Evaluation of the *Oculomedin* gene in the etiology of primary open angle and exfoliative glaucoma

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**Purpose:** Glaucoma is a disease of the retinal ganglion cells leading to reduction of peripheral vision. It is often associated with an increase in intraocular pressure, leading to mechanical stress of tissues. The oculomedin gene is activated by such stretching and is therefore a candidate for causing glaucoma.

**Methods:** The coding sequence and part of the promoter was screened for sequence variants in Swedish cohorts of primary open angle glaucoma, exfoliative glaucoma, and matched controls.

**Results:** Only rare variants were detected in the patient material.

**Conclusions:** There was no evidence that the oculomedin gene participates in the etiology of glaucoma.

Glaucoma is the second most common cause of blindness in the world. The symptoms involve an irreversible loss of the peripheral vision due to a degeneration of the retinal ganglion cells and cupping of the optic nerve head. The mechanism behind the disease is largely unknown, but genetic components have been identified [1-9]. So far, two genes have been cloned and shown to contain mutations in families and cases with primary open angle glaucoma (POAG). The *TIGR/MYOC* gene is induced by treatment with glucocorticoids and contains mutations in families with juvenile glaucoma and a fraction of cases with POAG [4,10]. The *Optineurin* gene is mutated in some forms of adult onset POAG, mostly with normal tension [9]. The cloned and mapped genes, however, cannot account for all the heritability.

Glaucoma is often associated with an increase in the intraocular pressure (IOP) [11]. The regulation and increase of IOP is not fully understood but it has been suggested that the trabecular meshwork (TM) cells are important. One gene proposed as a candidate gene for glaucoma is the *Oculomedin* (*OCLM*) or Trabecular meshwork-Inducible Stretch Response gene (*TISR*). The function of the gene is unknown, but it is induced by cyclic stretching in TM cells. The TM is located in the iridocorneal angle which is the principal site of aqueous outflow from the eye. It is proposed that the trabecular cells sense the intraocular pressure and regulate the aqueous outflow. The gene is expressed in the TM and retina, but not in other tissues. *Oculomedin* translates into a small protein containing 44 amino acids. The gene has homology to neuromedin K and an ALU repeat in the 5' UTR [12]. It is a valid candidate for glaucoma by the analogy to the *TIGR/MYOC* gene.

The most common form of glaucoma is POAG but there are several secondary variants. Among these is exfoliative glaucoma, in which there are pseudoexfoliative deposits in the anterior segment of the eye and on the lens capsule. Exfoliative glaucoma is found worldwide but is particularly common in the middle and northern part of Sweden and is often associated with a higher IOP and a more aggressive form of the disease [13].

To scan for sequence variants in a DNA fragment different methods can be utilised. In this study we used denaturing High-Performance Liquid Chromatography (dHPLC), in which the retention time at the critical melting temperature is compared between homo- and heteroduplexes.

The aim of this study was to screen the coding region of the oculomedin gene for mutations and allelic variants in Swedish POAG and exfoliative glaucoma patients, and controls matched for age, sex, and ethnic origin. The gene has been analysed in POAG patients of Chinese origin. In this study, 110 cases of POAG and 108 controls were screened for sequence changes in the exon as well as in the 5' and 3' UTR. No variant with an association to glaucoma was found [14]. This relatively small study in one ethnic group can not necessarily be expected to cover all patients of other ethnic origin, hence it was relevant to examine the gene in a larger case material of European origin. Also, exfoliative glaucoma cases has previously not been screened for this gene and may, in view of the higher IOP associated to the variant, be a stronger candidate for association.

**METHODS**

**Subjects:** Patients with either POAG or exfoliative glaucoma were recruited at the out-patient clinic at the department of Ophthalmology, University Hospital, Uppsala and the Department of Ophthalmology, Tierps Hospital, Tierp. The controls were matched for age, sex, and ethnic origin to the glaucoma patient groups and glaucoma was excluded by a screening examination including intraocular pressure measurement and ophthalmoscopy of the optic disc. After giving informed con-
sent, peripheral blood was collected from 200 patients from each group. The study has been approved by the local Research Ethics Committee.

**Polymerase Chain Reaction (PCR):** Genomic DNA was extracted from 8-10 ml of peripheral blood using the salt precipitation method [15]. A 388 bp fragment containing the translated region of *Oculomedin* was amplified using the primers 5'-TGT AAA TCA TTG CTT CAG GTT-3' as forward and 5'-GTC TAT GCT CTT CAC ACA GGT-3' as reverse. Amplification was performed using standard protocols.

**Denaturing High-Performance Liquid Chromatography (dHPLC):** All 400 patients and 200 controls were screened using dHPLC. To maximize the yield of heteroduplex fragments in the PCR product, the samples were denatured and slowly reannealed (95 °C to 65 °C over 30 min) after PCR. The dHPLC analyses were performed using the ProStar Helix DHPLC System (Varian Inc., Walnut Creek, CA, USA) with a Helix DNA 75 mm x 3.0 mm column with C18 alkylated silica as stationary phase. As mobile phase Varian BufferPack A (100 mM TEAA, pH 7.0, 0.1 mM EDTA) and Varian BufferPack B (100 mM TEAA, pH 7.0, 0.1 mM EDTA, 25% (v/v) acetonitrile) were used. For the analysis, 5 µl crude PCR product was injected onto the column and the DNA was eluted using an increasing gradient of the organic solvent. Recommendations for oven temperature were obtained from the Stanford University Homepage (http://insertion.stanford.edu/melt.html) and temperatures for the analysis were chosen from those recommended and by running a temperature gradient. The results were detected by UV absorbance at 260 nm and analysed using Star 5.5 software (Varian Inc.).

