Journal of Genetic Syndromes & Gene Therapy
ISSN: 2157-7412

Lavaris et al., J Genet Syndr Gene Ther 2016, 7:1
DOI: 10.4172/2157-7412.1000285

Research Article Open Access

Association of *Superoxide Dismutase* 2 Polymorphism Rs4880 and Open-Angle Glaucoma in a Greek Patients' Cohort

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Abstract

Purpose: Glaucoma is a multifactorial optic neuropathy and leading cause of visual impairment and blindness. Multigenic inheritance hypothesis is being investigated over the past decades and numerous mainly causative and synergic polymorphisms have been revealed. Aim of this study is to investigate whether *superoxide dismutase 2 (SOD2)* polymorphism rs4880 is associated with primary open angle glaucoma (POAG) in Greek population.

Materials and method: This is a case control study of 106 POAG patients and 120 thoroughly examined, unrelated, healthy control subjects of Greek origin, surveyed for *SOD2* polymorphism rs4880 and potential correlation to POAG.

Results: *SOD2 rs4880* polymorphism showed no statistically significant difference between POAG patients and healthy controls. Mean intraocular pressure (IOP) of both eyes of the heterozygous (T/C) group was found significantly higher than in homozygous (T/T) group (19.13 ± 0.60 *vs.* 17.59 ± 0.33, p = 0.02). When we compared the IOP in each eye separately, the (T/C) and (C/C) carriers had significantly higher IOP on their left eye compared to the (T/T) carriers [(T/C) 18.79 ± 0.56 *vs.* (T/T) 17.2 ± 0.36, p = 0.02 and (C/C) 20.75 ± 2.14 vs. (T/T) 17.2 ± 0.36, p = 0.03).

Conclusion: Our study did not find any significant association between *SOD2 rs4880* polymorphism and POAG. Mean IOP of the polymorphic (C) allele carriers was found significantly higher than in homozygous (T/T) group. As we cannot reject the possibility that oxidative stress might be a crucial factor for the POAG development further studies may be needed to confirm the importance of *SOD2* gene in POAG pathogenesis.

Keywords: *Superoxide dismutase 2*; Primary open angle glaucoma; Glaucoma genetics

Introduction

Glaucoma is a term defining a group of ocular disorders with multi-factorial etiology characterized by progressive optic neuropathy, featuring retinal ganglion cells (RGC's) apoptosis and irreversible visual impairment and blindness. It is categorized according to whether it is primary or secondary (a causative factor is recognized), whether it is congenital or acquired, and whether the irido-corneal angle is narrow (close angle glaucoma) or wide (open angle glaucoma). Open angle glaucoma (OAG) corresponds to 74% of all glaucoma cases and primary open angle glaucoma (POAG) is the most common form [1]. In 2013, the number of people between 40 to 80 years old with glaucoma worldwide was 64.3 million, tending to increase to 76 million by 2020 [2]. The exact etiology of glaucoma remains obscure. However, over the past decades, several studies concerning glaucoma inheritance have been conducted and demonstrate that genetic factors contribute to the pathogenesis of POAG [3].

Genome wide association studies have defined a number of potentially causative genes, such as *Optineurin (OPTN)* on chromosome 10p14-15 [4], *Myocilin (MYOC)* on chromosome 1q24-25 [5] , and WD repeat domain 36 *(WDR36)* on chromosome 5q21-22 [6]. However, recent studies have identified new genetic variations that may contribute to POAG pathogenesis: among them, *Caveolin 1 and 2 (CAV1, CAV2)* on chromosome 7q31 [7]; *tank binding kinase 1 (TBK1)* [8,9], *transmembrane and coiled-coil domains 1 (TMCO1)* on chromosome 1q24 [10,11], *S1 RNA binding domain 1 (SRBD1)* [12], *(CDKN2B-AS1)* [11,13].

