**RESEARCH ARTICLE**

*Bicaudal C* mutation causes *myc* and TOR pathway up-regulation and polycystic kidney disease-like phenotypes in *Drosophila*

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**Abstract**

Progressive cystic kidney degeneration underlies diverse renal diseases, including the most common cause of kidney failure, autosomal dominant Polycystic Kidney Disease (PKD). Genetic analyses of patients and animal models have identified several key drivers of this disease. The precise molecular and cellular changes underlying cystogenesis remain, however, elusive. *Drosophila* mutants lacking the translational regulator Bicaudal C (BicC, the fly ortholog of vertebrate BICC1 implicated in renal cystogenesis) exhibited progressive cystic degeneration of the renal tubules (so called “Malpighian” tubules) and reduced renal function. The BicC protein was shown to bind to *Drosophila* (*d-*) *myc* mRNA in tubules. Elevation of *d-Myc* protein levels was a cause of tubular degeneration in *BicC* mutants. Activation of the Target of Rapamycin (TOR) kinase pathway, another common feature of PKD, was found in *BicC* mutant flies. Rapamycin administration substantially reduced the cystic phenotype in flies. We present new mechanistic insight on *BicC* function and propose that *Drosophila* may serve as a genetically tractable model for dissecting the evolutionarily-conserved molecular mechanisms of renal cystogenesis.

**Author summary**

Polycystic kidney disease (PKD) is a degenerative, potentially lethal, genetic malady that affects 12.5 million people world-wide for which there is no cure. In the kidney, PKD causes the formation of prominent, fluid-filled cysts the growth of which damages progressively kidney function. Crucial to PKD development, mutations in the *PKD1* and *PKD2* genes cause renal cystic degeneration via factors and mechanisms that are only partially known. This manuscript reports novel insights into the molecular mechanisms of the evolutionarily conserved RNA binding protein BicC, which has been implicated in vertebrate cystic kidney diseases. The BicC mutants of the fruit fly *Drosophila melanogaster* recapitulate crucial characteristics of PKD. A clear link between BicC and PKD has begun to emerge, in part because both *PKD1* patients and *Pkd1* mice exhibit reduced BicC function. This first in kind *Drosophila* model of renal cystogenesis offers strong potential to
decipher the complex mechanisms of the molecular and cellular changes causing renal
cyst formation.

Introduction

Maintenance of structural and functional integrity of the kidney is a complex, crucial task pre-
sided over by the activity of numerous genes. Renal cyst formation can result from the muta-
tion of at least one of over 57 genes [1,2]. Multiple, clinically relevant, forms of cystic kidney
disease exist, exhibiting different modalities of genetic inheritance: syndromic, non-syndro-
mic, dominant, and recessive [2]. Autosomal Dominant Polycystic Kidney Disease (ADPKD) is
the most common cause of end-stage renal failure, affects 12.5 million people world-wide
and has an incidence of 1–2 cases per 2000 live births world-wide [1]. Mutations in the PKD1
and PKD2 genes account for the majority of the genetic lesions in ADPKD patients [3,4].
ADPKD causes the loss of polarity in the cells of the renal tubule epithelium and the develop-
ment of fluid-filled cysts and interstitial fibrosis in the kidney [1,5,6,7]. Several animal models
have been studied to identify the pathways and the complex changes that eventually lead to
renal cystogenesis [8]. Among these, activation of the mammalian (m) TOR pathway was
found in various forms of renal cystic pathologies, including human ADPKD cysts, autosomal
recessive PKD, and rodent models of PKD and nephronophthisis [5,6,7,9,10,11]. TOR is a con-
served serine threonine kinase central to controlling cellular anabolic processes via protein
translation, ribosome biogenesis, nutrient sensing/transport and mitochondrial metabolism
[12,13,14]. TOR is often dys-regulated in disease [12,15]. The stages and pathological progres-
sion of PKD cystogenesis are known [1]. Although genetic analyses have indicated that some
signaling pathways are altered in PKD tissue, the precise molecular and cellular changes
underlying cystogenesis in PKD and other diseases remain elusive.

Mutations in the Bicaudal C (BicC) gene in many vertebrates are associated with the devel-
opment of renal cysts [16,17,18,19]. BicC proteins are a family of RNA binding factors of
which the prototypical member, BicC, was demonstrated to be necessary for establishing the
correct anterior-posterior polarity of the embryo of the fruit fly Drosophila melanogaster
[20,21]. Subsequent studies reported that BicC functions to establish polarity of the oocyte,
and consequently of the embryo. During oogenesis BicC recruits the CCR4-NOT deadenylase
to its target mRNAs and regulates cytoplasmic polyadenylation, eventually affecting transla-
tion [22,23,24].

Evolutionarily conserved from Drosophila to vertebrates, BicC is abbreviated as Bicc1 and
BICC1 for mouse and humans respectively [20]. Several lines of evidence have implicated
Bicc1 and BICC1 in renal function and cystogenesis. Mutations in Bicc1 as well as in its Xenopus
and zebrafish orthologs result in cystic kidneys [16,19,25,26]. In humans, two BICC1 muta-
tions were identified in patients with cystic kidney dysplasia [17]. Bicc1 can associate with the
Ank and NPHP proteins in a complex important for the development of the zebrafish pro-
nephros [27]. The Bicc1/Ank/NPHP complex was implicated in human nephronophthisis, a
cystic kidney disease characterized by multiple extra-renal manifestations [28]. Moreover, a
30% reduction in Pkd2 mRNA levels has been observed in Bicc1 mutant mice [25]. Despite the
numerous vertebrate BicC animal models, the precise molecular mechanisms underlying
BicC-dependent kidney degeneration are largely unknown.

The Drosophila renal function is carried out by two pairs of renal tubules, specifically called
Malpighian tubules [29], and anatomically separated nephrocytes, that resemble vertebrate
podocytes [30]. The fly Malpighian tubules are regarded as morphologically and functionally
equivalent to the vertebrate renal tubule. Fly and vertebrate tubules both are composed of a tubular epithelium, contain distinct physiological regions, produce primary urine and reabsorb some solutes [31,32]. The transcriptomes of the Malpighian tubule and the human renal tubule are also remarkably similar [33]. Several fly models exist for various kidney diseases [34] because of the evolutionary conservation of renal function; however, no Drosophila models have been described for cystic kidney disease.

Herein, we report that Drosophila BicC mutants developed cysts in the Malpighian tubules and provide novel evidence of the mechanism of BicC regulation. BicC was found to bind directly the d-myc mRNA. Characteristic of cystic tubule degeneration and PKD, BicC mutants exhibited both d-myc up-regulation and TOR pathway activation. Moreover, underscoring conserved causative mechanisms, rapamycin, a TOR inhibiting drug known to ameliorate PKD defects in vertebrates, was shown to be effective in reducing the defects observed in BicC mutant flies. Notably, BicC was significantly down-regulated in PKD tissue, both in patients and in mouse Pkd1 models, indicating that BicC is genetically downstream of PKD1. Therefore, Drosophila BicC mutants appear to provide a valid model to dissect the molecular and cellular aspects of cyst biology.

