**Drosophila Pkd2 Is Haploid-insufficient for Mediating Optimal Smooth Muscle Contractility***

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Humans heterozygous for PKD1 or PKD2 develop autosomal dominant polycystic kidney disease, a common genetic disorder characterized by renal cyst formation and extrarenal complications such as hypertension and vascular aneurysms. Cyst formation requires the somatic inactivation of the wild type allele. However, it is unknown whether this recessive mechanism applies to life-threatening vascular aneurysms, which could involve weakening of the endothelial lining or surrounding vascular smooth muscle cells (SMCs). *Drosophila Pkd2* at 33E3 (*Pkd2*) encodes a PKD2 family of Ca\(^{2+}\)-activated Ca\(^{2+}\)-permeable cation channels. We show here that loss-of-function *Pkd2* mutations severely reduced the contractility of the visceral SMCs, which was restored by anodine-induced perturbation of intracellular Ca\(^{2+}\) stores. Consistent with this, *Pkd2* interacted strongly with a ryanodine receptor mutation, causing a synergistic reduction of larval body wall contraction rate that is normally regulated through Ca\(^{2+}\) oscillation during excitation-contraction coupling in the skeletal muscle. These results suggest that PKD2 cooperates with the ryanodine receptor to promote optimal muscle contractility through intracellular Ca\(^{2+}\) homeostasis. Further genetic analysis indicated that *Pkd2* is strongly haploinsufficient for normal SMC contractility. Since Ca\(^{2+}\) homeostasis is a conserved mechanism for optimal muscle performance, our results raise the possibility that inactivation of just one PKD2 copy is sufficient to compromise vascular SMC contractility and function in *Pkd2* heterozygous patients, thus explaining their increased susceptibility to hypertension and vascular aneurysms.

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disease affecting ~1/1,000 of the general population (1). Mutations in two disease-causing genes, *PKD1* and *PKD2*, account for nearly all incidences of ADPKD. The main characteristic of ADPKD is progressive cyst formation in the kidney that results in renal failure at high frequencies (2). In addition, there are many extrarenal complications in ADPKD such as cyst formation in the liver and pancreas (3, 4). ADPKD is also a disease of the blood vessels (5). Systemic cardiovascular abnormalities include hypertension, cardiac valve defects, colonic diverticulae, and vascular aneurysms of the intracranial, coronary, aortic, thoracic, and splenic blood vessels (1, 2, 5). Sudden intracranial aneurysms sometimes occur in asymptomatic young adults with *PKD1* or *PKD2* mutation, and this significantly contributes to elevated morbidity and mortality associated with ADPKD (1, 5).

ADPKD patients are heterozygous individuals (*PKD1*/+−) with respect to the *PKD1* or *PKD2* locus. Molecular analyses of cyst-forming cells in the kidney indicate that they are homozygous mutant (*PKD1*/−−) clones resulting from the inactivation of the wild type gene copy by somatic mutation (6–8). Analogous to mutations in tumor suppressor genes such as the retinoblastoma gene, ADPKD is dominantly transmitted from one generation to the next, but cyst formation involves a recessive mechanism that requires a second gene inactivation event. This mechanism of cystogenesis is supported by a transgenic mouse model (*Pkd2*W25/−−) that involves an unstable *Pkd2*W25 allele (9). In this case, *Pkd2*W25/−− mice developed kidney cysts earlier and faster than *Pkd2*W25/*Pkd2*W25 mice. Renal cysts formed in *Pkd2*W25/−− mice invariably arise from renal tubular cells that do not produce PKD2 protein (9). Thus, somatic inactivation of *PKD2* expression from both genomic copies is necessary and sufficient to trigger renal cyst formation. However, it is questionable whether this “two-hit” recessive mechanism explains many of the extrarenal phenotypes and cardiovascular pathologies of ADPKD. Some of the extrarenal phenotypes such as cardiac valve defects may originate in development early in life. Adult *Pkd2*/+− mice show reduced long term survival in the absence of cystic disease or renal failure, indicating that a 50% reduction of gene dosage may be sufficient to cause some of the abnormalities associated with ADPKD (9).

