sachdeva, Manisha Rathi, and Jitender Phogat have provided patient samples and other details. Chand Singh Dhull conceptualized the study along with Mukesh Tanwar. Mukesh Tanwar also performed computational analysis. Minakshi Vashist and Mukesh Tanwar has written the manuscript. All authors read and finalized the manuscript.

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Availability of data and materials

MYOC reference sequence ENSG00000034971; CLUSTALW; ClinVar; gnomAd;

Ethics approval and consent to participate

The study was approved by the Institutional Review Board of Pt. B.D. Sharma University of Health Sciences Rohtak (HR) India, and participating individuals or their guardians gave written informed consent

Consent for publication:

Not Applicable. Patients' anonymity is strictly maintained.

Competing Interests:

The authors declare that they have no competing interests

Author Details

[1] Laboratory for Human Molecular Genetics, Department of Genetics, Maharshi Dayanand University, Rohtak (HR) India – 124001

[2] Regional Institute of Ophthalmology, Pt. B.D. Sharma University of Health Sciences, Rohtak (HR) India – 124001

[3] Human Molecular and Cytogenetic Laboratory, Department of Genetics, Maharshi Dayanand University, Rohtak (HR) India – 124001

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Tables

Table 1: Showing MYOC Primers Sequence (5′-3′)

S.No.	Primer Name	Primer Sequence	Product Size in basepairs
1	MYC1.1F	5′ TATTTTCTAAGAATCTTGCTGGCAG 3′	394 bp
2	MYC1.1R	5′ TGGATTCATTGGGACTGGC 3′	
3	MYC1.2F	5′ AAGCCTCACCAAGCCTCTG 3′	341 bp
4	MYC1.2R	5′ GCCTGGTCCAAGGTCAATT 3′	
5	MYC1.3F	5′ CTGGAGGCCACCAAAGCT 3′	448 bp
6	MYC1.3R	5′ AGAAAGGGCAGGCAGGGA 3′	
7	MYC2F	5′ CATAGTCAATCCTTGGGCCA 3′	381 bp
8	MYC2R	5′ CTGCAGACCTGCTCTGACAAG 3′	
9	MYC3.1F	5′ TCTGAATTTACCAGGATGTGGAG 3′	424 bp
10	MYC3.1R	5′ GTCAATGTCCGTGTAGCCAC 3′	
11	MYC3.2F	5′ GGACAGTTCCCGTATTCTTGG 3′	430 bp
12	MYC3.2R	5′ GCTTGGAGGCTTTTCACATC 3′	
13	MYC3.3F	5′ CAAGACCCTGACCATCCCA 3′	411 bp
14	MYC3.3R	5′ GCCCCAAATCACAAGAAAAC 3′	

Table 2: Showing clinical phenotypes and MYOC nucleotide variants identified in this study

