NDT
Nephrology Dialysis Transplantation

Original Article

Early renal and vascular changes in ADPKD patients with low-grade albumin excretion and normal renal function

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Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) shows an increase in both urine monocyte chemoattractant protein-1 (MCP-1) and carotid intima–media thickness (CIMT) before changes in serum creatinine concentration. Although microalbuminuria is an index of disease progression, data on whether renal alterations and vascular remodelling are already present at normal or minimally increased levels of urine albumin excretion in early stages of the disease are lacking.

Methods. Forty-eight ADPKD patients (24.8 ± 0.8 years) with normal renal function (MDRD 108.1 ± 3.1 ml/min) and 21 age-matched controls were studied in a cross-sectional study. The urine albumin/creatinine ratio (UACR) above the upper range of controls (6.8 mg/g) was taken as the predictor of renal alterations and vascular remodelling. Urine MCP-1, MCP-1 fractional excretion (FE_{MCP-1}), endothelial-dependent vascular relaxation (EDVR), aortic pulse-wave velocity (Ao-PWV) and CIMT were chosen as biological markers.

Results. No differences between ADPKD with UACR ≤ 6.8 mg/g and controls were observed in urine MCP-1 (77.7 ± 13.9 versus 57.8 ± 6.3 ng/g), FE_{MCP-1} (91 ± 19 versus $74 \pm 8\%$) and CIMT (0.47 ± 0.06 versus 0.44 ± 0.07 mm), respectively. Conversely, ADPKD with UACR >6.8 mg/g showed values that were different from the two other groups. In addition, patients with UACR >6.8 and <20 mg/g showed greater values for urine MCP-1, FE_{MCP-1} and CIMT (131.8 ± 21.7 ng/g, $159 \pm 31\%$ and 0.55 ± 0.05 mm, respectively), as compared with patients with UACR ≤ 6.8 mg/g. The same pattern was found in a subset of normotensive ADPKD patients. No differences were found in EDVR and Ao-PWV.

Conclusion. In young ADPKD patients, normal levels of UACR suggest that renal interstitium is comparable to that in healthy subjects and indicate an absence of subtle atherosclerotic changes in the carotid arteries. Likewise, early renal and vascular changes may be present at UACR below the levels defined as microalbuminuria.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common Mendelian hereditary kidney disorder, responsible for ∼7% of dialysis patients in Argentina [1]. After a long phase of arterial hypertension and microalbuminuria, there appears a rather abrupt and relentless fall of kidney function, leading to end-stage renal disease in >50% of patients [2,3]. Whereas monitoring of disease progression through glomerular filtration rate (GFR) changes is well established, few studies have dealt with the clinical significance of renal and vascular markers in early phases of the disease. Thus, Cowley *et al*. found increased expression of monocyte chemoattractant protein-1 (MCP-1) in a rat model of ADPKD [4]. This was followed by the demonstration of augmented urinary excretion of MCP-1 in adult patients with ADPKD before appreciable increases in serum creatinine or total urinary protein excretion [5]. In addition, numerous reports have established that microalbuminuria is a frequent sign of the disease, associated with cardiovascular manifestations and progressive renal failure [6–8]. More recently, altered intima–media thickness of carotid arteries (CIMT), impaired endothelial-dependent vascular relaxation (EDVR) and ultrasound-defined changes in cardiac structures have been reported in normotensive ADPKD patients as well [8–11]. However, no studies have been designed to early assess the meaning of higher levels of albuminuria within the normal range and to explore the systemic nature of the disease. One approach is to look for the association of kidney and vascular markers in a single population of ADPKD patients when GFR is still normal.

We report here a novel relationship among urine albumin excretion (UAE), urine MCP-1 and vascular structure in a

cross-sectional study of young ADPKD patients with normal renal function. The results suggest that normal UAE as seen in healthy controls is a predictor of normal renal and vascular status and that increased UAE below microalbuminuric levels may be associated with early manifestations of the disease.

Methods

Patient selection

Both ADPKD Caucasian patients with no associated renal disease and controls were recruited at the Instituto de Investigaciones Médicas Alfredo Lanari of Universidad de Buenos Aires and Hospital Universitario Austral of Universidad Austral, Buenos Aires, Argentina, during 2005 through 2007. The protocol was approved by the IRB of both institutions, and each subject gave written informed consent. Inclusion criteria were age below 35 years and independently of blood pressure status, absence of overt proteinuria and estimated GFR >80 ml/min/1.73 m^2, as given by the abbreviated MDRD equation [12]. ADPKD was diagnosed by ultrasound, according to established criteria [13].

