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Molecular Association of angiotensin type II receptorgene (AT2R) with Diabetic type2 patients in women in Iraq

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Abstract : Objectives: This study aimed to investigate the molecular association of angiotensin type II receptor gene (AT2R)with diabetic disease in women (because AT2R gene is located on the chromosome X at the locus Xq23–26, therefore only women patients were taken in this study[5]).

Methods: Study include patient samples consisted of 70 patients, while the control group consisted of 40 healthy blood donors. Blood that collected in EDTA tubes, stored in - 40˚C (deep freeze) in order to be used later in DNA extraction for genomic analysis. It was estimated the molecular association of AT2R gene with diabetic patients using PCR-RFLP technique. The AT2R polymorphism was genotyped using polymerase chain reaction technique (PCR) and RFLP, and the PCR product with 120-bp.

ResultsThe results show the genotype of AT2Rgene in the two study groups control and patients (the control were 40 samples while the patients were 70 samples), AA homogenotype represented (120bp), AT heterogenotypere presented (120bp, 91bp,and 29bp) and TT homogenotype represented (19bp,and 29bp)(6)**.**For patient groups the allele frequency of(A) variant allele was 0.41 , but(T) allele variant frequency was 0.59 according to Hardy-Wienberg equation. While for control groups the allele frequency of (A) variant allele was 0.4 , but (T) allele variant frequency was 0.6 according to Hardy- Wienberg equation. Results show that the *P-value* of the AA, At, and TT genotype of AT2Rgene in the two study groups control and patients has no significant value ($p \leq 0.05$).

Conclusion : Results indicate that there is no significant association btween the AT2R gene and diabetes.

Key words : Molecular Association, angiotensin type II receptor gene (AT2R), Diabetic type2.

Introduction

Environmental and genetic factors play important roles in the mechanisms involved in the development of T2DM. An association study of candidate genes, viz., fatty acid binding protein 2 (FABP2), uncoupling protein type 1 gene (UCP1), protein phosphatase type 1 (PP1G), β3 adrenergic receptor (β3AR), VDR, was carried out on T2DM patients. Studies on genes inducing susceptibility to T2DM have been carried out by various groups in different populations. Environmental and genetic factors play important roles in the mechanisms involved in the development of T2DM. An association study of candidate genes, viz., fatty acid binding protein 2 (FABP2), uncoupling protein type 1 gene (UCP1), protein phosphatase type 1 (PP1G), β3 adrenergic receptor (β3AR), VDR, was carried out on T2DM patients. Studies on genes inducing susceptibility

to T2DM have been carried out by various groups in different populations. An association study of candidate genes, viz., fatty acid binding protein 2 (FABP2), uncoupling protein type 1 gene (UCP1), protein phosphatase type 1 (PP1G), β3 adrenergic receptor (β3AR), VDR, and angiotensin type 1 receptor (AT1R) was carried out on T2DM patients[1].

The rennin-angiotensin system (RAS) plays a central role in the regulation of physiological pathways in human body[2]. In case of diabetic patients hyperglycemia stimulate the elevation level of tissue angiotensin II which induces oxidative stress, glomerular hyperfiltration, endothelial damage, thrombosis, inflammation and vascular remodeling[3]. Angiotensin II binds to two main types of receptors. The angiotensin type 1 receptor (AT1R) mediates vasoconstriction and the proliferative action of angiotensin II, while the type 2 receptor (AT2R) inhibits cell proliferation and mediates apoptosis and works as a cardio protective agent against AT1R[2,4].

The AT2R gene is located on the chromosome X at the locus Xq23–26. The AT2R gene consists of three exons and two introns. A common AT2R polymorphism is located within intron 1, 29 bp before the start of exon2, therefore only women patients were taken in this study[5].

Materials and Methods

Subjects

The patient sample consisted of 70 patients. They all gave their written informed consent for the study after its nature had been fully explained. The study was approved by the ethics committees. The control group consisted of 40 healthy blood donors. All samples were obtained from Merjan hospital in Hilla city.

Collection of the blood samples:

Fasting blood samples (fasting at least 8 h) were taken from all participants. All of the collected blood samples were transported on dry ice at prearranged intervals to laboratory. The blood samples were collected into ethylene diaminetetra acetic acid (EDTA) tubes. Blood that collected in EDTA tubes, stored in - 40˚C (deep freeze) in order to be used later in DNA extraction for genomic analysis.

PCR Amplification of AT2R

The AT2R polymorphism was genotyped using the primers of 5'-GGA AGG TAG AAC ATA CAT TAA ATG-3' and 5'-AGA GAA ACA GCA GCT AAA GAA TT-3', with annealing temperature 60ºC. The PCR product with 120-bp was digested with *EcoRI* restriction enzyme. In the presence of GG allele two fragments with 91- and 29-bp fragments were produced, while in the presence of AA allele the 120-bp fragment remained intact[5,6].

Statistical Analysis:

Genetic analysis was performed using Chi-square($\chi 2$) test. P values less than (0.05) is considered significant and less than (0.01) is considered highly significant.

Results

The PCR product of AT2R gene amplification was 120 bp[6] figure (1).

