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Evaluation of common polymorphisms of eNOS gene and ACE gene in autosomal dominant polycystic kidney disease patients and their association with hypertension and renal failure

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ABSTRACT

Introduction: Autosomal dominant polycystic kidney disease (ADPKD) is the most hereditary renal disease that leads to end-stage renal disease (ESRD).
Objectives: Since there is no available parameter to assess the clinical course of ADPKD and its outcome, yet, the aim of our study was evaluation of the association of common polymorphisms of eNOS and ACE genes with clinical manifestations (kidney failure and hypertension) in ADPKD.
Patients and Methods: Seventy-five ADPKD patients and 100 control subjects participated in our study. Around 7.5 cc of whole blood was taken from each participant and sent to the genetic laboratory. DNA was obtained from them by the phenol chloroform extraction and ethanol precipitation techniques. Then genotyping for I/D polymorphism of ACE gene and Glu298 ASP and T786C polymorphisms of eNOS gene was performed by PCR electrophoresis and molecular evaluation by special primers for two genes.
Results: The frequency of DD polymorphism of ACE gene and TC polymorphism of T786C of eNOS were considerably elevated in ADPKD individuals than control subjects. No significant difference between groups regarding Glu298 ASP polymorphisms of eNOS gene was detected. In ADPKD patients, 29 patients (39%) had hypertension, 5 patients (6.7%) had diabetes and 43 patients (57%) had glomerular filtration rate (GFR) below 60 mL/min/1.73 m^2. The polymorphisms of ACE and eNOS genes were not meaningfully different regarding diabetes, high blood pressure, GFR and plasma creatinine in ADPKD individuals ($P>0.05$).
Conclusion: In our study, we could not find any association between polymorphisms of ACE and eNOS genes with renal insufficiency and hypertension in ADPKD patients.

Implication for health policy/practice/research/medical education:
In a study on 75 ADPKD individuals and 100 control subjects, we found, the frequency of DD polymorphism of ACE gene in ADPKD patients was meaningfully higher than that in the control group; however the polymorphism of this gene was not significantly different in patients with and without hypertension or renal insufficiency in ADPKD patients. Therefore, despite the increased activity of the renin-angiotensin-aldosterone system in these patients, it seems that the ACE gene polymorphism is not able to determine the progression of blood pressure and renal disease in these patients. However, more studies are necessary on this subject.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a late-onset systemic disorder that presents with bilateral renal cysts, cysts in other organs such as the liver, seminal vesicles, pancreas and arachnoid membranes, vascular abnormalities including intracranial aneurysms, aortic root dilatation and thoracic aortic dissection, mitral valve prolapse, and also herniation of the abdominal wall. The renal manifestations include hypertension, renal pain, and renal failure (1). ADPKD is the most common

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hereditary renal disease, with an estimated prevalence of approximately 1: 1000 (2, 3). Although ADPKD is a hereditary illness, at least 10% of families have new mutations (1).

Approximately 50% of patients with ADPKD have end-stage renal disease (ESRD) of up to 60 years of age (1, 4, 5), accounting for about 8%-10% of patients undergoing renal replacement therapy (2). On the other hand, the early onset of hypertension is a major manifestation in these patients, with 60% of them having hypertension before decrease of glomerular filtration rate (GFR) (6). In addition, high blood pressure is an important parameter in the development of chronic renal failure (CRF) in these patients (3,6-8). Some studies have suggested that hypertension causes cysts to grow faster and thus can rupture in cases such as intracranial aneurysms. Therefore, early treatment of hypertension can be associated with reduced morbidity and mortality in individuals with ADPKD (4).

The pathogenesis of high blood pressure and the progression of kidney disease in ADPKD patients is related with increased kidney volume and cyst density in both children and adults (9). Recent studies have shown that renal function remains relatively stable as long as the kidney volume is less than 1500 cubic centimeters, while a rapid decline in renal function can lead to renal replacement therapy. It is believed that distortion of renal structure could lead to tubular structural destruction and dysfunction, resulting in the activation of the renin-angiotensin-aldosterone (RASS) system, which increases blood pressure prematurely (4,5,7,10-12). Therefore, RAAS antagonists can prevent cell proliferation and inflammation and control hypertension in these patients (11,12). In addition, it has been reported that the prevalence of hypertension in the general population differs in the polymorphisms of DD, DI and II of the ACE gene (13).

