Impact of maternal environment on PKD

Impact of prenatal and postnatal maternal environment on nephron endowment, renal function and blood pressure in the Lewis polycystic kidney rat

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Short title: Impact of maternal environment on PKD
Impact of maternal environment on PKD

Abstract

Maternal insufficiency during fetal development can have long lasting effects on the offspring, most notably on nephron endowment. In polycystic kidney disease (PKD), variability in severity of disease is observed and maternal environment may be a modifying factor. In this study, we firstly established that in a rodent model of PKD, the Lewis Polycystic Kidney (LPK) rat, nephron numbers are 25% lower compared to wildtype animals. We then investigated the effects of prenatal and postnatal maternal environment on phenotype and nephron number. LPK pups born from and raised by homozygous LPK dams (control) were compared with LPK pups cross-fostered onto heterozygous LPK dams to improve postnatal environment, LPK pups from and raised by heterozygous LPK dams to improve both prenatal and postnatal environment and LPK pups from and raised by Wistar Kyoto-LPK heterozygous dams to improve both prenatal and postnatal environment on a different genetic background. Improvement in both prenatal and postnatal environment improved postnatal growth, renal function and reduced blood pressure, most notably in animals on the different genetic background. Animals with improved postnatal environment only showed improved growth and blood pressure, but to a lesser extent. All intervention groups showed increased nephron number compared to control LPK. In summary, prenatal and postnatal environment had significant effect in delaying progression and reducing severity of PKD, including nephron endowment.
Impact of maternal environment on PKD

**Keywords:** Polycystic kidney disease; maternal environment; nephron endowment; blood pressure; body weight.

**Introduction**

The link between developmental programming and disease in adult life was initially established in a series of landmark epidemiological studies that highlighted low birth weight contributing to risk of disease in adulthood, including hypertension, kidney disease and diabetes (Barker, 2007; Gluckman, 2008; Hoy, 2005) and it is now well established that changes in maternal environment during the critical prenatal and postnatal developmental periods can have long lasting effects. The kidneys are particularly prone to perturbations during development and insults are reflected by glomerulomegaly and a decrease in nephron endowment in the offspring (Dorey, 2014; Hoy, 2010). Further, nephron endowment or nephron number is permanent, such that no new nephrons can be formed after nephrogenesis (Bertram, 2014). In humans, the 34-week gestation point marks the completion of nephrogenesis, and thus assessing nephron number provides an insight into the overall success of fetal and kidney development *in utero*. In contrast, nephron development in rodent continues until around postnatal days 7-10 (Dickinson, 2005), and as such, both prenatal and postnatal environment can affect nephron number, renal function, and the development of hypertension in adult life (Singh, 2011; Singh, 2012; Wlodek, 2007).
Impact of maternal environment on PKD

Polycystic kidney disease (PKD) and nephronophthisis (NPHP) are characterised by the development cystic kidneys, ultimately progressing to end stage renal disease (ESRD) [Grantham, 2008 #4975; Chapman, 2015 #4976]. In addition to renal decline, manifested in decreased glomerular filtration rate and proteinuria, extra-renal manifestations such as hypertension, may also be present. In monogenic conditions such as PKD and NPHP, significant clinical heterogeneity and inter/intra familial variability is observed [Otto, 2008 #3065]. For instance, in a study of a family spanning over 3 generations, affected members exhibited large differences in severity and disease manifestations, such as age of onset of ESRD, despite having the same mutation in PKD1 [Perrichot, 2001 #4979]. As such, disease severity and prognosis are not dictated by the mutation alone [Devuyst, 2003 #4980]. Fetal developmental programing may be a factor that influences disease severity in PKD [Barker, 1992 #4981; Dipple, 2000 #4982], with a Danish study showing low birth weight was associated with an earlier onset of ESRD in PKD patients [Orskov, 2012 #4985]. If maternal environment is an important factor in the ultimate expression of PKD phenotype, it will have an important implication in genetic counselling and clinical treatment of PKD patients.

Based on the above, we hypothesize that in a rodent model of cystic kidney disease, animals will show a reduced nephron number compared to wildtype controls. We further predict that an improvement in the prenatal and/or postnatal environment will confer overall healthier offspring, with improvements in rates of body weight
Impact of maternal environment on PKD

gain, clinical phenotypes such as renal function and blood pressure, cystic parameters and importantly nephron number. To examine our hypothesis, we have used the Lewis Polycystic Kidney (LPK) rat, which is a rodent model of NPHP featuring a spontaneous missense mutation in the Nek8 gene (McCooke, 2012 #3482), with phenotypic similarities to the juvenile cystic kidney (jck) mouse model of PKD (Liu, 2002 #5068). The disease progression of LPK has been studied extensively in our previous work where we show hypertension developing from 6 weeks of age and renal failure at 12 weeks of age (Phillips, 2007 #2456). We firstly sought to determine if there was a deficit in nephron number in homozygous LPK rats compared to Lewis wildtype controls. We then compared LPK rats raised under different prenatal and postnatal environmental conditions, taking advantage of heterozygous LPK dams who are clinically normal and the technique of cross-fostering pups. An additional group of LPK rats bred and raised on a non-Lewis background (Wistar Kyoto WKY) with heterozygous mothers were used to investigate improved prenatal and postnatal environment on a distinct genetic background.

