Therapeutic Apheresis and Dialysis

Therapeutic Apheresis and Dialysis 2016; ••(••):••–••
doi: 10.1111/1744-9987.12458
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Toll-Like Receptors in the Progression of Autosomal Dominant Polycystic Kidney Disease

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Abstract: Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary cause of chronic kidney disease. The intriguing role of innate immune system and inflammation become a target for potential therapeutic approach to slow progression. When toll-like receptors (*TLRs*) signaling and their receptors activate, they start a cascade of intracellular signaling that induces the production of the inflammatory cytokines and chemokines. Thus, we aim to investigate the association of *TLRs* between progression of ADPKD. Ninety ADPKD patients and ninety matched controls were enrolled this prospective study and were followed during 3 years. *TLR-2* and *TLR-4* gene polymorphisms and expressions were measured. Hypertension was diagnosed with ambulatory blood pressure monitoring. Rapid progression was defined as sustained decline in estimated glomerular filtration rate (eGFR) of more than 5 mL/min per 1.73 m^2 per year. *TLR-4Asp299Gly* polymorphisms were significantly different between patient and control group ($P < 0.05$). Also, *TLR-2* and *TLR-4* gene expressions were significantly different between the ADPKD patients and the control subjects ($P < 0.05$). The expression levels of both *TLR-2* and *TLR-4* were found to be higher in the rapid progression groups comparing the slow progression group ($P < 0.05$). *TLR-2* gene expression, hypertension and uric acid were found to be independent risk factors in identifying rapid progression in ADPKD patients. *TLR-2* and *TLR-4* gene expressions are associated with rapid progression in ADPKD patients. *TLRs* may play a role in the progression of ADPKD. **Key Words:** Inflammation, Polycystic kidney disease, Toll-like receptors.

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary cause of end stage renal disease (1). Early occurrence of hypertension and progression to chronic kidney disease without any intervention are the prominent features of the disease. Kidney enlargement, activation of the renin angiotensin aldosterone system, inflammation, oxidative stress and endothelial dysfunction are also contributing factors to the progression of ADPKD (2).

Several animal and in vitro studies have shown the role of cytokines in the development of interstitial inflammation leading to kidney injury and progression in ADPKD. High levels of these pro-inflammatory cytokines in the cystic epithelium are correlated with interstitial macrophage accumulation (3–5). It has been demonstrated that increased urinary monocyte chemoattractant protein-1 excretion, which appeared to precede elevation in serum creatinine and the development of proteinuria was found in ADPKD patients (4). It has been recently shown that higher plasma levels of IL-6, IL-8 and systemic inflammation markers were found in ADPKD patients with normal renal function (6–8).

Toll-like receptors (*TLRs*) are an emerging family of receptors that recognize pathogen-associated molecular patterns and promote the activation of leukocytes and intrinsic renal cells (9). TLRs have an extracellular recognition domain comprising leucine-rich repeats, a transmembrane region and an intracellular Toll/IL-1-receptor signaling domain (TIR). Upon ligand binding and subsequent formation of a sustainable homodimer or heterodimer, the TIR domain functions as a scaffold for the TIR domains of various

Received January 2016; revised April 2016.
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adaptor molecules. Binding and dimerization initiate downstream signaling that leads to the secretion of pro-inflammatory cytokines and, in the case of endosomal TLRs, of type I interferons via various pathways (10,11). Toll-like receptors (TLRs), nucleotide-binding oligomerization domain receptors (NLRs) and PYD domains-containing protein 3 (NLRP3) inflammasome regulate inflammatory and repair processes in the kidneys. These receptors are protective in the host defense and play a crucial role in self-perpetuating tissue damage in inflammatory and immune-mediated diseases. It has been shown that both TLR2 and TLR4 signaling and also genetic polymorphisms have deleterious effect on several inflammatory kidney disorders such as urinary tract infections, lupus nephritis, acute kidney injury, renal transplant rejection, glomerulonephritis and diabetic nephropathy (12–15). *TLR 2* and *TLR*4 play distinct roles in the pathogenesis of renal fibrosis; *TLR 2* initiates proinflammatory responses, whereas *TLR 4* mediates both proinflammatory and pro-fibrotic pathways (16).