Results

Impaired BicC function induces renal cystogenesis in Drosophila

As seen by light microscopy, wild-type Malpighian tubules were thin and elongated, with regular diameter. This appearance was constant over thirty days of adult life (Fig 1A–1C). In contrast, BicC mutant tubules from Df(2L)RA5/BicCΔYC33 and Df(2L)RA5/BicCΔIIF34 flies (subsequently referred to as BicCΔYC33 and BicCΔIIF34 respectively) exhibited deformations from early age (1 day, Fig 1D–1F and 1G–1I, respectively). Disorganization of the tubular epithelial cells and irregularly distributed enlargements were consistently observed in the BicC mutant flies. The Malpighian tubule malformations were reminiscent of the defects observed in various forms of human PKD [35]. At an early age the tubules appeared severely affected and presented large, often numerous cysts in both BicCΔYC33 and BicCΔIIF34 flies (Fig 1D, 1G and 1J). When the BicC mutant flies approached 14 days of age, both tubules displayed irregularities, appeared enlarged, and presented numerous deformities (Fig 1E, 1H and 1K). Prominent enlargements closely resembling cysts appeared filled with fluid and floating dark crystalline particles that were not observed in the wild-type controls that were raised in parallel under identical conditions. Dense, amorphous, material could also be seen in long sections of the BicC tubules and -rarely- in short sections of the wild-type tubules from old flies. Unlike wild type, that maintained thin, elongated Malpighian tubules, at 30 days, BicC flies displayed multiple cysts in both tubules and prominent deformities of the anterior terminal tubule (Fig 1C, 1F, 1I and 1L). Additionally, extra tubular budding and branching were observed exclusively in BicC mutant tubules (Fig 1J and 1L). To better characterize the defects and the progression of the phenotype over time, we scored the incidence of cystic deformations in Malpighian tubules dissected from 50 flies (100 anterior and 100 posterior tubules) of each genotype and at different ages. Both pairs of Malpighian tubules displayed cysts, especially in the terminal and intermediate regions (observed in 42–100% of cases, Fig 2A–2C), which was reminiscent of PKD that is reported to affect preferentially the terminal section of the nephron [36,37]. Akin to PKD [36] the BicC tubules also presented extra branching (9–46% of cases). The cysts of the BicC Malpighian tubules appeared to become more numerous over time, albeit the deaths of many flies influenced the representation of the different classes (Fig 2D).

Considering that the BicC mutation causes renal cystogenesis in vertebrates [16,17,19,25,26], immunostaining was performed using species-specific antibodies to characterize
expression and distribution of the BicC protein in wild-type Malpighian tubules and of its ortholog Bicc1 in kidney sections from C57BL/6 wild-type adult mice. Drosophila BicC was expressed in the Malpighian tubule epithelium (Fig 3A–3D). Similarly, mouse Bicc1 was found at highest levels in the renal tubule epithelium, with a much lower signal in the medullar region (Fig 3E–3H). Thus, consistent with the homologous primary structure [20], the Drosophila and the mouse BicC proteins are both expressed in the renal tubules, suggesting a possible functional conservation.

BicC is necessary for proper Malpighian tubule function in Drosophila

Cyst formation and interstitial fibrosis cause chronic kidney disease and kidney failure by age 55 in 50% of all PKD patients [8]. To investigate if the morphological defects in BicC mutant Malpighian tubules had phenotypic consequences, we assessed the viability of wild-type and mutant adult flies. Compared to wild-type, both BicCΔ/YC33 and BicCΔ/IIF34 mutant flies displayed impaired survival, with population decrease by day 9 to 91.5 ± 1.9% and 57 ± 6.6% survival for BicCΔ/YC33 and BicCΔ/IIF34 respectively, contrasted to 99.5 ± 1% survival for wild-type flies (n = 200; Fig 4A). Populations of BicCΔ/YC33 and BicCΔ/IIF34 mutants reached 50% survival at 28 and 16 days respectively, compared to 48 days for wild-type flies. Notably, BicCΔ/+ heterozygotes displayed similar survival to wild-type (S1 Fig) and were not further analyzed. Impaired viability correlated with renal degeneration. Malpighian tubules dissected from moribund flies were misshapen, displayed large fragile cysts, and often contained prominent impacted material (Fig 4B).
Malpighian tubule function was tested by placing flies in a cornmeal medium containing 0.5M NaCl. The hypertonic medium acts as a stress that may be used to reveal diminished Malpighian tubule function. Under the high-salt conditions survival of the wild-type was greatly impaired, with populations reaching 50% in ~2.2 days and 0% at 4–5 days. Compared to wild-type, \textit{BicC}^{Δ/YC33} and \textit{BicC}^{Δ/IIF34} fly lifespans were shortened, with populations steadily declining from exposure to high salt medium and reaching 50% survival at 1.8 and 1 days respectively (Fig 4C, \textit{n} = 150). Differential survival did not appear to be due to starvation, because all genotypes appeared to feed normally on high salt food stained with food dye (S2 Fig). Taken together, our observations indicate that \textit{BicC} is required for proper renal tubule function in \textit{Drosophila}.

Fig 2. Quantification of the Malpighian tubule cystic phenotype of \textit{BicC} flies. Malpighian tubules from wild type, \textit{BicC}^{Δ/YC33} and \textit{BicC}^{Δ/IIF34} (50 flies each) were dissected at age one day (A), 10 days (B), and 30 days (C) and scored phenotypically \textit{ex vivo}. Shown are the percentages of tubules affected in the terminal, intermediate, and proximal regions, as well as the observed extra tubular branching. Anterior and posterior tubules were scored separately. Tubular cyst number from the same sample is shown (D). The occurrence of \textit{BicC}^{Δ/YC33} tubules displaying at least four cysts appeared to increase over time. The same class in the \textit{BicC}^{Δ/IIF34} tubules was more numerous at one day of age, was less represented at ten days, and increased again in the older flies (Fig 2D), possibly reflecting mortality of the more severely affected flies (see text).
Fig 3. **BicC expression in renal tissue.** Confocal section of *Drosophila* Malpighian tubules with BicC immunofluorescent staining (red, A, C, D) and DAPI nuclear staining (blue B, C) show that BicC is expressed in the principal cells. Boxed area in (A) is shown enlarged in (D). Epifluorescence microscopy of a 5 μm kidney section from C57BL/6 mice shows Bicc1 accumulation in the cells lining the renal tubule (red, E, G, H). DNA, DAPI (blue, F, G). Panel (H) is an enlargement of the boxed area in (E). Scale bars: 20 μm.

https://doi.org/10.1371/journal.pgen.1006694.g003

Fig 4. **BicC flies exhibit severe renal defects and impaired survival.** (A) Survival curves of fly populations (n = 200, standard deviations are shown) of wild-type (wt), BicCΔYC33 (Δ/YC33) and BicCΔIIF34 (Δ/IIF34). Compared to wild-type, both BicCΔYC33 and BicCΔIIF34 flies have impaired survival, reaching 50% respectively at 48, 28, and 16 days after adult eclosion. (B) Malpighian tubules dissected from a moribund BicCΔIIF34 fly showed severe deformities with large cysts containing apparent impacted materials; scale bar: 100 μm. (C) BicCΔYC33 and BicCΔIIF34 flies exhibited sensitivity to salt stress. Fly populations (wild-type, BicCΔYC33, BicCΔIIF34, n = 150, standard deviations are shown) placed in vials containing cornmeal agar with 0.5M NaCl were at a disadvantage and had shorter life span than the wild-type controls.

https://doi.org/10.1371/journal.pgen.1006694.g004
In the *Drosophila* ovary BicC is a negative regulator of translation and functions by first binding to its mRNA targets [20,24]. To examine BicC role(s) in the Malpighian tubule, RNA-protein (RNP) complexes were immunoprecipitated from extracts of Malpighian tubules from wild-type flies with either BicC antisera or a non-immune serum. RNA extracted from small aliquots of the input extracts (respectively ΔBicC and wild-type) also produced distinct clear PCR products. Representative immunoblots of extracts of Malpighian tubule (four pairs per lane) from wild-type (wt), BicCΔ/YC33 and BicCΔ/IF34 flies probed for d-Myc and tubulin (left). Corresponding graphs of means ± standard deviations of d-Myc levels relative to tubulin (right). Values were calculated from independent biological replicates (n, indicated) and p values (Student’s t test) are shown (right). Myc/tubulin ratios were normalized to the wild-type average. d-Myc protein levels were generally higher in BicC mutants and increased with age.

