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Novel mutation in the *MYOC* gene in primary open angle glaucoma patients

EDITOR—Glaucoma is the world's leading cause of irreversible blindness[1] and is characterised by progressive optic disc cupping with corresponding visual field loss. Both intraocular pressure (IOP) and positive family history are risk factors for the development of the disease.[2] Juvenile open angle glaucoma (JOAG) is a subtype of open angle glaucoma characterised by an early onset (10 to 35 years of age) and autosomal dominant inheritance with high penetrance,[3] a characteristic which has led several authors to investigate affected families in an attempt to identify a gene or genes associated with this condition.[4–10] With the use of genetic linkage analysis in families with JOAG, a genetic locus (GLC1A) was recognised on chromosome 1q21-q31.[4] The gene associated with GLC1A has been identified and it codifies a 57 kDa protein named trabecular meshwork induced glucocorticoid response protein (TIGR),[10] also known as myocilin (MYOC).[11] The *MYOC* gene is composed of three exons of 604, 126, and 785 bp, respectively.[12] During screening for mutations in the *MYOC* gene in 25 unrelated Brazilian patients with JOAG, an unreported mutation (Cys433Arg) was detected, present in seven of them.

Patients were followed at the Glaucoma Service of the State University of Campinas, Brazil. They underwent an ocular examination, including gonioscopy by Posner lens, applanation tonometry, slit lamp biomicroscopy, optic nerve evaluation, and automated perimetry (Humphrey 630, program 30-2). JOAG was defined as the presence of characteristic bilateral optic nerve damage and visual field loss in the presence of an open angle in subjects younger than 36 years of age. Each patient included in this study came from different families according to interview data. The study was approved by the Ethics Committee of the State University of Campinas. At the time of the ocular examination, the mean age of JOAG patients was 25.52 years (SD 6.99), ranging from 10 to 35 years, and the mean IOP was 29.96 mm Hg (SD 13.00). Thirteen patients (52%) were male and 12 patients (48%) were female; 13 (52%) were white, 11 (44%) were black, and one (4%) was Asian. Some of the patients had been taking antiglaucomatous medication or had undergone surgical procedures for IOP control and showed IOP levels lower than 22 mm Hg at the time of examination. Fourteen patients (56%) had a positive family history.

Genomic DNA from peripheral blood (withdrawn from the antecubital vein) was prepared using DNAzol™ Reagent according to the manufacturer's instructions (GIBCO/BRL, Gaithersburg, MD). Primers were designed to amplify the three exons of the *MYOC* gene, according to the reported sequence (GenBank accession numbers U85257 and Z98750). Exon 3 was the first to be analysed, since this is the region where the majority of the mutations have been found.[12] SSCP analysis was performed on a Pharmacia PhastSystem (Pharmacia, Uppsala, Sweden) using a 20% homogeneous polyacrylamide gel. Samples with abnormal mobility shifts were sequenced using Thermo Sequenase Cycle Sequencing Kit with γ-^{32}P ATP (Amersham Life Science Inc, Cleveland, Ohio), according to the manufacturer's instructions.

Seven patients (28%) showed a point mutation in exon 3, a heterozygous T to C transition at nucleotide position 1374 leading to a cysteine (Cys) 433 for arginine (Arg) substitution. The SSCP pattern shows two abnormal mobility shifts (figs 1 and 2). Analysis through SSCP and restriction digestion with *Fok*I was performed in 130 control samples of different ethnic origin in order to discard the possibility of the new substitution being a polymorphism. Cleavage of the approximately 195 bp amplified fragment with *Fok*I generates three fragments of 13, 50, and 132 bp in size in the absence of the mutation, and in its presence the 132 bp fragment is cleaved into two additional 33 and 99 bp fragments. The mutation was not present in any of the samples. The Cys433Arg mutation was present in five (71%) white patients and two (29%) black patients. Six patients (85%) of those with the mutation had a positive familial history of glaucoma. The mean age of patients harbouring this mutation was 27.00 years (SD 6.02), ranging from 15 to 35 years and the mean IOP was 39.13 mm Hg (SD 12.62). In contrast, JOAG patients without the mutation had a mean age of 24.02 (SD 7.46) and a mean IOP of 25.65 mm Hg (SD 11.68) ($p>0.05$). In another patient, a previously reported mutation (Pro370Leu) was identified.[13]

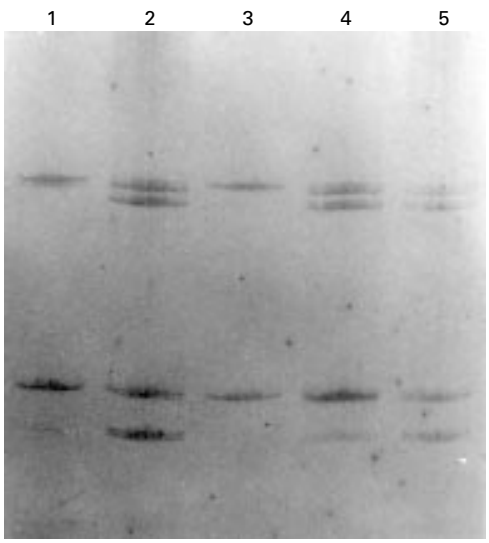

Figure 1 Silver stained SSCP gel showing the 195 bp PCR product encompassing amino acids 412 to 476 of the MYOC gene. Lane 1: control sample. Lanes 2-5: samples of JOAG patients. Samples 2, 4, and 5 show abnormal mobility shifts as compared with the control.