Additionally, release of nitric oxide (NO) by endothelial cells plays an important role in the control of local hemodynamics and systemic hypertension. Accordingly, the disturbance in the production of NO in endothelial cells is an important finding for defective vasodilatation and thus hypertension in ADPKD patients (14). In these patients, there is also an impaired endothelial-dependent vasodilation, which can be an early and strong marker for progression of renal disease (6,15). This damage results from the reduction of NO production by endothelial nitric oxide synthase (eNOS) (16) which is encoded by the eNOS gene (17). Therefore, the eNOS gene polymorphisms can alter renal vascular endothelial function, creating kidney failure and hypertension in these patients by effect on NO production (6).

Although high blood pressure and kidney failure in ADPKD individuals require prediction and precise monitoring, the factors associated with the severity of renal disease in these patients have not yet been identified and there is not a good indicator for predicting the clinical course of the disease and its outcomes. Various studies have reported different intra-familial and inter-familial phenotype variations of ADPKD that indicate involvement of both environmental and genetic factors in the disease (18). On the other hand, the polymorphism of the ACE and eNOS genes is different in various populations since it is associated with high blood pressure (19), which may be helpful in predicting the clinical course of these patients (20). However, the relationship between the above-mentioned gene polymorphisms with high blood pressure and kidney failure in ADPKD patients has been different in many studies (21) and is still controversy. Thus, the aim of this study was to evaluate the association between the common polymorphisms of eNOS and ACE genes with clinical manifestations (high blood pressure and kidney failure) in these patients.

Patients and Methods

Study protocol

This case-control study was performed on 75 patients with ADPKD over 18 years of age who referred to Hasheminejad hospital in Tehran (2015 to 2016). Diagnosis of ADPKD was based on patient family history and kidney ultrasound. In addition, 100 attendants of patients who did not mention a specific problem were randomly selected as the control group. First, the study design was explained to all participants and a written consent form received from all of them. Then, participants' data including age, gender, weight, height, BMI (body mass index), history of hypertension (the participants who had a history of taking blood pressure pills), presence or absence of diabetes and IHD (ischemic heart disease), and serum creatinine level were recorded using a checklist. Their blood pressure was taken at least 2 separate visits in Hasheminejad clinic and BP ≥140/90 mm Hg was considered hypertensive. GFR was measured by Cockcroft–Gault formula. None of them had history of IHD. They had not any rise in their serum creatinine level at least for the past 3 months. GFR less than 60 mL/min was considered CRF, since GFR more than 60 mL/min was considered normal.

Subsequently, 7.5 cc of whole blood sample was received from each participant which 5 cc inserted in EDTA solution and 2.5 cc in RNA later or TRIzol solution. The samples were refrigerated at -70°C until sending to the laboratory. Specimens were sent to the genetic laboratory for genetic evaluation. In the laboratory, DNA was extracted using phenol chloroform solution and ethanol deposition techniques (9). Then, genotyping was performed for the I/D polymorphism of ACE gene and Glu298 ASP and T786C polymorphisms of the eNOS gene using PCR electrophoresis and molecular evaluation with specialized primers of these two set genes (Figure 1A-1C).

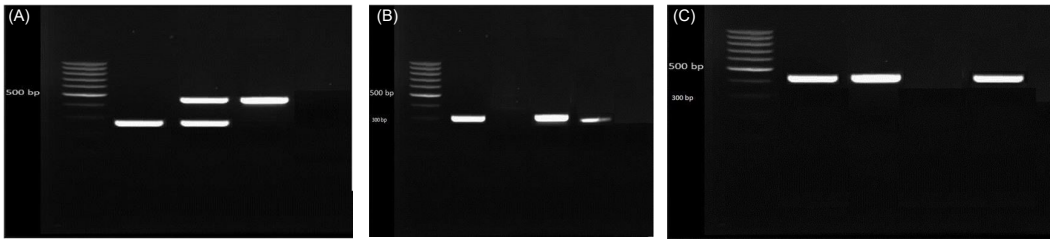

Figure 1. (A) ACE I/D, (B) eNOS Glu298 ASP, (C) eNOS T786C polymorphisms in our study.