https://doi.org/10.1371/journal.pgen.1006694.g005

**Fig 5.** BicC regulates *d-myc* expression in the Malpighian tubule. *(A)* RT-PCR of RNA immunoprecipitated with either non-immune control serum (NI, lane 1) or BicC antiserum (αBicC, lane 2). A product specific for *d-myc* was amplified exclusively from the BicC immunoprecipitate, indicating that BicC regulates *d-myc* mRNA in the Malpighian tubule. Similar to the situation in the ovary [24], BicC primers used as positive control also yielded a specific product only in the immunoprecipitate. In contrast, tubulin 84B primers did not produce any amplification product, showing that the immunoprecipitation was specific (negative control). Lane 3: PCR negative control (−, no cDNA). Lane 4: PCR positive control (+, cDNA, Malpighian tubule total cDNA). *(B)* RT-PCR of control RNA immunoprecipitation from wild type and Df(2L)IRAS/Df(2L)Osp29 (BicC null, ΔBicC) Malpighian tubules. For both extracts RNP particles (RNPs) were captured with either non-immune control serum (NI, lane 1, 3) or BicC antisera (αBicC, lane 2, 4). *d-myc*-specific amplification products were exclusively observed for wild type extracts with the RNP captured by the BicC antiserum, but not with those captured by the non-immune serum. Neither non-immune nor BicC antisera recovered *d-myc* containing particles. (C) Renal cystogenesis in the *BicC* fly.
that the experimental conditions were selective for RNP complexes specifically containing BicC. Importantly, all primers amplified the expected size products when the reaction was spiked with cDNA synthesized from total RNA extracted from wild-type Malpighian tubules. To confirm the selective enrichment of the \textit{d-myc} mRNA we repeated the RNP immunoprecipitation with immune and non-immune sera using extracts from flies either wild type or containing two overlapping deletions that remove the \textit{BicC} gene, \textit{Df(2L)RA5} and \textit{Df(2L)Osp29 (ABicC)}. While a \textit{d-myc}-specific PCR product was obtained from the wild type RNA associated with the BicC antiserum, no such product was observed for the RNPs recovered from the BicC deleted extract (Fig 5B). The immunoprecipitation with the non-immune serum yielded no amplification product from both extracts, suggesting that the BicC protein and the \textit{d-myc} RNA may interact \textit{in vivo}.

In the ovary, BicC regulates negatively the expression of its own targets. Therefore, the levels of d-Myc protein in wild-type and \textit{BicC} Malpighian tubules were compared relative to tubulin. Quantitative immunoblots of extracts from four pairs of Malpighian tubules which were dissected from flies of different ages (0, 6, 30 days old), revealed that tubules from \textit{BicC} \textit{ΔYC33} mutants contained from two to seven times more full-length d-Myc protein than wild-type tubules from flies of identical age that were raised in parallel (Fig 5C, \(p = 0.0074, 0.0100, 0.0204\) respectively). d-Myc levels in \textit{BicC} \textit{ΔYC34} tubules were also significantly above those of wild-type in the 6 and 30 days old samples (Fig 5C, \(p = 0.0021\) and \(0.0078\) respectively).

c-Myc is upregulated in PKD kidneys [38,39,40,41,42,43] and its overexpression can induce renal cystogenesis in mice [39,40]. To characterize the consequences of d-Myc over-expression in the Malpighian tubules and to pinpoint how elevated d-Myc may contribute to the \textit{BicC} cystic renal phenotypes, we overexpressed d-Myc both in principal and stellate cells (the main cell types of wild-type Malpighian tubules) of wild-type animals using the \{\textit{UAS-d-Myc}\} \textit{32} [44] transgene and cell-specific Gal4 drivers \textit{c42} and \textit{c724} [45] to drive expression in principal and stellate cells respectively. Very few individuals over-expressing \textit{d-myc} survived to adulthood. These rare escapers exhibited severely deformed Malpighian tubules, which were swollen and disorganized (compare Fig 6A–6C with D–F and G–I). Compared to \textit{BicC} \textit{ΔYC33}, Malpighian tubules over-expressing d-Myc were severely deformed and, unlike the tubules of \textit{BicC} mutant flies, did not appear to have extra tubular branching. d-Myc immunostaining (Fig 6A, 6D and 6G) and immunoblots of Malpighian tubule extracts from these flies (Fig 6J and 6K) confirmed that d-Myc was indeed substantially overexpressed relative to controls (3–34 X, \(p = 0.0440\)). Consistent with the observed severity of the \textit{d-myc} over-expression defects, Myc levels in the Malpighian tubules of such flies were up to an order of magnitude higher relative to the \textit{BicC} mutants.

To verify if the Malpighian tubule cysts of the \textit{BicC} flies were due at least in part to the observed up-regulation of d-Myc, we tested if cystogenesis could be suppressed by expression of a dsRNA transgene, \textit{TRiP.JF01762}, [46,47] that targeted \textit{d-myc} in both principal and stellate cells. The resulting flies (denoted as \textit{BicCY33; mycRNAi}) were dissected and Malpighian tubules from the various genotypes were examined by light microscopy to assess tubular morphology. Compared to the sibling \textit{BicCY33} flies that displayed prominent cysts (Fig 7A–7C), \textit{BicCY33; mycRNAi} flies exhibited substantial morphological rescue (Fig 7D and 7E). Consistent with the results shown in Fig 5C, immunoblots of Malpighian tubule extracts from these genotypes revealed that, relative to tubulin, d-Myc levels in the \textit{BicCY33} tubules were increased over two-fold compared to the wild-type (\(p = 0.0264\)). Compared to wild-type, \textit{d-myc} RNAi slightly reduced d-Myc levels when induced in a wild-type background. In contrast, RNA interference in the \textit{BicCY33; mycRNAi} flies reduced d-Myc to the levels of the wild-type and transgene heterozygote controls (Fig 7F and 7G). Myc RNAi in the \textit{BicCY33} tubules reduced the incidence of cysts in the terminal and in the proximal tubule and could decrease swollen tubular sections, compared to Malpighian tubules from sibling
BicC YC33/YC33 flies (Fig 7H). These results were confirmed with an independent line, TRiP, JF01761, which reduced Myc expression less efficiently than TRiP.JF01762 (S3 Fig). Staining with DAPI indicated that, in contrast to BicC YC33/YC33 tubules, which appeared enlarged with irregular distribution of principal cell nuclei, in the thinner-looking tubules from BicC YC33/YC33; myc RNAi flies, cell nuclei appeared more regularly distributed and evenly shaped (Fig 7, compare I with J and K). We conclude that d-Myc up-regulation contributes substantially to the BicC cystic tubule phenotype.

