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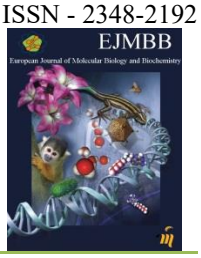

POLYMORPHISM RS12997 IN THE 3' UNTRANSLATED REGION OF ACTIVIN A RECEPTOR TYPE 1 GENE IS ASSOCIATED WITH NORMAL-TENSION GLAUCOMA

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ABSTRACT

Aim of the study: To determine association of rs12997 (A>G) and rs1043784 (T>C) located in the 3'untranslated region (UTR) region of *ACVR1* and *BMP6* genes, respectively, in normal-tension glaucoma (NTG) patients of Saudi origin. Materials and Methods: In a case-control study, rs12997 and rs1043784 genotyping was performed in 250 non-glaucoma controls and 102 NTG patients using the TaqMan real-time PCR assays. Statistical analysis was performed to evaluate any allelic/genotype association of polymorphisms with the disease or its related clinical markers. Results: Rs12997[G] allele frequency was significantly (p=0.035) higher in cases (0.42) than controls (0.33). The rs12997 genotypes showed significant association with NTG in co-dominant, recessive, and log-additive models. The rs12997 G/G genotype exhibited a significant two-fold increased risk of NTG independent of age, sex, and rs1043784 genotype in binary logistic regression analysis (odds ratio=2.12, 95% confidence interval=1.05–4.25, p=0.034). Rs1043784 polymorphism showed no significant association with NTG. Both the polymorphisms showed no association with intraocular pressure, cup/disc ratio, and number of antiglaucoma medications. Conclusion: Rs12997 polymorphism in the 3'UTR region of *ACVR1* is significantly associated with NTG in a Saudi cohort. However, further studies are needed in a large population-based cohort, potentially with age and gender-matched controls, and in different ethnicities to validate these findings.

INTRODUCTION

The role of members of the transforming growth factor-β (TGF-β) superfamily of growth factors in glaucoma pathogenesis is well documented [1]. The TGF-β family also includes bone morphogenetic proteins (BMPs), activins, and other signaling molecules. Activin A receptor type I (*ACVR1*), also known as ALK2, is a member of the BMP signaling pathway that encodes a BMP type I receptor and is linked to a wide variety of pathologies, including glaucoma [2].

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BMP6 is an AVCR1 ligand and plays a significant role in the pathogenesis of age-related macular degeneration, retinal pigment epithelial cells protection against oxidative damage, and apoptosis [3]. Similar to TGF-β cytokine, signaling by BMP ligands involves the interaction of type I and type II transmembrane serine/threonine kinase receptors via SMAD1/5/8 (canonical) or p38/JNK/MAP kinase (non-canonical) pathways [4]. Besides, these molecules are abundantly expressed in the human eye tissues [5].

Our group has previously investigated the association of polymorphisms rs12997 (A>G) and rs1043784 (T>C) located in the 3'untranslated region (UTR) region of *ACVR1* and *BMP6* genes, respectively,

and for the first time reported a significant association of rs12997 polymorphism in primary open-angle (POAG), angle-closure, and pseudo exfoliation glaucoma [6, 7]. This evidence strongly supports a general role for this genetic variant in TGF-β/BMP signaling-related glaucoma pathogenesis. Considering this shared etiology, and recognizing that IOP elevation is absent in NTG, we hypothesize that this polymorphism may contribute to NTG pathogenesis through a mechanism independent of elevated IOP. Therefore, using an independent cohort of Saudi NTG patients in this study we examined the association of the *ACVR1* rs12997 (A>G) and *BMP6* rs1043784 (T>C) polymorphisms with NTG susceptibility.

MATERIALS AND METHODS

Study design and Ethical considerations:

We performed a retrospective case-control genetic association study. The study was approved by our Institutional Review Board/Ethics Committee and adhered to the tenets of the Declaration of Helsinki for human research (protocol number #09–657). Written informed consents were obtained from all the participating individuals.

Study cohort:

Participants of Saudi origin were recruited from the glaucoma clinic of our University Hospital. The NTG patients (n = 102) had adult-onset primary open-angle glaucoma with normal intraocular pressure (IOP) (less than 21 mmHg measured by Goldmann tonometry) in the presence of visual field loss and/or glaucomatous optic disc changes were included in this study. Ethnicity-matched controls of Saudi origin (n=250) were greater than 40 years of age, with normal IOP (<21 mmHg), healthy optic disc (cup/disc <0.5), open angles on gonioscopy, and free from any form of glaucoma on examination.

Genotyping of rs12997 and rs1043784:

DNA extracted from whole EDTA blood using QIAamp DNA Blood Mini Kit (Cat. No. 51306, Qiagen, Hilden, Germany) were subjected to genotyping using the commercially available real-time PCR-based TaqMan® SNP genotyping assays, ID# C___7545093_10 and C___2064624_20, respectively (Catalog number: 4351379, Applied Biosystems Inc., Foster City, CA, USA) under the recommended PCR conditions on ABI 7500 (Applied Biosystems Inc.) as described previously [8].

