Archives of Razi Institute, Vol. 77, No. 6 (2022) 2365-2370


DOI: 10.22092/ARI.2022.358736.2294

***Original Article***

# Angiotensin-Converting Enzyme 2 (*ACE2*) Gene Polymorphism in Patients with Type 2 Diabetes (T2DM) in Thi-Qar, Iraq

**Shamki Fayyad, N[1], Risan Al-Rikabi, H[1]***

*1. Biology Department. College of Education for Pure Science, University of Thi-Qar University, Thi-Qar, Iraq*

Received 18 May 2022; Accepted 8 June 2022
Corresponding Author: hassanalrkaby@utq.edu.iq

**Abstract**

The increased levels of blood glucose associated with genetic and/or environmental risk factors have been known as Type 2 diabetes mellitus (T2D). T2D is categorized as a dangerous clinical syndrome for global public health. It has been well-documented that several candidate genes are functionally associated with T2D. One of the candidate genes linked to T2D is named Angiotensin-converting enzyme 2 (ACE2). Therefore, this study aimed to evaluate the relationship between ACE2 gene polymorphism and the development of T2D in Iraq. This study includes 150 participants (100) T2D patients and 50 healthy participants as a control group. Through the analysis of the recorded data, 78% were nonsmokers, while the rest were smokers. The current study found 61% of T2D patients suffer from hypertension (*P*-value=0.028). In this study, 80% of patients have the GG genotype. However, 26% have the GA genotype without a significant difference between patients and the control group (OR=0.78). Mutations in the ACE2 gene were recorded in the gene bank with accession numbers LC656363, LC656364, LC656365, LC656366, LC656367 and LC656368. The current study showed a relationship between mutations and polymorphisms in the *ACE2* gene and type 2 diabetes mellitus. The polymorphisms, deletion and insertion mutations had an important role in the pathophysiology of type 2 diabetes mellitus. The study recorded some mutations in the Clinical Variation website at NCBI's National Center for Biotechnology Information. The current study showed a relationship between smoking and the risk of developing type 2 diabetes. The current study's findings indicated that mutations affecting the three encoded genes could directly cause impaired insulin production in people with type 2 diabetes.

**Keywords:** ACE2, PCR, Type 2 diabetes mellitus, Thi Qar population

## 1. Introduction

Diabetes is one of the most common diseases in developed and developing countries. Scientific studies have shown that 8-5% of individuals have diabetes. The elevated levels of blood glucose associated with genetic and/or environmental risk factors have been known as Type 2 diabetes mellitus (T2D). T2D is categorized as a dangerous clinical syndrome for global public health. It affects millions of people worldwide with a rapid increase in prevalence and incidence (1). During recent decades rapid urbanization and increasingly sedentary lifestyles have led to a dramatic increase in the global prevalence of T2DM (2, 3).

Genetic polymorphism is an accidental change(s) in the DNA sequence between individuals, groups or populations. One type of polymorphism is known as single nucleotide polymorphism (SNPs), which is the replacement of one nucleotide in a specific sequence in the genome. It has been approved that ethnic differences possibly influenced the occurrence of SNPs such as *IL-6* polymorphisms in European and Asian populations (4). Three million SNPs have been

identified in humans, about one million of which are used to see if there is an association between the polymorphisms and susceptibility to diabetes and cancer (5). According to the International Diabetes Federation, approximately 452 million people currently live with diabetes worldwide, and the number is expected to rise to 691 million by 2045 (6). Type 2 diabetes is a clinical syndrome that causes a high level of diabetes. Diabetes mellitus (DM) is a polygenic disorder and may also be called a heterogeneous abnormality that requires lifelong care and is characterized by chronic symptoms such as hyperglycemia or defective macromolecular metabolism due to impaired secretion or effect of a hypoglycemic hormone or both and points to multifactorial etiology as a significant predisposing factor (7).

It has been well-documented that several candidate genes are functionally associated with T2D. One of the candidate genes linked to T2D is named the renin-angiotensin-aldosterone system (RAAS). The essential product of RAAS is called Angiotensin-converting enzyme 2 (ACE2), which has been recently identified as a potent target for the treatment and prevention of T2D. The gene encoding ACE2 is located on chromosome Xp22, extends along 39.98 kb of genomic DNA, and contains 20 introns and 18 exons. One of the prominent characteristics of the ACE2 gene is a high degree of genetic polymorphism exhibition. Some of the ACE2 gene polymorphisms are highly linked to T2D. Previously published reports revealed that ACE2 gene polymorphisms showed a high degree of genetic heterogeneity linked to T2D; it is essential to know that not all variants show a link with T2D risk.

Therefore, this study aimed to evaluate the relationship between ACE2 gene polymorphism and the development of T2D in Iraq.

