Normal Tension Glaucoma is not Associated With the Interleukin -1α (− 889) Genetic Polymorphism

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Background: Factors other than intraocular pressure are likely to play a role in the pathogenesis of glaucomatous optic neuropathy, particularly in individuals with normal tension glaucoma (NTG). Recent laboratory evidence has shown that there are potential similarities between Alzheimer disease and NTG in cellular apoptosis leading to neurodegeneration. IL-1α (− 889) T allele polymorphism has been found to increase the risk of developing Alzheimer disease. The aim of this study was to test in a Chinese cohort the hypothesis that IL-1α (− 889) polymorphism is associated with NTG.

Methods: One hundred sixty-two unrelated patients with NTG were recruited and compared with 167 controls in a Chinese population. Genomic DNA was amplified by polymerase chain reaction, followed by enzymatic restriction fragment length polymorphism technique. Patients and controls were genotyped for the C/T polymorphism at position − 889 of the IL-1α gene promoter region.

Results: There was no significant difference in the frequency of IL-1α (− 889) alleles or genotypes in the NTG population compared with that in the control group.

Conclusions: We conclude that C/T polymorphism at position − 889 of the IL-1α gene promoter region does not increase the risk of developing NTG. However, further studies on NTG are necessary to investigate the genetic basis and factors involved in the development of the neurodegenerative process.

Key Words: intraocular pressure (IOP), optic neuropathy, normal tension glaucoma (NTG), Alzheimer disease, interleukin -1α (− 889) genetic polymorphism

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Glaucoma is a progressive optic neuropathy characterized by optic nerve degeneration and visual field defect often related to elevated intraocular pressure (IOP). The disease affects over 67 million people worldwide and is the second largest cause of blindness.[1] 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). NTG is a subtype of primary open angle glaucoma (POAG) and accounts for approximately 20% to 50% of all POAG.[2] Patients with NTG have IOPs that are within the statistically normal range and the disease usually presents late in life when visual field defects have already occurred. For early diagnosis of NTG, a genetic approach to the identification of risk factors seems very promising.

Vickers et al[3] have presented evidence that the neuronal pathology of Alzheimer disease may be attributable to an aberrant regenerative response of nerve cells triggered by the gradual compression and physical damage to axons within β-amyloid plaques that form in the brain. Similar evidence also indicates that there is β-amyloid build-up in retinal ganglion cells in rats with experimental glaucoma.[4,5] In this regard, glaucoma may be viewed as a chronic neurodegenerative disease similar to Alzheimer disease, and a slow buildup of β-amyloid in the ganglion cells may eventually trigger cell death and optic nerve axon loss. There is also evidence that the interleukin 1α (IL-1α) protein may act to promote the development of β-amyloid deposits.[6–9]

A C/T polymorphism at position − 889 of the IL-1α gene promoter region has been reported to be associated with Alzheimer disease, and the IL-1α (− 889) T allele polymorphism has been found to increase the risk of developing Alzheimer disease.[10–12] In the IL-1α gene, the IL-1α (− 889) T allele has been shown to increase the protein level with respect to the IL-1α (− 889) C allele.[13]

Given the potential similarities in cellular events leading to neurodegeneration between Alzheimer disease and glaucoma, we hypothesized that the IL-1α (− 889) polymorphism may be a genetic factor predisposing affected individuals to glaucoma due to its effect on IL-1 protein expression. We, therefore, decided to investigate the distribution of the IL-1α (− 889) polymorphism in patients with NTG and compare them with a healthy control population.

METHODS

Subjects

Written informed consent was obtained from all study subjects before enrollment. The study was conducted with the approval of the Human Study Committee of Taichung Veterans General Hospital. Subjects were recruited from the outpatient clinic in the Department of Ophthalmology at Veterans General Hospital, Taichung, Taiwan from January 2004 to January 2006. NTG patients were invited to participate in the study when they came to the clinic for previously scheduled visits, and were enrolled after consent. Normal control subjects were recruited during their visits to the outpatient clinic for various other reasons.

All participants received comprehensive ophthalmologic examinations including visual acuity testing with refraction, IOP measurement (Goldmann applanation tonometer), visual field test (Humphrey 30-2), slit-lamp examination, and dilated slit-lamp stereo biomicroscopy. Comprehensive ophthalmologic history and longitudinal follow-up data were also obtained for each individual. The definition of NTG included characteristic arcuate, Bjerrum, Seidel, and/or paracentral scotoma and/or nasal step on Humphrey 30-2 with reference to Anderson's[14] criteria for minimal abnormality in glaucoma; corresponding cupping of optic nerve heads and/or nerve fiber layer defects on stereo biomicroscopy; open anterior chamber angles on gonioscope; and absence of a secondary cause for glaucomatous optic neuropathy such as a previously raised IOP after trauma, a period of steroid administration, or uveitis were required. Patients also did not have evidence of high myopia or congenital ocular abnormality, and had no other cause than glaucoma for disc changes and visual field loss. Patients with NTG had IOPs without treatments that were consistently 21 mm Hg or less on diurnal test and follow-up.

