



# Investigation of the association between interleukin-1 $\beta$ polymorphism and normal tension glaucoma

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**Purpose:** In normal tension glaucoma (NTG), factors other than elevated intraocular pressure are likely to have a role in the pathogenesis of optic neuropathy. The potential similarities in cellular apoptosis leading to neurodegeneration between Alzheimer's disease and NTG were shown in recent studies. The interleukin-1 $\beta$  (IL-1 $\beta$ ; -511) and IL-1 $\beta$  (+3953) polymorphisms were found to increase risk with Alzheimer's disease. The purpose of this study was to test the hypothesis that the IL-1 $\beta$  polymorphism is associated with NTG in the Chinese population.

**Methods:** This is a cohort study in a Chinese population that involved 231 people with NTG and 245 healthy controls. Genomic DNA was amplified by a polymerase chain reaction, followed by the enzymatic restriction fragment length polymorphism technique. Patients and controls were genotyped for the C/T polymorphism at position -511 and +3953 of the IL-1 $\beta$  gene. Genotypes for NTG and control groups were compared for statistically significant differences.

**Results:** There was no significant difference in genotype frequency or allele frequency distribution of the IL-1 $\beta$  gene polymorphisms (position -511 and +3953) between NTG patients and the control group ( $p > 0.3$ ).

**Conclusions:** Our study showed no evidence for an association between the IL-1 $\beta$  (-511) and IL-1 $\beta$  (+3953) polymorphisms and NTG. The IL-1 $\beta$  gene polymorphisms (position -511 and +3953) may not play a key role in NTG pathogenesis in Chinese population.

Glaucoma is a degenerative optic neuropathy characterized by loss of retinal ganglion cells, cupping of the optic nerve head, and visual field defects often related to elevated intraocular pressure. The disease affects approximately 70 million people worldwide and is the second most common cause of blindness [1]. Factors other than IOP are likely to have a role in the pathogenesis of glaucomatous optic neuropathy, particularly in individuals with normal tension glaucoma (NTG). NTG is a subtype of primary open angle glaucoma (POAG) and accounts for one-third of all cases of POAG [2]. Patients with NTG show IOP measurements within the statistically normal range, and these patients usually present late in life after a visual field defect has occurred. A genetic approach is needed to identify those at risk of developing NTG.

Recent laboratory evidence shows a connection between abnormal autoimmunity and NTG, suggesting NTG may be a glaucomatous condition affected by antibodies damaging retinal tissue and inducing apoptosis. Wax et al. [3] found deposition of IgG, IgA, and retinal antibodies in the retinal ganglion cell of NTG patients. Fellman et al. [4] noted that patients with NTG and rheumatoid disease have a high level of serum antibodies to rhodopsin and heat shock protein 60.

Shinji et al. [5] found interleukin-1 $\beta$  (IL-1 $\beta$ ) plays an important role in mediating ischemic and excitotoxic damage in the retina in glaucoma. Proinflammatory cytokines, such as IL-1, as well as other indicators of microglial activation, have been suggested as drivers of neuropathological changes in several neurodegenerative conditions.

Vickers et al. [6] presented evidence that the neuronal pathology of Alzheimer's disease contributes to an aberrant regenerative response of nerve cells triggered by the gradual compression and physical damage to axons within beta-amyloid plaques that form in the brain. Glaucoma may be a chronic neurodegeneration like Alzheimer's disease, because similar evidence also indicates there is beta-amyloid build-up in retinal ganglion cells in rats with experimental glaucoma [7,8]. In this regard, glaucoma may be viewed as a chronic neurodegenerative disease similar to Alzheimer's disease, and a slow build up of beta-amyloid in the ganglion cell may eventually trigger cell death and optic nerve axon loss. There is evidence that the IL-1 protein may act to promote the development of beta-amyloid deposits [9-12].

Researchers have noted several polymorphic regions in the IL-1 $\beta$  gene. A C/T polymorphism at position -511 of the IL-1 $\beta$  gene in the promoter region has been reported to be associated with Alzheimer's disease, with the IL-1 $\beta$ (-511) T allele polymorphism found to increase the risk for late-onset Alzheimer's disease [13-15]. The other polymorphism in IL-1 $\beta$  (at position +3953 in exon 5) has shown an association with the risk for Alzheimer's disease [16].