Among other factors, oxidative stress has been implicated as a potential risk factor in glaucoma pathogenesis [14-17]. *Superoxide dismutase (SOD)* genes encode antioxidant enzymes that play a significant role in oxidative stress defense, by clearing reactive oxygen species (ROS) [14]. Among *SOD* genes, *SOD2* encodes manganese superoxide dismutase (MnSOD), a primary mitochondrial antioxidant enzyme that protects cells form oxidative stress by dismutating superoxide (O2(.-)) to hydrogen peroxide and oxygen preventing mitochondrial dysfunction and eukaryotic cells premature apoptosis. Previous studies have investigated a mutation at c.47T > C (rs4880 polymorphism) in the *SOD2* gene and its potential correlation with POAG; results though remain controversial [18-20]. Because of: the important role of mitochondria in retinal ganglion cells apoptosis, the increasing evidence of oxidative stress involvement in many pathologic conditions [21,22] including glaucoma, the presence of Mn-SOD enzyme in mitochondria and controversial results from previous studies, we decided to investigate whether this mutation is a risk factor for POAG in Greek population.

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Received December 04, 2015; **Accepted** February 01, 2016; **Published** February 08, 2016

Citation: Lavaris A, Gazouli M, Kitsos G, Brouzas D, Moschos MM (2016) Association of *Superoxide Dismutase* 2 Polymorphism Rs4880 and Open-Angle Glaucoma in a Greek Patients' Cohort. J Genet Syndr Gene Ther 7: 285. doi:10.4172/2157-7412.1000285

Materials and Method

Patients

Forty-four (44) inherited and sixty-two (62) sporadic cases of POAG and 120 unaffected, unrelated healthy control subjects, with a free history of ophthalmological or systematic diseases were recruited from the 1st Department of Ophthalmology, University of Athens and had blood sample taken for genetic analysis. All participants have given their informed consent and the study protocol has been approved by the institute's ethics committee.

The clinical examination protocol included: anterior segment examination by slit lamp, including gonioscopy; fundus examination and optic disc evaluation with cup/disc ratio measurement with a stereoscopic fundus lens (Volk Optical Inc., Mentor, Ohio). Intraocular pressure (IOP) was assessed using Goldmann applanation tonometer (Haag Streit AG, Bern, Switzerland). Automated perimetry was performed with Humphrey Automated Field Analyzer (Humphrey Inc., San Leandro CA) using 30-2 SITA protocol in order to assess visual field defects in both glaucomatic and control groups. No glaucomatic defects were detected in controls.

Genotyping

Genomic DNA was extracted from peripheral blood using the NucleoSpin® Blood kit according the manufacturer instructions (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Polymerase Chain Reaction (PCR) was performed as previously described using the primers sense 5'-GCC CAG CCT GCG TAG ACG GTC CC-3', and anti-sense 5'-TGC CTG GAG CCC AGA TAC CCC AAG-3' where the underlined nucleotide represents the deliberate primer mismatches designed to introduce artificial restriction site [20]. The 110 bp PCR amplified product was cleaved 10U of the *BsuRI (HaeIII)* (Thermo Scientific, Waltham, MA, USA).

Statistical Analysis

Genotype frequencies were compared with the chi-square with Yate's correction using S-Plus (v. 6.2, Insightful, Seattle, WA). Odds ratios (ORs) and 95% confidence intervals (CIs) were obtained with GraphPad (v. 3.00, GraphPad Software, San Diego, CA). Hardy–Weinberg equilibrium was verified by calculating the expected frequencies and numbers and was tested separately in patients and controls using the goodness-of-fit Chi square test. The student's *t* test was used to compare means between the groups regarding continuous variables and Mann Whitney U test was used whenever indicated. The p values are all two-sided, and p values of <0.05 were considered to be significant.

Results

A total of 106 POAG cases and 120 healthy unrelated controls were recruited in this study. The patients group consisted of 44 inherited POAG patients and 62 sporadic cases, 42 males and 64 females with mean age of 67.06 ± 11.15 years old, all initially diagnosed with POAG at least 10 years before recruiting into this study. Controls' group consists of 120 individuals, 56 males and 64 females with mean age of 69.99 ± 13.76 years old. IOP measurements were significantly higher in POAG patients than in healthy controls ($p < 0.0001$). Both groups' demographic characteristics are presented on Table 1. The Hardy–Weinberg equilibrium of the rs4880 SNPs was tested in all patients, and no statistically significant deviation was observed. The genotype and allele frequencies were not found to be significantly associated with POAG susceptibility (Table 2). As indicated in Figure 1, comparing the heterozygous (T/C) and the homozygous mutated (C/C) genotypes to the wild-type (T/T) group in terms of the mean IOP of both eyes the (T/C) group has presented with a significantly higher mean IOP than in (T/T) group (19.13 ± 0.60 *vs.* 17.59 ± 0.33, $p = 0.02$). Even if the IOP was increased, no significant difference was observed with (C/C) carriers (19.63 ± 1.77, $p = 0.17$) maybe due to the small number of carriers. When we compared the IOP in each eye separately, the (T/C) and (C/C) carriers have significantly higher IOP on their left eye compared to the (T/T) carriers [(T/C) 18.79 ± 0.56 *vs.* (T/T) 17.2 ± 0.36, $p = 0.02$ and (C/C) 20.75 ± 2.14 vs. (T/T) 17.2 ± 0.36, p=0.03).