Ethical approval

The research followed the tenets of the Declaration of Helsinki. The ethics committee of Iran University of Medical Sciences approved this study (IR. IUMS. REC1395.9311402002). Accordingly, written informed consent was taken from all participants before any intervention. This study is extracted from the nephrology fellowship thesis of Bahareh Madadi at this university.

Statistical analysis

SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, N.Y., USA) was used to analyze the data. Frequency and percentage as well as mean and standard deviation were used to describe the variables. Chi-square test and independent samples *t* test were used for data analysis and comparison between groups, respectively. A *P* value of less than 0.05 was considered statistically significant.

Results

Forty-five patients (60%) in the ADPKD group and 48 participants (48%) in the control group were female. The mean (SD) of age of ADPKD and control groups was 44 ± 15 years and 41 ± 11 years, respectively. The two groups did not have a significant difference in age and gender. There was no significant difference between the two groups in terms of height, weight and BMI too (Table 1). In the ADPKD group, 46 patients (61%) had hypertension and 5 (6.7%) had diabetes, while none of the control people were hypertensive and had diabetes (Table 1). Forty-two patients (56%) had GFR <60 mL/min/1.73 m^2 in the ADPKD group (Table 1).

Table 2 compares the polymorphisms of ACE and eNOS genes between ADPKD and control groups. In the case of ACE gene, the most common polymorphism in the ADPKD group was DD (43%), while in the control group was ID (53%), which was a significant difference between the two groups ($P<0.001$). For the T786C polymorphism of the eNOS gene, the most common type in the ADPKD type was TC (57%), whereas in the control group was TT (68%), which also had a significant difference between the groups ($P<0.001$). However, in both ADPKD and control groups, the most common type of Glu298 ASP polymorphism of eNOS gene was GG (56% and 61%, respectively), with no significant difference between the two groups was detected ($P=0.311$).

Comparing the different characteristics of the two groups of ADPKD patients with and without hypertension, we found that hypertensive ADPKD patients were more male, had higher age and serum creatinine level, and also they had more GFR <60 mL/min/1.73 m^2 (74% versus 28%) (Table 3). However, the polymorphism of ACE and eNOS genes did not differ significantly between them (Table 4 and Figure 2A-2C). In addition, the polymorphism of these genes in ADPKD patients with and without decreased GFR was not significantly different (Table 5).

Table 1. Comparison of characteristics of ADPKD patients and control groups

	ADPKD patients (n=75)	Control group (n=100)	*P* value
Gender			
Male	30 (40%)	52 (52%)	0.115*
Female	45 (60%)	48 (48%)	
Age, year	44 ± 15	41 ± 11	0.110**
Height, cm	168 ± 10	169 ± 7	0.537**
Weight, kg	72 ± 16	74 ± 12	0.447**
BMI, kg/m^2	25.5 ± 5.5	25.9 ± 4	0.566**
Hypertension	46 (61%)	0 (0%)	<0.001*
Diabetes	5 (6.7%)	0 (0%)	0.019*
Creatinine, mg/dL	3.6 ± 3	0.96 ± 0.1	<0.001*
GFR status			
Normal (≥60 mL/min/1.73 m^2)	33 (44%)	99 (99%)	<0.001*
Low (<60 mL/min/1.73 m^2)	42 (56%)	1 (1%)	

* Chi-square test; ** Independent samples *t* test.
GFR, Glomerular filtration rate.

Table 2. Comparison of the polymorphisms of ACE and eNOS genes between the two groups

		ADPKD patients (n=75)	Control group (n=100)	*P* value*
ACE gene polymorphisms	DD	32 (43%)	24 (24%)	<0.001
	II	25 (33%)	23 (23%)	
	ID	18 (24%)	53 (53%)	
eNOS gene polymorphisms (Glu298 ASP)	TT	7 (9%)	14 (14%)	0.311
	GG	42 (56%)	61 (61%)	
	GT	26 (35%)	25 (25%)	
eNOS gene polymorphisms (T786C)	TC	43 (57%)	25 (25%)	<0.001
	CC	15 (20%)	7 (7%)	
	TT	17 (23%)	68 (68%)	

* Chi-square test.