Patient ID	Presenting Complaint	IOP in mm Hg at presentation (Rt/Lt)	Current Optical Cupping (Rt/Lt)	Current Vision (Rt ; Lt)	Identified Variants (H: homozygous; h:heterozygous)
P1	Heaviness of Forehead, halos around lights, Watering in the eyes	25/18	0.6:1/0.5:1	6/6/ ; 6/6	--
P2	Heaviness of Forehead, redness of eyes	27/17	0.7:1/0.5:1	6/36 ; 6/6	p.R76K (H)
P3	Blurring of vision	28/16	0.6:1/0.5:1	6/18 ; 6/12	p.R76K (H)
P4	Blurring of vision	14/24	0.5:1/0.9:1	6/6 ; 6/12	--
P5	Heaviness of Forehead, Vision problem	26/34	0.8:1/0.9:1	6/18 ; 6/18	p.P370L (h)
P6	Blurring of vision	21/23	0.7:1/0.7:1	6/18 ; 6/18	--
P7	Headache, severe Vision loss day by day	20/18	0.9:1/0.9:1	3/60; 1/60 VF<10^0	p.[(Leu215Pro; Lys216Ile)] (h)
P8	Pain in eyes	22/21	0.4:1/0.7:1	6/6 ; 6/6	--
P9	Heaviness of Forehead, sudden vision problems during night	25/24	0.6:1/0.6:1	6/6 ; 6/9	p.R76K (H)
P10	Redness of eyes, Vision Loss	33/33	0.8:1/0.4:1	HM ; 5/60	p.R76K (h)
P11	Low vision, watering in eyes, heaviness of forehead	22/22	0.8:1/0.7:1	6/60 ; 6/36	
P12	Heaviness of Forehead, halos around light with vision problems, redness of eyes	22/25	0.9:1/0.8:1	PL -ve ; 6/18	p.G122A (H), p.P370L (h),
P13	Heaviness of Forehead, watering, redness of eyes	27/19	0.7:1/0.5:1	6/12; 6/6	p.R136I (h), p.K484= (h)
P14	Heaviness of forehead, Vision loss	24/20	0.8:1/0.8:1	6/9; 6/18	p.K484= (h)
P15	Heaviness of forehead, Vision loss	22/22	0.7:1/0.7:1	6/60; 6/60	--
P16	Watering in eyes, Severe vision loss in both eyes	49/38	0.9:1/0.7:1	PL -ve ; 6/36	--
P17	vision loss in both eyes	30/30	0.7:1/0.7:1	6/12; 6/9	p.K484= (h)
P18	Heaviness of Forehead, redness of eyes, severe vision loss	35.8/14.3	Opaque media/0.8:1	PL+ ; 6/6	--
P19	Heaviness of forehead, Vision loss	36/40	0.8:1/TC	6/18 ; HM	p.R76K (H), p.K484= (h)
P20	Heaviness of Forehead, redness of eyes, watering in eyes	24/12	0.8:1/0.5:1	6/6 OU	p.R76K (h), p.K484= (h)
P21	Heaviness of Forehead	23/23	0.6:1/0.6:1	6/6; OU	p.K484= (h)
P22	Vision loss	23/23	0.6:1/0.9:1	6/9; HM	p.R76K (H)
P23	Heaviness of forehead, Redness of eyes	18.9/23.8	0.7:1/0.7:1	6/9 ; 6/9	p.R76K (H), p.K484= (h)
P24	Pain in eyes	13.4/14.6	0.8:1/0.7:1	6/6 ; 6/6	p.S238N (h)
P25	Heaviness of Forehead, vision loss	20/28	0.7:1/0.7:1	6/12 ; 6/18	p.K484= (h)
P26	Heaviness of Forehead, watering in eyes	22/20	0.7:1/0.7:1	6/9 ; OU	p.R76K (H), p.K484= (H)
P27	Heaviness of Forehead, vision loss	10.2/14.6	HC/0.68:1	6/18 ; 6/24	p.R76K (H), p.K484= (h)
P28	Heaviness of Forehead	22/20	0.7:1/0.7:1	6/18 ; 6/18	p.Q48H (h), p.R76K (H), p.K484= (h)
P29	Pain in eyes, sudden blindness some times for few seconds	12.2/17.3	0.8:1/0.8:1	6/9 OU	--
P30	Heaviness of Forehead, redness of eyes	22/24	0.7:1/0.6:1	6/12 OU	p.K484= (h)
P31	Heaviness of Forehead	26/14	0.6:1/0.5:1	6/6 OU	p.K484= (h)
P32	Vision loss	27/24	0.9:1/0.9:1	PL -ve ; FC	p.K484= (h)
P33	Heaviness of Forehead, watering in eyes	26/20	0.8:1/0.4:1	6/36 ; 6/18	--
P34	Heaviness of Forehead	24/27	0.6:1/0.7:1	6/6 OU	p.K484= (h)
P35	Vision problem during night driving	22/22	0.7:1/0.4:1	6/12; 6/9	--
P36	Heaviness of Forehead, watering in eyes, vision problems	28/28	0.4:1/0.7:1	6/24 ; 6/60	p.R76K (H), p.S238N (h), p.R422H (h), p.K484= (h),
P37	Watering in eyes, vision problems	28/14	0.9:1/0.3:1	6/36 ; 6/18	p.E14= (h)
P38	Heaviness of Forehead	25/24	0.6:1/0.7:1	6/6 OU	p.E14= (h), p.K484= (h)
P39	Vision loss, halos around light	14.6/15.9	0.6:1/0.5:1	6/9 ; 6/6	p.R76K (h), p.E14= (h), p.K156K (h), p.K484= (h)
P40	Heaviness of Forehead , Vision loss	12/12	0.7:1/0.8:1	6/6; 6/9	p.R76K (h), p.K484= (h)
P41	Vision loss, watering in eyes	26/26	0.8:1/0.8:1	5/60; 3/60	p.K484= (h)
P42	Halos around light, scattered vision	28/22	0.8:1/0.8:1	6/36 OU	p.P370L (h), p.K484= (h)
P43	Pain and watering in the yes	26/22	0.6:1/0.6:1	6/6 OU	p.K484= (H)
P44	Pain in eyes, vision loss	17/28	0.5:1/0.9:1	6/6 ; PL+	p.K484= (h)
P45	Redness of eyes, watering, vision problem	25/20	0.7:1/0.7:1	6/36; 6/18	p.R76K (h), p.S173T (h), p.R200Kfs*16/p.L215X,
P46	Headache	25/23	0.8:1/0.7:1	6/9; 6/9	--
P47	Vision loss in left eye, watering	17.3/18.6	0.7:1/0.8:1	6/9 ; HM	p.K484= (H)
P48	Watering in eyes, vision problems	21/26	0.8:1/0.8:1	6/9 ; 6/18	p.K484= (H)
P49	Vision problems	Soft eye/25	Hazy cornea OU	6/60; 6/36	p.K484= (h)
P50	Watering in eyes, vision loss	17.3/30.4	0.7:1/0.8:1	6/9 ; FC	p.K484=(h)
P51	Blurred vision, Watering in eyes, heaviness of forehead	23.5/16	0.5:1/0.7:1	6/12 ; 6/6	p.R76K (h)
P52	Watering in eyes, heaviness of forehead	22/17.8	0.6:1/0.7:1	6/18 ; 6/12	p.K484= (h)
P53	Watering in eyes, heaviness of forehead	16/22.4	0.7:1/0.7:1	6/18 ; 6/6	p.R76K (H)
P54	Hazy cornea, Watering in eyes, headache	23.6/14.3	0.8:1/0.5:1	6/24 ; 6/36	p.Q19K (h)
P55	Redness of eyes, headache	21/23	0.6:1/0.7:1	6/12 ; 6/6	--
P56	Redness of eyes, heaviness of forehead	24.6/20.6	0.8:1/0.6:1	6/36 ; 6/18	--
P57	Pain in both eyes	23.2/19	TC/0.6:1	6/36; 6/6	--
P58	Pain in right eye, Heaviness of Forehead	23/15.9	0.6:1/0.6:1	6/6 ; 6/6	--
P59	Hazy cornea, Pain in eyes, Heaviness of Forehead	28/20	0.8/0.8	6/36 ; 6/12	--
P60	Slight pain and irritation in eyes, Heaviness of Forehead	22.3/17.3	0.5:1/0.7:1	6/6 ; 6/6	--
P61	Headache, No vision in right eye	15.9/24.6	0.5:1/0.7:1	PL -ve; 6/18	p.R76K (h); p.F430=
P62	No vision in left eye, headache	17.7/8.8	0.5:1/TC	6/18 ; PL -ve	
P63	Headache, watering in eyes	20.6/24.4	0.7:1/0.6:1	6/6 ; 6/6	p.R76K (h)
P64	Pain in eyes	26.1/22.3	0.7:1/0.8:1	6/12 ; 6/12	p.R76K (h)
P65	Redness and pain in eyes	22.4/18.9	0.5:1/0.6:1	6/18 ; 6/18	p.R76K (h)
P66	Heaviness of forehead	20.6/28	0.7:1/0.7:1	6/6 ; 6/24	
P67	Hazy cornea, Heaviness of forehead	22.9/17.3	0.5:1/0.6:1	6/6 ; 6/12	p.R76K (h); p.Y347=;
P68	Redness of eyes, Heaviness of forehead	15/22.7	0.7:1/0.5:1	6/12 ; 6/18	p.R76K (H)
P69	Pain in eyes	23.6/18.9	0.6:1/0.5:1	6/12 ; 6/36	p.R76K (h)

