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Original Paper

Endothelial Nitric Oxide Synthase Gene Expression Is Associated with Hypertension in Autosomal Dominant Polycystic Kidney Disease

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Key Words
Endothelial nitric oxide synthase · Hypertension · Polycystic kidney disease

Abstract
Background/Aims: Early occurrence of hypertension is the prominent feature of autosomal dominant polycystic kidney disease (ADPKD). The role of angiotensin-converting enzyme *(ACE)* gene polymorphism and endothelial nitric oxide synthase *(eNOS)* gene polymorphism in the clinical course of ADPKD is not well understood. However, data about the expression of these genes are lacking. Thus, we aimed to investigate the polymorphisms and expressions of both the *ACE* and *eNOS* genes that affect hypertension in ADPKD. ***Methods:*** Whole blood samples were obtained from all participants. *ACE* and *eNOS* gene polymorphisms and their expressions were analyzed in 78 ADPKD patients and 30 controls. Gene expressions were assessed by quantitative real-time PCR. Twenty-four-hour blood pressure monitoring was performed for the diagnosis of hypertension in all study participants. ***Results:*** *eNOS* expression and the estimated glomerular filtration rate were found to be significantly higher in ADPKD patients without hypertension than in those with hypertension. Each unit of increase in *eNOS* expression led to a 0.88-fold decrease (95% CI: 0.80–0.96) in the risk of hypertension in multiple logistic regression analysis. ***Conclusions:*** *eNOS* gene expression is independently predictive of hypertension in the ADPKD population. This study showed, for the first time, a novel link between *eNOS* gene expression and hypertension in ADPKD.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic cause of chronic kidney disease. Hypertension occurs in the early stages of the disease without evidence of renal functional deterioration [1, 2]. In addition, hypertension is also associated with both rapid progression to end-stage renal disease (ESRD) and increased cardiovascular events in the ADPKD population [3, 4]. The precise mechanism of hypertension has not been clarified yet. Many factors including activation of the systemic renin-angiotensin system (RAS) due to cyst compression, fluid retention as a result of impaired sodium handling, inflammation, endothelial dysfunction due to decreased nitric oxide synthesis, hyperuricemia and also, according to our recent investigations, intrarenal RAS activation independent of systemic RAS have been reported as causes of the hypertension development in ADPKD [5–9]. Unfortunately, there is no available index to predict the outcomes and clinical course of ADPKD.

The release of nitric oxide via endothelial cells plays a crucial role in the control of local hemodynamics and systemic blood pressure [10, 11]. Thus, it has been proposed that gene coding for endothelial nitric oxide synthase *(eNOS)* could have a modifying effect on hypertension. Indeed, the association of *eNOS* gene polymorphism and hypertension has been well established [12].

Early vascular changes and endothelial dysfunction have been shown in ADPKD patients in our previous studies [8, 13]. Moreover, endothelium-dependent relaxation is impaired and endothelial synthase activity is decreased in this population [14, 15]. In addition, depending on the role of RAS in ADPKD, angiotensin-converting enzyme (ACE) activity, which is controlled by the *ACE* gene, has been thought to be associated with disease severity. Therefore, *ACE* gene polymorphism has become a target. Consequently, many authors have focused on the *eNOS* and *ACE* gene polymorphisms to clarify the temporal relationship between the clinical presentation and genetic variability of ADPKD. However, studies have reported conflicting results, and there is no consistent or direct evidence to show the effect of these genes on the clinical aspects of ADPKD as yet [16–19]. Currently, there are no consistent data about the expression of these genes in the ADPKD population to be found in the literature. In this regard, we hypothesized whether *eNOS* and *ACE* gene polymorphisms and also their expressions may have an effect on the course of ADPKD. We compared both hypertensive and normotensive ADPKD patients with healthy subjects with regard to *eNOS* (Glu298Asp, intron 4 VNTR) and *ACE* gene (DD/DI/II) polymorphisms and the expression of these genes.

