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The Association between TGF-β1 G915C (Arg25Pro) Polymorphism and the Development of Primary Open Angle Glaucoma: A Case-Control Study

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ABSTRACT

The purpose of the current study was to identify the potential association between Single Nucleotide Polymorphism (SNP) TGFβ1 +915 (C or G) in codon 25 and Primary Open Angle Glaucoma (POAG).
Overall, 88 cases with POAG and a control group of 52 healthy individuals were recruited from the First Ophthalmology Department of Athens University. DNA was isolated from whole blood samples and genotype frequencies for the polymorphism rs1800471 (G915C, Arg25Pro) of the TGF-β1 gene were assessed.
Genotype distribution frequencies for the polymorphism rs1800471 (G915C, Arg25Pro) of the TGF-β1 gene were not statistically different between patients with POAG and control subjects.
The present study failed to determine any significant genotypic association with POAG, despite the fact that the presence of the C allele was scarcely increased in the POAG when compared with the control group.

KEY WORDS

Single Nucleotide Polymorphism (SNP); Glaucoma, Open-Angle; TGF-β1 Gene

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INTRODUCTION

Glaucoma is defined as progressive optic neuropathy, which is commonly related to high Intraocular Pressure (IOP), Extracellular Matrix (ECM) remodeling, and ocular vascular changes. Transforming Growth Factor-beta (TGF-β) is a multifunctional peptide, belonging to a family of cytokines present in many cell types, involved in regulating proliferation, differentiation, adhesion, migration, and a number of other functions. Receptors of TGF-β are present in many cells, and the TGF-β protein regulates many other growth factors, both positively and negatively. In processes of wound healing and scarring, TGF-β plays a central role, throughout the body [1-3],

and is present in normal Aqueous Humor (AH) [4, 5] with significantly high levels in the AH of glaucomatous patients, as indicated in numerous studies of the past 20 years [6-17]. A number of studies [17-19] suggest that the outflow system of the human eye, especially the Trabecular Meshwork (TM), is sensitive to TGF-β; the biological effects in TM is indicated by the high levels of TGF-β found in the AH of glaucomatous patients. Transforming Growth Factor-beta stimulates fibroblast activities, as the most potent growth factor in AH [20, 21]. It has been hypothesized that the pathogenesis of POAG, leading to pathological alterations with subsequent aqueous outflow deficiency, is due to accumulated damage to the TM and Schlemm's canal [22], such as chronic scarring and fibrosis of the TM. Both TGF-β2 and TGF-β1 seem to be involved in the pathogenesis of glaucomas, where TGF-β2 is the predominant isoform in normal human AH. However, there are significantly increased levels of TGF-β2 in the AH of patients with POAG [6], and significantly high aqueous levels of latent and active TGF-β1 in patients with exfoliative glaucoma and exfoliation syndrome [23]. Furthermore, numerous studies have claimed that TGF-β1 influences the TM of patients with POAG. Transforming Growth Factor-beta mainly limits aqueous outflow with subsequent elevation of IOP and increases risk of clinically significant optic neuropathy [24-29].

The pathogenesis of neurodegenerative, ocular, and vascular diseases has been shown to involve TGF-β signaling, as well as remodeling of ECM [30, 31]. Dysfunctional TGF-β signaling seems to be involved, partially, in glaucoma pathogenesis, since there seems to be an overlap between the cascade of pathogenesis and responses caused by TGF-β in cells and tissues. Hence, a potential therapeutic target in glaucoma might be the modulation of TGF- β response in cells and tissues [32]. It is important to mention that a number of polymorphisms have been identified for the TGF-β1 encoding gene, which is located on chromosome 19q13. Allelic variations have been found in the 5' flanking region of the TGF-β1 gene, such as those at positions –988, –800, and –509, while others are located in the coding region (i.e. codons 10 and 25 of exon 1, and codon 263 of exon 5). Furthermore, a C insertion has been observed in the 5' untranslated region at position +72 [33]. Transforming Growth Factor-beta 1 production varies from one person to another, and this is partly related to the polymorphism of the TGF-β1 gene at codons 10 and 25 [34]. These changes have potential functional importance by modulating TGF-β1 production. A polymorphism detected at codon 10 is expressed as a change of the amino acid Leu to the Pro, while another polymorphism at codon 25 is due to Pro's replacement by Arg [33]. In the current study, the authors aimed at identifying the potential association between the Single Nucleotide Polymorphism (SNP) TGF-β1 +915 (C or G) in codon 25, and POAG. This polymorphism is one of the most studied and has been targeted for potential correlation with pathological conditions yet has not been investigated for its association with ocular pathology [35-37].

