Journal of Molecular and Cellular Cardiology 58 (2013) 199–208

Contents lists available at SciVerse ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Original article

Polycystin-2 mutations lead to impaired calcium cycling in the heart and predispose to dilated cardiomyopathy

Jere Paavola [a,b], Simon Schliffke [a,c], Sandro Rossetti [d], Ivana Y.-T. Kuo [a], Shiaulou Yuan [e], Zhaoxia Sun [e], Peter C. Harris [d], Vicente E. Torres [d], Barbara E. Ehrlich [a,*]

[a] Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA
[b] Minerva Foundation Institute for Medical Research, Biomedicum Helsinki 2U, Tukholmankatu 8, 00290 Helsinki, Finland
[c] Department of Anatomy II: Experimental Morphology, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany
[d] Division of Nephrology and Hypertension, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA
[e] Department of Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

ARTICLE INFO

Article history:
Received 20 July 2012
Received in revised form 4 January 2013
Accepted 17 January 2013
Available online 30 January 2013

Keywords:
Zebrafish
Polycystin-2
ADPKD
Calcium
Heart
Cardiomyopathy

ABSTRACT

Mutations in *PKD1* and *PKD2*, the genes encoding the proteins polycystin-1 (PC1) and polycystin-2 (PC2), cause autosomal dominant polycystic kidney disease (ADPKD). Although the leading cause of mortality in ADPKD is cardiovascular disease, the relationship between these conditions remains poorly understood. PC2 is an intracellular calcium channel expressed in renal epithelial cells and in cardiomyocytes, and is thus hypothesized to modulate intracellular calcium signaling and affect cardiac function. Our first aim was to study cardiac function in a zebrafish model lacking PC2 (*pkd2* mutants). Next, we aimed to explore the relevance of this zebrafish model to human ADPKD by examining the Mayo Clinic's ADPKD database for an association between ADPKD and idiopathic dilated cardiomyopathy (IDCM). *Pkd2* mutant zebrafish showed low cardiac output and atrioventricular block. Isolated *pkd2* mutant hearts displayed impaired intracellular calcium cycling and calcium alternans. These results indicate heart failure in the *pkd2* mutants. In human ADPKD patients, we found IDCM to coexist frequently with ADPKD. This association was strongest in patients with *PKD2* mutations. Our results demonstrate that PC2 modulates intracellular calcium cycling, contributing to the development of heart failure. In human subjects we found an association between ADPKD and IDCM and suggest that *PKD* mutations contribute to the development of heart failure. This article is part of a Special Issue entitled "Calcium Signaling in Heart".

1. Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disease affecting approximately 1 in 500 people. It is characterized by renal cysts and numerous extra-renal manifestations [1]. Cardiovascular problems are a major cause of morbidity and a leading cause of mortality in patients with ADPKD [2,3]. Hypertension [4], left ventricular hypertrophy [5], arterial aneurysms [6], and cardiac valve abnormalities [7,8] are associated with the disease. Interestingly, young ADPKD patients with normal blood pressure and renal function exhibit early vascular changes and bi-ventricular diastolic dysfunction [9,10]. The cellular and molecular etiologies of these early changes remain elusive.

Idiopathic dilated cardiomyopathy (IDCM) is a relatively common heart disease characterized by dilated ventricles and weakened systolic function [11]. It is the most frequent form of non-ischemic cardiomyopathy, affecting approximately 1 in 2500 people [12,13]. An estimated 25–50% of the cases are hereditary [14–16]. The genetic background is varied; to date over two dozen chromosomal loci and disease genes have been linked to IDCM [17]. However, no direct evidence links ADPKD to IDCM.