Study protocol

We used a cross-sectional design that compared the magnitude of UAE with renal and vascular profiles in ADPKD patients. The cut-off for the urine albumin/urine creatinine ratio (UACR) of 6.8 mg/g was taken from the upper limits of age-matched healthy controls. We defined two broad categories of UAE: normal UACR (non detectable to $\leq$6.8 mg/g) and increased UACR ($>$6.8 mg/g). In addition, patients with higher levels of albuminuria within the normal range (UACR $>$6.8–$<$20 mg/g) were classified as intermediate UAE. The rationale of this approach rests on the increasing association between intermediate UAE levels and cardiovascular events in vascular non-diabetic disorders [14–16]. Urine MCP-1 and fractional excretion of MCP-1 (FE$_{MCP-1}$) were considered as outcomes of renal status, and CIMT, EDVR of the brachial artery and aortic pulse-wave velocity (Ao-PWV) as vascular markers. To avoid hypertension as a potential confounder, a subset of ADPKD patients with normal blood-pressure (BP) was separately analysed. Normal BP was defined as office registers $<$140/90 mmHg and/or diurnal average of 24-h BP monitoring (SPACELAB 90217, Issaquah, WA, USA) $<$135/85 mmHg, with no antihypertensive treatment.

Biochemical parameters

Fasting blood samples were taken under disodic citrate 3.5% for MCP-1 and frozen at $-20°$C after centrifugation. Fresh early morning midstream urine specimens were collected, in women in the middle of the menstrual cycle. Microscopy of the urinary sediment was immediately performed thereafter, and the sample was discarded in the case of more than one leucocyte with or without concomitant microorganisms or two erythrocytes per high-power field ($\times$400) [17]. Urine was titrated if necessary to pH 6–8, and supernatants were frozen at $-20°$C until albumin and MCP-1 assays were obtained. UAE was measured with a competitive chemiluminescent enzyme immunoassay (Immulite/Immulite 1000 Albumin, DPC, Los Angeles, CA, USA). The calibration range was 2.5–60 μg/ml, and the analytical sensitivity was 1.0 μg/ml. In our laboratory, the coefficient of variation (CV) was 4.0 and 6.5% at 4.0 and 51.0 μg/ml, respectively, and the recovery was 98 $\pm$ 5% (mean $\pm$ SEM, $n = 3$). Plasma and urine MCP-1 were measured by an enzyme-linked immunosorbent assay using methods recommended by the manufacturer (Biosource International, Amarillo, CA, USA), as already described [5]. The sensitivity was 20 pg/ml, and the recovery was 110 $\pm$ 7% (mean $\pm$ SEM, $n = 5$). Urine MCP-1 was expressed as ng/g of urine creatinine, and FE$_{MCP-1}$ was estimated by (urine MCP-1/serum MCP-1)/(urine creatinine/plasma creatinine) $\times$ 100. The total urinary protein was determined with a quantitative colorimetric method based on the use of pyrogallol red (Proti U/LCR, Wiener lab, Rosario, Argentina) using procedures recommended by the manufacturer. The sensitivity was 1 mg/dl, and the standard curve was lineal up to 125 mg/dl. The inter-assay CV were 6.3 and 4.6% at 6.25 and 50 mg/dl, respectively. Results were expressed in mg/g urinary of creatinine. To test tubular protein reabsorption, α_1-microglobulin in the urine was quantified by the immunoturbidimetric method with a Tina-Quant α_1-microglobulin

Table 1. Baseline characteristics

Characteristics	Controls ($n = 21$)	ADPKD patients ($n = 48$)
Age (years)	26.4 $\pm$ 1.0	24.8 $\pm$ 0.8
Body mass index (kg/m^2)	21.8 $\pm$ 0.7	22.6 $\pm$ 0.5
Sex (M/F)	12/9	22/26
Hypertensive/normotensive	0/21	20/28*
GFR estimated by MDRD[a] (ml/min/1.73 m^2)	111.1 $\pm$ 6.1	108.1 $\pm$ 3.1

[a]Modification of Diet in Renal Disease. Data are mean $\pm$ SE.
*$P < 0.001$ versus controls.

kit (Cobas Integra, Roche Diagnostics GmbH, Manheim, Germany) using the Architect ci8200 (Abbott Diagnostics, IL, USA). The sensitivity was 2.0 mg/l and the standard curve was lineal up to 200 mg/dl. The CV was 6% at 17 mg/dl. Values were expressed as mg/g urine creatinine. Plasma and urine creatinine were determined by the alkaline picrate colorimetric assay.

