Polycystin 2-dependent cardio-protective mechanisms revealed by cardiac stress

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Abstract

Although autosomal dominant polycystic kidney disease (ADPKD) is characterized by the development of multiple kidney cysts, the most frequent cause of death in ADPKD patients is cardiovascular disease. ADPKD is linked to mutations in *pkd1* or *pkd2*, the genes that encode for the proteins polycystin 1 and polycystin 2 (PC1 and PC2, respectively). The cardiovascular complications have been assumed to be a consequence of renal hypertension and activation of renin/angiotensin/aldosterone (RAAS) pathway. However, the expression of PC1 and PC2 in cardiac tissue suggests additional direct effects of these proteins on cardiac function. We previously reported that zebrafish lacking PC2 develop heart failure, and that heterozygous $Pkd2^{+/-}$ mice are hypersensitive to acute β-adrenergic receptor (βAR) stimulation. Here we investigate the effect of cardiac stress (prolonged continuous βAR stimulus) on $Pkd2^{+/-}$ mice. After βAR stimulation for 7 days, wildtype (WT) mice had increased left ventricular mass and natriuretic peptide (ANP and BNP) mRNA levels. The WT mice also had upregulated levels of PC2 and chromogranin B (CGB, an upstream regulator of BNP). Conversely, $Pkd2^{+/-}$ mice had increased left ventricular mass, but natriuretic peptide and CGB expression levels remained constant. Reversal of the increased cardiac mass was observed in WT mice 3 days after cessation of the βAR stimulation, but not in $Pkd2^{+/-}$ mice. We suggest that cardiac stress leads to upregulation of the PC2-CGB-BNP signaling axis, and this pathway regulates the production of cardio-protective natriuretic peptides. The lack of a PC2-dependent cardio-protective function may contribute to the severity of cardiac dysfunction in $Pkd2^{+/-}$ mice and in ADPKD patients.

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1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD), characterized by the bilateral development of multiple renal cysts, is the most common form of polycystic kidney disease and one of the most frequent inherited monogenic disorders [47]. ADPKD is a consequence of mutations in one of two genes, *pkd1* or *pkd2*, that code for the proteins polycystin 1 (PC1) and polycystin 2 (PC2), respectively. PC1 is a transmembrane protein with a large extracellular N-terminal domain and a C-terminal cytoplasmic domain with many binding partners, including PC2 [40]. PC2, also known as transient receptor potential cation channel (TRPP2) is a nonselective cation channel primarily localized in ER, but also found in plasma and ciliary membranes [17, 52]. PC1 and PC2 form a heteromeric molecular complex in the plasma and ciliary membranes, where it is proposed that PC1 serves as a mechanosensor and PC2 functions as a calcium permeable channel [36]. Mutations in *pkd1* are found in 85% of ADPKD cases, which typically have an earlier onset and more severe phenotype than those with mutations in *pkd2*, accounting for 15% of cases [13]. Regardless of the causative mutation, ADPKD is associated with a high risk of cardiovascular complications, where 90% of ADPKD patients exhibit cardiac hypertrophy at the time of death [8, 34]. This risk of cardiovascular complications in patients with ADPKD is higher than that expected in the general population [26, 35]. Previous studies have documented early development of left ventricular hypertrophy (LVH) in ADPKD patients, especially in young normotensive ADPKD patients who have no renal deficits in comparison to the age matched general population [4, 6, 35, 41, 48]. However, in a more recent study, the prevalence of hypertrophy in ADPKD patients (age range 15–49) was found to be 4%, instead of ~40% as previously described [1, 38]. The lower prevalence may be related to the use of a more accurate diagnostic technique in the HALT-PKD study (magnetic resonance imaging), updated guidelines for the diagnosis of LVH, and the inclusion of a population where nearly 60% of the cohort was already receiving pharmacological treatment for hypertension [1]. In addition to LVH, an association of idiopathic dilated cardiomyopathy and ADPKD has been reported in humans, with a higher coexistence in subjects with *pkd2* than *pkd1* mutations [35].

The cellular and molecular mechanisms leading to the onset of cardiac dysfunction associated with ADPKD have been assumed to be a consequence of compromised kidney function and hypertension. Renal hypertension is thought to arise primarily from activation of the renin/angiotensin/aldosterone (RAAS) pathway because of the decreased vasculature function associated with a large cystic load, which then leads to the cardiac structural and functional changes [50]. However, PC1 and PC2 are also expressed in cardiac tissue, suggesting that mutations to polycystins that alter their function could elicit a direct effect on cardiac performance.

The first experimental data supporting an impact of cardiac-localized PC2 on cardiac function was the demonstration that PC2 directly regulated the open probability of the main

intracellular cardiac calcium channel, the ryanodine receptor (RyR) [2]. Subsequent studies showed that zebrafish lacking PC2 had hearts with altered calcium signaling and were prone to develop cardiac abnormalities [35]. More relevant to human ADPKD, where patients are heterozygous and can have decreased expression of PC2, heterozygous mice (Pkd2$^{+/-}$ mice) have decoupled calcium-contraction coupling and an enhanced inotropic response to acute isoproterenol (ISO) stimuli, even though these animals do not have either hypertension or renal cysts [21]. A comparison of 1 and 9 month old Pkd2$^{+/-}$ mice showed that the cardiac pathology was progressive [20]. These studies provide evidence for the importance of PC2 function in the heart and of the existence of a cardiac phenotype in Pkd2$^{+/-}$ mice in the absence of renal functional impairment. The role of PC1 has also been studied. Mice overexpressing PC1 develop cardiac anomalies, such as severe left-ventricular hypertrophy, marked aortic arch distention and/or valvular stenosis and calcification [22]. In contrast, mice lacking PC1 in the heart (cardiac-specific PC1 knockout mice) present with decreased ejection fraction at baseline, but the cardiac function does not worsen with pressure overload caused by transverse aortic constriction [37]. However, mutations in PC1 can disrupt the interaction between PC1 and PC2, which would lead to altered PC2 function and cardiac effects in humans.