Methods

Animals

The LPK animals used for this study arose as an autosomal recessive mutation in Nek8 from a colony of wildtype Lewis (+/+) strain (LEW/SsNArC) rodents at the Animal Resource Centre, Perth WA, Australia (McCooke, 2012 #3482). All males used for LPK breeding were homozygous for the LPK mutation (−/−). Homozygous female
Impact of maternal environment on PKD

LPK and heterozygous female LPK (LPK +/-) females were used for breeding and cross fostering, and an additional control heterozygous maternal line (WKY-LPK +/-) was established using WKY/NCrlArc dams crossed to LPK -/- males.

The animals were housed in cages in room with controlled temperature at approximately 23°C and 12-hour light/dark cycle. All animals were provided with food and water ad libitum.

Experimental design

Study One: Lewis born and raised by their biological dams (wildtype Lewis, n = 4 dams) and LPK born and raised by their biological homozygous LPK dams (n = 4 dams) were used for stereological estimation of glomerular number. A total of 5 pups (all male) from each strain were used. Animals were weaned at age 3 weeks. At age 12 weeks, animals were euthanased (isofluorane 5% in 100% oxygen and then decapitated) after which the kidneys were removed and fixed with 4% paraformaldehyde for 24 hours. They were then stored in 70% ethanol until manual paraffin embedding under standard histological conditions and processing as detailed below.

Study Two: The following four groups of LPK offspring were established (Table 1):
Impact of maternal environment on PKD

(i) Pups born from and raised by their biological LPK dams, being animals with a poor prenatal and postnatal phenotype due to the full PKD phenotype and renal disease in the dams (control),

(ii) Pups born from LPK dams cross-fostered as a whole litter onto heterozygous LPK dams, thereby providing improved postnatal environment (LPK:Pn),

(iii) Pups born from and raised by their biological heterozygous LPK dams, thereby providing improved prenatal and postnatal environment (LPK:Pr/Pn),

(iv) Pups born from and raised by their biological heterozygous WKY-LPK dams, being an alternative group testing for improved prenatal and postnatal environment on a distinct genetic background [Consortium, 2008 #4990] (WKY-LPK:Pr/Pn).

In the cross-fostered group (LPK:Pn) offspring were cross-fostered 1 day after birth, while in all other groups pups remained with the biological dam. All animals were weaned at age 3 weeks. At age 5 weeks, animals were tested for the homozygous LPK mutation by palpation for the presence of enlarged kidneys. Only homozygous offspring were used for the study and were selected from each litter at random to achieve a minimum of n = 6 males and 6 females for each group before being transported from the breeding facility to the research institution, where they were housed in standard housing with environmental enrichment.

Experimental procedures
Impact of maternal environment on PKD

Between the ages of 6 to 16 weeks, pups from each group were tracked weekly for rate of growth (body weight measurement) and fortnightly for renal function (urine analysis) and hypertension [systolic blood pressure (SBP)]. Animals were allowed to age out to 16 weeks of age to allow full phenotypic discrimination of the effect prenatal and postnatal maternal environment.

**Urine analysis**

Animals were placed into individual metabolic cages for up to 4 hours to allow adequate collection of urine. Urine samples were analysed for urinary protein: creatinine ratio (UPC) using automated IDEXX VetTest Chemistry Analyser, an automated processor using enzymatic-based dry-slide technology (IDEXX Laboratories, ME, USA). When protein was below the levels of the machine sensitivity (< 0.05 mmol/L), UPC was recorded as 0.

