ORIGINAL ARTICLE

Genetic Association of *AGT* Polymorphism in patients with Dilated Cardiomyopathy from Punjabi population of Pakistan

MUHAMMAD IKRAM ULLAH[1], NAZISH MEHMOOD AISHA[2], MUHAMMAD SHAKIL[2], *TAYYABA SAMREEN[3], ASMA IDREES[3], SHABBIR HUSSAIN[4], IRFAN SHAUKAT[5]*

[1]Department of Clinical Laboratory Sciences, Jouf University, Sakaka, Aljouf, Kingdom of Saudi Arabia.
[2]Department of Biochemistry, Services Institute of Medical Sciences (SIMS), Lahore, Pakistan.
[3]School of Allied Health Sciences, Punjab Institute of Cardiology, Lahore, Pakistan.
[4] Department of Biochemistry University of Health Sciences, Lahore, Pakistan
[5] Faculty of Medicine, University of Lorraine, Nancy, France.
Correspondence to Dr. Muhammad Ikram Ullah, E-mail: ikram.uhs12@gmail.com

ABSTRACT

Background: Dilated cardiomyopathy (DCM) is a myocardial disease due to ventricular dilatation and systolic dysfunction. The angiotensinogen (*AGT*) gene mediates the protein angiotensinogen which is further cleaved by different enzymes to produce active enzyme angiotensin-II. The protein affects the contractile activity and heart rate and can play role in hypertension pathogenicity.
Aim: To detect *AGT* gene polymorphisms in Dilated Cardiomyopathy patients from Punjabi population.
Methods: A retrospective case-control study was conducted on 35 patients diagnosed for DCM cardiomyopathy and 42 ethnically-matched healthy controls. Genotype of *AGT* was carried out in patients and controls by DNA sequencing following the PCR amplification of specific oligonucleotide sequence. The information collected from each patient was age, gender, hypertension, the age of onset, left ventricular ejection fraction (LVEF), left ventricular internal diameter end diastole (LVIDD) and smoking. The whole blood was collected for the genomic DNA extraction and DNA sequencing of the *AGT* gene was performed.
Results: The results demonstrated an association between SNP rs699 (235T>C) and the DCM patients (*P* <0.006) than the controls. On the other hand, there were no significant associations observed between rs4762 (174C>T) and rs11568053 (235G>A) and DCM in patients.
Conclusion: The results conclude that genetic polymorphism SNP rs699 (M235T) of the *AGT* gene might be a higher-risk allele to cause the dilated cardiomyopathy phenotype.
Keywords: Dilated cardiomyopathy, *Angiotensinogen* (*AGT*) gene, Single Nucleotide Polymorphism, Pakistan

INTRODUCTION

Dilated cardiomyopathy (DCM) is a myocardial abnormality in which ventricular dilatation and impaired function of systole have been documented[1]. In cardiomyopathy, the left ventricle (LV) is more severely affected resulting in failed heart, the DCM affected enlarged and weakened heart. It cannot proficiently pump the flow of blood from the ventricle due to a process called remodeling[2].

The prevalence rate of Dilated cardiomyopathy is variable from 1 in 2,500 to 7 in 100,000 of the worldwide[3,4]. The onset of DCM may occur at any stage of age from new born to adulthood and it generally affects the adults during the 4th to 6th decade of life[5]. The genetic predisposition of DCM disease is documented in approximately 40% of heart diseases[6].

The DCM associated genes are categorized into various classes which encode for synthesis of various cellular proteins like structural proteins, nuclear envelope, sarcomere proteins, unclassified and ion-channel proteins[7]. The renin-angiotensin-aldosterone-system (RAAS) has the regulatory activity in cardiac function, electrolyte balance and blood pressure regulation. It is also important mechanism to modify the expression level of left ventricular hypertrophy [8]. The human angiotensinogen (*AGT*) gene is involved in production of angiotensin II, recognized to affect the hypertrophy of cardiac myocytes[9].