One of the patients who showed the Cys433Arg mutation had the family investigated for its presence. Nine subjects were studied and four harboured the Cys433Arg mutation (fig 3). Three of them had glaucoma and one had ocular hypertension without optic nerve or visual field damage. The other members who did not have the disease were not affected with the Cys433Arg mutation.

In order to discriminate between a founder effect and a de novo recurrence, haplotype analysis was performed in the six patients with a positive family history who had the new mutation and in one family using four microsatellite markers, mapped at band 1q21-25, closely linked to the GLC1A locus, D1S210, D1S2790, D1S1619, and NGA19. Polymerase chain reaction (PCR) was carried out following standard procedures and primer sequences were obtained from the Genome Data Bank. For allele scoring, PCR products were size fractionated on a 6% polyacrylamide-urea gel and autoradiographed. The analysis of four microsatellite markers showed that the Cys433Arg mutation is associated with a common haplotype, suggesting that these patients inherited their mutation from a common ancestor. A pedigree of the family analysed can be seen in fig 3, depicting a potential disease haplotype.

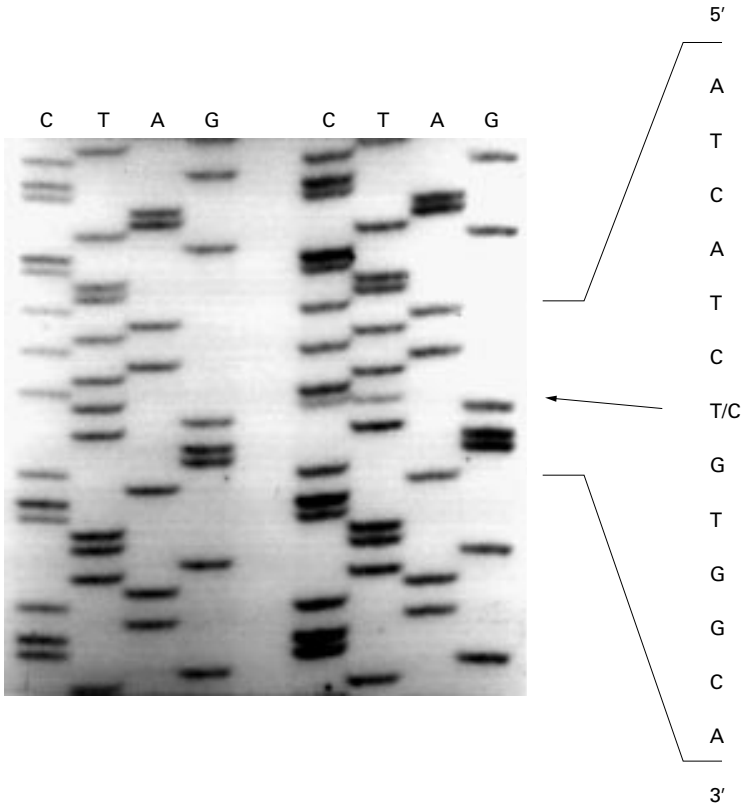

Figure 2 Direct sequencing of the PCR product showing a T-C substitution at codon 433 of the MYOC gene, which changes the amino acid Cys (TGT) to Arg (CGT). The arrow indicates the exact location of the mutation.

