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Original Paper

Association of Angiotensin Converting Enzyme Gene Deletion/Deletion Genotype with Risk of Autosomal Dominant Polycystic Kidney Disease: A Single-center Study from Iranian Azeri Turkish Population

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Introduction. Autosomal Dominant Polycystic kidney disease (ADPKD) is defined as one of the most common genetic disorders and the cause of kidney failure or end-stage kidney disease (ESKD). Several studies have shown that renin-angiotensin system has an important role in pathogenesis of ADPKD. The aim of this study was to examine the association between the angiotensin converting enzyme (ACE) gene Deletion/Deletion (D/D) polymorphism and risk of ADPKD among Iranian patients from west Azerbaijan province of Iran.

Methods. This case-control study was conducted on 40 patients and 72 controls. Genetic polymorphism of the ACE gene was determined using polymerase chain reaction (PCR) and electrophoresis.

Result. The frequency (frequency%) of ACE gene I/I, I/D, D/D genotypes were 5 (12.5%), 12 (30%), 23 (57.5%) in cases and 16 (22.22%), 30 (41.67%), 26 (36.11%) in controls, respectively. The frequency (frequency%) of ACE gene I and D alleles were 22 (27.5%) and 58 (72.5%) in cases and, 62 (43.06%) and 82 (56.94%) in controls, respectively. Statistical analysis indicated that there were significant differences among the cases and controls regarding ACE gene D/D genotypes (P = .028). The ACE gene D/D genotype was associated with increased ADPKD susceptibility with an OR of 2.39, (95%) CI = (1.09–5.28), and P = .028. But in the case of ACE gene, I/I and I/D genotypes, there were no statistically significant differences between cases and controls (P > .05). Considering allelic comparison, the ACE gene D allele was associated with ADPKD susceptibility with an OR of 1.99, (95%) CI = (1.1–3.6), and P = .021.

Conclusion. Our findings suggest that ACE gene D/D genotype was associated with ADPKD.

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INTRODUCTION

Autosomal Dominant Polycystic kidney disease (ADPKD, OMIM ID: 173900) is a hereditary condition characterized by the formation of kidney cyst, leading to progressive enlargement of kidneys and kidney failure with the occurrence of 1 in 1,000 childbirths. At least 10% of cases with end-stage kidney disease (ESKD) are due to ADPKD. Symptoms

and signs of this disease include abdominal pain, hematuria, and high blood pressure. ADPKD is diagnosed through renal imaging using CT and MRI scans, as well as ultrasonography.[1] ADPKD results in the development of cysts in extrarenal organs, including liver, pancreas, spleen, and thyroid. It is associated with inguinal hernias, colonic diverticula, heart valve dysfunction, ovarian cysts,[3] prostatic cysts,[4] epididymal cysts, seminal vesicle cysts,[5] as well as abnormalities in arachnoid membrane, spinal meningeal and connective tissue.[6] *PKD1* (MIM 601313) and *PKD2* (MIM 173910) genes account for 85% and 15% of cases with ADPKD, respectively.[7] *PKD1 and PKD2* genes are located on chromosomes 16p13.3 and 4q21, respectively.[7] *PKD1* gene codes polycystin-1 protein with 4302 aa through 46 exons, while *PKD2* gene polycystin-2 protein is with 968 aa through 15 exons codes.[7] The role of polycystin and the polycystin-associated mutations remains unclear in pathogenesis of the ADPKD.[8,9] Polycystin-1 protein as an adhesive G protein coupled receptor (GPCR), is characterized by several domains and sites including GPCR autoproteolysis-inducing (GAIN) domain, GPCR proteolysis site (GPS), G protein binding domain (GBD) and C-terminal cleavage site.[10] Polycystin-1 is defined as a receptor protein for cell-cell or cell-matrix connections having central roles in the balancing of cell proliferation, morphogenesis and apoptosis.[11,12] Polycystin-2 protein acts as an ion channel that regulates the intra-cellular concentration of calcium ions. A modified Polycystin-2 protein leads to an increased diameter of kidney tubules and formation of kidney cysts.[13] Polycystin-1 proteins by forming a complex with polycystin-2 proteins exert its regular function regarding various mechanisms such as cAMP, G-protein-coupled signaling pathways and Ca^{2+} signaling regulation.[10-13]