Subjects and Methods

Study Population

ADPKD patients who were registered by the Medical Faculty of Erciyes University in the Turkish Society of Nephrology Polycystic Kidney Disease Working Group Registry were evaluated for this study between June 2012 and October 2013. The study was approved by the local ethics committee. All participants were included after signing written informed consent forms. ADPKD patients who were known to have reduced glomerular filtration rates (GFR; <30 ml/min) and those with cardiovascular disorders were excluded from the study. The patients were also scanned for hypertension by ambulatory blood pressure monitoring for the diagnosis of hypertension. Finally, 78 ADPKD patients (with and without hypertension) and 30 healthy subjects were eligible for the study.

The enrolled patients were reevaluated for biochemical parameters as described below. The estimated GFR (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [20].

Biochemical Measurements

Blood samples were taken from the vein of the antecubital fossa, with subjects in a seated position and following a 20-min rest after 12 h of fasting. Glucose, creatinine, and lipid profiles were determined using standard methods.

DNA Extraction and Genotyping

Samples of about 2 ml of blood with EDTA were obtained from all participants. Genomic DNA was extracted from peripheral blood mononuclear cells using standard methods with a High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). The final DNA concentration was determined with a NanoDrop 2000 spectrophotometer (Thermo Scientific). All genetic studies were performed in the Genome and Stem Cell Center at Erciyes University (GENKOK).

The *eNOS* Glu298Asp polymorphism was determined by using a PCR-restriction fragment length polymorphism-based protocol with the following primer sequences: forward 5′-CATGAGGCTCAGCCCCAGAAC-3′ and 5′-AGTCAATCCCTTTGGTGCTCAC-3′. DNA samples from each patient and control were amplified in a volume of 50 μl reaction mixture containing 200 ng of DNA, 2.5 mM $MgCl_2$, a deoxyribonucleotide mix (2.5 mM each), oligonucleotide primers (10 pmol each), and *Taq* DNA polymerase (2.5 U/μl). The reaction mixture was subjected to 30 cycles at 95°C for 1 min, annealing at 60°C for 1 min, and extension at 70°C for 1 min. The PCR products were checked on 2.5% agarose gel, and only the 206-bp *eNOS* product was digested overnight at 37°C with *Mbo*I restriction endonuclease enzyme. The enzymatic digestion generates 1 fragment of 206 bp from the normal allele and 2 fragments of 119 and 87 bp from the mutant allele [21].

Two oligonucleotide primers (forward 5′-AGGCCCTATGGTAGTGCCTT-3′ and reverse 5′-TCTCTTAGTGCTGTGGTCAC-3′) were used to amplify a 27-bp repeat sequence in intron 4 of the *eNOS* gene. The 50-μl reaction mixture contained 50 ng genomic DNA, 10× PCR buffer, 0.2 mM of each dNTP, $MgCl_2$ (1.5 mM), *Taq* DNA polymerase (1 U/ml), and 10 pmol of each primer. The reaction mixture was subjected to 35 cycles at 94°C for 30 s, annealing at 56°C for 1 min, and extension at 72°C for 1 min. The PCR products were analyzed by 2% agarose gel stained with ethidium bromide.

The *ACE* gene I/D polymorphism was amplified only by the PCR method. The 50-μl final volume of the PCR mixture consisted of 50 ng genomic DNA, 10× PCR buffer, 0.2 mM of each dNTP, $MgCl_2$ (1.5 mM), *Taq* DNA polymerase (1 U/ml), and 10 pmol of each primer [22]. The *ACE* deletion polymorphism is characterized by a 190-bp fragment, whereas the presence of the insertion leads to a 490-bp fragment.

mRNA Expression Studies by Quantitative Real-Time PCR

Whole blood samples were obtained from all participants for evaluating mRNA expression. mRNA expression profiling was performed with total RNA extraction from leukocytes by the TRIzol-based extraction method. The final RNA concentration was determined with a NanoDrop 2000 spectrophotometer. We used a Transcriptor High Fidelity cDNA Synthesis Kit (Roche) for cDNA synthesis. *eNOS* and *ACE* gene expressions were assessed by quantitative real-time PCR with a RotorGene Q PCR cycler (Qiagen). β-Actin was used as the endogenous reference.