The *Drosophila* genome has four *Pkd2* or *Pkd2-like* genes (*CG6504, CG13762, CG9472, and CG16793*). *CG6504*, also known as *Pkd2* (10) or *almost there* (11), was analyzed in this study. The protein encoded by *CG6504* is most closely related to the human *PKD2* family of proteins (~40% amino acid identity to *PKD2* and 39% identity to *PKD2L1*). The remaining three fly *PKD2-like* proteins show 19–27% amino acid identity to human *PKD2* or *PKD2L1* (11). *CG6504* encodes a protein of 924 amino acids that contains essential features of the *PKD2* channel family, including six transmembrane segments, a pore-forming sequence (P-loop), and a C-terminal coiled-coil domain. All mammalian *PKD2* proteins examined exhibit non-selective cation channel activity (12–16). Expression of the cloned *CG6504 Pkd2* cDNA (GenBank™ accession number AY283154) in *Drosophila* cultured S2 cells that do not express...
the endogenous Pkd2 also produces a Ca\(^{2+}\)-activated cation current," suggesting that the fly Pkd2 also has the capacity to function as a calcium-activated cation channel. Mammalian Pkd2 has been shown to localize on primary cilia of many cell types, and cilia are involved in sensing a variety of extracellular chemical or mechanical signals (17–19). For example, cilia-associated PKD2 of mouse kidney epithelia has been shown to mediate calcium influx in response to fluid flow (17). Similar to these, the fly Pkd2 is abundantly present on sperm flagella, a type of motile cilia (10, 11). Pkd2 mutant sperm are motile but fail to congregate at the anterior uterus (10) and the storage organ in the female (10, 11), which results in a severe reduction of male fertility. Thus, it appears that the fly Pkd2 functions on sperm flagella to sense directional cues in the female reproductive tract.

Besides their association with cilia, mammalian PKD1 and PKD2 are expressed in vascular smooth muscle cells (SMCs) (20–23). Vascular fragility and leakage have been reported in Pkd1−/− and Pkd2−/− mouse models (20, 24, 25). In Pkd2+/− mouse vascular SMCs, there is a small (17%) but statistically significant reduction of intracellular calcium (26). While these phenotypes implicate the vascular smooth muscle cells, it is not known whether Pkd2 has specific functional roles in these cells. Here we show by in vivo functional assays that the fly Pkd2 (CG6504) is haploinsufficient for optimal smooth muscle contractility. Mutations in the fly Pkd2 also weakly affected the contractility of skeletal muscles. Furthermore we show evidence that the fly Pkd2 mediates muscle contractility via calcium homeostasis through a functional coupling with the calcium-releasing ryanodine receptor channel on the sarcoplasmic reticulum. Our results are consistent with the recent finding that Ca\(^{2+}\) influx through ciliary PKD2 in the kidney epithelia is accompanied by Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) storage organelles through the ryanodine receptor (17). Propagation of calcium signaling through coupling of PKD2 with the ryano-dine receptor may represent an evolutionarily conserved aspect of Pkd2 function. Haploid insufficiency of Pkd2 for optimal smooth muscle contractility provides a plausible explanation for systemic vascular complications such as hypertension and aneurysms observed in ADPKD patients.