For the vascular studies, subjects abstained from smoking and exercise, fasted for 12 h before testing and without medication, were kept in a quiet room and in a supine position for 10 min. An echocardiograph Vivid 5 (GE/VingMed Ultrasound, Norway) with a multiple lineal array 10 MHz flat transducer was used. CIMT was measured according to ASE guidelines in a well-defined wall segment of the posterior wall of the common carotid artery, 1 cm below the carotid bulb [18]. Sequential intra-observer measurements showed a correlation coefficient $>$0.8. Values for each patient were expressed in millimetres and represented the mean between left- and right-carotid artery measurements. EDVR and Ao-PWV determinations were performed according to international guidelines [19,20]. Thirty ADPKD patients and 14 controls accepted to participate in the protocol for vascular studies.

Statistical analysis

Assuming a clinically relevant mean difference between groups of 470 pg/mg and 0.13 mm in both urine MCP-1 and CIMT, with an average SD of 420 pg/mg and 0.12 mm, respectively [5,10], we needed 15 cases in each group to obtain 80% power and 5% significance. Biochemical and anthropometrical values are presented as means $\pm$ SEM and vascular studies as means $\pm$ SD. Statistical analysis was performed using the SPSS software (SPSS, Chicago, IL, USA). Non-parametric tests (Mann–Whitney or Kruskal–Wallis) were used for comparisons between two or more groups, and the χ^2 test was employed for comparisons of dichotomic characteristics. $P < 0.05$ was considered as significant.

Results

Patients' characteristics

As shown in Table 1, 48 young patients and 21 healthy controls, with a mean age of $\sim$25 years, were the basis of this study. They did not differ in BMI, sex and estimated GFR. A higher proportion of mild hypertension was present in the ADPKD group ($\sim$42%).

Relationship between UACR, urine MCP-1 and CIMT

Figure 1 shows that in the ADPKD group with normal UACR ($n = 22$), urine MCP-1 (77.7 $\pm$ 13.9), FE$_{MCP-1}$ (91 $\pm$ 19) and CIMT (0.47 $\pm$ 0.06) were not different from controls (57.8 $\pm$ 6.3 ng/g, 74 $\pm$ 8% and 0.44 $\pm$ 0.07 mm, respectively). Conversely, patients with increased UACR (25.2 $\pm$ 4.0 mg/g, $n = 26$) showed higher figures than the two other groups (214.8 $\pm$ 46.5 ng/g, 278 $\pm$ 86% and 0.56 $\pm$ 0.07 mm, respectively) (Figure 1), (unadjusted $P < 0.005$,
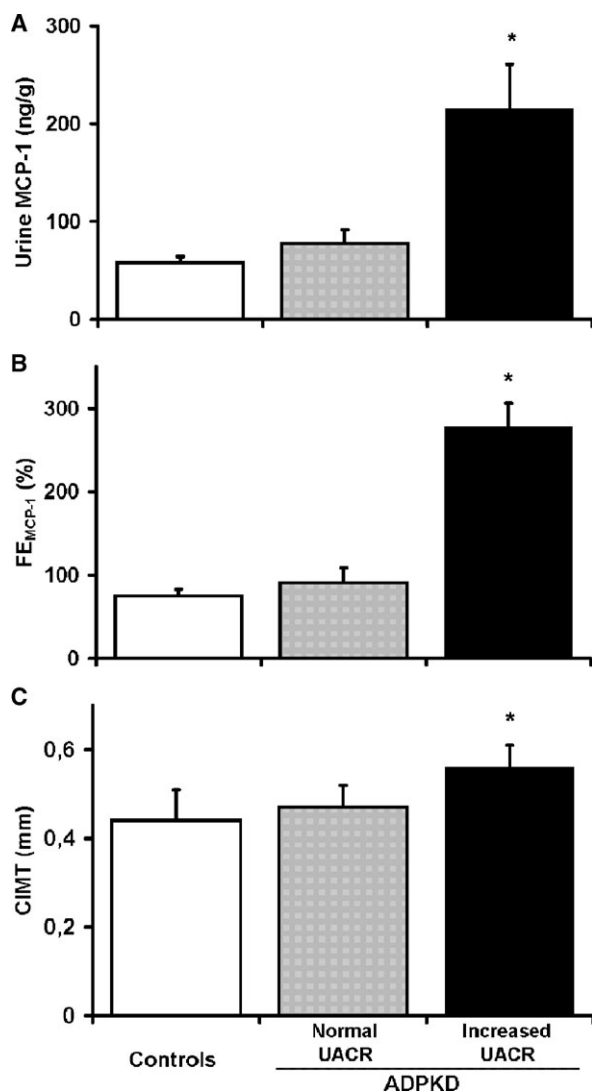
Fig. 1. Urinary excretion of MCP-1, FE_{MCP-1} and carotid intima–media thickness in ADPKD patients and controls, according to urine albumin. Abbreviations: UACR is the urine albumin/creatinine ratio (mg/g). Data are mean ± SE for urine monocyte chemoattractant protein (MCP-1) (panel A) and fractional excretion of MCP-1 (FE_{MCP-1}) (panel B). Data are mean ± SD for carotid intima–media thickness (CIMT) (panel C). Normal UACR (from non-detectable to ≤6.8) and increased UACR (>6.8 mg/g). *$P < 0.05$ versus ADPKD patients with normal UACR and controls.