Ambulatory Blood Pressure Measurements

Twenty-four-hour blood pressure monitoring was performed using a Del Mar Medical Ressurometer Model P6 (Del Mar Reynolds, Irvine, Calif., USA), and the results were assessed using the manufacturer's computer software. Ambulatory measurements were conducted once every 15 min from 7 a.m. until 11 p.m., and once every 30 min from 11 p.m. until 7 a.m. Evaluation was performed taking the mean values of day and night blood pressures into account. Hypertension was considered to be present if the average systolic pressure was ≥130 mm Hg and/or the average diastolic pressure was ≥80 mm Hg for a whole day or if the individual was taking antihypertensive medication.

Statistical Analysis

A histogram and Q-Q plots were examined, and the Shapiro-Wilk test was used to check data normality; the Levene test was used for variance homogeneity. Values are expressed as frequencies and percentages, means and standard deviations or medians and interquartile ranges. Pearson's χ^2 analysis and Fisher's exact test were used to compare the differences between categorical variables. The independent-samples t test and the Mann-Whitney U test were used to compare the differences between numerical variables. One-way analysis of covariance was used to analyze the data by adjusting the p values by age. Univariate and multiple logistic regression analyses were performed to identify the risk factors for having hypertension in polycystic

Table 1. Comparison of demographical, *ACE* and *eNOS* gene polymorphism, and expression data between the control and ADPKD patients

Variables	Healthy controls (n = 30)	ADPKD patients (n = 78)	p
Age, years	41±11.1	44±13.4	0.410
Gender			
Male	20 (66.7)	37 (47.4)	0.073
Female	10 (33.3)	41 (52.6)	
ACE			
Normal	5 (16.7)	18 (23.1)	0.034
Heterozygous	9 (30.0)	39 (50.0)	
Homozygous	16 (53.3)	21 (26.9)	
eNOS Glu298Asp			
Normal	12 (40.0)	43 (55.1)	0.086
Heterozygous	12 (40.0)	30 (38.5)	
Homozygous	6 (20.0)	5 (6.4)	
eNOS intron 4			
Normal	10 (33.3)	60 (76.9)	<0.001
Heterozygous	20 (66.7)	15 (19.2)	
Homozygous	0 (0.0)	3 (3.8)	
ACE expression	5.25 (2.31–51.63)	2.10 (1.30–5.99)	0.001
eNOS expression	3.08 (0.46–53.08)	0.09 (0.02–1.93)	<0.001

Values are expressed as n (%) for χ^2 analysis, medians (IQR) for the Mann-Whitney U test, and mean ± SD for age.

kidney disease patients. Logistic regression analysis was performed for both crude and age-adjusted data. Odds ratios were calculated with 95% confidence intervals (CI). Significant variables at the 0.10 level were considered in the multiple model, and backward elimination was applied using the Wald statistic. Also, a receiver operating characteristic (ROC) curve was generated for the *eNOS* expression value in predicting hypertension in ADPKD patients. The area under the ROC curve was calculated with a 95% CI. A cutoff value was also determined using the Youden index, and the sensitivity and specificity values were calculated for this value. Analyses were conducted using R 3.0.2 software. A p value <5% was considered statistically significant.