TOR pathway upregulation contributes to the cystic phenotype in BicC mutant flies

The activation of the mTOR pathway underlies many forms of renal cystogenesis, including both autosomal dominant and recessive PKD [6,7,48,49]. Since the observation that cystic progression was reduced in ADPKD patients undergoing post-transplantation immune suppressant therapy with rapamycin [7], the rapamycin derivatives Everolimus and Sirolimus have been examined in clinic and animal models to ameliorate kidney conditions and to delay cystic growth in the short term [50,51,52,53,54]. In light of the observed cystic defects in the BicC

Fig 6. d-Myc overexpression in the Malpighian tubule causes severe cellular and tubular defects. Confocal sections of d-Myc immuno-staining of Malpighian tubules from wild-type flies (A-C), BicC YC33/YC33 flies (D-F), and flies with d-Myc Gal4-driven over-expression in both principal and stellate cells (Gal4>d-myc, G-I). d-Myc (red), DNA (DAPI, blue), d-Myc over-expression in the Malpighian tubules caused severe defects. The irregular shape and density of nuclei are suggestive of tissue disorganization. All images in this panel were captured with identical settings and the signal for d-Myc staining in the Gal4>d-myc tubule (G) was saturated, indicating higher levels of d-Myc over-expression relative to those induced by BicC mutation. Scale bar: 50 μm. (J) Representative immunoblot of extracts of Malpighian tubules dissected from wild-type (wt) flies, flies heterozygote for the Gal4-d-myc construct (Gal4/+), flies over-expressing d-myc c724/+/; c42/Gal4>d-myc (Gal4>d-myc) probed for d-Myc and α-tubulin. (K) Graph of means ± standard deviations of d-Myc levels relative to tubulin from five independent biological replicas per each genotype. Values were normalized to the wild-type average; p values (Student’s t test) are shown. d-myc (d-Myc/tubulin) over-expression in Malpighian tubules ranged from three to over 30 times the levels in tubules of control flies.

https://doi.org/10.1371/journal.pgen.1006694.g006
Fig 7. d-myc knockdown can rescue the Malpighian tubule defects of BicC mutants. Light microscopy of dissected Malpighian tubules from sibling BicC^{YC33/YC33} (A, B, C, control) and BicC^{YC33/YC33}, d-myc^{RNAi} (D, E) flies in which RNAi was induced in both principal and stellate.
cells with the c42 and c724 drivers respectively. d-myc RNAi rescued greatly the morphology of the Malpighian tubule. Boxed regions in (A) and (D) are shown enlarged respectively in (B, C) and (E). (F) Representative d-Myc and tubulin immunoblots of extracts from five Malpighian tubules dissected from flies of the following genotypes: wild-type (wt); heterozygotes for the Gal4 driver c724 and c42 constructs (Gal4/+); heterozygotes for the d-mycRNAi construct (mycRNAi/+); myc RNAi driven by c724 and c42 (Gal4>mycRNAi); BicCΔ/C33/YC33 homozygotes (BicCΔ/C33); myc RNAi driven in BicCΔ/C33 homozygotes (BicCΔ/C33, Gal4>mycRNAi). The latter two genotypes were sibling flies from the same crosses. (G) Corresponding graph summarizing quantitative immunoblots of means ± standard deviations of d-Myc/tubulin ratios from three independent biological replicates per each genotype. Values were normalized to the wild-type average. The p value (Student's t test) is shown for the BicC mutants. Reducing d-myc expression in BicCΔ/C33/YC33 mutants restored the d-Myc protein to control levels. (H) Cystic scoring of the BicCΔ/C33/YC33, Gal4/+ (red, n = 100) and BicCΔ/C33/YC33, Gal4/mycRNAi (blue) sibling flies. Results are shown for the lines TRiP.JF01762 (dark blue, n = 100) shown above, and TRiP.JF01761 (light blue, n = 80, see supplemental information). RNAi-induced d-Myc reduction decreased cystic deformities in the terminal and intermediate tubules. Results for the anterior and posterior tubules are shown separately. (I-K) Confocal sections of Malpighian tubules dissected from BicCΔ/C33/YC33 flies (I, control) and BicCΔ/C33/YC33, mycRNAi flies (J, K) stained with DAPI. The distribution of cell nuclei in BicCΔ/C33/YC33 tubules (l) appeared disturbed (compared with wild-type in Figs 3B and 6B) with disrupted cell arrangement and cystic enlargements. Defects were largely rescued by reducing d-myc expression via RNAi specifically in the principal and stellate cells of the Malpighian tubules (J, K). Scale bar: 100 μm.

https://doi.org/10.1371/journal.pgen.1006694.g007

mutant flies, we tested if administration of rapamycin to adult flies could modify such cystic phenotype. The BicCΔ/IIF34 flies that were administered solvent alone (vehicle) exhibited the expected decreased fitness and rapid population decline relative to wild-type controls (50% survival at ~11 days; Fig 8A).

In contrast, BicCΔ/IIF34 mutants that were treated with 15 μM rapamycin exhibited substantial rescue, with no death observed before 10 days and 50% survival extended nearly two-fold to ~22 days (compare Fig 8A and 8B). Populations of BicC flies that were administered rapamycin eventually declined (Fig 8B), reminiscent of the short-term effectiveness of rapamycin treatment in rodent models of renal cystic disease [54]. Consistent with the observed rescue, the Malpighian tubules that were dissected from rapamycin-treated BicCΔ/IIF34 flies showed substantial improvements compared to those from vehicle-treated BicCΔ/IIF34 flies of the same age with rare, minimal-sized cysts and no extra branching, even at older ages (Fig 8C and 8D). Similar to observations of both Drosophila [55] and other treated organisms [56,57,58], rapamycin administration extended wild-type longevity.

Phosphorylation of the mTOR downstream target p70 ribosomal S6 kinase has been observed in both ADPKD human tissue and in mouse PKD models [7]. Comparative analysis of the Drosophila p70-S6 kinase [59] (hereby d-S6K) in the tubules from rapamycin or vehicle treated flies revealed that BicCΔ/IIF34 mutant tubules contained twice as much phosphorylated d-S6K relative to total d-S6K than wild-type (Fig 8E). In contrast, rapamycin-treated sibling flies, processed in identical conditions and in parallel, exhibited a similar ratio of phosphorylated vs. un-phosphorylated d-S6K (Fig 8E).

An evolutionarily-conserved functional link between BicC, renal cystogenesis, and PKD

Two BicC point mutations were identified in patients with cystic kidneys [17]. To test if BicC was altered in the context of the molecular defects underlying PKD, we analyzed publicly available microarrays from PKD1 ADPKD patients [60] and found that BicC was significantly downregulated in cystic relative to healthy renal tissue (p = 0.0196). This suggested that BICC1 may be genetically downstream of PKD1 in kidneys. Consistent with this possibility, we observed a severe decrease in Bicc1 protein levels in kidneys of Pkd1-/- newborn mice compared to their Pkd1+/+ siblings (Fig 9, similar to reports of whole embryos in [61]).

Discussion

In this work, we show that BicC mutation in Drosophila induced cellular and molecular defects typical of renal cystogenesis and PKD and that both human ADPKD tissue and Pkd1-/- mice
are characterized by decreased BiC function, which is likely to contribute to their characteristic cystic degeneration. BiC mutation in Drosophila caused progressive cystic degeneration of

Fig 8. TOR upregulation and rapamycin rescue of BiC mutant flies. (A) Control wild-type (wt) and BiCΔ/IF34 (Δ/IF34) flies that were administered equal volumes of solvent (ethanol, vehicle) showed the characteristic impaired survival of BiC mutants compared to wild-type. (B) In contrast, sibling flies of both genotypes that were administered rapamycin exhibited markedly improved survival of the BiC mutants compared to control flies that were administered vehicle. A, B *n = 200, standard deviations are shown. Rapamycin administration did not compromise survival of the wild-type flies. Rapamycin appeared to induce almost complete rescue at early time points and substantial rescue over time. Malpighian tubules of vehicle (C) and rapamycin-treated (D) BiCΔ/IF34 flies showed marked morphological rescue with fewer cysts and more regular tubule structure. (E) Total and phosphorylated S6K immunoblots of extracts from wild type and BiCΔ/IF34 Malpighian tubules from flies administered rapamycin or vehicle for 8 days post-eclosion indicated apparent mitigation of the TOR up-regulation in the BiC mutants.

https://doi.org/10.1371/journal.pgen.1006694.g008

Fig 9. BiC1 downregulation in Pkd1−/− mice. (A) In accordance to our analyses of human microarrays of PKD patients, BiC1 immunoblots of kidney extracts from newborn littermates Pkd1+/+ and Pkd1−/− showed that all Pkd1−/− renal tissues exhibited considerable BiC1 downregulation. GAPDH: loading control.

https://doi.org/10.1371/journal.pgen.1006694.g009

are characterized by decreased BiC function, which is likely to contribute to their characteristic cystic degeneration. BiC mutation in Drosophila caused progressive cystic degeneration of
the renal tubule with remarkable similarity to the defects typical of human PKD. 