$P < 0.04$ and $P < 0.01$, respectively). In addition, urine MCP-1, FE_{MCP-1} and CIMT were higher in the group with intermediate UACR ($n = 13$), as compared with patients with normal UACR ($n = 22$) (Table 2). To assess the role of hypertension, patients with normal and high BP were compared (Table 3). Figure 2 shows that increased UACR in normotensive ADPKD patients was also accompanied by higher values of the chemokine, FE_{MCP-1} and CIMT. The same pattern was observed in hypertensive patients (Figure 2), but those with normal UACR were ∼4 years younger than the group with increased UACR (Table 3), precluding a final analysis of the role of BP on albumin excretion in the hypertensive group. Overall, the results suggest that high levels of albuminuria below the cut-off point for microalbuminuria are early markers of renal and vascular alterations in ADPKD, independently of BP status.

Total urine protein and α_1-microglobulin in urine

The total urine protein was not different among all ADPKD patients and controls (45.9 ± 4.3 and 46.5 ± 4.3 mg/g) as already observed by others [5]; in addition, the total urine protein was not different between patients with increased UACR and normal UACR (53.9 ± 4.2 and 41.2 ± 7.4 mg/g). Urinary α_1-microglobulin was detected in one (4.6 mg/g) out of 21 controls and one (4.3 mg/g) out of 48 patients, suggesting an intact protein tubular reabsorption.

EDVR and Ao-PWV

EDVR of the brachial artery was 12.5 ± 8.9% in ADPKD patients and 14.2 ± 8.0 in controls (P N.S.). Similar results were observed for Ao-PWV (6.6 ± 1.6 and 7.1 ± 2.6 m/s, respectively). Lastly, values for EDVR and Ao-PWV were not related to either UACR or BP status. These observations would thus indicate that the increase observed in CIMT was not accompanied with evident damage in EDVR and Ao-PWV in the groups of patients studied here, even when considering the hypertension effect.

Discussion

We present here, in a young ADPKD cohort with normal renal function, novel data on the association of UACR

Table 2. Renal and vascular markers across different levels of urine albumin excretion

	ADPKD Patients		
Renal and vascular markers	Normal UACR ($n = 22$)	Intermediate UACR ($n = 13$)	Microalbuminuria ($n = 13$)
MCP-1[a] (ng/g)	77.7 ± 13.9 (22)*	131.8 ± 21.7 (13)**	287.5 ± 76.5 (13)
FE_{MCP-1}[b] (%)	91 ± 19 (22)*	159 ± 31 (13)	377 ± 153 (13)
CIMT[c] (mm)	0.47 ± 0.06 (14)*	0.55 ± 0.05 (10)	0.57 ± 0.07 (6)

UACR is the urine albumin/creatinine ratio (mg/g). Data are mean ± SE for [a]monocyte chemoattractant protein, [b]urine fractional excretion of MCP-1 and means ± SD for [c]carotid intima–media thickness.
Normal UACR (from non-detectable to ≤6.8), intermediate UACR (>6.8 – ≤20), microalbuminuria (>20 mg/g).
*$P < 0.04$ versus ADPKD patients with intermediate UACR and microalbuminuria.
**$P < 0.04$ versus ADPKD patients with microalbuminuria.

