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Association of TNF- α and TNF- β Gene Polymorphisms with Primary Open Angle and Primary Angle Closure Glaucoma

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1. Introduction

Glaucoma is a complex disease that comprises a group of heterogeneous optic neuropathies characterized by a progressive degeneration of the optic nerve head and visual field defects. It starts with unnoticeable blind spots at the edges of field vision progressing to the tunnel vision and finally leading to blindness (Quigley & Broman, 2006). The disease process is insidious, and the central vision is usually not lost until the disease is advanced, a significant proportion of individuals remain either undiagnosed or undertreated. The most common forms of glaucoma are age-related, generally beginning in midlife and progressing slowly but relentlessly as the age advances (A.T.G.S.R.S.G., 2009). If detected early enough, disease progression can be slowed with drug and/or surgical treatment, emphasizing the importance of identifying the disease in its earliest stages.

Glaucoma is broadly classified into primary and secondary glaucoma based on their etiology and aqueous humor dynamics (Shields, 2005). Based on anatomy of the anterior chamber (gonioscopy), primary glaucomas are further classified as primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG). Depending on the time of onset, glaucoma is also termed as infantile, juvenile and adult type. Rarely glaucoma may be found in babies at birth this form of glaucoma called congenital glaucoma. Secondary glaucomas are characterized by the involvement of predisposing ocular or systemic diseases such as uveitis, trauma, or diabetes thereby resulting in an alteration of aqueous humor dynamics. Pseudoexfoliation glaucoma (XFG) and pigmentary glaucoma (PG) are the most frequently reported type of secondary glaucoma (Shields, 2005).

Glaucoma affects 70 million people and is the second leading cause of blindness worldwide. It is estimated that by the year 2020, this number would rise to around 79.6 million (Quigley & Broman, 2006). The prevalence of glaucoma varies widely across the different ethnic groups (He et al., 2006; Wong et al., 2006; Sakata et al., 2007; Cedrone et al., 2008; Vijaya et al., 2008a; 2008b; Pekmezci et al., 2009) and is significantly higher in blacks (4.7%) as compared to in the white (1.3%) population (Kwon et al., 2009). Primary open angle glaucoma is the predominant disease among whites and Africans (Tielsch et al., 1991; Klein et al., 1992; Rotchford & Johnson, 2002; Rotchford et al., 2003; Varma et al., 2004; Friedman et al., 2006; Sakata et al., 2007) while PACG is a major form of glaucoma in Asians (Hu et al.,

1989; Jacob et al., 1998; Foster & Johnson, 2001; Casson et al., 2007). Epidemiological studies reveal that the prevalence of POAG around the world varies by about 2 orders of magnitude with the lowest estimates found among Eskimos (0.06%) residing in Alaska (Arkell et al., 1987) and the highest prevalence among African-derived people living in the Caribbean (7.1–8.8%) (Mason et al., 1989; Leske et al., 1994). PACG is leading cause of the most of the bilateral glaucoma related blindness in Singapore, China, and India. It has been estimated that PACG blinds more people than POAG worldwide (Dandona et al., 2000; Foster et al., 2000). According to a recent study glaucoma is the major cause of blindness in Saudi Arabia. The prevalence of both POAG and PACG is higher in western region of Saudi Arabia as compared to other Asian countries (Eid et al., 2009). To date no national study has been undertaken to determine the exact prevalence of glaucoma in this country.

Elevated intraocular pressure (IOP) is a major risk factor in glaucoma which is supported by the fact that experimentally induced elevation of IOP leads to glaucoma in animals (Levkovitch-Verbin et al., 2002). The etiology of raised IOP and resulting glaucoma is not fully understood. Fluid formed by the ciliary body (aqueous humor) is removed by the trabecular outflow pathways, which includes the trabecular meshwork, the juxtacanalicular connective tissue, the endothelial lining of Schlemm's canal, and the collecting channels and the aqueous veins (A.T.G.S.R.S.G., 2009). The IOP is dependent on the rate of fluid removal, which under normal conditions matches the rate of formation. In most patients with glaucoma, the rate of fluid removal declines so that it no longer keeps pace with the rate of formation resulting in the increased IOP. The increase in IOP has been associated with genetic factors, age and oxidative stress (Tschumper & Johnson, 1990; Leske et al., 1995; Green et al., 2007). Although elevation of intraocular pressure (IOP) is often related to the optic nerve damage in glaucoma, factors other than IOP are likely to play a role in the pathogenesis of glaucomatous optic neuropathy, particularly in individuals with normal tension glaucoma (NTG).

The loss of retinal ganglion cells (RGC) is the typical pathology in glaucoma. Progressive loss of optic nerve axons and RGCs result in characteristic optic nerve atrophy and visual field defects in glaucoma patients. A number of hypotheses have been put forward that describe the sequence of events responsible for triggering ganglion cell degeneration in glaucoma. These include compromised blood flow of the optic nerve, mechanical compression due to raised IOP, loss of neurotrophic factors, autoimmune mechanisms, nitric oxide-induced injuries to the optic nerve and glutamate excitotoxicity (Mozaffarieh et al., 2008). A combination of these factors may be involved in causing glaucomatous RGC loss. Animal models also confirmed that acute IOP elevation causes blockage of brain-derived neurotrophic factor (BDNF) transport and may contribute to neuronal death (Pease et al., 2000). On the other hand the mechanisms involved in the pathophysiology and development of PACG are rather complicated and involve the anatomy of the angle, and the spatial and structural relationships between the lens and the anatomy of the angle, the iris, and the lens. Most mechanisms for PACG impute to the increasing lens thickness during aging in a relatively small eye and a shallow anterior chamber. Acute PACG has been attributed to morphometric characteristics of the eye. Human sclera undergoes active remodeling during the development of myopia. Biochemical assays from highly myopic eyes show markedly reduced amounts of biochemical markers for collagen and glycosaminoglycans, when compared with the similar sclera of emmetropic eyes. This remodeling process occurs in concert with an increase in the production and enhanced activation of collagen degrading enzymes, particularly matrix metalloproteases (MMPs) (McBrien & Gentle, 2001). The net effect of these changes is a loss of scleral tissue at the posterior pole of the eye.

Growing evidence obtained from clinical and experimental studies strongly suggests the involvement of the immune system in glaucoma (Helpert & Grosskreutz, 2002; Wax & Tezel, 2002; Tezel & Wax, 2000; 2003). Paradoxically the role of immune system in Glaucoma has been described as either neuroprotective or neurodestructive. A balance between beneficial immunity and harmful neurodegeneration may ultimately determine the fate of RGCs in response to various stresses in glaucomatous eye (Tezel & Wax 2007). T-cell-mediated immune response may initially be beneficial to limit neurodegeneration (Schwartz & Kipnis, 2001, 2002; Kipnis et al., 2002). However, a failure to properly control aberrant, stress-induced immune response likely converts the protective immunity to an autoimmune neurodegenerative process that can facilitate the progression of neurodegeneration in some, if not all, glaucoma patients (Tezel & Wax, 2007). Expansion and secondary recruitment of circulating T cells through an antigen mediated process is supported by the evidence of abnormal T-cell subsets (Yang et al., 2001a) and increased production of serum autoantibodies to different optic nerve and retina antigens in many glaucoma patients (Tezel et al., 1998, 1999; Wax et al., 1998a, 1998b; Yang et al., 2001b) Furthermore, initial *in vivo* studies support the feasibility of eliciting an experimental autoimmune model of glaucomatous neurodegeneration in which RGCs progressively die in specific antigen-immunized animals by exhibiting a pattern of neuronal damage similar to that of human glaucoma (Wax & Tezel, 2009). As the role of the immune system in glaucoma is one of the surveillance, in which signal pathways of the immune system regulate cell death in response to conditions that stress retinal neurons in glaucoma. Recent studies have focused on immunological changes occurring in glaucoma pathogenesis and possible preventive therapies based along those lines have been proposed. The major targets of interest are cytokines, as these inflammatory mediators play an important role in the pathogenesis of glaucoma and may regulate RGC survival or death (Tezel & Wax, 2004).

The role of tumor necrosis factor (TNF), an important proinflammatory cytokine is a subject of interest in glaucoma studies. Both TNF- α , produced mainly by monocytes and activated macrophages and TNF- β , produced mainly by activated T-cells, play important immunoregulatory roles in various diseases. TNF- α is up regulated in several neurodegenerative disorders including optic nerve microglia and astrocytes in glaucoma (Yuan & Neufeld, 2000; 2001). It has been suggested that cell death mediated by TNF- α is a contributing factor in the progression of neurodegeneration in glaucoma (Tezel et al., 2001). TNF- α plays a critical role in optic neuropathy by initiating the inflammatory pathways through the induction of NOS-2 in astrocytes (Yuan & Neufeld, 2000). It has been observed that ischemic or pressure-loaded glial cells produce TNF- α which results in oligodendrocytes death and the apoptosis of RGC leading to glaucomatous neurodegeneration (Tezel & Wax, 2000). Furthermore, TNF- α directly induces apoptosis through TNF- α receptor-I (TNFRI) also known as death receptor which is mainly localized on the retinal ganglion cells (Tezel et al., 2001). The substantial role of TNF- α /TNFRI in the pathogenesis of glaucoma has also been supported by experimental study on mouse model of glaucoma in which induction of ocular hypertension resulted in a rapid up regulation of TNF- α followed by loss of optic nerve oligodendrocytes (Nakazawa et al., 2006). Moreover, intravitreal TNF- α injection in normal mice mimicked glaucoma like RGCs degeneration whereas anti-TNF- α neutralizing antibody or deleting the TNF- α gene blocked the deleterious effects of ocular hypertension (Nakazawa et al., 2006). Tezel et al. (2004) also reported that unilateral optic nerve crush injury in mice resulted in significantly less glial

activation, TNF- α production and retinal ganglion cell death in *TNFR1* -knockout mice. In fact, histopathologic studies have also shown increased immunostaining for TNF- α and TNFR1 in the glaucomatous optic nerve head as compared to age-matched control samples (Yan et al., 2000; Yuan & Neufeld, 2000). Interestingly, a new antiglaucoma drug (GLC756) significantly suppressed LPS-induced TNF- α production (Laengle et al., 2006a, 2006b). These observations further confirm the hypothesis of an involvement of the TNF- α /TNFR1 signaling pathway in the pathogenesis of glaucoma.

The genes for TNF- α (OMIM 191160) and TNF- β also known as lymphotoxin- α (LT- α , MIM 153440), located within the MHC III region of chromosome 6, shows close linkage to the HLA class I (*HLA-B*) and class II (*HLA-DR*) genes. Studies on monozygotic twins and their first-degree relatives, using *ex vivo* endotoxin stimulated whole blood samples, have provided evidence that 60% of variation in the production capacity of TNF- α appears to be genetically determined (Westendorp et al., 1997). Several polymorphisms within the promoter region of *TNF- α* and the intron 1 polymorphism of TNF- β , in particular, have been associated with altered levels of circulating TNF- α (Sharma et al., 2006; 2008).