Received on 17-09-2018
Accepted on 30-12-2018

For the *AGT* (OMIM: 106150), numerous single nucleotide polymorphisms (SNPs) alterations have been detected in the promoter, exons and introns of the gene. The *ATG* contains 5 exons and 4 introns spanning 12kb[10]. It is located on long arm of chromosome 1 at position 1q42.2. Previously, a study of African-American population proposed the involvement of the *AGT* haplotypes containing -217A, -532T, -793A and -1074T nucleotides that may increase the transcriptional activity of the *AGT* and contribute in human hypertension[11]. In another study, an association of the *AGT* M235T polymorphism with the hypertension and myocardial infarction has been demonstrated[12]. In Czech population, a genotype variation (M235T) of *AGT* has been associated to high risk of dilated cardiomyopathy with heart failure and with ischemic heart disease[13]. Also, a study of Canadian-Caucasians demonstrated the strong association of variant allele (*AGT* T235) of *AGT* with the risk of heart failure[14]. A report of Indian population documented significant variation of allele 235T DCM patients although association has not been established with either increase in LVED or decrease in LVEF[15]. Jakubiak et al. reported another M174 allele was more prevalent in heart failure patients than controls[14]. The association of T174 polymorphism of the *AGT* between the patients and controls of Indian population has been not found[15]. Tiret et al. reported an association between *AGT* gene T174M polymorphism and hypertension[16]. Mital et al. reported a significant association of rs11568053 (M235L) with DCM in the United States and Canadian population[17].

The objective of the current study was to investigate three different single nucleotide polymorphisms (SNPs) of *AGT* gene, -235 T>C and -235 G>A, -174C>T for connection with susceptibility and severity to Dilated Cardiomyopathy in a Punjabi population.

METHEDOLOGY

Ethical permission of this study was obtained from the Punjab Institute of Cardiology and University of Health Sciences, Lahore, Pakistan.

Subject selection and sample collection: Case-control study was performed between thirty five cases of DCM attending the OPD of Punjab Institute of Cardiology, Lahore, Pakistan and forty two ethnically matched healthy controls without any history for heart abnormality, hypertension and diabetes. The DCM patients who have ejection fraction (EF) less than of 40% were recruited for the study. Patients with other causes of DCM i.e., coronary artery disease, hypertrophy, and ischemia were excluded from this study.

A total 5 ml of whole blood was collected from all subjects in EDTA containing vacutainers for the DNA extraction and was kept at 4 till further use. The genomic DNA extraction was performed by the standard non-enzymatic salting out method[18] and was stored at -20 ℃ till further analysis.

Detection of Genetic polymorphism by DNA sequencing: The genotyping was performed by the PCR amplification of the genomic DNA using the primer sets of *AGT* as shown in Table. 1, followed by the cycle sequencing of purified DNA. For 25 µl PCR reaction, the mixture was prepared by adding DNA 1 µl, unique forward and reverse primer (10-20 pmol) each of 1 µl, nuclease-free water about 10 µl, and 4 µl of DMSO (Dimethyl sulfoxide) in 8 µl of ready to use 2X Master mix (WizPure, Korea South). The PCR conditions are described earlier[19]. The visualization of amplified products was done on 2% agarose gel and then the purification was carried out with ethanol-precipitation to remove the excess of primers and nucleotides. The purified PCR-products were then subjected to the cycle sequencing according to the instructions manual at the respective annealing temperatures and were directly sequenced on automated Genetic analyzer (Applied Biosystems, USA). The analysis of the sequencing data was carried out by BioEdit v6.1.

Statistical analysis: The data was entered and analysis was performed by SPSS version 21.0. The comparison of numerical variables between cases and controls was done by Student's t-test and the χ^2-test was applied to analyze comparison of the categorical variables. Frequencies of genotype and allele distribution in patients and controls were carried out by Pearson Chi-square test and Fischer exact test. Odds ratio and Confidence interval were specified accordingly. Analysis of results was considered statistically significance when the p-value was < 0.05.

RESULTS

In the present study, clinical history in 35 patients were examined including age, gender, onset of disease, EF, LVIDD, lipid profile, response to drug, smoking habit, hypertension and diabetes. Mean age of DCM patient was 49.70±12.99 (Mean±S.D), while age for the onset of disease was 43.59±14.10. On the basis of gender, frequency of DCM in male was higher (74.1%) as compared to the females. Demographic data and clinical features of patients are shown in table 2. Results of EF and LVIDD were highly significant in this report. The levels of serum HDL-Cholesterol and Tri-glycerides were considerably higher in DCM patients than controls as presented in table 3.

DNA sequencing was carried out for genotyping analysis. The frequency of CC genotype for SNP rs699 (-235 T>C) in DCM patients (40.7%) was higher as compared to control (8.6%) subjects (Fig. 1). The chi-square test of independence showed that genotype homozygous CC was significantly associated with DCM in patients (χ^2= 9.48, P = 0.006).