It has been demonstrated that the activity of the renin–angiotensin–aldosterone system (RAAS) has been increased in cardiovascular diseases[14] and ADPKD.[15]Angiotensin-converting enzyme (ACE) has an important role in the RAAS. This enzyme converts the angiotensin I (Ang I) to Ang II. Ang II is understood to be liable for most of the effects of the RAAS. Inhibitors of ACE cause a reduction in blood pressure and eventually heart/kidney failure.[16] The human *ACE* gene (OMIM 106180) is located on chromosome 17q23.3, and consists **of** 26 exons and 25 introns.[17,18] One of the well-known sequence variants of the ACE gene is the 287 bp insertion/deletion (ACE I/D) in intron 16. ACE I/D (dbSNP rs4646994) is an I/D of an Alu repeat sequence in intron 16 of the *ACE* gene.[19,20] Carriers of the ACE D allele exhibit high production of circulating and tissue ACE mRNA expression and Ang II, as well as an increased degradation level of bradykinin.[21] So, the ACE enzyme activity is associated with an increased level in individuals with the DD genotype, an intermediate level in individual with ID genotype and a decreased level in individual with II genotype.[22] Furthermore, the results of several studies have proposed that patients with ADPKD carrying the deletion/deletion genotype of the ACE gene are at increased risk of kidney failure, indirectly.[23] However, variant data for *ACE* gene in the Iranian population is poorly studied. In this regard, Malakoutian *et al.* could not find any association between polymorphisms of ACE and eNOS genes with renal insufficiency and hypertension in ADPKD patients.[24] In this regard, this study aimed to assess the association between the *ACE* gene D/D sequence variants and the risk of autosomal dominant polycystic kidney disease in a group of Iranian population (West Azerbaijan Province, Iran).

MATERIALS AND METHODS

This study was conducted on 40 patients and 72 controls. All individuals were selected by an expert specialist according to strict criteria as described previously.[9] The study participants were informed about the aims, contents, and methods of the study. The patients were recruited from the Imam Khomeini University Hospital (Urmia, Iran). 4-5 ml of total blood was taken to Falcon™ 15 mL conical centrifuge tubes containing 500 microliters of 0.5 M EDTA as anticoagulant. The Falcon tubes were kept at -20 ºC for DNA extraction. The salting-out method was used for the extraction of the genomic DNA from Blood Samples.[25] DNA samples was kept at -80 ºC for further enquiry. ACE Genotypes were detected by means of Polymerase Chain Reaction (PCR). The PCR reaction was performed in 25-µl volumes containing 100 ng of DNA, 1x reaction buffer, 0.5 µl of each primer (5 pmol), 200 µmol of dNTPs, 0.2 unit of Taq DNA polymerase, and 1.5 mmol MgCl2 (Genefanavaran, Tehran, Iran); using the primers 5′-ctg gag acc act ccc atc ctt

tct-3′ and 5′-gat gtg gcc atc aca ttc gtc aga t-3′ by the following program: initial denaturation at 95 °C for 10 min, followed by Denaturation at 94 °C for 60 secs, annealing at 60 °C for 60 secs, and extension at 72 °C for 60 secs (35 cycles); The PCR was completed at 72 °C for 7-min.[18] The 490 bp (I allele) and 190 bp (D allele) PCR products were subjected to electrophoresis on a 2% agarose gel accompanied with DNA safe Stain (Cinna Gen, Tehran, Iran) and visualized by using a UV-trans illuminator.

Statistical Analysis

Statistical power was detected approximately 70% (two-tailed, a = 0.10) with at least 37 individuals in each group. Straight counting was used to detect the frequencies regarding ACE gene I and D alleles and I/I, I/D, D/D genotypes. The x^2 or Fisher's exact tests were performed to match the frequencies between study groups. Hardy-Weinberg equilibrium (HWE) test was employed for evaluating the genotypes distribution in controls. Statistical Package for the Social Sciences (SPSS) version 20.0 and Microsoft Excel 2016 were used for statistical analysis and calculation of the x^2 and P-value, the odds ratio (OR), and the 95% confidence interval (CI). Two-sided tests by means of power (1- β):90% were performed. A p-value of less than 0.05 was considered significant; while a p-value greater than 0.05 was considered not significant.

