Journal of Clinical Laboratory Analysis 17:259–263 (2003)

Insulin-Like Growth Factor-II Gene Polymorphism Is Associated With Primary Open Angle Glaucoma

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Hypoxia and ischemia play important roles in the onset and progression of glaucoma. Insulin-like growth factors (IGF) are important neurotrophic agents that respond to hypoxia-ischemia. In this study, we enrolled 60 primary open angle glaucoma (POAG) patients and 104 healthy volunteers from the China Medical College Hospital. Among the polymorphism of IGFs gene, exon 9 *Apa* I C/T gene polymorphism is the most frequently seen. The polymorphism was observed following polymerase chain reaction based restriction analysis used to to resolve the relationship between IGF-II exon 9 *Apa* I C/T gene polymorphism and POAG. The distribution of the IGF-II exon 9 gene polymorphism showed statistical differences in the distribution of genotype frequencies between POAG patients and normal controls (P=0.010). The odds ratio of C/C homozygote was 0.266 (95% confidence interval=0.636 ~ 0.111). IGF-II is an important neurotrophic agent and regulates the suffering of POAG. C/C homozygote of IGF-II exon 9 *Apa* I C/T gene polymorphism is a useful marker of POAG in Chinese. J. Clin. Lab. Anal. 17:259–263, 2003.

Key words: exon; hypoxia; ischemia; neurotrophic; nitric oxide

INTRODUCTION

It has been noted that insulin-like growth factors (IGF) have the ability to limit neuronal damage elicited by experimental hypoxia-ischemia conditions, which suggests an ability of IGFs to limit free radical generation (1–3). IGFs promote neurite outgrowth and are transported backward from muscle to nerve cell body in vivo (1,3–5). Pharmacological depletion of endogenous target-derived IGFs in vivo reduces neuron survival by up to 30% (4). According to the neurotrophic hypothesis (4,6), developmental neuron death results from the competition between neurons that limit the amount of neurotrophic support present in the target. The importance of target-derived trophic support for the survival of developing neurons is also well known (1,4–6). Trophic factors may be derived from various compartments in the neuron's environment, such as: glial cells (7); systemic circulation (8); and the vicinity of the nucleus (9,10). IGFs is one of the important neuron trophic factors and administration of IGFs increases nerve cell survival in vivo during naturally occurring neuron death (4,5,11). IGFs mRNA also increase during some tissue differentiation (11,12).

IGF-II gene expression is higher in brain and spinal cord than in other tissues of the adult rat (12) and is closely correlated with the development of neural synapses (13). The local administration of IGFs increases the regeneration rate of motor and sensory nerves in rats, whereas anti-IGFs antiserum decreases the regeneration rate. Physiological concentrations of these ligands can enhance neurite formation by acting directly on cultured sensory (14), sympathetic (15), and motor neurons (15).

Moreover, microtubules composed of α- and β-tubulins are major components of the axonal cytoskeleton. IGFs can increase α- and α-tubulin gene expression in a cloned cell line as a consequence of increased stability of transcripts during neurite growth

Grant sponsor: China Medical University Hospital.

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Received 12 June 2003; Accepted 20 June 2003
DOI 10.1002/jcla.10106
Published online in Wiley InterScience (www.interscience.wiley.com).

(16), indicating that a biochemical pathway leading to neurite formation is activated (17).

IGFs promote survival in some cell lines through two distinguishable PI3-kinase-regulated pathways that culminate in expression of the cyclin-dependent kinase inhibitor, p21. In previous studies, incubation with IGFs or transfection with active PI3-kinase led to rapid induction of MyoD and p21, and forced expression of protein maintained viability in the absence of growth factors (18). The endogenous or exogenous IGF-II was also cytoprotective against cytokine-induced cell apoptosis (2–5). Additionally, in the pancreas, an increase in inducible nitric oxide synthesis (iNOS) within β-cells may involve an induction of islet cell apoptosis. IGF-I can inhibit iNOS induction in some tissue (2) and interfere with cytokine-stimulated nitric oxide (NO) synthesis.

Glaucoma is a complex disease. Both mechanical and vascular hypotheses have been proposed as the mechanism. Regardless of the mechanism, the ganglion cells ultimately die, which represents the final common pathway of glaucomatous vision loss (19). Several studies have demonstrated that retinal ganglion cells die during the course of POAG by a form of cell death known as apoptosis (19, 20, 21). Many ideas have been proposed to resolve this optic nerve death and design a treatment for POAG, but there is still no definite conclusion for a POAG treatment. IGF-II is one of many important nerve trophic factors. IGF-II is also closely related to nerve death during apoptosis and has the ability to interfere with cytokine-stimulated NO, which is also suspected to be an important agent in the glaucomatous neuropathy. In IGF-II, the most commonly noted polymorphism is the exon 9 gene polymorphism. We investigated the relationship between the IGF-II exon 9 gene polymorphism and POAG.