There are no data on the role of both *TLR 2* and *TLR 4* gene polymorphisms and expressions in the clinical course of patients with ADPKD. Thus, we aimed to investigate the potential role of *TLRs* via the inflammation pathway in the progression of ADPKD patients.

PATIENTS AND METHODS

Study population

Between February 2012 and February 2015, 90 patients were enrolled in the study from the Turkish Society of Nephrology Polycystic Kidney Disease Working Group Registry in Kayseri, Turkey. The diagnosis of ADPKD was established on clinical grounds and family history and confirmed by the ultrasonographic criteria described by Pei et al. (17). After the study group was assembled, patients were asked to participate after receiving informed consent. Enrolled patients were re-evaluated in terms of systemic inflammation, urinary tract stones and infection. Urinary infection was defined as an infection only if confirmed by one or more positive urinary cultures. None of the patients showed any signs of either stones or infection. Based on these facts, we recruited a matched control group ($n = 90$) from people who were admitted to our family medicine center for routine check-up. Inclusion criteria were no known diseases and not currently taking any drugs. We did not enroll hypertensive patients to our study according to baseline 24 h evaluation of hypertensive state by using singular ABPM. However, 35 patients (35%) were slightly non-dipper hypertensive according to night-time measurements. We also measure blood pressure annually at the clinic visits and totally 15 patients were excluded from the study during follow-up.

The enrolled patients were followed up for 3 years and biochemical parameters were recorded annually. The estimated GFR (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (18). 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. All samples were collected at the beginning of the study.

The definition of rapid progression may vary according to country or region. However, a general definition of progression may be used, which includes a change of category of eGFR or albuminuria or both, as well as numeric changes over an established period of time. After the baseline evaluation, 90 patients were followed up for a period of 24 months. Rapid progression was defined as a sustained decline in eGFR of more than 5 mL/min per 1.73 m^2 per year according to KDIGO guidelines (19). This study was conducted in accordance with the Helsinki Declaration of the World Medical Association.

DNA isolation and genotyping studies for TLR-2 (−196 to −174del) and TLR-4 (Asp299Gly) gene polymorphisms

Two milliliter venous blood samples with ethylenediaminetetraacetic acid (EDTA) were taken from patients and the control group to study gene polymorphisms. The standard method was used for the extraction of genomic DNA samples according to the manufacturers' instructions (Roche, Mannheim, Germany). Polymorphism at *TLR-2* –196 to–174del was investigated by the polymerase chain reaction (PCR) method using specific primers, a forward primer 5′-CACGGAGGCAGCGAGAAA-3′ and a reverse primer 5′-CTGGGCCGTGCAAAGAAG-3′. PCR was carried out in a reaction volume of 50 μL containing 50 ng of genomic DNA, 10 pmoles of each primer, 2.5 mM $MgCl_2$, a deoxyribonucleotide mix (2.5 mM each), and *Taq* DNA polymerase (2.5 U/μL). Following the initial denaturation at 95 °C for 5 min, amplification was carried out for 35 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 40 s and extension at 72 °C for 40 s. The final extension was prolonged to 7 min. PCR products were visualized under UV following electrophoresis using 4% agarose gel stained with ethidium bromide. The 286 base pair (bp) PCR product corresponded to *TLR2* ins/ins, and the 264 bp product corresponded to homozygous deletion while the 286 bp and 264 bp bands belonged to heterozygous individuals.

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The PCR-restriction fragment length polymorphism (RFLP) technique was carried out in order to identify the *TLR4* (Asp299Gly; rs4986790) genotype in patient and control groups. A 50 µL total reaction volume with 10 pmol of both 5′-GATTAGCATACTTAGACTACTACCTCCATG-3′ forward primer and 5′-GATCAACTTCTGAAAAAGCATTCCCAC-3′ reverse primer, 50 ng of genomic DNA, 10 pmoles of each primer, 2.5 mM $MgCl_2$, a deoxyribonucleotide mix (2.5 mM of each), and *Taq* DNA polymerase (2.5 U/µL, Fermentas, Germany) was used for genotyping. The PCR conditions for *TLR4* polymorphism were as follows: after an initial denaturation step at 95 °C for 5 min, amplification was carried out by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, followed by a final elongation cycle at 72 °C for 5 min. Ten microliters of *TLR4* PCR products was digested with *Nco I* restriction endonuclease (New England Biolabs, USA) at 37 °C overnight and then analyzed by 4% agarose gel electrophoresis. A 252 bp band corresponded to *TLR4* Asp, a 224 bp band corresponded to homozygous Gly, while 252 bp and 224 bp bands belonged to heterozygous individuals (Fig. 1).