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NTG may be a chronic neurodegeneration like Alzheimer's disease. Given the potential similarities in cellular events leading to neurodegeneration between Alzheimer's disease and glaucoma, we hypothesized that the IL-1 $\beta$  (-511) and IL-1 $\beta$  (+3953) polymorphisms, because of the effect on IL-1 protein expression, may predispose affected individuals to glaucoma. We therefore sought to investigate the distribution of IL-1 $\beta$  (-511) and IL-1 $\beta$  (+3953) polymorphisms in NTG patients and compare them with a healthy control population.

## METHODS

**Subjects:** Subjects were recruited at the outpatient clinic in the Department of Ophthalmology at the Veterans General Hospital, Taichung, Taiwan from January 2004 to February 2007. NTG patients were approached as they visited the clinic for previously scheduled visits and were enrolled after consenting to participate in the study. Normal control subjects were recruited during their visits to the outpatient clinic for various other reasons. Written informed consent was obtained from all study subjects prior to enrollment. The study was carried out with the approval of the Human Study Committee of the Veterans General Hospital.

All participants received comprehensive ophthalmologic examinations including visual acuity testing with refraction, IOP measurement, Humphrey 30-2, slit lamp examination, and dilated slit lamp stereobiomicroscopy. Comprehensive ophthalmologic history and longitudinal follow data were also obtained for each individual. The definition for NTG included the presence of typical glaucomatous optic neuropathy with compatible visual field defects (arcuate, Bjerrum, Seidel and/or paracentral scotoma and/or nasal step on Humphrey 30-2), open anterior chamber angle, and absence of any contributing ocular or systemic disorders. Patients with NTG had untreated IOP measurements that were consistently 21 mmHg or lower on diurnal test and at follow-up.

Unrelated control subjects were recruited from clinic patients who were seeking treatment for senile cataract, floater, refractive errors, or itchy eye. All normal control subjects had no systemic disease and no family history of glaucoma. They were excluded from glaucoma using the same criteria of diagnosis as the NTG patients after the same ophthalmic examination procedure.

**DNA preparation and genotype identification:** Blood samples were collected from each subject (5 ml) and genomic DNA was isolated using the Qiagen QiaAmp Blood mini kit (Qiagen, Valencia, CA). IL-1 $\beta$  C(-511)T and C(+3953)T genotyping of genomic DNA were determined with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. A 304 bp PCR fragment of the IL-1 $\beta$  (-511) in the promoter region was amplified using the following primers: F5'-TGG CAT TGA TCT GGT TCA TC-3' and R5'-GTT TAG GAA TCT TCC CAC TT-3'. PCR conditions were as follows: a denaturing step of 95 °C for 10 min, then 35 cycles of 95 °C for 45 s, 60 °C for 45 s, 72 °C for 1 min, and a final incubation at 72 °C for 5 min. The products were digested with *Bsu*36I (New England Biolabs, Inc., Beverly, MA) at 37 °C for 3 h and were run on ethidium bro-

mid-stained 2% agarose gel. This gave products that either remained intact (C allele) or were cut into two fragments of 190 and 114 bp (T allele).

The polymorphic region containing the *Taq*I (New England Biolabs, Inc.) restriction site at position +3953 within exon 5 of the IL-1 $\beta$  gene was amplified using the following primers: F5'-GTT GTC ATC AGA CTT TGA CC-3' and 5'-TTC AGT TCA TAT GGA CCA GA-3'. The PCR conditions were the same as described in the previous. The products were digested with *Taq*I at 65 °C for 3 h. *Taq*I digestions of the 249 bp fragments were cut into two fragment of 135 and 114 bp (allele C) or remained intact (allele T).

**Statistical analysis:** Genotype and allele frequencies between the control and NTG groups were compared using the chi-square test and Fisher's exact test, respectively. Age and gender were compared between the control and NTG groups using the Student's t-test and Fisher's exact test, respectively. Odds ratios were computed to assess the strength of association between the presence of each genotype and the clinical diagnosis of NTG. A p value of less than 0.05 was defined to be of statistical significance. All statistical analyses were performed using SPSS 10.0 (SPSS Inc., Chicago, IL). The power calculation was carried out according to the method by Schlesselman [17].

