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Hypertension

ORIGINAL ARTICLE

Endothelium-Specific Deficiency of Polycystin-1 Promotes Hypertension and Cardiovascular Disorders

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BACKGROUND: Autosomal dominant polycystic kidney disease is the most frequent hereditary kidney disease and is generally due to mutations in *PKD1* and *PKD2*, encoding polycystins 1 and 2. In autosomal dominant polycystic kidney disease, hypertension and cardiovascular disorders are highly prevalent, but their mechanisms are partially understood.

METHODS: Since endothelial cells express the polycystin complex, where it plays a central role in the mechanotransduction of blood flow, we generated a murine model with inducible deletion of *Pkd1* in endothelial cells (*Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$) to specifically determine the role of endothelial polycystin-1 in autosomal dominant polycystic kidney disease.

RESULTS: Endothelial deletion of *Pkd1* induced endothelial dysfunction, as demonstrated by impaired flow-mediated dilatation of resistance arteries and impaired relaxation to acetylcholine, increased blood pressure and prevented the normal development of arteriovenous fistula. In experimental chronic kidney disease induced by subtotal nephrectomy, endothelial deletion of *Pkd1* further aggravated endothelial dysfunction, vascular remodeling, and heart hypertrophy.

CONCLUSIONS: Altogether, this study provides the first in vivo demonstration that specific deletion of *Pkd1* in endothelial cells promotes endothelial dysfunction and hypertension, impairs arteriovenous fistula development, and potentiates the cardiovascular alterations associated with chronic kidney disease. **(*Hypertension*. 2022;79:2542–2551. DOI: 10.1161/HYPERTENSIONAHA.122.19057.)** • **Supplemental Material**

Key Words: autosomal dominant polycystic kidney disease ■ chronic kidney disease ■ ciliopathies ■ endothelial dysfunction ■ hypertension ■ polycystin

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic disorder of the kidney, affecting around 4 in 10 000 subjects in the general population.[1,2] In ≈80% of cases, the genetic variations responsible for ADPKD are located in *PKD1*, encoding polycystin-1.[3] In addition to the consequences of ADPKD on the kidney, extrarenal and particularly cardiovascular disorders are highly prevalent, including arterial hypertension, left ventricular hypertrophy, and arterial aneurysms, which worsen patient prognosis despite improvement in their overall management.[4,5] Increasing evidence suggests that the presence of abnormal polycystins at the vascular level may be one of the main factors in cardiovascular manifestations in ADPKD. The polycystin complex is found at the base of the primary cilium on vascular endothelial cells (ECs) and plays a central role in the mechanotransduction of blood flow stimuli. In particular, cell studies have shown that polycystins regulate nitric oxide release in response to the increase in shear stress.[6,7] We confirmed this finding in ADPKD

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NOVELTY AND RELEVANCE

What Is New?

Endothelial polycystin deficiency causes endothelial dysfunction, which leads to hypertension.
Endothelial polycystin deficiency aggravates endothelial dysfunction and left ventricular hypertrophy associated with chronic kidney disease.
Endothelial polycystin complex is required for the normal development of arteriovenous fistula.

What Is Relevant?

The experimental design of this study allows to draw pathophysiological conclusions on the direct role of the endothelium in CV disorders of autosomal dominant polycystic kidney disease (ADPKD), which is not possible in the clinical setting.

These results challenge our classical pathophysiological understanding of hypertension in ADPKD, considered to be mainly related to renin angiotensin system activation by the kidney cystic disease and secondary decrease in glomerular filtration rate.

Clinical/Pathophysiological Implications?

Repeated screening for hypertension in children from ADPKD families is required, even in the absence of kidney cysts.
Special attention should be paid during surgery and follow-up of patients with ADPKD after creation of an arteriovenous fistula to reduce the risk of thrombosis.
Therapeutic strategies targeting the endothelium may be of particular interest in patients with ADPKD.

Nonstandard Abbreviations and Acronyms

ADPKD	autosomal dominant polycystic kidney disease
AVF	arteriovenous fistula
CKD	chronic kidney disease
EC	endothelial cell
GFR	glomerular filtration rate

patients by showing a major impairment of nitric oxide-dependent flow-mediated dilatation of conduit arteries at an early stage of the disease.[8] Given the implications of endothelial dysfunction in the cardiovascular and kidney complications of nephropathies,[9] analyzing the specific impact of endothelial polycystin deficiency at both levels is important. We hypothesized that polycystin 1 deficiency at the endothelial level plays a central role in hypertension and the cardiovascular complications of ADPKD. To study this hypothesis, we developed a murine model with endothelium-specific deletion of *Pkd1*. We studied the impact of endothelial polycystin 1 deficiency on vascular function, blood pressure and heart hypertrophy in absence and in presence of chronic kidney disease (CKD) and evaluated its impact on the maturation of arteriovenous fistula.