With regard to PACG a number of lines of evidence have suggested that both genetic and environmental factors contribute to the development of PACG (Lowe, 1972). There are various published studies that suggested a genetic basis of the PACG (Aung et al., 2008a; 2008b). PACG, as a common complex disease, has become an important target for association studies in recent years. Genetic heterogeneity is illustrated by the several loci in many glaucoma causing genes identified to date. Single nucleotide polymorphisms in several genes such as matrix metalloproteinases-9 (MMP-9), Cytochrome P450 1B1 (CYP1B1), membrane-type frizzled-related protein (MFRP), methylenetetrahydrofolate reductase gene (MTHFR), myocilin (MYOC), calcitonin receptor-like gene (CALRL), endothelial nitric oxide synthase (eNOS), heat shock protein 70 (HSP70), retinal homeobox gene (CHX10), have been associated with PACG (Wang et al., 2006; Chakrabarti et al., 2007; Michael et al., 2008; 2009, Aung et al., 2008; Dai et al., 2008; Cong et al., 2009; Cao et al., 2009, Ayub et al., 2010). However, ethnic variations exist in the association between these polymorphisms and PACG, and many cases such association has not been replicated in subsequent research (Wang et al., 2006; Aung et al., 2008a). Moreover, the biological significance of the some associated single nucleotide polymorphisms (SNPs) in the development of PACG is still unknown (Michael et al., 2008). PACG has been reported to be associated with genes related to regulation of axial length and structural remodeling of connective tissues, such as *MFRP*, *MMP-9*, and *MTHFR* genes (Michael et al., 2008; Wang et al., 2006; Aung et al., 2008a; 2008 b; Wang et al., 2008). It has also been reported that TNF- α is capable of inducing both increased synthesis and activity of matrix metalloproteinases (MMPs) (Rajavashisth et al., 1999; Siwik et al., 2000; Uchida et al., 2000) therefore the polymorphism in TNF genes might be associated with regulation of axial length and structural remodeling of connective tissues and ultimately to the development of PACG.

Several polymorphisms have been reported in the promoter region of TNF- α gene. One of the best described single nucleotide polymorphisms (SNPs) is located at nucleotide position -308 within the TNF- α promoter region (rs1800629) and affects a consensus sequence for a binding site of transcription factor AP-2 (Abraham & Kroeger, 1999). TNF- α promoter polymorphism leads to a less common allele-A (allele 2) which has been associated with increased TNF- α production in vitro (Braun et al., 1996; Wilson et al., 1994) and higher rate of TNF- α transcription than wild type GG genotype (Wilson et al., 1997; Jeong et al., 2004). This

polymorphism has been linked to increased susceptibility to several chronic metabolic degenerative, inflammatory and autoimmune diseases (Cuenca et al., 2001). It has also been reported to be involved in increased susceptibility to different eye diseases including diabetic retinopathy and glaucoma (Limb et al., 1999; Yoshioka et al., 2006; Huang et al., 2009). TNF- α is involved in T-cell dependent B-cell responses, T-cell proliferation, natural killer (NK)-cell activity and dendritic cell maturation. The previously published data indicate that the host's ability to produce TNF- α may play an important role in vulnerability to glaucoma.

Of interest, G/A polymorphism at nucleotide position -308 within the human TNF- α promoter region is associated with elevated TNF levels, disease susceptibility, and poor prognosis in several diseases (Messer et al., 1991; Wilson et al., 1992; Galbraith & Pendey, 1995; Patino-Garcia et al., 2000). Adenine at position -308 makes the TNF- α promoter a much more powerful transcription activator than guanine (Messer et al., 1991). The TNF- α is up regulated in several neurodegenerative disorders including multiple sclerosis, Parkinson's disease, Alzheimer's disease (Shohami et al., 1999; Yan et al., 2000; Liu et al., 2006) and in optic nerve microglia and astrocytes in glaucoma patients (Yuan & Neufeld, 2000; 2001]. Increased TNF- α level have been associated with a poor prognosis after trauma in the brain (Munoz-Fernandez & Fresno, 1998; Ertel et al., 1995), whereas a decrease in TNF- α is known to reduce nerve damage. In a rat cerebral ischemic model, exogenous TNF- α was found to exacerbate focal ischemic injuries, whereas blocking endogenous TNF- α was neuroprotective (Shohami et al., 1996).

TNF- β resembles to TNF- α in terms of several biological activities including apoptosis and gives rise to a similar pro-inflammatory response and has been shown to play a critical role in pathogenesis of many diseases. TNF- β gene polymorphism at nucleotide position 252 within first intron (A252G) (rs909253) affects a phorbol ester-responsive element. The presence of G at this position defines the mutant allele known as TNF- β * 1 (allele-1) which is less frequent allele in white subjects and is associated with higher TNF- α and TNF- β production (Messer et al., 1991; Abraham et al., 1993). Association of TNF- β +252 A/G polymorphism has been reported with various autoimmune disorders including Gravis' disease (Kula et al., 2001) idiopathic membranous glomerulonephritis, IgA nephropathy, insulin dependent diabetes mellitus (Medcraft et al., 1993), myasthenia gravis (Zelano et al., 1998), asthma diathesis (Albuquerque et al., 1998), SLE with nephritis (Lu et al., 2005), systemic sclerosis (Pandey & Takeuchi, 1999), plaque psoriasis (Vasku et al., 2000), rheumatoid arthritis (Takeuchi et al., 2005), myocardial infarction in patients with rheumatoid arthritis (Panoulas et al., 2008), and type 1 diabetes (Boraska et al., 2009). Recently TNF- β +252 A/G polymorphism is reported to be associated with both susceptibility to and mortality from sepsis (Watanabe et al., 2010; Tiancha et al., 2011).

A few studies have been undertaken to determine the association of TNF- α polymorphisms and glaucoma in different part of the world (Limb et al., 1999; Yoshioka et al., 2006; Huang et al., 2009). The results of these studies on association of TNF- α polymorphism with POAG are inconsistent. These differences in findings have been attributed to variation in sample size, ethnicity and method of diagnosis (Lin et al., 2003; Mossbock et al., 2006; Tekeli et al., 2008; Razeghinejad et al., 2009). To date there is no report available on the association between TNF- β polymorphism and glaucoma. The joint analysis of polymorphism at TNF- α and TNF- β given that both are involved in the expression of TNF- α and in a suggested mechanism for autoimmune diseases, will provide further insight into the pathogenesis of glaucoma and help in developing effective therapeutic agents. Therefore the present study

was undertaken to determine the possible association of TNF- α -308 and TNF- β +252 gene polymorphisms with POAG and PACG in Saudi population.

2. Methods

2.1 Subjects

The present study was undertaken to evaluate the association of TNF- α and TNF- β alleles and genotypes in Saudi primary glaucoma patients. A total of 200 unrelated Saudi patients with Primary Glaucoma [primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG)] were recruited from ophthalmology clinic of the Riyadh Military Hospital (RMH), Saudi Arabia. The patient group consisted of 94 males and 106 females, with age at diagnosis ranging from 25 to 78 years (mean \pm SD: 58 ± 14.4). The control group consisted of 200 unrelated subjects, 100 males and 100 females, ages ranging from 25 to 68 years (mean \pm SD: 55 ± 11.6). The diagnosis of primary glaucoma was based on comprehensive clinical examination as mentioned below.

2.2 Preliminary examination

The preliminary examination was performed in ophthalmology clinic of RMH. Detailed demographic data and medical history were recorded for each case and control subject. The preliminary examination included oblique flashlight test, anterior segment evaluation by slit lamp, Goldmann applanation tonometry, and direct fundoscopy (all examinations performed by resident/consultant ophthalmologists). The optic disc was examined after each pupil was dilated with 2 drops of tropicamide, unless contraindicated by the presence of a shallow anterior chamber depth at the slit lamp examination. The vertical cup-disc ratio (VCDR) was estimated for each eye. The rim border was determined based on the course of the blood vessels and the gradation of color, shadows, and texture (Spaeth, 1993). A suspect appearance of the optic disc (glaucomatous-appearing optic disc; GAOD) was defined in eyes with VCDR ≥ 0.6 , asymmetry of the VCDR between the two eyes ≥ 0.2 , focal thinning of the neuroretinal rim, localized or diffuse retinal nerve fiber layer defect, and/or optic disc hemorrhage. A glaucoma specialist confirmed the presence of a GAOD in the participants. Fundus photography, automated perimetry, and gonioscopy were not part of the preliminary examination protocol.

2.3 Detailed examination

Patients with GAOD status and/or intraocular pressure (IOP) measurements > 21 mm Hg at the screening underwent a definitive examination. At first each subject underwent a comprehensive ophthalmic examination, including review of medical history, best corrected visual acuity (BCVA) (using Snellen distance vision chart), retinoscopy and subjective refraction (when visual acuity $< 20/30$), slit lamp biomicroscopy, IOP measurement (using Goldmann applanation tonometry), standard automated perimetry tests, gonioscopy (using Sussman four-mirror lens), and fundoscopy examination. Gonioscopy was performed by a consultant (glaucoma specialist). Indentation gonioscopy with four-mirror Posner lens was performed in eyes with an anatomically narrow angle. The angle was graded according to the Scheie scheme (Scheie, 1957) and the peripheral iris contour, degree of pigmentation, presence of peripheral anterior synechiae, and other angle abnormalities were recorded. When the gonioscopy showed no contraindications, dilated direct fundoscopy and slit lamp

biomicroscopy of the optic disc (using a 78-D lens) were performed. The VCDR was determined independently in a masked fashion by two glaucoma specialists and cases of disagreement were resolved by consensus between the two graders during the definite examination visit. There was a good agreement between the two graders in determining VCDR (0.7 or more). The presence of any notching, optic disc hemorrhages, or nerve fiber layer defects was documented. Initially, most of the standard automated perimetry tests were performed with the Octopus 101 perimeter (Haag-Streit Octopus, Switzerland). In an attempt to avoid the influence of learning effect in visual field (VF) results, these VF examinations were not considered for assessing visual function status.

The patients were further subjected to a second VF examination using a Humphrey visual field perimeter (Humphrey Visual Field Analyzer; Carl Zeiss Meditec, Inc., Dublin, CA), with the 24-2 full-threshold or SITA (Swedish interactive threshold algorithm) standard strategy. The visual function status was determined based on this single VF test, and only reliable VF examinations were considered for analysis. An abnormal VF examination was determined by the presence of one of the following criteria: (1) glaucoma hemifield test (GHT) result outside normal limits and (2) the presence of a cluster of ≥ 3 contiguous points in the pattern deviation (PD) probability plot with $P < 5\%$ or worse (within the same hemifield) (Foster et al., 2002). A reliable visual field test was defined as an examination with less than 33% of fixation losses, false positive and false negative. The glaucoma specialist verified whether the VF defects were consistent with glaucoma.

2.4 Criteria for glaucoma diagnosis

Glaucoma was diagnosed according to the International Society of Geographical and Epidemiologic Ophthalmology (ISGEO) classification, which uses three levels of evidence (Foster et al 2002). Briefly, in category 1, diagnosis was based on structural and functional evidence. It required CDR or CDR asymmetry ≥ 97.5 th percentile for the normal population or a neuroretinal rim width reduced to ≥ 0.1 CDR (between 11- and 1-o'clock or 5- and 7-o'clock) with a definite VF defect consistent with glaucoma using the Swedish interactive threshold algorithm 30-2. Category 2 was based on advanced structural damage with unproved field loss. This included those subjects in whom VFs could not be determined or were unreliable, with CDR or CDR asymmetry ≥ 99.5 th percentile for the normal population. Lastly, category 3 consisted of persons with an IOP ≥ 99.5 th percentile for the normal population, whose optic discs could not be examined because of media opacities.

2.5 Glaucoma classification

Participants who fulfilled any of the three categories of evidence mentioned earlier were classified as having POAG or PACG

POAG: Anterior chamber angles open and appearing normal by gonioscopy, typical features of glaucomatous optic disc as defined earlier, and visual field defects corresponding to the optic disc changes.

