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Polymorphism of Insulin-Like Growth Factor-II Gene in Primary Open Angle Glaucoma and its Effect on Treatment

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Abstract: Different genetic loci reported to contribute to the susceptibility of eyes to primary open angle glaucoma/normal transient glaucoma have been identified and at least 15 loci, from juvenile open angle glaucoma gene to glaucoma 1 open angle gene, have been linked to primary open angle glaucoma. Thus, it is reasonable to examine the association between primary open angle glaucoma and insulin like growth factor II gene polymorphisms and to investigate effect of polymorphism on the type of treatment. The present study included 80 cases with primary open angle glaucoma and 40 age and sex-matched controls. Cases subdivided into two equal groups: The first included 40 cases that were medically controlled and the second included 40 cases that were surgically treated. All subjects had full ophthalmologic examination and were investigated for IGF-II polymorphism. GG was reported in 10, 7.5 and 45% in subgroup A, B and control groups, respectively; while GA was reported in 50.0, 75.0 and 32.5% in the same order and finally AA was reported in 40, 35.0 and 22.5% in the same order; with significant increase of GG and decrease of both GA and AA in control group when compared to either of study subgroups A or B. At the same time, there was no significant difference between subgroups A or B as regard Genotyping. The odds ratio was significantly different between study and control groups. The odds ratio of G/G homozygote is 0.31 (95% CI = 0.12-0.76). IGF-II gene polymorphism is associated with POAG. The most reasonable mechanism may be linked to oxidative stress. No effect of IGF-II polymorphism on treatment was observed.

Key words: Insulin like growth factor, polymorphism, open angle glaucoma

INTRODUCTION

Glaucoma is a complex disease, as it comprises a group of heterogeneous optic neuropathies characterized by a progressive degeneration of the optic nerve head and subsequent visual field defects. It was estimated to affects 70 million people and is considered the second leading cause of blindness worldwide. By the year 2020, it is estimated that, this number would rise to around 79.6 million (Quigley and Broman, 2006). Glaucoma starts gradually and as central vision is usually not lost until the disease is advanced, a significant number of individuals remain either undiagnosed or undertreated. Most common forms of glaucoma are age-related, starting in midlife and progress slowly. Early detection of glaucoma can slow disease progression with drug and/or surgical treatment (Fan and Wiggs, 2010).

The common clinical types of glaucoma include primary open-angle (POAG) and primary Angle-closure Glaucoma (PACG). The POAG is characterized by obstruction of the aqueous humour pathway (leading to optic disc cupping and visual field loss) due to trabecular meshwork degeneration. Obstruction prevents aqueous humour exit with subsequent increase of IOP which is often thought to damage the optic nerve (Sihota *et al*., 2008). Elevated IOP alone cannot explain the pathophysiology of POAG and the disease is multifactorial. Anatomical risk factors for the diagnosis of the PACG include shallowness and the narrow angle of the anterior chamber (Bonomi *et al*., 2000). Typically, adult-onset POAG is inherited in a multifactorial or complex fashion. It is estimated that family history of glaucoma accounts for a one-to ten-fold risk among the first-degree relatives of an affected individual (Green *et al*., 2007). It is well known that the risk for developing a multifactorial disease is greatly influenced by the occurrence of allelic polymorphisms in a variety of genes (Buentello-Volante *et al*., 2013).

Different genetic loci reported to contribute to the susceptibility of eyes to POAG/NTG have been identified

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and at least 15 loci, from GLC1A to GLC1O, have been linked to POAG (Shields, 2011). Worldwide, three genes have been identified: The myocilin (MYOC) gene (Stone *et al*., 1997), the optineurin (OPTN) gene (Rezaie *et al*., 2002) and the WD repeat domain 36 (WDR36) gene (Monemi *et al*., 2005). In addition to the candidate genes, many other genes have been proposed to be associated with POAG.