Mutations in two genes are known to account for ADPKD [18,19]. *PKD1* mutations account for ~85% and *PKD2* mutations for ~15% of cases [20]. *PKD1* and *PKD2* encode the proteins polycystin-1 (PC1) and polycystin-2 (PC2), respectively. PC1 is a transmembrane protein that interacts with PC2, which is a non-selective calcium-regulated cation channel [21,22]. PC2 is a member of the TRP family (TRPP2) [23] and is primarily found on the endoplasmic/sarcoplasmic reticulum (E/SR)

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; APD, action potential duration; AV, atrioventricular; bpm, beats per minute; dpf, days post fertilization; CICR, calcium-induced calcium release; ER, endoplasmic reticulum; hpf, hours post fertilization; IDCM, idiopathic dilated cardiomyopathy; InsP3R, inositol 1,4,5-trisphosphate receptor; LTCC, L-type calcium channel; LVEF, left ventricular ejection fraction; NCX, sodium–calcium-exchanger; PC1, polycystin-1; PC2, polycystin-2; PKD1, polycystic kidney disease 1; PKD2, polycystic kidney disease 2; RyR2, cardiac ryanodine receptor; SR, sarcoplasmic reticulum; TRPP2, polycystin-2; TWA, T-wave alternans; WT, wildtype.

* Corresponding author at: Yale University School of Medicine, Sterling Hall of Medicine, Room B207, 333 Cedar St., New Haven, CT 06520-8066, USA. Tel.: +1 203 737 1158; fax: +1 203 737 2027.
E-mail address: barbara.ehrlich@yale.edu (B.E. Ehrlich).

0022-2828/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.yjmcc.2013.01.015

membrane and in primary cilia [24,25], where it colocalizes with PC1. Most evidence points to PC2 as an intracellular calcium channel that participates in the regulation of intracellular calcium concentration [26–28]. However, a PC2-like protein appears to function as a channel on the plasma membrane in rat ventricular cardiomyocytes [29]. In addition, PC2 is a modulator of the cardiac ryanodine receptor (RyR2) [30]. RyR2, which is a calcium release channel found on the SR membrane, is crucial for calcium-induced calcium release (CICR), which is a prerequisite for excitation–contraction coupling. Altered RyR2 function is seen in heart failure [31], and in patients carrying mutations in RyR2 [32]. PC2 interacts with RyR2, stabilizing the closed state and inhibiting the release of calcium [30]. Loss of inhibition of RyR2 by PC2 in PC2-deficient cardiomyocytes results in a higher frequency of spontaneous calcium oscillations, reduced SR calcium stores, and reduced calcium transient amplitude compared with wildtype (WT) cells [30].

In recent years, zebrafish (*Danio rerio*) have emerged as a powerful model for studying genetic mechanisms of human cardiovascular diseases [33–35]. We studied cardiac function in a zebrafish model of ADPKD that lacks PC2 [36]. PC2 is ubiquitously expressed in the zebrafish [37,38] including in muscles where it is expressed in a sarcomeric pattern, strongly suggesting localization on the SR [37]. Our first goal was to determine whether cardiac function is altered in PC2-deficient fish by in vivo monitoring of cardiac performance. Next, we aimed to examine potential underlying causes by studying intracellular calcium cycling and action potentials. Importantly, to determine whether the results of our zebrafish studies are relevant to human ADPKD, we examined the Mayo Clinic ADPKD database. The observation that ADPKD and IDCM coexisted with high frequency had previously suggested a possible association between these two conditions [39]. However, this connection remains unexplored. We hypothesized that the PC2-deficient fish would exhibit altered calcium handling and cardiac dysfunction. We also hypothesized that PKD2 patients would have an increased risk of heart failure compared to non-PKD patients, due to the direct interaction of PC2 with intracellular calcium cycling proteins.

2. Materials and methods

2.1. Zebrafish and morpholino injections

The zebrafish line *pkd2/hi4166*, which lacks expression of the protein PC2, has been described previously [36]. Morphant embryos were obtained through antisense morpholino oligonucleotide injection into WT eggs at one-cell stage. Fish homozygous for the *pkd2* mutation (referred to as *pkd2* mutants) were compared to unrelated WT fish in all experiments, unless otherwise stated.

