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Graphical abstract

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Title: The Tryptophan Metabolizing Enzyme Indoleamine 2,3-Dioxygenase 1 Regulates Polycystic Kidney Disease Progression

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Conflict of Interest
K.H. receives royalties for industry use of the Pkd1RC/RC mouse model in concordance with Mayo Clinic Ventures regulations (Mayo Technology Case #2012-144).
Abstract 200 words

Autosomal dominant polycystic kidney disease (ADPKD), the most common monogenic nephropathy, is characterized by phenotypic variability exceeding genic effects. Dysregulated metabolism and immune cell function are key disease modifiers. The tryptophan metabolites, kynurenines, produced through IDO1, are known immunomodulators. Here, we study the role of tryptophan metabolism in PKD using an orthologous disease model (C57Bl/6J Pkd1^RC/RC). We found elevated kynurenine and IDO1 levels in Pkd1^RC/RC kidneys versus wildtype. Further, IDO1 levels were increased in ADPKD cell lines. Genetic Ido1 loss in Pkd1^RC/RC animals resulted in reduced PKD severity as measured by %kidney weight/body weight and cystic index. Consistent with an immunomodulatory role of kynurenines, Pkd1^RC/RC;Ido1^-/- mice presented with significant changes in the cystic immune microenvironment (CME) versus controls. Kidney macrophage numbers decreased and CD8⁺ T cell numbers increased, both known PKD modulators. Also, pharmacological IDO1 inhibition in Pkd1^RC/RC mice and kidney specific Pkd2 knockout mice with rapidly progressive PKD resulted in less severe PKD versus controls with similar changes in the CME as in the genetic model. Our data suggest that tryptophan metabolism is dysregulated in ADPKD and that its inhibition results in changes to the CME and slows disease progression, making IDO1 a novel therapeutic target for ADPKD.
Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common, life threatening genetic kidney disease(1, 2). It is characterized by progressive kidney cyst growth leading to organ failure and accounts for 5-10% of end stage kidney disease cases worldwide(3, 4). Tolvaptan, a vasopressin receptor antagonist, is the only FDA approved therapy for ADPKD, which slows cyst growth but also impairs quality of life, underscoring the need for alternative therapies(5, 6). ADPKD is predominantly caused by mutations to PKD1 or PKD2, but manifests with significant inter- and intrafamilial phenotypic variability that exceeds genic or allelic effects(7-9). Disease variability has been linked to genetic modifiers, kidney injury, and environmental/lifestyle factors(7, 10-12).

Accumulating evidence suggests that metabolic reprogramming is a key feature of ADPKD(13-15). In the HALT-PKD Study A population, overweight/obesity were shown to be strong independent risk factors of total kidney volume increase and eGFR decline(10). Correlatively, preclinical studies in PKD models have shown that mild-to-moderate caloric restriction, time restricted feeding, or ketogenic diet ameliorate kidney cyst growth(16-18). Indeed, targeting specific metabolic pathways found to be dysregulated in PKD such as glycolysis, fatty acid oxidation, and arginine, glutamine, and methionine metabolism alleviated cystic kidney disease in PKD models(19-24).

Data from our group and others suggest that tryptophan metabolism may also play a role in ADPKD progression(25, 26). Tryptophan is catabolized to kynurenine via tryptophan 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO1, IDO2, Supplemental Figure 1). While TDO is predominantly expressed in the liver, IDO1/2 are expressed in kidney epithelial- and immune cells, with IDO1 having higher catalytic activity for tryptophan(27-29). Kynurenine and/or its metabolite, kynurenic acid, are known drivers of oxidative stress, dysregulated calcium homeostasis, and mitochondrial dysfunction(30-32). As such, they are considered uremic toxins; their serum levels correlate with chronic kidney disease (CKD) severity(33). Interestingly, serum metabolomics of participants of the Modification of Diet in Renal Disease Study showed that levels of kynurenic acid were selectively elevated in ADPKD patients compared to other CKD patients. Also, we recently published that ADPKD patients have higher
plasma kynurenine concentrations compared to healthy subjects and levels further increased with disease progression(25, 26).

In cancer, a disease with multiple parallels to PKD, plasma kynurenine levels and tumor expression of IDO1 negatively correlate with cancer survival and clinical outcome(34-38). Elevated kynurenine levels and IDO1 upregulation promote an immunosuppressive microenvironment and thus allow tumor escape from immune destruction. This includes suppression of anti-tumorigenic CD8+ T cells through upregulation of the immune checkpoint PD-1/PD-L1, generation of pro-tumorigenic regulatory T cells (T_{Reg}), and promotion of tumor-associated M2 macrophage polarization(39, 40). Indeed, inhibition of tryptophan metabolism via IDO1 inhibitors has been FDA approved for multiple cancers either as mono- or combination therapy with anti-PD-1(38). Interestingly, recent data by others and us suggest that both innate and adaptive immune cells, such as M2-like macrophages and CD8+ T cells, are also important modulators of kidney cyst growth in PKD murine models(41-43). However, the functional impact of the tryptophan pathway on cyst growth and its potential as a therapeutic target in ADPKD have not been clearly established.

Here, we utilize an orthologous ADPKD1 model to study tryptophan metabolism in PKD(41, 44, 45). We found both tryptophan metabolites and IDO1 expression to be elevated in ADPKD1 mice correlative with disease progression. Genetic loss and pharmaceutical inhibition of IDO1 slowed cyst growth in our model. This was associated with reduced numbers of kidney M2-like resident macrophages and T_{Reg}, reduced expression of PD-1/PD-L1, and an increase of CD8+ T cell numbers within the adaptive immune cell population. Together, our data implicate tryptophan metabolism as a novel modifier of ADPKD progression and suggest that FDA approved IDO1 inhibitors may represent a new treatment approach for ADPKD.
Results

Tryptophan metabolism via IDO1 is dysregulated in Pkd1<sup>RC/RC</sup> mice

To determine whether tryptophan metabolism is abnormally regulated in murine PKD, we performed metabolomic analyses of metabolites within the tryptophan pathway using the orthologous ADPKD model C57Bl/6J Pkd1 p.R3277C (Pkd1<sup>RC/RC</sup>)(41, 44, 45). Comparing kidney metabolites of strain-, age-, and sex-matched wildtype (WT) mice, we found that tryptophan levels remained stable throughout PKD progression (3 to 9 months [mo]) and comparable to WT levels (Table 1, Supplemental Figure 1, Supplemental Table 1). However, the immunosuppressive metabolites kynurenine/kynurenic acid were significantly upregulated in Pkd1<sup>RC/RC</sup> kidneys correlative with disease severity and independent of sex (Table 1, Supplemental Table1)(46). Similarly, levels of xanthurenic acid, a kynurenic acid metabolite, were elevated compared to WT. Consistent with increased production of kynurenic and xanthurenic acid, Pkd1<sup>RC/RC</sup> kidneys displayed significantly decreased levels of picolinic acid compared to WT (Table 1, Supplemental Table 1). Picolinic acid is an isomer of nicotinic acid, a derivative of nicotinamide, which was also significantly decreased and has been shown to slow cyst growth and improve kidney function in two PKD models(47). No changes were observed in levels of anthranilic acid and quinolinic acid (data not shown). Overall, these data highlight that tryptophan metabolism is dysregulated in Pkd1<sup>RC/RC</sup> kidneys with a shift towards the production of known immunosuppressive metabolites.