Previous studies have not addressed the longer-term cardiac stress models that would elicit cardiac hypertrophy in Pkd2 mutant models. Patients with ADPKD are known to have elevated catecholamine levels [28], which, in animal models, are known to both acutely and chronically result in cardiac dysfunction [14, 19, 39, 45, 46]. In this study we examine the cardiac phenotype in Pkd2$^{+/-}$ mice after prolonged activation of the adrenergic pathway, which, at sustained moderate concentrations, is known to cause cardiac hypertrophy, but not necrosis, inflammatory infiltrate or fibrosis [19, 39]. We hypothesized that Pkd2$^{+/-}$ mice lack some of the compensatory mechanisms after cardiac stress. We report that both WT and Pkd2$^{+/-}$ mice had increased cardiac mass in the absence of renal cysts after being subjected to sustained ISO treatment. Intriguingly, WT mice responded with a substantial increase in PC2 expression and an upregulation of the cardio-protective natriuretic peptides [33], whereas Pkd2$^{+/-}$ mice did not. These results suggest that PC2 has a cardio-protective role and that the absence of this protein diminishes the ability of the heart to compensate for stress.

2. MATERIAL AND METHODS

2.1 Animals

Pkd2$^{+/-}$ mice were obtained from the laboratory of Dr. S. Somlo (Yale University School of Medicine). The Pkd2$^{+/-}$ mice were on a >98% pure C57/Bl6 background. Pkd2$^{+/-}$ were bred with C57/Bl6 mice, obtained from Charles River Laboratories. Animals were housed under a 12-h light/dark cycle. All mouse studies were performed with male mice, using WT littermates as controls. The animals were 5 weeks old by the beginning of the experimental procedures. Animals were genotyped at 3 weeks of age with primers described previously [21]. None of the Pdk2$^{+/-}$ mice had any evidence of renal cysts, consistent with previously published literature [20, 51]. The Yale University Animal Ethics committee (IACUC)

approved the animal housing conditions and experimental procedures conducted in this study.

2.2 Micro-osmotic pump implant

Mice received general anesthesia consisting of a vapor mixture of 2.5% sevoflurane, 5% nitrous oxide and 95% oxygen. Additionally, 0.1 ml of 1% lidocaine was used as a local anesthetic and injected subcutaneously in the interscapular area. Micro-osmotic pumps (Alzet) were filled with 25μg × g body weight (BW) of DL-hydrochloride-isoproterenol (diluted in 0.9% phosphate buffered saline and 0.5mmol/L ascorbic acid) or only the diluent. An aseptic incision was made and the micro-osmotic pump was subcutaneously implanted in the back of the mice. The drinking water was supplemented with 1 mg/ml ibuprofen for the following 48 hours after the micro-osmotic pump implantation. The pump content was delivered constantly over a period of seven days at a rate of 25 μg/g body weight/day.

2.3 Echocardiograms

Mice were anesthetized with 1% isoflurane in oxygen vapor and then placed on a heated procedure board at 37°C. Stable sonographic images (both B and M mode) were acquired and analyzed using Vevo 770 and Vevo2100 (VisualSonics). Systolic and diastolic left ventricle wall thicknesses and chamber dimensions were measured using M-mode images. The ejection fraction and fractional shortening were calculated using the included software [16]. Data gathering and analysis was performed with the outcome assessor and data analyzer blinded to treatment allocation.

2.4 Left ventricular hemodynamic measurements

Mice were anesthetized with an intraperitoneal mixture of ketamine (100 mg/kg body weight) and xylazine (5 mg/kg) and placed on a heated pad at 37°C. The trachea was orally intubated and connected to a volume-controlled ventilator at a respiratory rate of 100 breaths/min of room air. A 1.2-French conductance pressure catheter (Scisense) was inserted via the right carotid artery and advanced into left ventricle to measure left ventricular (LV) function. LV pressure signals were continuously monitored and digitally recorded at 1000 Hz (Sciences Inc). Baseline hemodynamic parameters were recorded for 10 min following stabilization after carotid artery catheter implantation. Systolic and diastolic blood pressure, left ventricular peak pressure and high-fidelity positive and negative dP/dt (dP/dtmax and dP/dtmin) values were calculated using LabScribe2 software (iWorx, CB Sciences Inc) [16]. A dobutamine stress test was performed in addition to baseline hemodynamic measurements. A solution of dobutamine was infused via the left jugular vein at graded doses (0.75, 1.25, 2.0, and 4.0 μg/kg body weight), each concentration over a period of 3 minutes. The same parameters were evaluated.

2.5 mRNA and protein studies

Left ventricles (LV) were isolated from anesthetized mice and flash-frozen in liquid nitrogen. Total RNA was extracted using an RNEasy kit supplemented with a protease K digestion step (Qiagen). Total RNA concentration was measured with a Nanodrop Spectrophotometer (Thermo Scientific). The mRNA was reverse-transcribed into cDNA by

using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Primers specific for ANP, BNP and 18S were used for semi-quantitative real-time PCR with TaqMan Gene Expression Master Mix (Applied Biosystems) reagents. A 7500 Fast-Real Time PCR System (Applied Biosystems) was used to measure mRNA expression. Levels of mRNA were normalized to internal control 18S rRNA and expressed as mean fold change of expression. Fold change in mRNA transcript levels was determined by using the 2− CT method.