Unrelated control subjects were recruited from those attending the clinic for condition of senile cataract, floater, refractive errors, or itchy eye. Normal control subjects had no systemic disease and no family history of glaucoma. They were diagnosed as nonglaucomatous using the same criteria as those for 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 a Quiagen QiaAmp Blood mini kit (Quiagen, Valencia, CA). IL 1α C (−889) T genotyping of genomic DNA was determined with the polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) technique on blood samples obtained for routine clinical work according to standard procedures.[15] PCR fragments of the IL-1α promoter region (−889) were amplified using the following primers: 5′-GCATGCCATCACACCTAGTT-3′ and 5′-TTACATATGAGCCTTCCATG-3′ as upstream and downstream primers, respectively. These primers amplified the region of IL-1α from −1062 to −869 [194 base pairs (bp)]. The PCR products were digested with *Nco*I (New England Biolabs, Inc, Beverly, MA) overnight at 37°C and were run on ethidium bromide-stained 6% polyacrylamide gels for 45 minutes at 200 V and detected under UV light. The IL-1α (−889) C allele showed 2 fragments (1 fragment of 178 bp and one of 16 bp), and the IL-1α (−889) T allele showed an intact fragment of 194 bp (Fig. 1). We sequenced the 5′ regulatory region (containing the promoter) of the IL-1α gene in selected individuals with each genotype (TT, CT, and CC) to confirm the validity of the PCR-RFLP assay. The sequences of IL-1α from −899 to −879 were CCAGGCAACA(C or T)CATTGAAGGC. Complete matching results of the genotype were obtained. To ensure accuracy, we performed each test 3 times for each sample.

Statistical Analysis

The Hardy-Weinberg equilibrium for each polymorphism sample was tested by χ^2 test. Genotype and allele frequencies between the control and NTG groups were compared using the χ^2 test and Fisher exact test, respectively. Age and sex were compared between the control and NTG groups using the Student *t* test and Fisher exact test, respectively. Odds ratios were computed to assess the strength of association between each genotype and the clinical diagnosis of NTG. A *P* value of less than 0.05 was defined as statistically significant. All statistical analyses were performed using SPSS

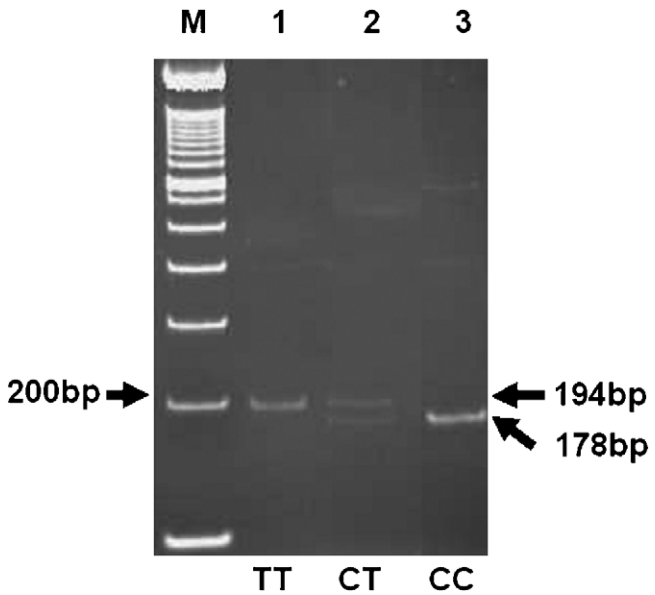

FIGURE 1. Results of studies of polymorphism including *Nco*I digestion of PCR products and analysis by 6% acrylamide electrophoresis. Lane M: DNA ladder 100 bp, lane 1: IL-1α (−889) T/T homozygote; lane 2: IL-1α (−889) T/C heterozygote; and lane 3: IL-1α (−889) T/T homozygote. The T allele is an undigested single band: 194 bp and the C allele has 2 fragments: 178 and 16 bp.