BicC mutant flies exhibited enlarged Malpighian tubules and fluid-filled cysts from the time of their hatching. Cyst number, size and severity increased over time. Moreover, the terminal and collector tubes appeared to be particularly affected, with frequent extra branching. Compared to wild-type flies, BicC mutants displayed reduced longevity and greater sensitivity to salt stress. Malpighian tubules dissected from moribund BicC flies were severely degenerated, indicative of compromised renal function.

We found that the BicC protein was similarly expressed in the cells of the renal tubular epithelium in both Drosophila and mice. BicC is an RNA binding protein that downregulates post-transcriptional expression of its target mRNAs in Drosophila [20,24]. Evidence for a link between BicC and renal cysts was obtained by immunopurification of BicC RNP particles from wild-type flies, which were found to contain specifically enriched d-myc mRNA, with up-regulation of Myc being a hallmark of cystic proliferation. Consistent with the profile of cysts, d-Myc protein was elevated in the Malpighian tubules of BicC flies. Moreover, d-Myc over-expression in the Malpighian tubules produced very deformed cystic tubules in rare survivors. The effects of d-Myc over-expression were more severe than BicC defects, likely attributable to the much higher d-Myc levels in the former (3-34X) than the latter (2-7X). Consistent with the possibility that the d-myc up-regulation in BicC mutants greatly contributes to the cystic phenotype, d-myc depletion via RNAi restored wild-type d-Myc protein levels in BicC tubules and substantially–albeit not completely–improved morphology. Therefore, de-regulation of other, still unknown, BicC targets is likely to contribute to the cystic degeneration of the renal tubule.

As in human and mice PKD tissue, Drosophila Malpighian tubules from BicC flies displayed elevated d-S6K phosphorylation, compared to wild-type, indicating TOR pathway activation. Of note, d-S6K phosphorylation may amplify the effects of d-myc over-expression [63]. The ratio between phosphorylated and un-phosphorylated d-S6K of BicC tubules was found to be twice as high as the wild-type, but rapamycin treatment restored wild-type levels. Consistently, rapamycin administration to the BicC flies markedly improved survival and reduced cystogenesis, albeit fly populations declined over the long term, as reported previously for PKD patients and rodent cystic models, indicating that repression of the TOR pathway rescued the cystic defects of the BicC Malpighian tubules. Rapamycin treatment was well-tolerated by wild-type flies, which displayed increased longevity. In vertebrate cystic models of PKD, long-term treatment with rapamycin has proven challenging because of habituation and possible toxicity [54,62]. Application of the BicC flies to elucidate the molecular mechanisms of cyst formation may thus empower the design of more effective therapeutic strategies.

Taken together, our findings are congruent with studies of vertebrate BicC: BicC function is needed for kidney homeostasis in vertebrates, mutations cause renal cysts in zebrafish, Xenopus and mice [16,19,26], and two BicC mutations were found in patients with infantile, unilateral renal cystic dysplasia [17]. Zebrafish BicC was also recently found in a protein complex with NPHP proteins [27] that are implicated in nephronophthisis [28]. This evidence suggests that evolutionarily conserved BicC functions are crucial for the physiology of the renal tubule and highlight the need for further study of the mechanism(s) and targets of BicC in the kidney and specifically in the renal tubule. Because decreased BicC expression was a feature of both microarrays from human PKD1 tissue and Pkd1−/− mouse kidney extracts, a strong nexus between the BicC and PKD phenotypes is beginning to emerge. BicC-mediated cellular functions such as post-transcriptional regulation of mRNAs may be part of evolutionarily-conserved pathways that are critical for kidney homeostasis, the malfunction of which leads to renal cystogenesis and PKD. The Drosophila Malpighian tubule promises to be a useful and genetically tractable system for further exploring the cellular and molecular changes that contribute to cystic kidney diseases and PKD.
Methods
Fly lines and survival assays
Flies were grown on cornmeal agar (Jazzmix) at room temperature (~23 degrees) and aged as indicated. For salt stress the cornmeal food was supplemented with crystalline NaCl to reach a final concentration of 0.5M NaCl. Food coloring was added to test feeding. Rapamycin (LC Laboratories) was dissolved in absolute ethanol and added to the fly food (final 15μM rapamycin, 1.5% ethanol). Unlike Oregon R wild-type flies that were maintained by standard methods, BicC flies were generated by crossing CyO balanced stocks containing Df(2L)RA5 (Bloomington Stock Center) and BicC YC33 or BicC IIF34 mutations[22] respectively and retrieving non-Cy progeny. The BicC YC33 and BicC IIF34 mutations gave rise to truncated proteins in the ovary, of which the former was present in low amounts (S4 Fig) and initially missed (see Fig 1 in [22]). The BicC IIF34 allele gave rise to the most severe phenotypes in both the Malpighian tubules and the ovary, suggesting it may behave as dominant negative. Because Df(2L)RA5 double balanced flies are semi-lethal, the RNA interference transgenes and Gal4 drivers were crossed into BicC YC33 homozygotes. Note the BicC YC33 homozygotes were previously used in [24] to study ovarian development. BicC null flies were obtained by crossing Df(2L)RA5 and Df(2L)Osp29 (Bloomington Stock Center). Survival assays: for each genotype four cornmeal agar vials with 50 females and 12 males were set up. Flies were passed in fresh vials every three (normal and rapamycin-containing food) or one (high salt food) days and survivors counted. Percentage survival rates with standard deviations were plotted against time (Excel). For the rapamycin rescue, one day old flies (raised in Jazzmix) were placed in vials containing rapamycin or the same volume of solvent (ethanol, 1.5% final, vehicle control) and moved to fresh vials every three days.

d-myc RNAi: the Gal4 drivers for the principal and stellate cells, c42 and c724 [45] and Gal4-inducible RNAi constructs targeting d-myc, y1 v1; p[w+tm6b=UAS-Myc.Z]132 (Bloomington Drosophila Stock Center) were induced via the c724 and c42 drivers at 18˚C.

Immunoblots
Malpighian tubules were micro-dissected in PBS. Four tubule pairs per each lane were lysed in RIPA, 1x Complete (Roche), 2X Laemml sample buffer at 100˚C, resolved on 10% SDS Laemmli polyacrylamide gels (PAGE) and transferred to PVDF. Mice from Pkd1 were backcrossed on C57BL/6J background for several generations and genotyped as in [64]. The protocols for in vivo mouse experiments were reviewed and approved by the Institut de recherches cliniques de Montreal—IRCM Animal Care Committee (ACC #2014–26), which follow the regulations and requirements of the Canadian Council on Animal Care (CACC). Dissected neonatal kidneys from Pkd1+/− and control littermates were homogenized and lysed in RIPA buffer, 2mM PMSF, 2μg/ml aprotinin, and cleared by centrifugation. Aliquots of 25 μg were loaded in each well, resolved by 10% SDS-PAGE and transferred to nitrocellulose. Drosophila immunoblots were probed with anti d-Myc antibody (rabbit, Santa Cruz Biotechnology, 1:2,000) or anti Thr398-phosphorylated p70 d-S6 Kinase (Cell Signaling, 1:1000), anti-d-S6K (1199, gift of G. Thomas) and anti α tubulin (mouse, 12G10, Developmental Studies Hybridoma Bank, 1: 50,000). Mouse immunoblots were probed with anti Bic1 (Aviva Systems Biology) polyclonal and anti-GAPDH (Sigma) antibodies. Signal was revealed via
horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch) and chemiluminescence on film (mouse) or recorded by ChemiDoc (BioRad) and analyzed (Image Lab 5.2, Excel, Drosophila). In this study the d-Myc positive band with electrophoretic mobility ~73 kDa (consistent with the expected d-Myc molecular weight) was quantified. Values were expressed as d-Myc/tubulin or d-S6K/tubulin intensity ratio, normalized to the average of the wild-type and plotted as means ± standard deviation. Where appropriate, p values were determined by Student’s t test. Similar trends were obtained utilizing actin for normalization. Antibody controls are shown in S5 Fig.