## 2. Materials and Methods

### 2.1. Samples Collection

One hundred blood samples (4 ml) were collected from individuals who had type 2 diabetes at the Diabetes and Endocrinology Center in Thi Qar Governorate, Iraq. As the control group, 50 (4 ml) blood samples were collected from healthy people. All the blood samples were placed in tubes containing the anticoagulant substance (EDTA) and kept at 20°C. An information form was completed for the two participant groups about the following information: age, smoking, area of residence, blood pressure, and family history (Tables 1 and 2).

**Table 1.** Distribution of control groups and patients according to smoking

| Smoking | Control Group | | Patient Group | | *P*-value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| Smoked | 11 | 22% | 39 | 39% | |
| No Smoked | 39 | 78% | 61 | 61% | 0.037* |
| Total | 50 | 100% | 100 | 100% | |

$^{2}\chi$= 4.33 df=1 *P*-value≥0.05

**Table 2.** Distribution of the two groups of patients according to stress

| Hypertension | patient group | | *P*-value |
|---|---|---|---|
| | N | % | |
| Yes | 39 | 39% | 0.028* |
| No | 61 | 61% | |
| Total | 100 | 100% | |

$^{2}\chi$= 4.84 df=1 *P*-value≥0.05

### 2.2. DNA Extraction

DNA extraction from patient and healthy samples was done using a commercial kit, according to the DNA Extraction protocols provided by Geneaid Company (Korean origin).

### 2.3. Polymerase Chain Reaction (PCR)

PCR technique was used to amplify the ACE2 gene using specific primers as follows: (F-5-TGGGGAAACTTAACTGGGCT-3 and R-5-GGTTGGCAGAC ATCAGCTCATA-3). The primers were designed from the GenBank website of the National Center for Biotechnology Information (NCBI) using Primer3Plus software. The primers were used for the first time in this study, and the Macrogene Company prepared them. The PCR condition with a 20µl reaction mixture is shown in table 3.

**Table 3.** PCR Condition for amplification of gene ACE2

| No. | Steps | Temperature | Time | No. of cycle | Size (bp) |
|---|---|---|---|---|---|
| 1 | Initial denaturation | 95 | 10 m. | 1 | |
| 2 | Denaturation<br>Annealing<br>Extention | 95<br>59<br>72 | 30 sec.<br>30 sec.<br>35 sec. | 30 | 549 |
| 3 | Final extension | 72 | 10 m. | 1 | |

## 3. Results

The recorded data about different ACE2 gene genotypes and alleles frequency in patients and healthy group are tabulated in tables 4 and 5, where the percentage of the genotype (GG) in the healthy group (control) was 54%, while in the patient's group it was 80%. On the other hand, the genotype (GA) in the healthy group was 30% compared to the patient's group, which shows 26% frequency for the GA genotype. These changes were insignificant compared to the genotype (GG) (OR = 0.78). The results showed that the frequency of the genotype (AA) in the healthy group was 16% compared to the Patient group, which showed a percentage of 14%. These changes were insignificant compared to the genotype (GG) (OR=0.78). As for the frequency of alleles, where the frequency of the (G) allele in the healthy group was (69%) it represents the frequency of 73% inpatient group, while the frequency of the (A) allele was (31%) in the healthy group and (27%) in the patient's group without significant differences when comparing the frequency of alleles (OR=0.82) and as shown in table 4.

The forward sequence of the ACE2 gene sequence was relied on in the alignment process compared to the sequence of the National Center for Biotechnology Information (NCBI), and genetic changes or mutations were identified depending on the difference from the sequence of the NCBI (NG_012575.2) about the study samples. Where the analysis program (Mutation Surveyor V.5.1.2) was used. The results of the nucleotide sequence analysis of the ACE2 gene for people with type 2 diabetes showed the presence of 6 polymorphic sites, four variable sites in the group of patients and two in the comparison group at different sites. As shown in tables 4 and 5.

**Table 4.** Frequency of genotypes and alleles of the ACE2 gene for both patient and comparison groups

| genotypes | Control group | Patients group | OR | 95%CI |
|---|---|---|---|---|
| GG | 27(%54) | 60(%80) | 1.0 | --------- |
| GA | 15(%30) | 26(%26) | 0.78 | 0.35-1.70 |
| AA | 8(%16) | 14(%14) | 0.79 | 0.29-2.09 |
| Total | 50(%100) | 100(%100) | | |
| **Allele frequency** | | | | |
| G | 69(%)69 | 146(%76) | 1.0 | ---------- |
| A | 31(%31) | 54(%24) | 0.82 | 0.48-1.39 |
| Total | %100 | 200(%100) | | |