Western blot analysis of whole kidney homogenates showed increased expression of IDO1 in kidneys of Pkd1<sup>RC/RC</sup> mice compared to WT (Figure 1A). Similar increases were observed in 9-12 cells (PKD1<sup>-/-</sup>) compared to renal cortical epithelial cells (RCTE; PKD1<sup>+/+</sup>, Figure 1B). The expression levels of IDO1 could further be amplified by stimulating RCTE or 9-12 cells with interferon gamma (IFNγ, Figure 1B). IFNγ is recognized as a highly potent inducer of IDO1 via signal transducer and activator of transcription-1 (STAT-1) and/or nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling(48, 49). Importantly, we recently reported Ifnγ to be elevated in Pkd1<sup>RC/RC</sup> kidneys and multiple murine studies accentuate aberrant activation of most STAT proteins in PKD kidneys (41, 50). Further, we found IDO1 to be expressed in primary epithelial cells derived from individual kidney cysts of ADPKD
patients (Figure 1C). We confirmed elevated levels of IDO1 by immunofluorescence (Figure 1D). In WT mice, IDO1 expression was sparsely detected within interstitial cells and not in the kidney epithelium. However, Pkd1RC/RC mice showed increased levels of IDO1 expression in cyst lining- and interstitial cells. Based on the literature, interstitial cells expressing IDO1 are likely macrophages or dendritic cells (DCs), which have been shown to upregulate IDO1 in various types of cancer resulting in increased tumorigenesis(36, 49). These data suggest that the observed dysregulation of tryptophan catabolism may be attributed to overexpression of IDO1 within the cystic epithelia as well as in kidney immune cells.

Genetic loss of Ido1 alleviates PKD severity and corrects tryptophan metabolism abnormalities

To functionally establish a role for tryptophan metabolism and IDO1 in PKD pathogenesis, we crossed C57Bl/6J Pkd1RC/RC mice with C57Bl/6J Ido1-/- mice. We aged the resulting second filial generation animals (Pkd1RC/RC;Ido1+/+ or Pkd1RC/RC;Ido1-/-) to 3mo or 6mo of age and evaluated histopathological/physiological features commonly analyzed in murine PKD studies (Figure 2, Supplemental Figure 2, Supplemental Table 2). At 3mo of age we observed no difference in gross histological appearance, percent kidney weight normalized to body weight (%KW/BW), or cystic volume/index between Pkd1RC/RC;Ido1+/+ or Pkd1RC/RC;Ido1-/- mice (Figure 2A-C, Supplemental Figure 2A). However, at 6mo of age Pkd1RC/RC;Ido1+/+ mice presented with decreased cystic kidney disease compared to Pkd1RC/RC;Ido1-/- mice, independent of sex (Figure 2A-C, Supplemental Figure 2A, Supplemental Table 2). Indeed, it appears that cyst growth from 3 to 6mo of age in Pkd1RC/RC;Ido1-/- mice was halted compared to controls, as evidenced by a significant “reduction” in kidney cyst count and size (Figure 2C). We did not detect a difference in fibrotic burden or kidney function (BUN levels, Supplemental Figure 2B, C, Supplemental Table 2).

Consistent with Ido1 loss, we observed significantly decreased levels of the immunosuppressive metabolite kynurenine in Pkd1RC/RC;Ido1-/- animals compared to controls and independent of sex (Figure 2D, Supplemental Table 2). Indeed, the kynurenine levels of 6mo old Pkd1RC/RC;Ido1-/- mice were comparable to WT levels (Supplemental Figure 2D). Neither tryptophan nor kynurenic acid were
significantly altered between the two *Ido1* genotypes in the setting of PKD, but we observed a possible trend towards decreased kynurenic acid levels at 6mo of age in *Pkd1*<sup>RC/RC;Ido1<sup>+/−</sup></sup> animals versus controls, which was more pronounced in males versus females(Figure 2D, Supplemental Table 2).

*Ido1* loss is associated with a CME favorable to halt cyst progression

We compared kidney immune cell types of *Pkd1*<sup>RC/RC;Ido1<sup>+/-</sup></sup> and *Pkd1*<sup>RC/RC;Ido1<sup>−/−</sup></sup> mice at 3mo and 6mo of age using flow cytometry of kidney single cell suspensions (Figure 3, Supplemental Figure 3)(41, 42, 51). Overall, we found that the observed decreased PKD severity in *Pkd1*<sup>RC/RC;Ido1<sup>−/−</sup></sup> mice compared to *Pkd1*<sup>RC/RC;Ido1<sup>+/−</sup></sup> mice at 6mo of age was associated with lower numbers of kidney immune cells (CD45<sup>+</sup>, Figure 3A) and a significant decrease in infiltrating (F4/80<sup>lo</sup>; CD11b<sup>+</sup>) and resident (F4/80<sup>hi</sup>; CD11b<sup>+</sup>) macrophages, neutrophils (GR1<sup>+</sup>), DCs (CD11b<sup>+</sup>; CD11c<sup>+</sup>) and natural killer cells (NK cells, NKp46<sup>+</sup>) compared to control (Figure 3B, Supplemental Figure 3A-C). While less is known about the role of neutrophils, DCs, or NK cells in PKD, ample evidence supports a role of “M2-like” macrophages driving kidney/liver cyst growth in murine PKD models(42, 43, 52-58). With respect to adaptive immune cells, we did not find a change in overall T cell (TCRβ<sup>+</sup>, CD4<sup>+</sup>, or CD8<sup>+</sup>) numbers associated with the less severe disease observed in *Pkd1*<sup>RC/RC;Ido1<sup>−/−</sup></sup> mice compared to *Pkd1*<sup>RC/RC;Ido1<sup>+/−</sup></sup> mice at 6mo of age (Figure 3C). However, the distribution of T cell subtypes changed, with 6mo old *Pkd1*<sup>RC/RC;Ido1<sup>−/−</sup></sup> mice having significantly more CD8<sup>+</sup> T cells compared to control (Figure 3D). Given our previously published findings that CD8<sup>+</sup> T cells play an anti-cystogenic role in the *Pkd1*<sup>RC/RC</sup> model, this increase in CD8<sup>+</sup> T cell numbers supports a reduction in cyst severity in PKD *Ido1<sup>−/−</sup>* animals compared to control(41). This increase of CD8<sup>+</sup> T cells is counterbalanced by a decrease in double negative T cells, whose functional role has not been studied in PKD, but we and others have reported that their numbers increase in ADPKD compared to control in *Pkd1*<sup>RC/RC</sup> kidneys as well as ADPKD patient kidneys (Supplemental Figure 3D)(51, 59).