P70	Redness or eyes	17.3/23.3	0.7:1/0.7:1	6/36 ; 6/36	p.R76K (h)
P71	Watering in eyes, pain in eyes, heaviness of forehead	20.6/28.6	0.6:1/0.4:1	6/9 ; 6/24	p.R76K (h)
P72	Redness of eyes, heaviness of forehead	17.3/25.8	0.8:1/0.5:1	6/9 ; 6/9	--
P73	Hazy cornea, watering in eyes, redness of eyes and pain in eyes	20/24.6	0.8:1/0.6:1	FC ; 6/24	--
P74	Redness of eyes, watering in eyes, heaviness of forehead	17.3/22.9	0.8:1/0.7:1	6/6 ; 6/6	--
P75	Hazy cornea left eye	14.6/30.4	0.5:1/0.7:1	6/6 ; 6/12	--
P76	Pain in right eye	25.4/19	0.7:1/0.5:1	6/36 ; 6/6	--
P77	Problem in sunlight	25/25	0.4:1/0.7:1	6/6 ; 6/6	--
P78	Low vision	24.2/17.3	0.8:1/0.8:1	6/6 ; 6/6	--
P79	Redness of eyes	17.3/20.6	TC /0.8:1	PL +ve; 6/9	--
P80	heaviness of forehead, Vision problems	26/25	0.6:1/0.6:1	6/36 ; 6/36	--