OR: Odd Rations 95% CI Confidence Interval

**Table 5.** Shows mutations in the ACE2 gene for patients with T2DM

| Mutation | Location | Location on the nucleotide | Type | F | Accession number |
|---|---|---|---|---|---|
| Ins A14819 | Exon 4 | X 15592396 | Insertion | %1 | LC656363 |
| T>TA14891 | Exon 4 | X 15592321 | Transversion | %1 | LC656364 |
| A>G14829 | Exon 4 | X 15592383 | Transitions | %1 | LC656365 |
| G>T14581 | Exon 4 | X 15592036 | Transversion | %1 | LC656366 |
| G>A14987 | Exon 4 | X 15592225 | Transitions | %1 | LC656367 |
| G>A14987 | Exon 4 | X 15592225 | Transitions | %1 | LC656368 |

## 4. Discussion

Regarding the ACE2 gene G8790A polymorphism at the beginning of the intron (the fourth base of the third intron), the SNP may cause changes in mRNA montage by alternative splicing and affect gene expression (3, 8). In previously published studies conducted by Benjafield, Wang (9) and Huang, Yang (10), their findings noted the paucity of studies in this regard and a muscular imbalance of the G8790A polymorphism has already been described with two other SNPs in this gene.

The current study's results agree with the study previously published by Doris (11), which demonstrated unprecedented findings on the genetic nature and polymorphism of the ACE2 gene and hypertension. The results of the current study based on the analysis of the ACE2 gene (G8790A rs2285666) showed the presence of six-point mutations in people with type 2 diabetes when compared with the reference sequence (NG_012575.2), and the sequences containing these mutations were recorded in (NCBI BANK).

The nitrogenous base adenine (Ins A 14819) was entered into the Clinical Variation site with accession number (LC656363). SNPs (X15592396) were replaced with the nitrogenous base (T>TA14891) and recorded with accession number (LC656364) on the single nucleotide site SNPs (X15592321), and the nitrogenous base was replaced at the site (A>G14829) and recorded with accession number (LC656365) on the SNPs site (X15592383). The nitrogen base (G>T14581) was also replaced and registered with the accession number (LC656366). The nucleotide SNPs are located on chromosomes (X15592036). In the healthy group, the following variations were recorded where the nitrogenous base was replaced at two sites (G>A14987) and recorded with the following accession numbers (LC656367, LC656368) on the single nucleotide SNPs (X15592225).

The recorded data in the current study was in agreement with the results of a study conducted by Alghamri, Weir (12), which showed that genetic mutations of the ACE2 gene increase the risk of atherosclerosis and left heart remodelling. ACE-2 has also been associated with the development of hypertension, kidney disease, and DM, and the G8790A (rs2285666) single nucleotide polymorphism was associated with the risk of T2DM (13, 14).

On the other hand, the recorded data in the current study was in agreement with the results of a study previously conducted by Elihimas Júnior, Elihimas (15), which showed that smoker patients with T2DM were at risk of developing chronic kidney disease, and therefore smoking cessation is one of the leading medical recommendations for diabetic patients. It also agreed with the results of a study conducted by Zhang, Jia (16) on the effect of the *RAGE* gene on patients with a complication of diabetes mellitus and chronic kidney disease, where smoking had an apparent effect on those with this disease. It also differed from the results of Cai, Li (17), where there was no recorded effect of the *RAGE* gene smoking for Chinese patients with T2DM.

The more substantial effect of ACE2 interaction with obesity and smoking in males with high blood pressure compared to females may be attributed to different reasons, one of which is the localization of the gene on the X chromosome. Specific and thus leave two active alleles in females versus one in males (18, 19). It also agreed with the study by Liu, Li (20) on the ACE2 gene polymorphism and its relationship to heart disease in patients with type 2 diabetes.

The current study showed a relationship between mutations and polymorphisms in the *ACE2* gene and type 2 diabetes mellitus. The polymorphisms, deletion and insertion mutations had an essential role in the pathophysiology of type 2 diabetes mellitus. The study recorded some mutations in the Clinical Variation website at NCBI's National Center for Biotechnology Information. The current study showed a relationship between smoking and the risk of developing type 2 diabetes. The current study's findings indicated that mutations affecting the three encoded genes could directly cause impaired insulin production in people with type 2 diabetes.

## Authors' Contribution

Study concept and design: H. R. A.
Acquisition of data: N. S. F.
Analysis and interpretation of data: N. S. F.
Drafting of the manuscript: H. R. A.
Critical revision of the manuscript for important intellectual content: N. S. F.
Statistical analysis: H. R. A.
Administrative, technical, and material support: N. S. F.