Table 3. Comparison of different characteristics between ADPKD patients with and without hypertension

	ADPKD patients without HTN (n=29)	ADPKD patients with HTN (n=46)	*P* value
Gender			
Male	5 (17%)	25 (54%)	0.001*
Female	24 (83%)	21 (46%)	
Age, year	36 ± 15	50 ± 12	<0.001**
BMI, kg/m^2	23.8 ± 5.0	26.7 ± 5.7	0.058**
Creatinine, mg/dL	2.5 ± 3.1	4.3 ± 2.8	0.015*
GFR status			
Normal (≥60 mL/min/1.73 m^2)	21 (72%)	12 (26%)	<0.001*
Low (<60 mL/min/1.73 m^2)	8 (28%)	34 (74%)	

* Chi-square test; ** Independent samples *t* test.
GFR, Glomerular filtration rate; HTN: hypertension.

Table 4. Comparison of the polymorphism of ACE and eNOS genes between ADPKD patients with and without hypertension

		ADPKD patients without HTN (n=29)	ADPKD patients with HTN (n=46)	*P* value*
ACE gene polymorphisms	DD	12 (41%)	20 (44%)	0.766
	II	11 (38%)	14 (30%)	
	ID	6 (21%)	12 (26%)	
eNOS gene polymorphisms (Glu298 ASP)	TT	4 (14%)	3 (6%)	0.559
	GG	15 (52%)	27 (59%)	
	GT	10 (34%)	16 (35%)	
eNOS gene polymorphisms (T786C)	TC	17 (59%)	26 (56%)	<0.489
	CC	4 (14%)	11 (24%)	
	TT	8 (27%)	9 (20%)	

* Chi-square test.
HTN: hypertension.

Table 5. Comparison of the polymorphism of ACE and eNOS genes between ADPKD patients with and without decreased GFR

		ADPKD patients with GFR>=60 (n=33)	ADPKD patients with GFR<60 (n=42)	*P* value*
ACE gene polymorphisms	DD	15 (46%)	17 (41%)	0.861
	II	11 (33%)	14 (33%)	
	ID	7 (21%)	11 (26%)	
eNOS gene polymorphisms (Glu298 ASP)	TT	5 (15%)	2 (5%)	0.298
	GG	18 (55%)	24 (57%)	
	GT	10 (30%)	16 (38%)	
eNOS gene polymorphisms (T786C)	TC	18 (55%)	25(59%)	0.318
	CC	5 (15%)	10 (24%)	
	TT	10 (30%)	7 (17%)	

* Chi-square test.
GFR, Glomerular filtration rate.

Discussion

The findings of this study showed that patients with ADPKD were more likely to have hypertension and renal failure than the control group. We also found that the polymorphism of the ACE gene and the T786C polymorphism of the eNOS gene differed significantly in ADPKD patients with the control group but the Glu298 ASP polymorphism of the eNOS gene did not differ with the control group. Additionally, hypertensive ADPKD patients were more male, with a higher age, and had significant renal insufficiency. However, the polymorphisms of ACE and eNOS genes between ADPKD patients with and without hypertension and also in patients with and without decreased GFR were not significantly different. Therefore, the polymorphism of the ACE and eNOS genes can help in the diagnosis of ADPKD patients, however, it seems that it is not a helpful marker in predicting the hypertension status and severity of kidney disease in these patients.