Note:

3'UTR: 3' Untranslated Region

MYOC sequence variants in Untranslated Regions, introns has not been considered
H: Homozygous h: heterozygous Rt: Right eye Lt: Left Eye OU: Both Eyes NS: Normal Shifting
TC: Total cupping HC: Hazy Corneal Media NS: Normal Shifting HM: Hand movement FC: Finger Counting PL-ve: No perception of light
Red Color: Pathogenic variant
Blue Color: not sure about the pathogenicity
Green Color: Non-pathogenic variant

Table3: Identified sequence variants and pathogenicity predictions of missense variants

Sr. No.	Identified Change	Genomic Position & Base Change	cDNA position	Codon Change	Amino Acid Change	No. of Patients with the change (H: homozygous h: heterozygous)	Present in controls or not	Presence in gnomAD Database	Presence in ClinVar Database	Pathogenicity Predictions			
										SIFT	PROVEAN	PolyPhen2	Mutati Taste
1	p.Glu14=	g.171652570G>A	c.42	GAG>GAA	p.E14=	03 (h)	No	Yes	No	Not Applicable			
2	p.Gln19Lys	g.171652557C>A	c.55	CAG>AAG	p.Q19K	01(h)	No	No	No	tolerated	neutral	benign	polymorp
3	p.Gln48His	g.171652468G>T	c.144	CAG>CAT	p.Q48H	01 (h)	No	Yes	Yes	tolerated	neutral	benign	disease-causing
4	p.Arg76Lys	g.171652385G>A	c.227	AGA>AAA	p.R76K	11 (H), 13 (h)	Yes	Yes	Yes	not tolerated	Neutral	benign	polymorp
5	p.Gly122Ala	g.171652247G>C	c.365	GGC>GCC	p.G122A	01 (H)	No	No	No	tolerated	Neutral	benign	polymorp
6	p.Arg136Ile	g.171652205G>T	c.407	AGA>ATA	p.R136I	01 (h)	No	No	No	not tolerated	neutral	benign	polymorp
7	p.Lys156=	g.171652144G>A	c.468	AAG>AAA	p.K156=	01(h)	No	No	No	Not Applicable			
8	p.Ser173Thr	g.171652094G>C	c.518	AGC>ACC	p.S173T	01 (h)	No	No	No	tolerated	neutral	possibly damaging	polymorp
9	p.Arg200Lysfs*15 or p.Arg200Lys*Ter*15	g.171652013delG	c.599	Frameshift mutation	p.R200KTer*15	01 (H)	No	No	No	Not Applicable			
10	p.[(Leu215Pro; Lys216Ile)]	g.171607823T>C, g.171607822G>C,	c.644 c.645	CTG>CCC	p. [(L215P;K216I)]	01 (h) As compound heterozygous	No	Yes	No	not tolerated	deleterious	probably damaging	disease-causing
11		g.171607820A>T, g.171607819G>T	c.647 c.648	AAG>ATT			No	No	No	not tolerated	deleterious	probably damaging	disease-causing
12	p.Ser238Asn	g.171638614G>A	c.713	AGT>AAT	p.S238N	02 (H)	No	No	No	tolerated	neutral	benign	polymorp
13	p.Tyr347=	g.171636399T>C	c.1041	TAT>TAC	p.Y347=	01(h)	No			Not Applicable			
14	p.Pro370Leu	g.171605471C>T	c.1109	CCG>CTG	p.P370L	03 (h)	No	No	Yes	not tolerated	deleterious	probably damaging	disease-causing
15	p.Arg422His	g.171636175G>A	c.1265	CGT>CAT	p.R422H	01 (h)	No	Yes	No	tolerated	neutral	probably damaging	disease-causing
16	p.Phe430=	g.171636150C>T	c.1290	TTC>TTT	p.F430=	01 (h)	No	No	No	Not Applicable			
17	p.Lys484=	g.171635988G>A	c.1452	AAG>AAA	p.K484=	01 (h)	No	Yes	No	Not Applicable			