**Systolic blood pressure**

Tail cuff plethysmography was used to determine SBP (IITC Life Science, CA, USA). Animals were acclimatised to restraint and the recording protocol for one week prior to the first measurement. Animals were placed in a restrainer within a preheated chamber at 32 °C and an automatic inflatable cuff placed around the tail. Using photoelectric sensors within the automatic inflatable cuff, real-time systolic readings were recorded using IITC software (IITC Life Science). The average of at least three measurements was taken to determine SBP measurement.
Impact of maternal environment on PKD

Blood and tissue collection

At study endpoint (16 weeks), final body weight was determined immediately prior to sacrifice. Half of the animals from each group were euthanized with pentobarbitone sodium (60mg/kg i.p.) (Virbac, NSW, Australia) for collection of tissue for estimation of glomerular number and determination of cystic parameters. Blood was collected via cardiac puncture into lithium heparin and the animal was then perfused through the base of the left ventricle with 200 ml 0.9% w/v saline followed by same volume of in 4% paraformaldehyde (PFA) (Sigma Aldrich, MO, USA) diluted in dissolved in 0.01M phosphate buffered saline (PBS) (pH 4.2) at room temperature. The kidneys were post-fixed in 4% PFA overnight at 4 °C and then transferred to 70% ethanol until further analysis. The remaining animals from each group were euthanized with 5% isofluorane in 100% oxygen and decapitated for trunk blood collection in BD Vacutainer® PST™ II tubes with lithium heparin and the kidneys, heart and left ventricle were dissected and relative weights determined. For this reason, relative body weight data is from half the total number of animals used in the study overall.

Blood samples were centrifuged at 2000 rpm for 5 min and the supernatant collected for determination of plasma creatinine, urea, albumin, total protein, and globulin using an IDEXX VetTest Chemistry Analyser (IDEXX Laboratories).

Estimation of glomerular number and cystic index
Impact of maternal environment on PKD

The right kidney was processed through ascending concentrations of ethanol and then Histochoice (Amesco, OH, USA) before final embedding in paraffin (Leica, Wetzlar, Germany). The entire kidney was then transversely sectioned at 10 µm, and based on the total number of sections, 10 pairs of sections dispersed evenly throughout the kidney were systemically selected for estimation of nephron number as previously described using sections stained with lectin peanut agglutinin (PNA) [Cullen-McEwen, 2011 #4368]. Images of the kidney were acquired at 5x magnification (Zeiss, Oberkochen, Germany) and from the selected 10 section pairs, appearing and disappearing PNA-positive glomeruli were marked by superimposing consecutive images. The sum of appearing and disappearing PNA-positive glomeruli of all 10 pairs was used to estimate the total nephron number using the equation:

\[ N_{\text{glom}} = \text{SSF} \times \frac{1}{2} \times \frac{1}{2} \times Q \]

where \( N_{\text{glom}} \) is the estimated total nephron number, SSF is the reciprocal of the total sections per kidney (the number of sections between each section pairs) and \( Q \) is the sum of all PNA-positive glomeruli appearing and disappearing between the reference and lookup section.

To determine cystic index, imaged sections (4 per animal) were analysed using Fiji [Schindelin, 2012 #4016]. Images were thresholded and the ‘analyse particles’ function used to measures cyst to kidney area ratio and the average size of each cyst (mm²). The calyx area was excluded from analysis.
Impact of maternal environment on PKD

Statistical analysis

Results are expressed as mean ± SEM and statistical significance was set at P ≤ 0.05. For study one, an unpaired t-test was used to compare Lewis and LPK. For study two, preliminary general linear model (GLM) analysis of variance was first performed on the whole data set (all groups, both sexes) to identify potential sex or group effects, including their interaction, and data was also tested for assumption of equal variances (Levene Statistic). If a sex effect or interaction was identified upon preliminary analysis, the data was the analysed separately for each sex otherwise the data was pooled. For single point variables, a One-way analysis of variance (ANOVA) was then performed based on intervention groups, followed by Tukey post-hoc analysis if indicated. Data that displayed unequal variance were analysed using Welch ANOVA, followed by Games-Howell post-hoc analysis. Longitudinal data (body weight, SBP and UPC) was analysed using two-way ANOVA, testing for intervention group and age effects followed by Tukey post-hoc analysis as indicated. Linear regression modelling to test for any relationship between bodyweight and renal function was undertaken with the plasma urea or creatinine as the dependent variable and bodyweight at 6 and 16 weeks as the independent variables, using a stepwise selection method of entry and listwise exclusion of missing values, with male and female data analysed separately due to the established sex effect on bodyweight. Adjusted R² value and beta standardized regression coefficients (beta: β) values are provided as an indicator of the relative influence of the body weight
Impact of maternal environment on PKD parameter. Pearson correlation coefficients were derived from the model, using a one-tailed test for significance ($p \leq 0.05$).

Data was analysed using IBM Statistical Package for the Social Sciences (v22, SPSS; Chicago, Illinois USA) and GraphPad Prism software (version 6, La Jolla, CA, USA).