Microscopy and immunofluorescence
Micro-dissected Malpighian tubules were photographed on a Leica MZ FLIII Fluorescence Stereomicroscope with Leica MZ series 10X/21B Widefield Adjustable eyepieces equipped with a Canon DS126201 EOS 5D MARK II, using visible light. Canon raw files were converted into TIF format (Adobe Lightroom 3.2). Images were merged and processed with Adobe Photoshop CS5. Immunofluorescence: dissected Malpighian tubules were fixed (20 min. in 4% paraformaldehyde 1X PBS 0.1% Tween-20), washed in PBT, incubated with primary antibody (anti-BicC [24] 1:1000, anti-d-Myc monoclonal 1:20, gift of Dr. Bruce Edgar) followed by Alexa Fluor 488 or 546 conjugated secondary antibodies (Molecular Probes) and DAPI, mounted (ProLong Gold, Molecular Probes), and imaged (Zeiss LSM710 confocal microscope). Acquired images were exported as TIF files (ZEN 2012), and processed with Adobe Photoshop CS5. 5 μm thick section of paraffin-embedded, formaldehyde-fixed kidneys from adult mice were de-paraffinized, treated with NaBH₄ for 30 min, washed with TBS, 1% SDS (5 min) and washed as described above prior to blocking (mouse IgG) and probing with 1:30 anti-Bicc1 polyclonal (Aviva Systems Biology), or blocking buffer (control), followed by Alexa-Fluor 546-conjugated secondary antibody and DAPI. Sections were mounted in ProLong Gold. Antibody controls are shown in S5 Fig.

Immunoprecipitation and RNA assays
250 μl of packed, microdissected wild-type Malpighian tubules were lysed in ice-cold buffer (25 mM Hepes pH 6.8, 50 mM KCl, 1 mM MgCl₂, 1 mM DTT, 125 mM sucrose, 100 U/ml RNasin (Promega) and cleared by centrifugation (10,000 g, 10 min, 4°C). The supernatant was brought to 0.2% Triton-X100, pre-cleared with protein A Sepharose, and incubated (2.5 hours, 4°C) with 25 μl of protein A Sepharose Fast Flow (GE Healthcare) pre-conjugated to either Bic-C anti-serum or non-immune control serum. The beads were washed in binding buffer, with 0, 250, 500, 600, mM NaCl and rinsed in 250 mM NaCl containing buffer, treated with proteinase K, extracted with phenol-chloroform and precipitated with ethanol. For the control RNA immunoprecipitation 150 Malpighian tubules from each genotype were lysed and processed as above. Extracts were divided equally and incubated with either non-immune or anti-BicC immune conjugates. RNA from the immunopurified RNPs was extracted with GENEzol (Geneaid) following recommended procedures. Recovered RNA was reverse transcribed with Superscript II and random primers (both Invitrogen) following the manufacturer’s recommendations. RT-PCR was performed with 1/10 of the reactions and the following gene specific primers: d-Myc (Forward, F: 5’CGATCGCAGACGCAGATATA3’, Reverse, R: 5’GGCGGTT ATTAATGACCT3’), tubulin 84B (F: 5’TTCAGTTTGTCAAGCCTTAGC3’, R: 5’CTG AAAGGGTGGTGAAGGAGTC3’), BicC (F: 5’ATAGCTTTCCGCAAAACAGC 3’, R: 5’ AAGGCAACTACGCTTTGGA3’), d-S6K (F: 5’ CAGTCAGAGATCCCTTCTCATA GT 3’, R: 5’ CGTGTAGTGATGCCAGTTT 3’), d-TOR (F: 5’ AGCTCTTTCGCTGTCGCA AT 3’, R: 5’ TCCAGTACGGTGGCTCGC 3’).
Supporting information

S1 Fig. Wild type and BicCΔ/+ heterozygote flies have similar survival. Survival assays of populations of wild-type (wt) and BicC heterozygotes for the BicC deletion (Δ/+; n = 200, with standard deviations) showing that BicC hemizygotes and wild-type flies displayed similar survival. (TIF)

S2 Fig. Flies were fed on high salt vials containing green food dye for 30 hours, frozen and mounted on modeling clay for photography. For image clarity legs were clipped. (TIF)

S3 Fig. (A) Representative d-Myc and tubulin immunoblots of extracts from five Malpighian tubules dissected from flies of the following genotypes: d-myc RNAi driven in BicCYC33/YC33 homozygotes (BicCYC33; Gal4>mycRNAi); BicCYC33/YC33 homozygotes (BicCYC33, sibling to the previous flies); wild-type (wt); heterozygotes for BicCYC33 and the c42 Gal4 driver. The TRiP line used in these assays was JF01761. (B) Corresponding graph summarizing quantitative immunoblots of means ± standard deviations of d-Myc/tubulin ratios from independent biological replicas per each genotype (n, indicated). Values were normalized to the wild-type average; p values (Student’s t test) are shown for the BicC mutants and rescued flies. For the latter, significance was computed compared to wild type (top) and BicC mutant (below). Reducing d-myc expression in BicCYC33/YC33 mutants decreased the d-Myc protein. (TIF)

S4 Fig. BicC alleles BicCYC33 and BicCIF34 produce truncated proteins. BicC immunoblots of extracts from dissected ovarian stages 1–9 (20 μg/lane) of the following genotypes: OreR (wild type, wt, lane 1); BicCA/YC33 (lane 2); BicCA/IF34 (lane 3); w EGFPnos (E, Forrest et al. 2004, lane 4); w EGFPnos; BicCA/YC33 (E; BicCYC33, lane 5); w EGFPnos; BicCA/IF34 (E; BicCA/ IF34, lane 6) show that both BicCA/YC33 and BicCA/IF34 flies produced truncated BicC proteins (asterisks), compared to the full-length BicC protein found in wild type and the w EGFPnos ovaries (lanes 1 and 4, respectively). The smudge at ~100 kDa in lane 6 was due to spill over from the sample in the next well. (TIF)

S5 Fig. Antibody controls. (A) Whole d-Myc immunoblot for the gel in Fig 7F. d-Myc is indicated with an asterisk. The molecular size marker is shown (colorimetric image, left) and corresponding sizes are specified. Red indicates areas of over-exposure. (B) Immunoblot of Malpighian tubule extracts from mycPo/dm1, OreR (wt), Gal4>TRiP.JF01761, Gal4>TRiP.JF01762. (A, B): rabbit polyclonal anti-d-Myc. (C) Confocal section of Myc immunostaining of OreR (wt) with anti-Myc monoclonal B10. (D) Confocal section of Myc immunostaining of mycPo/dm1 Malpighian tubules with anti-d-Myc monoclonal B10; mycPo/dm1 was obtained by crossing strong hypomorphic mutants [44,65]. (E) Epifluorescence microscopy of a 5 μm kidney section from C57BL/6 mice shows Bic1 accumulation in the cells lining the renal tubule and DNA (DAPI, blue). This is the same panel shown in (Fig 3E–3G). (F) A 5 μm kidney section from C57BL/6 mice was processed in parallel and identical conditions to (E), except for the addition of the primary anti-Bic1 antibody. All image pairs were captured in identical conditions and the corresponding samples processed in parallel. (G) Epifluorescence microscopy of a mosaic Malpighian tubule displaying a single cell expressing a long dsRNA targeting BicC (Valium 20 P{TRiP.HMS01407}) and marked by GFP co-expression, surrounded by neighbouring wild-type cells. The clone boundaries are indicated. (C-G): DNA (DAPI), blue. Scale bars indicated. (TIF)
S1 Text. Supporting methods describing the d-myc alleles in S5 Fig and procedures for making protein extracts and immunoblots from dissected ovaries as displayed in S4 Fig with associated references.