Table 3. Baseline characteristics of ADPKD patients according to levels of urine albumin and blood pressure (BP) status

Characteristics of patients	Normal BP		High BP	
	Normal UACR[a] (n = 12)	Increased UACR[a] (n = 16)	Normal UACR[a] (n = 9)	Increased UACR[a] (n = 11)
Age (years)	25.2 ± 1.2	25.2 ± 1.3	22.7 ± 1.2*	26.6 ± 1.2
Body mass index (kg/m^2)	21.1 ± 0.5	23.5 ± 1.4	23.6 ± 0.8	21.9 ± 0.5
Sex (M/F)	5/7	4/12	6/3	7/4
Mean blood pressure (mmHg)	92.1 ± 2.99	93.2 ± 2.45	98.9 ± 5.86	105.1 ± 5.97
GFR estimated by MDRD[b] (ml/min/1.73 m^2)	110.5 ± 4.4	101.0 ± 6.1	108.4 ± 4.3	102.5 ± 4.0

[a]UACR is the urine albumin/creatinine ratio (mg/g), [b]Modification of Diet in Renal Disease. Data are mean ± SE.
Normal UACR (from non-detectable to ≤6.8), increased UACR (>6.8 mg/g).
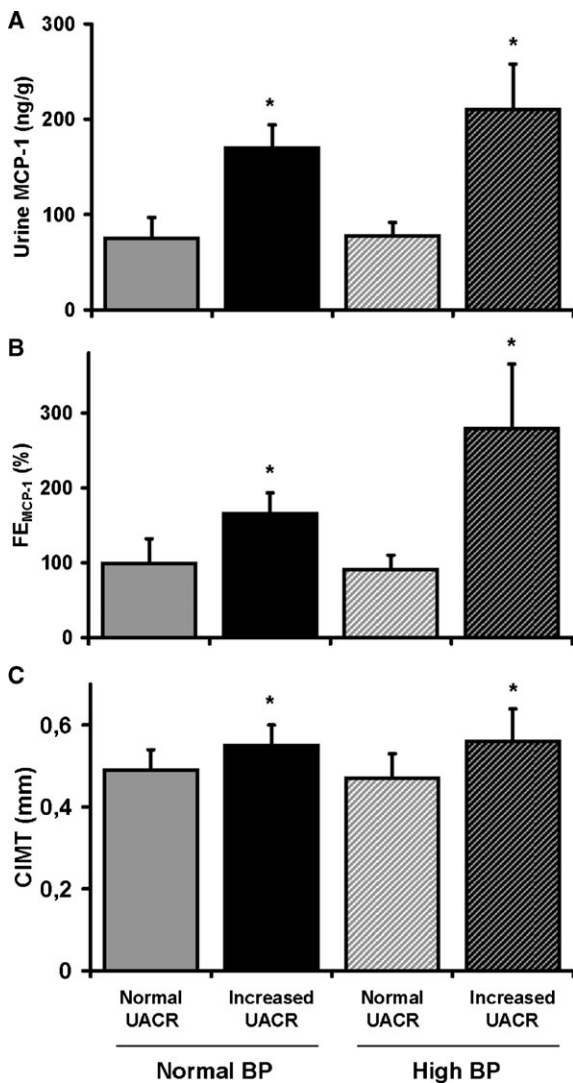*$P < 0.05$ versus high BP patients with increased UACR.

Fig. 2. Urinary excretion of MCP-1, FE_{MCP-1} and carotid intima–media thickness in ADPKD patients and controls, according to urine albumin and blood pressure status. Abbreviations: UACR is the urine albumin/creatinine ratio (mg/g) and BP is blood pressure. Data are mean ± SE for urine monocyte chemoattractant protein (MCP-1) (panel A) and fractional excretion of MCP-1 (FE_{MCP-1}) (panel B). Data are mean ± SD for carotid intima–media thickness (CIMT) (panel C). For normal and high BP, see the text. Normal UACR (from non-detectable to ≤6.8) and increased UACR (>6.8 mg/g). *$P < 0.05$ versus ADPKD patients with normal UACR.