Statistical analysis:

Statistical analysis was performed using SPSS version 22 (IBM Inc., Chicago, Illinois, USA) and SNP Stats online software (https://www.snpstats.net/start.htm). Pearson's Chi-square analysis and Fisher's test (where applicable) were used to test Hardy-Weinberg Equilibrium (HWE), gender distribution, allele and genotype associations. Normality testing of continuous variables was performed and accordingly group comparisons were done using the Mann-Whitney U test (2-groups comparison) and Kruskal-Walli's test (3-groups comparison). Regression analysis was performed to test the effects of multiple factors (age, sex, genotypes) on glaucoma outcome. A $p<0.05$ (2-tailed) was considered significant. Bonferroni's correction was used to adjust for multiple testing and corrected p-value was considered where applicable.

RESULTS

Age and gender distribution among the study groups were non-significant ($p>0.05$) (**Table 1**). The control group showed no significant deviation from Hardy Weinberg Equilibrium ($p>0.05$). As shown in Table 1, the minor rs12997[G] allele frequency was 0.33 and 0.42 in controls and cases, respectively, and significantly different ($p=0.035$); whereas, rs1043784[C] allele frequency distribution was non-significant ($p=0.920$) in cases (0.18) and controls (0.15).

The *ACVR1* rs12997 genotypes showed significant association with NTG in co-dominant, recessive, and log-additive models. No significant association was observed for *BMP6* rs1043784 genotypes (**Table 2**).

The rs12997 G/G genotype exhibited a significant two-fold increased risk of NTG independent of age, sex, and rs1043784 genotype in binary logistic regression analysis (odds ratio=2.12, 95% confidence interval=1.05–4.25, $p=0.034$) (**Table 3**). However, clinical parameters such as intraocular pressure, cup/disc ratio, and the number of antiglaucoma medications, markers of disease severity, showed no significant genotype association with both variants.

Table 1: Demographic characteristics and distribution of allele frequencies of rs12997 in *ACVR1* and rs1043784 in *BMP6* genes in normal-tension glaucoma and controls.

Characteristics	NTG (n=150)	Controls (n=250)	Odds ratio	95% confidence interval	*p*-value
Age in years (SD)	57.9 (12.8)	59.8 (11.5)	-	-	0.056
Male/Female, n	51/51	136/114	1.01	0.67 – 1.52	0.454
Allele Frequency					
Rs12997 (*ACVR1*)					
A	0.58	0.67	1	Reference	**-**
G	0.42	0.33	**1.43**	**1.02 – 2.00**	**0.035**
Rs1043784 (*BMP6*)					

T	0.82	0.85	1	Reference	-
C	0.18	0.15	1.21	0.78 – 1.87	0.383

Significant odds ratio and p-value in bold.
NTG, normal-tension glaucoma.

Table 2: Genotype analysis of polymorphisms rs12997 in *ACVR1* and rs1043784 in *BMP6* under different genetic models.

SNP ID / Model	Genotype	Subjects		Odds ratio (95% confidence interval)	*p*-value
		Controls, n (%)	NTG, n (%)		
rs12997					
Co-dominant	A/A	110 (44.4)	41 (40.2)	1.00	**0.012**†
	A/G	111 (44.8)	37 (36.3)	0.89 (0.53-1.50)	
	G/G	27 (10.9)	24 (23.5)	**2.38 (1.24-4.60)***	
Dominant	A/A	110 (44.4)	41 (40.2)	1.00	0.470
	A/G-G/G	138 (55.6)	61 (59.8)	1.19 (0.74-1.89)	
Recessive	A/A-A/G	221 (89.1)	78 (76.5)	1.00	**0.003**‡
	G/G	27 (10.9)	24 (23.5)	**2.52 (1.37-4.62)**	
Log-additive	---	---	---	**1.40 (1.01-1.93)**	**0.044**
rs1043784					
Co-dominant	T/T	184 (73.6)	73 (71.6)	1.00	0.440
	C/T	57 (22.8)	22 (21.6)	0.97 (0.55-1.71)	
	C/C	9 (3.6)	7 (6.9)	1.96 (0.70-5.46)	
Dominant	T/T	184 (73.6)	73 (71.6)	1.00	0.700
	C/T-C/C	66 (26.4)	29 (28.4)	1.11 (0.66-1.85)	
Recessive	T/T-C/T	241 (96.4)	95 (93.1)	1.00	0.200
	C/C	9 (3.6)	7 (6.9)	1.97 (0.71-5.45)	
Log-additive	---	---	---	1.18 (0.79-1.77)	0.420

*** A/A vs. G/G p-value=0.0085**
† p-value=0.032 adjusted for age and sex
‡ p-value=0.010 adjusted for age and sex
NTG, normal-tension glaucoma.
Significant odds ratio and p-value in bold. Bonferroni corrected p-value is 0.01.

