A novel Asp380Ala mutation in the GLC1A/myocilin gene in a family with juvenile onset primary open angle glaucoma

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Abstract
Glucoma describes a clinically and genetically heterogeneous group of diseases that result in optic neuropathy and progressive loss of visual fields. A gene for juvenile onset primary open angle glaucoma (JOAG) has recently been mapped to 1q21-31. Mutations in the trabecular meshwork induced glucocorticoid response gene (TIGR, also known as myocilin or the GLC1A locus) have been found to cause both juvenile and later onset primary open angle glaucoma. Family TCD-POAG1 is a Spanish kindred, which segregates JOAG in an autosomal dominant fashion. This family was found to be linked to the previously identified GLC1A locus on chromosome 1q. Direct sequencing of the TIGR/myocilin gene showed a heterozygous A to C transition in codon 380, resulting in the substitution of alanine for aspartic acid (Asp380Ala). This substitution created a Sp6I restriction site, which segregated with the JOAG phenotype and permitted rapid screening of all members of the family. This restriction site was not present in 60 controls. (J Med Genet 1998;35:957–960)

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The term glaucoma encompasses a heterogeneous group of optic neuropathies which bring about loss of visual field and which, if left untreated, can lead to total blindness. Glaucoma has been subdivided into various categories, depending on factors such as the age of onset, the shape of the iridocorneal angle, and whether or not the glaucoma is primary or secondary in nature. The most frequently observed form of the disease is primary open angle glaucoma, or POAG, which affects approximately 2% of the population over the age of 45 years. Characteristic of POAG include atrophy of the optic nerve as a result of degeneration of the retinal ganglion cells and resulting progressive loss of visual field. Losses in visual field are often preceded and accompanied by raised intraocular pressures; however, the trabecular meshwork is usually normal in appearance. POAG varies in its age of onset and has been classified (somewhat arbitrarily) into juvenile onset primary open angle glaucoma (JOAG) and the more frequently observed later onset form, chronic primary open angle glaucoma (COAG). Several loci have been implicated in the aetiology of various forms of glaucoma. Regions implicated in adult onset POAG have been mapped to chromosomes 2cen-q13 and 3q21-24. The pigment dispersion syndrome, of which JOAG is a feature, has recently been mapped to 7q35-q36. A form of POAG also cosegregates with the nail patella syndrome (NPS) locus on 9q34. Open angle glaucoma associated with iris and iridocorneal angle abnormalities has been linked to 4q25 and 6p25. Primary congenital glaucoma has been mapped to 1p36, 2p21, and the terminal region of 6p. The cytochrome P4501B1 gene has been found to be mutated in cases of primary congenital glaucoma that map to 2p21. The first locus for the condition (named GLC1A) was placed on 1q21-31 by a large number of studies and the critical region was eventually narrowed to 3 cM. Recently, mutations in the TIGR gene (trabecular meshwork inducible glucocorticoid response protein), which maps on 1q, have been associated with glaucoma in both JOAG and COAG patients. TIGR has been shown to be identical to an independently isolated myosin-like protein named myocilin, which was identified from a retina specific cDNA library and was found to be expressed in the interconnecting cilia of photoreceptor cells. (Note: previous usage of the acronym TIGR led to the suggestion that the gene be referred to as myocilin and/or GLC1A. Henceforth the gene is referred to as myocilin in this text.)

Myocilin is encoded by a gene with three exons and two introns and is 504 amino acids in length. Putative structural features include various specific promoter motifs, a signal sequence for secretion, putative sites thought to be involved in cell–cell and glycoprotein interactions, an N-terminal hydrophobic region, a myosin-like domain, a leucine zipper, probably involved in homodimerisation, and a C-terminal olfactomedin-like domain. To date, the vast majority of the mutations have been confined to exon 3, which encodes the olfactomedin-like C-terminal domain.