PACG: At least two of the criteria mentioned: glaucomatous optic disc damage or glaucomatous visual field defects in combination with anterior chamber angle partly or totally closed, appositional angle closure or synechiae in angle, absence of signs of secondary angle closure (e.g., uveitis, lens related glaucoma, microspherophakia, evidence of neovascularization in the angle and associated retinal ischemia or congenital angle anomalies). Patients with signs of intracranial disease that would cause optic nerve atrophy

in x-ray, computerized tomography or magnetic resonance imaging were excluded. An anatomically narrow-angle eye should have pigmented trabecular meshwork not visible in $\geq 270^\circ$ of the iridocorneal angle (as assessed by nonindentation gonioscopy) and/or the presence of peripheral anterior synechiae not explained by other causes but a primary angle-closure process.

2.6 Sample collection

Venous blood was collected from the patients (POAG, PACG) and controls and stored at - 20°C before extraction of DNA. The study protocol was approved by the Ethics Committee of the Hospital.

2.7 Genotyping

2.7.1 PCR amplification

Genomic DNA was extracted from the peripheral blood of patients and controls using QIAamp[®] DNA mini kit (Qiagen CA, USA). TNF- α and TNF- β genes were amplified using amplification refractory mutation systems (ARMS)-PCR methodology (Perry et al., 1999) to detect any polymorphism involved at position -308 of TNF- α and +252 in Interon1 of TNF- β gene respectively. The set of primers used to amplify target DNA in the promoter region of TNF- α and TNF- β genes are summarized in Table 1.

Locus	Generic (antisense) primer	Sense primers
TNF- α (G308A)	5'-TCT CGG TTT CTT CTC CAT CG-3'	5'-ATA GGT TTT GAG GGG CAT GG-3' 5'-AAT AGG TTT TGA GGG GCA TGA-3'
TNF- β (A252G)	5'-AGA TCG ACA GAG AAG GGG ACA-3'	5'-CAT TCT CTG TTT CTG CCA TGG-3' 5'-CAT TCT CTG TTT CTG CCA TGA-3'

Table 1. Showing sets of primers used to amplify the TNF- α and TNF- β to detect polymorphism

PCR amplification was carried out using Ready to Go PCR Beads (Amersham Biosciences, USA). Reaction consisted of 10 temperature cycles of denaturation for 15 s at 94 °C, annealing for 50 s at 65 °C and extension for 40 s at 72 °C. Then 25 cycles of denaturation for 20s at 94 °C, annealing for 50 s at 59 °C, extension for 50s at 72 °C. Final extension was performed at 72 °C for 7 m. A positive control was included in the PCR assay by amplification of the human growth hormone (HGH) gene. The amplified product for various samples were separated on the 1.5 % agarose gel, stained with ethidium bromide and photographed.

2.7.2 Statistical analysis

The differences in allele/ genotype frequencies between patients and controls were analyzed by the Fisher’s exact test. *P* values less than 0.05 were considered significant. The strength of the association of disease with respect to a particular allele/genotype is expressed by odd ratio interpreted as *relative risk* (RR) following the Woolf’s method as outlined by Schallreuter et al. (1993). It is calculated only for those Alleles/ genotypes which are increased or decreased in patients as compared to control group. The RR in this study has been calculated for all the subjects.

$$RR = (a \times d) / (b \times c) \text{ where,}$$

(a) = number of patients with expression of allele or genotype
(b) = number of patients without expression of allele or genotype
(c) = number of controls with expression of allele or genotype
(d) = number of controls without expression of allele or genotype
Etiologic Fraction (EF): The EF indicates the hypothetical genetic component of the disease. The values between 0 and 1 are of significance. EF is calculated for positive association only where RR> 1 (Savejgaard et al., 1983).

$$EF = (RR - 1) f / RR, \text{ where } f = a / a + c$$

Preventive Fraction (PF): The PF indicates the hypothetical protective effect of one specific allele/ genotype for the disease. PF is calculated for negative association only where RR< 1 (Savejgaard et al., 1983). Values < 1.0 indicate the protective effect of the allele/genotype against the manifestation of disease.

$$PF = (1 - RR) f / RR (1 - f) + f, \text{ where } f = a / a + c$$

3. Results

In this case control study TNF-α and TNF-β genes were amplified to determine the allele and genotype frequencies in equal number of patients (200) and unrelated matched controls (200). The patient group consisted of POAG (135) and PACG (65) cases. The molecular analysis of the blood specimen of the patients and controls was performed in the same laboratory and at the same time. The investigator was blind to the phenotype of the subjects at the time of molecular analysis. Later on the results were separated for patient and control groups and analyzed for the determination of the frequencies of genotypes and alleles. Allelic frequencies and genotype distributions of both TNF-α and TNF-β gene polymorphisms differ between primary glaucoma (including both POAG and PACG cases) and control subjects (Tables 2-9).
The results of the genotypes and alleles distribution of TNF-α (-308) polymorphism in primary glaucoma (including total POAG and PACG cases) are summarized in Table 2. The frequency of GA (-308) genotype was significantly higher (P=0.02) while the frequency of GG (-308) genotype was lower in total primary glaucoma patients as compared to controls (P=0.01).

Genotype/ Allele	Glaucoma (N=200)		Control (N=200)		P-value	RR	EF*/PF
	N	%	N	%			
GG	85	42.5	110	55	0.01‡	0.60	0.23
GA	100	50.0	76	38	0.02‡	1.63	0.22*
AA	15	7.5	14	7	0.85	1.08	0.04*
G-allele (TNF-α 1-allele)	270	67.5	296	74	0.04‡	0.73	0.15
A-allele (TNF-α 2-allele)	130	32.5	104	26	0.04‡	1.37	0.15*

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction;
‡ statistically significant

Table 2. Genotype and allele frequencies of (G-308A) TNF-α variants in primary glaucoma patients and matched controls

No significant difference was noticed in distribution of AA genotype among the patients and controls ($P=0.85$). Frequency of allele A was found to be significantly increased in glaucoma patient while that of allele G was higher in control group ($P=0.04$).The higher frequencies of genotype GA and allele A in primary glaucoma patients as compared to controls indicated that the genotype GA and allele A are susceptible to the primary glaucoma ($RR=1.63$, $EF=0.22$ and $RR=1.37$, $EF=0.15$ respectively). The genotype GG and allele G being higher in controls indicating their protective nature for primary glaucoma ($RR=0.60$, $PF=0.23$ and $RR=0.73$, $PF=0.15$ respectively).

The results of $TNF-\alpha$ -308 polymorphism for total primary glaucoma cases were then stratified into POAG and PACG groups. The distribution of alleles and genotypes of $TNF-\alpha$ (-308) polymorphism in POAG are shown in Table 3.

Genotype/ Allele	Open Angle Glaucoma (N=135)		Control (N =200)		P-value	RR	EF*/PF
	N	%	N	%			
GG	59	43.7	110	55	0.04‡	0.64	0.16
GA	68	50.4	76	38	0.03‡	1.66	0.19*
AA	8	5.9	14	7	0.82	0.84	0.06
G-allele ($TNF\alpha$ 1- allele)	186	68.9	296	74	0.16	0.83	0.07
A-allele ($TNF\alpha$ 2 -allele)	84	31.1	104	26	0.16	1.21	0.07*

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; ‡ statistically significant

Table 3. Genotype and allele frequencies of (G-308A) $TNF-\alpha$ variants in open angle glaucoma patients and controls

The frequency of genotype GA was significantly higher in POAG patients ($P=0.03$) while the frequency of GG was lower in POAG as compared to controls ($P=0.04$). The frequency of allele A was higher whereas the frequency of allele G was lower in PAOG patients as compared to control. However, the differences were not statistically significant for both alleles ($P=0.16$). The higher frequency of GA genotype in POAG indicated that the genotype GA of $TNF-\alpha$ -308 polymorphism might be susceptible to POAG ($RR=1.66$, $EF=0.19$) while the increased frequency of genotype GG in controls as compared to POAG patients indicated that genotype GG is resistant to POAG ($RR=0.64$, $PF=0.16$).

The frequencies of alleles and genotypes of $TNF-\alpha$ (-308) polymorphism in PACG is given in Table 4. The frequency of genotype GG was significantly lower in PACG as compared to controls ($P=0.04$). Although the frequency of genotype GA and AA was higher in PACG, the difference was not statistically significant ($P=0.11$ and $P=0.42$ respectively). The frequency of allele A was significantly increased in PACG as compared to controls (0.04) indicating that the allele A might be susceptible to the PACG ($RR=1.56$, $EP= 0.11$) while allele G might be protective ($RR=0.64$, $PF=0.11$) in Saudi patients with PACG. Similarly the lower frequency of genotype GG in PACG as compared to controls indicated that GG is protective for PACG ($RR=0.55$, $PF=0.14$).

Comparison of the distribution frequency of genotypes and alleles of $TNF-\alpha$ (-308) polymorphism between the POAG and PACG type of glaucoma is summarized in Table 5. The results of the genotype and allele distribution in the two types of glaucoma (POAG and PACG) show similar pattern without insignificant differences.

Genotype/ Allele	Angle closure glaucoma (N=65)		Control (N=200)		P- value	RR	EF*/PF
	N	%	N	%			
GG	26	40	110	55	0.04‡	0.55	0.14
GA	32	49.2	76	38	0.11	1.58	0.11*
AA	7	10.8	14	7	0.42	1.60	0.12*
G-allele (TNF α 1-allele)	84	64.6	296	74	0.04‡	0.64	0.11
A-allele (TNF α 2-allele)	46	35.4	104	26	0.04‡	1.56	0.11*

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction;
‡, statistically significant

Table 4. Genotype and allele frequencies of (G-308A) TNF- α variants in angle closure glaucoma patients and controls

Genotype/Allele	Open angle glaucoma (135) N (%)	Angle closure glaucoma (65) N (%)	P-value
GG	59 (43.7)	26 (40)	0.76
GA	68 (50.4)	32 (49.2)	0.88
AA	8 (5.9)	4 (10.8)	0.25
G-allele (TNF α 1-allele)	186 (68.9)	84 (64.6)	0.42
A-allele (TNF α 2-allele)	84 (31.1)	46 (35.4)	0.42

Table 5. Comparison of genotype/ allele frequencies of TNF- α (G308A) polymorphism between POAG and PACG

Our studies on TNF- β gene polymorphism at position +252 of intron1 showed that the frequency of GG was significantly higher in primary glaucoma (including total POAG and PACG cases) as compared to controls ($P=0.001$) while the frequency of GA genotype was significantly lower in glaucoma patients ($P=0.001$). Allele-G was predominantly distributed in glaucoma patients ($P=0.001$) whereas frequency of allele A was significantly higher in control group ($P= 0.011$). Though there was difference in the distribution of AA genotype among the primary glaucoma and control, the difference was not statistically significant ($P=0.25$) (Table 6).

Genotype/Allele	Glaucoma (N=200)		Control (N=200)		P-value	RR	EF*/PF
	N	%	N	%			
GG	69	34.50	28	14	0.001‡	3.235	0.491*
GA	99	49.50	148	74	0.001‡	0.344	0.432
AA	32	16.00	24	12	0.253	1.396	0.161*
G-allele (TNF β 1-allele)	237	59.25	204	51	0.011‡	1.396	0.152*
A-allele (TNF β 2-allele)	163	40.75	196	49	0.011‡	0.715	0.152

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction;
‡, statistically significant

Table 6. Genotype and allele frequencies of TNF- β (LT α)- interon1 +252 variants in primary glaucoma patients and matched controls

The increased frequencies of genotype GG and allele G of TNF-β (LTα) - interon1 +252 polymorphism in patients group indicated that genotype GG/allele G is susceptible to the primary glaucoma (RR=3.235, EF=0.491and RR=1.396, EF=0.152 respectively) whereas the decreased frequencies of the genotype GA and allele A in glaucoma patients showed their resistant/ refractory nature (RR=0.344, PF=0.432 and RR=0.715, PF=0.52 respectively).