MATERIALS AND METHODS

Reverse Transcription-PCR Analysis—For Fig. 1A, stage-specific mRNA samples were isolated from wild type (Oregon R) embryos (0–20 h), adult males, and adult females by using the FastTrack™ 2.0 kit (Invitrogen). First strand cDNA synthesis was done with embryonic mRNA (4 μg) and adult male and female mRNAs (1 μg each) by 300 ng of oligo(dT) primer at 42 °C for 1.5 h by following the SmartTM protocol for rapid amplification of cDNA ends (Clontech). PCR amplification of Pkd2 cDNA used primers Pkd2.1 and Pkd2.4 (5'-GCAGGCGTTCTTCTTATGAAAAC-3' and 5’-CTGCCTTCGGTGTATCAACCCCATTT-3') that produce a 1,043-bp amplification product. The same pair of primers amplifies a 1,400-bp genomic fragment. By following Fig. 1C, the primers used were Pkd2.1 and Pkd2.8 (5’-GGCGCGCTCTTCTTTAGAAAAC-3' and 5’-TAAAGCCGACGCTTTCTTGGGAGCA-3'). The templates were from the Drosohila Rapid-scan™ gene expression panel (Oregene Technologies, Inc.) that contains equal amounts of pre-made first strand cDNA from embryonic, larval, pupal, and adult stages at four 10-fold serial diluted quantities (10 ng, 1 μg, 100 pg, and 10 pg), thus allowing a rough estimation of Pkd2 mRNA abundance during development.

Genetics of Pkd2—Several Pkd2 mutant alleles were generated by a targeted knock-out protocol (27). The allele used in this study is Pkd2iso3, that was derived from the integration of a mutated Pkd2 donor into the endogenous Pkd2 locus (10). Pkd2iso3 contains a tandem duplication of Pkd2 with one copy carrying the BamHI and the other copy carrying the MunI frameshift mutations (see Fig. 3A, 3B). The wild type Pkd2 gene encodes a polypeptide of 924 amino acids. The MunI muta-

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8 Venglarik, C. J., Guo, Z., and Lu, X., J. Am. Soc. Nephrol., in press.
cells was done by crossing flies carrying the Pkd2-Gal4 transgenic line to flies carrying a UAS-GFP reporter.

RESULTS

Developmental Expression of Pkd2—We determined the developmental expression pattern of Pkd2 using antibodies recognizing the fly PKD2. Tissue survey by immunostaining indicates that the male testis is the only organ where PKD2 is abundantly expressed (10). Due to low abundance of PKD2 in most somatic tissues, the protein was detectable in the male testis (Fig. 1, A and B). To facilitate the identification of the fly PKD2, tissue survey by immunostaining indicated that the male testis is the only organ where PKD2 is abundantly expressed (10).

Immunostaining showed that PKD2 was expressed in visceral SMCs of the gut is grossly analogous to mammalian blood vessels (26), PKD2 in fly visceral SMCs of the gut and the skeletal muscles of both sexes (40 heads/lane, Fig. 1A). Consistent with this, PKD2 was detectable in more concentrated head extracts of other cells where the protein was detectable in the male testis (Fig. 1B). The Pkd2 mRNA level in the embryo was extremely low. Coinciding with early testis development, the level began to rise in third instar male larvae and persisted at high levels in male pupae and male adults (Fig. 1C).

Immunostaining showed that Pkd2 was expressed in visceral SMCs of the gut and the skeletal muscles of both sexes (Fig. 2). As in mammalian vascular SMCs (26), Pkd2 in fly visceral SMCs appeared predominantly in the cytoplasm surrounding the nucleus (Fig. 2A). To facilitate the identification of other cells where Pkd2 may be expressed, we generated transgenic flies carrying the Pkd2 promoter cloned upstream of the coding region for the yeast transcriptional factor Gal4. This Pkd2-Gal4 transgene was used to drive the expression of UAS-GFP reporter to locate the cells where the endogenous Pkd2 is expressed. Several Pkd2-Gal4 insertions showed green fluorescent protein (GFP) expression in the testis, pharynx muscle, and SMCs surrounding the esophagus (Fig. 2E) and in the hindgut. The role of Pkd2 in these muscle cells is demonstrated by functional assays below.