RESULTS

The study findings are summarized in Table 1. The frequency (frequency%) of ACE gene I/I, I/D, D/D genotypes were 5 (12.5%), 12 (30%), 23 (57.5%) in cases and 16 (22.22%), 30 (41.67%), 26 (36.11%) in controls, respectively. The frequency (frequency%) of ACE I and D alleles were 22 (27.5%) and 58 (72.5%) in cases and, 62 (43.06%) and 82 (56.94%) in controls, respectively. The genotypes distribution was constant with HWE regarding ACE gene I/I, I/D, D/D genotypes in controls ($X^2 = 1.6 < 3.84$, $P = .44 > 0.05$ with $d_f = 2$). Statistical analysis indicated that there were significant variations among the cases and controls regarding ACE DD genotypes ($P = .028$). The ACE D/D genotype was associated with increased ADPKD susceptibility with an OR of 2.39, (95%) CI = (1.09–5.28), and $P = .028$. But in the case of ACE I/I and I/D genotypes, there were no statistically significant differences between cases and controls ($P > .05$). Considering allelic comparison, the ACE D allele was associated with ADPKD susceptibility with an OR of 1.99, (95%) CI = (1.1–3.6), and $P = .021$.

DISCUSSION

ADPKD is a systemic disorder characterized by the development of multiple renal cysts, ultimately resulting in kidney failure. ADPKD is known as multifactorial diseases; and it is understood that there are several risk factors influencing the ADPKD development.[8,9] Hypertension, as a primary sign, is a significant factor influencing the course of kidney failure in ADPKD.[26] Recent studies have showed that irrelevant activation of RAAS is the foremost reason in the pathogenesis of hypertension in patients with ADPKD.[26,27] The RAAS may contribute to ADPKD development by activating signaling pathways within the kidney cysts to promote growth and deregulate epithelial passage.[28] The ACE gene D/D genotype increases the level of serum ACE and the risk of developing renal failure.[29] *ACE* as the first modifier gene, plays an important role on the development of ADPKD. Also, several other genes and genetic polymorphisms such as angiotensinogen (AGT), and angiotensin-II type 1 receptor (AT1R) genes increase the susceptibility to ADPKD. These genes may act synergistically with ACE D/D genotypes to increase the risk of end-stage renal disease.[30-33] In this study, we matched the frequency of ACE gene I and D alleles and I/I, I/D, D/D genotypes,

Table 1. Distribution of ACE I/D polymorphisms in patients and controls in this study

ACE	Cases F (%F)	Controls F (%F)	OR (95% CI)	χ^2	*P*
II	5 (12.5)	16 (22.22)	0.5 (0.17-1.49)	1.6	.206
ID	12 (30)	30 (41.67)	0.6 (0.26-1.37)	1.49	.221
DD	23 (57.5)	26 (36.11)	2.39 (1.09-5.28)	4.78	.028
I	22 (27.5)	62 (43.06)	0.5 (0.28-0.91)	5.31	.021
D	58 (72.5)	82 (56.94)	1.99 (1.1-3.6)	5.31	.021

F: Frequency

in a group of patients with ADPKD compared to a control group to evaluate which may be associated with ADPKD. Regarding the ACE I and D alleles and I/I, I/D, D/D genotypes, we support the hypothesis that the ACE D/D genotype increased risk for developing ADPKD. Subsequent studies have yielded conflicting results.[30-36] Our results are consistent with some studies; which have shown that the ACE gene D allele and D/D genotype are significant risk factors for developing ADPKD.[30-33] But our findings are not in accordance with others.[34-36] These contradictions can be due to gene-gene and gene-environmental factors. In turn, around 2,322 and 278 sequence variants of *PKD1* and *PKD2* genes have been recorded in the Autosomal Dominant Polycystic Kidney Disease Mutation Database (PKDB, http://pkdb.mayo.edu; January 2024). The Human Gene Mutation Database (HGMD) has reported 1,177 and 211 sequence variants of the *PKD1* and *PKD2* genes, respectively (http://www.hgmd.cf.ac.uk). So far, 1225 pathogenic (likely pathogenic and pathogenic) mutations have been reported in the *PKD1* gene, including 277 missense mutations (https://pkdb.mayo.edu/variants). Also, 196 pathogenic (likely pathogenic and pathogenic) mutations have been reported in the *PKD2* gene, including 23 missense mutations (https://pkdb.mayo.edu/variants). In the case of *ACE* gene, the Human Gene Mutation Database (HGMD) has reported 59 sequence variants (http://www.hgmd.cf.ac.uk, January 2024). It has been demonstrated that majority of these variants are single nucleotide polymorphism that are located within promoter or the other regulatory elements.[37,38] Our study had some limitations regarding low samples size as well as tested genes such as AGT and AT1R. Study with more details could clarifies the pathogenesis of ADPKD.

CONCLUSION

Our findings suggest that ACE D/D genotype was associated with ADPKD.

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COMPETING INTEREST

The authors have no competing interest to declare.

ETHICAL APPROVAL

All stages of this study were approved by Urmia University of Medical Sciences Committee for Ethics in Biomedical Research (IR.UMSU.REC.1402.250).

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