On stratification of results for two types of glaucoma (POAG and PACG), the distribution of genotypes and allele of TNF-β (LTα) - interon1 +252 polymorphism retained almost the same pattern in POAG and PACG as was in the results for the total primary glaucoma group (Tables 7, 8). The frequency of genotype GG and allele G were higher in POAG as compared to controls ($P=0.001$ and $P=0.05$ respectively) while the frequencies of genotype GA and allele A were higher in controls ($P=0.001$ and $P=0.05$ respectively).

The increased frequencies of genotype GG (RR=3.28, EF=0.435) and allele G (RR=1.376, EF=0.119) in POAG as compared to controls (Table 7) indicated that genotype GG and allele G are susceptible to the POAG in Saudis. The reduced frequencies of genotype GA (RR=0.326, PF= 0.386) and allele A (RR= 0.726, PF=0.119) in the POAG patients as compared to controls showed that genotype GA and allele A of TNF-β intron1+252 polymorphism are resistant for POAG.

Genotype/ Allele	Open angle glaucoma (N=135)		Control (N=200)		P-value	RR	EF*/PF
	NO.	%	NO.	%			
GG	47	34.81	28	14	0.001‡	3.280	0.435*
GA	65	48.15	148	74	0.001‡	0.326	0.386
AA	23	17.04	24	12	0.20	1.506	0.164*
G-allele (TNFβ 1-allele)	159	58.89	204	51	0.05‡	1.376	0.119*
A-allele (TNFβ 2-allele)	111	41.11	196	49	0.05‡	0.726	0.119

N, number of subjects; RR, Relative risk; EF, etiological fraction; PF, preventive fraction;
‡ statistically significant

Table 7. Genotype and allele frequencies of TNF-β (LTα) - interon1+252 variants in Open angle glaucoma patients and matched controls

The distribution of genotypes and alleles of TNF-β intron1+252 polymorphism is shown in Table 8. The frequency of genotype GG was higher in PACG as compared to controls ($P=0.001$) while the frequencies of genotype GA was higher in controls ($P=0.001$). The higher frequencies of genotype GG in PACG as compared to controls indicated that genotype GG is susceptible to the disease (RR=3.142, EF=0.299) while genotype GA might be refractory for PACG as the frequency of GA was higher in controls RR=0.385, PF=0.229). The frequency of allele G was also higher in PACG and that of allele A was lower in PACG as compared to controls however, the difference in the frequency distribution of allele A and G was not significant in PACG ($P=0.08$).

Comparison of the distribution frequency of genotypes and alleles of TNF- β (LT-α) interon 1+252 polymorphism between the POAG and PACG is summarized in Table 9. The results clearly indicated that the genotype and allele distribution in the two types of glaucoma (POAG and PACG) have similar pattern with minor insignificant differences.

Genotype/Allele	Angle closure glaucoma (N=65)		Control (N=200)		P-value	RR	EF*/PF
	NO.	%	NO.	%			
GG	22	33.85	28	14	0.001‡	3.142	0.299*
GA	34	52.30	148	74	0.001‡	0.385	0.229
AA	9	13.85	24	12	0.67	1.078	0.041*
G-allele (TNF β 1-allele)	78	60.00	204	51	0.08	1.441	0.084*
A-allele (TNF β 2-allele)	52	40.00	196	49	0.08	0.693	0.084

N, number of subjects; RR, Relative risk; EF, etiological fraction; PF, preventive fraction;
‡, statistically significant

Table 8. Genotype and allele frequencies of TNF- β (LT- α) interon1+252 variants in angle closure glaucoma patients and matched controls

Genotype/Allele	Open angle glaucoma (135)	Angle closure glaucoma (65)	P-value
	N (%)	N (%)	
GG	47 (34.81)	22 (33.85)	1.00
GA	65 (48.15)	34 (52.30)	0.65
AA	23 (17.04)	9 (13.85)	0.68
G-allele (TNF β 1-allele)	159 (58.89)	78 (60.00)	0.45
A-allele (TNF β 2-allele)	111 (41.11)	52 (40.00)	0.45

Table 9. Comparison of genotype/ allele frequencies of TNF- β (G252 A) polymorphism in POAG and PACG

The comparison between the associations of TNF- α 308 polymorphism in glaucoma patients in various ethnic populations is given in Table 10. The distribution trend shows ethnic variations. TNF - α (-308) polymorphism is strongly associated with POAG in Saudis, Chinese and Iranian but not in Caucasians and Japanese.

4. Discussion

As early as 1873, Dooremal for the first time observed that the eye is one of the immune privileged region of the body which was later described by Streilein (1999). Multiple factors have been suggested to contribute to the immune privilege of the eye (Niederkorn, 2006) such as the blood- aqueous- barrier and the fact that eyes do not have a lymph drainage system. Aqueous humor is for example, capable of suppressing cytokine production by activated T-lymphocytes (Mochizuki et al., 2000).
The anterior chamber of the eye has an active immunomodulation (Streilein, 2003). It has also been suggested that autoimmune damage to the optic nerve may occur directly by autoantibodies or indirectly by a “mimicked” autoimmune response to a sensitizing antigen, which in turn injures retinal ganglion cells (Wax, 2000). In a study by Joachim et al. (2007) IgG antibody levels were found to be significantly higher in the aqueous humor of patients with POAG and PEXG as compared to controls, suggesting the role of multiple immune factors in glaucoma.

Study	Ethnicity	No. of Patients	Glaucoma	TNF - α (308) polymorphism
Present study	Saudis	200	Primary glaucoma (POAG +PACG)	Associated with TNF - α -308 GA
Lin et al., 2003	Chinese	60	POAG	Associated with TNF - α -308 AA
Fan et al., 2010	Chinese	405	POAG	Associated with TNF - α -308G
Razeghinejad et al., 2009	Iranian	223	POAG	Associated with TNF - α -308A
Funayama et al., 2004	Japanese	194	POAG	No association
Mossbock et al., 2006	Caucasian	114	POAG	No association

Table 10. Association of TNF- α 308 polymorphism in glaucoma patients in various ethnic populations

Growing evidence obtained from clinical and experimental studies over the past decade strongly suggests the role of immune system in the pathogenesis of various types of glaucoma. It has been observed that immune mediators are not usually the primary causative agents but play a critical role in the progression of the disease (Funayama et al., 2004; Joachim et al., 2007). In addition, it has been reported that components of the immune system involved in the pathogenesis of glaucoma are also involved in neurodegeneration following brain injury. In such cases inflammation occurs in response to glutamate, reactive oxygen species (ROS), nitric oxide (NO), and cytokines including tumor necrosis factor- α (TNF- α), which are released from activated microglia or macrophages (Schwartz, 2007). As glaucoma is a disease of old age and involves optic nerve neuropathy, it has been proposed that both genetic as well as epigenetic factors are involved in the progression of the disease. Such factors cause a decrease in the cellular viability and self renewal capacity, which results in the generation of dysfunctional microglia. Such age-related attrition may contribute to the development of neurodegenerative diseases by diminishing glial neurosupportive functions. Secondary degeneration by the immune components leads to the neurodegenerative injury in glaucoma (Levkovitch-Verbin et al., 2003).

TNF- α is considered to be a neuroprotective component of the immune system, because it activates the ubiquitous transcription factor NF- κ B through binding to the high affinity TNF receptors (TNF-R2), which in turn mediates the expression of a wide range of genes essential for neuronal survival. Contrary to its neuroprotective role, TNF- α can also serve as a neurodegenerative factor when it binds to the low affinity death receptors TNF-R1 and activates the mitochondria mediated apoptotic pathway (Lilienbaum & Israel, 2003; Marchetti et al., 2004, Tezel & Yang, 2005). Thus a delicate balance between the two pathways determines the survival of the cell, and any shift in equilibrium might have deleterious effects. An increased expression of TNF- α can shift the balance toward TNF-R1 signaling, as seen in glaucoma, and thus promote retinal ganglion cell death.

In the present study we found a strong association of GA genotype of TNF- α (-308) polymorphism with primary glaucoma, suggesting its role in the pathogenesis of the glaucoma. The results of our study showing the association between TNF- α -308 polymorphism and glaucoma are in agreement with some of the published data on Asian

populations. A recent study from Iran showed that inheritance of high producer TNF- α -308 A allele is susceptibility factor for development of POAG (Razeghinejad et al., 2009). A highly significant association of POAG and TNF- α -308 AA genotype has also been observed in the Chinese population (Lin et al., 2003). These investigators suggested that the close association between TNF- α 308 A/AA and POAG could be used as genetic marker for the disease mapping and identifying subjects susceptible to glaucoma. On the other hand in another study from China the frequency of allele G of TNF- α 308 was higher in patient group than controls and it was suggested that variants in TNF were risk factors for POAG in the Chinese population (Fan et al., 2010). The variations in association of TNF- α -(308) polymorphism with POAG (Table 10) could be due to racial differences, sample size, poorly characterized controls and clinical heterogeneity between different samples. Khan et al. (2009) found a close association between TNF- α 308 polymorphism and pseudoexfoliation glaucoma (PEXG, a type of secondary glaucoma) in Pakistani population. These findings clearly suggest that transcriptional regulation of TNF- α is essential to circumvent the deleterious effects of over expression by transcriptional up regulation. The results of this study clearly suggest that excessive production of TNF- α associated with the G-308A polymorphism may have an important role in the development of glaucoma and may act as a genetic susceptibility factor driven by a high TNF- α expression, which would subsequently lead to immune responses causing the onset and /or progression of the disease. This hypothesis is further supported by the findings of several earlier investigators showing upregulation of the expression of TNF- α and TNF- α receptor-1 in the retina and optic nerve head in glaucomatous eyes (Yan et al., 2000; Tezel et al., 2001; Yuan & Neufeld, 2000). Agarwal et al. (2000) reported that the G to A transition at position -308 results in a six- to sevenfold increase in transcription of TNF- α as compared to normal basal level. Similarly Abraham & Kroeger (1999) demonstrated the functionality of TNF- α -308 polymorphism in the reporter gene assays with a significant up regulation of up to five fold in the constructs of TNF1 (G-allele) and TNF2 (A-allele). A recent study by Sawada et al. (2010) demonstrated that TNF- α levels were significantly elevated in the aqueous humor of glaucoma patients as compared to controls.

Contrary to our findings on Saudi population, studies on Japanese and Austrian Caucasian populations showed no association between TNF- α polymorphism G-308A and primary glaucoma (Funayama et al., 2004; Mossbock et al., 2009). These differences may be attributed to the variation in ethnicity, sample size, poorly characterized controls and clinical heterogeneity of the patients. However, Funayama et al. (2004) noticed a definite interaction between polymorphism in Optineurin (OPTN) and TNF- α genes that would increase the risk for glaucoma in Japanese which indirectly suggest the participation of immune mechanism in glaucoma. The variation in Japanese population to some extent may be attributed to the fact that G-308 A polymorphism is very rare in this population (Allen, 1999).

The role of immune system in glaucoma is further substantiated by the fact that TNF- α mediated activation of matrix metalloproteinases (MMPs) in aqueous humor of the eye results into optic neuropathies. Several *in vitro* studies have shown that TNF- α is capable of inducing both increased synthesis and activity of MMPs (Rajavashisth et al., 1999; Siwik et al., 2000; Uchida et al., 2000). The MMPs are a family of zinc-dependent endopeptidases that have been reported to cause the degradation of the extracellular matrix (ECM) (Stamenkovic, 2003) and involved in wide range of normal and pathological conditions. Uncontrolled activation of MMPs is counterbalanced by specific tissue inhibitors of

metalloproteinases (TIMPs), and a delicate balance of MMPs and TIMPs is required for physiologic ECM turnover. An impaired balance between MMPs and TIMPs may thus contribute to the development of glaucoma (Schlötzer-Schrehardt et al., 2003; Määtä et al., 2005). The higher frequency of Allele-A in our patients clearly suggest increased secretion of $\text{TNF-}\alpha$ which lead to increased synthesis and activity of MMPs. Increased expression of MMPs has been associated with structural remodeling of connective tissues of eye which might lead to the development of PACG (Papp et al., 2006). Further studies are warranted to confirm the role of MMPs in $\text{TNF-}\alpha$ mediated genesis of glaucoma and other eye diseases.