Figures

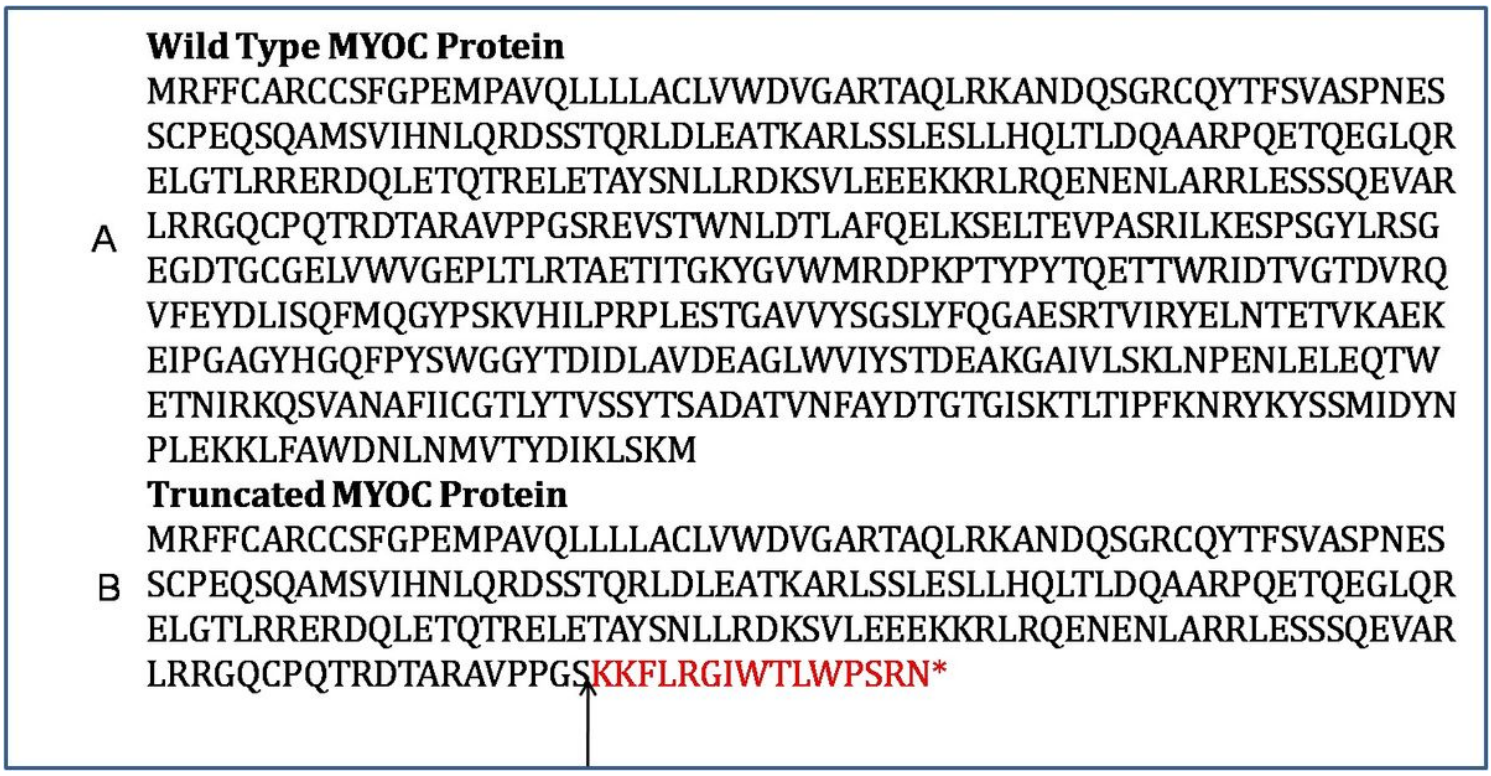

Figure 1

Showing amino acid sequence of human myocilin protein A: Wild-type MYOC protein. B: Truncated MYOC protein of 214 amino acids (black arrow shows the position after which frameshift occurs and red letters show amino acids after frameshift).