Protein was extracted from 15 mg of the LV by homogenization in 500μl RIPA buffer (25 mM Tris. HCl pH 7.4, 150 mM NaCl, 5 mM EDTA, 1% NP-40, 0.1 % SDS, 1.0 % sodium deoxycholic acid) supplemented with protease and phosphatase inhibitors (5 μg/ml leupeptin, 5 μg/ml aprotinin, 1 μg/ml pepstatin A, 1 mM PMSF, 1 mM benzamidine, 1 mM Na_3VO_3, and 10 mM NaF). The homogenate was centrifuged at $10,000 \times g$ for 10 min at 4°C. Protein concentrations were determined using the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). Equal amounts of total of protein (30–100 μg) were run on SDS/ PAGE gels (Novex, Life Technologies). Protein was transferred to PVDF membranes by wet transfer. Membranes were blocked and exposed to the following primary antibodies against RyR (Affinity BioReagents), βAR-1, βAR-2, SERCA2a and α-tubulin (Abcam), PC2 (gift of Dr. Stefan Somlo, Yale University) and CGB (BD Bioscience). Specific bands were visualized using chemiluminescence (Amersham, Freiburg, Germany). Densitometric analysis of Western Blots was performed using NIH ImageJ.

2.6 Statistical analysis

Statistical analyses were performed by using GraphPad Prism software (version 6). Groups were compared by using repeated measures, two-way ANOVA, performed followed by Tukey post hoc tests. All data are expressed as means ± SEM. Differences are considered significant when $P < 0.05$. *, **, *** and **** mean $p<0.05$, 0.01, 0.001 or 0.0001, respectively. Each experimental group had 3–5 mice.

3. Results

3.1 Polycystin 2 expression is increased after cardiac stress induced by activation of the adrenergic pathway

Here we examined how 5 week old WT and Pkd2$^{+/−}$ mice react to cardiac stress after implanting microosmotic pumps that released saline (control) or ISO (to induce cardiac stress) for 7 days. This age was chosen to ensure that the animals had similar baseline characteristics and expression of the calcium handling proteins [20]. Moreover, these mice had similar acute responses to a challenge with ISO [20]. In initial studies with a range of prolonged ISO treatments (10–50 mg/day for 7 days), we unexpectedly found that there was a noticeable upregulation of PC2 in the WT mice, which was not observed in the Pkd2$^{+/−}$ mice (Supplemental Fig. 1). This result lead us to hypothesize that the upregulation of PC2 is cardio-protective and that decreased expression of PC2 would be deleterious. To test this idea more rigorously, we moved to a micro-osmotic pump model that evenly dispenses ISO at a constant infusion rate, and in a concentration below one that has previously reported to cause myocardium lesions (i.e. no necrosis). High acute concentrations of ISO and other

catecholamines in various animal models are reported to result in necrosis within 60 mins [45, 46].

As would be expected from our initial experiments (Supplemental Fig. 1), PC2 expression was increased after the 7 days of sustained ISO infusion in WT mice, whereas in the Pkd2^{+/−} stressed mice, the PC2 protein expression was not statistically different from control (Fig. 1A). We also analyzed the expression level of β-adrenergic receptor type 1 ($\beta_1 AR$), β-adrenergic receptor type 2 ($\beta_2 AR$), the sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a), and ryanodine receptor (RyR) in WT and Pkd2^{+/−} mice (Fig. 1B–E). The sustained administration of ISO induced a significant decrease in the $\beta_1 AR$ expression by the same amount in both WT and Pkd2^{+/−} mice compared with the respective saline control groups (Fig. 1B). The decrease in $\beta_1 AR$ expression is consistent with the response to prolonged adrenergic stress [25]. In contrast, the sustained administration of ISO had no effect on $\beta_2 AR$ expression in WT mice, but increased expression in Pkd2^{+/−} mice compared to the Pkd2^{+/−} control group (Fig. 1C). The sustained administration of ISO reduced the SERCA2a protein expression by the same amount in both WT and Pkd2^{+/−} mice (Fig. 1D). RyR expression was unchanged in both WT and Pkd2^{+/−} mice (Fig. 1E).

3.2 Adrenergic stress increases cardiac mass in both WT and Pkd2^{+/−} mice

An echocardiographic examination of all four groups of mice showed that there were changes in myocardial morphological parameters in the stressed mice consistent with prolonged ISO treatment. ISO treatment increased the ratio between heart and body weight in WT and Pkd2^{+/−} mice (Fig. 2A and Supplemental Table 1). Consistent with this observation, the cardiac left ventricular mass also increased after the sustained administration of ISO in both WT and Pkd2^{+/−} mice (Fig. 2B) when compared with their respective control groups. The sustained administration of ISO also induced an increase in systolic and diastole thicknesses in the left ventricular posterior wall when compared with their respective control groups (Fig. 2C and D). However, the changes in both WT and Pkd2^{+/−} mice were comparable (Fig. 2). Other cardiac parameters were unaltered (Supplemental Fig. 2). Consistent with previously published results using similar ISO concentration in mice [19, 39], no differences in cell death or fibrosis was observed in histological analysis (data not shown).

3.3 Unchanged blood pressure in Pkd2^{+/−} mice following cardiac stress

Importantly, administration of ISO can also affect blood pressure, and it is generally assumed that the cardiac pathologies in ADPKD arise from renal hypertension, which elevates circulating catecholamines (adrenergic receptor agonists). In examining the systemic effect of the sustained administration of ISO, we found no difference in systolic or diastolic arterial pressure among the experimental groups (Fig. 3A and B), although the sustained administration of ISO induced tachycardia (even under anesthesia) in both WT and Pkd2^{+/−} mice (Fig. 3C). Isoproterenol has been shown to increase mean arterial blood pressure by 10 mmHg from baseline when constantly infused subcutaneously at 20 μg/g BW per day for 5 consecutive days in mice [14]. Therefore, interference in the blood pressure measurements by the anesthesia cannot be completely excluded.

Although this dose of ISO was sufficient to cause an increase in cardiac mass at this time point, there was no decrease in ejection fraction (EF) between the two stressed groups (Supplemental Fig. 2I). Systolic and diastolic left ventricular internal diameters were also unaltered (Supplemental Fig. 2C and D).

After the sustained administration of ISO was completed, the response to an acute stress was measured using dobutamine, an agonist that predominantly targets β_1AR, but also has weak affinity for β_2AR. Qualitatively, Pkd2$^{+/-}$ mice and WT mice responded in the same manner to dobutamine injection in both control and ISO treated (Supplemental Fig. 3). However, consistent with the sustained administration of ISO, the magnitude of the rate of rise of left ventricular systolic pressure (Supplemental Fig. 3A), and rate of decline of diastolic pressure (Supplemental Fig. 3B) were significantly different between control and stress treatment in both groups, Pkd2$^{+/-}$ and WT mice.