Kynurenines have been reported in the cancer literature to support immune escape of tumors via engagement of the PD-1/PD-L1 immune checkpoint and differentiation of CD4<sup>+</sup> T cells into
immunosuppressive TRegs (60, 61). In PKD, we found activation of the PD-1/PD-L1 checkpoint as well as increased numbers of TRegs (CD4+/FoxP3+) in Pkd1RC/RC kidneys correlative to disease progression (Supplemental Figure 4, unpublished) (62). We observed a downward trend in kidney TReg numbers in Pkd1RC/RC;Ido1−/−animals compared to control and a significant decrease in PD-L1 expression on the kidney epithelium (EpCAM+/PD-L1+), macrophages (F4/80+/PD-L1+), and PD-1 on CD8+ T cells at 6mo of age (Figure 3E, F, Supplemental Figure 3E).

Pharmaceutical inhibition of IDO1 slows cyst growth in a slowly and rapidly progressive model of PKD as well as changes the CME towards an anti-cystogenic composition

We treated 1mo C57Bl/6J Pkd1RC/RC mice with 400mg/kg 1-Methyl-tryptophan (1-MT) via oral gavage twice daily for 3 weeks (63, 64). 1-MT is a synthetic tryptophan analog/IDO pathway inhibitor (65-67). Pkd1RC/RC mice treated with 1-MT versus control displayed significantly less severe PKD as apparent by histology, %KW/BW, cyst volume/index, and cyst number (Figure 4A-C, Supplemental Figure 5A, Supplemental Table 3). This treatment effect occurred independent of sex (Supplemental Table 3). Treated mice also showed a significant reduction in kidney kynurenic acid levels versus controls (Figure 4D, Supplemental Table 3). Kynurenine levels, however, remained unchanged, which may be explained by the dynamic catabolic balance between kynurenine and kynurenic acid. Treatment did not impact cyst size, fibrotic burden, or kidney function (Figure 4C, Supplemental Figure 5A, Supplemental Table 3).

We confirmed the therapeutic efficacy of 1-MT using the juvenile induced C57Bl/6J Pax8rtTA;TetO-cre;Pkd2flx/flx model (68-70). In this model, kidney-specific loss of Pkd2 was induced via intraperitoneal (IP) injection of doxycycline at postnatal day (P) 10 and 11. The mice present with rapidly progressive PKD with a 50% survival rate of 38.5 days (Supplemental Figure 6A-D). Generally, females have less rapidly progressive kidney disease than males (Supplemental Figure 6A, B). As for Pkd1RC/RC mice, tryptophan metabolism was dysregulated in this model, as highlighted by significantly increased kynurenic acid levels in the kidney correlative with disease severity, although no difference in kynurenine was noted (Supplemental Figure 6E). Pax8rtTA;TetO-cre;Pkd2flx/flx mice were treated with 100mg/kg 1-
MT from P12 to P21 via daily IP injections (71-73). *Pax8*rtTA;TetO-cre;*Pkd2*flx/flx mice treated with 1-MT presented with a significant reduction in %KW/BW as well as cyst- and fibrotic volume/index compared to control. Cyst size, number, and BUN trended towards a therapeutic effect of reduced PKD severity upon 1-MT treatment (Figure 5A-D, Supplemental Figure 6F). Treatment efficacy was noted independent of sex, although males showed a better response to 1-MT treatment compared to females (Supplemental Table 4). 1-MT treatment resulted in a trend towards reduced kidney kynurenic acid levels but did not change tryptophan or kynurenine levels (Figure 5E, Supplemental Figure 6G, Supplemental Table 4).

As for the genetic *Ido1* knockout model, we evaluated the impact of 1-MT treatment on the kidney immune microenvironment in *Pkd1*RC/RC mice. Immune cell- (CD45+), NK cell-, DC-, neutrophil-, and macrophage numbers decreased in kidneys of 1-MT-treated animals versus controls, although not all declines reached significance (Figure 4E, Supplemental Figure 5B-D). Interestingly, upon 1-MT treatment, the number of kidney resident macrophages (F4/80hi; CD11b+) preferentially decreased, while the number of kidney infiltrating macrophages (F4/80lo; CD11b+) remained unchanged (Figure 4E). 1-MT treatment, similarly to genetic loss of *Ido1*, resulted in a shift of the T cell (TCRβ+) population towards CD8+ T cells and a decrease of double negative T cell numbers within the kidney; no changes were detected in overall T cell numbers or subtypes (Figure 4F, Supplemental Figure 5F). 1-MT treated animals also had significantly reduced expression of PD-L1 on macrophages (F4/80+), and a trend towards reduced expression on epithelial cells (EpCAM+) (Figure 4G, Supplemental Figure 5E). We also observed a significant decrease in T_reg (CD4+/FoxP3+) numbers within the kidneys of 1-MT treated *Pkd1*RC/RC mice compared to control, which reinforces the idea of kynurenines driving T_reg differentiation (Figure 4G). Together, these data provide preclinical support for use of IDO1 inhibitors as a PKD therapeutic and highlight that pharmaceutical inhibition of IDO1 mimics the immunomodulatory phenotypes observed in the genetic model.
Discussion

Tryptophan and its metabolites regulate a variety of physiological processes including cell growth/maintenance and neuronal function. However, >95% of tryptophan is a substrate for the kynurenine pathway, which controls hyperinflammation and long-term immune tolerance(74, 75). While IDO1 plays a minor role in metabolizing tryptophan to kynurenine under normal conditions, IDO1 levels and IDO1-dependent tryptophan metabolism in macrophages, DCs, and epithelial cells is potently induced by inflammatory signals. These include IFNγ, interleukin 6, and tumor necrosis factor α, all of which have been found by others and us to be elevated in murine models of PKD or ADPKD patient cyst fluid(41, 75-77). Indeed, our in vitro studies confirm that treatment with recombinant IFNγ induces IDO1 expression in cystic epithelial cells. Consistently, we observed increased levels of kynurenines and IDO1 correlative to disease severity and increased IDO1 staining in interstitial cells as well as the cystic epithelium of Pkd1RC/RC mice. These results parallel our recent findings that kynurenine and kynurenic acid are significantly accumulated in plasma collected from children and adults with ADPKD compared to healthy subjects(78).