MATERIALS AND METHODS

From the department of ophthalmology at the China Medical University Hospital, we enrolled POAG patients that were seen from May to July 2000. All patients in this study received serial ophthalmic examinations including intraocular pressure (IOP), visual acuity, automated perimetry, gonioscopy, optic disc examination, and retinal examination. The volunteers in the control group were examined by the same ophthalmologist. Volunteers suspected of having glaucoma were excluded from the study. Patients with ocular diseases other than POAG were excluded from this study. Patients included in this study were POAG patients who fulfilled one of the criteria from both the visual field and the optic disc categories listed below.

The visual field criteria were:1) at least two abnormal visual field tests by Humphrey automated perimetry, as defined by computer-based objective criteria; and 2) the presence of one or more absolute defects in the central visual field 30°, with ophthalmologic interpretation as glaucomatous visual field loss.

The optic disc criteria (optic disc damage present in fundus photographs) were: 1) either a horizontal or vertical cup-to-disc ratio of 0.6 or more; and 2) narrowest remaining neuroretinal rim was 20% or fewer disc diameters.

Patients with disc and field changes other than POAG were excluded.

In this study, we investigated the IGF-II polymorphism in all subjects. The prevalence of the polymorphism was compared between the control group and patient groups. Odds ratios were used to calculate the frequencies of the different alleles. This study was carried out with the approval of the Human Study Committee of the China Medical College Hospital. Informed consent was obtained from all patients who participated in this study.

The primer for IGF-II gene exon 9 was 5′-CCACAAGGCAATGAGATAACA-3′) and (5′-AGGGTAAGCAGCAAGAGAGC-3′). PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer, Foster City, CA,). The PCR product of 169-bp was mixed with two units *Apa* I (New England Biolabs, Beverly, MA); two fragments of 102-bp and 67-bp will be present on 3% agarose gel electrophoresis if the product is digestible. We compared the statistical analysis of the allelic frequency distribution in this polymorphism in the control and POAG patient groups. Results were considered statistically significant when the probability of findings occurring by chance was less than 5% ($P < 0.05$).

RESULTS

In this study, 104 healthy volunteers and 60 patients with POAG were enrolled. The POAG patients ranged in age from 20–70 years old (mean: 55 years) and were unrelated. There were 30 females and 30 males. The volunteers ranged in age from 52 to 71 years old (mean: 50 years), and were free from any ophthalmic diseases. The volunteers were all Chinese and not related to each other. There were 52 females and 52 males. All patients were followed up between 2 and 8 years, with an average follow-up period of 5 years. Ten of the patients received trabeculectomy and two of these 10 patients received trabeculectomy twice (at different sites). Fifty patients in the POAG group controlled intraocular pressure with topical drugs. Each patient used an average of 1.3 types

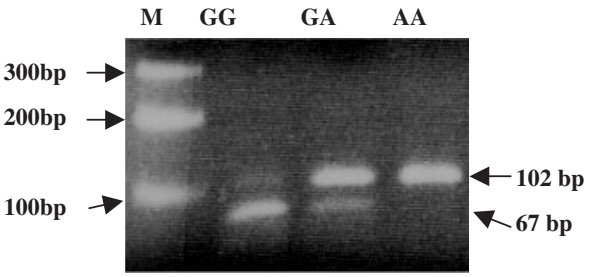

Fig. 1. The PCR products of IGF-II exon 9 gene polymorphism present on 3% agarose gel electrophoresis. M: marker. Line 2: "C/C" homozygote, two digestable bands were 102-bp and 67-bp on the gel. Line 3:"C/T" heterozygote. Line 4: "C/T" heterozygote, "T" was an indigestible site and the fragment was 102-bp.

of antiglaucomatous drugs. Nine patients did not require drugs to control IOP following trabeculectomy.

If the product can be digested, two bands will be presented on the gel and reveal two fragments of 102-bp and 67-bp. The polymorphism was divided into digestible (G/G homozygote), indigestible (A/A homozygote), and G/A heterozygote (Fig. 1). The frequencies of the genotypes in the POAG group and the control group are shown in Table 1. The allelic frequency of "A" and "G" was 49.2% and 50.8% in the control group, respectively and 52.5% and 47.5% in the POAG group, respectively (Table 2). The distribution of the IGF-II exon 9 gene polymorphism was compared using the chi-square test and the result showed statistical differences in the distribution of genotype frequencies between POAG patients and normal controls ($P<0.01$).

The odds ratio was also significant different between two groups when comparing the frequency of GG and GA genotype. The odds ratio of G/G homozygote is 0.266 (95% confidence interval = 0.636 ~ 0.111). This means that individuals with G/G homozygote have 3.7 (1/0.266 = 3.7) times greater chance than G/A heterozygote of suffering from POAG. The odds ratio was still significant when analyzed by methods of regression according to age (Table 3), which means that age does not influence the result. We also calculated "power" for a test of the null hypothesis by the Statistical Package for Social Science (SPSS, Chicago, IL). There is a power of 81% to yield a statistically significant result in this sample size.