METHODS

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Mice

A murine C57/BL6J model with an inducible endothelial-specific deletion of *Pkd1* (*Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$) was generated by crossing *Pkd1*$^{fl/fl}$ mice and mice with tamoxifen-inducible Cre/ERT2 driven by the EC-specific *Cdh5* promoter (*Cdh5-Cre*^{ERT2}; Figure S1; Supplemental Methods).[10–12] *Cre* recombinase activity was induced either after birth or at month 4 in separate experiments, to differentiate the effects of endothelial *Pkd1* deletion with and without potential long-term functional and structural impacts.[13,14] Validation of the transgenic model was performed using *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$-*26R*-tomato mice and RNA expression of *Pkd1* on isolated EC[15,16] (Figure S1; Supplemental Methods). Experiments were performed on male mice only to reduce potential variability related to hormonal cycles. A timeline of the experiments performed is presented in Figure S3. Experiments were approved by the national animal ethics committee (CENOMEXA C2EA-54).

Blood Pressure

Measurements of systolic blood pressure were performed in the fifth month after birth in male mice. Invasive measurements were performed in conscious mice after insertion of a micromanometer-tipped catheter (SPR 407, Millar Instruments) in the right carotid artery under isoflurane anesthesia (2%, Baxter). Mice were allowed to recover, and pressure was assessed 30 minutes after recovery from anesthesia. Noninvasive measurements of systolic blood pressure were performed by tail-cuff plethysmography (CODA, Kent Scientific Corporation) in conscious and trained mice and consisted in 2 series of 10 cycles of measurements.

Cardiovascular Parameters

Echocardiography was performed in male mice using standardized procedures, as described in Supplemental Methods. Endothelial function was assessed on small vessel myograph and arteriograph at sacrifice in 6-month old mice.[17,18] The mesentery was placed in cold oxygenated Krebs buffer. A 1.5 to 2.0 mm segment of first order of mesenteric resistance artery segment was mounted on a myograph (DMT). After normalization, endothelium-dependent relaxation to acetylcholine (Ach: 10^{-9} to 3.10^{-5} mol/L) and endothelium-independent relaxation

to sodium nitroprusside (SNP: 10^{-9} to 3.10^{-5} mol/L) were performed in segments preconstricted with phenylephrine (Phe: 10^{-5} mol/L). A 2 to 3 mm segment of third mesenteric resistance artery segment was mounted on an arteriograph (DMT, Denmark). Only vessels with Phe (10^{-5} mol/L) induced constriction >40% and subsequent Ach (10^{-5} mol/L) induced relaxation >50% were considered suitable for analysis and were included in experiments. The dilatory response to stepwise increases in intraluminal flow (from 3 to 100 µL/min) was assessed in vessels preconstricted with Phe (10^{-5} mol/L).

Experimental Chronic Kidney Disease

To determine whether reduced glomerular filtration rate (GFR) may potentialize cardiovascular consequences due to the endothelial deletion of *Pkd1*, subtotal nephrectomy (5/6 Nx) was performed in 2-month old male *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$mice, as previously described (Supplemental Methods; Figure S3).[19]

Experimental Arteriovenous Fistula

Two-month old male *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$mice were used. Anesthesia was administered using xylazine (10 mg/kg; Rompun 2%, Bayer, France) and ketamine (100 mg/kg; Ketamine 1000, Virbac, France) and pain control was provided with buprenorphine (0.05 mg/kg; Buprecare, Axience) intraperitoneally. Arteriovenous fistula (AVF) was created between aorta and IVC with a 25-gauge needle as previously described.[20] Successful creation of an AVF was confirmed at D1 by increase of downstream IVC blood flow on doppler ultrasound. The mice were sacrificed at D14 or D28 for analysis.

Statistical Analyses

Statistical analyses were performed with GraphPad Prism 8.0.2 (GraphPad Software, La Jolla, CA). Data were expressed as mean values±SEM. The normality of the data and homogeneity of variances were verified by Shapiro-Wilk and Bartlett tests. Differences between groups were analyzed by Student *t* test, 2-way ANOVA and Tukey post hoc tests or repeated-measures ANOVA and Holm-Sidak post hoc tests. All *P* values were 2-tailed with statistical significance indicated by a value of $P<0.05$.

RESULTS

Development of a Mouse Line With Endothelium-Specific Deletion of *Pkd1*

We generated a conditional polycystin-1 knockout *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ line in C57BL/6J mice, in which polycystin-1 knockout was induced by tamoxifen-inducible *Cre*^{ERT2} expression under the endothelial cell-specific Cdh5 promoter (Figure S1A). The specificity and efficiency of the knockout was verified. First, *ROSA26*$^{td\text{-}Tomato}$ mice were crossed with *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice. Confocal imaging of heart and kidney sections obtained from the generated *iCdh5*-cre*/ERT2-26R-tomato* mice showed that Tomato expression was only present in vascular EC, as illustrated by its colocalization with the EC marker podocalyxin (Figure S1B). In addition, *Pkd1* mRNA expression was nearly abolished in CD31+ cells isolated from the heart of *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice (Figure S1C), as compared with control mice. These data confirm that *Pkd1* is conditionally and precisely silenced in EC of *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice. Electron microscopy analysis of the thoracic aorta showed rare primary cilia, and shorter endothelial cell surface microvilli in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice compared with control mice. No significant differences were observed between groups regarding kidney weight (5.8±0.6 mg/g in *Pkd1*$^{fl/fl}$ mice and 5.8±0.4 mg/g in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice), kidney structure evaluated on Masson trichrome staining (including the absence of cysts), and plasma creatinine (Figure 4E).