In cancer, kynurenine/kynurenic acid and IDO1 levels regulate immunosuppression through suppression of anti-tumorigenic immune cells (DCs, NK-, and effector T cells), expansion of protumorigenic immune cells (TReg, M2 macrophages, and myeloid-derived suppressor cells), and upregulation of immune checkpoints (PD-1|PD-L1, CTLA4|CD80/86)(61, 79, 80). To date, the functional role of DCs, NK cells, and the different CD4+ T cell subtypes in PKD progression have not been well defined. However, urinary CD4+ T cell numbers have been shown to correlate with eGFR decline in ADPKD patients, and our recent publication suggests that granzyme-B or IFNγ-producing T cells may be critical to mediating injury-driven PKD(59, 81). Similarly, kidney M2-like macrophages are known to drive PKD, and we have shown that loss of CD8+ T cells enhances cyst growth in the Pkd1RC/RC model(41, 52-56, 82-84). Here, we further provide data that kidney TReg numbers are increased in Pkd1RC/RC mice compared to wildtype, suggesting they may play a role in PKD. Since genetic loss of Ido1/1-MT treatment in our PKD model not only reduced kidney kynurenine/kynurenic acid levels, but also slowed cyst growth,
we hypothesized inhibition of immunosuppressive pathways to be an underlying mechanism. In line with this hypothesis, we observed decreased numbers of kidney macrophages and increased numbers of CD8+ T cells within the adaptive immune cell population (TCRβ+) in the kidneys of Pkd1RC/RC Ido1 knockout/1-MT treated animals compared to controls. Further, we observed downregulation of PD-1/PD-L1 expression and a reduction of TReg numbers, all favorable for a CME that supports slowed cyst growth. We also observed fewer DCs, NK cells, and neutrophils in Pkd1RC/RC mice when tryptophan metabolism was inhibited, but the functional impact of these microenvironmental changes is less clear. A key limitation of our CME analyses is that we only investigated immune cell numbers and not their function. It is also unclear whether our observed changes in CME composition are directly due to IDO1 inhibition or a consequence of reduced PKD severity. Future mechanistic studies are needed to disjoin these two observations and decipher cell types critical to IDO1-mediated PKD pathogenesis. Conditional, cell type specific Ido1 loss in the setting of PKD would establish which cells are key producers of kynurenine/kynurenic acid as well as outline if the pathogenic impact of IDO1 overexpression and/or high kynurenine/kynurenic acid levels is predominantly mediated by immunosuppression or cell autonomous effects within the cystic kidney epithelium. To that extent, kynurenines may drive pro-proliferative pathways within the kidney epithelium through binding to the aryl hydrocarbon receptor (AhR), as has been described in cancer(85). AhR interacts with a multitude of proteins found to be key modulators of PKD, including mechanistic target of rapamycin, mitogen-activated protein kinases, sirtuin-1, and NF-κB(86-90). Interestingly, we found that treatment with 1-MT decreased the proliferative rate of 9-12 cells over RCTE cells, suggesting that the therapeutic effect of IDO1 inhibition may go beyond immunomodulation (Supplemental Figure 7).

There are several other limitations in this study. Firstly, data suggest kidney accumulation of both kynurenine and kynurenic acid driving PKD pathology and immunosuppression. Future studies will need to test if individual administration of either of these tryptophan metabolites to murine PKD models is sufficient to modulate kidney cyst growth and/or immune cell function. In addition, other tryptophan metabolites might be critical to the pathology. For example, both 3-hydroxyanthranilic acid and quinolinic
acid have been shown to lead to effector T cell apoptosis, hence contributing to an immunosuppressive milieu(91). Neither of these metabolites however, were significantly altered in our model of ADPKD. Secondly, it is unclear whether differential IDO1 levels are the sole driver of kynurenine/kynurenic acid accumulation in ADPKD. Indeed, analysis of key tryptophan metabolizing enzymes in kidneys of $Pkd1^{RC/RC}$ versus wildtype mice revealed significantly elevated levels of IDO2 and kynurenine amino transferase (KAT) as well as decreased levels of kynurenine 3-monooxygenase (KMO, $p=0.08$) and kynureninase (KYNU, $p=0.01$), all potentially contributing to kynurenine/kynurenic acid accumulation in the kidney (Supplemental Figure 1, 8). We also did not assay IDO1 enzymatic activity, which could be enhanced in the setting of ADPKD. A detailed dissection of the different tryptophan catabolism pathway-junctions and evaluation of enzyme activities will be required to better delineate which aspect of dysregulated tryptophan metabolism is critical to driving kidney cyst growth. These studies are essential to better understand how to ideally target the pathway for clinical translation. Finally, a better understanding of what drives increased IDO1 expression in PKD would facilitate therapeutic targeting of the pathway. While we show that IFN$\gamma$ may contribute to increased IDO1 levels, there are multiple other pathways of IDO1 induction that were not assayed. For example, cyclooxygenase-2 (COX2) and prostaglandin E2 (PGE2) activate IDO transcription via the phosphatidylinositol-3-kinase pathway(49). Interestingly, levels of both COX2 and PGE2 are increased in PKD models and patient cyst fluid, and inhibition of COX2 has been shown to ameliorate PKD in $Anks6^{+/+}$ (Han:SPRD Cy/+ ) rats, a non-orthologous ADPKD model, and in $Pkd2^{WS25/-}$ mice. However, the selective COX2 inhibitor celecoxib did not impact disease severity in $Pkd1^{RC/RC}$ mice(92-94). In addition, in a series of tumor cell lines, IDO1 expression was shown to be driven by an autocrine positive feedback loop via the activation of AhR by kynurenine(49). We did not evaluate AhR-dependent transcripts within this study.