MATERIALS and METHODS

The current study was conducted during years 2009 to 2010 at the First Ophthalmology Department of University of Athens in G. Gennimatas Hospital, after obtaining ethical approval from the Institutional Ethical Committee. A total of 88 cases with POAG and 52 healthy controls (to serve as the positive control group) were recruited from the First Ophthalmology Department of Athens University (Table 1), following explanation of the research objectives and obtaining their informed consent. Concerned ophthalmologists performed the clinical examination of all patients. Only patients, who had glaucoma in both eyes, with one of the eyes with previous glaucoma surgery history being either trabeculectomy or tube-shunt surgery, were included in the patient group. Glaucoma diagnosis was based on glaucomatous optic nerve and visual field changes. Patients with any underlying ocular disorders, other than glaucoma, or a history of previous ocular surgery, other than a glaucoma surgery, were not included. Using a special form, details of the clinical, epidemiological, and ocular variables of each patient were carefully recorded.

Table 1: Characteristics of the Study Subjects

Demographic characteristics	POAG n = 88	Controls n = 52
Age range (years)	42-83	43-84
Males	46	24
Females	42	28

POAG = Primary Open Angle Glaucoma, n = Number.

Single Nucleotide Polymorphism Technical Analysis

DNA was isolated from whole blood samples with high pure Polymerase Chain Reaction (PCR) template preparation kit (Roche Life Science), according to the manufacturer's instructions. Genotype frequencies for the polymorphism rs1800471 (G915C and Arg25Pro) of the TGF-β1 gene were assessed with an assay described previously [38], using a Light Cycler instrument (Roche Life Science). In details, 80 ng of isolated DNA was used as the template for the amplification of a 523-bp segment that included codon 25, with the use of primers

Arg25Pro-for (5'- CTA GGT TAT TTC CGT GGG - 3') and Arg25Pro-rev (5'- CCT TGG CGT AGT AGT CG-3') at a concentration of 0.5 µM. Labeled probes (5'-GCT ACC GCT GCT GTG GCT ACT GGT GCT-3'-fluorescein and LC-Red640-5'-ACG CCT GGC CCG CCG-Ph-3') were used at a concentration of 0.2 µM, along with 3 mM of $MgCl_2$, 5% (v/v) DMSO, and 1X LC-FastStart DNA master hybridization probes (Roche Life Science). Samples were initially heated to 95°C for 10 minutes and subsequently submitted to 45 cycles at 95°C for 10 seconds, 59°C for 10 seconds, and 72°C for 20 seconds. Melting curve analysis included a denaturation step for 1 minute at 95°C, a hybridization step of 30 seconds at 40°C, and then ramping to 80°C at a rate of 0.2°C/second. The fluorescein-labeled probe was designed to hybridize to nucleotides 2012 to 2038 of the TGF-β1 gene (GenBank: X05839) while the LC-Red640-labeled probe corresponded to the adjacent region, at 2040 to 2054, of the aforementioned gene. The detection of the alleles was carried out with the implementation of the Fluorescence Resonance Energy Transfer (FRET) principle. Typically, the melting curve of a DNA homozygous for Arg25 presented a single peak at 51°C, while in case of heterozygosity, 2 peaks, at 51°C and 65°C, were observed (Fig 1). In the current study no Pro25 homozygous individuals were identified. Data were analyzed using the SPSS software, version 17.0 (SPSS Inc., Chicago, IL). All tests were 2-tailed and differences with P values of less than 0.05 were considered significant. For estimation of the association of SNP with the development of POAG, the Fisher's exact test was applied.