Deletion of Endothelial *Pkd1* Induces Arterial Hypertension

Noninvasive systolic blood pressure was significantly increased in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice (Figure 1A). This difference was confirmed by invasive measurements (Figure 1B). To discriminate between direct functional effects of the endothelial deficiency in *Pkd1* and hemodynamic consequences of chronic vascular remodeling, blood pressure was measured after the Cre recombinase was induced late in life by injection of tamoxifen at 4 months in a subset of in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$mice. The latter experiment similarly demonstrated that systolic blood pressure was significantly higher in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice compared with control *Pkd1*$^{fl/fl}$mice (Figure 1C).

Deletion of Endothelial *Pkd1* Impairs Vascular Function in Resistance Arteries

To further investigate whether hypertension in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice may be related to primary functional alterations of the vasculature, ex vivo analyses were performed on resistance arteries. Impaired mesenteric artery flow-mediated dilatation was demonstrated in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ compared with *Pkd1*$^{fl/fl}$ mice (Figure 2A). Vascular dysfunction of resistance arteries in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice was further confirmed by the impaired mesenteric vasorelaxation to acetylcholine (Figure 2B). A mildly decreased vasorelaxation to SNP was also observed in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice (Figure 2C). Furthermore, mesenteric artery wall thickness was increased in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice while external diameter was similar in both groups, showing inward hypertrophic remodelling in 6-month old *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice induced at birth (Figure 2D and 2E). In mice with induction of the Cre recombinase later in life, impaired flow-mediated dilatation was similarly demonstrated in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice compared with control *Pkd1*$^{fl/fl}$mice, in the absence of

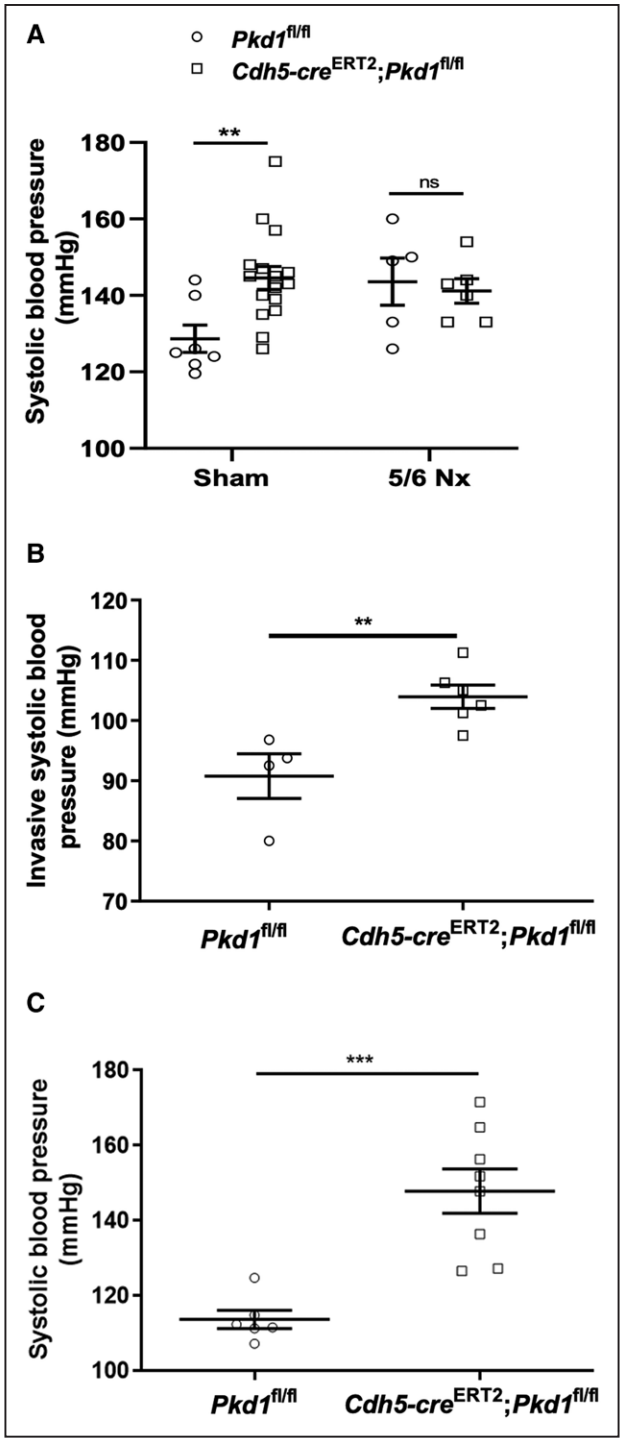

Figure 1. Effects of endothelium-specific deletion of *Pkd1* and subtotal nephrectomy on blood pressure.
Noninvasive systolic blood pressure in 5-month old *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice induced at birth, without and with subtotal nephrectomy (**A**). Invasive systolic blood pressure in 5-month-old *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice induced at birth (**B**). Noninvasive systolic blood pressure in 5-month-old *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice with late induction of Cre recombinase (**C**). **$P<0.01$.

significant vascular remodeling (Figure 2F; Figure S4), demonstrating the direct functional impact of endothelial *Pkd1* deficiency.