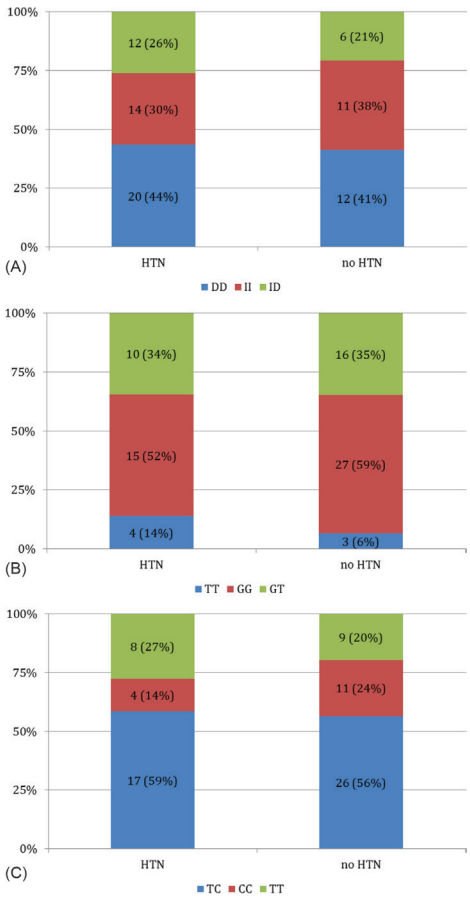

Figure 2. Comparison of (A) ACE polymorphisms, (B) Glu298 ASP polymorphism of eNOS gene, (C) and T786C polymorphism of eNOS gene in ADPKD patients with and without HTN.

Several studies have shown, an endothelial dysfunction due to NO production disturbances in ADPKD patients (22). Similarly, human and animal models of this disease have confirmed changes in endothelium-dependent vasoconstriction (16). However, the results of various studies on the relationship between the severity of kidney disease or hypertension in ADPKD patients and the eNOS gene polymorphism are different. Persu et al assessed the

effect of the Glu298Asp polymorphism on the onset age of renal failure in ADPKD patients and concluded that it was accompanied by a 5-year decrease in mean age of renal failure in male patients. This condition could be due to decreased NOS activity, thereby reducing the endothelial production of NO (17). However, this effect was not observed in women, which is said to be due to the ability to stimulate endothelial NO production by estrogen (22). Tazon-Vega et al reported that NOS3 polymorphisms have little involvement in the ADPKD renal outcomes (8). On the other hand, Stefanakis et al reported that in ADPKD individuals, the Glu298Asp polymorphism of the NOS3 gene was associated with the onset of ESRD and the T allele was associated with an earlier progression to ESRD (23). Dasar et al showed that the prevalence of GG, GT and TT genotypes of NOS3 gene in ADPKD patients with kidney failure were 66.7%, 33.3% and 0%, respectively, in ADPKD patients without kidney failure were 78.6%, 19%, 2.4%, and in control group were 64.3%, 35.7% and 0% respectively, while there was no significant difference between the groups. In addition, there was no significant relationship between Glu298Asp polymorphism and the onset age of ESRD (24). Ramanathan et al also showed that NOS3 tag SNPs do not play a role in the progression of renal failure in ADPKD patients (25). In a review article about relationship of the eNOS gene polymorphism with the progression of renal disease in ADPKD patients, Xue et al reported that the GG genotype of the Glu298Asp variant reduces the progression of renal failure. However, there was no positive relationship of this genotype with hypertension or presence of renal failure (6). Kocyigit et al also reported that, the expression of the eNOS gene is independently predictive of hypertension in ADPKD patients, while there is no link between eNOS gene polymorphism and hypertension in these patients (20). The relationship between other eNOS gene polymorphisms and renal failure in ADPKD patients has also been reported (26-28). Our study showed that the T786C polymorphism of the eNOS gene in ADPKD patients was different with the control group, but the Glu298 ASP polymorphism did not differ with the control group. Additionally, eNOS gene polymorphism was not significantly different between ADPKD patients with and without hypertension and also in patients with and without GFR reduction. Differences in the results of the above studies may indicate differences in the studied populations and differences in the method of study. Since, it seems that, the commonly occurring Glu298ASP polymorphism of the eNOS gene, is associated with high blood pressure in the general population, however, is not able to predict the course of disease and hypertension in ADPKD patients.