Results

The demographical features, polymorphisms, and expression data from patients and controls are summarized in table 1. Briefly, of the 78 patients, 47.4% were male with a mean age of 44.4 ± 13.4 years. The 30 controls had a mean age of 41.1 ± 11.1 years, and 66.7% of them were male. There were no significant differences in *eNOS* Glu298Asp polymorphism between the groups. However, there were significant differences in *ACE* and *eNOS* intron 4 polymorphisms between the two groups. Also, there were significant differences in *ACE* [2.10 (1.30–3.99) vs. 5.25 (2.31–51.63), p = 0.001] and *eNOS* [0.09 (0.02–1.93) vs. 3.08 (0.46–53.08), p < 0.001] expression between the ADPKD patients and the control subjects. Additionally, 24-hour ambulatory blood pressure monitoring confirmed the status of all study participants (table 2).

eNOS expression and the eGFR were found to be significantly higher in ADPKD patients without hypertension than in those with hypertension (table 3; fig. 1). On the other hand, levels of uric acid and triglycerides were higher in patients with hypertension than in nonhypertensive patients. There were no significant differences in other parameters between the two groups (table 3). In univariate analysis, *eNOS* expression negatively correlated with

Table 2. Data from ambulatory BP measurements of the study subjects

Parameters	ADPKD patients without HT (n = 31)	APDKD patients with HT (n = 47)	Healthy controls (n = 30)	p
Systolic BP				
24 h	115.4±5.4	127.0±8.0*,†	110.8±6.4	<0.001
Daytime	120.4±6.9	132.3±8.1*,†	117.6±8.1	<0.001
Nighttime	107.4±5.3	126.5±8.2*,†	104.1±6.3	<0.001
Diastolic BP				
24 h	74.2±3.8	87.8±6.9*,†	71.8±4.5	<0.001
Daytime	79.1±4.8	91.0±6.9*,†	76.9±5.3	<0.001
Nighttime	69.8±4.6*	85.6±7.1*,†	67.0±4.3	<0.001
Mean BP				
24 h	87.4±4.0*	99.7±5.0*,†	84.8±4.6	<0.001
Daytime	92.6±4.7	104.7±5.1*,†	90.4±5.4	<0.001
Nighttime	82.3±4.4*	98.6±5.3*,†	79.2±4.4	<0.001

Average values (mm Hg) are shown. BP = Blood pressure; HT = hypertension. * $p < 0.05$ versus healthy controls; † $p < 0.05$ versus ADPKD patients without HT.

average 24-hour mean blood pressure (r = –0.275, p = 0.028), average daytime diastolic blood pressure (r = –0.294, p = 0.021), and average daytime systolic BP (r = –0.279, p = 0.026).

An increase of 1 year in age led to a 1.06-fold increase in the risk of hypertension (95% CI: 1.02–1.10) in univariate logistic regression analysis and a 1.02-fold risk increase (95% CI: 0.96–1.07) in multiple logistic regression analysis. Each unit of increase in eGFR led to a 0.96-fold decrease in the risk of hypertension (95% CI: 0.94–0.98) in univariate logistic regression analysis and a 0.95-fold risk decrease (95% CI: 0.92–0.98) in multiple logistic regression analysis.

After univariate analysis, age, *eNOS* Glu298Asp, eGFR, uric acid, and triglycerides were found to be statistically significant. These variables were taken into a separate model, and backward elimination was applied using the Wald statistic. After the analysis, age, *eNOS* expression, and eGFR were found to be independent risk factors. Moreover, each unit of increase in *eNOS* expression led to a 0.91-fold decrease in the risk of hypertension (95% CI: 0.85–0.98) in univariate logistic regression analysis and a 0.88-fold risk decrease (95% CI: 0.80–0.96) in multiple logistic regression analysis (table 4).

The ROC analysis of the *eNOS* expression values in predicting hypertension in ADPKD patients is given in figure 2. The area under the ROC curve was found to be 0.65 (0.51–0.80). A 79.6% sensitivity and 58.3% specificity were obtained for the cutoff value of 0.56.

Discussion

The present study reveals several interesting and novel findings regarding *eNOS* and *ACE* gene polymorphisms and their expression in the ADPKD population. While the *eNOS* GluAsp298 polymorphism did not show a significant difference, *ACE* and *eNOS* intron 4 gene polymorphisms were significantly different between ADPKD patients and controls. Importantly, both *ACE* and *eNOS* expression was significantly decreased in ADPKD patients compared to normal subjects. Moreover, we demonstrated that hypertensive ADPKD patients had low levels of *eNOS* gene expression in comparison with normotensive ones. Indeed, we showed for the first time that *eNOS* gene expression was independently predictive of hypertension in the ADPKD population.