Endothelial Deletion of *Pkd1* Impacts the Development of Arteriovenous Fistula

To further understand the vascular impact of endothelial *Pkd1* deletion, we studied adaptation to the hemodynamic disturbances induced by an aorto-cava AVF in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice. After surgery survival was not significantly different between groups (87% and 86% in *Pkd1*$^{fl/fl}$ and *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice, respectively). While in *Pkd1*$^{fl/fl}$ mice venous blood flow increased significantly over 14 days (9.1 versus 43.6 µl/s; $P=0.005$) in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$mice this increase was not significant (8.88 versus 26.97 µl/s, Figure 3A). Similarly, downstream vein velocity (Figure 3B), shear stress (Figure 3C), and diameter (Figure 3D) presented a disturbed development in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice as compared with controls. Downstream vein thickness, a hallmark of AVF remodeling, was significantly reduced in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice compared with *Pkd1*$^{fl/fl}$ mice at D14 and D28 (Figure 3E). Histology of AVF, presented in Figure S5, showed that neointima formation, a physiological response to the aggression of the vascular wall, was marginally decreased in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice. In addition, transcriptional analyses revealed that the expression of CCL2 and MMP-2 were significantly increased in the arterialized vein of *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice compared with *Pkd1*$^{fl/fl}$ counterparts (Figure S5).

Endothelial Deletion of *Pkd1* Aggravates the Cardiovascular Phenotype Associated With CKD

Heart weight and plasma creatinine were not significantly different between 6-month old *Pkd1*$^{fl/fl}$ and *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice (Figure 4A and 4E). Accordingly, echocardiographic investigation of systolic (Figure 4B and 4C) and diastolic (Figure 4D) heart function found no significant difference between *Pkd1*$^{fl/fl}$ and *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice in the absence of CKD. After 5/6 Nx, although *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice presented a milder decrease in kidney function, cardiac hypertrophy was aggravated in this group compared with *Pkd1*$^{fl/fl}$ counterparts (Figure 4A). Echocardiography showed diastolic and systolic dysfunctions in both groups after 5/6 Nx, without significant difference between *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice (Figure 4B through 4D). Systolic blood pressure was not different between groups after 5/6 Nx (Figure 1A).

Subtotal nephrectomy induced an impairment in flow-mediated dilatation which was further aggravated in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$mice compared with *Pkd1*$^{fl/fl}$ counterparts (Figure 2A), as was vasorelaxation to acetylcholine (Figure 2B), showing an additive effect of GFR reduction and endothelial *Pkd1* deficiency on endothelial dysfunction. No impact was observed on the endothelial-independent vasorelaxation to sodium