Insulin/IGF resistance and oxidative stress promote cell loss and neurodegeneration (De La Monte and Wands, 2005). In addition, insulin/IGF have important roles in regulating myelin maintenance in both peripheral and central nervous systems (D'Ercole and Ye, 2008; Chesik *et al*., 2008). In the brain, oligodendrocytes maintain myelin via insulin/IGF signaling. Similarly, in the peripheral nervous system, Schwann cells utilize IGF signalling for myelinogenesis and myelin maintenance (Pillion *et al*., 1992; Ogata *et al*., 2006). Pharmacological depletion of endogenous target-derived IGFs *in vivo* reduces neuron survival upto 30% (Stewart and Rotwein, 1996). Thus, we hypothesized that, insulin like factors polymorphism are associated with development of primary open angle glaucoma.

The purpose of this study is to present the results of the association between POAG and insulin like growth factor II gene polymorphisms in a sample of Egyptian population) and to investigate if there is an effect of polymorphism on type of treatment.

According to researchers best of knowledge studies evaluating Insulin-like growth factor gene polymorphism in the field of ophthalmology are scarce, although many studies linked IGF-I and II polymorphism to development of many disease process, e.g., Diabetes (McCann *et al*., 2004), hepatocellular carcinoma (Tang *et al*., 2006), esophageal adenocarcinoma (Zhao *et al*., 2009). In addition, it is the first time to investigate the effect of polymorphism on treatment in such cases.

MATERIALS AND METHODS

After approval of the study protocol by the Local Ethical Committee and obtaining patients consent, adult-onset POAG patients and control subjects were recruited at Ophthalmology Department Benha University in the period from January 2012 to July 2013. The present study included 80 cases presented with POAG and 40 age and sex-matched controls cases were subdivided into two equal groups according to provided treatment: The first subgroup included 40 cases who were medically controlled and the second subgroup included 40 cases who failed medical control and were surgically treated.

Inclusion criteria:

- The inclusion criteria for the case group were initial IOP (before treatment) above 21 mm Hg, suggestive of glaucoma
- The inclusion criteria for the control group were IOP below 21 mm Hg, open anterior chamber angle, optic nerve and visual fields without abnormalities suggestive of glaucoma
- The visual field criteria were:
  - At least two abnormal visual field tests by Humphrey automated perimetry, as defined by computer-based objective criteria
  - The presence of one or more absolute defects in the central visual field 301, with ophthalmologic interpretation as glaucomatous visual field loss
- The optic disc criteria (optic disc damage present in fundus photographs) were:
  - Either a horizontal or vertical cup-to-disc ratio of 0.6 or more
  - Narrowest remaining neuroretinal rim was 20% or fewer disc diameters

Exclusion criteria:

- Patients with history of surgery, uveitis, trauma or secondary glaucoma
- Patients with malignant or autoimmune diseases
- Patients with disc and field changes other than POAG

All subjects had full ophthalmologic examination and were investigated for IGF-II polymorphism. The prevalence of the polymorphism was compared between the control group and patient subgroups.

DNA was extracted from whole blood samples using the QIAamp® DNA minikit (Qiagen, USA) following the manufacturer's instructions. The primer for IGF-II gene exon 9 was (5' - CACAAGGCAATGAGATAACA-3') and (5'-AGGGTAAGCAGCAAGAGAGC-3'). The PCR amplification was done by a programmable thermal cycler GeneAmp PCR System MyGeneTM Series Gradient (Thermal Cycler Model MG96G, Long Gene Scientific Instrument Co., Ltd). The PCR product of 169 bp was mixed with two units Apa I; two fragments of 102 and 67 bp were present on 3% agarose gel electrophoresis if the product is digestible and results were as the following: GG: Homozygote, two digestible bands (67 bp); GA: Heterozygote (102 and 67 bp) and AA: Homozygote, two indigestible fragment was 102 bp.

Statistical analysis: The collected data was organized, tabulated and statistically analyzed using Statistical Package for Social Sciences (SPSS) version 20 (IBM, SPSS Inc, USA). Data was summarized for quantitative data using the mean, standard deviation and range while proportion was used for qualitative variables. The difference between two means was statistically analyzed using the students (t) test and for comparison between more than two means, the one way analysis of variance (ANOVA) test was used. For qualitative data the Chi square (χ^2) square was used as a test of significance. Significance was adopted at $p<0.05$ for interpretation of results.