Table 3. Comparison of polymorphisms and laboratory features between the ADPKD patients with and those without hypertension

Variables	Hypertension		p	p[a]
	absent (n = 31)	present (n = 47)		
Gender				
Male	12 (50.0)	26 (48.1)	0.880	0.470
Female	12 (50.0)	28 (51.9)		
Age, years	37.78±13.02	47.38±12.74	0.004	–
ACE				
I/D	14 (58.3)	25 (46.3)	0.564	0.917
I/I	4 (16.7)	14 (25.9)		
D/D	6 (25.0)	15 (27.8)		
ACE expression	1.96 (0.90–3.92)	2.14 (1.50–3.99)	0.468	0.196
eNOS Glu298Asp				
Normal	9 (39.1)	34 (63.0)	0.025	0.384
Heterozygous	14 (60.9)	16 (29.6)		
Homozygous	0 (0)	4 (7.4)		
eNOS intron4				
Normal	19 (79.2)	41 (75.9)	0.496	0.806
Heterozygous	5 (20.8)	10 (18.5)		
Homozygous	0 (0)	3 (5.6)		
eNOS expression	1.55 (0.06–6.20)	0.07 (0.02–0.36)	0.033	*<0.004*
eGFR, ml/min/1.73 m^2	99.8 (84.01–108.39)	64.6 (47.30–88.58)	<0.001	*<0.001*
Glucose, mg/dl	90.00 (84.00–96.00)	94.50 (87.50–103.00)	0.073	0.498
Uric acid, mg/dl	5.09±1.64	6.86±1.76	0.001	*<0.027*
Sodium, mm	139.00 (138.00–140.00)	140.00 (137.00–141.00)	0.758	0.939
Potassium, mm	4.17±0.43	4.40±0.50	0.062	0.287
Calcium, mg/dl	9.34±0.48	9.30±0.58	0.777	0.804
Phosphorus, mg/dl	3.40 (2.90–3.70)	3.40 (3.10–3.80)	0.986	0.810
Albumin, g/dl	4.00 (3.80–4.30)	4.00 (3.80–4.20)	0.346	0.636
Triglyceride, mg/dl	105.00 (84.00–143.00)	141.00 (107.00–194.00)	0.014	*<0.032*
Total cholesterol, mg/dl	189.00 (159.00–208.00)	195.00 (177.00–220.00)	0.341	0.727
HDL cholesterol, mg/dl	48.10 (38.00–56.00)	40.00 (33.00–46.00)	0.022	0.079
LDL cholesterol, mg/dl	114.40 (87.40–137.00)	119.20 (83.60–140.40)	0.609	0.952
Proteinuria, g/day	0.15 (0.10–0.29)	0.17 (0.12–0.30)	0.406	0.382

Values are expressed as n (%), means ± SD, or medians (IQR). HDL = High-density lipoprotein; LDL = low-density lipoprotein.
[a] p values adjusted for age; values in italics are significant.

Hypertension occurs in the early stages of the disease in the ADPKD population that lead to a diagnosis and is often related to progression to ESRD and increased cardiovascular problems [2]. Although the activation of systemic RAS due to local cyst pressure on the renal vasculature has been a widely accepted hypothesis with regard to the development of hypertension in ADPKD, the evidence of early vascular changes and endothelial dysfunction in normotensive ADPKD patients showed the necessity of further studies in this area [5, 13–15]. Furthermore, recent studies have shown that intrarenal RAS activation, hyperuricemia, impaired nitric oxide generation, inflammation, and increased oxidative stress could be associated with the development of hypertension in ADPKD patients [8, 9, 13, 15, 23]. In addition, genetic predisposition might be related to early occurrence of hypertension. Hence, Schrier et al. [24] reported that the likelihood of hypertension in patients with ADPKD is significantly greater if their parents are hypertensive. Otherwise, intrafamilial heterogeneity has been shown in disease expression, and discrepancies between twins have also been reported [25, 26]. Currently, the underlying reasons for both