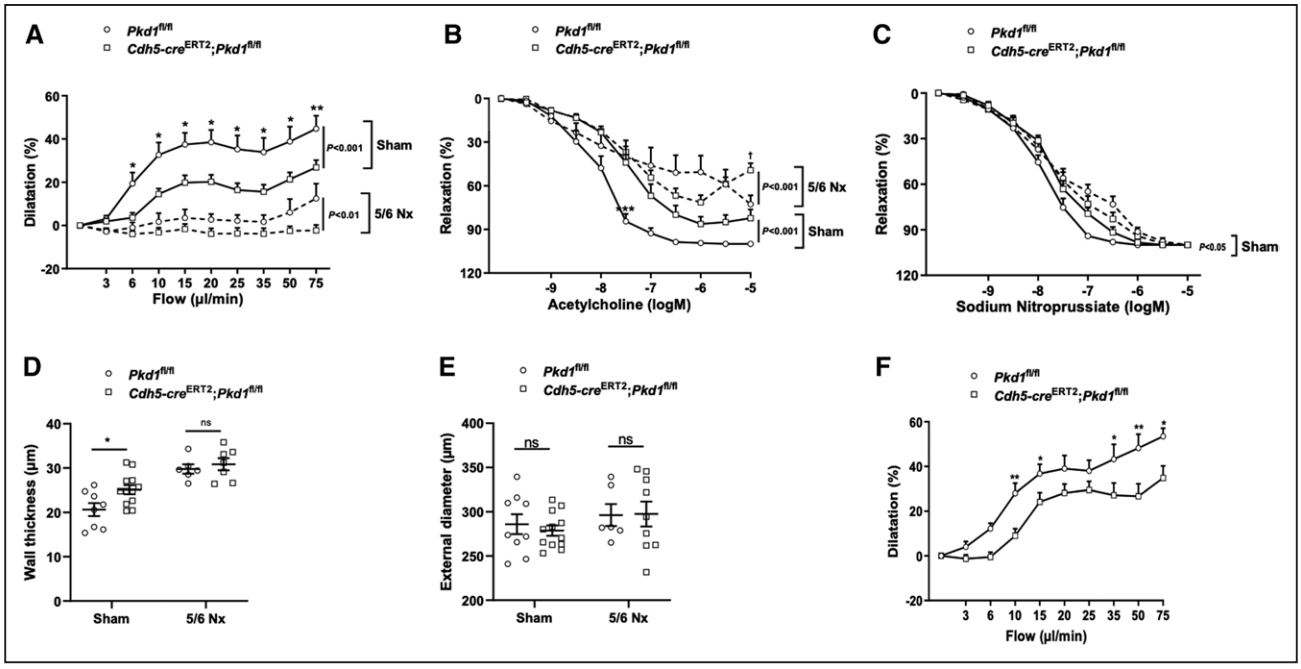

Figure 2. Effects of endothelium-specific deletion of *Pkd1* and subtotal nephrectomy on arterial function and structure.
Flow-mediated dilatation (**A**) (*Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ n=13+6Nx, *Pkd1*$^{fl/fl}$ n=10+6), vasorelaxation in response to acetylcholine (**B**) (*Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ n=10+12Nx, *Pkd1*$^{fl/fl}$ n=12+6), endothelium-independent vasorelaxation in response to sodium nitroprussiate (**C**) (*Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ n=9+10Nx, *Pkd1*$^{fl/fl}$ n=8+6), arterial wall thickness (**D**), and arterial external diameter (**E**) in mesenteric arteries of 6-month old *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice, with early late induction of the *Cre* recombinase, without and with subtotal nephrectomy. Flow-mediated dilatation (**F**) in mesenteric arteries of 6-month-old *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice with late induction of the *Cre* recombinase. ***P*<0.05, ***P*<0.01, ****P*<0.001 *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ vs *Pkd1*$^{fl/fl}$.

nitroprusside (Figure 2C), or on arterial structure assessed by the external diameter and vascular wall thickness (Figure 2D and 2E).

DISCUSSION

ECs are a cornerstone of vascular homeostasis.[21,22] This study provides the demonstration that deletion of *Pkd1* in EC promotes endothelial dysfunction, hypertension, and resistance artery remodeling, impairs arteriovenous fistula development, and potentiates cardiovascular disease associated with CKD.

ADPKD is characterized by a broad variety of cardiac and vascular disorders, independently of the onset of CKD.[3,4,23,24] We and others have shown that patients with ADPKD present endothelial dysfunction at an early stage of the disease, in the absence of decreased kidney function and of classical cardiovascular risk factors.[8,25] However, definite demonstration of the mechanism of these vascular disorders is not possible in patients due to the systemic nature of the genetic disease which leads to multiple potential confounders, including renin-angiotensin-aldosterone system activation, increased oxidative stress, and decreased GFR as a consequence of the progressive cystic burden. To resolve this issue, we generated mice with an inducible deletion of *Pkd1* specifically in EC targeted by *Cdh5-Cre*, a highly specific and reliable reporter gene for EC. In comparison, *Pdgfb-Cre* or *Tie2-Cre* mice show significant leaking of the *Cre* recombinase outside EC, especially in hematopoietic cells.[12,26,27] In this study, we demonstrated colocalization of the *Cre* recombinase in EC, as shown by colocalization of the *Tomato* reporter fluorescence and podocalyxin in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$*-26R-tomato* mice. Furthermore, our results show that *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice present a near total abolishment of *Pkd1* mRNA expression in CD31+ cells isolated from the heart.

We found that after an early induction in life, *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice presented no significant alteration of growth and survival. In addition, *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice did not exhibit changes in kidney weight, structure, and function, and no kidney cyst was observed even 6 months after induction with tamoxifen, as was previously demonstrated in *Tie2-Cre-Pkd1*$^{del/del}$ mice.[10] This demonstrates that, as expected, endothelial *Pkd1* deletion alone is not sufficient to promote kidney cystogenesis, but does not exclude an indirect potentiating effect when *Pkd1* expression is also altered in other kidney cells. Thus, in contrast to total inactivation of *Pkd1* in the postnatal period, which induces kidney cysts and dysfunction depending on the genetic variation, the hetero or homozygosity and the time of induction of the transgene, our model allows to specifically study the impact of endothelial *Pkd1* deficiency in the absence of significant confounding kidney changes.[28,29]