DISCUSSION

The pathophysiology of POAG is not precisely known. POAG is a multifactorial disease (20–22) and the assumption that a single gene is responsible is not reasonable. POAG may be the result of multiple and interactive genetic and environmental effects. There is a well-recognized increased riskthat family members of

TABLE 1. The distribution of IGF II polymorphism in healthy control subjects and POAG patients

	GG	GA	AA	Total
POAG	18 (30.0%)	21 (35.0%)	78 (35.0%)	60 (100%)
Control	13 (12.5%)	57 (49.5%)	34 (34.9%)	104 (100%)
Total	31 (18.9%)	78 (47.6%)	55 (33.5%)	164 (100%)

χ^2 test; $P=0.010$.

TABLE 2. The allelic frequencies in healthy subjects and POAG patients

	A	G
POAG group	63 (52.5)	57 (47.5)
Control group	59 (49.2)	61 (50.8)

TABLE 3. Age-adjusted tests for the genotypes of IGF II gene polymorphism

	Odds ratio (95% CI)[a]	
Genotype	Unadjusted	Age-adjusted
AA	1.7 (0.8–3.5)	1.8 (0.8–3.7)
GA	1	1
GG	3.8*(1.6–9.0)	4.1*(1.7–10.2)

[a]CI denotes confidence interval.
*$P<0.05$.

patients with POAG will also develop the disease. Currently, there is a lack of information regarding the genetics of the disease, and, thus the molecular biology of glaucoma is currently under close investigation.

Studies of ganglion cell death in animal and human models have demonstrated many similarities to the morphological changes seen in apoptosis, such as chromatin condensation, formation of apoptotic bodies, and DNA fragmentation (20,23). POAG is death of optic nerves and the death of glaucomatous optic nerves has been proven to be by apoptosis (21). IGFs regulate peripheral nerve regeneration, and IGFs are neurotrophic factors that have recently been implicated in the pathogenesis of some neuropathies. It has been noted that the action of IGF-II doesnot act in a cell cycle-specific manner, but instead acts by recruiting quiescent cells into G1 (24). In one study, the programmed cell death induced by serum deprivation has been shown to be reversible by simultaneous addition of IGF-II (25).

Furthermore, members of the IGFs axis have unique temporal and spatial mRNA and protein expression patterns in embryos from different vertebrate species, indicating that these genes have distinct and tightly regulated functions in the development of specific tissues. These expression patterns often colocalize with known areas of apoptosis. IGF-II is also a strong

candidate for therapeutic intervention in dystrophinopathies. IGF-II ameliorates the dystrophic phenotype and coordinately down-regulates programmed cell death. Action of IGFs is also modified by IGF binding proteins (IGFBPs), a family of secreted proteins that bind both IGF-I and IGF-II with high affinity. In addition, a number of studies have indicated that the IGF-II receptor, a single-chain transmembrane glycoprotein also known as the cation-independent mannose δ-phosphate receptor that is involved in transport of lysosomal enzymes (8, 18, 26), modulates IGF-II action by removing the growth factor from the extracellular environment. Endogenous IGF-II functions as a critical survival factor during the transition from proliferation to the differentiation in some cells.

Overexpression of IGF-II has also been observed in a wide variety of other malignancies (27). Apoptosis in N-myc2-expressing αML cells is strongly inhibited by IGF-II. IGF-II gene expression is also strongly up-regulated during hepatic carcinogenesis in virally infected animals and has been speculated to be part of an autocrine growth-stimulatory pathway.

In this study, IGF-II proved a useful genetic marker for POAG. Because glaucomatous optic neuropathy is a type of apoptosis and IGF-II is closely related to the pathway of apoptosis and nerve protection, we suspected that IGF-II plays a role in the regulation of apoptosis in the optic neuron. The GG genotype of IGF-II may change the apoptosis state in the optic neurons when confronted with stresses, such as the increase in intraocular pressure, the ischemic sign, and toxic amino acid (e.g., glutamate). Nevertheless, we do not intend to say that IGF-II is a direct cause but we are suggesting an association between the two. This means that other factors must be added to determine the result and the exact role of the GG type IGF-II gene may be resolved by proteinmonics in the future. Single nucleotide polymorphisms (SNP) have important implications in human genetic studies. The screening for such alleles helps in the detection of a genetic predisposition to disease. The presence of a specific SNP allele can be implicated as a causative factor of a genetic disorder. Furthermore, understanding the associated polymorphism is expected to increase the understanding of the course of disease. Our studies are ongoing in order to determine the relationship between SNPs and glaucoma. Knowing the genetic pathway of apoptosis is important for designing new treatments for glaucoma.

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