The frequency of CC allele for SNP rs4762 (-174 C>T) in DCM patients was 88.9% and in controls it was 94.3% (Fig. 2). The frequency of heterozygous CT genotype for SNP rs4762 (-174 C>T) in DCM patients and controls was 11.1% & 5.7% respectively. The chi-square test of independence showed that the genotype CT was not significantly associated with DCM in patients (χ^2= 1.33, P = 0.428)

The frequency of GA genotype for SNP rs11568053 in DCM patients and controls was 11.5% and 11.1% respectively (Figure 3). The frequency of GG genotype for *AGT* in DCM patients and controls was 88.5% and 88.9% respectively. The chi-square test of independence showed that genotype GA was not significantly associated with DCM in patients (χ^2= 2.094, P = 0.418). The results are summarized in table 4.

Table 1: Primers sequence of *the AGT* gene amplified in patients with DCM.

Primer sequence		**Amplicon (bo)**	**Annealing temperature**
AGT (T174 M)			
Forward	TACAGGCAATCCT GGGTGTTCCTTG	404	65
Reverse	AGCAGAGAGGTTT GCCTTACCTTG		
AGT (M235T)			
Forward	CAGGGTGCTGTCC ACACTGGACCCC	165	65
Reverse	CCGTTTGTGCAGG GCCTGGCTCTCT		

Table 2: Clinical parameters for DCM patients.

Parameters		n
Smoking (%)	Smokers	15 (44.4%)
	Non-smokers	20 (55.6%)
Alcohol (%)	Alcoholic	0 (0%)
	Non-alcoholic	35 (100%)
Diabetes (%)	Diabetic	13 (37%)
	Non-diabetic	22 (63%)
Hypertension	Hypertensive	26 (74.1%)
	Nonhypertensive	9 (25.9%)
Psychic Status	Normal	28 (81.5%)
	Anxiety	3 (7.4%)
	Depression	4 (11.1%)
Drug response	Yes	31 (88.9%)
	No	4 (11.1%)

Table 3: Comparison of lipid profile in DCM patients and controls.

Variales	n=35	Controls	P value
Cholesterol	149.96 ± 35.57	148.26 ± 28.20	0.840
HDL	35.48 ± 8.63	43.79 ± 6.42	0.000*
LDL	93.88 ± 38.02	90.29 ± 22.68	0.667
Tri-glyceride	148.88 ± 54.25	112.91 ± 18.40	0.002*

Table 4: Genotype frequencies of the of the *AGT* polymorphisms in DCM patients and control subjects

Genotype	n=35	Controls	X^2	P value
rs699T>C				
CC	15(40.7%)	4 (8.6%)	9.48	0.006*
TT	20(59.3%)	38(91.4%)		
rs4762C>T				
CC	32(88.9%)	40(94.3%)	1.333	0.428
CT	3(11.1%)	2(5.7%)		
rs11568053G>A				
GA	4(11.5%)	4(11.1%)	2.094	0.418
GG	31(88.5 %)	38(88.9%)		

Fig.1:. Sequencing Chromatograms of exon 2 of AGT gene (**a**) the wild type sequence (**b**) representing the sequence of homozygous patient carrying base change ATG/ACG that results in SNP rs699 i.e. Met235Thr. Arrow indicates the base change. Underline shows codon change.

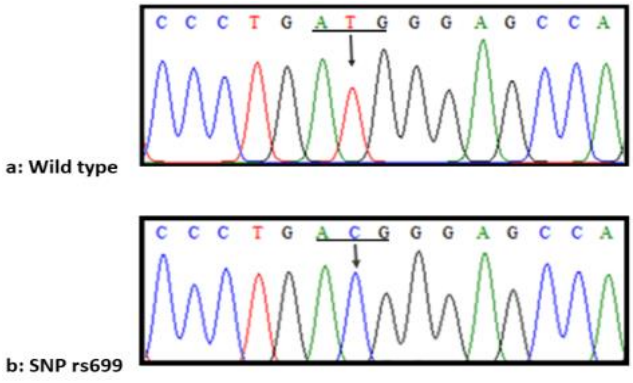


Fig. 2: Sequencing Chromatograms of exon 2 of AGT gene (**a**) the wild type sequence (**b**) representing the sequence of heterozygous patient carrying base change, ACG/ATG that results in Thr174Met, indicated by arrow. Underline shows codon change.