RNA isolation, reverse transcription-PCR and quantitative real-time PCR

Total RNA extractions were performed using TRIzol reagent (Roche) from venous blood samples according to the manufacturer's instructions. Total RNA was treated with DNase I (Fermentas, Vilnius, Lithuania) to remove genomic DNA contamination from samples. RNAs were stored at –80 °C until use. Quality controls of the eluted RNA were carried out using a Biospec-Nano spectrophotometer (Shimadzu Biotech, Kyoto, Japan). First-strand cDNA synthesis was performed with 1 microgram of the eluted RNA from each sample using a cDNA synthesis kit from Roche (High Fidelity cDNA synthesis kit) according to the provided protocol. cDNA was amplified for 10 min at 29 °C, and for 5 min at 85 °C. For Real-Time Ready Catalog Assay (LOT 900016408, Roche) 1 µL of each primer (20 pmol/µL), Light Cycler 480 Probes Master Mix and 5 µL of cDNA were added to each well of the PCR plate. The quantitative Real-Time PCR reactions were performed using a Roche Light Cycler 480 for the *TLR-2* and *TLR-4* genes. The cDNA was validated by PCR amplification of the *ACTB* gene (β-actin). The cycling conditions were as follows: 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 s and 60 °C for 30 s, 72 °C for 1 min and a final cooling phase consisting of one cycle of 30 s at 40 °C. All the samples were run in duplicate and the mean for each sample was used for statistical evaluations. The Quantitative Real Time PCR data were normalized using the $2^{\ CT}$ method.

Ambulatory blood pressure measurements

Twenty-four-hour blood pressure monitoring was performed using a Del Mar Medical Pressurometer Model P6 (Del Mar Reynolds, Irvine, CA, 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 (24 h) or if the individual was taking antihypertensive medication. We evaluated the ABPM as mean arterial pressure (MAP) values. MAP: [(Diastolic blood pressure + (Systolic blood pressure-Diastolic blood pressure) / 3)]

Statistical analysis

Histogram and q-q values were examined and the Shapiro–Wilk test was performed to assess data normality. The Levene test was used to assess variance homogeneity. To compare the differences between

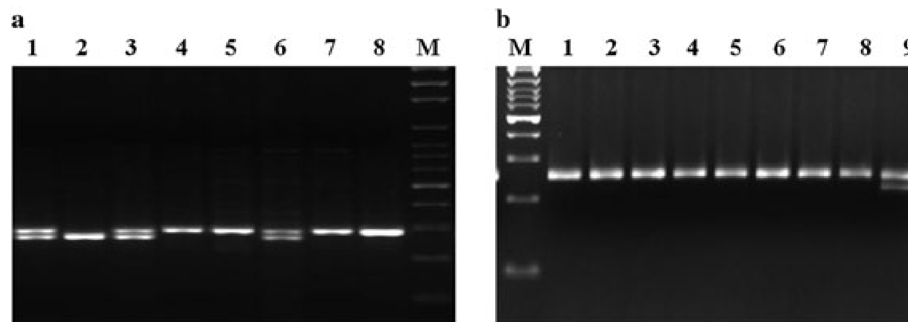

FIG. 1. Electrophoretic patterns of the fragments generated by polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (RFLP) for the polymorphisms. A: *TLR-2* -196 to -174 del, lane 4, 5, 7, 8: ins/ins; lane 1, 3, 6: ins/del; lane 2: del/del B: *TLR-4*Asp299Gly, lanes 1–8 wild type, lane 9: heterozygote genotype. M, Molecular weight marker of 100 bp.