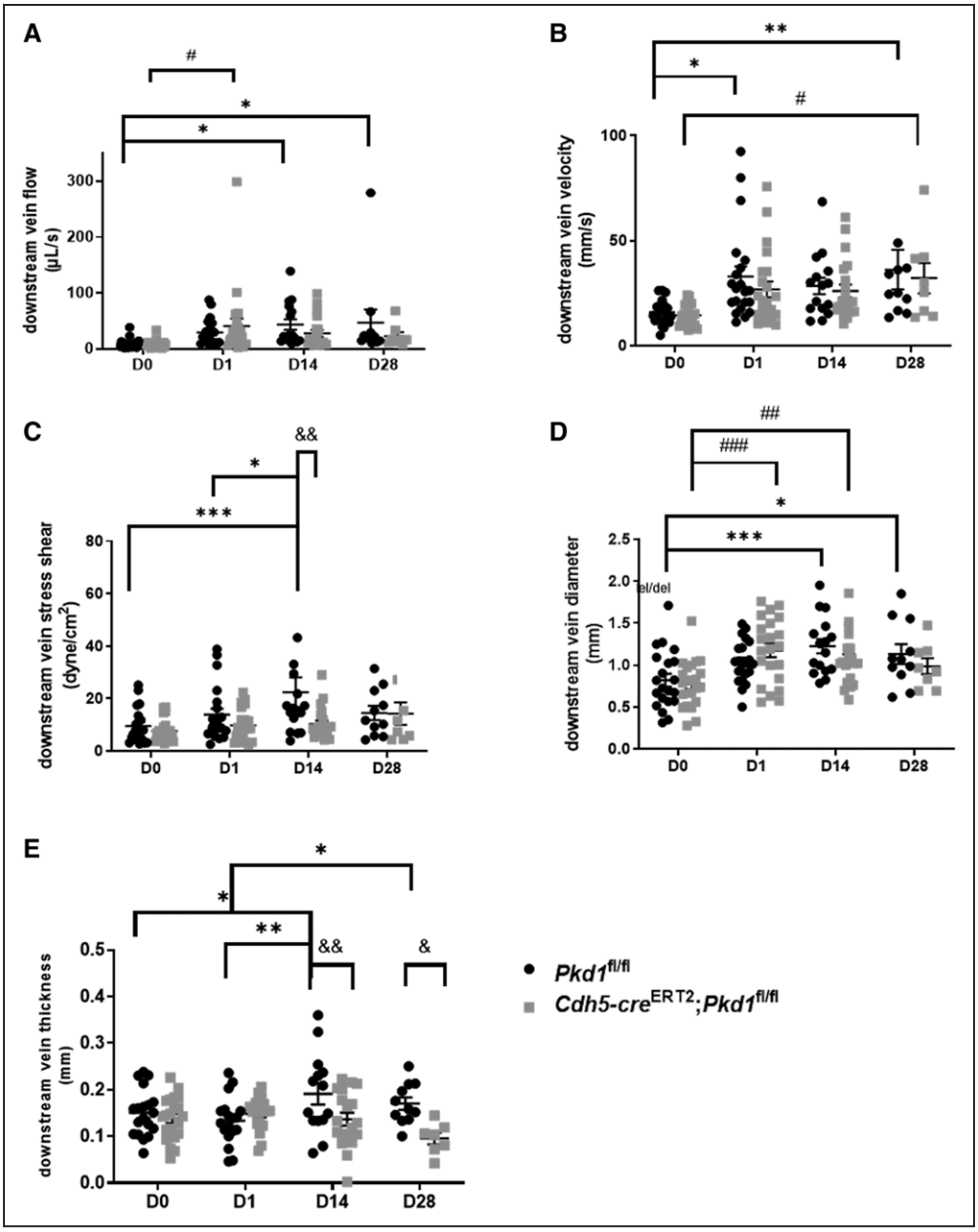

Figure 3. Effects of endothelium-specific deletion of *Pkd1* on the development of aorto-cava arteriovenous fistula.
Downstream vein flow (**A**), velocity (**B**), shear stress (**C**), diameter (**D**), and wall thickness (**E**) in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ (n=23 at D0, 22 at D1, 22 at D14, 18 at D28) and *Pkd1*$^{fl/fl}$ mice (n=21 at D0, 21 at D1, 16 at D14, 10 at D28). *P<0.05, **P<0.01, ***P<0.001 *Pkd1*$^{fl/fl}$ vs *Pkd1*$^{fl/fl}$ #P<0.05, ##P<0.01, ###P<0.001 *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ vs *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$, &&P<0.01 *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ vs *Pkd1*$^{fl/fl}$.

Hypertension is a common and early manifestation in ADPKD, but its pathophysiology is still debated.[4,30–34] The association between hypertension and kidney cysts, found in adolescent and adult patients with ADPKD, does not imply exclusive causality between the kidney cystic burden and hypertension in ADPKD. Indeed, the growth of kidney cysts occurs with the progressive increase in loss of heterozygosity in epithelial cells, and in parallel with the increase of the extrarenal expression of the disease, including in the arteries. In this study we demonstrate that endothelial deletion of *Pkd1* alone is sufficient to cause arterial hypertension, as shown by invasive and noninvasive measurements of blood pressure. Indeed, in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice, hypertension occurred independently of alterations in kidney function and kidney structure. Since the endothelium is a major regulator of vasomotor tone in resistance arteries, which in turn is a key determinant of blood pressure, we investigated whether the increase in blood pressure shown in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice was associated with endothelial dysfunction. We demonstrated reduced flow-mediated dilatation in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice, over a wide range of blood flow variation, which is consistent with findings in patients with ADPKD.[8,25] Both