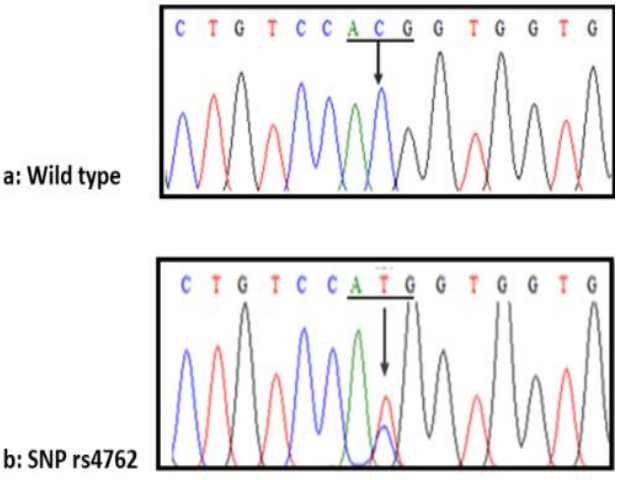
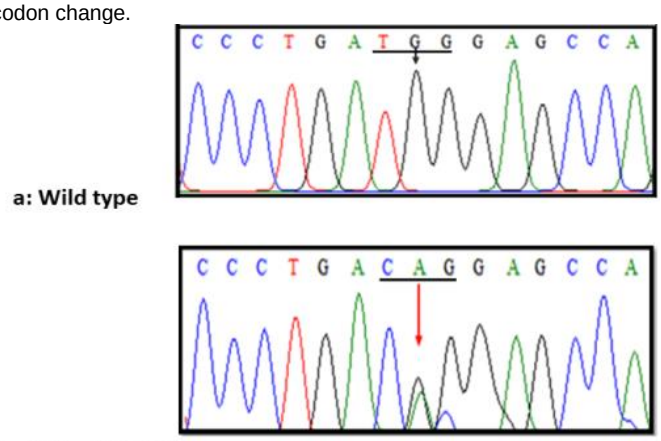


Fig. 3: Sequencing Chromatograms of exon 2 of AGT gene (**a**) the wild type sequence (**b**) representing the sequence of heterozygous patient carrying base change, ATG/ATA that results in SNP rs11568053 i.e. Met235Leu, indicated by arrow. Under line shows codon change.



DISCUSSION

The DCM is characteristic of the left ventricular (LV) enlargement and abnormal systolic function. This may result in an impaired heart function which leads to high mortality and morbidity rates including heart failure. Mutations/nucleotide variations in more than 30 different genes are associated with this disease[20].

Angiotensinogen (*AGT*) gene (OMIM: 106150) mediates the synthesis of angiotensinogen. The tissue for *AGT* encoded proteins like pre-angiotensinogen (precursor of angiotensinogen) expression is present in the liver and catabolized by the renin enzyme when blood pressure is low. Then the synthesis of angiotensin-I protein is regulated by angiotensin converting enzyme (ACE) and cleaved to make the angiotensin-II which is functionally active. This protein affects cardiac contractility and heart rate. Thus, it can involve in pathogenesis of hypertension[9].

According to available data, the current study of Punjabi population of Pakistan about the detection of *AGT* variations in DCM patients is the first ever report from this ethnic group. In present study, the genotype frequency of M235T was significantly higher in DCM patients than the controls. While, no significant association observed for genotype frequencies of T174M and M235L polymorphisms with DCM patients and healthy controls. The results of present study are consistent with previous study from North Indian population in which the allele frequency of 235T was significantly higher in DCM patients[15]. Also, the association of this polymorphism 235T has been associated with disease risk in other ethnic groups like in Czech, Canadian-Caucasian populations[3,14], Italian population and Japanese groups[12]. Although, in other populations, no genetic association has been observed with the disease itself or its severity in French population[21] and Japanese population[22].

In our population, association of genotype frequencies of *AGT* T174M polymorphism has been not found which is consistent to the previous reports of different populations like in North Indian population[15], Canadian-Caucasians[14]. In contrast, a study of French population has been

established for the association T174M of *AGT* gene polymorphism and hypertension[16].

For another polymorphism M235L of *AGT*, no genotype and allele frequencies have been associated between patients and controls in this study although a previous report determined the significant association of rs11568053 (M235L) with DCM in the United States and Canadian populations[17].

CONCLUSION

In conclusion, our results show that the 235T allele frequency of *AGT* increases the DCM risk in a Punjabi population of Pakistan. Further studies are necessary to identify the particular genotype change and the mechanism underlying the association between 235T allele and DCM susceptibility in Pakistani and other populations on large scale.

Acknowledgment: We are thankful to the individuals who participated in this study. We are also grateful for the University of Health Sciences, Lahore, Pakistan for providing the research facilities.

Conflict of Interest: All authors declared no conflicts of the interest.