In our study the GG genotypes of $\text{TNF-}\beta$ (+252) polymorphism were significantly over-represented in glaucoma patients as compared to the controls ($P=0.001$) while GA genotype was significantly higher in controls as compared to glaucoma patients ($P=0.001$) indicating that GG, genotypes at +252 were susceptible to glaucoma ($\text{RR}=3.235$, $\text{EF}=0.491$), whereas GA was found to be refractory ($\text{RR}=0.344$, $\text{PF}=0.432$). Similarly the higher frequency of allele-G of $\text{TNF-}\beta$ (+252) polymorphisms in patients ($P=0.011$) indicated that Allele G is associated with glaucoma. $\text{TNF-}\beta$ (+252) polymorphism has earlier been reported to be associated with a number of autoimmune diseases (Albuquerque et al., 1998; Zelano et al., 1998; Pandey et al., 1999; Vasku et al., 2000; Kula et al., 2001; Lu et al., 2005; Takeuchi et al., 2005; Panoulas et al., 2008; Boraska et al., 2009; Watanabe et al., 2010; Tiancha et al., 2011) however, this is the first report on the association between $\text{TNF-}\beta$ polymorphism and glaucoma. The presence of allele G at +252 position defines the mutant allele also known as $\text{TNF-}\beta^*1$ (allele-1) is reported to be associated with higher $\text{TNF-}\alpha$ and $\text{TNF-}\beta$ production (Messer et al., 1991; Abraham et al., 1993). Therefore the higher frequencies of allele G and genotype GG of $\text{TNF-}\beta$ (+252) along with increased genotype GA of $\text{TNF-}\alpha$ (308) polymorphism might act in tandem to increase $\text{TNF-}\alpha$ secretion in ocular tissue of the patients that might lead to the development of glaucoma.

Several studies have shown the relationships between increased $\text{TNF-}\alpha$ levels and several ocular diseases including retinopathy (Doganay et al., 2002; Zorena et al., 2007). Sugita et al. (2007) detected significantly elevated levels of $\text{TNF-}\alpha$ and $\text{TNF-}\alpha$ receptors in the ocular fluid of patients with active uveitis. In an animal model of high intraocular pressure, elevation of $\text{TNF-}\alpha$ precedes the loss of retinal ganglion cells and oligodendrocytes. In addition, retinal cell degeneration has been observed by administering of $\text{TNF-}\alpha$ even without the elevated intraocular pressure (Nakazawa et al., 2006). $\text{TNF-}\alpha$ contributes to this process by adversely affecting oligodendrocytes (Butt & Jenkins, 1994) which increases the susceptibility of axons to excitotoxicity in the optic nerve head and retinal ganglion cell death (Coleman, 2005). It has been observed that both mRNA and protein levels of $\text{TNF-}\alpha$ or $\text{TNF-}\alpha$ receptor-1 (TNF-R1) are raised in the retina of glaucomatous eyes as compared to normal eyes, and therefore it was suggested that cell death mediated by $\text{TNF-}\alpha$ is a contributing factor in the neurodegeneration in glaucoma (Tezel et al., 2001). Using animal models, Nakazawa et al. (2006) showed that ocular hypertension or increased intraocular pressure induces $\text{TNF-}\alpha$ upregulation in the retina, which in turn leads to RGC degeneration. It has also been observed that anti- $\text{TNF-}\alpha$ antibodies can prevent death of RGCs suggesting that reducing the expression of $\text{TNF-}\alpha$ would be beneficial in treating glaucoma.

5. Conclusion

This study clearly showed that the $\text{TNF-}\alpha$ (-308) and $\text{TNF-}\beta$ (+252) polymorphisms are significantly associated with the susceptibility to primary glaucoma (POAG and PACG) in

Saudis and could be used as a genetic marker for disease mapping. To the best of our knowledge this is the first study showing an association of TNF- β (+252) polymorphism with POAG and PACG. Immune hypotheses of glaucoma go very well with the findings of this study performed on the polymorphisms of TNF- α and of TNF- β genes. These cytokines might play a crucial role in the signaling glaucomatous neurodegeneration. The TNF- α (G-308A) and TNF- β (A+252G) polymorphisms alter and modulate the production of cytokines such as TNF- α which is consistently proposed to be involved in the RGC degeneration. Moreover, TNF has been found to be associated with the regulation of glial cells and stimulation of the synthesis and secretion of nerve growth factors (NGF). Therefore understanding the role of gene polymorphism like TNF- α (-308) and TNF- β (+252) in various ethnic populations could be helpful in predicting the predisposition to glaucoma and might help in developing novel therapeutic strategies for the management of this disease.

6. References

- Abraham, L.J. & Kroeger, K.M. (1999). Impact of the -308 TNF promoter polymorphism on the transcriptional regulation of the TNF gene: relevance to disease. *Journal of Leukocyte Biology*, 66, 562-566, ISSN 0741-5400
- Abraham, L.J., French, M.A.H. & Dawkins, R.L. (1993). Polymorphic MHC ancestral haplotypes affect the activity of tumour necrosis factor-alpha. *Clinical & Experimental Immunology*, 92, 14-18, ISSN 0009-9104
- Agarwal, P., Oldenburg, M.C., Czarneski, J.E., Morse, R.M., Hameed, M.R., Cohen, S. & Fernandes, H. (2000). Comparison study for identifying promoter allelic polymorphism in interleukin 10 and tumor necrosis factor alpha genes. *Diagnostic Molecular Pathology*, 9, 158-164, ISSN 1052-9551
- Albuquerque, R.V., Hayden, C.M., Palmer, L.J., Laing, I.A., Rye, P.J., Gibson, N.A., Burton, P.R., Goldblatt, J., Lesouef, P.N. (1998). Association of polymorphism within the tumour necrosis factor (TNF) genes and childhood asthma. *Clinical and Experimental Allergy*, 28, 578-584, ISSN 0954-7894
- Allen, R.D. (1999). Polymorphism of the human TNF- α promoter random variation or functional diversity? *Molecular Immunology*, 36, 1017-1027, ISSN 0161-5890
- Appropriateness of Treating Glaucoma Suspects RAND Study Group. (2009). For which glaucoma suspects is it appropriate to initiate treatment? *Journal of Ophthalmology*, 116, 710-716, ISSN 2090-0058
- Arkell, S.M., Lightman, D.A., Sommer, A., Taylor, H.R., Korshin, O.M. & Tielsch J.M. (1987). The relevance of glaucoma among Eskimos of northwest Alaska. *Archives of Ophthalmology*, 105, 482-485, ISSN 0003-9950
- Aung, T., Lim, M.C., Wong, T.T., Thalamuthu, A., Yong, V.H., Venkataraman, D., Venkataraman, A., Chew, P.T., Vithana, E.N. (2008a). Molecular analysis of CHX10 and MFRP in Chinese subjects with primary angle closure glaucoma and short axial length eyes. *Molecular Vision*, 14, 1313-1318, ISSN 1090-0535
- Aung, T., Victor, H.K., Lim, M.C., Venkataraman, D., Toh, J.Y., Chew, P.T. & Vithana, E.N. (2008b). Lack of association between the rs2664538 Polymorphism in the MMP-9

- Gene and Primary angle Closure Glaucoma in Singaporean Subjects. *Journal of Glaucoma*, 17, 257-258, ISSN 1057-0829
- Ayub, H., Khan, M.I., Micheal, S., Akhtar, F., Ajmal, M., Shafique, S., Ali, S.H.B., Den Hollander, A.I., Ahmed, A., Qamar, R. (2010). Association of eNOS and HSP70 gene polymorphism with glaucoma in Pakistani cohorts. *Molecular Vision*, 16, 18-25, ISSN 1090-0535
- Boraska, V., Zeggini, E., Groves, C.J., Rayner, N.W., Skrabic, V., Diakite, M., Rockett, K.A., Kwiatkowski, D., McCarthy, M.I. & Zemunik, T. (2009). Family based analysis of tumor necrosis factor and lymphotoxin-alpha Tag polymorphism with type 1 diabetes in the population of South Croatia. *Human Immunology*, 70, 159-9, ISSN 0198-8859
- Braun, N., Michel, U., Ernst, B.P., Metzner, R., Bitsch, A., Weber, F. & Riechmann, P. (1996). Gene polymorphism at position -308 of the tumor-necrosis-factor-alpha (TNF-alpha) in multiple sclerosis and its influence on the regulation of TNF-alpha production. *Neuroscience Letters*, 215, 75-78, ISSN 0304-3940
- Butt, A.M. & Jenkins, H.G. (1994). Morphological changes in oligodendrocytes in the intact mouse optic nerve following intravitreal injection of tumor necrosis factor. *Journal of Neuroimmunology*, 51, 27-33, ISSN 0165-5728
- Cao, D., Lu, X., Guo X., Cong, Y., Huang, J. & Mao, Z. (2009). Investigation of the association between CALCRL polymorphisms and primary angle closure glaucoma. *Molecular Vision*, 15, 2202-2208, ISSN 1090-0535
- Casson, R.J., Newland, H.S., Muecke, J., McGrover, S., Abraham, L., Shein, W.K., Selva, D. & Aung T. (2007). Prevalence of glaucoma in rural Myanmar: the Meiktila Eye study. *British Journal of Ophthalmology*, 91, 710-714, ISSN 1468-2079
- Cedrone, C., Mancino, R., Cerulli, A., Cesareo, M. & Nucci, C. (2008). Epidemiology of primary glaucoma : prevalence, incidence, and blinding effects. *Progress in Brain Research*, 173, 3-14, ISSN 0079-6123
- Chakrabarti, S., Devi, K.R., Komatireddy, S., Kaur, K., Parikh, R.S., Mandal, A.K., Chandrasekhar, G. & Thomas, R. (2007). Glaucoma-Associated CYP1B1 mutations share similar haplotypes backgrounds in POAG and PACG phenotypes. *Investigative Ophthalmology & Visual Science*, 48, 5439-5444, ISSN 0146-0404
- Coleman, M. (2005). Axon degeneration mechanisms: commonality amid diversity. *Nature Reviews Neuroscience*, 6, 889-898, ISSN 1471-003X.
- Cong, Y., Guo, X., Liu, X., Cao, D., Jia, X., Xiao, X., Li, S., Fang, S. & Zhang, Q. (2009). Association of the single nucleotide polymorphisms in the extracellular matrix metalloprotease- 9 gene with PACG in southern China. *Molecular Vision*, 15, 1412-1417, ISSN 1090-0535
- Cuenca, J., Perez, C., Aguirre, A., Schiattino, I. & Aguillon, J.C. (2001). Genetic polymorphism at position -308 in the promoter region of the tumor necrosis factor (TNF): implications of its allelic distribution on susceptibility or resistance to diseases in the Chilean population. *Biological Research*, 34, 237-241, ISSN 0716-97.
- Dai, X., Nie, S., Ke, T., Liu, J., Wang, Q. & Liu, M. (2008). Two variants in MYOC and CYP1B1 genes in a Chinese family with primary angle closure glaucoma. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, 25, 493-496, ISSN 1003-9406