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groups, Pearson's χ^2 analysis was applied for categorical variables; the independent samples *t*- test and Mann–Whitney *U*-tests were applied for continuous variables. Univariate and multiple logistic regression analyses were applied to identify the risk for each factor and to determine the independent risk factors. Significant variables at $P < 0.10$ were included in multiple models and backward elimination was performed using the Wald statistic. Odds ratios were calculated with 95% confidence intervals. Analyses were conducted using R 3.1.1 (www.r-project.org). A *P*-value less than 5% was considered statistically significant.

RESULTS

The demographic features, polymorphisms, and expression data from patients and controls are summarized in Table 1. Briefly, of the 90 patients, 45% were male with a mean age of 43.8 ± 13.1 years. The 90 controls had a mean age of 45.3 ± 12.6 years, and 36% of them were male. There were no significant differences in *TLR-2196-174del* polymorphism between the groups. However, there were significant differences in *TLR-4Asp299Gly* polymorphisms between the two groups. Also, there were significant differences in *TLR-2* and *TLR-4* gene expressions between the ADPKD patients and the control subjects ($P < 0.05$) (Fig. 2). Additionally, while there was significant difference between rapid and slow progression group in terms of eGFR at the end of follow-up (57 ± 31 mL/min per 1.73 m^2 vs. 69 ± 28 mL/min per 1.73 m^2, $P < 0.05$, respectively), however, eGFR levels were similar in both groups at the beginning of the study (75 ± 28 mL/min per 1.73 m^2 vs. 78 ± 30 mL/min per 1.73 m^2, $P > 0.05$, respectively)

The expression levels of both *TLR-2* and *TLR-4* were found to be higher in the rapid progression group ($P < 0.05$) (Fig. 3). All of the mean arterial blood pressure (daytime, nighttime and 24 h) were found to be higher in the rapid progression group ($P < 0.05$). A one unit increase in *TLR-2* and *TLR-4* expressions led to a 1.17- and 1.08-fold rapid progression risk increase, respectively ($P < 0.05$) (Table 2). Additionally, *TLR-2* gene expression, hypertension and uric acid were found to be independent risk factors in identifying rapid progression of ADPKD patients.

DISCUSSION

This study, for the first time, was undertaken to further the potential role of *TLRs* gene polymorphism and expressions in the progression of ADPKD. Three major findings are presented based on the study results. Firstly, only *TLR-4 Asp299Gly* polymorphisms are different between ADPKD patients and controls; however both *TLR-2* and *TLR-4* gene expressions are significantly different. Secondly, increased *TLR-2* and *TLR-*

TABLE 1. *Comparison of demographical, TLR-2 and TLR-4 gene polymorphism, and expression data between the control and autosomal dominant polycystic kidney disease (ADPKD) patients*

	Groups			
Variable	Control (n = 90)	Patient (n = 90)	*P*-value	OR (95%CI)
Gender				
Female (%)	58 (64)	49 (55)	0.227	1.00
Male (%)	32 (36)	41 (45)		1.78 (0.70–4.54)
Age (years)	45.3 ± 12.6	43.86 ± 13.1	0.630	0.99 (0.96–1.03)
eGFR†, mL/min per 1.73 m^2	98.1 ± 17.3	77.0 ± 32.1	<0.001	1.63 (1.41–1.85)
BMI (kg/m^2)	23.7 ± 3.5	25.6 ± 4.1	0.120	1.16 (1.06–1.26)
Mean arterial BP (mm Hg)				
24 h	83 ± 4.9	95 ± 5.8	<0.001	1.45 (1.30–1.60)
Daytime	89 ± 5.4	102 ± 6.2	<0.001	1.53 (1.35–1.73)
Nighttime	77 ± 4.5	91 ± 5.5	<0.001	1.75 (1.47–2.09)
TLR-2				
196-174del				
Normal	60 (66.0)	65 (72)	0.330	1.00
Heterozygous	30 (34.0)	21 (24)		0.53 (0.19–1.48)
Homozygous	0 (0.0)	4 (4)		–
Expression	0.65 (0.46–0.81)	1.01 (0.77–1.81)	<0.001	44.26 (4.81–407.59)
TLR-4				
Asp299Gly				
Normal	81 (90)	90 (100.0)	0.015	1.00
Heterozygous	9 (10)	0 (0.0)		–
Expression	1.03 (0.76–1.36)	1.37 (0.91–2.49)	0.012	2.42 (1.06–5.54)

†Calculated by the CKD-EPI formula. Values are expressed as *n* (%), mean ± SD or median (1st–3^{rd}quartiles). BP, Blood pressure; BMI, Body mass index; OR, Odds ratio; CI, Confidence interval.