REFERENCES

1. Burkett EL, Hershberger RE. Clinical and genetic issues in familial dilated cardiomyopathy. J Am Coll Cardiol 2005; 45: 969-81.
2. Taylor MR, Fain PR, Sinagra G, Robinson ML, Robertson AD, and Carniel E Natural history of dilated cardiomyopathy due to lamin A/C gene mutations. J Am Coll Cardiol 2003; 41: 771-80.
3. Codd MB, Sugrue DD, Gersh BJ, Melton LJ. 3rd Epidemiology of idiopathic dilated and hypertrophic cardiomyopathy. A population-based study in Olmsted County, Minnesota, 1975-1984. Circulation 1989; 80: 564-72.
4. Miura K, Nakagawa H, Morikawa Y, Sasayama S, Matsumori A, Hasegawa K et al. Epidemiology of idiopathic cardiomyopathy in Japan: results from a nationwide survey. Heart 2002; 87: 126-30.
5. Hershberger RE, Morales A, Siegfried JD. Clinical and genetic issues in dilated cardiomyopathy: A review for genetics professionals. Genetics in Medicine 2010; 12: 655
6. Hershberger RE, Hedges DJ, Morales A 2013. Dilated cardiomyopathy: the complexity of a diverse genetic architecture. Nat Rev Cardiol, 10: 531-547.
7. Dellefave L and McNally EM. The genetics of dilated cardiomyopathy. Curr Opin Cardiol 2010; 25:198-204.
8. Griendling KK, Murphy TJ & Alexander RW. Molecular biology of the renin-angiotensin system. Circulation 1993; 87: 1816-28.
9. Sadoshima J, Xu Y, Slayter HS, Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. Cell 1993; 75: 977-84.
10. Jeunemaitre X, Inoue I, Williams C, Charru A, Tichet J, Powers M et al. Haplotypes of angiotensinogen in essential hypertension. Am J Human Genet 1997; 60: 1448-60.
11. Kumar A, Li Y, Patil S. A haplotype of the angiotensinogen gene is associated with hypertension in African Americans. Clin Exp Pharmacol Physiol 2005; 32: 495-502.
12. Ishigami T, Umemura S, Iwamoto T, Tamura K, Hibi K, Yamaguchi S et al. Molecular variant of angiotensinogen gene is associated with coronary atherosclerosis. Circulation 1995; 91: 951-54.
13. Goldbergova MP, Spinarova L, Spinar J, Parenica J, Siskova L, Groch, L, et al. Difference in angiotensinogen haplotype frequencies between chronic heart failure and advanced atherosclerosis patient new prognostic factor? Physiol Res 2011; 60: 55-64
14. Jakubiak ZM, De Denus S, Dube´ MP, Be´langer F, White M, Turgeon J, et al. Ten renin-angiotensin system-related gene polymorphisms in maximally treated Canadian-Caucasian patients with heart failure. Br J Clin Pharmacol 2008; 65: 742
15. Rani B, Kumar A, Bahl A, Sharma R, Prasad R, Khullar M. Renin-angiotensin system gene polymorphisms as potential modifiers of hypertrophic and dilated cardiomyopathy phenotypes. Mol Cell Biochem 2017; 427: 11.
16. Tiret L, Mallet C, Dorent R, Schwartz K, Cambien F, Komajda M et al. Lack of association between polymorphisms of eight candidate genes and idiopathic dilated cardiomyopathy: the CARDIGENE study. J Am Coll Cardiol 2000; 35: 29-35.
17. Mital S, Chung W, Colan S, Sleeper L, Manlhiot C, Arrington C. Renin-Angiotensin-Aldosterone Genotype Influences Ventricular Remodeling in Infants with Single Ventricle. Circulation 2011; 123: 2353-62.
18. Suguna S, Nandal DH, Kamble S, Bharatha A and Kunkulol R. Genomic DNA isolation from human whole blood samples by non-enzymatic salting out method. Int J pharm pharm Sci 2014; 6: 198-99.
19. Ullah MI, Nasir A, Ahmad A, Harlalka GV, Ahmad W, Hassan MJ, Baple EL, Crosby AH, Chioza BA. Identification of novel L2HGDH mutation in a large consanguineous Pakistani family- a case report. BMC Med Genet 2018; 20: 25.
20. Hershberger RE and Siegfried JD. State of the Art Review 2011. Update: clinical and genetic issues in familial dilated cardiomyopathy. J. Am. Coll. Cardiol 2011; 57(16): 1641-49.
21. Mallet C, Poirier O, Nicaud V, Millaire A, Bouhour JB, Roizes G et al. Lack of association between polymorphisms of eight candidate genes and idiopathic dilated cardiomyopathy: the CARDIGENE study. J Am Coll Cardiol 2000; 35: 29-35.
22. Yamada Y, Ichihara S, Fujimura T, Yokota M. Lack of association of ACE and AGT genes with non-familial hypertrophic or dilated cardiomyopathy. Am J Hypertens 1997; 10: 921-28.