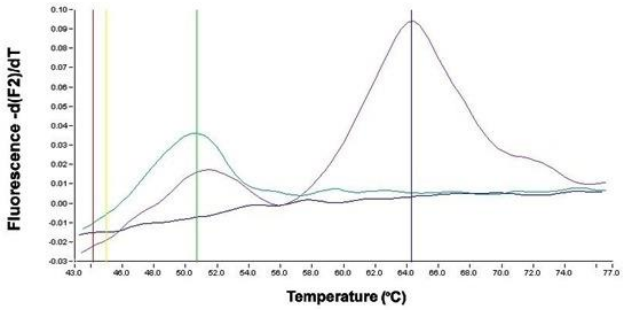

Figure 1. Melting Curve Analysis of a Heterozygous Sample for the Arg25Pro Polymorphism (purple line) and of a Homozygous Arg25 Sample (green line)

Both alleles present typical melting points at 51°C (Arg25) and 65°C (Pro25). A no template negative control was included in the analysis (blue line).

RESULTS

In the current analysis, genotype frequencies for the rs1800471 polymorphism (G915C, Arg25Pro) of the TGF-β1 gene were determined. No significant differences in genotype distribution could be established between patients with POAG and control individuals (Table 2). Although the presence of the C allele was slightly increased in the POAG when compared with the control group, this difference was not statistically significant.

Table 2. Genotype Distribution and Allele Frequency in Cases with Primary Open Angle Glaucoma and the Corresponding Control Group

	POAG	Control	P value
Arg25Arg (GG)	72 (81.8%)	45 (86.5%)	0.638
Arg25Pro (GC)	16 (18.2%)	7 (13.5%)	
Total	88 (100%)	52 (100%)	
C allele frequency	0.091	0.067	0.653

POAG = Primary Open Angle Glaucoma; Arg25Arg (GG), Arg25Pro (GC): Genotype Frequencies for the rs1800471 Polymorphism (G915C, Arg25Pro) of the TGF-β1 Gene.

The data are expressed as frequencies (percentages). *P* values were assessed by Fisher's exact test.

DISCUSSION

According to the findings of the present study, no significant differences in genotype distribution frequencies for the rs1800471 polymorphism (G915C and Arg25Pro) of the TGF-β1 gene could be established between patients with POAG and control subjects. Management of glaucoma is essential for maintaining retinal health and normal vision. Therefore, the discovery of mechanisms involved in glaucoma is essential for the development of preventive strategies and effective therapies. Zhao et al. [27] examined changes in gene and protein expression of human TM cells following exposure to TGF-β1 and TGF-β2. Both isoforms resulted an overexpression of ECM protein-encoding genes. Specifically, following TGF-β1 exposure, the increase in expression of cytoskeletal tropomyosin 1α and proteins was more pronounced, and the redox enzyme thioredoxin reductase 1 expression was decreased. Transforming Growth Factor-beta seems to influence IOP with a mechanism that seems to involve the contraction of TM cells, which may be affected by TGF-β1. In vitro, the application of TGF-β1 in a culture of bovine TM cells in collagen gel, resulted in contraction of the collagen gel, which was dose-dependent [28]. Transforming Growth Factor-beta 1 triggers actin stress fibers formation in TM cells, mediated by protein kinase C and

`Rho GTPase [28], and influences contraction of TM cells and hence AH outflow facility. In vitro, action of the TGF-β1 increases human TM cell expression of connective tissue growth factor [39] and elastin production from TM cells, which could potentially play a role in outflow resistance [40]. A myofibroblast-like phenotype is induced by TGF-β1 in TM cells. This is evident by an increase in α-Smooth Muscle Actin (αSMA) expression and production, which is dose-dependent. Human TM cells, which are αSMA-positive, have a spindle shape and contain stress fibers. In vitro, these cells signify an increase in contractility and a decrease in outflow facility [24]. Altered actin cytoskeletal fibers contribute in the pathophysiology of both primary open angle and steroid-induced glaucomas [41-43].