## Ethics

Approval for the research study was obtained from the University of Thi-Qar University, Thi-Qar, Iraq ethics board (project approval number 20215478)

## Conflict of Interest

The authors declare that they have no conflict of interest.

## References

1. Danaei G, Singh GM, Paciorek CJ, Lin JK, Cowan MJ, Finucane MM, et al. The global cardiovascular risk transition: associations of four metabolic risk factors with national income, urbanization, and Western diet in 1980 and 2008. Circulation. 2013;127(14):1493-502.
2. Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U, Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Res Clin Pract. 2014;103(2):137-49.
3. Huldani H, Jasim SA, Bokov DO, Abdelbasset WK, Shalaby MN, Thangavelu L, et al. Application of extracellular vesicles derived from mesenchymal stem cells as potential therapeutic tools in autoimmune and rheumatic diseases. Int Immunopharmacol. 2022;106:108634.
4. Karki R, Pandya D, Elston RC, Ferlini C. Defining "mutation" and "polymorphism" in the era of personal genomics. BMC Med Genom. 2015;8(1):1-7.
5. Teama S. DNA polymorphisms: DNA-based molecular markers and their application in medicine. Genetic Diversity and Disease Susceptibility. 2018;25.
6. Cho N, Shaw J, Karuranga S, Huang Y, da Rocha Fernandes J, Ohlrogge A, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271-81.
7. Leslie RD, Palmer J, Schloot NC, Lernmark A. Diabetes at the crossroads: relevance of disease classification to pathophysiology and treatment. Diabetologia. 2016;59(1):13-20.
8. Yang M, Zhao J, Xing L, Shi L. The association between angiotensin-converting enzyme 2 polymorphisms and essential hypertension risk: A meta-analysis involving 14,122 patients. J Renin Angiotensin Aldosterone Syst. 2015;16(4):1240-4.
9. Benjafield AV, Wang WY, Morris BJ. No association of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms with essential hypertension. Am J Hypertens. 2004;17(7):624-8.
10. Huang W, Yang W, Wang Y, Zhao Q, Gu D, Chen R. Association study of angiotensin-converting enzyme 2 gene (ACE2) polymorphisms and essential hypertension in northern Han Chinese. J Hum Hypertens. 2006;20(12):968-71.
11. Doris PA. Hypertension genetics, single nucleotide polymorphisms, and the common disease: common variant hypothesis. Hypertension. 2002;39(2):323-31.
12. Alghamri MS, Weir NM, Anstadt MP, Elased KM, Gurley SB, Morris M. Enhanced angiotensin II-induced cardiac and aortic remodeling in ACE2 knockout mice. J Cardiovasc Pharmacol. 2013;18(2):138-51.
13. Olegovich Bokov D, Jalil AT, Alsultany FH, Mahmoud MZ, Suksatan W, Chupradit S, et al. Ir-decorated gallium nitride nanotubes as a chemical sensor for recognition of mesalamine drug: a DFT study. Mol Simul. 2022:1-10.
14. Zhou J-B, Yang J-K. Meta-analysis of association of ACE2 G8790A polymorphism with Chinese Han essential hypertension. Journal of the Renin-Angiotensin-Aldosterone System. 2009;10(1):31-4.
15. Elihimas Júnior UF, Elihimas HCdS, Lemos VM, Leão MdA, Sá MPBdO, França EETd, et al. Smoking as risk factor for chronic kidney disease: systematic review. J Bras Nefrol. 2014;36:519-28.
16. Zhang Y, Jia N, Hu F, Fan N, Guo X, Du H, et al. Association of single-nucleotide polymorphisms in the RAGE gene and its gene-environment interactions with diabetic nephropathy in Chinese patients with type 2 diabetes. Oncotarget. 2017;8(57):96885.
17. Cai W, Li J, Xu J-X, Liu Y, Zhang W, Xiao J-R, et al. Association of 2184AG polymorphism in the RAGE gene with diabetic nephropathy in Chinese patients with

type 2 diabetes. J Diabetes Res. 2015;2015.
18. Ansari MJ, Jasim SA, Taban TZ, Bokov DO, Shalaby MN, Al-Gazally ME, et al. Anticancer drug-loading capacity of green synthesized porous magnetic iron nanocarrier and cytotoxic effects against human cancer cell line. J Clust Sci. 2022:1-11.
19. Elsheikh M, Casadei B, Conway G, Wass J. Hypertension is a major risk factor for aortic root dilatation in women with Turner's syndrome. Clin Endocrinol. 2001;54(1):69-73.
20. Liu C, Li Y, Guan T, Lai Y, Shen Y, Zeyaweiding A, et al. ACE2 polymorphisms associated with cardiovascular risk in Uygurs with type 2 diabetes mellitus. Cardiovasc Diabetol. 2018;17(1):1-11.