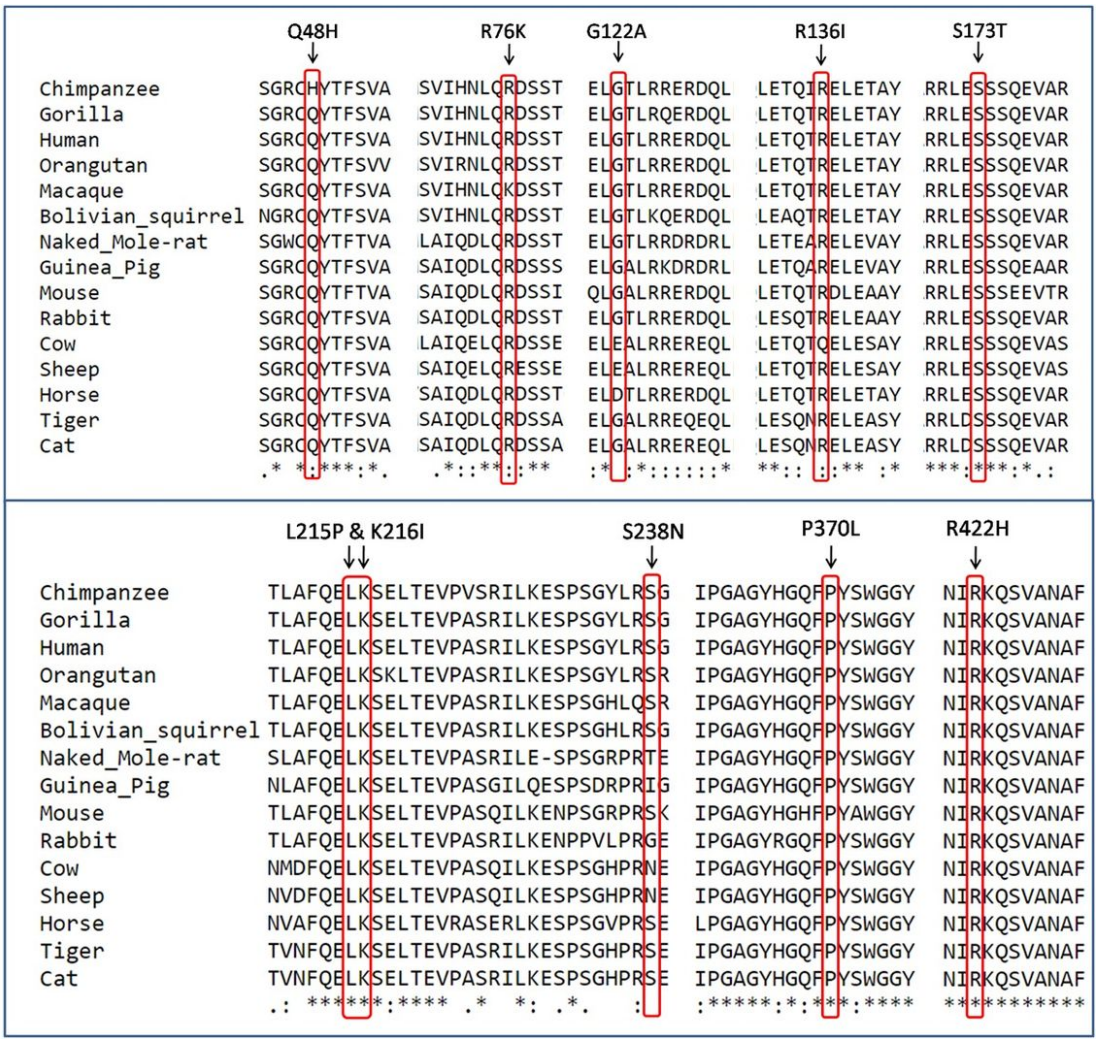

Figure 2

a: Multi sequence alignment of the human MYOC protein with the protein from different species Arrow indicates the position of mutated amino acids at position 48, 76, 122,136 and 173. b: Multi sequence alignment of the human MYOC protein with the protein from different species. Arrow indicates the position of mutated amino acids at position 215, 216, 238, 370 and 422

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFileforDNAseqChromatogramsandpedigree.pdf