Visceral Smooth Muscle Functions Are Severely Affected by Pkd2 Mutations—The Drosophila gut is a monolayered endothelial tube ensheathed by circular and longitudinal fibers of visceral smooth muscle cells (31). At the cellular level, the Drosophila gut is grossly analogous to mammalian blood vessels except that the gut lumen has no valves. Food intake into the gut is mediated by pharynx contraction. Once inside, the food is propelled along the gut via the contraction of the esophagus and midgut. Later waste is excreted out of the anus via the contraction of the hindgut. To determine whether Pkd2 has a functional role in pharyngeal and visceral smooth muscles, we determined the rates of food intake and waste excretion as these rates reflect the contractility of the pharyngeal and visceral smooth muscles (29).

The Pkd2 mutation analyzed was the Pkd2\textsuperscript{ko67} allele we generated by the integration of a mutated Pkd2 donor sequence into the endogenous locus (10). Pkd2\textsuperscript{ko67} contains two Pkd2 copies with one copy carrying a BamHI frameshift mutation and the other copy carrying a MunI frameshift mutation (Fig. 3, B and denoted by * in A). We chose to use Pkd2\textsuperscript{ko67} in our assays because it carries a donor-derived white\textsuperscript{iso3} eye marker gene, which conveniently allowed us to isogenize Pkd2\textsuperscript{ko67} into the w\textsuperscript{118}; iso2; iso3 isogenic strain (28). Genomic Southern analyses confirmed the general structure of Pkd2\textsuperscript{ko67} and identified two chromosomal deletion lines, Df(2L)prd1.7 and Df(2L)Prl1.3;iso3 (33A01-02;33E). Df(2L)prd1.7 contains two chromosomal deletion lines, Df(2L)prd1.7 and Df(2L)Prl1.3;iso3 (33A01-02;33E) do not delete the Pkd2 locus (Fig. 3C).

To determine the food intake rates, newly hatched larvae were fed with blue yeast paste (dyed with digestion-resistant bromphenol blue) for 0.5 or 1 h, and those containing blue food in the hindgut were scored. To avoid developmental age differences, newly hatched larvae were used in the assay as was done previously for measuring the reduced muscle function of Drosophila ryanodine receptor (Ryr) mutants (29). Similar to another published report (29), 83 ± 3.6% of the wild type larvae were ingestion-positive by 0.5 h (Fig. 4A). In contrast, ingestion rates were reduced by over half (−56%, p < 0.0001) of the wild type rate for Pkd2\textsuperscript{ko67}/Pkd2\textsuperscript{ko67} and Pkd2\textsuperscript{ko67}/Df(2L)prd1.7 homozygous mutants (36 ± 1.3 and 34.9 ± 1.0%, respectively; Fig. 4A). Pkd2\textsuperscript{ko67}/+ and Df(2L)prd1.7/+ heterozygous larvae also...
showed reduced ingestion rates (53.7 ± 1.5 and 52.6 ± 6.3%, respectively) by a little over one-third (~35%, p < 0.0001) of the wild type rate. This indicates that Pkd2 is haploinsufficient for optimal food intake functions (Fig. 4A).

To measure the rates of waste excretion, newly hatched larvae were fed with bromphenol blue-dyed yeast paste for 4 h, and those that contained the blue food in the hindgut were then transferred to dye-free food and scored for the complete loss of the blue food in the gut in 1 h. The rate of waste excretion depends on food passage along the entire digestive tract and thus is the best measurement for overall contractility of the visceral smooth muscles. In this "pulse-chase" experiment, 51.6 ± 1.0% of the wild type larvae had completed the blue food excretion in 1 h. In contrast, very few of the Pkd2^{ko67}/Pkd2^{ko67} and Pkd2^{ko67}/Df(2)2Liprd1.7 mutant larvae (8.5 ± 0.8 and 5.8 ± 0.2%, respectively) had completed the blue food excretion within the same amount of time, indicating that the excretion rates were severely reduced by over 80% of the wild type rate (Fig. 5A). The excretion rate of Pkd2^{ko67} heterozygotes (24.2 ± 4.9%) was nearly half of the wild type rate. The Df(2L)liprd1.7+ heterozygote was more severe than the Pkd2^{ko67}+ heterozygote (14.5 ± 3.6%). This could be because Pkd2^{ko67} has some residual activity or the Df(2L)liprd1.7 chromosome carries background-modifying mutations since it was not isogenized. Our analyses showed that Pkd2 is strongly haploinsufficient for optimal waste excretion functions (Fig. 5A).