3.4 Natriuretic peptide expression is unaltered in Pkd2$^{+/-}$ mice after cardiac stress

Cardiac hypertrophy is associated with increases in natriuretic peptides [49]. Analysis of the left ventricular mRNA levels of the natriuretic peptides ANP and BNP showed an increase in the WT mice (Fig. 4), as expected. Despite evident increases in cardiac mass in the Pkd2$^{+/-}$ stressed mice (Fig. 2), the expression of mRNA for ANP and BNP did not change in comparison with the control Pkd2$^{+/-}$ group (Fig. 4A and B). Short-term cardiac stress resulting in transient cardiomyopathies can be reversible (for example, Takotsubo syndrome [44]). Therefore, we reasoned that cessation of the administration of ISO would unmask the effects of not having an ANP/BNP response in the Pkd2$^{+/-}$ mice. Consistent with this idea, WT mice monitored 3 days after cessation of administration of ISO showed a regression of the increased mass back to control values (Fig. 5), as seen by other groups [9]. In contrast, the Pkd2$^{+/-}$ mice monitored at the same time still retained the increased cardiac mass (Fig. 5).

These data lead us to propose that the elevation of PC2 is cardio-protective in the WT mice. To explore this idea further, we examined possible upstream calcium-dependent proteins in the ANP/BNP activation pathway. One such molecule is the calcium-sensitive binding protein chromogranin B (CGB), where in ventricular myocytes, rises in CGB expression is upstream of the increases in BNP expression [12]. We found that after the sustained administration of ISO the expression of CGB is higher in the stressed WT group when compared to its control (Fig. 6). However, the amount of CGB protein did not rise in Pkd2$^{+/-}$ mice after the sustained administration of ISO (Fig. 6). These results are consistent with the lack of an increase in BNP mRNA and that CGB expression is upstream of BNP (Fig. 4B) [12].

4. Discussion

In this series of experiments, we show that PC2 may play a crucial role in the mechanism of protection after prolonged cardiac stress. We compared responses of WT mice and Pkd2$^{+/-}$ mice in a β-adrenergic model of cardiac stress. Both groups showed increase in cardiac mass in response to prolonged stress. Intriguingly, the Pkd2$^{+/-}$ mice lacked upregulation of the cardio-protective natriuretic peptides, whereas the WT mice had increased expression of the

natriuretic peptides. As WT mice had increased PC2 expression (a calcium channel), as well as an increase in the downstream calcium binding protein, CGB (which regulates BNP [12]), we suggest that this protective calcium dependent pathway is activated in WT mice, whereas it is disrupted in the Pkd2$^{+/-}$ mice. As previously described, the cardiac mass began to return to pre-treatment levels in WT mice 3 days after termination of the prolonged adrenergic stress [9] but the decrease of cardiac mass in the Pkd2$^{+/-}$ mice was less, indicating either a delayed or an inability to return to baseline. These results are the first to demonstrate that decreased PC2 levels are associated with maladaptation after cardiac stress without renal cysts. Moreover, these data support the idea that increased PC2 expression is cardio-protective, as it may be upstream of the activation of natriuretic peptides. Therefore, loss of cardiac PC2 expression will have deleterious functional effects on many organ systems, in addition to the kidney, as a consequence.

It has been assumed that the high incidence of cardiac hypertrophy associated with ADPKD arises as a consequence of renal cysts and hypertension. However, the mice included in this study had neither renal cysts nor hypertension. Instead, our most striking observation was that, using a model of cardiac stress, Pkd2$^{+/-}$ mice do not exhibit rises in the natriuretic peptide signaling pathway. Moreover, once the adrenergic stress was stopped, the Pkd2$^{+/-}$ mice sustained an increase in cardiac mass compared to WT mice. These findings suggest that a contributing cause to the high incidence of hypertrophy observed in ADPKD arises from lack of natriuretic peptides. Several extracellular stimuli, including the distension of cardiomyocytes and prolonged adrenergic stress, lead to the production and secretion of natriuretic peptides [7]. Relevant to the findings presented here, mice lacking natriuretic receptor-A (NPR-A), the receptor for ANP and BNP, develop cardiac hypertrophy and fibrosis independent of their blood pressure [33]. It is well described that ANP and BNP inhibit hypertrophic cellular signaling by modulating different targets such as CaMKII, ERK, and a complement of transcription factors, such as GATA-4, activator protein-1 (AP-1), AP-2, and a cAMP-responsive element (CRE) associated with cardiac hypertrophy [30, 31, 33, 43]. Consistent with the beneficial role of natriuretic peptides, the increase of cardiac mass observed in the WT mice regressed after cessation of adrenergic stimulation, whereas Pkd2$^{+/-}$ mice (which did not have an elevation in natriuretic peptides) showed a slow reversion of the hypertrophy after removal of the stimulus (Fig. 5A and B).

Why is the natriuretic peptide pathway not activated in the Pkd2$^{+/-}$ mice? The lack of upregulation of the natriuretic peptides in the Pkd2$^{+/-}$ mice after adrenergic stress may be due to the difference in calcium signaling. PC2, which can act as an intracellular calcium release channel, is upregulated in the WT mice. Intracellular calcium levels can regulate the expression of the natriuretic peptides ANP and BNP [18, 23]. For example, we previously demonstrated that cardiac hypertrophy is associated with altered BNP transcription through a calcium-dependent pathway involving expression of CGB through regulation of the calcium-sensitive transcription factor NFkB [12]. Consistent with this pathway, the levels of ventricular CGB in the WT mice studied here using adrenergic conditions also had an increase in CGB compared to baseline (Fig. 5C and 6D) and a related increase in the natriuretic peptides [33]. Conversely, the Pkd2$^{+/-}$ mice did not show an increase in CGB, presumably because the changes in intracellular calcium are diminished in cells with mutated or lacking PC2 [11]. These results suggest that the upregulation of PC2 expression

is cardio-protective and is directly linked to production of the natriuretic peptides. However, the effects on natriuretic peptides is likely to be one of several additional downstream mechanisms of PC2-associated cardio-protection, including effects of the PC2-CGB axis.