- Dandona, L., Dandona, R., Srinivas, M., Mandal, P., John, R.K., McCarty, C.A., et al. (2000). Open angle glaucoma in an urban population in southern India, the Andhra Pradesh eye disease study. *Ophthalmology*, 107, 1702-1709, ISSN 2090-004X
- Doganay, S., Evereklioglu, C. Er, H., Türköz, Y, Sevinç , A., Mehmet, N. & Savli, H. (2002). Comparison of serum NO, TNF- α , IL-1 , sIL-2R, IL-6 and IL-8 levels with grades of retinopathy in patients with diabetes mellitus. *Eye*, 16,163-170, ISSN 0950-222X
- Eid, T.M., El -Harwary, I. & El- Menawy, W. (2009). Prevalence of glaucoma types and legal blindness from glaucoma in the Western region of Saudi Arabia: a hospital based study. *International Ophthalmology*, 29, 477-483, ISSN 0165-5701.
- Ertel, W., Keel, M., Bonaccio, M., Steckholzer, U., Gallati, H., Kenney, J.S. & Trentz,O(1995). Release of anti-inflammatory mediators after mechanical trauma correlates with severity of injury and clinical outcome. *Journal of Trauma*, 39, 879-887, ISSN 0022-5282
- Fan, B.J., Liu, K., Wang, D.Y., Tham, C.C., Tam, P.O., Lam, D.S. & Pang, C.P. (2010). Association of polymorphisms of tumor necrosis factor and tumor protein p53 with primary open-angle glaucoma. *Investigative Ophthalmology & Visual Science*, 51, 4110-4116, ISSN 0146-0404
- Foster, P.J. & Johnson, G.J. (2001). Glaucoma in China: how big is the problem? *British Journal of Ophthalmology*, 85, 1277-1282, ISSN 0007-1161
- Foster, P.J., Oen, F.T., Machin, D., Ng, T.P., Devereux, J.G., Johnson, G.J., Khaw, P.T. & Seah, S.K. (2000). The prevalence of glaucoma in Chinese residents of Singapore: a cross-sectional population survey of the Tanjong Pagar district. *Archives of Ophthalmology*, 118, 1105-1111, ISSN 0003-9950
- Foster, P.J., Buhrmann, R., Quigley, H.A. & Johnson, G.J. (2002). The definition and classification of glaucoma in prevalence surveys. *British Journal of Ophthalmology*, 86, 238-242, ISSN 0007-1161
- Friedman, D.S., Jampel, H.D., Munoz, B. & West, S.K. (2006).The prevalence of open angle glaucoma among blacks and whites 73 years and older: the Salisbury eye evaluation glaucoma study. *Archives of Ophthalmology*, 124, 1625-1630, ISSN 0003-9950
- Funayama, T., Ishikawa, K., Ohtake, Y., Tanino, T., Kurosaka, D., Kimura, I., et al. (2004).Variants in optineurin gene and their association with tumor necrosis factor-alpha polymorphisms in Japanese patients with glaucoma. *Investigative Ophthalmology & Visual Science*, 45, 4359-4367, ISSN 0146-0404
- Galbratith, G.M. & Pendey, J.P. (1995). Tumor necrosis factor alpha (TNF-alpha) gene polymorphism in alopecia areata. *Human Genetics*, 96, 433-436, ISSN 0340-6717
- Green, C.M., Kearns, L.S., Wu, J., Barbour, J.M., Wilkinson, R.M., Ring, M.A., et al. (2007). How significant is a family history of glaucoma? Experience from the glaucoma inheritance study in Tasmania. *Clinical & Experimental Ophthalmology*, 35, 793 -799, ISSN 2155-9570
- Halpern, D.L. & Grosskreutz, C.L. (2002). Glaucomatous optic neuropathy: mechanisms of disease. *Ophthalmology Clinics of North America*, 15, 61-68, ISSN 0896-1549

- He, M., Foster, P.J., Huang, W., Zheng, Y., Freidman, D.S, Lee, P.S. & Khaw, P.T. (2006). Prevalence and Clinical characteristics of Glaucoma in Adult Chinese: A population based study in Liwan District, Guangzhou. *Investigative Ophthalmology & Visual Science*, 47, 2782-2788, ISSN 0146-0404
- Hu, Z., Zhao, Z.L. & Dong, F.T. (1989). An epidemiological investigation of glaucoma in Beijing and Shun Yi county. *Chinese Journal of Ophthalmology*, 25, 115-118, ISSN 0412-4081
- Huang, P., Zhang, S.S. & Zhang, C. (2009). The two sides of cytokine signaling and glaucomatous optic neuropathy. *Journal of Ocular Biology, Diseases, and Informatics*, 2, 78-83, ISSN 1936-8437
- Jacob, A., Thomas, R., Koshi, S.P., Braganza, A. & Muliylil, J. (1998). Prevalence of primary glaucoma in an urban south Indian population. *Indian Journal of Ophthalmology*, 46, 81-86, ISSN 0301-4738
- Jeong, P., Kim, E.J., Kim, E.G., Byun, S.S., Kim, C.S. & Kim, W.J. (2004). Association of bladder tumors and GA genotype of -308 nucleotide in tumor necrosis factor-alpha promoter with greater tumor necrosis factor-alpha expression. *Urology*, 64, 1052-1056, ISSN 1677-5538
- Joachim, S.C., Wuenschig, D., Pfeiffer, N. & Grus, F.H. (2007). IgG antibody patterns in aqueous humor of patients with primary open angle glaucoma and psuedoexfoliation glaucoma. *Moleuclar Vision*, 13, 1573-1579, ISSN 1090-0535
- Khan, M.I., Michael, S., Akhtar, F., Naveed, A., Ahmed, A. & Qamar, R. (2009). Association of ABO blood groups with glaucoma in the Pakistani population. *Canadian Journal of Ophthalmology*, 44, 582-586, ISSN 1715-3360
- Kipnis, J., Mizrahi, T., Hauben, E., Shaked, I., Shevach, E., Schwartz, M. (2002). Neuroprotective autoimmunity: naturally occurring CD4+ CD25+ regulatory T cells suppress the ability to withstand injury to the central nervous system. *Proceedings of the National Academy of Sciences*, 99, 15620-15625, ISSN 0027-8424
- Klein, B.E., Klein, R., Sponsel, W.E., Frank ,T., Cantor, L.B., Martone, J. & Menage, M.J. (1992). Prevalence of glaucoma. The Beaver Dam Eye study. *Journal of Ophthalmology*, 99, 1499-1504, ISSN 2090-004X
- Kula, D., Jurecka-Tuleja, B., Gubala, E., Krawczyk, A., Szpak, S., Jarzab, M. (2001). Association of polymorphism of LT alpha and TNF genes with Graves disease. *Folia Histochem Cytobiol*, 39 Suppl 2: 77-8, ISSN 0258-851X
- Kwon, Y.H., Fingert, J.H., Kuehn, M.H. & Alward, W.L. (2009). Primary open angle glaucoma. *New England Journal of Medicine*, 360, 1113-1124, ISSN 0028-4793
- Laengle, U.W., Markstein, R., Pralet, D., Seewald, W. & Roman, D. (2006a). Effect of GLC756, a novel mixed dopamine D1 receptor antagonist and dopamine D2 receptor agonist, on TNF-alpha release in vitro from activated rat mast cells. *Experimental Eye Research*, 83, 1335-1339, ISSN 0014-4835
- Laengle, U.W., Trendelenburg, A.U., Markstein, R., Nogues, V., Provencher-Bollinger, A. & Roman, D. (2006b). GLC756 decreases TNF-alpha via an alpha2 and beta2 adrenoceptor related mechanism. *Experimental Eye Research*, 83, 1246-1251, ISSN 0014-4835

- Leske, C., Connell, M., Wu, Y., Hyman, G. & Schachat, A.P. (1995). Risk factors for open-angle glaucoma. The Barbados Eye Study. *Archives of Ophthalmology*, 113, 918-924, ISSN 0003-9950.
- Leske, M.C., Connell, A.M., Schachat, A.P. & Hyman, L. (1994). The Barbados Eye Study. Prevalence of Open Angle Glaucoma. *Archives of Ophthalmology*, 112, 821-829, ISSN 0003-9950
- Levkovitch-Verbin, H., Quigley, H. A., Martin K.R., Valenta, D., Baumrind, L.A. & Pease, M.E. (2002). Translimbal laser photocoagulation to the trabecular meshwork as a model of glaucoma in rats. *Investigative Ophthalmology & Visual Science*, 43, 402-410, ISSN 0146-0404
- Levkovitch-Verbin, H., Quigley, H.A., Martin, K.R., Zack, D.J., Pease, M.E. & Valenta, D.F. (2003). A model to study differences between primary and secondary degeneration of retinal ganglion cells in rats by partial optic nerve transection. *Investigative Ophthalmology & Visual Science*, 44, 3388-3393, ISSN 0146-0404
- Lilienbaum, A. & Israel, A. (2003). From calcium to NF-kappa B signaling pathways in neurons. *Molecular and Cellular Biology*, 23, 2680-2698. ISSN: 1098-5549
- Limb, G.A., Soomro, H., Janikoun, S., Hollifield, R.D. & Shilling J. (1999). Evidence for control of tumour necrosis factor - α (TNF- α) activity by TNF receptors in patients with proliferative diabetic retinopathy. *Clinical and Experimental Immunology*, 115, 409-414, ISSN 0009-9104
- Lin, H.J., Tsai, F.J., Chen, W.C., Shi, Y.R., Hsu, Y. & Tsai, S.W. (2003). Association of tumor necrosis factor alpha -308 gene polymorphism with primary open-angle glaucoma in Chinese. *Eye*, 17, 31-34, ISSN 0950-222X
- Liu, B. (2006). Modulation of microglial pro-inflammatory and neurotoxic activity for the treatment of Parkinson's disease. *The American Association of Pharmaceutical Scientists Journal*, 8, 606-621, ISSN 1550-7416
- Lowe, R.F. (1972). Primary angle-closure glaucoma. Inheritance and environment. *British Journal of Ophthalmology*, 56, 13-20, ISSN 0007-1161
- Lu, L.Y., Cheng, H.H., Sung, P.K., Tai, M.H., Yeh, J.J., Chen, A. (2005). Tumor necrosis factor - β +252A polymorphism is associated with systemic lupus erythematosus in Taiwan. *Journal of the Formosan Medical Association*, 104, 563-570, ISSN 0929-6646
- Määtä, M., Tervahartiala, T., Harju, M., Airaksinen, J., Autio-Harmainen, H. & Sorsa, T. (2005). Matrix metalloproteinases and their tissue inhibitors in aqueous humor of patients with primary open-angle glaucoma, exfoliation syndrome, and exfoliation glaucoma. *Journal of Glaucoma*, 14, 64-69, ISSN 1057-0829
- Marchetti, L., Klein, M., Schlett, K., Pfizenmaier, K. & Eisel, U.L. (2004). Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-Methyl-D-aspartate receptor activation: Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. *Journal of Biological Chemistry*, 279, 32869-32881, ISSN 0021-9258
- Mason, R.P., Kosoko, O., Wilson, M.R., et al. (1989). National survey of the prevalence and risk factors of glaucoma in St. Lucia, West Indies. Part I. Prevalence findings. *Ophthalmology*, 96, 1363-1368, ISSN 2090-004X