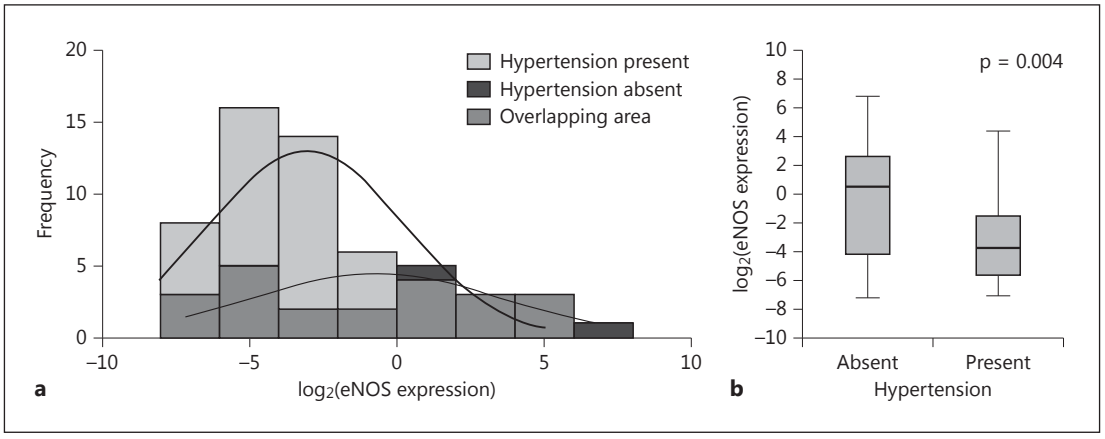

Fig. 1. a Distribution of $\log_2$(eNOS expression) values between ADPKD patients with and those without hypertension. **b** Box plot showing the change of eNOS expression values between ADPKD patients with and those without hypertension.

the variability and early occurrence of hypertension in ADPKD patients remain to be elucidated.

Expression levels of human genes vary extensively between individuals. Genetic, epigenetic, and environmental factors determine phenotypic variability, including susceptibility to disease or treatment outcome [27]. Cellular characteristics and functions are largely determined by gene expression, and expression levels differ between individuals. However, it is not clear how these levels are regulated. While many *cis*-acting DNA sequence variants in promoters and enhancers that influence gene expression have been identified, only a few polymorphic *trans*-regulators of human genes are known [28].

The role of RAS and specifically ACE activity in ADPKD is an intriguing issue as it relates to the therapeutic potential of ACE inhibitors in the control of hypertension. However, Ecder and Schrier [29] concluded that the activation of RAS contributes to the progression of renal disease in ADPKD independently of its effect on blood pressure. Moreover, an increased plasma ACE activity has been demonstrated in essential hypertension and cardiovascular disease [30, 31]. The *ACE* insertion/deletion (I/D) polymorphism is strongly associated with the level of circulating enzymes. This polymorphism of the *ACE* gene can result in different levels of ACE. The homozygous polymorphism for the D allele (DD genotype) is associated with higher serum ACE levels and hypertension in humans [32, 33]. Depending upon the relation between RAS and ADPKD, many authors investigated whether *ACE* gene polymorphisms in this population affect the clinical course and progression of renal disease. Some authors demonstrated an association between the *ACE* DD genotype and progression to ESRD in ADPKD [34, 35], although others did not find such a relationship [18, 36]. The meta-analysis of 13 studies recently conducted by Pereira et al. [37] showed that *ACE* gene polymorphism has no effect on the progression of renal disease in ADPKD. Additionally, Ecder et al. [18] reported that *ACE* gene polymorphism was not associated with blood pressure in their cohort. In conjunction with this study, our study showed that *ACE* gene polymorphism was not significantly different between hypertensive ADPKD patients and normotensive ones. Recently, He et al. [30] showed that *ACE* mRNA expression was not associated with essential hypertension in a Chinese population. To the best of our knowledge, there are no available data on *ACE* gene expression and its relation to hypertension in ADPKD patients. Therefore, this study aimed to investigate the association between *ACE* gene expression and hypertension in ADPKD. While ADPKD patients showed significantly decreased *ACE* expression compared to control