Since the excretion rate was most strongly affected in Pkd2 mutants, we determined whether expression of wild type Pkd2 cDNA from a Ga4-inducible UAS-F3112 rescue transgene was expressed by using muscle Ga4 driver 24B (32) that is expressed in visceral smooth muscles of the gut. One copy of 24B and UAS-F3112 restored the excretion function of Pkd2^{ko67}/Pkd2^{ko67} larvae back to the wild type level (Fig. 5B). The ingestion function was only partially rescued by 24B and UAS-F3112 due to the low expression level of 24B in the pharyngeal muscle (Fig. 4B). These results suggest that reduced Pkd2 activity severely reduces the contractility of visceral smooth muscles.

**Skeletal Muscle Function Is Weakly Affected by Pkd2 Mutations**—The skeletal muscle of the body wall mediates Drosophila larval locomotion. When larvae crawl on the surface of agar substrate, forward movement is initiated by body wall contraction at the posterior tail region. The contraction wave is propagated anteriorly until it reaches the mouth hook, a double tooth-like hook located in the larval mouth. Forward movement is completed by the extension of the larval head in a stereotypical “up, forward, and down” pattern. Head down motion is often associated with the insertion of the mouth hook into the agar substrate. This leaves mouth hook marks on the agar surface.

The locomotive behavior of Pkd2 mutant larvae was grossly normal. BWC rates of Pkd2^{ko67}/Pkd2^{ko67} and Pkd2^{ko67}/Df(2L)liprd1.7 larvae were similar to that of the wild type larva (42.5 ± 1.0 BWCs/min, n = 30). However, the frequency and depth of the mouth hook marks left along the traveling path, which reflect the force of skeletal muscle contraction, were reduced in the mutant. The average number of mouth hook marks made by a wild type larva was 86.5 ± 2/min (n = 20) of active forward movement and that made by the Pkd2^{ko67}/Pkd2^{ko67} larva was 65.5 ± 1/min, a 24% reduction (n = 20, p < 0.0001). In addition to reduced frequency of mouth hook marks, Pkd2^{ko67}/Pkd2^{ko67} or Pkd2^{ko67}/Df(2R)Prd1.7 larvae made shallower mouth hook marks than wild type control larvae (Fig. 6, A versus B). For quantification purposes, any hook marks larger and deeper than those shown in Fig. 6B
Bars driver only weakly expresses in the pharynx. 

... weakly rescue the mutant phenotype possibly because the 24B Gal4 driver only weakly expresses in the pharynx. Bars represent S.E. from three separate measurements (n = 150 larvae/experiment, repeated three times, p < 0.0001 at 0.5 h).

were counted as deep hook marks. Pkd2ko67/Pkd2ko67 larvae made approximately −15% fewer deep hook marks (n = −9,000) as compared with wild type larvae based on this criterion (10 male and 10 female were used in the assay). Thus it appears that loss of Pkd2 activity only slightly reduces the performance of the body wall skeletal muscle. Interestingly this weak effect of Pkd2 mutation was greatly augmented by a small reduction of the ryanodine receptor activity (see below).

Pkd2 affects Ca2+ homeostasis by cooperating with the ryanodine receptor—Optimal muscle performance revolves around Ca2+ homeostasis. The maintenance of Ca2+ homeostasis requires the concerted action of Ca2+ channels on the cell surface and on the membrane of the intracellular calcium storage organelle, the sarcoplasmic reticulum. One of the important sarcoplasmic reticulum Ca2+ channels is the ryanodine-inhibitable ryanodine receptor (Ryr), which is involved in excitation-contraction coupling of the skeletal muscle (33, 34). Ryr mutations in mouse, Caenorhabditis elegans, and Drosophila show that ryanodine receptors have conserved roles in muscle contraction (29, 33–35). Drosophila has a single Ryr gene that is involved in regulating rapid Ca2+ oscillation that ultimately influences the rate of larval BWC (29).