- McBrien ,N.A. & Gentle, A. (2001). Timp -2 regulation of MMP-2 activity during visually guided remodeling of the tree shrew sclera in lens-induced myopia. *Investigative Ophthalmology & Visual*, 42 Suppl: 314, ISSN: 0146-040
- Medcraft, J., Hitman, G.A., Sachs, J.A., Whichelow, C.E., Raafat, I. & Moore, R.H. (1993). Autoimmune renal disease and tumour necrosis factor beta gene polymorphism. *Clinical Nephrology*, 40, 63-68, ISSN 0301-0430
- Messer, G., Spengler, U., Jung, M.C., Honold, G., Biomer, K., Pape, G.R., et al. (1991). Polymorphic structure of the tumor necrosis factor (TNF) locus: an NcoI Polymorphism in the first intron of the human TNF-beta gene correlates with a variant amino acid in position 26 and a reduced level of TNF-beta production. *Journal of Experimental Medicine*, 173, 209-219, ISSN:1540-9538
- Micheal, S., Qamar, R., Akhtar, F., Khan, W.A. & Ahmed, A. (2008). C677T polymorphism in the methylene tetrahydrofolate reductase gene is associated with primary closed angle glaucoma. *Molecular Vision*, 14, 661-5, ISSN 1090-0535
- Micheal, S., Qamar, R., Akhtar ,F., Khan, M.I, Khan, W.A. & Ahmed, A. (2009).MTHFR gene C677T and A1298C polymorphisms and homocystein levels in primary open angle and primary closed angle glaucoma. *Molecular Vision*, 15, 2268-2278, ISSN 1090-0535
- Mochizuki, M., Sugita, S., Ishikawa, N. & Wantanabe, T.(2000). Immunoregulation by aqueous humor. *Cornea*, 19, S24-5, ISSN: 0277-3740
- Mossbock, G., Weger, M., Moray, M., Renner, W., Haller-Schober, E.M., Mattes, D., Schmut, O., Wegscheider, B. & El-Shabrawi, Y. (2006). TNF-promoter polymorphisms and primary open-angle glaucoma. *Eye*, 20, 1040-1043, ISSN 0950-222X
- Mozaffarieh, M., Greishaber, M.C. & Flammer, J. (2008). Oxygen and blood flow: Players in the pathogenesis of glaucoma. *Molecular Vision*, 14, 224-233, ISSN 1090-0535
- Munoz-Fernandez, M.A., Fresno, M. (1998). The role of tumor necrosis factor, interleukin 6, interferon- and inducible nitric oxide synthase in the development and pathology of the nervous system. *Progress in Neurobiology*,56, 307-340, ISSN 0301-0082
- Nakazawa, T., Nakazawa, C., Matsubara, A., Noda, K., Hisatomi, T., She, H., Michaud, N., Hafezi-Moghadam, A., Miller, J.W. & Benowitz, L.I. (2006). Tumor necrosis factor- α mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *Journal of Neuroscience*, 26, 12633-12641, ISSN 0270-6474.
- Nieder Korn, J.Y. (2006). See no evil, hear no evil, do no evil: the lessons of immune privilege. *Nature Immunology*, 7, 354-359, ISSN 1529- 2908
- Pandey, J.P. & Takeuchi, F. (1999). TNF- α and TNF-beta gene polymorphism in systemic sclerosis. *Human Immunology*, 60, 1128-1130, ISSN 0198-8859
- Panoulas, V.F., Nikas, S.N., Smith, J.P.,Douglas, K.M., Nightingale, P.,Milionis, H.J., Treharne, G.J., Toms, T.E., Kita, M.D., Kitas, G.D. (2008). Lymphotoxin 252A>G polymorphism is common and associates with myocardial infarction in patients with Rheumatoid Arthritis. *Annals of the Rheumatic Diseases*, 67, 1550-1556, ISSN 0003-4967
- Papp, A.M., Nyilas, R., Szepesi, Z., Lorinez, M.L., Lorinez, M.L., Takacs, E., Abraham , I., Szilagyi, N., et al. (2007). Visible light induces matrix metalloproteinase-9 expression in rat eye. *Journal of Neurochemistry* ,103, 2224-2233, ISSN 0022-3042

- Patino-Gracia, A., Sotillo- Pineiro, E., Modesto, C. & Sierrasesumaga, L. (2000). Analysis of the human tumour necrosis factor-alpha(TNF α) gene promoter polymorphisms in children with bone cancer, *Journal of Medical Genetics*, 37, 789-792, ISSN 0148-7299
- Pease, M.E., Mc Kinnon, S.J., Quigley, H.A., Kerrigan-Baumrind, L.A. & Zack, D.J. (2000). Obstructed axonal transport of BDNF and its receptor TrkB in experimental glaucoma. *Investigative Ophthalmology & Visual Science*, 41, 764-774, ISSN 0146-0404
- Pekmezci, M., Vo, B., Lim, A.K., Hirabayashi, D.R., Tanaka, G.H., Weinreb, R.N. & Lin, S.C. (2009). The characteristics of Glaucoma in Japanese Americans. *Archives of Ophthalmology*, 127, 167-171, ISSN 0003-9950
- Perrey, C., Turney, S., Pravica, V., Howell, W.M. & Hutchinson, I.V. (1999). ARMS-PCR methodologies to determine IL-10, TNF- α , TNF- β and TGF- β 1 gene polymorphisms. *Transplant Immunology*, 7, 127-128, ISSN 0966-3274
- Quigley, H.A. & Broman, A.T. (2006). The number of people with glaucoma worldwide in 2010 and 2020. *British Journal of Ophthalmology*, 90, 262-267, ISSN 1468-2079.
- Rajavashisth, T.B., Liao, J.K., Galis, Z.S., Tripathi, S., Laufs, U., Tripathi, J., Cahi, N.N., Xu, X.P., Jovinge, S., Shah, P.K. & Libby, P. (1999). Inflammatory cytokines and oxidized low density lipoproteins increase endothelial cell expression of membrane type 1-matrix metalloproteinase. *Journal of Biological Chemistry*, 274, 11924-11929, ISSN 0021-9258
- Razeghinejad, M.R., Rahat, F. & Kamali-Sarvestani, E. (2009). Association of TNFA -308 G/A and TNFRI +36 A/G gene polymorphisms with glaucoma. *Ophthalmic Research*, 42, 118-124, ISSN 0030-3747
- Rotchford, A.P., Kirwan, J.F., Muller, M.A., Johnson, G.J. & Roux P. (2003). Temba glaucoma study: a population - based cross- sectional survey in urban South Africa. *Journal of Ophthalmology*, 110, 376-382, ISSN 2090-004X
- Rotchford, A.P. & Johnson, G.J. (2002). Glaucoma in Zulus: a population -based cross sectional survey in a rural district in South Africa. *Archives of Ophthalmology*, 120, 471-478, ISSN 0003-9950
- Sakata, K., Sakata, L.M., Sakata, V.M., Santini, C., Hopker, L.M., Bernardes, R., Yabumoto, C. & Moreira, A.T. (2007). Prevalence of Glaucoma in a South Brazilian Population: Projeto Glaucoma. *Investigative Ophthalmology & Visual Science*, 48, 4974-4979, ISSN 0146-0404
- Savejgaard, A., Platz, P. & Ryder, L.P. (1983). HLA and disease-1982: a survey. *Immunological Reviews*, 70:193-218, ISSN 0105-2896
- Sawada, H., Fukuchi, T., Tanaka, T. & Abe, H. (2010). Tumor necrosis factor-alpha concentrations in the aqueous humor of patients with glaucoma. *Investigative Ophthalmology & Visual Science*, 51, 903-906, ISSN 0146-0404
- Schallreuter, K.U., Levenig, C., Kuhl, P., Loliger, C., Hohl-Tehari, M. & Berger, J. (1993). Histocompatibility antigens in vitiligo: Hamburg study on 102 patients from Northern Germany. *Dermatology*, 187, 186-192, ISSN 1167-1122
- Scheie, H.G. (1957). Width and pigmentation of the angle of the anterior chamber, a system of grading by gonioscopy. *Archives of Ophthalmology American Medical Association*, 58, 510-512, ISSN 0096-6339

- Schlötzer-Schrehardt, U., Lommatzsch, J., Kuchle, M., Konstas, A.G. & Naumann, G.O. (2003). Matrix metalloproteinases and their inhibitors in aqueous humor of patients with pseudoexfoliation syndrome/glaucoma and primary open-angle glaucoma. *Investigative Ophthalmology & Visual Science*, 44, 1117-1125, ISSN 0146-0404
- Schwartz, M. & Kipnis, J. (2002). Autoimmunity on alert: naturally occurring regulatory CD4(+) CD25(+) T cells as part of the evolutionary compromise between a "need" and a "risk". *Trends in Immunology*, 23, 530-534, ISSN 1471-4906
- Schwartz, M. & Kipnis, J. (2001). Protective autoimmunity: regulation and prospects for vaccination after brain and spinal cord injuries. *Trends in Molecular Medicine*, 7, 252-258, ISSN 1471-4914
- Schwartz, M. (2007). Modulating the immune system: a vaccine for glaucoma. *Canadian Journal of Ophthalmology*, 42, 439-441, ISSN 1715-3360.
- Sharma, S., Ghosh, B. & Sharma, S.K. (2008). Association of TNF polymorphisms with sarcoidosis, its prognosis and tumour necrosis factor(TNF)-alpha levels in Asian Indians. *Clinical & Experimental Immunology*, 151,251-259, ISSN 0009-9104
- Sharma, S., Sharma, A., Kumar, S., Sharma, S.K. & Ghosh, B. (2006). Association of TNF haplotypes with asthma, serum IgE levels, and correlation with serum TNF-alpha levels. *American Journal of Respiratory Cell and Molecular Biology*, 35, 488-495, ISSN 1040-0605
- Shields, M.B. (2005). *Shields' Textbook of Glaucoma*. 4th ed. Lippincott Williams and Wilkins publishers, ISBN 13: 978-1-60831-630-4 New York, USA
- Shohami, E., Bass, R., Wallach, D., Yamin, A. & Gallily, R. (1996). Inhibition of tumor necrosis factor alpha (TNF- α) activity in rat brain is associated with cerebroprotection after closed head injury. *Journal of Cerebral Blood Flow & Metabolism*, 16, 378-384, ISSN 0271-678X
- Shohami, E., Ginis, I. & Hallenbeck, J.M. (1999). Dual role of tumor necrosis factor alpha in brain injury. *Cytokine & Growth Factor*, 10, 119-130, ISSN 1359- 6101
- Siwik, D.A., Chang, D.L. & Colucci, W.S. (2000). Interleukin-1 beta and tumor necrosis factor-alpha decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. *Circulation Research*, 86, 1259-1265, ISSN: 0009-7330
- Spaeth, G.I. (1993). *Direct Ophthalmoscopy*, In: Varma, R., Spaeth, G.I., Parker, K.W., eds. *The optic Nerve in Glaucoma*, JB Lippincott Co, ISBN 0-397-51069-1, 127-135, Philadelphia, USA
- Stamenkovic, I.(2003). Extracellular matrix remodeling: the role of matrix metalloproteinases. *Journal of Pathology*, 200, 448-464, ISSN 0022-3417
- Sterilein, J.W. (2003). Ocular immune privilege: The eye takes a dim but practical view of immunity and inflammation. *J Leukocyte Biology*, 74, 179-85, ISSN 0741-5400
- Streilein, J.W. (1999). Immunoregulatory mechanisms of the eye. *Progress in Retinal and Eye Research*, 18, 357-370, ISSN 1350-9462
- Sugita, S., Takase, H., Taguchi, C. & Mochizuki, M. (2007). The role of soluble TNF receptors for TNF-alpha in uveitis. *Investigative Ophthalmology & Visual Science*, 48, 3246-3252, ISSN 0146-0404