**Results**

*Study one*

In our previous work, we have detailed the marked difference in phenotype between the Lewis wildtype and LPK animals. [Phillips, 2007 #2456]. A representative image of the LPK cystic renal phenotype is provided in Figure 1. Nephron numbers were significantly lower in the LPK compared to Lewis controls, corresponding to an approximately 25% reduction in nephron endowment (Lewis 18564 ± 980 vs. LPK: 14095 ±453, $p < 0.05$).

*Study two*

**Body weight**

Weekly body weights for the different groups across the study period (6 – 16 weeks) is illustrated in Figure 2. Sex differences were present and there were significant age and group effects (all $p < 0.001$). Over all ages, both male and female control LPK weighed less than all other groups. In the male LPK rats, this difference in body weight was statistically significant compared to all other groups at 14, 15 and
Impact of maternal environment on PKD

16 weeks, while at age 6 weeks their body weight was significantly less than that of the WKY-LPK:Pr/Pn group. The female LPK weighed significantly less than all other groups at 6 and 15 weeks of age. Over all ages, male and female LPK:Pn also were of lower body weight than the WKY-LPK:Pr/Pn and LPK:Pr/Pn groups though this was not statistically significant at any specific age.

**SBP**

Multivariate GLM analysis of overall SBP indicated significant age and group effects (p ≤ 0.001) but no sex effect (p = 0.206). The combined data set is illustrated in Figure 3. Blood pressure increased with age in all groups and was overall significantly greater in the LPK animals compared to the animals with improved prenatal and postnatal environments (LPK: Pr/Pn and WKY-LPK:Pr/Pn). This was most evident at age 16 weeks, where the LPK animals had significantly higher blood pressure than all other groups including pups that had been cross-fostered to improve the postnatal environment only (LPK:Pn).

**Urine**

Fortnightly UPC ratio for the different groups across the study period is illustrated in in Figure 4. A significant sex and age effect (both p < 0.05) was present. In males and females there was a significant increase in UPC in all groups as the animals aged, consistent with a decline in renal function. In the males there was also a group effect (p < 0.001), with UPC being greater in the LPK vs. WKY-LPK:Pr/Pn
Impact of maternal environment on PKD

across all age data combined, and the LPK:Pn showing higher UPC than both the
LPK: Pr/Pn and WKY-LPK: Pr/Pn groups across all age data combined. This was
most evident at age 12 weeks when the UPC in the male LPK:Pn was greater than all
other groups.

Organ weights at post-mortem

Kidney to body weight ratio

A sex/group interaction was present (p = 0.003) when comparing KW/BW ratio.
In the males, normalised kidney weight was not significantly different between
groups however in the females, KW/BW ratio was significantly less in WKY-LPK:
Pr/Pn group (p < 0.001), indicative of reduced kidney enlargement in these animals
(Figure 5A).

Heart to body weight ratio

Heart weight data normalised to body weight was analysed to determine the
extent of cardiac hypertrophy within experimental groups. Analysis identified both a
sex and group effect (p = 0.006 and p < 0.001, respectively). In the male animals, the
HW/BW ratio in the WKY-LPK:Pr/Pn was significantly less compared to all other
groups (p < 0.05; Figure 5B). In female animals, HW/BW ratio in the WKY-LPK:Pr/Pn
was significantly less compared to the control LPK group (Figure 5B). In order to
determine if the observed cardiac hypertrophy was related to left ventricular
hypertrophy, left ventricular weight was assessed normalised against body weight.
Impact of maternal environment on PKD

There was no sex effect between groups (p=0.066), however, consistent with the heart weight data, there was a reduced ratio of LV/BW in the WKY-LPK:Pr/Pn animals compared to all other groups (p < 0.05; LPK: 0.42 ± 0.02, LPK:Pn: 0.38 ± 0.01, LPK:Pr/Pn: 0.40 ± 0.01, WKY-LPK:Pr/Pn: 0.32 ± 0.00. n = 3 male/ 3 females per group except for the LPK:Pn female group where n = 5).

Plasma biochemistry

Group data summarising plasma biochemistry results is presented in Table 2. Assessment of plasma total protein and globulin did not yield any sex differences or group differences amongst the four groups (all p>0.05). Plasma albumin demonstrated a sex effect but no group effect, with albumin being less in male than female animals across all groups (25 ± 0.6 vs. 27.7 ± 0.7 g/L, p = 0.005). Plasma urea levels showed a significant difference between groups but no sex effect (p = 0.087) and thus data were pooled, with levels being significantly less in WKY-LPK:Pr/Pn animals compared to the LPK and the LPK:Pn groups. Urea was significantly less in the LPK:Pr/Pn compared to the LPK:Pn (both p < 0.05). Plasma creatinine demonstrated both a sex and group effect (both p < 0.001). Overall, creatinine levels were significantly more in male animals compared to female animals (118.8 ± 7.5 vs. 86.1 ± 5.4 µmol/L, p = 0.001). In the male animals, the WKY-LPK:Pr/Pn animals demonstrated significantly lower levels compared to both the control LPK and
Impact of maternal environment on PKD

LPK:Pn groups however there was no significant difference between groups in the female cohort.