It remains unclear why in our genetic model of IDO1 loss, we did not observe a phenotypic difference in PKD severity until 6mo of age compared to control or why some of the observed changes in the CME differed between genetic loss or pharmacological inhibition of IDO1. Regarding the latter, it is interesting that only pharmacological inhibition of IDO1 reduced the number of kidney TRegs, which we
hypothesize to be drivers of cyst growth. Similarly, pharmacological inhibition of IDO1 resulted in a selective reduction of resident macrophages and not infiltrating macrophages, whereas genetic loss decreased both populations within the kidney. The data on the contribution of infiltrating macrophages to PKD progression are inconsistent, and a single study suggests that resident macrophages promote cyst growth in a non-orthologous model of PKD(56, 58, 95). The role of these populations has not been studied in the slowly progressive Pkd1<sup>RK/RC</sup> model. Further, our data suggest that treatment with 1-MT is more effective in slowing PKD in our Pkd1<sup>RK/RC</sup> versus genetic Ido1 loss. This could be due to treatment with 1-MT impacting additional pathways. For example, 1-MT has been shown to have some affinity for IDO2, which is also increased in levels within the ADPKD kidney (Supplemental Figure 8)(96). Further, 1-MT has been shown to directly modulate AhR activity directly as well as mTORC1/autophagy and FoxP3 expression in T cells, all of which are of relevance to PKD(97).

Interestingly, like annual change in kidney growth and eGFR decline in ADPKD patients, kynurenine levels have also been positively associated with body mass index(10, 98, 99). Since tryptophan is an essential amino acid, the impact of various dietary regimens on kynurenine levels has been studied in animal models. Caloric restriction and a ketogenic diet resulted in downregulation of kynurenines, both of which have also been found to slow PKD progression in murine models(16-18, 100, 101).

In conclusion, our data provide convincing evidence that the kynurenine pathway and IDO1 are dysregulated in PKD and that targeting the pathway provides a novel therapeutic platform for disease treatment. 1-MT (NLG-8189) as well as other IDO1 inhibitors such as epacadostat which has higher affinity for IDO1 than 1-MT are being tested in multiple clinical trials for cancer. These inhibitors have been found to be well tolerated with minimal toxicity(102, 103). While the antitumor efficacy of IDO1 inhibitors alone was found to be limited, studies report synergistic effects if combined with immune checkpoint inhibitors(102, 103). Our data strongly support the testing of FDA approved IDO1 inhibitors in long-term preclinical ADPKD studies, with a goal of clinical translation. Similarly, we believe that the impact of dysregulated metabolism on immune cell function in PKD warrants further investigation and
could emerge as a promising new therapeutic platform for combination treatment approaches with epithelial-centric drugs such as tolvaptan.
Concise Methods

Full methods are available in the Supplemental Material.

Mouse models

The homozygous C57Bl/6J p.R3277C (Pkd1RC/RC)(41, 44, 45) model was crossed with C57Bl/6 Ido1 knock-out mice (Ido1^−/−, Jackson Laboratory, stock #005867) to generate C57Bl/6 Pkd1RC/RC;Ido1^−/− and C57Bl/6 Pkd1RC/RC;Ido1^+/+ animals. The C57Bl/6J Pax8rtTA;TetO-cre;Pkd2flx/flx model was received from the NIH NIDDK PKD Research Resource Consortium(68). Pkd2 loss was induced by IP injection of doxycycline at 40mg/kg on P10 and 50mg/kg on P11.

IDO1 inhibition

One-month-old C57Bl/6J Pkd1RC/RC mice were treated by oral gavage for three weeks: twice daily with 400mg/kg 1-Methyl-D-tryptophan (Sigma-Aldrich, #452483, IDO1 inhibitor) or 0.5% hydroxypropyl methyl cellulose, 0.1% Tween 80 (control). P12-21-old C57Bl/6J Pax8rtTA;TetO-cre;Pkd2flx/flx mice were IP injected daily with 100mg/kg 1-MT or buffered PBS (1 N HCl adjusted to pH 7.0 with sterile PBS and 1 M NaOH; control).

Human samples

De-identified ADPKD patient cyst cells were obtained from the Baltimore Polycystic Kidney Disease Research and Clinical Core Center (NIDDK, P30DK090868).

Cell culture

Cell lines (RCTE & 9-12) used have been previously described(104). Cyst cells and cell lines were grown in DMEM/F12 50/50 +L-glutamine, 15nM HEPES (Corning Inc. +10% fetal bovine serum (VWR International) and 1% penicillin-streptomycin (Corning).
IFNγ stimulation

RCTE and 9-12 cells were treated for 24 hours with recombinant human IFNγ (PeproTech, #300-02) at 100ng/ml or PBS control.

Cell viability assay

RCTE & 9-12 cells were seeded in 96-well plate for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT, Thermo Fischer Scientific. #M6494) assay. Cells were treated with vehicle (PBS) or 1-methyl-D-tryptophan dissolved in PBS (1-MT, Sigma-Aldrich, #452483). Following, cells were incubated in MTT solution (5mg/mL) and purple formazan was measured per manufacturer instructions.

Kidney function analyses

Blood urea nitrogen was measured following the manufacturer’s protocol (QuantiChrom Urea Assay Kit, BioAssay Systems, # 501078333).

Histomorphometric analysis

Kidney cystic index, cyst size, and cyst number were analyzed using a custom-built NIS-Elements AR v4.6 macro (Nikon). Fibrotic area was analyzed from picrosirius red-stained kidney sections and visualized using an Olympus BX41 microscope (Olympus Corporation) with a linear polarizer. Images were obtained and quantified as previously described(41).

