European Journal of Medical Genetics 56 (2013) 292–296

Contents lists available at SciVerse ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

Short clinical report

A *de novo MYOC* mutation detected in juvenile open angle glaucoma associated with reduced myocilin protein in aqueous humor

John Kuchtey [a], Uttio Roy Chowdhury [b], Colby C. Uptegraft [a], Michael P. Fautsch [b], Rachel W. Kuchtey [a,*]

[a] *Vanderbilt Eye Institute, Vanderbilt University, 2311 Pierce Avenue, Nashville, TN 37232, USA*
[b] *Department of Ophthalmology, Mayo Clinic, Rochester, MN 55905, USA*

ARTICLE INFO

Article history:
Received 7 October 2012
Accepted 10 March 2013
Available online 19 March 2013

Keywords:
Aqueous humor
de novo mutation
Glaucoma
Myocilin

ABSTRACT

MYOC mutations were originally identified in patients with juvenile open angle glaucoma (JOAG). Cell culture and mouse studies suggest that *MYOC* mutations cause glaucoma through a dominant-negative effect on myocilin protein secretion. We tested this hypothesis with patient samples in this study. Glaucoma and control patients underwent complete ocular examination. DNA samples from glaucoma patients, unaffected relatives and controls were used for DNA sequencing of *MYOC*. Aqueous humor (AH) samples from glaucoma and control patients were obtained at the time of surgery. Myocilin protein in AH was detected by quantitative Western blot analysis. A *de novo* Val251Ala mutation of *MYOC* was found to segregate with disease in a family with autosomal dominant JOAG. Myocilin protein was detected in all control AH samples but was nearly undetectable in AH samples from a patient heterozygous for the Val251Ala mutation. Our results using human patient samples are consistent with a dominant-negative effect of pathogenic *MYOC* mutations on myocilin secretion.

1. Introduction

Primary open angle glaucoma (POAG) is the leading cause of irreversible blindness due to optic nerve damage. An important risk factor for POAG is elevated intraocular pressure (IOP) which is determined by the balance of the flow of aqueous humor (AH) into and out of the anterior chamber of the eye [1]. In POAG, the rate of AH production is not affected and elevated IOP is caused by increased resistance of AH outflow through the trabecular meshwork (TM), a filtering structure composed of alternating layers of extracellular matrix and TM cells. The precise mechanism of increased resistance to AH outflow through the TM is not well understood [2].

Myocilin contains an olfactomedin-homology domain and is a secreted protein with unknown functions. The myocilin gene (*MYOC*) is located within the glaucoma locus *GLC1A* that was identified in a family with an early-onset form of glaucoma, juvenile open angle glaucoma (JOAG) inherited in an autosomal dominant manner [3]. Screening of candidate genes within *GLC1A* revealed mutations in *MYOC* segregating with disease in several JOAG families [4]. Although initially discovered in patients with JOAG, mutations in *MYOC* also account for 2–4% of POAG cases [4–6]. More than 70 disease-causing mutations in *MYOC* have been identified, the vast majority of which occur in exon 3 which encodes the olfactomedin-homology domain [7].

Disease-causing mutations in *MYOC* result in intracellular retention of the normally secreted protein, as shown *in vitro* with cell lines transfected with mutant *MYOC* [8,9]. The non-secretion phenotype is a dominant-negative effect, since co-expression of mutant and wild-type myocilin in cell lines results in intracellular retention or reduced secretion of both forms [10]. The accumulation of myocilin in the endoplasmic reticulum (ER) has suggested that an ER stress response is likely the disease mechanism for *MYOC* mutations [11].

To date, only a few AH samples have been used to investigate possible effects of *MYOC* mutations [8], in part due to the difficulty of obtaining AH samples from the relatively rare patients with pathogenic *MYOC* mutations. In this study, we investigated the presence of myocilin in the AH of a JOAG patient with a disease-causing *MYOC* mutation.