While there was a significant negative relationship between body weight and both urea and creatinine at 6 and 16 weeks, in the regression model, body weight at 16 weeks was the only significant predictor variable for either urea or creatinine in male and female animals. Regression outputs are provided in Table 3 and individual data points are illustrated in Figure 6.

Cystic index and estimation of nephron number

Renal morphology in all groups showed large kidneys with cystic lesions distributed throughout (Figure 7). Preliminary GLM analysis of cystic index and cyst area both demonstrated a group effect (p=<0.001) but no sex effect (p=0.53 and 0.839 respectively. Post-hoc analysis indicated that both cystic index and cyst area were significantly less in the WKY-LPK:Pr/Pn compared to all other groups (p<0.05, Table 4).

Preliminary analysis of estimated nephron number indicated a group effect (p=0.001) but no sex effect (p = 0.417). Post-hoc analysis indicated estimated nephron numbers were significantly lower in the control LPK compared to all other groups, corresponding to an approximately 27% reduction in nephron number (Figure 7).

Discussion
Impact of maternal environment on PKD

The development of cystic kidneys, as seen in PKD and NPHP, eventuates in the progressive loss of renal function and is accompanied by extra-renal manifestations such as hypertension [Chapman, 2015 #4976]. There is considerable phenotypic variation in the presentation of PKD, consistent with the influence of environment and other modifying factors influencing disease outcomes [Edmunds, 2001 #4993]. A number of diseases including renal, cardiovascular and metabolic disease can be ‘programmed’ in utero [Gluckman, 2008 #4971;Hales, 2003 #4994], with insult during either prenatal and/or postnatal development inducing permanent damage that can influence future disease onset, severity and progression [Eriksson, 1999 #4995]. In the present study, we firstly determined that LPK rats had a significant nephron deficit compared to their wildtype Lewis counterparts. We then investigated the impact of improved prenatal and/or postnatal environment on nephron endowment and associated disease severity and progression in the rodent model. The impact of an improved postnatal environment was assessed by the cross fostering of pups from homozygous LPK dams to healthy heterozygous LPK dams, while the impact of improved prenatal and postnatal environment was assessed by the inclusion of pups bred from and raised by heterozygous LPK dams. In addition, we included an additional group of pups bred from heterozygous WKY-LPK dams, assessing the impact of a healthy prenatal and postnatal environment on a different genetic background.
Impact of maternal environment on PKD

Rats from a healthy prenatal and postnatal environment showed a significant improvement in body weight gain, blood pressure and renal function indices. Rats which were cross-fostered, and therefore given an improved postnatal environment, also showed an improvement in phenotype, though not as marked as the animals born and raised by under conditions of improved prenatal and postnatal environment. All the intervention groups showed a significant increase in nephron number compared to the control LPK. Overall, we observed a change from least to most disease affected phenotype with WKY-LPK:Pr/Pn ≥ LPK:Pr/Pn > LPK:Pn >> control LPK. An important consideration in the interpretation of this data is that only one group (LPK:Pn) were cross-fostered, which in itself is a postnatal environmental manipulation, that may induce physiological and epigenetic alterations (McCarty, 2017 #5122). Despite this confounder however, our results strongly support the hypothesis that an improved prenatal and/or postnatal environment is able to prevent a nephron deficit at 16 weeks of age with associated reductions in disease severity in the LPK model of PKD.

While cystic expansion is inevitable in PKD, anything that increases the risk of renal disease is of particular clinical relevance. A low birth weight has been linked to increased risk of CKD. This is likely to be due in part to the fact low birth weight is strongly associated with a reduction in nephron endowment (Hoy, 2005 #4972;Luyckx, 2005 #4998). Whilst we were not able to collect birth or weaning weight in our study we did measure nephron endowment and this was reduced in
Impact of maternal environment on PKD