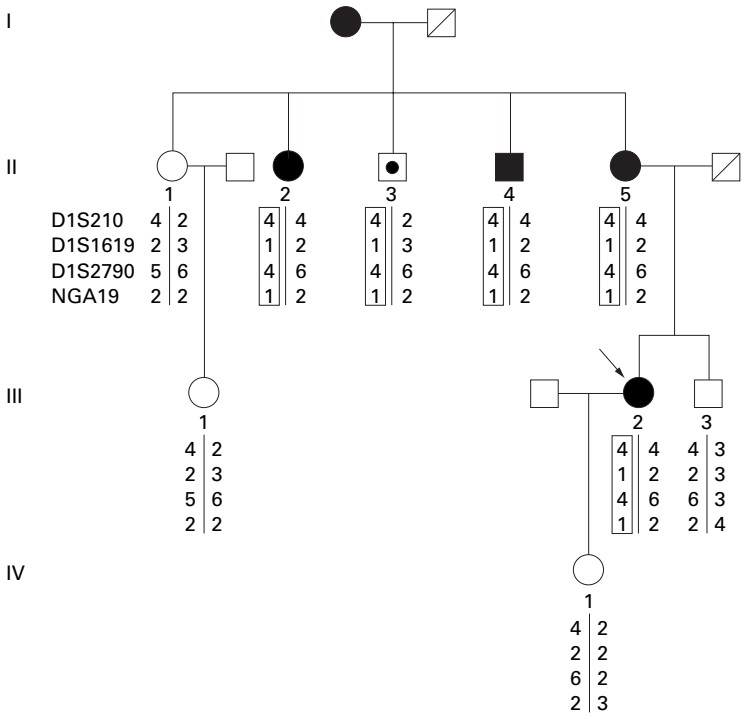

Figure 3 Pedigree of a JOAG patient's family (the proband is indicated with an arrow). Solid symbols indicate affected subjects, all harbouring the Cys433Arg mutation. II.3, indicated with a dotted symbol, has not developed glaucoma, but has very high IOP levels and also shows the Cys433Arg mutation. Four microsatellite markers, located in the vicinity of the GLC1A locus, were used for haplotype analysis. The pedigree shows the genotypes at polymorphic microsatellite loci as well as a potential disease haplotype shown in the boxes.

Since the first description of the *MYOC* gene (then called the *TIGR* gene) by Stone *et al*[10] as one of the genes related to open angle glaucoma at the GLC1A locus, several mutations have been described among patients with open angle glaucoma.[10 11 13 14]

During the screening of exon 3 of the *MYOC* gene in our JOAG patients, we identified a mutation at amino acid 433 (exon 3) which encodes for an arginine instead of a cysteine in 28% of the patients. The mutation is located in a highly conserved amino acid sequence, the olfactomedin (OLFM)-like domain, a region where most of the mutations have been identified.[12] In fact, the cysteine residue 433 is of particular interest, as it is located within the most conserved region between species, from *C elegans* to humans. As in other olfactomedins, it is likely that this cysteine residue is involved in protein oligomerisation by disulphide linked polymer formation.[15 16] According to Nguyen *et al*,[16] oligomerisation of the MYOC protein could be an important feature in the obstruction of the trabecular meshwork. It is possible that MYOC dimers or polymers are linked to form a higher molecular mass structure via a cysteine-cysteine formation, similar to that predicted in olfactomedin by Yokoe and Anholt.[15] Therefore, mutations in this domain may alter protein oligomerisation and lead to IOP elevation.[16]

Clinically, the Cys433Arg mutation seems to be closely related to glaucoma; it was found in three glaucomatous relatives of one patient and in a relative with ocular hypertension. This change was not found in our control group of 130 unrelated subjects or in any healthy subject studied, indicating that it is a probable disease causing mutation, which, to our knowledge, has not yet been described. Affected patients tend to present with the disease during the third decade, with very high IOPs (high 30s). Hence, the phenotype-genotype correlation is closer to that which has been reported for the Pro370Leu mutation,[17] which also determines a severe disease with an early onset, in contrast with the Gln368Stop mutation, which is associated with later onset of glaucoma.[14]

Haplotype analysis showed that the six patients with a positive familial history who harboured the mutation had the same disease associated haplotype, indicating that this mutation has probably arisen from a common ancestor, as shown for the Asn480Lys mutation[17 18] and for the 1177GACA→T mutation.[19]

Although the mechanisms involved in the association between the *MYOC* gene, POAG, and JOAG are not completely understood,[14] it is not unreasonable to expect that the pathophysiology of these diseases will be elucidated, leading to better treatments. Furthermore, it may be used as a screening test to identify susceptible subjects long before the development of optic nerve damage, allowing early treatment and possibly avoiding the disease related visual impairment. Finally, it may be possible to modify the *MYOC* gene in order to inhibit phenotypic changes induced by mutations, thereby ultimately halting the development of glaucoma.

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Three novel *SALL1* mutations extend the mutational spectrum in Townes-Brocks syndrome

EDITOR—Townes-Brocks syndrome (TBS, MIM 107480) was first described by Townes and Brocks[1] in 1972 as an association of imperforate anus, supernumerary thumbs, malformed ears, preauricular tags, and sensorineural hearing loss. Several additional familial as well as isolated cases have been reported.[2] TBS is caused by mutations of the putative zinc finger transcription factor gene *SALL1*.[3] All *SALL1* mutations identified to date in TBS patients are located 5' of the first double zinc finger encoding region.[4] Three of these are nonsense mutations at two different positions. The mutation 826C>T was found in three unrelated sporadic cases, and at position 1115 one patient carried an adenine (1115C>A) and another a guanine (1115C>G) instead of a cytosine. All seven other reported mutations are short frameshift deletions of 1, 2, 7, or 10 base pairs.[4]