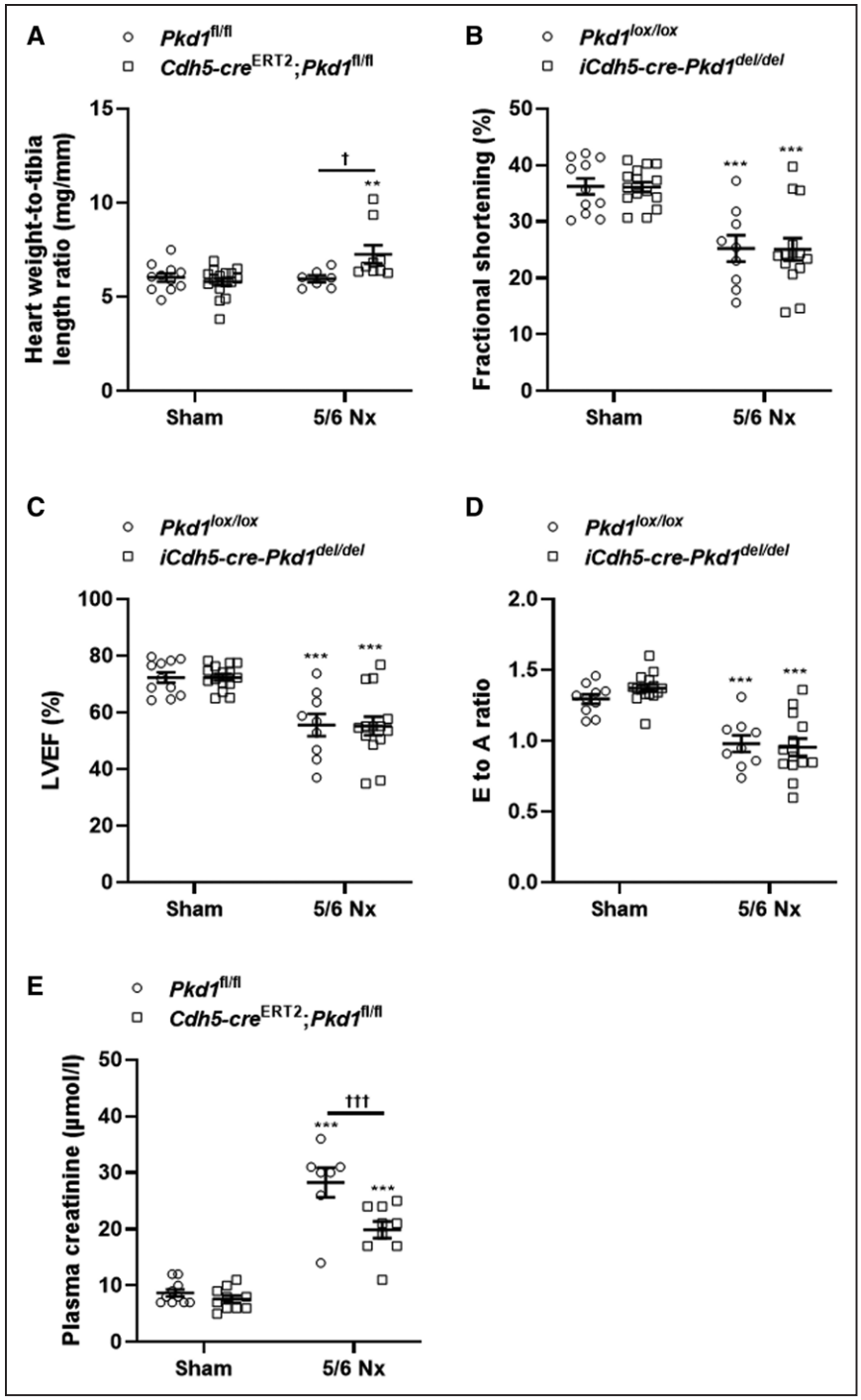

Figure 4. Effects of endothelium-specific deletion of *Pkd1* and subtotal nephrectomy on the heart. Heart weight-to-tibia-length ratio (**A**), fractional shortening (**B**), and left ventricular ejection fraction (**C**) as echocardiographic assessments of systolic function, E/A (**D**) as echocardiographic assessment of diastolic function, and plasma creatinine (**E**) in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and *Pkd1*$^{fl/fl}$ mice, without and with subtotal nephrectomy. **$P<0.05$, **$P<0.01$, ***$P<0.001$ 5/6 Nx vs sham counterparts, †$P<0.05$, ††$P<0.01$, †††$P<0.001$ *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ vs *Pkd1*$^{fl/fl}$.

hypertension and endothelial dysfunction were present in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice independently of the timing of induction of the *Cre* recombinase (at birth or after 4 months). Their presence in mice with the late induction, which have preserved arterial wall structure, excludes the role of vascular remodeling in these functional alterations, demonstrating the primitive functional impact of *Pkd1* deficiency in the endothelium. Vascular dysfunction was further supported by the presence of an impaired vasorelaxation to acetylcholine in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice. A mildly decreased vasorelaxation to sodium nitroprusside was also observed, which could be related to the remodelling of the media in resistance arteries as a possible consequence of hypertension. Indeed, vascular wall thickness was increased in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice induced at birth compared with control *Pkd1*$^{fl/fl}$ mice, but this difference was not observed when the *Cre* recombinase was induced later in life. Patients with ADPKD present artery intima-media thickening, which is recognized as an independent predictor of cardiovascular events.[35,36] Taken together, our results point endothelial dysfunction of resistance arteries specifically due to

defective polycystin-1-dependent mechanosensing in EC as a direct and central cause of hypertension in ADPKD. Interestingly, these results extend recent findings from a study using a similar mouse genetic approach, which showed that *Pkd2* deficiency in EC also promotes endothelial dysfunction assessed by flow-mediated dilatation and high blood pressure[37] and thus supports the complementary role of polycystin-1 and polycystin-2 in blood flow sensing and mechanotransduction.[6] The functional role of polycystins in arteries is complex and also involves smooth muscle cells. Indeed, as shown by Sharif-Neaini et al,[38] in vascular smooth muscle cells, the polycystin-1/polycystin-2 ratio controls pressure sensing by regulating the opening of stretch-activated ion channels and deletion of *Pkd1* alone reduces arterial myogenic tone.