A number of in vivo studies have also shown the effect of TGF-β1 in glaucoma with different mechanisms. In glaucomatous eyes, Thrombospondin-1, which activates TGF-β, influences the juxtacanalicular region of TM [44]. Transforming Growth Factor-beta 1 and dexamethasone enhance the expression of thrombospondin-1 in TM [45]. Furthermore, in vitro TGF-β1 exposure increases TM-inducible glucocorticoid response protein gene expression and TM cell myocilin [46]. Finally, IL-6 expression, which is induced by TGF-β1, results in transcriptional activation of the TGF-β1 promoter [47], and may serve an IOP regulator by controlling AH outflow. There is also evidence of the role of TGF-β in structural changes of lamina cribrosa. In the glial cells around the lamina cribrosa in an animal glaucoma model, elevated TGF-β1 and TGF-β2 levels, suggested the potential role of TGF-β in lamina cribrosa remodeling [48]. An in vitro study by Kirwan et al. [49] suggested that in glaucoma, an increased activation of TGF-β1 in the lamina cribrosa may cause optic nerve head remodeling. Two of the most studied TGF-β1 polymorphisms are located at codons 10 and 25. The homozygous Arg/Arg genotype at codon 25 and the presence of the Pro allele at codon 10 has been associated with increased TGF-β1 production [50]. This study aimed at evaluating whether TGF-β1 gene +915 (C or G) in codon 25 polymorphisms has a role on the development of POAG. The association of the TGFβ1 -509C > T SNP with POAG in patients from India was analyzed in a study by Sripriya et al. [35]. The statistical analysis did not suggest any significant difference in the distribution of allele and genotype frequencies and the study showed no association between the TGFβ1-509C > T polymorphism and POAG. In the current study, AS failed to find any significant genotypic association with POAG, despite the fact that the presence of the C allele was scarcely increased in the POAG when compared with the control group.

Sandhya et al. [36] investigated the TGFβ1 codon 10 polymorphism in patients with myopia from a South Indian sample. They found that individuals with the CC genotype might carry gender-specific risk for myopia progression, yet a strong association with high myopia was not detected. The current study failed to identify any significant genotypic association with POAG, despite the fact that the presence of the C allele was scarcely increased in POAG when compared with the control group. However, this difference was not statistically significant. A larger study and a review of the genetics of glaucoma did not manage to identify abnormalities in the genes encoding TGF-β [51, 52]. An active TGF-β1 isomer, which was transferred with adenovirus resulted in a decrease in αSMA, as shown by Robertson et al. [53]. However, anatomic changes resembled greater Primary Angle Closure Glaucoma (PACG) than POAG [53]. Inatani et al. [11] showed that in the AH of eyes with POAG, the level of the biologically active TGF-β2 was higher compared with eyes with PACG, pseudoexfoliative Glaucoma (XFG), and secondary glaucoma associated with uveitis. Multiple isoforms of TGF-β have also been measured by a number of studies. There are differences in the effect of TGF-β2, TGF-β1, and TGF-β3 in different types of glaucoma. More specifically, in POAG, only TGF-β2 is significantly elevated whereas in other forms of glaucoma, TGF-β1 and TGF-β3 show greater elevation [12, 13].

Transforming Growth Factor-beta 1 and TGF-β2 proteins seem to be potential new targets for glaucoma treatment as they have been proved as modulators of ECM remodeling, aqueous outflow facility, and inflammation in glaucomatous eyes. Analysis of the other polymorphisms in the regulatory region of the TGF-β1 gene could provide better understanding of the role of TGF-β in POAG pathogenesis. However, as stated by a recent review [54], due to the stronger correlation of TGF-β2 with the pathogenesis of POAG, TGF-β2 may be a more promising target for future investigation of polymorphisms. To the best of the author's knowledge, this is the first report that has examined this specific polymorphism and its association with POAG. Limitations of the study are the small number of patients and the limited number of polymorphisms examined. Further studies are required in order to establish specific relationships. Studies with extensive data on glaucoma may provide better opportunities in this field.

DISCLOSURE

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