2. Materials and methods

This study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Vanderbilt

* Corresponding author. Tel.: +1 615 936 7190; fax: +1 615 936 1540.
E-mail address: Rachel.kuchtey@vanderbilt.edu (R.W. Kuchtey).

1769-7212/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmg.2013.03.002

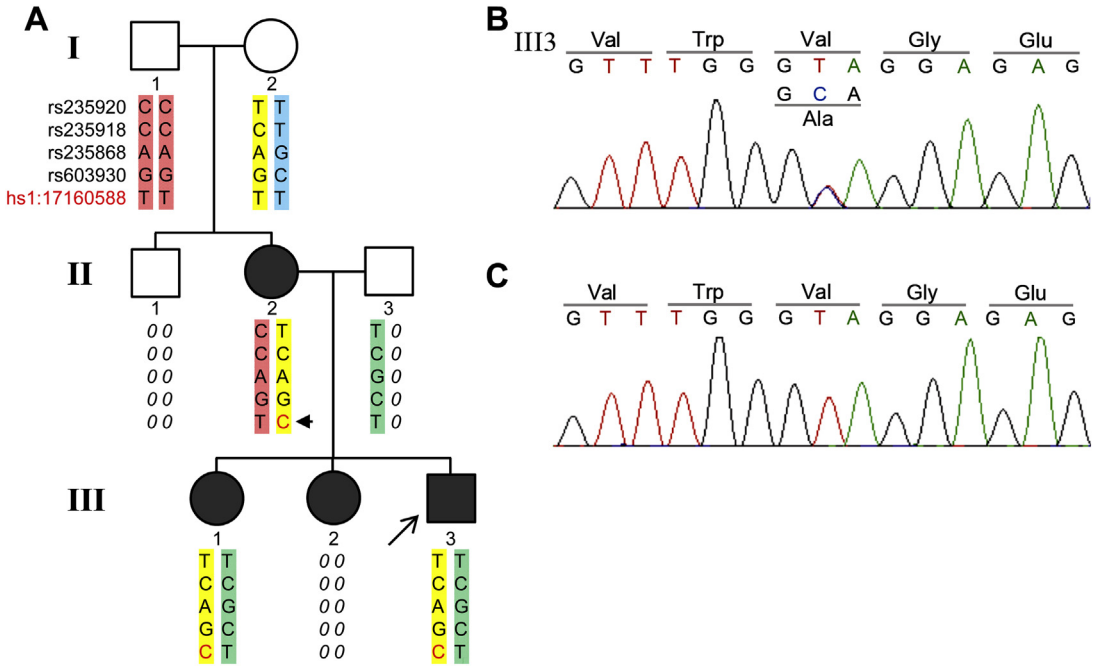

Fig. 1. Pedigree of patients with juvenile open angle glaucoma (JOAG) caused by a *de novo MYOC* Val251Ala mutation. Autosomal dominant inheritance of disease is shown in the three-generation pedigree (A). Affected members are represented with filled symbols, with the proband indicated by an arrow. DNA samples were unavailable from family members II-1, II-3 and III-2. Two grandparents (I-1 and I-2) in the first generation were disease free, but their daughter (II-2) and three grandchildren (III-1, III-2 and III-3) were all affected. Sequencing of *MYOC* revealed that all affected individuals in the pedigree (II-2, III-1 and III-3) carry a heterozygous T > C substitution, resulting in an amino acid change Val251Ala (B). Two available normal individuals (I-1 and I-2) in the pedigree have homozygous allele TT in the location (C). Haplotype analysis of 4 polymorphic SNPs within *MYOC* revealed that the T > C substitution is a *de novo* mutation (arrow head) arising in II-2 and transmitted in an autosomal dominant fashion (A). Chromosomal location of the mutation and rs numbers for the genotyped SNPs are indicated (A).

Medical Center. Other than unavailable family members in the 3-generation pedigree (II1, II3 and III2, Fig. 1A), all patients underwent complete ocular examination, including control subjects. Inclusion criteria for JOAG were absence of secondary causes, age of onset < 40 years, elevated IOP > 21 mm Hg, open iridocorneal angles and glaucomatous optic nerve damage with associated visual field defects.