the LPK control animals compared to wild type Lewis. Having established that the LPK exhibit reduced nephron number, a key outcome of this study was therefore determination of the effect of improved prenatal and postnatal maternal environment on nephron number in the LPK genetic model of PKD. Various studies have shown the kidney as being most vulnerable to a suboptimal environment during development in utero [Moritz, 2008 #5003]. Perturbations during fetal development are reflected through a reduction in kidney weight or more importantly, nephron number. Such congenital nephron loss causes the remaining nephrons to undergo hypertrophy to maintain adequate glomerular filtration. Such changes can predispose the kidney to glomerulosclerosis and proteinuria, perpetuating as kidney disease in adult life [Hostetter, 1981 #5123; Simons, 1993 #5124]. Risk of hypertension and kidney disease increases with time [Luyckx, 2013 #5004]. In the case of PKD, at the time of pregnancy, LPK dams are hypertensive, have renal dysfunction and are anaemic [Phillips, 2007 #2456]. Their disease state will affect the fetal oxygen supply, negatively impact placental growth, and the likely increase in stress hormones will also impact fetal development [Fowden, 2006 #5125; Graignic-Philippe, 2014 #5126]. Further studies to elucidate the cause and mechanism of intrauterine impairment will further our understanding of nephrogenesis and phenotypic variability observed in PKD patients. Given that nephron number can be normalised by improving postnatal environment in rodent models of maternal environment insufficiency [Wlodek, 2007 #3471], we
Impact of maternal environment on PKD

hypothesized that an improved either prenatal and/or postnatal environment could recover nephron number in the PKD model. Notably, our results demonstrated that a healthy postnatal environment did in fact have such an effect, with the animals with improved postnatal environment having significantly higher nephron counts than the control LPK animals. Clinically, this highlights the importance of maternal environmental conditions for humans at risk of PKD, as nephron endowment is set by 34 weeks’ gestation, and ensuring the health of the mother during the late gestation period could translate to delaying the onset or slow the progression of PKD in any children that inherit the genetic disorder.

With specific reference to body weight and postnatal growth, the body weight of male and female control LPK over the study period was significantly less than the other groups, and this was evident at the study start age of 6 weeks. Results from the cross-fostered animals indicated that an improvement in the postnatal environment was able to partially reverse this deficit in body weight, however the greatest gain was seen in those animals who had a healthy prenatal and postnatal environment. In addition, we observed that across the cohorts as a whole, lower body weight by study end point was correlated with increased both plasma urea and creatinine. While this relationship is an association only, it does provide insight into the relationship between body weight and the degree of severity of CKD. At the age used for breeding in this study (12-14 weeks), homozygous LPK dams are already exhibiting signs of renal disease (Phillips 2007), and will have a compromised uterine
Impact of maternal environment on PKD and lactation environment for the pups, and therefore compromising fetal development. The kidneys are particularly vulnerable to perturbations during fetal development, and this is in turn associated with an increased risk of renal disease in adult life [Vikse, 2008 #4997; Luyckx, 2005 #4998]. Indeed, control LPK and cross-fostered animals exhibited reduced renal function when considering the UPC results and plasma urea and creatinine in comparison to those animals which had improved prenatal and postnatal environments.

Whilst development of hypertension has important implications in the normal population, it is of special clinical interest in PKD where the majority of PKD patients develop hypertension and early establishment of hypertension is associated with poor prognosis [Schrier, 2009 #3959]. In our study, a poor prenatal and postnatal environment was associated with the most severe hypertensive phenotype, noting that tail-cuff plethysmography was used to determine blood pressure in this study, and further changes may have been noted between the intervention groups if more direct methodology such as telemetry had been used to determine this parameter [Van Vliet, #5120]. Our results also indicated a reduction in both heart and left ventricular weight to body weight ratio in the WKY-LPK:Pr/Pn group. The explanation for this effect could be two-fold. It may be a primary outcome, associated with the improved prenatal and postnatal developmental conditions, with comparable results evident in human studies where poor fetal development is proportionally correlated with cardiac dysfunction [Crispi, 2008 #4999]. It may also
Impact of maternal environment on PKD

be a secondary effect, associated with the lower blood pressure in the WKY-LPK:Pr/Pn group, given the critical and well established impact of hypertension as a maladaptive response of the left ventricular mass to cardiac workload in hypertension [Tomek, 2017 #5128]. Future studies would be required to delineate the relative impact of these different contributors.