On the other hand, it has been shown that the DD genotype of ACE gene in humans is associated with higher serum levels of ACE and hypertension (29, 30). O'Donnell et al also reported in the Framingham heart study participants, that the polymorphism of the ACE gene is related only to men with hypertension and diastolic blood pressure (13). Baboolal et al (31) and Pérez-Oller et al (32) reported that the DD genotype of the ACE gene was associated with the progression of renal failure in ADPKD patients. Tazon-Vega et al also reported that ACE polymorphism has a brief effect on the onset age of renal failure in these patients (8). However, in other studies, no relationship between ACE polymorphism and hypertension or kidney disease course was detected. Likewise, Lee et al found no association between the I/D polymorphism of ACE gene and hypertension or renal failure in ADPKD patients (33). Similarly, Schiavello et al also failed to establish a relationship between the ACE gene polymorphism with the severity of the ADPKD phenotype in several different races (34). Ecder et al failed to establish a relationship between ACE gene polymorphism and hypertension in these patients (35) and Metra et al also found no association between ACE gene polymorphisms and kidney disease progression (36). Pereira et al in a meta-analysis of 13 studies showed that the ACE gene polymorphism does not affect the progression of renal disease in ADPKD patients (37). Kocyigit et al also reported no association between ACE gene polymorphism and hypertension in ADPKD patients (20). Our study also showed that, the frequency of DD polymorphism of ACE gene in ADPKD patients was significantly higher than that in the control group, however the polymorphism of this gene was not significantly different in patients with and without hypertension or renal insufficiency. Therefore, despite the increased activity of the renin-angiotensin-aldosterone system in these patients, it seems that, the ACE gene polymorphism is not able to determine the progression of blood pressure and renal disease in these patients.

Finally, we can conclude that current studies on the gene polymorphism of the renin-angiotensin-aldosterone system and its role in kidney failure and hypertension in ADPKD patients have been performed in different populations with different designs. Hence, the results of various studies are different. Additionally, it appears that more proportion of patients is necessary to investigate the relationship of polymorphisms and manifestations of ADPKD. However, considering the results of the above studies, it can be concluded that, currently no specific polymorphism that can predict the course of the disease in ADPKD patients was existed.

Conclusion

The findings of this study showed that the polymorphism of the ACE gene and the T786C polymorphism of the eNOS gene differed in ADPKD patients with the control group, but the Glu298 ASP polymorphism of the eNOS gene was not different from the control group. However,

the polymorphism of ACE and eNOS genes between was not significantly different in ADPKD patients with and without hypertension or decreased GFR. Therefore, although the polymorphism of the ACE and eNOS genes can help in the diagnosis of ADPKD patients, it seems that it is not a helpful marker for predicting the course of the disease. However, more studies are necessary on this subject.

Limitations of the study

Our investigation was conducted on a limited sample size. Accordingly, additional investigations should be carried out on larger population regarding this subject.

Authors' contribution

TM and BM; data collection and manuscript drafting. AE; genetic study. All authors read and signed the final paper.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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References