Table 3: Regression analysis to determine the effect of age, sex, and polymorphisms on the risk of normal-tension glaucoma

Group Variables	B	SE	Wald	Odds ratio (95% confidence interval)	*p*-value
Age	0.020	0.013	2.415	0.98 (0.95 – 1.00)	0.120
Sex	-0.185	0.248	0.554	0.83 (0.51 – 1.35)	0.457
rs12997	---	---	6.467	---	**0.039**
A/G	-0.140	0.273	0.264	0.87 (0.50 – 1.48)	0.607
G/G	0.752	0.355	4.480	**2.12 (1.05 – 4.25)**	**0.034**
rs1043784	---	---	0.060	---	0.970
T/C	0.129	0.550	0.055	1.13 (0.38 – 3.34)	0.815
C/C	-0.013	0.306	0.002	0.98 (0.54 – 1.80)	0.966
Constant	-0.496	0.770	0.415	1.642	0.520

Significant odds ratio and p-value in bold.

DISCUSSION

Members of the complex TGF-β/BMP signaling pathway have been implicated in the maintenance of TM homeostasis and glaucoma pathogenesis [9]. Thus, it is likely that any alterations in the expression of genes involved in BMP signaling pathway as a result of genetic polymorphism(s), may have functional consequences and thereby influence the disease risk [4]. Accordingly, similar to the previously published findings in other forms of glaucoma [6, 7], NTG was significantly associated with rs12997 in *ACVR1* but not with rs1043784 in *BMP6*.

The MAF of rs12997[G] observed in our Saudi cohort (0.33 in controls) was comparable to the Europeans (0.28) and Asians (0.34), but different (lower) than the Africans and African American population (0.84) as noted in the Allele Frequency Aggregator (ALFA) database,

indicating ethnic variability. The exact molecular mechanism(s) by which the *ACVR1* variant may influence NTG risk is unknown. The causal role of *ACVR1* mutations has been extensively studied in fibrodysplasia ossificans progressiva (FOP), a rare genetic disease characterized by progressive heterotopic ossification [2, 10]. Interestingly, few FOP patients having *ACVR1* mutation were reported to exhibit childhood glaucoma [11], supporting its role in glaucoma. Vascular endothelial dysfunction is believed to be one of the mechanisms responsible for NTG development and progression [12]. Likewise, increased endothelin-1 (ET-1) levels and polymorphisms in this gene have been reported to be associated with NTG [12, 13]. ET-1 promotes vasoconstriction by interacting with its receptors [12]. Interestingly, ALK2 (*ACVR1*) was reported to be an essential receptor in ET-1 production, which was blocked by ALK2 (*ACVR1*) knockdown [14]. Since rs12997 is located in the 3'UTR region, *ACVR1* expression may be regulated by specific miRNAs, affecting mRNA stability. Thus, the mutant allele might exhibit increased expression of *ACVR1* and plausibly affect ET-1 production and the risk of NTG. Other potential mechanism for *ACVR1* in glaucoma may include trabecular meshwork modulation via regulation of Wnt signaling [15]. *ACVR1* has also been reported to function as a tumor suppressor gene in the mouse lens [16]. Further in-vitro and molecular studies are needed to support this hypothesis. Unlike *ACVR1*, no association was detected for rs1043784 in *BMP6* gene, suggesting that *BMP6* may not have a major role in glaucoma pathogenesis. However, the plausible role of other polymorphism(s) in *BMP6* cannot be ruled out.

In conclusion, our study reports a significant association of variant rs12997 in the 3UTR region of *ACVR1* with NTG in an independent Saudi cohort, validating the critical role of this polymorphism and TGF-β/BMP signaling pathway in glaucoma. The consistent association of rs12997 in *ACVR1* in NTG, and other types of glaucoma [6, 7] suggests an essential role that this variant/gene may have and the presence of a common underlying molecular mechanism in glaucoma pathogenesis, independent of IOP status, perhaps through its involvement in ET-1 production or miRNA-mediated expression regulation, providing novel pathogenetic insights into the IOP-independent factors involving this form of glaucoma. However, the study is limited by sample size and the lack of functional validation. Future in vitro mechanistic studies are essential to confirm the proposed role of *ACVR1* rs12997 polymorphism in NTG via ET-1 and miRNA/mRNA regulation, and large-scale, multi-ethnic cohorts must be investigated to validate this polymorphism as a strong genetic biomarker of NTG susceptibility across wider populations.

DECLARATIONS

Ethical statement:

The study adhered to the Declaration of Helsinki principles and was approved by the institutional review board committee at the College of Medicine, King Saud University, Riyadh, Saudi Arabia.

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Conflict of interests:

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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