In PKD, the expansion of cysts is correlated with loss of renal parenchyma and renal function[Grantham, 2006 #3049] and larger kidney size has been positively correlated with a more rapid progression to end-stage renal failure[Halvorson, 2010 #5127]. When assessing kidney mass, a decrease in kidney to body weight ratio was evident in the female animals in the WKY-LPK:Pr/Pn cohort, while an improvement in both cystic index and cyst area was seen in this group as a whole, indicating an improvement in the renal structural phenotype in association with improved prenatal and postnatal conditions specifically on the different genetic background. That the improvement in cystic index was not reflected in kidney mass in the male WKY-LPK:Pr/Pn may be due to males having a more severe disease phenotype that is less responsive to intervention than their female counterparts, as supported by our plasma creatinine data from this and our previous studies[Phillips, 2007 #2456] and consistent with results from human studies and other animal models of PKD [Gretz, 1995 #1466;Silbiger, 2003 #4996]. This may be due to PKD associated factors or be an inherent feature of female rats, which have recently been shown to have a distinct pattern of transporters along the nephron that confers a protective phenotype.
Impact of maternal environment on PKD including resistance to ischaemia and oxidative stress [Veiras, 2017 #5036], features we have previously reported in the LPK rat model [Ding, 2012 #3334; Ow, 2014 #4086] animals. We must be careful with the interpretation of sex effects arising from this study however, as relatively small number of animals were in each treatment group/sex cohort. We do believe this was countered by our undertaking of a preliminary GLM analysis of variance to identify group or sex effects, including their interaction, using the power of the whole cohort of animals prior to subsequent ANOVA and post-hoc analysis. However, future studies will require additional numbers to extend the results currently presented.

We further found that renal function, as assessed by UPC ratio, declined at a slower rate in the WKY-LPK:Pr/Pn with a similar pattern seen in the LPK:Pr/Pn. There was some variability however in the UPC data, and this may have been associated with urine only being collected for a short time frame (equivalent to a spot collection) rather than over a 24hr period. This is a limitation of the study and further, precluded any final estimation of creatinine clearance as a further indicator of renal function. At study endpoint, however, both serum urea and creatinine, further indicators of renal function, were elevated in control LPK group and LPK:Pn compared to offspring with improve prenatal and postnatal environment. Overall, these results indicate that similar to the changes we saw in blood pressure, an improvement in postnatal environment alone was not sufficient to improve renal function.
Impact of maternal environment on PKD

Notably in this study, the pups from the WKY-LPK heterozygous dams showed overall the best phenotype including a notable reduction in cystic index. This suggests that the different genetic background, being the WKY strain, conferred an additional protective effect. The WKY and Lewis rat strains are phylogenetically quite distinct [Canzian, 1997 #5000] and as such, differing genes may have interacted with to modulate disease phenotype [Consortium, 2008 #4990;Mrug, 2015 #5001]. Genetics and the impact of modifier genes is an important consideration in determining disease severity and prognosis. In PKD, modifier genes may impact age of onset of ESRD, presentation of extra-renal manifestation such as cardiovascular disease, liver cyst, retinal degeneration, and brain malformation [Harris, 2009 #3061;Singh, 2015 #5002]. In a recessive mouse model of PKD for example, Kif12 has been identified as a modifier gene, accelerating disease effects such as increased kidney to body weight ratio and cyst size [Mrug, 2015 #5001]. The challenge with identifying genetic modifiers is their non-pathogenic nature under normal conditions, thereby rendering them difficult to isolate. In our present study, we did not perform genetic linkage analysis to identify genetic modifiers did not isolate the NEK8 mutation onto the WKY background. An additional factor that must be considered in the interpretation of this result, and that of the other intervention groups is that a limited number of dams were used to generate the offspring. Additional breeding and intensive genetic linkage studies will therefore need to be carried out to identify genetic factors contributing to phenotypic disease variability.
Impact of maternal environment on PKD

In summary, we have shown for the first time that PKD is associated with a decrease in nephron number and that improvement in prenatal and postnatal environment positively influences disease phenotype in a rodent model of PKD. Whilst improvement in either prenatal or postnatal environment can confer positive disease prognosis, it is the compounded effect of both that appears to provide maximal reduction in disease severity. Furthermore, genetic background and a hypothesised influence of modifier genes could also be an important consideration. Future research to identify beneficial, disease-minimising genetic modifiers alongside the ongoing development of education and lifestyle strategies to improve gestational conditions for PKD gene carrying individuals will be of great importance.

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Impact of maternal environment on PKD

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Conflicts of Interest

None.

Ethical Statement

The authors assert that all procedures contributing to this work comply with the ethical standards of the National Health and Medical Research Council Australian code of practice for the care and use of animals for scientific purposes (2013) and were approved by Animal Ethics Committee of Macquarie University.
Impact of maternal environment on PKD