TABLE 1. Genotype and Allele Frequencies of IL-1α (−889)

	NTG (%) n = 162	Control (%) n = 167	χ^2	*P*
Genotype				
C/C	118 (73%)	125 (75%)		
C/T	41 (25%)	40 (24%)	0.34	0.84
T/T	3 (2%)	2 (1%)		
Allele				
C	277 (86%)	290 (87%)	0.24	0.62
T	47 (15%)	44 (13%)		

There was no significant difference in the IL-1α genotype ($P = 0.84$) or allele ($P = 0.62$) frequencies between the NTG group and controls. The frequencies of the IL-1α (−889) T allele were no higher in NTG patients than in normal controls ($P = 0.65$).

10.0 (SPSS, Inc, Chicago, IL). The power calculation was performed according to the method devised by Schlesselman.[16]

RESULTS

In our study, 162 patients with NTG and 167 normal controls were enrolled. The mean age was 69 years for the NTG patients (range 32 to 84) and 70 years for the controls (range 30 to 85). There was no difference between the control and NTG groups in age ($P > 0.05$, *t* test) or sex ($P > 0.05$, Fisher exact test).

The genotype and allele frequencies of the IL-1α polymorphism at position −889 of the promoter region in NTG and control subjects are presented in Table 1. There was no significant difference in the IL-1α genotype ($P = 0.84$) or allele ($P = 0.62$) frequencies between the NTG group and controls. Moreover, the frequencies of the IL-1α (−889) T allele were no higher in NTG patients than in normal controls ($P = 0.65$). The distribution of genotypes in the population of patients and controls was consistent with the Hardy-Weinberg equilibrium, with no significant detectable differences between the expected and the observed numbers.

DISCUSSION

Factors other than IOP are likely to have a role in the pathogenesis of glaucomatous optic neuropathy, particularly in individuals with NTG.

There is also evidence that the IL-1α protein may act to promote the development of β-amyloid deposits in Alzheimer patients.[6–9] The death of retinal ganglion cells in glaucoma involving chronic amyloid-β neurotoxicity mimics Alzheimer disease at the molecular level. Our study was designed to determine whether patients with NTG had a higher risk of developing the disease as a result of their ILα-1 genotype.

In a case-control study, Cartwright et al[17] found that 30% of patients with NTG had at least one immune-related disease. They speculated that immunopathogens may inflict damage and that NTG might be an autoimmune disease.[17] IL-1 has been shown to play an important role in mediating ischemic and excitotoxic damage in the retina.[18] The association between the degree of immune responses and the development of glaucomatous optic nerve degeneration may be fluid, and in certain cases may result in retinal ganglion cell death through an aberrant immune signaling process.

Contribution of the genetic polymorphisms to NTG has not been studied extensively, and it is likely that multiple genes may be associated with the disease. Mutations in myocilin and optineurin genes have been implicated in NTG,[19,20] and mutation in the optineurin gene was initially reported in 16.7% of families with hereditary POAG, most of whom had NTG.[19] Recently, Aung et al[21] reported that NTG is associated with polymorphisms of the OPA-1 gene on chromosome 3, which is responsible for dominant optic atrophy. However, these 3 genes cannot interpret the overall inheritance susceptibility of NTG pathogenesis. Other associations involved in the development of NTG await further investigation.

In this study of a Chinese population, no association between IL-1α (−889) genetic polymorphism and NTG was observed. The allele frequency in our control population was different from that observed in white populations.[10–12] Specifically, the frequency of the IL-1α (−889) T allele was lower in our population. IL-1α (−889) C/T polymorphism has been reported to be associated with Alzheimer disease, and the IL-1α (−889) T allele polymorphism has been found to increase the risk of developing Alzheimer disease in whites.[10–12] However, no association between the IL-1α (−889) T allele polymorphism and Alzheimer disease was noted in Chinese[22,23] or Korean[24] populations. The IL-1α (−889) T allele is rarer in Asians than in whites. This phenomenon may be due to ethnic difference, which may lead to controversial results. Although there have been several studies on IL-1α (−889) polymorphism and Alzheimer disease, further studies with NTG cohorts of different ethnic backgrounds are needed to confirm this association. The lack of an association of the common polymorphisms of the IL-1α (−889) locus with NTG in the Chinese population in our study suggests that this factor may not have a significant role in the pathogenesis of optic neuropathy.

Occasionally, the lack of association may be a false-negative result due to a type II error and low statistical power of the sample. In our study, complete ocular examinations were performed on patients with NTG and controls. The statistical power over 80% suggests that the probability of detecting a difference can be believed.

CONCLUSIONS

In conclusion, we found no significant associations between polymorphisms in the IL-1α (−889) locus and NTG in a Chinese population. However, the possibility of other mutations or sequence changes in the IL-1α gene cannot be excluded in Chinese NTG patients. Further studies on NTG are necessary to investigate the genetic basis and factors involved in the development of the neurodegenerative process.

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