Table 4. Univariate and multiple logistic regression analyses in identifying hypertension in patients with ADPKD

Variables	Univariate regression		Multiple regression, OR (95% CI)
	crude OR (95% CI)	adjusted OR (95% CI)[a]	
Gender			
Male	1.00	1.00	–
Female	1.08 (0.41–2.82)	1.57 (0.53–4.65)	–
Age (years)	1.06 (1.02–1.10)	–	1.02 (0.96–1.07)
ACE			
I/D	1.00	1.00	–
I/I	1.96 (0.54–7.12)	0.83 (0.19–3.60)	–
D/D	1.40 (0.44–4.43)	0.92 (0.26–3.21)	–
ACE expression	0.98 (0.95–1.01)	0.98 (0.95–1.02)	–
eNOS Glu298Asp			
Normal	1.00	1.00	–
Heterozygous	0.30 (0.11–0.85)	0.26 (0.08–0.84)	–
Homozygous	–	–	–
eNOS intron4			
Normal	1.00	1.00	–
Heterozygous	0.93 (0.28–3.09)	0.76 (0.21–2.74)	–
Homozygous	–	–	–
eNOS expression	0.95 (0.90–1.00)	0.91 (0.85–0.98)	0.88 (0.80–0.96)
eGFR (ml/min/1.73 m^2)	0.96 (0.94–0.98)	0.96 (0.94–0.98)	0.95 (0.92–0.98)
Glucose (mg/dl)	1.02 (0.99–1.05)	1.01 (0.98–1.04)	–
Uric acid (mg/dl)	1.64 (1.19–2.26)	1.45 (1.02–2.05)	–
Calcium (mg/dl)	0.88 (0.35–2.16)	0.91 (0.34–2.43)	–
Phosphorus (mg/dl)	1.04 (0.46–2.38)	1.11 (0.45–2.74)	–
Albumin (g/dl)	0.45 (0.12–1.72)	0.75 (0.20–2.73)	–
Triglycerides[b] (mg/dl)	1.11 (1.01–1.22)	1.08 (0.99–1.18)	–
Total cholesterol[b] (mg/dl)	1.07 (0.95–1.21)	1.02 (0.92–1.13)	–
HDL cholesterol (mg/dl)	0.97 (0.92–1.01)	0.96 (0.93–1.01)	–
LDL cholesterol[b] (mg/dl)	1.05 (0.93–1.17)	1.01 (0.90–1.11)	–
Proteinuria (g/day)	6.56 (0.35–123.24)	4.18 (0.23–76.57)	–

OR = Odds ratio; HDL = high-density lipoprotein; LDL = low-density lipoprotein. [a] Adjusted for age. [b] OR are calculated for a 10-unit increase in related variables.

subjects, there were no significant differences between hypertensive ADPKD patients and normotensive ones.

Nitric oxide is generated via NOS, which plays an important role in the regulation of blood pressure [10, 11]. Endothelial dysfunction via impaired nitric oxide production could contribute to the pathogenesis of hypertension in ADPKD [14, 15]. Genetic polymorphisms in the promoter region of the *eNOS* gene may be responsible for the variations in the genetic control of plasma nitric oxide. DNA polymorphisms in the *eNOS* gene have been shown to be associated with constitutive eNOS expression [38]. On the other hand, the gene coding *eNOS* could be a modifier gene; hence, *eNOS* gene polymorphism has been shown to be associated with early renal functional decline in ADPKD patients [16]. In contrast, Walker et al. [17] did not find any correlation between renal disease severity and *eNOS* polymorphism in ADPKD. Moreover, one of the most common *eNOS* polymorphisms, Glu298Asp, has already been related to essential hypertension, a condition which is characterized by endothelial dysfunction [12]. Persu et al. [16] also found that Glu298Asp is strongly associated with renal outcomes in ADPKD. However, a similar observation was not reported with regard to intron 4 polymorphism. Indeed, the association between *eNOS* gene polymorphism and renal disease