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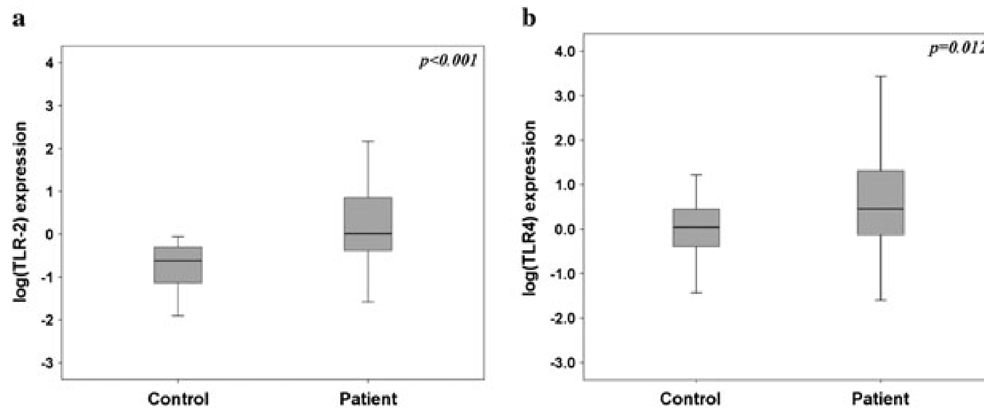

FIG. 2. Box plot showing the change of *TLR-2* and *TLR-4* expression values in peripheral mononuclear cells between autosomal dominant polycystic kidney disease (ADPKD) patients and control.

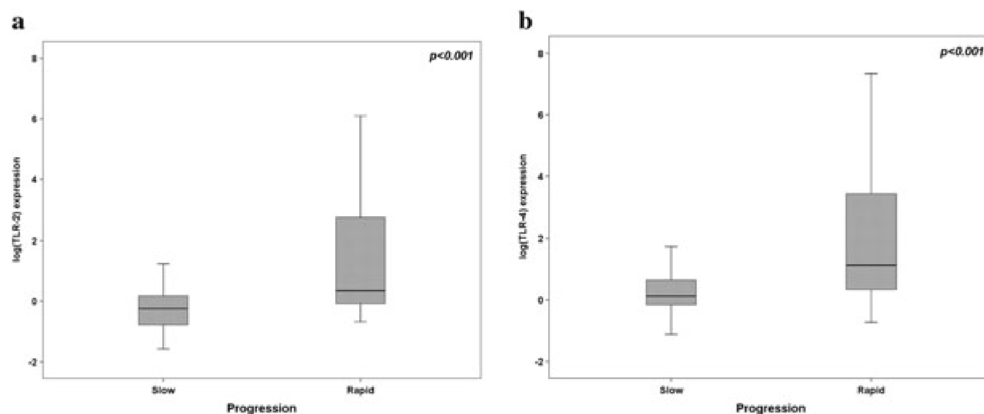

FIG. 3. Box plot showing the change of *TLR-2* and *TLR-4* expression values between rapid and slow progression groups.

4 gene expressions are related to the rapid progression of the disease; however, genetic profiling does not differ between the rapid and slow progression groups. Thirdly, *TLR-2* gene expression, hypertension and uric acid level are independently predicting the rapid progression of ADPKD.

Autosomal dominant polycystic kidney disease is one of the hereditary and progressive systemic disorders that results in end stage renal disease (1). Due to its systemic and genetic pattern, many authors have performed research on the early stage of the disease to find measurements for halting the disease progression (20,21). It has been recently shown that systemic inflammation is evident in early phase ADPKD patients and this inflammation may contribute to both cardiovascular disease and kidney function deterioration (7,8,22,23). It is thought that the activation of systemic and local immune responses contributes to renal dysfunction, but the mechanisms of activation are poorly understood (9). Otherwise, it has been well established that inflammation exists in chronic kidney disease and plays a crucial role in the progression of CKD (24,25).The prevention of this two-way inflammation process may be important in ADPKD patients in terms of slowing disease progression.