1. Harris PC, Torres VE. Polycystic Kidney Disease, Autosomal Dominant. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al, editors. GeneReviews®. Seattle (WA): University of Washington; 1993.
2. Wanic-Kossowska M, Posnik B, Kobelski M, Pawliczak E, Pawlaczyk K, Hoppe K, et al. The polymorphism of the ACE gene affects left ventricular hypertrophy and causes disturbances in left ventricular systolic/diastolic function in patients with autosomal dominant polycystic kidney disease. Sci World J. 2014;2014:707658. doi: 10.1155/2014/707658.
3. Chapman AB, Stepniakowski K, Rahbari-Oskoui F. Hypertension in autosomal dominant polycystic kidney disease. Adv Chronic Kidney Dis. 2010;17:153-63.doi: 10.1053/j.ackd.2010.01.001.
4. Ratnam S, Nauli SM. Hypertension in autosomal dominant polycystic kidney disease: a clinical and basic science perspective. Int J Nephrol Urol. 2010;2:294-308.
5. Gnanasambandan R, Elumalai R, Soundararajan P, Lakkakula BV. Angiotensinogen gene polymorphisms and progression of chronic kidney disease in ADPKD patients. Clin Exp Nephrol. 2016;20:561- 8. doi: 10.1007/s10157-015-1183-2.
6. Xue C, Zhou CC, Sun LJ, He Ll, Xu CG, Dai B, et al. Effects of endothelial nitric oxide synthase gene on end stage renal disease progression in autosomal dominant polycystic kidney disease: eNOS gene and ADPKD. Nephrology. 2014;19:630-7.doi:10.1111/nep.12310.
7. Sans Atxer L, Torra R, Fernandez Llama P. Hypertension in autosomal-dominant polycystic kidney disease (ADPKD). Clin Kidney J. 2013;6:457-63. doi:10.1093/ckj/sft031.
8. Tazon-Vega B, Vilardell M, Perez-Oller L, Ars E, Ruiz P, Devuyst O, et al. Study of candidate genes affecting the progression of renal disease in autosomal dominant polycystic kidney disease type 1. Nephrol Dial Transplant. 2007;22:1567-77. doi:10.1093/ndt/gfm036
9. Zafar I, Tao Y, Falk S, McFann K, Schrier RW, Edelstein CL. Effect of statin and angiotensin-converting enzyme inhibition on structural and hemodynamic alterations in autosomal dominant polycystic kidney disease model. Am J Physiol Renal Physiol. 2007;293:854-9. doi: 10.1152/ajprenal.00059.2007.
10. Torres VE, Abebe KZ, Chapman AB, Schrier RW, Braun WE, Steinman TI, et al. Angiotensin Blockade in Late Autosomal Dominant Polycystic Kidney Diseas. N Engl J Med. 2014;371:2267-76. doi: 10.1056/NEJMoa1402686.
11. Belibi FA, Edelstein CL. Novel targets for the treatment of autosomal dominant polycystic kidney disease. Expert Opin Investig Drugs. 2010;19:315-28.doi: 10.1517/13543781003588491.
12. Sun Y, Zhou H, Yang B-x. Drug discovery for polycystic kidney disease. Acta Pharmacologica Sinica. 2011;32:805-16. doi: 10.1038/aps.2011.29.
13. O'Donnell CJ, Lindpaintner K, Larson MG, Rao VS, Ordovas JM, Schaefer EJ, et al. Evidence for association and genetic linkage of the angiotensin-converting enzyme locus with hypertension and blood pressure in men but not women in the Framingham Heart Study. Circulation.1998; 97:1766-72. doi: 10.1161/01.cir.97.18.176.
14. Zhang YH. Neuronal nitric oxide synthase in hypertension – an update. Clin Hypertens. 2016;22.doi:10.1186/s40885-016-0055-8.
15. Al-Nimri MA, Komers R, Oyama TT, Subramanya AR, Lindsley JN, Anderson S. Endothelial-derived vasoactive mediators in polycystic kidney disease. Kidney Int. 2003; 63:1776-84. doi: 10.1046/j.1523-1755.2003.00913.x.
16. Wang D, Iversen J, Strandgaard S. Endothelium-dependent relaxation of small resistance vessels is impaired in patients with autosomal dominant polycystic kidney disease. J Am Soc Nephrol. 2000;11:1371-6.
17. Persu A, Stoenoiu MS, Messiaen T, Davila S, Robino C, El-Khattabi O, Mourad M, et al. Modifier effect of ENOS in autosomal dominant polycystic kidney disease. Hum Mol Genet. 2002;11:229-41. doi: 10.1093/hmg/11.3.229.
18. Rossetti S, Burton S, Strmecki L, Pond GR, San Millán JL, Zerres K, et al. The position of the polycystic kidney disease 1 (PKD1) gene mutation correlates with the severity of renal disease. J Am Soc Nephrol. 2002;13:1230-7. doi: 10.1097/01.asn.0000013300.11876.37.
19. Li J, Cun Y, Tang WR, Wang Y, Li SN, Ouyang HR, et al. Association of eNOS gene polymorphisms with essential hypertension in the Han population in southwestern China. Genet Mol Res. 2011;10:2202-12. doi: 10.4238/vol10-3gmr1160
20. Kocyigit I, Taheri S, Sener EF, Unal A, Eroglu E, Öztürk F, et al. Endothelial nitric oxide synthase gene expression is associated with hypertension in autosomal dominant polycystic kidney disease. Cardiorenal Med. 2014;4:269-79.