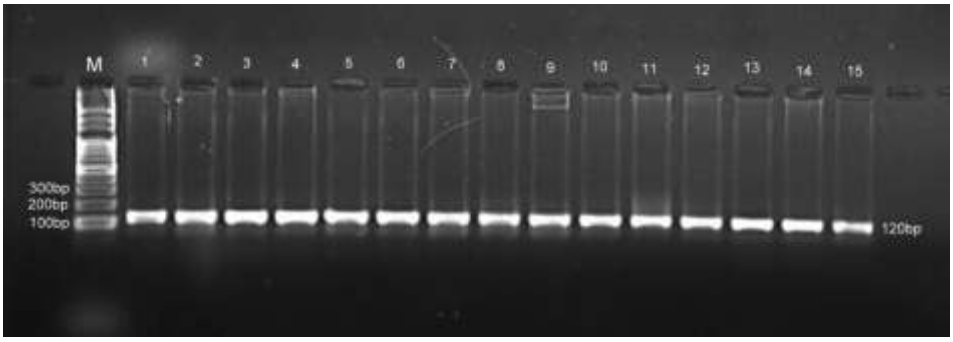

Figure (1)electrophoresis pattern of PCR product of AT2R gene, the optimum annealing temperature was 60°C

Results from figure (2) show the genotype of AT2Rgene in the two study groups control and patients (the control were 40 samples while the patients were 70 samples), AAhomogenotype represented (120bp), ATheterogenotype represented (120bp, 91bp,and 29bp) and TThomogenotype represented (19bp,and 29bp)[6]**.**

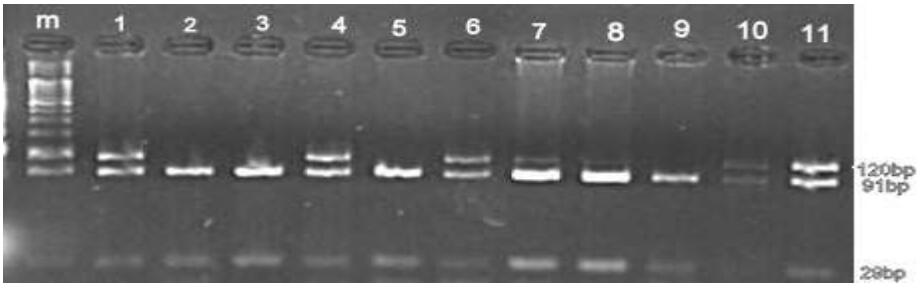

Figure (2)Electrophoresis pattern of PCR-RFLP by 2.5% agarose gel for PCR product (120bp) with restriction enzyme *EcoRI*. Lane M DNA ladder. Lane (1-4): control. Lane: (5-12) patients. Lane (1,4,6,7,10,11) heterozygote (Tt) genotype, Lane (2,3,5,8,9) homozygote (tt) genotype.

The genotype frequencies of AA, AT, and TT of AT2R gene polymorphism were 9(12.9%), 40(57.1%)and21(30%) in the patient group, while 4(10%), 24(60%) and 12 (30%) in the control group , table (1).

Table(1): Genotype and allele frequency of AT2R gene polymorphism

Genotype AT2R	Control N(40)	Patient T2DM N(70)	χ^2 (N=110)	*P* value	O.R.
AA	4(10)	9(12.9)	0.212	0.9	1.327
AT	24(60)	40(57.1)	0.211	0.646	0.888
TT	12(30)	21(30)	0.012	0.913	1
Total number	40(100%)	70(100%)			
Allele frequency					
Allele	**Control**		**Patient**		
A	0.4		0.41		
T	0.6		0.59		

Results from table(1) show that the *P-value* of the AA, At, and TT genotype of AT2Rgene in the two study groups control and patients has no significant value ($p \leq 0.05$).

Discussion

For patient groups the allele frequency of(A) variant allele was 0.41 , but(T) allele variant frequency was 0.59 according to Hardy-Wienberg equation. While for control groups the allele frequency of (A) variant allele was 0.4 , but (T) allele variant frequency was 0.6 according to Hardy- Wienberg equation.

The anti-proliferative actions of the AT2R offset the growth promoting effects mediated by the AT1R. It has been suggested that reduced transcription of the AT2R gene in the presence of A allele might produce clinical effects resulting from a decreased production of AT2R. Experimental antagonism of AT2R results in an increase in systolic blood pressure[5]. To the contrary, stimulation of the AT2R is associated with a vasodilator cascade involving increased production of bradykinin and nitric oxide [5,7]. In a more complicated system with a combined hypertension and insulin resistance, insulin and RAS may cross-talk with each other. After blocking AT1R signaling pathway, unbound angiotensin II can act on AT2R. The stimulation of AT2R can regulate insulin sensitivity at multiple sites of the insulin signaling pathway, and also regulate vascular remodeling in concert with insulin[8]. However further study is needed to clarify the direct cross-talk of insulin and angiotensin II mediated signaling through the AT1R and AT2R in glucose metabolism and vascular modeling. The AT2R A allele was reported to be associated with congenital anomalies of the kidney and urinary tract in a small study of males only[9]. The present study indicates a higher but not significantly different frequency of AT2R A allele in diabetic patients compared to controls. However, we observed an association between the risk of diabetic nephropathy with AA genotype of AT2R[5,11].

PCR product of AT2Rgene amplification was 120bp[6]. The Genotype of AT2Rgene polymorphism between the two group control and patient group were detected using PCR-RFLP technique. Results show the genotype of AT2R gene in the two study groups control and patients (the control were 40 samples while the patients were 70 samples). Results from table (1) indicate that there is no significant association between the AT2R gene and diabetes, and other researches ensure that there are other genes that have strong significant association with the diabetes[5,12].

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