**Sequence analysis:** Direct DNA sequence analysis was performed on samples showing positive results from the dHPLC analysis. All amplified PCR fragments used for sequence determination were purified using QIAquick spin columns according to the QIAquick PCR Purification Kit (QIAGEN®Inc., Valencia, CA, USA), and sequenced using ABI PRISM® Big-Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer®, Applied Biosystems, Foster City, CA, USA) and analysed on an ABI PRISM® 377 DNA sequencer (Perkin Elmer®). The results were analysed using Sequencing Analysis 3.3 (Applied Biosystems) and Sequencer 3.1 (Gene Codes Co. Ann Arbor, MI, USA).

### TABLE I. VARIANT ALLELES IN THE *Oculomedin* GENE

<table>
<thead>
<tr>
<th>Sequence alteration</th>
<th>Amino acid change</th>
<th>Location</th>
<th>POAG (n=200)</th>
<th>Exfoliative glaucoma (n=200)</th>
<th>Controls (n=200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-33T&gt;C</td>
<td>-</td>
<td>5'-UTR</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>104C&gt;G</td>
<td>S35C</td>
<td>Exon</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>154A&gt;G</td>
<td>-</td>
<td>3'-UTR</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

Allelic variants found in the *Oculomedin* gene in Swedish cohorts of primary open angle and exfoliative glaucoma, and unaffected controls. The sequence alteration, its corresponding amino acid change and location in the gene along with the number of patients in each cohort is presented. Sequence alterations are numbered using A in the start codon as base 1.

**Amplification-Refractory Mutation System (ARMS):** ARMS was used to distinguish between variant and normal alleles [16]. Allele specific oligonucleotide primers were designed for the polymorphism in the 5'-UTR region and the polymorphism in the coding region, and the patients and controls were screened for the variants. The second nucleotide from the 3' end was changed to decrease the hybridisation strength of the unmatched primer. The primers used for 104C>G were 5'-GAG TGG TAT TAT ATG GCT AGC-3' for the normal allele and 5'-GAG TGG TAT TAT ATG GCT AGG-3' for the variant allele together with the reverse PCR primer. For the -33T>C polymorphism the primers used were 5'-CTG AGT GTA TAT TTC-3' for the normal allele and 5'-CTG AGC AAC AGT GTA TAT TTC-3' for the variant allele, again together with the reverse PCR primer for the -33T>C polymorphism the primers used were 5'-CTG AGT GTA TAT TTC-3' for the normal allele and 5'-CTG AGC AAC AGT GTA TAT TTC-3' for the variant allele, again together with the reverse PCR primer.

**RESULTS**

In this study, 200 patients with POAG and 200 patients with exfoliative glaucoma along with 200 controls matched for age, sex, and ethnic origin, were screened for allelic variants in the *Oculomedin* gene (Table 1). In the POAG group, the only variant found was 104C>G which changes serine to cysteine, counting A in the start codon as base 1. In the exfoliative glaucoma group, the 104C>G polymorphism was found and also the change 154A>G in the 3'-UTR. In the control group a polymorphism in the 5'-UTR, -33T>C, was found in two instances and also the 104C>G polymorphism was found twice. This was not a significant difference compared to the case groups (P=0.5, Fishers exact test). As compared to the cDNA sequences posted to the NCBI databases (NM_022375, XM_015940, AF142063) our sequence also contained a synonymous change 84G>A (K28K) and a deletion 135+36delC in the 3'-UTR in all patients and controls screened. The interpretation is that the original sequences contained errors in these positions.

**DISCUSSION**

Increased intraocular pressure is one of the most important risk factors of glaucoma and although it is not a required symptom for the diagnosis, there is often an elevation of IOP in glaucoma patients [11]. The increased IOP could lead to the tissues, and thereby the cells, in the eye undergoing stress in the form of mechanical stretching. The discovery of a gene product induced by cyclic stretching in the tissue involved in regulation of intraocular pressure was interesting, since mutations in such a gene could very well give a phenotype that involves elevated pressure in the eye [12]. However, to be sure of this the function of the gene has to be established to ascertain that the induction is no secondary effect.
No association between allelic variants in oculomedin and Swedish patients with either POAG or exfoliative glaucoma was found. This is in concordance with previous studies, where no association was found between Chinese POAG patients and the oculomedin gene [16]. The difference in ethnicity between the groups, and the fact that neither had any viable association, reduces the chance of this gene being associated to glaucoma in another population. Also, our screen of two different types of glaucoma reduces the chance of this gene being involved in the etiology of glaucoma. Having said this, there is always the possibility that other aspects of the gene, such as the expression level, which were not investigated here, are involved.

The only change found in our study was the 104C>G polymorphism, which changes uncharged polar serine to likewise uncharged polar cysteine. This change gives no difference in charge, polarity, or size, but does change the hydroxyl group of serine to a sulfhydryl group in cysteine, thereby creating the possibility of a disulphide bond. This could change the folding of the protein, but since the change was found both in patients and controls, it’s not likely that the effect is pathological with regard to glaucoma. One change in the 5′-UTR region was found in two control subjects. This variant changed the T 33 base pairs upstream of the ATG to a C. Since this change was only found in 1% of the controls, there is no significant association between this variant and the glaucoma phenotype.

The sequence of the gene in the Swedish population differs from that in the NCBI databases. The mRNA sequences posted differed in two positions from that of our patients (NM_022375, XM_015940, AF142063). The changes 84G>A and 135+36delC were present in all patients and controls as compared to the sequence reported. The same sequence variants as the Swedish were reported in a previous study [16]. However, there were no differences compared to the Homo sapiens chromosome 1 working draft sequence (NT_004487) implying sequencing mistakes in the mRNA sequences posted.

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REFERENCES


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