Recently, studies of ADPKD have been focused on the potential role of both inflammation and immune system including cytokines, biomarkers, receptors and molecules. The substantial role of interstitial inflammation in renal dysfunction has been well-established in ADPKD patients (26). Authors have recently identified that the between the association of innate immune system progression of ADPKD. Mrug et al. (27) showed that higher levels of markers were expressed for alternatively activated macrophages, indicative of the immune responses, in severely affected mice than mildly affected ones. Zhou et al. (28) has demonstrated that CD14, the validated marker of activated macrophages, is highly upregulated in the kidneys of severely affected mice. Additionally, urine CD14 level was found in ADPKD patients and the level was correlated with the increased total kidney volume. CD14 is expressed at high levels in

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TABLE 2. *Univariate and multiple logistic regression analyses in identifying progression rate in patients with ADPKD*

Variable	Between group comparisons			Logistic regression analysis	
	Slow progression ($n=51$)	Rapid progression ($n=39$)	P-value	Univariate OR (95%CI)	Multiple OR (95%CI)
Gender					
Female (%)	30 (58.1)	14 (35.8)	0.093	1.00	–
Male (%)	21 (41.9)	25 (64.2)		2.27 (0.87–5.96)	–
Age (years)	42.21 ± 12.8	48.86 ± 12.7	0.034	1.04 (1.01–1.08)	–
Mean arterial BP (mm Hg)					
24 hour	92 ± 5.4	99 ± 6.0	<0.001	1.22 (1.11–1.33)	1.31 (1.15–1.79)
Daytime	99 ± 5.9	104 ± 6.3	<0.001	1.18 (1.08–1.29)	1.45 (1.33–1.90)
Nighttime	87 ± 5.2	95 ± 5.5	<0.001	1.33 (1.18–1.50)	1.79 (1.55–2.04)
TLR-2					
196-174del					
Normal	36 (70.5)	29 (75.9)	0.383	1.00	–
Heterozygous	13 (25.3)	10 (24.1)		1.55 (0.48–5.01)	–
Homozygous	2 (4)	0 (0.0)		–	–
Expression	0.85 (0.57–1.18)	1.27 (0.95–6.70)	<0.001	1.17 (0.98–1.41)	1.32 (1.01–1.71)
TLR-4					
Asp299Gly					
Normal	100 (100.0)	100 (100.0)	–	–	–
Heterozygous	–	–		–	–
Expression	1.10 (0.89–1.62)	2.19 (1.27–10.81)	0.001	1.08 (1.01–1.17)	–
BMI (kg/m^2)	24.9 ± 4.1	25.7 ± 4.4	0.293	1.09 (0.98–1.20)	–
Proteinuria(g/day)	0.12 (0.09–0.25)	0.33 (0.14–0.58)	0.002	4.64 (0.79–27.30)	–
Hs-CRP (mg/L)	3.45 (3.36–4.96)	4.13 (3.45–9.93)	0.003	1.22 (1.05–1.41)	–
Uric acid (mg/dL)	5.50 ± 1.74	6.61 ± 2.29	0.032	1.32 (1.03–1.70)	1.39 (1.05–1.84)
Glucose (mg/dL)	87.0 (80.0–95.0)	92.0 (80.0–98.0)	0.445	1.01 (1.00–1.03)	–
Albumin (g/dL)	4.16 ± 0.39	4.02 ± 0.41	0.153	0.41 (0.12–1.41)	–
BUN (mg/dL)	16.0 (12.0–24.0)	19.0 (15.0–28.0)	0.038	1.03 (0.99–1.07)	–
Hemoglobin (g/L)	13.87 ± 1.58	14.03 ± 1.86	0.683	1.06 (0.80–1.41)	–

Values are expressed as n (%), mean ± SD or median (1st to 3rdquartiles). CI, Confidence interval; OR, Odds ratio.

inflammatory cells such as macrophages, monocytes, non-myeloid cells and including cells of the kidney and liver. CD14 facilitates the binding of lipopolysaccharides to *TLR* to activate the innate immune system. It has been shown that upregulation of CD14 expression and increased levels of the proteolytically shed CD14 variant are indicative of immunological activation or cell injury in ADPKD. Thus, the renal tubule-derived CD14 could activate *TLR4* signaling either locally or in more distal segments of the nephron, long before the infiltration of inflammatory cells (29). The specific role of immunity in ADPKD pathogenesis is further supported by cystogenesis-inhibiting effects of immunosuppressive drugs (30–32). These results diverted us to seek the promising role of *TLRs* in the clinical course of patients with ADPKD.