<6.8 mg/g with an unaltered renal interstitium and an absence of subtle atherosclerotic changes in the carotid arteries. Likewise, we have found a relationship between urine MCP-1, CIMT and increased UACR, even below the levels classically defined as microalbuminuria. Thus, ADPKD patients with increased UACR have not only higher urine MCP-1 excretion than controls and patients with normal UACR (Figure 1A) but also a FE_{MCP-1} higher than 200% (Figure 1B). The data agree with the report of Zheng *et al.*, who presented strong evidence that MCP-1 generates within the tubule cells and is secreted into the urine. In that study, however, albumin excretion was not explored, and no relationship between the total urinary protein and urine MCP-1 was found in patients with serum creatinine <1.5 mg/dl [5]. In the current study, no association between the total urinary protein and the urine chemokine or between the total urinary protein and UAE was found either. This unexpected finding might be explained, at least in part, by the lower analytical sensitivity of the method employed here to measure the total protein (1.0 mg/dl), as compared with that of albumin (1.0 µg/ml). Taken together, these observations suggest that UAE and not the total urinary protein is a reliable marker for urine MCP-1 and hence of early renal interstitial inflammation.

The clinical value of microalbuminuria in ADPKD, defined as UACR ≥20 mg/g, is well established. Chapman *et al.* have demonstrated that both overt proteinuria and microalbuminuria were associated with higher mean arterial pressure, lower GFR, larger renal volume and a more aggressive course of the renal disease [6]. Later on, a high prevalence of microalbuminuria was found in normotensive adults and children with ADPKD [7,21], associated with the echocardiographic features of a pre-hypertensive state [8,10]. It seemed appropriate, therefore, to explore whether intermediate UACR between 6.8 and 20 mg/g was associated with subtle manifestations of the disease. We were able to detect in this group higher values for urine MCP-1, FE_{MCP-1} and CIMT, as compared with patients with normal UACR (Table 2). Furthermore, urine MCP-1 increased with urine albumin along the three UACR levels studied. These results suggest, together with the finding in non-ADPKD individuals who are non-diabetic and at high cardiovascular risk (15, 16), that the definition of normal albumin excretion should be reevaluated at the early stages of ADPKD.

Table 4. Vascular studies in controls and ADPKD according to levels of urine albumin and blood pressure (BP) status

Vascular measurements	Controls ($n = 14$)	ADPKD normal BP		ADPKD high BP	
		Normal UACR[a] ($n = 7$)	Increased UACR[a] ($n = 11$)	Normal UACR[a] ($n = 7$)	Increased UACR[a] ($n = 5$)
CIMT[b] (mm)	0.44 ± 0.07	0.49 ± 0.05	$0.55 \pm 0.05^*$	0.47 ± 0.06	$0.56 \pm 0.08^*$
EDVR[c] (%)	14.2 ± 8.0	14.7 ± 10.0	15.9 ± 7.0	6.38 ± 6.6	14.03 ± 12.3
Ao-PWV[d] (m/s)	7.1 ± 2.6	6.9 ± 1.6	6.8 ± 1.1	6.12 ± 1.6	7.60 ± 1.8

[a]UACR is the urine albumin/creatinine ratio (mg/g), [b]carotid intima–media thickness, [c]endothelial-dependent vascular relaxation, [d]aortic pulse-wave velocity.
Data are mean $\pm$ SD.
Normal UACR (from non-detectable to ≤ 6.8), increased UACR (>6.8 mg/g).
$^*P < 0.05$ versus ADPKD with normal UACR and control groups.

Our finding of a similar relationship between UACR and CIMT (Figure 1C), independently of BP status and together with a high urine MCP-1, also has no precedent. A previous study also described an increase in CIMT in a normotensive ADPKD population $\sim$10 years older than the one presented here, with no mention of urinary albumin excretion [10]. Of interest, UACR and CIMT revealed a parallel increase, with CIMT values levelling off unexpectedly at microalbuminuric levels (Table 2). Whether this dissociation was due to the low number of patients in this group or to an effect of antihypertensive drugs cannot be differentiated in the present study. Taken together, the presence of a significant increase in CIMT in normotensive patients with ADPKD may reflect not only an increased risk of cardiovascular disease but may also be an indicator of concomitant renal interstitial inflammation and cyst growth.