RESULTS

In the present study, age ranged from 44-69 years and there was no-significant difference between subgroup A, B or control groups (58.37±3.98, 58.65±3.42 or 28.00±5.53 years, respectively). Males represent 60% of subgroup A, 65% of subgroup B and 75% of the control group, with no significant difference. Diabetes was significantly increased in subgroups A and B (45 and 50%, respectively) in comparison to control group (22.5%) but there was no significant difference between medically and surgically treated subgroups. On the other hand, both hypertension and smoking showed non-significant distribution in studied groups (Table 1).

As regard genotyping, GG was reported in 10, 7.5 and 45% in subgroup A, B and control groups, respectively; while GA was reported in 50.0, 75.0 and 32.5% in subgroup A, B and control groups, respectively and finally AA was reported in 40, 35.0 and 22.5% in subgroup A, B and control groups, respectively; with significant increase of GG and decrease of both GA and AA in control group when compared to either of study subgroups A or B. At the same time, there was no significant difference between subgroups A or B as regard Genotyping (Table 2). The odds ratio was significantly different between study and control groups when comparing the frequency of GG and GA genotype. The odds ratio of G/G homozygote is 0.31 (95% confidence interval = 0.12-0.76). This means that individuals with G/G homozygote have 3.23 time greater chance than G/A heterozygote of subjects suffering from POAG. On the other hand GA/AA gene polymorphism was statistically insignificant (Table 3).

Table 1: Comparison between study and control group regarding to general characteristics

	Study group							
	Subgroup (A)		Subgroup (B)		Control group			
Characteristics	No.	%	No.	%	No.	%	Test	p-value
Age (Mean±SD; range)	58.37±3.98 (49-68)		58.65±3.42 (49-65)		58.00±5.53 (44-69)		0.21	0.80 NS
Gender (n,%)								
Male	24	60.0	26	65.0	30	5.0	2.10	0.35 NS
Female	16	40.0	14	35.0	10	25.0		
Diabetes	18	45.0	20	50.0	9.0	22.5	7.20	0.027*
Hypertension	16	40.0	17	42.5	19	47.5	0.47	0.78 NS
Smoking	8	20.0	10	25.0	9.0	22.5	0.28	0.86 NS

NS: Not significant, *Significant at $p<0.05$

Table 2: Comparison between study and control group regarding to IGF-II gene polymorphism

	Study group							
	Subgroup (A)		Subgroup (B)		Control group			
Genotyping	No.	%	No.	%	No.	%	Test	p-value
GG	4	10.0	3	7.5	18	45.0	21.70	<0.001*
GA	20	50.0	23	57.5	13	32.5		
AA	16	40.0	14	35.0	9	22.5		

*Significant at <0.05

Table 3: Odds ratio for gene polymorphism in study group (A and B subgroups)

Gene polymorphism	Odds ratio	95% CI	p-value
GG/GA	0.31	0.12-0.76	0.002*
GA/AA	0.96	0.64-1.15	0.79 (NS)

DISCUSSION

In last years, association studies have suggested many POAG-related genes with conflicting results either in single or multiple studies. For example, it was reported that, many patients with adult-onset POAG have affected family members, making model-independent and model-dependent linkage approaches to gene identification possible. Model-dependent linkage analyses using multiplex POAG pedigrees have yielded a number of potential POAG loci (GLC1A-GLC1H and GLC1L). Optineurin (OPTN) at GLC1E (10p15-p14) (Rezaie *et al*., 2002), although initially described as a POAG-causative gene, is primarily responsible for rare cases of familial Normal Transient Glaucoma (NTG), a type of open-angle glaucoma in which the optic nerve deteriorates despite normal IOP (Hauser *et al*., 2006).

The variations in association may be explained by racial differences, sample size, poorly characterized controls, or clinical heterogeneity between different populations (Rao *et al*., 2011). A recent review reported that a total of 27 POAG-related genes have been identified in association studies between 2005-2010 (Fuse, 2010).