Danger associated molecular patterns (DAMPs) may play important roles in their activation and that specific pattern recognition receptors (PRRs) may regulate the response of macrophages to DAMPs. Initially, DAMPs were expected to attest cell death. The PRRs that have been most intensely investigated as receptors for DAMPs are the *TLRs* (33). *TLRs* play a role in recognizing pathogen-associated molecular patterns and promote the activation of leukocytes and intrinsic renal cells. Moreover, expression of *TLR*-1,2,3,4 and 6 was shown in tubular epithelial cells suggesting that these *TLRs* might contribute to the activation of immune responses in tubulointerstitial injury. In addition, *TLR*-dependent activation of circulating immune cells could lead to cytokine production that either directly or indirectly contributes to renal injury (9).

Recently, the role of TLRs has been investigated in kidney diseases including acute renal failure, renal transplantation rejection, sepsis-induced renal failure, glomerulonephritis and urinary tract infection (9,12,34).

It has also been shown that *TLRs* have a role in the kidney aging process in animal models (35). However, the relationship between ADPKD and *TLRs* has not been clarified. Furthermore, we evaluated the role of *TLRs* in the progression of ADPKD. In our cohort of subjects who were at different stages of ADPKD, expression of *TLRs* was higher in the rapid progression group than in both the slow progression group and controls. These results suggest that *TLR* dependent inflammation may contribute to the progression of ADPKD. Our study supports the recently proposed hypothesis that inflammation is an upstream event in the pathogenesis of ADPKD and may be a contributory factor in its progression (7,26).

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Hypertension occurs in approximately 60% of patients with ADPKD before kidney function deterioration and it is associated with both glomerular filtration decline and cardiovascular complications (2). A cross-sectional study of 198 ADPKD patients demonstrated the strong relation between hypertension and decreased eGFR (36). A survival analysis of 506 ADPKD patients concluded that, patients with diagnosis of hypertension at age <35 progressed to ESRD 14 years earlier (51 vs 65 years old) than patients who were normotensive until >35 years old (37). Moreover, a prospective study with 198 ADPKD patients by following 100 months revealed that hypertension was an independent risk factor for CKD progression (38). Schrier et al. (39) have recently reported that both early-onset and severity of hypertension are associated with progression to ESRD in patients with ADPKD. Our study has similar findings to the literature that the increased blood pressure was positively correlated with rapid progression of this devastating disease. Our study showed that increased mean arterial blood pressure also associated with rapid progression.

Also, the present study demonstrated that serum uric acid level independently predicts the rapid progression of ADPKD. This finding suggests an independent effect of hyperuricemia on the progression of ADPKD and is consistent with studies in the general population that have indicated a role for uric acid in the development and progression of chronic kidney disease (40–42).

There are several limitations for this study. First, the control and patients groups did not match in terms of gender. Second, we did not perform genetic profiling of ADPKD patients whether they have PKD1 or PKD2 mutations. Third, our patients with ADPKD were normotensive according to 24-h evaluation of ABPM, but 35 patients (35%) were slightly non-dipper hypertensive according to nighttime measurements. Therefore, we could not completely exclude the effect of hypertension in disease progression. On the other hand, multivariate analysis showed the effect of TLR expression on ADPKD progression independently. Finally, we did not measure total kidney volume of the patients with ADPKD for the evaluation of progression.

CONCLUSIONS

This study showed the relationship between toll-like receptors and the clinical course of autosomal dominant polycystic kidney disease. *TLR-2* and *TLR-4* gene expression may be associated with rapid progression in our study participants. Further studies are needed to investigate the potential role of TLRs via inflammation and immune pathways in ADPKD patients.

Acknowledgements: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee, informed consent was obtained from all individual participants included in the study. This study was supported by Research Foundation of the Erciyes University (Project Number:TSA-2013-4583).

Conflict of Interest: None.

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