Although the BWC rate was normal for Pkd2ko67/Pkd2ko67 or Pkd2ko67/Df(2)Lprd1.7 larvae (42.5 ± 0.7 BWCs/min, n = 30), we noticed that the mutant larvae showed increased sensitivity to ryanodine (Fig. 6C). After being fed with yeast paste containing a low concentration of ryanodine (6 μM ryanodine for 30 min) that had no significant effect on the wild type larvae, the BWC rate of Pkd2ko67/Df(2)Lprd1.7 larvae was reduced by 19% (34.3 ± 1.4 BWCs/min, n = 30, p < 0.0001). To test the genetic interaction between Pkd2 and Ryr, we used the weak Ryr04913 allele. The BWC rate of Ryr04913/+ heterozygous larvae was nearly normal (41.0 ± 0.9 BWCs/min, n = 30, p > 0.02); however, that of Pkd2ko67/Ryr04913/Pkd2ko67/+ was reduced by 18% (33.6 ± 1.0 BWCs/min, n = 50, p < 0.0001) to a level nearly as low as Ryr04913/Ryr04913 homozygotes (32.0 ± 0.75 BWCs/min, n = 30). This is a strong synergistic reduction since the BWC rate of Ryr04913 homozygous null mutant was only reduced by 55% (19.0 ± 1 BWCs/min, n = 30) (29). These results strongly suggest that Pkd2 mediates optimal muscle contractility through intracellular Ca2+ homeostasis and cooperates with the ryanodine receptor in this process. This is consistent with the recent finding that Ca2+ influx through ciliary PKD2 in the kidney epithelia is accompanied by Ca2+ release from intracellular Ca2+ storage organelles through the ryanodine receptor (17).

**DISCUSSION**

This study demonstrated that Drosophila PKD2 functions in the skeletal and smooth muscle cells where it is required for optimal muscle contractility. During the course of this study, muscle fiber organization and attachment to the epidermis in Pkd2 mutants was found to be normal by visualizing GFP expressed in muscles of living Drosophila larvae (data not shown). Muscle ultrastructure determined by transmission electron microscopy was also found to be normal (data not shown). Thus, insufficient Pkd2 activity appears to compromise...
is consistent with a recent report that intracellular \(Ca^{2+}\) is reduced in \(Pkd2^{+/−}\) heterozygous mouse vascular SMCs, and these mice show higher rates of vascular complications and lethality than \(Pkd2^{+/+}\) control mice under an experimentally induced hypertension condition (26). Based on these data, we propose that haploinsufficiency of human PKD2 function leads to decreased vascular SMC contractility and function, and this may play a primary role in the development of a variety of vascular complications such as hypertension and aneurysms in ADPKD patients.

Our analyses also suggest that the muscle function of \(Pkd2\) is mediated through intracellular \(Ca^{2+}\) homeostasis that involves the synergistic actions of \(Pkd2\) and the intracellular calcium-releasing channel, the ryanodine receptor. A recent study shows that PKD1 and PKD2 are co-localized on primary cilia of cultured renal epithelia (17). Fluid flow across the epithelial layer causes cilium bending and triggers \(Ca^{2+}\) influx from the plasma membrane (17). Interestingly this \(Ca^{2+}\) influx through ciliary PKD2 in the renal epithelial culture is also accompanied by intracellular \(Ca^{2+}\) release from ryanodine-sensitive internal \(Ca^{2+}\) stores (17). Our observation of the strong genetic interaction between \(Pkd2\) and the ryanodine receptor mutation suggests that cooperation between PKD2 and the ryanodine receptor also occurs in the SMCs. PKD2 has been shown to localize on the endoplasmic reticulum membrane and the plasma membrane as well as on primary cilia (15, 17, 18, 23, 37, 38). In both mammalian and fly SMCs, PKD2 appears to be predominantly cytoplasmic (23). Thus, propagation of \(Ca^{2+}\) signaling through coupling of PKD2 with the ryanodine receptor may represent a common and evolutionarily conserved aspect of PKD2 function in both epithelial and non-epithelial cell types. However, it is possible that different molecular coupling mechanisms are involved between PKD2 and the ryanodine receptor depending on the subcellular location of PKD2 in different cell types.