Since microalbuminuria has been classically associated with BP status, both in non-ADPKD and ADPKD populations, there is the question whether the increase in both the chemokine and CIMT observed here were caused by arterial hypertension. Previous data of Kocaman *et al.* [10] and of the present study in normotensive patients (Table 3 and Figure 2) suggest that the concomitant renal and vascular changes are intrinsic to ADPKD. A further analysis of GFR in the different groups given in Table 3 is precluded by the fact that the MDRD equation underestimates GFR by $\sim$8.3 ml/min/1.73 m^2 and is less accurate in populations with normal or near normal GFR [22].

Since increased urine albumin may be the consequence of endothelial dysfunction and abnormal vascular permeability, the changes found in our study may be mediated by such a mechanism. Alternatively, since polycystins have been tracked down to vascular smooth muscle cell structures and the cilia of endothelial cells [23,24], vascular events cannot be excluded as a primary phenotype of the disease.

We were not able to find a decreased EDVR of the brachial artery, not even in the subset of normotensive patients (Table 4), which suggests that an intact NO-dependent relaxation of conduit arteries was present in our patients. Our results are thus compatible with previous data where preserved EDVR and impaired amplified pulse-wave reflection were found, supporting the role of small resistance arteries in the vascular phenotype of ADPKD [25–27]. Data from a previous report where EDVR was diminished should be interpreted as an altered smooth muscle response,

as shown in the endothelial-independent relaxation results [10]. Lastly, Ao-PWV was unchanged in our study, which agrees with a recent observation [27], indicating that viscous and elastic components of great blood vessels were not altered at the early stages of the disease.

Increased UAE may be the consequence, at least in part, of decreased albumin re-uptake at a tubular level. We measured here urine α_1-microglobulin, a protein that is freely filtered and reabsorbed at proximal tubular level. α_1-microglobulin was not increased above the control limits in ADPKD patients, in accordance with a previous report [28]. Although additional tubular markers have been found to be increased in ADPKD [29], a tubular mechanism seems to play a minor role in the increased UAE observed in ADPKD.

In summary, our study mainly showed that urinary albumin excretion in the range of a normal population predicts a renal interstitium comparable to that of healthy subjects and absence of vascular remodelling in young ADPKD patients with normal renal function. In addition, it suggests that low grade UAE, below the levels defined as microalbuminuria, is already associated with early pathological processes at renal and vascular levels. Whether the variables measured here could eventually be used as markers of disease progression is a hypothesis that needs further longitudinal exploration.

Conflict of interest statement. C.K. is involved in trials with TAKEDA-NOVARTIS and GILEAD. P.F. is the medical advisor of SERVIER. R.M. is PI in a phase III study on the use of Tolvaptan in ADPKD. Other authors have nothing to declare.

References

1. Iglesias DM, Martin RS, Fraga A *et al*. Genetic heterogeneity of autosomal dominant polycystic kidney disease in Argentina. *J Med Genet* 1997; 34: 827–830
2. Azurmendi P, Fraga A, Muchnik C *et al*. Progresión de la poliquistosis renal autonómica dominante. Influencia de polimorfismos de genes

de sintasa endotelial del óxido nítrico (ecNOS) y del sistema renina angiotensina. *Medicina (Buenos Aires)* 2004; 64: 139–142

3. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. *Lancet* 2007; 369: 1287–1301
4. Cowley BD Jr, Ricardo SD, Nagao S *et al*. Increased renal expression of monocyte chemoattractant protein-1 and osteopontin in ADPKD in rats. *Kidney Int* 2001; 60: 2087–2096
5. Zheng D, Wolfe M, Cowley BD Jr *et al*. Urinary excretion of monocyte-chemoattractant protein 1 in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 2003; 14: 2588–2595
6. Chapman AB, Johnson AM, Gabow PA *et al*. Overt proteinuria and microalbuminuria in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 1994; 5: 1349–1354
7. Martinez-Vea A, Gutierrez C, Bardaji A *et al*. Microalbuminuria in normotensive patients with autosomal-dominant polycystic kidney disease. *Scand J Urol Nephrol* 1998; 32: 356–359
8. Martinez-Vea A, Bardaji A, Gutierrez C *et al*. Exercise blood pressure, cardiac structure, and diastolic function in young normotensive patients with polycystic kidney disease: a prehypertensive state. *Am J Kidney Dis* 2004; 44: 216–223
9. 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: 1371–1376
10. Kocaman O, Oflaz H, Yekeler E *et al*. Endothelial dysfunction and increased carotid intima–media thickness in patients with autosomal dominant polycystic kidney disease. *Am J Kidney Dis* 2004; 43: 854–860
11. Oflaz H, Alisir S, Buyukaydin B *et al*. Biventricular diastolic dysfunction in patients with autosomal dominant polycystic kidney disease. *Kidney Int* 2005; 68: 2244–2249
12. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. *Am J Kidney Dis* 2002; 39(Suppl 1): S1–S266
13. Ravine D, Gibson RN, Walker RG *et al*. Evaluation of ultrasonographic diagnostic criteria for autosomal dominant polycystic kidney disease 1. *Lancet* 1994; 343: 824–827
14. Arnlov J, Evans JC, Meigs JB *et al*. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham Heart Study. *Circulation* 2005; 112: 969–975
15. Ruggenenti P, Remuzzi G. Time to abandon microalbuminuria? *Kidney Int* 2006; 70: 1214–1222
16. Forman JP, Fisher NDL, Schopick EL *et al*. Higher levels of albuminuria within the normal range predict incident hypertension. *J Am Soc Nephrol* 2008; 19: 1983–1988
17. Kasiske BL, Keane WF. Laboratory assessment of renal disease: clearance, urinalysis, and renal biopsy. In: Brenner BM (ed.). *The Kidney*. 6th edn. Philadelphia: W.B. Saunders, 2000, p. 1152
18. Roman MJ, Naqvi TZ, Gardin JM *et al*. American Society of Echocardiography; Society of Vascular Medicine and Biology. Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. *J Am Soc Echocardiogr* 2006; 19: 943–954
19. Corretti MC, Anderson TJ, Benjamin EJ *et al*. International Brachial Artery Reactivity Task Force. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol* 2002; 39: 257–265
20. Asmar R, Benetos A, Topouchian J *et al*. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995; 26: 485–490
21. Sharp C, Johnson A, Gabow P. Factors relating to urinary protein excretion in children with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 1998; 9: 1908–1914
22. Stevens LA, Coresh J, Feldman HI *et al*. Evaluation of the modification of diet in renal disease study equation in a large diverse population. *J Am Soc Nephrol* 2007; 18: 2749–2757
23. Qian Q, Li M, Cai Y *et al*. Analysis of the polycystins in aortic vascular smooth muscle cells. *J Am Soc Nephrol* 2004; 14: 2280–2287
24. Naulism SM, Kawanabe Y, Kaminsky JJ *et al*. Endothelial cilia are fluid shear sensors that regulate calcium signaling and nitric oxide production through polycystin-1. *Circulation* 2008; 117: 1161–1171
25. Clausen P, Feldt-Rasmussen B, Iversen J *et al*. Flow-associated dilatory capacity of the brachial artery is intact in early autosomal dominant polycystic kidney disease. *Am J Nephrol* 2006; 26: 335–339
26. Wang D, Iversen J, Wilcox CS *et al*. Endothelial dysfunction and reduced nitric oxide in resistance arteries in autosomal dominant polycystic kidney disease. *Kidney Int* 2003; 64: 1381–1388
27. Borresen ML, Wang D, Strandgaard S. Pulse wave reflection is amplified in normotensive patients with autosomal-dominant polycystic kidney disease and normal renal function. *Am J Nephrol* 2007; 27: 240–246
28. Flynn FV, Lapsley M, Sampson PA *et al*. Urinary excretion of beta-2 glycoprotein-1(apolipoprotein H) and other markers of tubular malfunction in 'non-tubular' renal disease. *J Clin Pathol* 1992; 45: 561–567
29. Casal JA, Hermida J, Lens XM *et al*. A comparative study of three biomarker tests in autosomal dominant polycystic kidney disease. *Kidney Int* 2005; 68: 948–954