doi:10.1159/000369105.
21. Ramanathan G, Ghosh S, Elumalai R, Periyasamy S, Lakkakula BV. Influence of angiotensin converting enzyme (*ACE*) gene rs4362 polymorphism on the progression of kidney failure in patients with autosomal dominant polycystic kidney disease (ADPKD). Indian J Med Res. 2016;143:748-55. doi: 10.4103/0971-5916.191992
22. Devuyst O.Variable renal disease progression in autosomal dominant polycystic kidney disease: a role for nitric oxide? J Nephrol. 2003;16:449-52.
23. Stefanakis N, Ziroyiannis P, Trygonis S, Lamnissou K. Modifier effect of the Glu298Asp polymorphism of endothelial nitric oxide synthase gene in autosomal-dominant polycystic kidney disease. Nephron Clin Pract. 2008;110:101-6. doi: 10.1159/000157623.
24. Dasar N, Ghaderian SMH, Azargashb E. Human Evaluation of the Glu298Asp polymorphism in NOS3 gene and its relationship with onset age of ESRD in Iranian patients suffering from ADPKD. Int J Mol Cell Med. 2012;1:105-12.
25. Ramanathan G, Periyasamy S, Lakkakula BV. NOS3 tagSNPs does not modify the chronic kidney disease progression in autosomal dominant polycystic kidney disease. Nephrology. 2014;19:537-41. doi: 10.1111/nep.12278.
26. Ramanathan G, Periyasamy S, Lakkakula BV. NOS3 tagSNPs does not modify the chronic kidney disease progression in autosomal dominant polycystic kidney disease. Nephrology (Carlton). 2014;19(9):537-41. doi: 10.1111/nep.12278.
27. Merta M, Reiterová J, Tesař V, Štekrová J, Viklický O. Influence of the endothelial nitric oxide synthase polymorphism on the progression of autosomal dominant polycystic kidney disease and IgA nephropathy. Ren Fail. 2002;24:467-75. doi: 10.1081/JDI-120006773.
28. Walker D, Consugar M, Slezak J, Rossetti S, Torres VE, Winearls CG, et al. The ENOS polymorphism is not associated with severity of renal disease in polycystic kidney disease 1. Am J Kidney Dis. 2003;41:90-4. doi: 10.1053/ajkd.2003.50027.
29. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J clin Invest. 1990;86:1343-6. doi: 10.1172/JCI114844.
30. Ji Ld, Zhang Ln, Shen P, Wang P, Zhang Ym, Xing Wh, et al. Association of angiotensinogen gene M235T and angiotensin-converting enzyme gene I/D polymorphisms with essential hypertension in Han Chinese population: a meta-analysis. J Hypertens. 2010;28:419-28. doi: 10.1097/HJH.0b013e32833456b9.
31. Baboolal K, Ravine D, Daniels J, Williams N, Holmans P, Coles GA, et al. Association of the angiotensin I converting enzyme gene deletion polymorphism with early onset of ESRF in PKD1 adult polycystic kidney disease. Kidney Int. 1997;52:607-13. doi: 10.1038/ki.1997.373.
32. Pérez-Oller L, Torra R, Badenas C, Milà M, Darnell A. Influence of the ACE gene polymorphism in the progression of renal failure in autosomal dominant polycystic kidney disease. Am J Kidney Dis. 1999;34:273-8. doi: 10.1016/s0272-6386(99)70355-0.
33. Lee KB, Kim UK, Lee CC. Association of the ACE gene polymorphism with the progression of autosomal dominant polycystic kidney disease. J Korean Med Sci. 2000;15:431-5. doi: 10.3346/jkms.2000.15.4.431.
34. Schiavello T, Burke V, Bogdanova N, Jasik P, Melsom S, Boudville N, et al. Angiotensin-converting enzyme activity and the ACE Alu polymorphism in autosomal dominant polycystic kidney disease. Nephrol Dial Transplant. 2001;16:2323-7. doi: 10.1093/ndt/16.12.2323.
35. Ecder T, McFann KK, Raynolds MV, Schrier RW. No effect of angiotensin-converting enzyme gene polymorphism on disease progression and left ventricular hypertrophy in autosomal dominant polycystic kidney disease. Am J Nephrol. 2003;23:466-70. doi: 10.1159/000074653.
36. Merta M, Reiterova J, Stekrova J, Rysava R, Rihova Z, Tesař V, et al. Influence of the alpha-adducin and ACE gene polymorphism on the progression of autosomal-dominant polycystic kidney disease. Kidney Blood Press Res. 2003;26:42-9. doi: 10.1159/000069768.
37. Pereira TV, Nunes ACF, Rudnicki M, Magistroni R, Albertazzi A, Pereira AC, et al. Influence of ACE I/D gene polymorphism in the progression of renal failure in autosomal dominant polycystic kidney disease: a meta-analysis. Nephrol Dial Transplant. 2006;21:3155-63.doi: 10.1093/ndt/gf1412.