Immunofluorescence labeling and quantification

Paraffin embedded tissues were processed and stained as previously described(41). Primary and secondary antibodies: 1⁰ anti-mouse IDO1 (clone mIDO-48; BioLegend, 1:50), 2⁰ AF594 goat anti-rabbit IgG (Life Technologies, 1:1000); 1⁰ anti-mouse E-cadherin (clone 36; BD Transduction Laboratories, 1:100), 2⁰ AF488 goat anti-rat IgG (Life Technologies, 1:1000). Sections were mounted with Vectashield mounting medium with DAPI and visualized with a Nikon Eclipse Ti microscope.
**Western blotting**

Whole cell lysates from cell lines, cells derived from human cysts, or homogenized mouse kidney tissue were prepared in RIPA cell lysis buffer with protease inhibitor (#P8340; Sigma Aldrich). Protein samples were separated by SDS-PAGE-electrophoresis and transferred to PVDF membranes. Membranes were incubated with primary antibodies overnight and detected using HRP-linked secondary antibodies and ECL detection reagents. Primary antibodies: IDO1 (clone mIDO-48; BioLegend #122402, 1:400), IDO2 (clone 1HC; Novus Biologicals #NBP2-21641, 1:500), Kynureninase/KYNU (clone 771312; R&D Systems #MAB7389, 1:500), Kynurenine 3-Monoxygenase/KMO (clone 2493A; R&D Systems #MAB8050-SP, 1:500), Kynurenine aminotransferase/KAT (clone C-7; Santa Cruz Biotechnology #sc-374531, 1:500), GAPDH (polyclonal FL-335, Santa Cruz Biotechnology #sc-25778, 1:500). Secondary: anti-rat-HRP (1:5,000), anti-rabbit-HRP (1:5,000), anti-mouse-HRP (1:10,000). Densitometry on X-ray films was quantified using ImageJ.

**Single cell suspension**

Single cell suspensions were prepared as previously described(41). Kidneys harvested from terminal dissection were mechanically dissociated and digested in DMEM/F12, Liberase TL, and DNasel at 37°C for 30 minutes. Dissociation was completed using an 18G needle and quenched using FA3 buffer. Cells were passed through a 100µm filter, incubated with red blood cell lysis buffer, and then passed through a 70µm filter. Cells were suspended in FA3 buffer for flow cytometry staining.

**Flow cytometry**

Flow cytometry was performed as previously described(41). Detailed methods, antibodies used, and gating strategies are outlined in the Supplemental Material. The blocked, viability dye-stained, cell suspension was split in two and used for different flow cytometry panels. Panel 1 (innate immune- and epithelial cells): CD45-FITC, CD11c-PE, F4/80-PE/Dazzle594, CD11b-PerCP-Cy5.5, Gr-1-PE/Cy7, PD-L1-APC, MHCII-DyLight680, EpCAM-APC-eFluor780, and NKp46-eFluor450. Panel 2 (T cells): CD44-
FITC, PD-1-PE, CD45-PE-CF594, TCRβ-PE-Cyanine5, CD69-PE-Cy7, CD8-AF700, CD4-APC/Cyanine7, Ki-67-APC, and FoxP3-eFluor450. Stained cells were analyzed on the Gallios Flow Cytometer. All data were analyzed using Kaluza Analysis v2.1 (Beckman Coulter).

Metabolomics - Liquid chromatography tandem mass spectrometry (LC/MS-MS)

Semi-quantitative targeted metabolomics was performed following a validated approach(26, 105). Kidney tissue was homogenized in 80% (v/v) cooled methanol, incubated for protein precipitation, dried in a SpeedVac concentrator centrifuge (Savant, ThermoFisher), and reconstituted in water/methanol (80:20 v/v). Selected multiple reaction monitoring of 250 metabolites using a positive/negative ion-switching high-performance liquid chromatography-tandem mass spectrometry (5500 QTRAP HPLC–MS/MS21) was used for analysis. MultiQuant (v2.1.1, Sciex) software was used for data processing of the 250 unique metabolites.

Kynurenines were analyzed using a modified protocol(106). Frozen tissue was homogenized in 0.5mL formic acid (10% in water)/methanol (30/70, v/v). The extraction solution was enriched with isotope labeled internal standards and samples were vortexed and centrifuged at 26,000xg for 20min. Supernatant was transferred into HPLC vials with glass inserts. LC-MS/MS was performed on an Agilent Technologies 1200 HPLC system connected to an ABSCIEX 5500 QTRAP mass spectrometer equipped with a turbo ion spray source operated in electrospray mode. LC separation was carried out on an Atlantis T3 3µm (2.1x50mm) column (Waters Corp.) using a mobile phase consisting of 0.1% formic acid in water (Solvent A) and acetonitrile (Solvent B). All analytes were detected in positive ion multiple reaction monitoring mode.

Data analysis was performed using MetaboAnalyst v4.0(107).

Statistical analysis

Data were analyzed using PRISM9 (GraphPad Software). Data are depicted as mean ± SEM or box plot with whiskers of 10-90th percentile; single data points are depicted. Analyses were performed as unpaired
t test or one-way ANOVA with Tukey’s multiple comparison test including ROUT (Q=1%) outlier testing. P-values are denoted by *(P<0.05), **(P<0.01), ***(P<0.001), and ****(P<0.0001).

Study approval

All animal procedures were performed in an AAALAC-accredited facility in accordance with the Guide for the Care and Use of Laboratory Animals(108) and approved by the University of Colorado Anschutz Medical Campus Institutional Animal Care and Use Committee (protocol #33, #685).
Author Contribution

DTN, EKK and KH designed the research study and BYG, MBC, and RAN provide guidance on the study design; DTN, EKK, ND, MLTM, ETC, JK, and KH conducted the experiments and acquired the data; DTN, JK, and KH analyzed the data; RAN, JK, and KH wrote the manuscript; EKK, ND, BYG, MBC and ETC provide feedback on the manuscript. Authorship order among DTN and EKK, co-first authors, was decided based on overall time and intellectual contribution to the study.
Acknowledgements

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The Pkd1^{RC:RC} mouse model was provided by the Mayo Clinic Robert M. and Billie Kelley Pirnie Translational Polycystic Kidney Disease Research Center (NIDDK P30 DK090728, Mayo Clinic Rochester MN, Dr. Peter C. Harris). Human, ADPKD patient, cyst-derived cells were received from the Baltimore Polycystic Kidney Disease Research and Clinical Core Center (NIDDK, P30DK090868, University of Maryland, Baltimore MD, Dr. Terry J. Watnick). The Pax8^{rtTA};TetO-cre;Pkd2^{flx/flx} model was received from the NIH NIDDK PKD Research Resource Consortium (U54 DK126114, University of Maryland, Baltimore MD, Dr. Terry J. Watnick). George S. DeBeck (Nikon Instruments Inc.) aided in the development of the histomorphometric analysis macro for the cystic kidney.
References