Our results did not demonstrate major changes in cardiac structure and function induced by endothelial *Pkd1* deficiency as previously shown for *Pkd2*.[37] In contrast, previous results suggested that the specific deletion of polycystin-1 in cardiomyocytes may play a critical role in regulating cardiac contractility.[23,39] Thus, although a longer follow-up may be needed, these results suggest that endothelial *Pkd1* deficiency is not a key player in the cardiac abnormalities observed in ADPKD patients before the onset of CKD.

Arteriovenous fistula is the preferred vascular access for hemodialysis in CKD patients. Interestingly, AVF also represents a relevant setting to study vascular adaptation, in which the anastomosis between a high-pressure arterial and a low-pressure venous system sharply increases blood flow rate, wall shear stress, and intramural tensile stress in the vein.[40] This normally leads to an increase in the vein diameter and in wall thickness to accommodate and decrease wall shear stress and tensile stress towards baseline values. The endothelium is considered to play a key role in the functional and structural adaptations of the AVF. In CKD, preliminary reports suggest that ADPKD patients present an abnormal maturation of AVF with an increased rate of early failure.[41] In the present study, we found that *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$mice present a decreased venous blood flow, shear stress, and diameter in experimental aorto-cava AVF. Interestingly, vascular wall thickness, which is predominantly driven by neointimal hyperplasia, was reduced in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$mice. These results show that endothelial *Pkd1* deficiency impacts the normal remodeling of AVF. The increase in vascular wall shear stress induced by AVF increases nitric oxide synthesis, a process which involves the polycystin complex.[6,8,42,43] nitric oxide, in turn, increases the expression of TGF bêta[44,45] and limits MMP2.[46,47] The expression of these mediators, which both play an important role in neointima synthesis, was significantly altered in the AVF of mice with endothelial *Pkd1* deficiency, providing a possible mechanistic explanation to the structural disorders observed.

In ADPKD, cyst growth progressively decreases kidney function, generally leading to end-stage kidney disease in the fifth or sixth decade. Since CKD is, per se, a major cause of cardiovascular disease, isolating the role of decreased GFR and of the specific cardiovascular impact of ADPKD in this context is challenging. To determine whether the endothelial deficiency of *Pkd1* may further aggravate the cardiovascular phenotype of CKD, we applied a model of CKD, subtotal (5/6) nephrectomy, to *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ and control *Pkd1*$^{fl/fl}$ mice. While this experimental model presents clear differences with the pathological processes occurring in kidneys from ADPKD patients, we deliberately chose a noncystic model of decreased GFR, to avoid confounders related to the systemic expression of altered polycystins in the latter case. The analysis of vascular function after 5/6 Nx showed strongly impaired vasomotor function in both groups. Although they presented a less severe decrease in kidney function, *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice exhibited a larger alteration of mesenteric artery flow-mediated dilatation and of vasorelaxation to acetylcholine than *Pkd1*$^{fl/fl}$ mice after 5/6 Nx. No difference was observed regarding endothelium-independent relaxation to sodium nitroprusside, further demonstrating that endothelial deletion of *Pkd1* aggravates the endothelial dysfunction associated with CKD. Although heart weight was comparable between groups at baseline, heart hypertrophy was potentiated in *Cdh5-Cre*^{ERT2};*Pkd1*$^{fl/fl}$ mice after 5/6 Nx, as a possible consequence of the preexisting hypertension. In patients with ADPKD, left ventricular hypertrophy has been observed in several studies. Its mechanism may beside hypertension involve disrupted expression of polycystins in the heart microvasculature.[23,48,49] Polycystin-1 deficiency in cardiomyocytes protected against heart hypertrophy in a model of transverse aortic constriction, suggesting that polycystin-1 differentially regulates cardiac remodelling depending on its cellular localization.[39] The main limitations of this study were the fact that blood pressure was not recorded at night and the possibility to extend findings from the experimental CKD model chosen to cystic CKD. In addition, the absence of female mice in the experiments could, given the impact of sexual hormones on the cardiovascular system, limit the generalizability of the results obtained from male mice.

PERSPECTIVES

The present study provides the first in vivo demonstration that endothelium-specific disruption of polycystin-1 promotes the development of vascular dysfunction and hypertension. Furthermore, this study shows that vascular dysfunction and cardiac hypertrophy associated with CKD are aggravated by the endothelial deletion of *Pkd1*. Altogether, these results establish the endothelium

as a significant contributor to cardiovascular disease in ADPKD and as a potential target for innovative therapies in this setting.

ARTICLE INFORMATION

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Disclosures

None.

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