	POAG (n = 106)	Controls (n = 120)	P value
Sex (male/female)	42/64	56/64	0.857
Age, yrs (mean ± S.D.)	67.06 ± 11.15	69.99 ± 13.76	0.08
Family history	44	-	-
Smoking (n, %)	24 (22.64)	21 (17.5)	0.404
IOP (mm Hg)	30.13 ± 5.71	16.21 ± 3.18	< 0.0001

Table 1: Demographic characteristics of patients and controls.

	Genotype	Controls (n = 120)	POAG patients (n = 106)	P; OR (95% CI)
rs4880				
	TT	82	74	1.00 (Reference)
	CT	34	28	0.88; 0.91 (0.50-1.65)
	CC	4	4	1.00; 1.11 (0.27-4.59)
	T allele C allele	198 42	176 36	1.0 (Reference) 0.90; 0.96 (0.59-1.57)

Table 2: Genotype and allele distributions of *SOD2* rs4880 polymorphism in POAG patients and controls.

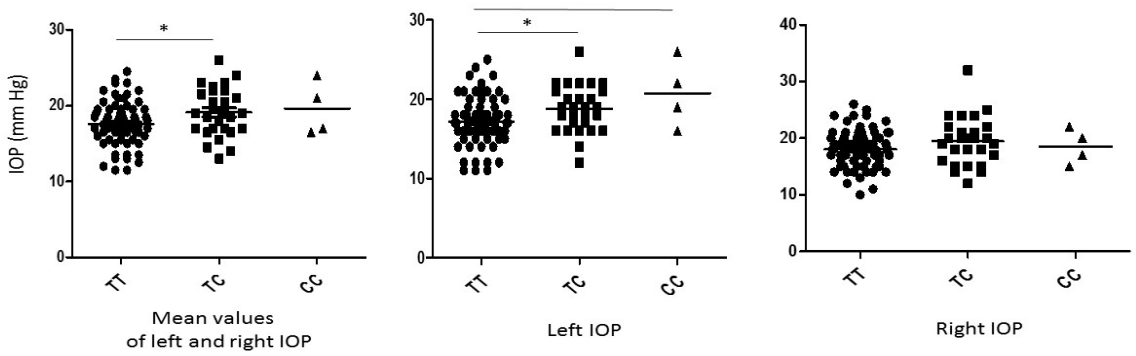

Figure 1: Pedigree of the affected patient.

Zhou et al.				
Genotype	Cases (n = 416) Frequency (%)	Controls (n = 997) Frequency (%)	p value	OR (95% CI)
TT	309 (0.743)	745 (0.748)	-	
TC	99 (0.238)	235 (0.236)	0.945	1.02 (0.77-1.33)
CC	8 (0.019)	16 (0.016)	0.655	1.21 (0.51-2.85)
T allele	717 (0.862)	1725 (0.866)	-	
C allele	115 (0.138)	267 (0.134)	0.813	1.04 (0.82-1.31)
Abu-Amerro et al.				
Genotype	Cases (n = 226) Frequency (%)	Controls (n = 403) Frequency (%)	p value	OR (95% CI)
TT	50 (22.1)	91 (22.6)	-	-
TC	115 (50.9)	202 (50.1)	0.916	1.04 (0.672-1.607)
CC	61 (27)	110 (27.3)	0.988	1.0 (0.617-1.653)
T allele	215 (47.6)	384 (47.6)	-	-
C allele	237 (52.4)	422 (52.4)	0.988	1.0 (0.791-1.271)
Celojevic et al				
	Cases (n = 185) Frequency (%)	Controls (n = 239) Frequency (%)		
T allele	47.7%	46.5%	0.75	1.05 (0.79-1.40)

Table 3: Genotype and allele frequencies of *SOD2* rs4880 as found by Zhou et al. [18], Abu-Amerro et al. [19] and Celojevic et al. [20].