## RESULTS

The study consisted of 231 NTG patients (127 men, 104 women) and 245 normal controls (135 men, 110 women). The mean age was 70 years for the NTG patients (range 31-85) and 71 years for the controls (range 30-85). There was no difference between the control and NTG groups in age ( $p > 0.05$ , t-test) and gender ( $p > 0.05$ , Fisher's exact test). The mean and standard deviation of the maximum IOP was  $17.5 \pm 2.1$  mmHg for the NTG patients and  $16.9 \pm 2.3$  mmHg for the control sub-

**TABLE 1. GENOTYPE AND ALLELE FREQUENCIES OF INTERLEUKIN-1 $\beta$  (-511) AND INTERLEUKIN-1 $\beta$  (+3953)**

Genotype	NTG (%) n= 231	Control (%) n=245	$\chi^2$	p-value
<b>IL-1<math>\beta</math> (-511)</b>				
Genotype				
C/C	70 (30.3%)	61 (24.9%)	1.77	0.4
C/T	108 (46.8%)	125 (51.0%)		
T/T	53 (22.9%)	59 (24.1%)		
Allele				
C	248 (53.7%)	247 (50.4%)	1.02	0.3
T	214 (46.3%)	243 (49.6%)		
<b>IL-1<math>\beta</math> (+3953)</b>				
Genotype				
C/C	214 (93.8%)	226 (92.2%)	0.03	0.98
C/T	16 (6.9%)	18 (7.3%)		
T/T	1 (0.4%)	1 (0.4%)		
Allele				
C	444 (96.1%)	470 (95.9%)	0.02	0.88
T	18 (3.9%)	20 (4.1%)		

There was no statistically significant difference in genotype frequency (GF) or allele frequency (AF) distribution of the two polymorphisms between NTG and control subjects. The breakdown was as follows: For IL-1 $\beta$  -511, the AF was  $p = 0.3$  and the GF was  $p = 0.4$ ; for IL-1 $\beta$  +3953, the AF was  $p = 0.88$  and the GF was  $p = 0.98$ .

jects. No deviations from Hardy-Weinberg equilibrium could be seen in NTG patients and control studies.

The genotype and allele frequencies of the IL-1 $\beta$  (-511) and IL-1 $\beta$  (+3953) polymorphism in NTG and control subjects are presented in Table 1. There was no statistically significant difference in genotype frequency (GF) or allele frequency (AF) distribution of the two polymorphisms between NTG and control subjects. (IL-1 $\beta$  -511 AF:  $p=0.3$ , GF:  $p=0.4$ ; IL-1 $\beta$  +3953 AF:  $p=0.88$ , GF:  $p=0.98$ )

The statistical power over 80% suggested that the probability of detecting a difference could be believable in this sample size.

## DISCUSSION

Mutations in three genes (myocilin, optineurin, and WDR36) have been implicated in NTG [18-22]. Mutation in the optineurin gene was initially reported in 16.7% of families with hereditary primary open angle glaucoma (POAG), with most of them having NTG [19]. Aung et al. [23] and Powell et al. [24] reported that NTG demonstrates an association with polymorphisms of the OPA1 gene on chromosome 3, which is responsible for dominant optic atrophy in the Caucasian population [25]. Many more important gene variants have recently been associated with glaucoma risk, such as apolipoprotein E (*APOE*) [26,27], endothelin receptor type A gene (*EDNRA*) [28,29], IL-1 $\alpha$  [30], methylenetetrahydrofolate reductase (*MTHFR*) [31,32], and beta-adrenergic receptors [33]. However, these genes cannot interpret the overall inheritance susceptibility of NTG pathogenesis. The other associations involved in the development of NTG should be further investigated.