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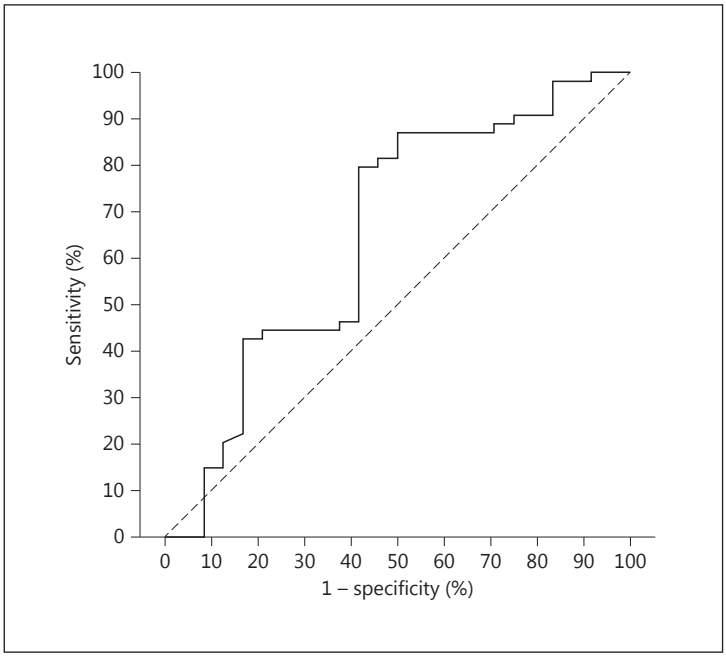

Fig. 2. ROC curve for eNOS expression values in predicting hypertension in ADPKD patients. The area under the ROC curve was found to be 0.65 (0.51–0.80). A 79.6% sensitivity and 58.3% specificity were obtained for the cutoff value of 0.56.

progression in ADPKD is yet to be elucidated, that is as to whether it relates to hypertension induction via impaired vasodilatation. These findings led us to investigate the role of Glu298Asp intron 4 polymorphisms and *eNOS* gene expression in an ADPKD population with regard to hypertensive state and also to make a comparison with healthy subjects. We found that *eNOS* gene expression was decreased in ADPKD patients compared to controls and that hypertensive ADPKD patients showed significantly lower levels even if compared to normotensive ones. Moreover, *eNOS* gene expression was independently predictive of hypertension in ADPKD according to our study results. Conclusively, for the first time, this study showed a novel link between *eNOS* gene expression and hypertension in ADPKD, which makes it highly valuable.

We suggest that screening of eNOS expression levels may predict hypertension and future cardiovascular events in the ADPKD population. Decreased eNOS expression may alert physicians to follow ADPKD patients closely with regard to early hypertension occurrence and cardiovascular events. Also, some medications which increase the nitric oxide level, such as nebivolol, may be preferable to other antihypertensive agents in this population.

The present study has some limitations. First, it is based on a small sample size and its study design is observational. Second, the number of controls did not match that of the ADPKD patients. Finally, protein levels were not assayed.

In conclusion, this study demonstrated that *ACE* and *eNOS* gene polymorphisms are not associated with ADPKD patients with regard to hypertension, while only *eNOS* gene expression is significantly decreased in hypertensive ADPKD patients in comparison with normotensive ones. Moreover, only *eNOS* gene expression is independently predictive of hypertension in the ADPKD population. This study may provide a background on the important role of *eNOS* gene expression in the development of hypertension in ADPKD patients.

Disclosure Statement

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