- Takeuchi, F., Nabeta, H., Hong, G.H., Kawasugi, K., Mori, M., Matsuta, K., Kuwata, S., Murayama, T., Nakano, K. (2005). The genetic contribution of the TNFa11 microsatellite allele and the TNFb +252*2 allele in Japanese RA. *Clinical & Experimental Rheumatology*, 23, 494-498, ISSN 0392-856X
- Tekeli, O., Turacli, M.E., Egin, Y., Akar, N. & Elhan, A.H. (2008). Tumor necrosis factor alpha-308 gene polymorphism and psuedoexfoliation glaucoma. *Molecular Vision*, 14, 1815-1818, ISSN 1090-0535
- Tezel, G. & Wax, M.B. (2007). Glaucoma. *Chemical Immunology and Allergy*, 92, 221-227, ISSN: 1660-2242
- Tezel, G. & Wax, M.B. (2004). The immune system and glaucoma. *Current Opinion in Ophthalmology*, 15, 80-84, ISSN 1040-8738
- Tezel, G. & Wax, M.B. (2003). Glial modulation of retinal ganglion cell death in glaucoma. *Journal of Glaucoma*, 12, 63-68, ISSN 1057-0829
- Tezel, G. & Yang, X. (2005). Comparative gene array analysis of TNF-alpha-induced MAPK and NF-kappaB signaling pathways between retinal ganglion cells and glial cells. *Experimental Eye Research*, 81, 207-217, ISSN 0014-4835
- Tezel, G. & Wax, M.B. (2000a). The mechanisms of hsp27 antibody-mediated apoptosis in retinal neuronal cells. *Journal of Neuroscience*, 20, 3552-3562, ISSN 0270-6474
- Tezel, G. & Wax, M.B. (2000b). Increased production of tumor necrosis factor-alpha by glial cells exposed to simulated ischemia or elevated hydrostatic pressure induces apoptosis in cocultured retinal ganglion cells. *Journal of Neuroscience*, 20, 8693-8700, ISSN 0270-6474
- Tezel, G., Edward, D. P. & Wax, M.B. (1999). Serum autoantibodies to optic nerve head glycosaminoglycans in patients with glaucoma. *Archives of Ophthalmology*, 117, 917-924, ISSN 0003-9950
- Tezel, G., Li, L.Y., Patil, R.V. & Wax, M.B. (2001). TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. *Investigative Ophthalmology & Visual Science*, 42, 1787-1794, ISSN 0146-0404
- Tezel, G., Seigel, G.M. & Wax, M.B. (1998). Autoantibodies to small heat shock proteins in glaucoma. *Investigative Ophthalmology & Visual Science*, 39, 2277-2287, ISSN 0146-0404
- Tezel, G., Yang, X., Yang, J. & Wax, M.B. (2004). Role of tumor necrosis factor receptor-1 in the death of retinal ganglion cells following optic nerve crush injury in mice. *Brain Research*, 996, 202-212, ISSN: 0006-8993
- Tiancha, H., Huiqin, W., Jiyong, J., Jingfen, J. & Wei, C. (2011). Association between lymphotoxin-? intron +252 polymorphism and sepsis: Ameta analysis. *Scandinavian Journal of Infectious Diseases*, 1-12, ISSN 0036-5548
- Tielsh, J.M., Sommer, A., Katz, J., Royall, R.M., Quigley, H.A. & Javitt, J. (1991). Racial variation in prevalence of primary open angle glaucoma. *Journal of the American Medical Association*, 266, 369-374, ISSN 0098-7484
- Tschumper, R.C. & Johnson, D.H. (1990). Trabecular meshwork cellularity: Differences between fellow eyes. *Investigative Ophthalmology & Visual Science*, 31, 1327-1331, ISSN 0146-0404

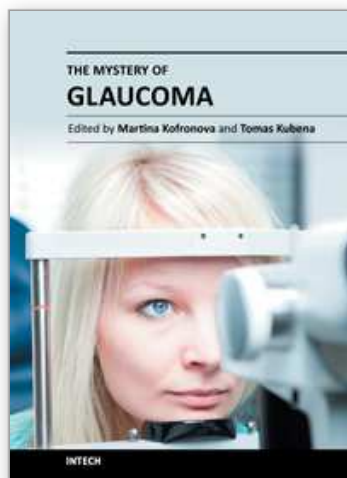
- Uchida, M., Shima, M., Shimoaka, T., Fujieda, A., Obara, K., Suzuki, H., Nagai, Y., Ikeda, T., Yamato, H. & Kawaguchi, H. (2000). Regulation of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) by bone resorptive factors in osteoblastic cells. *Journal of Cellular Physiology*, 185, 207-214, ISSN 0021-9541
- Varma, R., Ying-Lai, M., Francis, B.A., Nguyen, B.B., Deneen, J., Wilson, M.R., Azen, S.P. & Los Angeles Latino Eye study group . (2004). Prevalence of open angle glaucoma and Ocular hypertension in Latinos: the Los Angeles Latino eye study. *Journal of Ophthalmology*, 111, 1439-1448, ISSN 2090-004X
- Vasku, V., Vasku, A., Izakovicova Holla, L., Tschoplova, S., Kankova K, Benakova, N., Semradova, V. (2000). Polymorphism in inflammation genes (angiotensinogen, TAP1 and TNF-beta) in Psoriasis. *Archives of Dermatological Research*, 292, 531-534, ISSN 0340-3696
- Vijaya, L., George, R., Arvind, H., Baskaran, M., Ramesh, V., Raju, P., Kumaramanickavel, G. & McCarty, C. (2008a). Prevalence of primary angle- closure disease in an urban South Indian population and comparison with a rural population. The Chennai glaucoma study. *Journal of Ophthalmology*, 115, 655-660, ISSN 2090-004X
- Vijaya, L., George, R., Baskaran, M. , Arvind, H., Raju, P., Ramesh, V., Raju, P., Kumaramanickavel, G., & McCarty, C. (2008b). Prevalence of primary open angle glaucoma in an Urban South Indian population and comparison with a rural population. The Chennai glaucoma study. *Journal of Ophthalmology*, 115, 655-660, ISSN 2090-004X
- Wang, I.J., Chiang, T.H., Shih, Y.F., Lu, S.C., Lin, L.L., Shieh, J.W., Wang, T.H., Samples, J.R. & Hung PT.(2006). The association of single nucleotide polymorphisms in the MMP-9 genes with susceptibility to acute primary angle closure glaucoma in Taiwanese patients. *Molecular Vision*, 12, 1223-1232, ISSN 1090-0535
- Wantanabe, E., Buchman, T.G., Hirasawa, H., Zehnbauser, B.A. (2010). Association between lymphotoxin- α (tumor necrosis factor- β) intron polymorphism and predisposition to severe sepsis is modified by gender and age. *Critical Care Medicine*, 38,181-193, ISSN 0090-3493
- Wax, M.B. & Tezel, G. (2002). Neurobiology of glaucomatous optic neuropathy: diverse cellular events in neurodegeneration and neuroprotection. *Molecular Neurobiology*, 26, 45-55, ISSN 0893-7648
- Wax, M.B. (2000). Is there a role for the immune system in glaucomatous optic neuropathy? *Current Opinion in Ophthalmology*, 11, 145-150, ISSN 1040-8738
- Wax, M.B. & Tezel, G. (2009). Immunoregulation of retinal ganglion cell fate in glaucoma. *Experimental Eye Research*, 88,825-830, ISSN 0014-4835
- Wax, M.B., Barrett, D.A. & Pestronk, A. (1994). Increased incidence of paraproteinemia and autoantibodies in patients with normal pressure glaucoma. *American Journal of Ophthalmology*, 117, 561-568, ISSN 0002-9394
- Wax, M.B., Tezel, G.& Edward, P.D. (1998a). Clinical and ocular histopathological findings in a patient with normal-pressure glaucoma. *Archives of Ophthalmology*, 116, 993-1001, ISSN 0003-9950

- Wax, M.B., Tezel, G., Saito, I., Gupta, R.S., Harley, J.B., Li, Z. & Romano, C. (1998b). Anti-Ro/SS-A positivity and heat shock protein antibodies in patients with normal-pressure glaucoma. *American Journal of Ophthalmology*, 125, 145–157, ISSN 0002-9394
- Wax, M.B., Yang, J. & Tezel, G. (2001). Serum autoantibodies in patients with glaucoma. *Journal of Glaucoma*, 10, S22–24, ISSN 1057-0829
- Westendorp, R. G., Langermans, J.A., Huizinga, T.W., Elouali, A.H., Verweij, C.L., Boomsma, D.I. & Vandenbroucke, J.P. (1997). Genetic influence on cytokine production and fatal meningococcal disease. *Lancet*, 349, 170–173, ISSN 0140-6736
- Wilson, A.G., Di Giovine, F.S., Blakemore, A.I.F. & Duff, G.W. (1992). Single base polymorphism in the human tumor necrosis factor alpha (TNF- α) gene detectable by NcoI restriction of PCR product. *Human Molecular Genetics*, 1, 353, ISSN 0964-6906
- Wilson, A.G., Gordon, C., di Giovine, F.S., de Vries, N., van de Putte, L.B., Emery, P. & Duff, G.W. (1994). A genetic association between systemic lupus erythematosus and tumor necrosis factor alpha. *European Journal of Immunology*, 24, 191–195, ISSN 0014-2980
- Wilson, A.G., Symons, J.A., McDowell, T.L., McDevitt, H.O. & Duff, G.W. (1997). Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 3195–3099, ISSN-0027-8424
- Wong, T.Y., Loon, S.C., Saw, S.M. (2006). The epidemiology of age related eye diseases in Asia. *British Journal of Ophthalmology*, 90, 506–511, ISSN 0007-1161
- Yan, X., Tezel, G., Wax, M.B. & Edward, P. (2000). Matrix metalloproteinases and tumor necrosis factor alpha in glaucomatous optic nerve head. *Archives of Ophthalmology*, 118, 666–673, ISSN 0003-9950
- Yang, J., Patil, R.V., Yu H., Gordon, M. & Wax, M.B. (2001a). T cell subsets and sIL-2R/IL-2 levels in patients with glaucoma. *American Journal of Ophthalmology*, 131, 421–426, ISSN 0002-9394
- Yang, J., Tezel, G., Patil, R.V., Romano, C. & Wax, M.B. (2001b). Serum autoantibody against glutathione S-transferase in patients with glaucoma. *Investigative Ophthalmology & Visual Science*, 42, 1273–1276, ISSN 0146-0404
- Yoshioka, K., Yoshida, T., Takakura, Y., Umekawa, T., Kogure, A., Toda, H. & Yoshikawa, T. (2006). Relationship between polymorphisms 804C/A and 252A/G of lymphotoxin-alpha gene and -308G/A of tumor necrosis factor alpha gene and diabetic retinopathy in Japanese patients with type 2 diabetes mellitus. *Metabolism*, 55, 1406–1410, ISSN 0026-0494
- Yuan, L. & Neufeld, A.H. (2000). Tumor necrosis factor-alpha: a potentially neurodestructive cytokine produced by glia in the human glaucomatous optic nerve head. *Glia*, 32, 41–50, ISSN 1098-1136
- Yuan, L. & Neufeld, A.H. (2001). Activated microglia in the human glaucomatous optic nerve head. *Journal of Neuroscience Research*, 64, 523–532, ISSN 0360-4012
- Zelano, G., Lino, M.M., Evoli, A., Settesoldi, D., Batocchi, A.P., Torrente, I. & Tonali, P.A. (1998). Tumor necrosis factor beta gene polymorphism in myasthenia Gravis. *European Journal of Immunogenetics*, 25, 403–408, ISSN 0960-7420

Zorena, K., Mysliwska, J., Mysliwec, M., Balcerska, A., Hak, L., Lipowski, P., & Raczynska K. (2007). Serum TNF-alpha level predicts non-proliferative diabetic retinopathy in children. *Mediators of Inflammation*, (92196), 1-5, ISSN 0962-9351

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