Acknowledgments

We wish to thank C. Charbonneau (Institute for Research in Immunology and Cancer, IRIC) and D. Filion (Institut de recherches cliniques de Montréal, IRCM) for sharing their expertise in confocal microscopy and analyses; J. Pelletier (Université de Montréal), M. Therrien (IRIC) and their groups, particularly M. Lefrançois, for welcoming hospitality and shared resources; A. Kurbegovic (IRCM) for providing dissected mouse kidneys and histologic sections; D. Griffoni for the gift of d-Myc polyclonal antibody; B. Edgar and R. Eisenmann for the gift of d-Myc monoclonal; G. Thomas for the gift of anti-S6K, M. Therrien and P. Roux for the gift of anti-pS6K, the Developmental Studies Hybridoma Bank for the tubulin monoclonal; the Bloomington Stock Center for flies; M. Paliouras (Lady Davis Research Institute) and the Computational Systems Biology Group (NRC Biotechnology Research Institute) for performing the microarray analysis. L. Diao (Yale University) and N. Sonenberg (McGill University) for discussions.

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References

1. Harris PC, Torres VE (2009) Polycystic kidney disease. Annual review of medicine 60: 321–337. https://doi.org/10.1146/annurev.med.60.101707.125712 PMID: 18947299

2. Lancaster MA, Gleeson JG (2010) Cystic kidney disease: the role of Wnt signaling. Trends in molecular medicine 16: 349–360. https://doi.org/10.1016/j.molmed.2010.05.004 PMID: 20576469

3. Peters DJ, Sandkuilj LA (1992) Genetic heterogeneity of polycystic kidney disease in Europe. Contributions to nephrology 97: 128–139. PMID: 1633713

4. Rossetti S, Consugar MB, Chapman AB, Torres VE, Guay-Woodford LM, et al. (2007) Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology: JASN 18: 2143–2160. https://doi.org/10.1681/ASN.2006121387 PMID: 17582161
Dowling RJ, Topisirovic I, Fonseca BD, Sonenberg N (2010) Dissecting the role of mTOR: lessons from
15.
10.
Ibraghimov-Beskrovnaya O, Natoli TA (2011) mTOR signaling in polycystic kidney disease. Trends in
18.
12.
Maisonneuve C, Guilleret I, Vick P, Weber T, Andre P, et al. (2009) Bicaudal C, a novel regulator of Dvl
17.
13.
Bouvrette DJ, Sittaramane V, Heidel JR, Chandra sekhar A, Bryda EC (2010) Knockdown of bicaudal C
16.
19.
Saffman EE, Styhler S, Rother K, Li W, Richard S, et al. (1998) Premature translation of oskar in
21.
Mohler J, Wieschaus EF (1986) Dominant maternal-effect mutations of Drosophila melanogaster caus-
20.
Mahone M, Saffman EE, Lasko PF (1995) Localized Bicaudal-C RNA encodes a protein containing a
22.
Chicoine J, Benoit P, Gamberi C, Paliouras M, Simmons M, et al. (2007) Bicaudal-C recruits CCR4-
24.
Tran U, Zakin L, Schweickert A, Agrawal R, Doger R, et al. (2010) The RNA-binding protein bicaudal C
25.
Tran U, Pickney LM, Ozpolat BD, Wessely O (2010) The RNA-binding protein bicaudal C
23.
Mahone M, Saffman EE, Lasko PF (1995) Localized Bicaudal-C RNA encodes a protein containing a
20.
Kraus MR, Clauin S, Pfister Y, Di Maio M, Ulinski T, et al. (2009) Two mutations in human BICC1 result-
17.
Maisonneuve C, Guillere t I, Vick P, Weber T, Andre P, et al. (2009) Bicaudal C, a novel regulator of Dvl
11.
Fonseca BD, Smith EM, Yelle N, Alain T, Bushell M, et al. (2014) The ever-evolving role of mTOR in
12.
Fonseca BD, Smith EM, Yelle N, Alain T, Bushell M, et al. (2014) The ever-evolving role of mTOR in
10.1093/hmg/ddn325 PMID: 18845692
8.
Hartman TR, Liu D, Zilfou JT, Robb V, Morrison T, et al. (2009) The tuberous sclerosis proteins regulate
formation of the primary cilium via a rapamycin-insensitive and polycystin-1-independent pathway.
Human molecular genetics 18: 151–163. https://doi.org/10.1093/hmg/ddn325 PMID: 18845692
7.
Fischer DC, Jacoby U, Pape L, Ward CJ, Kuwertz-Broeking E, et al. (2009) Activation of the AKT/
mTOR pathway in autosomal recessive polycystic kidney disease (ARPKD). Nephrol ogy, dialysis,
transplantation: official publication of the European Dialysis and Transplant Association—European
Renal Association 24: 1819–1827.
5.
Bonnet CS, Aldred M, von Ruilcand C, Harris R, Sandford R, et al. (2009) Defects in cell polarity underlie
TSC and ADPKD-associated cystogenesis. Human molecular genetics 18: 2166–2176. https://doi.org/
10.1093/hmg/ddp149 PMID: 19321600
6.
Fischer DC, Jacoby U, Pape L, Ward CJ, Kuwertz-Broeking E, et al. (2009) Activation of the AKT/
mTOR pathway in autosomal recessive polycystic kidney disease (ARPKD). Nephrology, dialysis,
transplantation: official publication of the European Dialysis and Transplant Association—European
Renal Association 24: 1819–1827.
4.
Shillingford JM, Murchia NS, Larson CH, Low SH, Hedgepeth R, et al. (2006) The mTOR pathway is reg-
ulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. Proceed-
ings of the National Academy of Sciences of the United States of America 103: 5466–5471.
https://doi.org/10.1073/pnas.0509694103 PMID: 16567633
3.
Gamberi C, Lasko P (2012) The Bic-C family of developmental translational regulators. Comparative
medicine 60: 96–106. PMID: 20412683
2.
Mahone M, Saffman EE, Lasko PF (1995) Localized Bicaudal-C RNA encodes a protein containing a
KH domain, the RNA binding motif of FMR1. The EMBO journal 14: 2043–2055. PMID: 7538070
1.
Chicoine J, Benoit P, Gamberi C, Paliouras M, Simoneig M, et al. (2007) Bicaudal-C recruits CCR4-
NOT deadenylase to target mRNAs and regulates oogenesis, cytoskeletal organization, and its own
expression. Developmental cell 13: 691–704. https://doi.org/10.1016/j.devcel.2007.10.002 PMID:
17981137
0.
Tran U, Zakin L, Schweickert A, Agrawal R, Doger R, et al. (2010) The RNA-binding protein bicaudal C
regulates polycystin 2 in the kidney by antagonizing miR-17 activity. Development 137: 1107–1116.
https://doi.org/10.1242/dev.046045 PMID: 20215348
Renal cystogenesis in the BicC fly

PLOS Genetics | https://doi.org/10.1371/journal.pgen.1006694 April 13, 2017 18 / 20
26. Cogswell C, Price SJ, Hou X, Guay-Woodford LM, Flaherty L, et al. (2003) Positional cloning of jcpk/bpk locus of the mouse. Mammalian genome: official journal of the International Mammalian Genome Society 14: 242–249.