Analysis of the baseline data from the HALT-PKD study suggests that the decrease in LVH prevalence in human ADPKD subjects is a consequence of earlier detection and better guidelines for hypertension treatment [1]. In addition, the HALT-PKD study also showed a greater decline in the left-ventricularmass index in a rigorous blood-pressure treatment group compared to a standard blood-pressure treatment group [42], as expected from the proposed mechanisms for cardiac dysfunction associated to the renal hypertension in ADPKD. This relationship between LVH and hypertension draws a distinction between the human ADPKD and the mice included in this study which had neither renal cysts nor hypertension. Our most striking observation was that, using a model of cardiac stress, Pkd2$^{+/-}$ mice do not exhibit rises in the natriuretic peptide signaling pathway. This result is in accordance with a small clinical study, which did not show a significant difference of ANP basal levels in hypertensive ADPKD patients compared to essential hypertensive individuals, despite the higher number of patient with LVH in the ADPKD group (40% in ADPKD and 30% in control) [28]. However, in another small study, 21 ADPKD individuals with normal blood pressure and kidney function were compared to 12 unaffected subjects from the same families. No difference in ANP levels were observed at baseline, but the serum ANP was higher in ADPKD subjects after chronic higher sodium intake [5]. Further studies are needed to understand how the findings obtained with mice models will inform studies in human ADPKD populations.

The upregulation of PC2 is an intriguing finding, as it has not been reported previously in stressed ventricular cardiac tissue, although ER stress has been shown to lead to PC2 upregulation in renal ischemic models [53] and in cultured epithelial cells [27]. In addition to PC2 contributing to the generation of natriuretic peptides, the upregulation of PC2 may also be cardio-protective by the downstream effects of adrenergic stimulation. Adrenergic receptor stimulation activates cAMP, protein kinase A (PKA) and calcium/calmodulin-dependent protein kinase II (CaMKII). CaMKII, in turn, can phosphorylate RyR2 [24] and increase calcium release by RyR2. The constant hyperactivation of RyR2 leads to increased calcium release during systole and calcium leak during diastole. Collectively, the sustained increased calcium release from the sarcoplasmic reticulum (SR) can lead to SR stress [29]. Because PC2 binds to the RyR and can maintain the channel in the closed state [2, 32], an upregulation of PC2 would be expected to counteract the consequences of hyperphosphorylation and calcium leak from the RyR. Taken together, the upregulation of PC2 in the WT mice can counteract the deleterious signaling pathways associated with prolonged β-adrenergic activation.

Here, we have provided an initial characterization of the functional consequence of decreased PC2 in prolonged cardiac stress conditions. However, further studies are needed to explore how heart dysfunction as an initiating step, rather than a consequence, affects progression of kidney function impairment in ADPKD patients. Studies on BNP knock-out rats have shown a critical role of BNP on blood pressure control and the regulation of cardiac hypertrophy pathways, with young adult BNP knock-out rats developing left

ventricular cardiac hypertrophy that precedes hypertension. Moreover, the knock-out rats showed progressive nephropathy [15]. In addition to the natriuretic peptide system that we show here to be disrupted in stressed Pkd2$^{+/-}$ mice, it will be important to monitor other systems that are markedly disturbed in stress conditions, such as the catecholaminergic and RAAS systems. Indeed, high levels of aldosterone inhibit the expression of BNP in a mouse model of hypertension [3]. Along with this observation, it is shown that normotensive adult ADPKD patients, with well-preserved renal function, have higher plasma aldosterone levels when compared to their family members of the same age and sex [10].