Figures and Figure Legends

**Figure 1 | Pkd1RC/RC animals present with overexpression of IDO1.** (A) Western blot probing for IDO1 (left) and quantification (right) using wildtype (WT) and Pkd1RC/RC kidney homogenates, highlighting upregulation of IDO1 in Pkd1RC/RC kidneys compared to WT. (Quantification: N= 3males/3females, 9mo). (B) Western blot probing for IDO1 (left) and quantification (right) of cell lysates obtained from normal renal cortical epithelial cells (RCTE, WT for PKD1) or 9-12 cells (null for PKD1) ± IFNγ stimulation, confirming overexpression of IDO1 in PKD-relevant human cell lines compared to control. IDO1 expression levels were further increased by the cytokine IFNγ, which is known to be upregulated in PKD kidneys (N=4 per condition). (C) Western blot probing for IDO1 levels in epithelial cells obtained from individual cysts of ADPKD patients (each cyst was derived from a different patient, PKD genotype unknown). Most tested cysts show high levels of IDO1; relative to IDO1 levels in RCTE or 9-12 cells (exposure time to detect IDO1 in RCTE or 9-12 cells was insufficient but IDO1 is expressed in these cell lines [Figure 1B]). This provides direct clinical relevance for dysregulation of the tryptophan pathway in ADPKD patient kidneys. (D) Immunofluorescence images probing for IDO1 (red), e-cadherin (ECAD, green, epithelial cells), and DAPI (blue, nuclei). IDO1 is sparsely expressed in wildtype kidneys but upregulated in kidney cystic epithelial cells and interstitial cells of Pkd1RC/RC kidneys. Ido1 knock-out animals served as negative control. *references IDO1 positive interstitial cells in WT or Pkd1RC/RC, Ido1+/+ kidneys, #1 and #2.
references two different \( Pkd1^{RC/RC} \); \( Ido1^{+/+} \) animals. Scale bar = 50µm. Statistics: Graphs, mean ± SEM; Analyses, unpaired t test (A), one-way ANOVA (Kruskal-Wallis \( p=0.0373 \)) with FDR Benjamini and Hochberg multiple comparison (B). P-values *<0.05, comparisons with non-significant statistics are not shown.
Figure 2 | Genetic loss of *Ido1* slows cyst growth and reduces tryptophan catabolite levels in kidneys of an orthologous ADPKD1 model. (A) Kidney H&E cross sections of 3mo and 6mo old *Pkd1*<sup>RC/RC; Ido1<sup>+/+</sup></sup> (white) and *Pkd1*<sup>RC/RC; Ido1<sup>−/−</sup></sup> (yellow) kidneys showing overall decreased cystic disease severity in 6mo old PKD *Ido1* null versus *Ido1* wildtype (WT) animals. Scale bar: 500µm. Quantification of (B) %KW/BW, (C) computed cyst volume (cystic index [Supplemental Figure 2A] multiplied by kidney weight), cyst number, and cyst size in *Pkd1*<sup>RC/RC; Ido1<sup>+/+</sup></sup> (white) and *Pkd1*<sup>RC/RC; Ido1<sup>−/−</sup></sup> (yellow) animals, together providing statistical significance for reduced PKD severity in 6mo old PKD *Ido1* null versus *Ido1* WT animals. Red data point depicts the animal shown in (A). (D) Quantification of significantly altered tryptophan catabolites assayed via mass spectrometry in *Pkd1*<sup>RC/RC; Ido1<sup>+/+</sup></sup> (white) and *Pkd1*<sup>RC/RC; Ido1<sup>−/−</sup></sup> (yellow) kidneys. Loss of *Ido1* partially corrected the observed increased levels of the
immunosuppressive tryptophan catabolites, kynurenine and kynurenic acid, seen in \textit{Pkd1}^{RC/RC} kidneys (Supplemental Figure 2D). N= 5males (diamond), 4-7females (circle) per genotype and time point. Statistics: Graphs: mean ± SEM; Analyses: one-way ANOVA with Tukey’s multiple comparison test. P-value *<0.05, **<0.01, ***<0.001, ****<0.0001, comparisons with non-significant statistics are not shown.
Figure 3 | The kidney immune landscape of $\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{-/-}$ mice favors slowed PKD progression.

$\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{+/+}$ (white), $\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{-/-}$ (yellow); (A) Flow cytometry quantification of CD45+ immune cells within single cell suspensions of $\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{+/+}$ and $\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{-/-}$ kidneys showing an increase of CD45+ cells with PKD progression (3-6mo of age, $\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{+/+}$ mice) and a decrease in PKD $\text{Ido1}$ null versus wildtype (WT) animals at 6mo of age when reduced PKD severity was observed. (B) Representative flow cytometry plot indicating the gating strategy of infiltrating (F4/80lo; CD11b+) versus resident (F4/80hi; CD11b+) macrophages (left), and quantification (right), highlighting a significant increase of macrophages as disease progresses from 3-6mo of age in $\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{+/+}$ mice and a decrease at 6mo of age in $\text{Pkd1}^{\text{RC/RC}};\ Id\text{o1}^{-/-}$ mice versus WT. (C) Flow cytometry data quantification of all T cells (TCRβ+) and CD4+ or CD8+ subpopulations. The numbers of CD4+ or CD8+ cells in kidneys did not change significantly upon $\text{Ido1}$ loss. (D) Quantification of CD4+ and CD8+ T cell numbers as %TCRβ+.
cells, showing a shift in distribution of T cell subpopulations with an increase in CD8+ T cell numbers upon Ido1 loss and reduced PKD severity (6mo of age). (E) Representative flow cytometry plot (left) and quantification (right) of immune checkpoint ligand PD-L1 expression on kidney epithelial cells (plot and quantification, EpCAM+) and macrophages (quantification only, F4/80+) indicating reduced expression in 6mo old Pkd1^{RC/RC}; Ido1^{-/-} animals versus control (Pkd1^{RC/RC}; Ido1^{+/-}). (F) Quantification of immune checkpoint receptor PD-1 expression on CD8+ T cells showing a decrease in expression in 6mo old Pkd1^{RC/RC}; Ido1^{-/-} animals versus control (Pkd1^{RC/RC}; Ido1^{+/-}). Statistics: Graphs: box plot, whiskers 10-90th percentile; Analyses: one-way ANOVA with Tukey’s multiple comparison test. P-value *<0.05, **<0.01, ***<0.001, ****<0.0001, comparisons with non-significant statistics are not shown. N= 5males, 4-7females per genotype/time point.
Figure 4 | Treatment with a tryptophan analog shows therapeutic efficacy for halting slowly progressive ADPKD and is associated with changes in the immune microenvironment. Results obtained from Pkd1<sup>RC/RC</sup> mice treated with (blue) or without (white) 1-MT. (A) H&E cross sections. Quantification of (B) %KW/BW, (C) cystic volume (cystic index multiplied by KW), cyst number, and cyst size. (cystic index, fibrotic volume/index, and BUN can be found in Supplemental Figure 5) Pkd1<sup>RC/RC</sup>
mice treated with 1-MT show significantly reduced PKD severity compared to control (Cntrl.) (D) Quantification of significantly altered tryptophan catabolites assayed via mass spectrometry. 1-MT treatment significantly reduced levels of the immunosuppressive metabolite kynurenic acid. (E-F) Quantification of flow cytometry data of the 1-MT intervention experiment. 1-MT treated animals have (E) reduced numbers in overall immune cells (CD45⁺), and resident macrophages (F4/80^hi; CD11b⁺), but not infiltrating macrophages (F4/80^lo; CD11b⁺), and (F) increased numbers of CD8⁺ T cells as percent of all T cells (TCRβ⁺). (G) Expression of PD-L1 on macrophages (F4/80⁺) and numbers of T_{Reg} (CD4⁺/FoxP3⁺) are reduced, both suggesting a less immunosuppressed cystic microenvironment. Scale bars: 500µm, Treatment (1-MT): 4-7 weeks of age, N=3 males (diamond), 3 females (circle)/group. Statistics: Graphs: (B-D) mean ± SEM, (E-G) box plot, whiskers 10-90th percentile; Analyses: unpaired t test. P-value *<0.05, **<0.01, ***<0.001, ****<0.0001, comparisons with non-significant statistics are not shown.
Figure 5 | 1-MT treatment slows PKD progression in a rapidly progressive, inducible PKD2 model.