Here we describe a large Spanish family (TCD-POAG1) segregating autosomal dominant JOAG (fig 1) with a novel Asp380Ala mutation in the myocilin gene. All family

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members for whom DNA samples were available were clinically assessed at the Fundación Jimenez Diaz in Madrid, Spain. Initial diagnosis of affected subjects was made, in most cases, on the basis of raised intraocular pressures, which were often above 30 mm Hg and sometimes above 40 mm Hg. Medically uncontrolled raised intraocular pressures and continuing loss of visual field have necessitated drainage operations in many of the patients, usually within the third and fourth decades. Diagnosis of patients, extraction of DNA, PCR, direct sequencing, and linkage analysis were carried out as previously described.

Amplification and sequencing primers (5'-3') were as follows: TIGRA Fwd: GAACCTGGAACAAACCCTGGGA, TIGRA Revs: CATGGTCAATCATTAGCCG, TIGRB Fwd: ATACTCCTAGGCCACTGGA, TIGRB Revs: CATTGCTCGTG-TAGCCACC, TIGRex3.3 Fwd: TGGGCTACGACGGACAGTTC, TIGRex3.3 Revs: CATTGCTCGTGACTGCTTA.

An aliquot of each PCR product was digested with 20 units of Ssp1 (New England Biolabs) at 37°C overnight with the buffer supplied by the manufacturers. Samples were analysed on 2.5% ethidium bromide stained agarose gels.

Family TCD-POAG1 was found to show linkage to the previously identified GLC1A locus on chromosome 1q with a Zmax of 5.478 at 0% recombination with marker D1S2658 (not shown). Multipoint analysis gives a Zmax of just over 6 in the interval flanked by the markers D1S2658 and D1S2659. The reported involvement of the myocilin gene in the aetiology of glaucoma prompted the screening of the coding regions of the gene in affected members of this family. Direct sequencing of DNA of two affected family members showed a heterozygous A to C transition in exon 380 resulting in the substitution of alanine for aspartic acid (fig 2). This substitution created a Ssp1 restriction site which permitted rapid screening of all family members of the family and confirmed that the mutation segregated with the JOAG phenotype (fig 3). The restriction site was not present in 60 unrelated CEPH controls. Therefore, these data suggest that the Asp380Ala amino acid change is the cause of JOAG in this pedigree.
The fact that this substitution results in the replacement of an acidic and hydrophilic amino acid by a neutral and hydrophobic one is further evidence that the Asp380Ala mutation may disrupt the secondary structure of myocilin to pathogenic effect. Further evidence to this effect involves comparisons between myocilin and three related proteins from the bullfrog, rat, and C elegans: OLP, NOPR, and F11C3.24 This amino acid sequence alignment shows that the aspartic acid residue at position 380 of TIGR is conserved in all four proteins.24

The inheritance of JOAG has previously been described as autosomal dominant with reduced penetrance.1 3 There are no cases within this pedigree of persons who have inherited the disease mutation and who do not have glaucoma. However, four family members were initially diagnosed as affected and have since been found not to carry the Asp380Ala mutation. Subject V.30 was diagnosed with high IOP and glaucomatous field changes at 18 years and has been operated on to control the progression of disease. However, she has been found to have closed angle glaucoma. Subject V.8 was diagnosed as affected on the basis of mild visual field defects; however, her IOP was normal. Subject V.19 was diagnosed as affected on the basis of high IOP and asymmetrical cupping of the optic discs. Subject IV.10 was diagnosed at the age of 47 (later than is usual for the rest of the family) with high IOP and diffuse reduction of sensitivity in the visual fields. With the exception of V.30, the remaining three anomalous subjects have mild symptoms of glaucoma and, before mutational analysis, were classified as "unknown" for the purposes of linkage analysis. Given that younger members of the family are at high risk of developing glaucoma, intensive medical scrutiny might have resulted in the misdiagnosis of some of these people as affected on marginal grounds. Alternatively, given the mildness and later onset of symptoms observed in IV.10, V.8, and V.19 and the high frequency of later onset POAG in the population, it is entirely possible that they represent phenocopies of the more severe disease noted in those with the Asp380Ala mutation. A growing number of mutations in myocilin have now been implicated in both JOAG and adult onset POAG.5 24-30 33 Discovery of further mutations within this gene should assist in the identification of those at risk of developing glaucoma before the development of significant visual dysfunction. Further understanding of its biochemistry and functions may lead to the development of improved medication for glaucoma and possibly to the identification of further genes involved in its pathogenesis.