2.2. Zebrafish cardiac physiology

For cardiac output measurements, heart rates were counted and images of blood flow in the dorsal aorta were captured at 125 frames per second. Tracking of red blood cells and measurement of aorta diameter allowed stroke volume to be calculated. To study cardiac function, we found determining cardiac output based on measurements of aortic erythrocyte flow to be the most repeatable method. For measurements such as ventricular ejection fraction and fractional shortening, which are based on determining end-systolic and end-diastolic diameters of the long and short axes of the ventricle, positioning the heart in a standardized way proved difficult in vivo, complicating reliable measurement of these indices. Standardized positioning of the heart was partly complicated by edema in the *pkd2* mutant fish.

2.3. Intracellular calcium and action potential imaging, electrical pacing, and SR calcium measurement

For calcium imaging experiments, isolated hearts were loaded with fluo-4 AM, followed by de-esterification before imaging. For optical action potential recordings, isolated hearts were loaded with di-4-ANEPPS. After a baseline recording of spontaneous calcium transients, hearts were electrically stimulated by field pacing. The SR calcium content was determined in calcium-free Tyrode's solution by provoking calcium release with caffeine and thapsigargin.

2.4. ADPKD database and mutation screening

Use of the clinical Mayo Clinic ADPKD database and genotyping of research subjects were approved as part of a larger study of genotype–phenotype correlations in Polycystic Kidney Disease by the Mayo Institutional Research Board. The diagnosis of ADPKD was based on Ravine's criteria in the presence of a positive family history. In the absence of a family history, the criteria for diagnosing ADPKD required at least 20 bilateral renal cysts and absence of clinical findings suggesting the presence of a different cystic disease. A diagnosis of IDCM was made in patients with a LVEF<40%, exclusion of ≥50% obstruction of one or more coronary arteries, exclusion of active myocarditis or a primary or secondary form of heart muscle disease, and exclusion of advanced renal insufficiency (stage 4 or 5 chronic kidney disease). The entire coding and flanking intronic regions of *PKD1* and *PKD2* were screened for mutations by direct sequencing as previously described [20,40].

2.5. Statistical analysis

All values are presented as mean ± s.e.m., determined by Student's *t* test. A *P* value of <0.05 was considered statistically significant.

3. Results

3.1. Pkd2 mutant zebrafish lack cardiac expression of PC2

Pkd2 mutants show dorsal body curvature, making them easy to distinguish from fish lacking or carrying only one copy of the mutant allele (Figs. 1A and B). We found PC2 expression throughout the heart of WT fish (Fig. 1C), but not in the hearts of *pkd2* mutants (Fig. 1D) or fish injected with a morpholino silencing *pkd2* (Fig. 1E). These data verify that the *pkd2/hi4166* line lacks expression of the protein PC2.

3.2. In wildtype hearts PC2 localizes to the sarcoplasmic reticulum

In WT zebrafish hearts we found that PC2 localizes predominantly to the sarcoplasmic reticulum, as shown by the perinuclear co-localization with known SR markers Serca2 and BiP (Supplementary Fig. S1).

3.3. Pkd2 mutant zebrafish display impaired cardiac function

Pkd2 mutants had lower heart rates than their siblings with normal phenotype (Fig. 2A). Furthermore, this difference in heart rates became more pronounced with increasing age (Fig. 2B). This increasing difference in heart rates led us to study cardiac function in more detail. We determined cardiac output based on measurement of aortic erythrocyte flow (Fig. 2C). Heart rate (Fig. 2D) and stroke volume (Fig. 2E) in *pkd2* mutants were notably lower than in WT fish. As a result, cardiac output in WT fish was nearly double compared to that in *pkd2* mutants (16.7 ± 1.1 nl/min vs. 9.8 ± 0.7 nl/min, $P = 0.0002$) (Fig. 2F). During systole, peak velocities of erythrocytes in the aorta were similar in WTs and *pkd2* mutants, 1.94 ± 0.05 mm/s vs. 1.71 ± 0.09 mm/s, $P > 0.05$.

3.4. Pkd2 mutant zebrafish exhibit arrhythmia and edema

We observed an increase in pericardial and abdominal edema in the *pkd2* mutants. Analyzed data from videos demonstrate an increase in arrhythmias in the *pkd2* mutant hearts in vivo (Fig. 2G). In