In conclusion, prolonged cardiac stress in Pkd2$^{+/-}$ mice does not lead to the upregulation of natriuretic peptides. In contrast, WT mice displayed increased expression of the natriuretic peptides and an upregulation of PC2. Because the Pkd2$^{+/-}$ mice showed no change in natriuretic peptides, we propose that decreased expression of PC2 prevents the activation of the normal compensating cardiac mechanisms and, as a consequence, promotes the development of cardiac dysfunction and hypertrophy. In ADPKD patients, the dissociation of these compensating mechanisms may contribute to cardiovascular pathologies and/or abnormalities in ADPKD patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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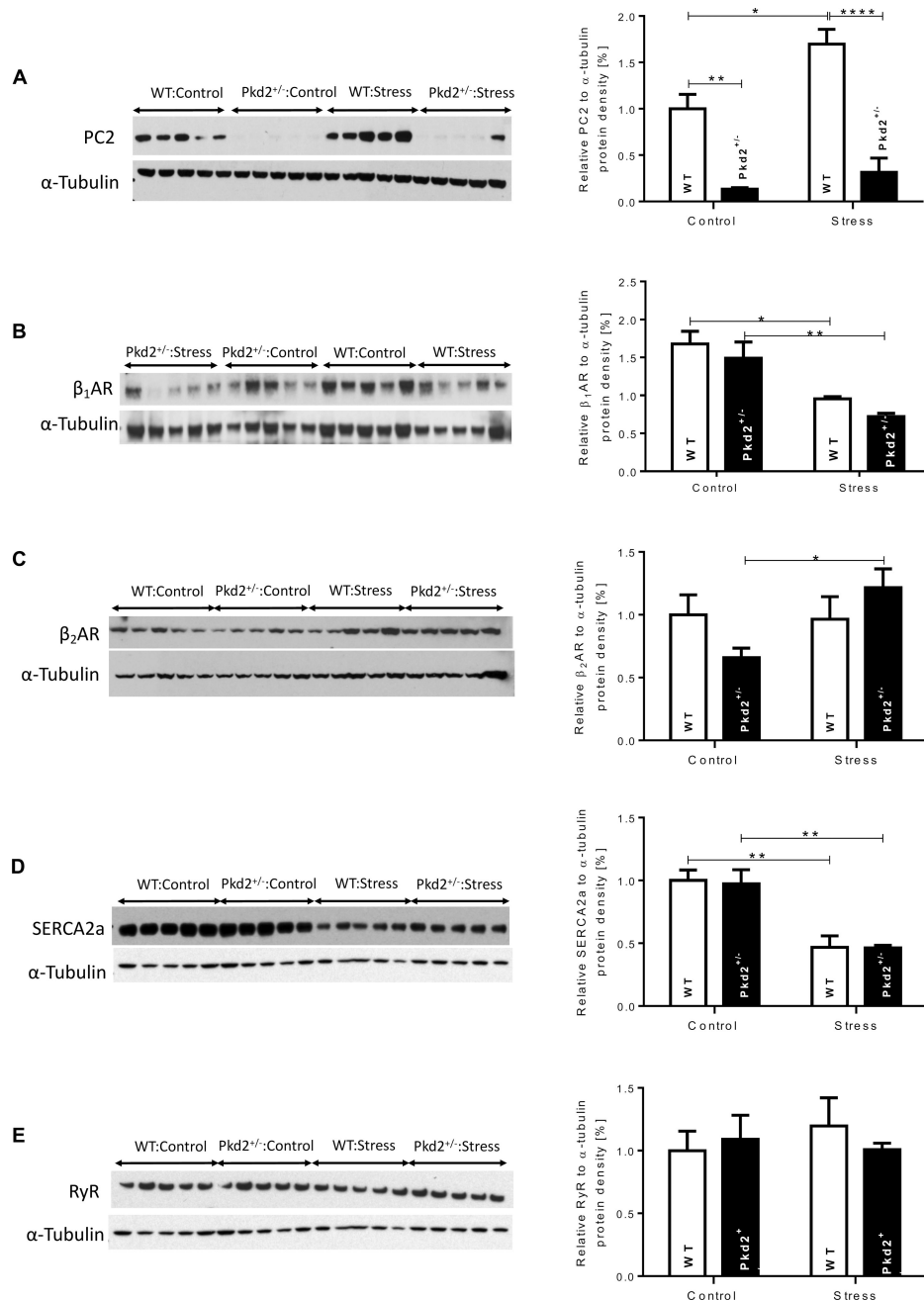
Fig. 1. Polycystin-2 (PC2), β_1-adrenoreceptor (β_1AR), β_2-adrenoreceptor (β_2AR), SERCA2a and ryanodine receptor (RYR) protein expression after prolonged β-adrenergic stress
Western blot analysis of PC2 (A), β_1AR (B), β_2AR (C), SERCA2a (D) and RyR (E) to alpha-tubulin expression in cardiac ventricular tissue of 6 week old male WT and Pkd2$^{+/-}$ mice after 7 days of control or isoproterenol (ISO: 25mg/kg/day) treatment and representative images of the blots. Each lane represents a different animal. Results are presented as the mean±sem. *, ** and **** mean p<0.05, 0.01 or 0.001, respectively. Two-way ANOVA, Tukey post test.
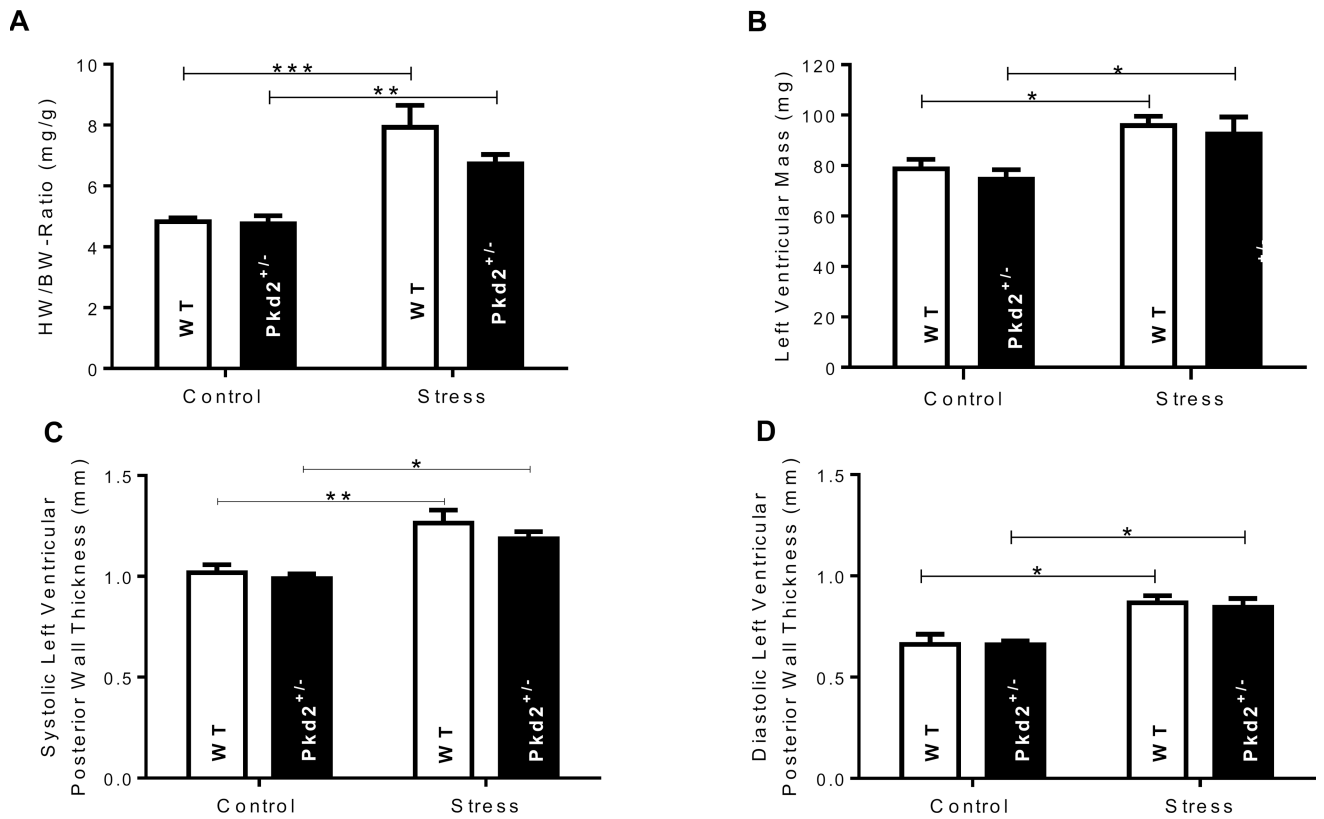
Fig. 2. Pkd2$^{+/-}$ and WT mice have increased left ventricular mass after prolonged β-adrenergic stress