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REFERENCES

1. Gahw, P. A. (1993) *N. Engl. J. Med.* 329, 332–342
2. Calvet, J. F., and Grantham, J. J. (2001) *Semin. Nephrol.* 21, 107–123
3. Perrone, R. D., Buthazer, R., and Terrin, N. C. (2001) *Am. J. Kidney Dis.* 38, 777–784
4. Perrone, R. D. (1997) *Kidney Int.* 51, 2022–2030
5. Arnaout, M. A. (2000) *Kidney Int.* 58, 2599–2610
6. Qian, F., Watnick, T. J., Onuchic, L. F., and Germino, G. G. (1996) *Cell* 87, 979–987
7. Watnick, T. J., Torres, V. E., Gandolph, M. A., Qian, F., Onuchic, L. F., Klinger, K. W., Landes, G., and Germino, G. G. (1998) *Mol. Cell* 2, 247–251
8. Pei, Y., Watnick, T. J., He, N. W., Wang, K., Liang, Y., Parfrey, P., Germino, G., and St. George-Hyslop, P. (1999) *J. Am. Soc. Nephrol.* 10, 1524–1527
9. Wu, G., D’Agati, V., Cai, Y., Markowitz, G., Park, J. H., Reynolds, D. M., Maeda, Y., Le, T. C., Hou, H., Jr., Kucherlapati, R., Edelmann, W., and Somlo, S. (1998) *Cell* 93, 177–188
10. Gao, Z., Ruder, D. M., and Lu, X. (2003) *Curr. Biol.* 13, 2175–2178
11. Watnick, T. J., Jin, Y., Matunis, E., Kernan, M. J., and Montell, C. (2003) *Curr. Biol.* 13, 2179–2184
12. Vassilev, P. M., Guo, L., Chen, X. Z., Segal, Y., Peng, J. B., Basora, N., Babakhanlou, H., Cruger, G., Kanazirska, M., Ye, C., Brown, E. M., Hediger, M. A., and Zhou, J. (2001) *Biochem. Biophys. Res. Commun.* 282, 341–350
13. Gonzalez-Perretti, S., Batielli, M., Kim, K., Essafi, M., Timpanaro, G., Moltabetti, N., Reisin, I. L., Arnaout, M. A., and Cantiello, H. F. (2001) *Proc. Natl. Acad. Sci. U. S. A.* 98, 1182–1187
14. Gonzalez-Perretti, S., Batielli, M., Kim, K., Essafi, M., Timpanaro, G., Moltabetti, N., Reisin, I. L., Arnaout, M. A., and Cantiello, H. F. (2002) *J. Biol. Chem.* 277, 24959–24966
15. Koulou, P., Cai, Y., Geng, L., Maeda, Y., Nishimura, S., Witzgall, R., Ehrlich, B. E., and Somlo, S. (2002) *Nat. Cell Biol.* 4, 191–197
16. Chen, X. Z., Segal, Y., Basora, N., Guo, L., Peng, J. B., Babakhanlou, H., Vassilev, P. M., Brown, E. M., Hediger, M. A., and Zhou, J. (2001) *Biochem.*
17. Nauli, S. M., Alenghat, F. J., Luo, Y., Williams, E., Vassilev, P., Li, X., Elia, A. E., Lu, W., Brown, E. M., Quinn, S. J., Ingber, D. E., and Zhou, J. (2003) Nat. Genet. 33, 129–137
18. McGrath, J., Somlo, S., Makova, S., Tian, X., and Brueckner, M. (2003) Cell 114, 61–73
19. Haycraft, C. J., Swoboda, P., Tsuulman, P. D., Thomas, J. H., and Yoder, B. K. (2003) Development 128, 1493–1505
20. Boulter, C., Mulroy, S., Webb, S., Fleming, S., Brindle, K., and Sandford, R. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 12174–12179