Recent evidence indicates a close link between chronic neurodegenerative disease and IL-1 $\beta$  expression. Polymorphism in the IL-1 gene clusters have been shown in myasthenia gravis, multiple sclerosis, Parkinson's disease, temporal lobe epilepsy with hippocampal sclerosis and Alzheimer's disease [34-38]. NTG may be a chronic neurodegeneration like Alzheimer's disease, considering the potential similarities in cellular events leading to neurodegeneration between Alzheimer's disease and glaucoma. There is evidence that IL-1 $\beta$  upregulates the production and processing of  $\beta$ -amyloid protein in neurons [39] as well as the expression and activity of acetylcholine esterase [40]. Furthermore, it has been shown that IL-1 $\beta$  activated microglia selectively killed cholinergic neurons in Alzheimer's patients [41]. The death of retinal ganglion cells in glaucoma involving chronic  $\beta$ -amyloid neurotoxicity mimics Alzheimer's disease at the molecular level [7]. Our study was designed to see whether patients with NTG had a higher risk of developing the disease as a result of their IL-1 $\beta$  genotype.

NTG has been considered a subtype of POAG, sharing many similar characteristics. However, some studies show a connection between abnormal autoimmunity and NTG, suggesting the disease is affected more by antibodies damaging retinal ganglion cells and inducing apoptosis [42]. Lin et al. [43] found that IL-1 $\beta$  (+3953) T allele was significantly more common in POAG patients than in control subjects in a Chi-

nese population. However, there was no association between the IL-1 $\beta$  (position -511 and +3953) polymorphisms and NTG in our study. The dissimilar findings revealed that the pathogenesis and effect of IL-1 $\beta$  may be different between NTG and POAG. IL-1 $\beta$  may not have a significant role in the pathogenesis of the optic neuropathy.

Genes coding for the two isoforms of IL-1 (IL-1 $\alpha$  and IL-1 $\beta$ ) and for the IL-1 receptor antagonist (IL-1RA) are located within the IL1 gene cluster at chromosomal locus 2q13 [44]. Polymorphisms within this gene cluster have been associated with a large variety of human diseases [45]. Some of these polymorphisms have been shown to alter the amount of IL-1 produced [46-49]. These allelic variants are known to alter function. The C/T polymorphism at position -511 in the promoter region of IL-1 $\beta$  regulates the production of IL-1 $\beta$  protein and in vitro synthetic capacity of C/C genotype carriers are lower than that of C/T of T/T carriers [50]. The homozygosity for the IL-1 $\beta$  (+3953T) allele has been associated with a fourfold increase in the production of IL-1 $\beta$  when compared to homozygosity for IL-1 $\beta$  (+3953C) allele [47].

Lack of association between IL-1 $\beta$  genetic polymorphism and NTG was observed in our study of a Chinese population. In our control group, the allele frequencies of the IL-1 $\beta$  (-511) were similar to those previously reported in other controls [43,51,52]. The frequency of IL-1 $\beta$  (-511) C allele and IL-1 $\beta$  (+3953) T allele were significantly lower among Chinese populations compared to Caucasians [53]. The observed negative association could be due to the low frequency of the IL-1 $\beta$  (-511) C allele and IL-1 $\beta$  (+3953) T allele in a Chinese population. Further studies with NTG cohort of different ethnic background are required to further define this association. We cannot exclude the possibility of IL-1 $\beta$  (-511) and IL-1 $\beta$  (+3953) polymorphism being associated with NTG in different ethnic population or the likelihood of an association between NTG and another IL-1 $\beta$  polymorphism. The lack of an association of the common polymorphisms of the IL-1 $\beta$  with NTG in Chinese population suggests this factor may not have a significant role in the pathogenesis of the optic neuropathy.

In conclusion, we found no significant associations between polymorphisms in the IL-1 $\beta$  (-511 and +3953) and NTG in a Chinese population. However, the possibility of other mutations or sequence change in the IL-1 $\beta$  gene cannot be excluded. Further genetic studies of NTG are necessary to investigate the development of the neurodegenerative process.

## ACKNOWLEDGEMENTS

This work was supported by grants from the Taichung Veterans General Hospital and Tunghai University (TCVGH-T-947802) Taichung, Taiwan, Republic of China. We express our sincere thanks to Dr. M. Elizabeth Fini at Bascom Palmer Eye Institute, University of Miami Miller School of Medicine for helpful comments during the preparation of this manuscript.

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