27. Yakulov TA, Yasunaga T, Ramachandran H, Engel C, Muller B, et al. (2015) Ank3 interacts with nephronophthisis proteins and is required for normal renal development. Kidney international.

28. Wolf MT, Hildebrandt F (2011) Nephronophthisis. Pediatric nephrology 26: 181–194. https://doi.org/10.1007/s00467-010-1585-z PMID: 20652329

29. Wang J, Kean L, Yang J, Allan AK, Davies SA, et al. (2004) Function-informed transcriptome analysis of Drosophila renal tubule. Genome biology 5: R69. https://doi.org/10.1186/gb-2004-5-9-r69 PMID: 15345053

30. Dow JA, Maddrell SH, Gortz A, Skaer NJ, Brogan S, et al. (1994) The malpighian tubules of Drosophila melanogaster: a novel phenotype for studies of fluid secretion and its control. The Journal of experimental biology 197: 421–428. PMID: 7852912

31. Baert L (1978) Hereditary polycystic kidney disease (adult form): a microdissection study of two cases at an early stage of the disease. Kidney international 13: 519–525. PMID: 713285

32. Harding MA, Gattone VH 2nd, Grantham JJ, Calvet JP (1992) Localization of overexpressed c-myc mRNA in polycystic kidneys of the cpk mouse. Kidney international 41: 317–325. PMID: 1552705

33. Lanoix J, D'Agati V, Szabolcs M, Trudel M (1996) Dysregulation of cellular proliferation and apoptosis mediates human autosomal dominant polycystic kidney disease (ADPKD). Oncogene 13: 1153–1160. PMID: 8808689

34. Trudel M, D'Agati V, Costantini F (1991) C-myc as an inducer of polycystic kidney disease in transgenic mice. Kidney international 39: 665–671. PMID: 1646908

35. Kurbegovic A, Cote O, Couillard M, Ward CJ, Harris PC, et al. (2010) Pkd1 transgenic mice: adult model of polycystic kidney disease with extrarenal and renal phenotypes. Human molecular genetics 19: 117–1189. https://doi.org/10.1093/hmg/ddp588 PMID: 20053665

36. Johnston LA, Prober DA, Edgar BA, Eisenman RN, Gallant P (1999) Drosophila myc regulates cellular growth during development. Cell 98: 779–790. PMID: 10499795

37. Sozen MA, Armstrong JD, Yang M, Kaiser K, Dow JA (1997) Functional domains are specified to single-cell resolution in a Drosophila epithelium. Proceedings of the National Academy of Sciences of the United States of America 94: 5207–5212. PMID: 9144216

38. Ni QJ, Liu LP, Binari R, Hardy R, Shim HS, et al. (2009) A Drosophila resource of transgenic RNAi lines for neurogenetics. Genetics 182: 1089–1100. https://doi.org/10.1534/genetics.109.103630 PMID: 19487563

39. Distefano G, Bocca M, Rowe I, Wodarczyk C, Ma L, et al. (2009) Polycystin-1 regulates extracellular signal-regulated kinase-dependent phosphorylation of tuberin to control cell size through mTOR and its...
downstream effectors S6K and 4EBP1. Molecular and cellular biology 29: 2359–2371. https://doi.org/10.1128/MCB.01259-08 PMID: 19255143

49. Becker JU, Saez AO, Zerres K, Witzke O, Hoyer PF, et al. (2010) The mTOR Pathway Is Activated in Human Autosomal-Recessive Polycystic Kidney Disease. Kidney & blood pressure research 33: 129–138.

50. Tao Y, Kim J, Schrier RW, Edelstein CL (2005) Rapamycin slows disease progression in a rat model of polycystic kidney disease. Journal of the American Society of Nephrology: JASN 16: 46–51. https://doi.org/10.1681/ASN.2004080660 PMID: 15663559

51. Wahl PR, Serra AL, Le Hir M, Molle KD, Hall MN, et al. (2006) Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association—European Renal Association 21: 598–604.

52. Wu M, Wahl PR, Le Hir M, Wackerle-Men Y, Wuthrich RP, et al. (2007) Everolimus retards cyst growth and preserves kidney function in a rodent model for polycystic kidney disease. Kidney & blood pressure research 30: 253–259.

53. Shillingford JM, Piontek KB, Germino GG, Weimbs T (2010) Rapamycin ameliorates PKD resulting from conditional inactivation of Pkd1. Journal of the American Society of Nephrology: JASN 21: 489–497. https://doi.org/10.1681/ASN.2009040421 PMID: 20075061

54. Torres VE, Boletta A, Chapman A, Gattone V, Pei Y, et al. (2010) Prospects for mTOR inhibitor use in patients with polycystic kidney disease and hamartomatous diseases. Clinical journal of the American Society of Nephrology: CJASN 5: 1312–1329. https://doi.org/10.2215/CJN.01360210 PMID: 20498248

55. Bjedov I, Toivonen JM, Kerr F, Slack C, Jacobson J, et al. (2010) Mechanisms of life span extension by rapamycin in the fruit fly Drosophila melanogaster, Cell metabolism 11: 35–46. https://doi.org/10.1016/j.cmet.2009.11.010 PMID: 20074526

56. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, et al. (2009) Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. Nature 460: 392–395. https://doi.org/10.1038/nature08221 PMID: 19587680

57. Medvedik O, Lamming DW, Kim KD, Sinclair DA (2007) MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in Saccharomyces cerevisiae. PLoS biology 5: e261. https://doi.org/10.1371/journal.pbio.0050261 PMID: 17914901

58. Powers RW 3rd, Kaeberlein M, Caldwell SD, Kennedy BK, Fields S (2006) Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes & development 20: 174–184.

59. Montagne J, Stewart MJ, Stocker H, Hafen E, Kozma SC, et al. (1999) Drosophila S6 kinase: a regulator of cell size. Science 285: 2126–2129. PMID: 10497130

60. Song X, Di Giovanni V, He N, Wang K, Ingram A, et al. (2009) Systems biology of autosomal dominant polycystic kidney disease (ADPKD): computational identification of gene expression pathways and integrated regulatory networks. Human molecular genetics 18: 2328–2343. https://doi.org/10.1093/hmg/ddp165 PMID: 19346236

61. Lian P, Li A, Li Y, Liu H, Liang D, et al. (2014) Loss of polycystin-1 inhibits BicC1 expression during mouse development. PLoS One 9: e88816. https://doi.org/10.1371/journal.pone.0088816 PMID: 24594709

62. Lieberthal W, Fuhr R, Andy C, Patel V, Levine JS (2006) Rapamycin delays but does not prevent recovery from acute renal failure: role of acquired tubular resistance. Transplantation 82: 17–22. https://doi.org/10.1097/01.tp.0000225772.22757.5e PMID: 16861938

63. Mitchell NC, Tchoukrieva EB, Chahal A, Woods S, Lee A, et al. (2015) S6 Kinase is essential for MYC-dependent rDNA transcription in Drosophila. Cellular signalling 27: 2045–2053. https://doi.org/10.1016/j.cellsig.2015.07.018 PMID: 26215099

64. Kurbegovic A, Trudel M (2013) Progressive development of polycystic kidney disease in the mouse model expressing Pkd1 extracellular domain. Human molecular genetics 22: 2361–2375. https://doi.org/10.1093/hmg/ddt081 PMID: 23439951

65. Gallant P, Shio Y, Cheng PF, Parkhurst SM, Eisenman RN (1996) Myc and Max homologs in Drosophila. Science 274: 1523–1527. PMID: 8929412