Blood samples were obtained from patients after written consent forms were signed. DNA extractions from whole blood were performed on a Gentra Systems AutoPure robot using Puregene chemistry (Qiagen, Inc., Valencia, CA). PCR primers (Table 1) based on *MYOC* sequence (genomic, GRCh37/hg19 assembly; cDNA, NM_000261.1) were used to amplify exons 1 and 3, including proximal intronic sequence and partial 5′ promoter region. *MYOC* was sequenced in a total of 20 JOAG patients, including the proband. An additional 43 controls were screened for the Val251Ala mutation. For haplotype analysis, PCR primers were designed to amplify regions within *MYOC* containing known SNPs (Table 1). PCR amplicons were sequenced using a 96-capillary ABI 3730xl DNA Analyzer (Life Technologies Corp., Carlsbad, CA). DNA sequence data was analyzed using Sequencher software, version 4.8 (Gene Codes Corp., Ann Arbor, MI). Novelty of identified *MYOC* variants was investigated by search of the variant databases NCBI dbSNP, Build 137 (Database of Single Nucleotide Polymorphisms, National Center for Biotechnology Information, National Library of Medicine), NHLBI Exome Variant Server (NHLBI GO Exome Sequencing Project, accessed September, 2012) and myocilin database, last updated on August 15, 2012.

AH samples were obtained from JOAG patients ($n = 3$) and controls ($n = 24$) at the time of glaucoma or cataract surgery by a single surgeon (RWK) at the beginning of the procedures. Glaucoma was ruled out in control subjects undergoing cataract surgery. To obtain AH, a 30-gauge needle mounted on a tuberculin syringe was inserted through the clear cornea at the limbus into the central portion of the anterior chamber and 50–100 μl AH was gently withdrawn. AH samples were frozen immediately after collection and stored at −80 °C. AH was also obtained from 3 cadaver eyes within 24 h post-mortem (N1, N2 and N3 in Fig. 2) from donors with no documented history of glaucoma. The age at time of death for cadaver eyes was 36, 38 and 32 years for N1, N2 and N3, respectively.

The amount of myocilin protein in AH samples from control and JOAG patients was determined by a quantitative Western blot method which includes recombinant myocilin standards in each gel, as previously described [12,13], using a rabbit-polyclonal

Table 1
Primers for PCR amplification of *MYOC* gene and Sanger sequencing. One pair of primers was used for promoter region and exon 1 including exon–intron junction, two pairs of primers were used for exon 3 including exon–intron junctions. Four sets of primers used for haplotype analysis by genotyping SNPs are shown with respective SNP rs numbers. Amplicon sizes are shown in base pairs (bp).

Name	Sequence (5′–3′)	Target	Amplicon size (bp)
M1.1F	GGTGCATAAATTGGGATGTTC	Promoter & exon 1	999
M1.1R	TTGTGCTAGCTGTGCAGTCTC		
M3-3F	TGCGATAACTGAGGCGTAGAG	Exon 3 (part 1)	997
M3-3R	GGAGGCTTTTCACATCTTGG		
M3-2F	CATTGACTTGGCTGTGGATG	Exon 3 (part 2)	992
M3.1R	CATCCTGCAATCACATCTCC		
MS5F	CTCAATGAGTTTGCAGAGTG	rs235920	399
MS5R	TCTGCTGTGCTGAGAGGTG		
MS89F	TGAACCTTTGCTCAGATTG	rs235918	399
MS89R	CCACTTTCACAAAAAGTGAC		
MS4F	GTCCTGAACACCTGAGAATC	rs235868	396
MS4R	GTAAGCAGGTTTAGGATTGG		
MS2F	CTCAGGCCCAACTGTTATC	rs603930	295
MS2R	AGAATTAGCTGGATGTGGTG		