Echocardiographic analysis of 6 weeks old male WT (white bar) and Pkd2$^{+/-}$ (black bar) mice after 7 days of isoproterenol (ISO: 25 mg/kg/day) or vehicle treatment. (A) Heart weight/Body weight ratio; (B) Left ventricular mass; (C) Systolic left ventricular posterior wall thickness; (D) Diastolic left ventricular posterior wall thickness. Results are presented as the mean±sem. * means p<0.05. Two-way ANOVA, Tukey post test.

A

B

C
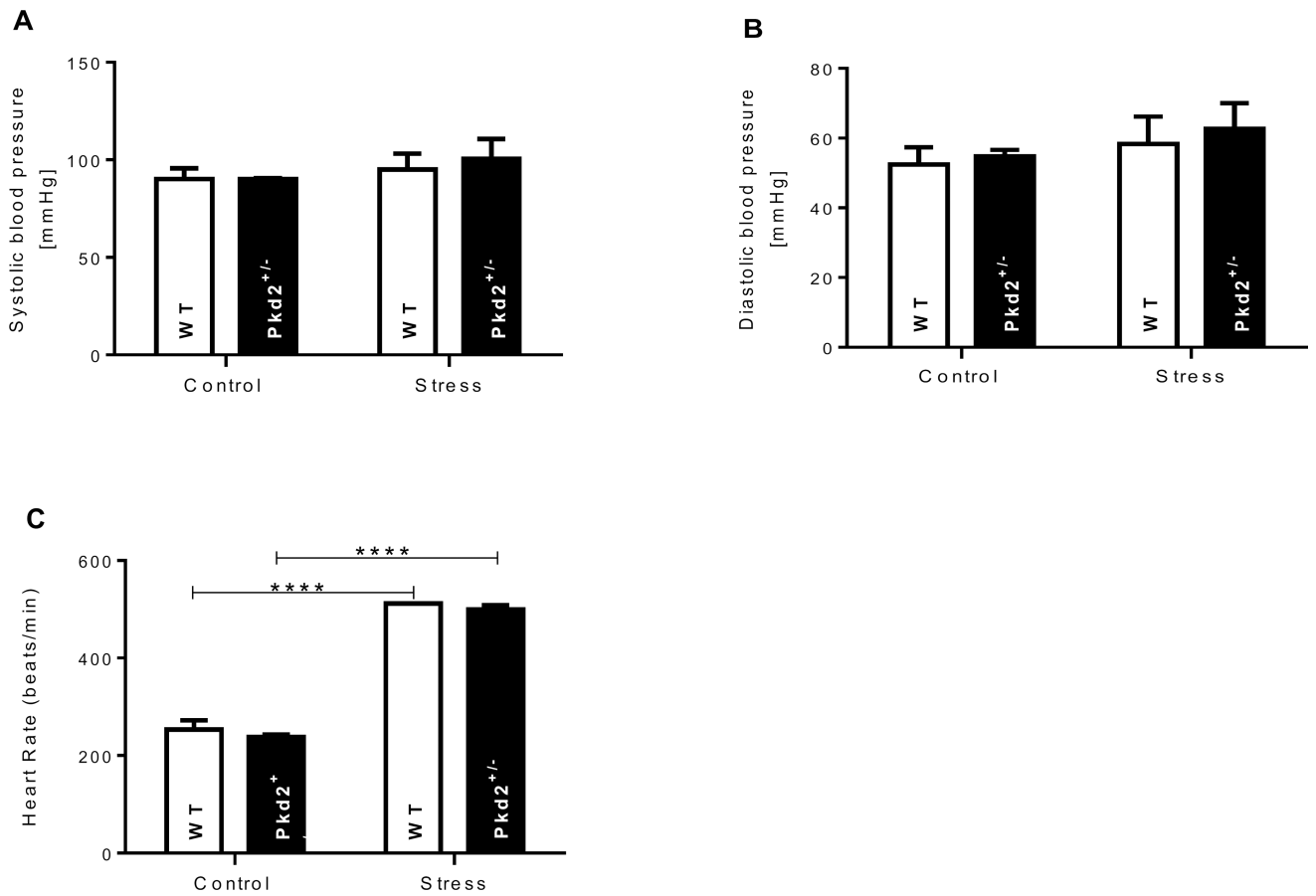
Fig. 3. WT and Pkd2$^{+/-}$ mice have unaltered blood pressure after prolonged β-adrenergic stress
Echocardiographic and hemodynamic analysis of 6 week old male WT (white bar) and
Pkd2$^{+/-}$ (black bar) mice after 7 days of isoproterenol (ISO; 25mg/kg/day) or vehicle
treatment. (A) Systolic blood pressure; (B) Diastolic blood pressure (C) Heart rate under
anaesthesia. Results are presented as the mean±sem. **** means $p < 0.001$. Two-way
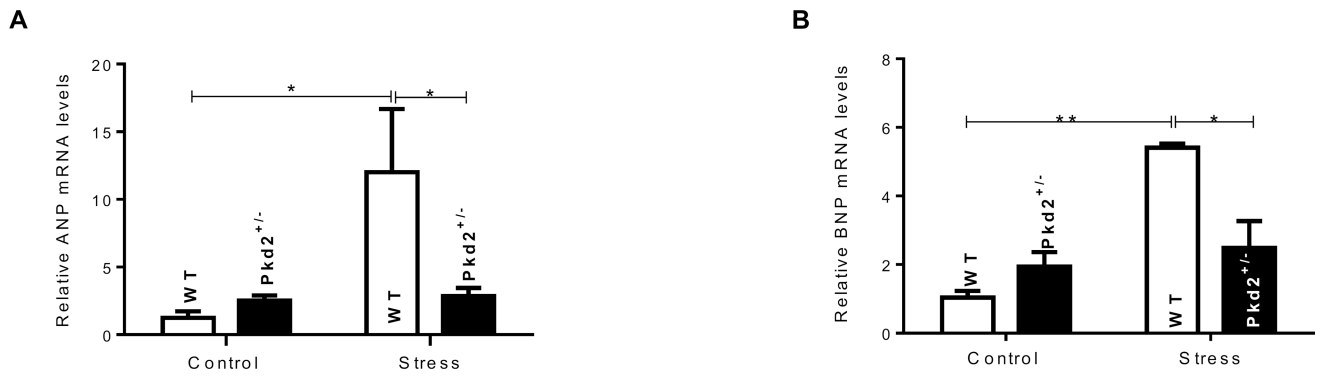ANOVA, Tukey post test.