21. Torres, V. E., Cai, Y., Chen, X., Wu, G. Q., Geng, L., Cleghorn, K. A., Johnson, C. M., and Somlo, S. (2001) J. Am. Soc. Nephrol. 12, 1–9
22. Griffin, M. D., Torres, V. E., Grande, J. P., and Kumar, R. (1997) J. Am. Soc. Nephrol. 8, 616–626
23. Qian, Q., Li, M., Cai, Y., Ward, C. J., Somlo, S., Harris, P. C., and Torres, V. E. (2003) J. Am. Soc. Nephrol. 14, 2280–2287
24. Kim, K., Drummond, I., Ibrahimbegovic-Bekrennaya, O., Klinger, K., and Arnout, M. A. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 1731–1736
25. Wu, G., Markowitz, G. S., Li, L., D’Agati, V. D., Factor, S. M., Geng, L., Tibara, S., Tuchman, J., Cai, Y., Park, J. H., van Adelsberg, J., Hou, H., Jr., Kucherlapati, R., Edelmann, W., and Somlo, S. (2000) Nat. Genet. 24, 75–78
26. Qian, Q., Hunter, L. W., Li, M., Marin-Padilla, M., Prakash, Y. S., Somlo, S., Harris, P. C., Torres, V. E., and Sieck, G. C. (2003) Hum. Mol. Genet. 12, 1875–1880
27. Rong, Y. S., Titen, S. W., Xie, H. B., Golic, M. M., Bastiani, M., Bandypadhyay, P., Olivera, B. M., Brodskey, M., Rubin, G. M., and Golic, K. G. (2002) Genes Dev. 16, 1568–1581
28. Hoskins, R. A., Phan, A. C., Naemuddin, M., Mapa, F. A., Ruddy, D. A., Ryan, J. J., Young, L. M., Wells, T., Kopczynski, C., and Ellis, M. C. (2001) Genome Res. 11, 1109–1113
29. Sullivan, K. M., Scott, K., Zucker, C. S., and Rubin, G. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 5942–5947
30. Sharma, Y., Cheung, U., Larsen, E. W., and Eberl, D. F. (2002) Genesis 34, 115–118
31. Bate, M. (1993) in The Development of Drosophila Melanogaster (Bate, M., and Martinez Arias, A., eds) Vol. II, pp. 1013–1090, Cold Spring Harbor Press, Cold Spring Harbor, NY
32. Brand, A. H., and Perrimon, N. (1993) Development 118, 401–415
33. Takeshima, H., Iino, M., Takekura, H., Nishi, M., Kuno, J., Minowa, O., Takano, H., and Noda, T. (1994) Nature 369, 556–559
34. Yamazawa, T., Takeshima, H., Shimuta, M., and Iino, M. (1997) J. Biol. Chem. 272, 8161–8164
35. Maryon, E. B., Saari, B., and Anderson, P. (1998) J. Cell Sci. 111, 2885–2895
36. Wernig, F., and Xu, Q. (2002) Prog. Biophys. Mol. Biol. 78, 105–137
37. Scheffers, M. S., Le, H., van der Bent, P., Leonhard, W., Prins, F., Spruit, L., Breuning, M. H., de Heer, E., and Peters, D. J. (2002) Hum. Mol. Genet. 11, 59–67
38. Newby, L. J., Streets, A. J., Zhao, Y., Harris, P. C., Ward, C. J., and Ong, A. C. (2002) J. Biol. Chem. 277, 20763–20773
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