Results obtained from Pax8rtTA;TetO-cre;Pkd2flx/flx mice treated with (purple) or without (white) 1-MT. (A) H&E cross sections. Quantification of (B) %KW/BW, (C) cystic volume (cystic index multiplied by KW), cyst number, and cyst size, and (D) fibrotic volume (cystic index, fibrotic index, and BUN can be found in Supplemental Figure 6). Pax8rtTA;TetO-cre;Pkd2flx/flx mice treated with 1-MT show significantly reduced PKD severity compared to control (Cntrl.). (E) Quantification of significantly altered tryptophan catabolites assayed via mass spectrometry. 1-MT treatment results in a trend towards reduced levels of the immunosuppressive metabolite kynurenic acid. Scale bars: 1mm, Treatment (1-MT): P12-21, N=5-6males (diamond), 4-5females (circle). Control: N=3-5males (diamond), 4females (circle). Statistics: mean ± SEM; Analyses: unpaired t test. P-value *<0.05, **<0.01, comparisons with non-significant statistics are not shown.
Table 1 | Levels of tryptophan metabolites in Pkd1<sup>RC/RC</sup> kidneys.

<table>
<thead>
<tr>
<th>Metabolite (Abundance/mg tissue [normalized])</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
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<tr>
<td><strong>Tryptophan</strong></td>
<td>2.23x10&lt;sup&gt;2&lt;/sup&gt;ʱ0.816x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>2.36x10&lt;sup&gt;2&lt;/sup&gt;ʱ0.759x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>1.94x10&lt;sup&gt;2&lt;/sup&gt;ʱ0.629x10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<tr>
<td><strong>Kynurenine</strong></td>
<td>1.96x10&lt;sup&gt;-4&lt;/sup&gt;ʱ0.804x10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>2.95x10&lt;sup&gt;-4&lt;/sup&gt;ʱ1.69x10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>2.87x10&lt;sup&gt;-4&lt;/sup&gt;ʱ2.43x10&lt;sup&gt;-4&lt;/sup&gt;</td>
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<tr>
<td><strong>Kynurenic acid</strong></td>
<td>1.10x10&lt;sup&gt;-2&lt;/sup&gt;ʱ0.476x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>0.735x10&lt;sup&gt;-2&lt;/sup&gt;ʱ0.291x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>0.688x10&lt;sup&gt;-2&lt;/sup&gt;ʱ0.371x10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<tr>
<td><strong>Xanthurenic acid</strong></td>
<td>2.92x10&lt;sup&gt;-2&lt;/sup&gt;ʱ0.766x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>4.17x10&lt;sup&gt;-2&lt;/sup&gt;ʱ3.42x10&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>1.59x10&lt;sup&gt;-2&lt;/sup&gt;ʱ0.600x10&lt;sup&gt;-2&lt;/sup&gt;</td>
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<tr>
<td><strong>Picolinic acid</strong></td>
<td>6.78x10&lt;sup&gt;-3&lt;/sup&gt;ʱ2.11x10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>6.07x10&lt;sup&gt;-3&lt;/sup&gt;ʱ1.20x10&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>7.58x10&lt;sup&gt;-3&lt;/sup&gt;ʱ2.17x10&lt;sup&gt;-3&lt;/sup&gt;</td>
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<tr>
<td><strong>Nicotinamide</strong></td>
<td>3.86±0.99</td>
<td>3.4±1.02</td>
<td>3.6±1.20</td>
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**Fold change**

<table>
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<th>6 months</th>
<th>9 months</th>
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<tr>
<td><strong>WT</strong></td>
<td>0.789</td>
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<tr>
<td><strong>Pkd1&lt;sup&gt;RC/RC&lt;/sup&gt;</strong></td>
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<td><strong>Fold change</strong></td>
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<td><strong>WT</strong></td>
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<tr>
<td><strong>Pkd1&lt;sup&gt;RC/RC&lt;/sup&gt;</strong></td>
<td>0.693</td>
<td>0.532</td>
</tr>
<tr>
<td><strong>Fold change</strong></td>
<td>0.720</td>
<td>0.598</td>
</tr>
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Mean ± SD, N = 5 males, 5 females per genotype and time point; Fold change: Pkd1<sup>RC/RC</sup> versus WT; ANOVA & Tukey’s multiple comparison, P-values: Pkd1<sup>RC/RC</sup> versus wildtype *<0.05, **<0.01, ***<0.001, ****<0.0001; 3-months Pkd1<sup>RC/RC</sup> versus 9-months Pkd1<sup>RC/RC</sup> #<0.05. Normalized abundance/mg tissue: Raw abundance peak area of each metabolite normalized to (i) peak area of 12 deuterated internal standards, (ii) peak area of all metabolites across a individual sample, (iii) individual tissue weight.