Fig. 4. Natriuretic peptide expression is unaltered in Pkd2$^{+/−}$ mice after prolonged β-adrenergic stress

qPCR analysis of left ventricular cardiac tissue from 6 weeks old male WT and Pkd2$^{+/−}$ mice after 7 days of isoproterenol (ISO: 25 mg/kg/day) or vehicle treatment. (A) Relative ANP (Atrial natriuretic peptide) gene expression; (B) Relative BNP (Brain natriuretic peptide) gene expression. Results are presented as the mean±sem. * and ** mean p<0.05 or 0.01, respectively. Two-way ANOVA, Tukey post test.
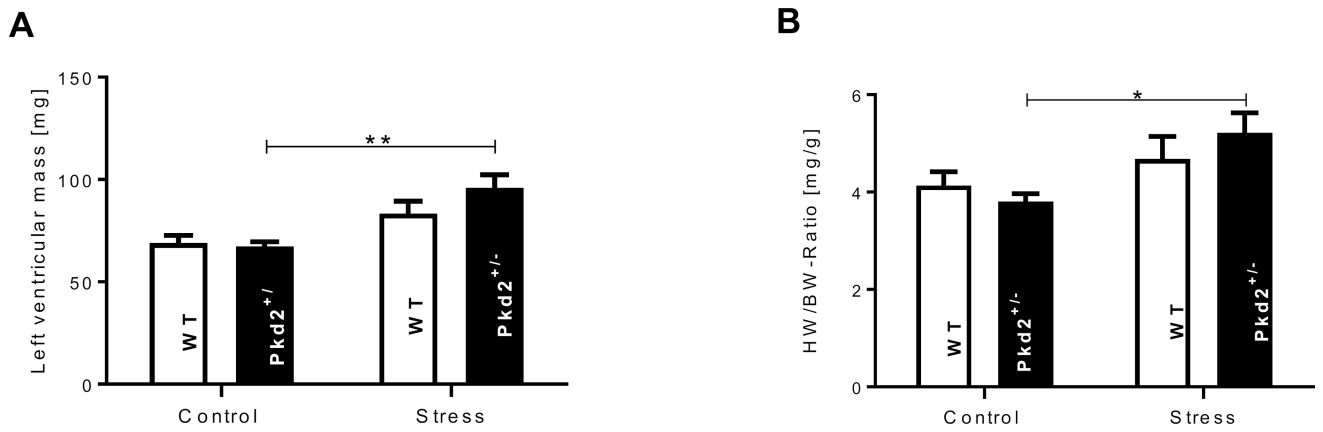
A

B

Fig. 5. Increased cardiac mass persists in Pkd2$^{+/}$-, but not WT, mice after termination of chronic β-adrenergic stress

Echocardiographic analysis of 6 week old male WT (white bar) and Pkd2$^{+/-}$ (black bar) mice after 7 days of isoproterenol (ISO; 25 mg/kg/day) or vehicle treatment and three days rest. (A) Left ventricular mass; (B) Heart weight to body weight ratio. Results are presented as the mean±sem. * and ** means p<0.05 or 0.01, respectively. Two-way ANOVA, Tukey post test.
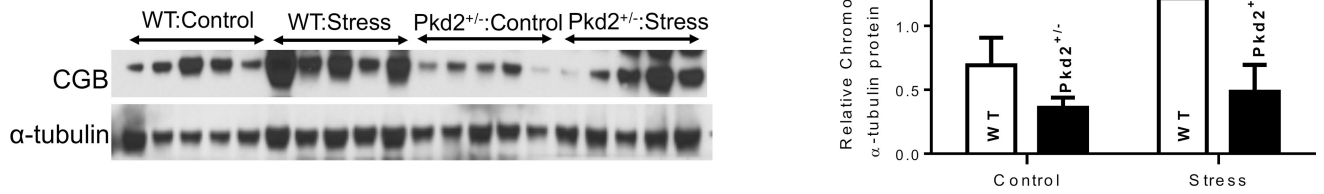
Fig. 6. Chromogranin B expression is not upregulated in Pkd2$^{+/-}$ mice after prolonged β-adrenergic stress

Western blot analysis of left ventricular cardiac tissue from 6 weeks old male WT and Pkd2$^{+/-}$ mice after 7 days of isoproterenol (ISO: 25 mg/kg/day) or vehicle treatment. Protein levels of CGB (chromogranin B) and representative images of the blots. Results are presented as the mean±sem. * means p<0.05. Two-way ANOVA, Tukey post test.