No significant differences between the groups were observed on IOP values of right eye [(T/T) 18.0 ± 0.37, (T/C) 19.46 ± 0.56, (C/C) 18.5 ± 1.55]. No association was found between genotypes and age of the patients tested.

Discussion

Despite the high incidence of glaucoma, the biological basis is not clearly understood and factors contributing to its progression have not yet been fully clarified. The elevated intraocular pressure (IOP) is one of the main risk factors for POAG [23]. RGCs, because of their high energy requirement, are heavily dependent on mitochondria for survival and function. It has been suggested that mitochondrial dysfunction involved in RGC loss in experimental animal models of glaucoma [24] and pre-existing mitochondrial dysfunction, can increase the susceptibility of RGC to stress from other risk factors, including raised IOP [25]. Among others, a basic risk factor for the prevalence and incidence of POAG is increasing age and it is likely that mitochondrial dysfunction may serve as one of the links between ageing and glaucoma [26,27]. The impact of genetic variations to the development of POAG has been demonstrated and disease-associated genes recognized [28]. Several evidences also suggest that interactions between genetic and environmental factors confer the multifaceted disease phenotypes of POAG [29,30]. This suggests that individuals carrying POAG-associated genetic polymorphisms might be more predisposed to the development of the disease when they are exposed to specific environmental factors like atmospheric pollutants, cigarette smoke, ultraviolet rays and radiation, by creating an imbalance between pro-oxidants and antioxidants, leading to oxidative stress [31].

Superoxide dismutases (SODs) are enzymes implicated in the protection against oxidative stress by detoxification of superoxide. *SOD2*, a member of SODs is one of the major superoxide scavengers in mitochondria, converses endogenously produced superoxide into hydrogen peroxide by protecting cells from reactive oxygen species (ROS) linked oxidative damage [14]. Increased expression of the *SOD2* gene was observed in the aqueous humour of POAG patients and in the ciliary body and iris tissue of subjects with pseudoexfoliation glaucoma [15,32]. *SOD2 rs4880* is a C to T substitution in its mitochondrion targeting sequence, resulting in a substitution of valine (Val) by alanine (Ala). Compared with *SOD2* Val variant, which is localized in the mitochondrial membrane, the Ala variant presents in the mitochondrial matrix, shows increased enzymatic activity [33]. The Val isoform of *SOD2* has been suggested that may lead to a decreased resistance against ROS produced in the mitochondria and to oxidative damage of proteins caused by less efficient SOD2 transport into the mitochondria [34]. Several studies suggested that a significant increase in oxidative stress may play a role in the pathogenesis of POAG [35,36].

Although the small sample size of our one-center study, a significant difference of mean IOP was observed between the heterozygous (T/C) and the homozygous (T/T) group; furthermore, the (T/C) and (C/C) carriers had significantly higher IOP on their left eye compared to the (T/T) carriers. Both, findings are very important, as IOP is one of the main risk factors for glaucoma [23]. Even if there are limited studies, and ethnic differences could explain the conflicting results from different genetic studies, our results concerning this polymorphism are in agreement with the findings of Celojevic et al. and Zhou et al. [18,20].

Recently as indicated in Table 3, Abu-Amero et al [19] investigated rs4880 polymorphism in POAG patients from Saudi Arabia and found that patients carrying this mutation had slightly increased intraocular pressure. Zhou et al [18] reported a trend of *SOD2* association with POAG, suggesting that *SOD2* might play a significant role in the development of POAG in the Chinese population. In contrary, Celojevic et al. [20] suggested that genetic variations in *SOD1, SOD2* and *SOD3* are not major contributors in POAG pathogenesis.

In conclusion, our study did not find any significant association between *SOD2 rs4880* polymorphism and POAG susceptibility, however we cannot reject the possibility that oxidative stress might be a crucial factor for the POAG since we observed that is implicated in the IOP levels, and other genes involved in the antioxidative defense mechanism should be tested.

Competing Interest

The authors declare that they have no competing interests.

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