The molecular changes responsible for glaucoma currently poorly understood, complicating the design of therapies based on the underlying disease mechanisms. However, most forms of glaucoma are inherited, either as common complex traits or as Mendelian autosomal dominant or recessive traits (Fan and Wiggs, 2010).

The purpose of this study is to present the results of the association between POAG and insulin like growth factor II gene polymorphisms in a sample of Egyptian population and for the first time to explore if this polymorphism can affect type of treatment of POAG.

According to researchers best of knowledge studies evaluating Insulin-like Growth Factor gene polymorphism in the field of ophthalmology are scarce, although many studies linke IGF-I and II polymorphism to development of many disease processes, e.g., Diabetes (McCann *et al*., 2004), hepatocellular carcinoma (Tang *et al*., 2006), esophageal adenocarcinoma (Zhao *et al*., 2009), cardiovascular diseases (Vaessen *et al*., 2001) and Alzheimer's disease (Messier and Teutenberg, 2005), age related macular degeneration (Chiu *et al*., 2011). All of which share the risk factors with POAG (Chiu *et al*., 2011). In short, results of the present study showed a difference between cases with POAG and control subjects as regard IGF-II gene polymorphism; but no effect of polymorphism on treatment type can be elicited.

We were able to identify a sole work by Tsai *et al*. (2003) who included 104 healthy volunteers and 60 patients with POAG to study IGF-II Polymorphism in POAG. The POAG patients ranged in age from 20-70 years old (mean: 55 years). There were 30 females and 30 males. The volunteers ranged in age from 52-71 years old (mean: 50 years) and were free from any ophthalmic diseases. This distribution of age and gender is quietly different from ours and may be attributed to different sample size or racial differences. In Tsai *et al*. (2003) study, the frequencies of the genotypes in the POAG group and the control group showed statistically significant difference (in glaucoma group, it was 30.0, 35.0 and 35.0% for GG, GA and GA while in control group, it was 12.5, 49.5 and 34.9% in the same order). The odds ratio was also significantly 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. These results are comparable to that of the present study.

They explained their results by linking IGF-II gene polymorphism to hypoxia and ischemia insults that play important roles in the onset and progression of glaucoma. Supporting this explanation, it had been reported that, increased markers of oxidative stress including carbonyls in proteins, lipid oxidation products and oxidized DNA bases have been reported in glaucoma (Sacca *et al*., 2005). Reactive Oxygen Species (ROS) might also cause glaucoma vascular complications through the mitochondrial dysfunction, formation of Advanced Glycation End-products (AGEs), pseudohypoxia, altered growth factor activity or dyslipoproteinemia. Moreover, decreased levels of antioxidants have been associated with glaucoma (Izzotti *et al*., 2006), suggesting that oxidative stress might play a causal role in this disease (Majsterek *et al*., 2011).

It has been demonstrated that primary antioxidant enzymes such as superoxide dismutase (SOD, scavenges superoxide anions), catalase (CAT, detoxifies hydrogen peroxide) and glutathione peroxidase (GPX, removes hydrogen peroxide and lipids peroxides) had an altered activity in POAG (Ghanem *et al*., 2010).

In another study, Chiu *et al*. (2011) were able to link genetic Polymorphisms of Insulin-like Growth Factor Axis Genes and Risk for Age-Related Macular Degeneration (AMD). They reported that, the single nucleotide polymorphism (SNP) rs2872060 in IGFR was significantly associated with the risk for advanced AMD and the association remained significant after stratification by the two types of the disease: Neovascularization and geographic atrophy. This study adds to the evidence that, IGF polymorphism played an important role in peripheral neuropathy and eye disease.

CONCLUSION

In short, results of the present study added to the evidence that IGF-II gene polymorphism is associated with primary open angle glaucoma. The most reasonable mechanism may be linked to oxidative stress that needed to be further confirmed in subsequent studies. In addition, it showed no effect of IGF-II gene polymorphism on treatment type.

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