References
Impact of maternal environment on PKD

Tables

*Table 1. Groups used for study two*

<table>
<thead>
<tr>
<th>Intervention group</th>
<th>Maternal prenatal</th>
<th>Maternal postnatal</th>
<th>Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (LPK)</td>
<td>LPK -/- (n=4)</td>
<td>LPK (-/-)</td>
<td>6 ♂ / 6 ♀</td>
</tr>
<tr>
<td>Postnatal improvement (LPK:Pn)</td>
<td>LPK -/- (n=5)</td>
<td>LPK +/- (cross-fostered)</td>
<td>6 ♂ / 10 ♀</td>
</tr>
<tr>
<td>Prenatal and postnatal improvement (LPK:Pr/Pn)</td>
<td>LPK +/- (n=6)</td>
<td>LPK +/-</td>
<td>6 ♂ / 6 ♀</td>
</tr>
<tr>
<td>Prenatal and postnatal improvement on a different</td>
<td>WKY-LPK +/- (n=2)</td>
<td>WKY-LPK +/-</td>
<td>6 ♂ / 6 ♀</td>
</tr>
<tr>
<td>genetic background (WKY-LPK:Pr/Pn)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Dams (maternal prenatal) were mated with homozygous Lewis Polycystic Kidney (LPK -/-) males. In those mateings using heterozygous dams (LPK +/-), only the homozygous LPK (-/-) offspring were included in the present study. Pn (postnatal), Pr (prenatal), WKY (Wistar Kyoto), n = number of dams from which offspring were derived. Litter size was 9 ± 0.9. Number of
Impact of maternal environment on PKD

offspring contributing to each study group is broken down into (male), (female). Only the LPK:Pn group were cross-fostered, all other groups remained with the maternal dam.
Impact of maternal environment on PKD

**Table 2.** *Plasma biochemical data at study end point*

<table>
<thead>
<tr>
<th>Group</th>
<th>Variable</th>
<th>LPK</th>
<th>LP:Pn</th>
<th>LPK:Pr/Pn</th>
<th>WKY-LPK:Pr/Pn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein (g/L)</td>
<td>54.8 ± 1.3</td>
<td>55.5 ± 1.1</td>
<td>54.4 ± 1.4</td>
<td>57.1 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Albumin (g/L)</td>
<td>25.8 ± 0.8</td>
<td>26.9 ± 0.8</td>
<td>26.2 ± 1.3</td>
<td>26.8 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Globulin (g/L)</td>
<td>29.4 ± 0.6</td>
<td>29.0 ± 1.0</td>
<td>28.3 ± 0.5</td>
<td>30.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Urea (mmol/L)</td>
<td>31.2 ± 2.2*</td>
<td>30.9 ± 1.4*,#</td>
<td>24.8 ± 1.1</td>
<td>22.3 ± 2.3</td>
</tr>
<tr>
<td>♂</td>
<td>Creatinine (µmol/L)</td>
<td>140.5 ± 13.3*</td>
<td>149.5 ± 6.5*,#</td>
<td>101.8 ± 6.8</td>
<td>83.2 ± 13.4</td>
</tr>
<tr>
<td>♀</td>
<td>Creatinine (µmol/L)</td>
<td>99.7 ± 16.3</td>
<td>98.2 ± 7.1</td>
<td>70.7 ± 2.8</td>
<td>68.0 ± 10.3</td>
</tr>
</tbody>
</table>

Values and are expressed as mean ± SEM. N values are as per Table 1. Preliminary analysis identified no sex effect and so male/female data were pooled except for the creatinine data where a sex effect was evident. (*) Significantly different to WKY-LPK:Pr/Pn, (#) significantly different to LPK:Pr/Pn, p < 0.05.
Impact of maternal environment on PKD

**Table 3. Outputs of linear regression modelling for bodyweight and renal function**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pearson’s correlation coefficient r*</th>
<th>Regression Model</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BW 6</td>
<td>BW 16</td>
<td>Adjusted R²</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>Urea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>-0.603</td>
<td>-0.729</td>
<td>0.508</td>
<td>-0.729</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Females</td>
<td>-0.409</td>
<td>-0.732</td>
<td>0.514</td>
<td>-0.732</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>-0.603</td>
<td>-0.654</td>
<td>0.399</td>
<td>-0.654</td>
<td>0.001</td>
</tr>
<tr>
<td>Females</td>
<td>-0.423</td>
<td>-0.752</td>
<td>0.545</td>
<td>-0.752</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Linear regression modelling demonstrating the relationship between plasma biochemistry indicators of renal function and bodyweight. Table provides Pearson’s correlation coefficients r* (all p < 0.05) and the linear regression model summary in male and female animals from all study groups for urea and creatinine (creat) as the dependent and body weight (BW) at age 6 and 16 weeks as the predictive variables. β: beta standardized regression coefficients. The number of data pairs for each correlation is indicated by italic superscript.
Impact of maternal environment on PKD

**Table 4. Cystic parameters**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LPK</td>
</tr>
<tr>
<td>Cystic index</td>
<td>0.612 ± 0.015</td>
</tr>
<tr>
<td>Average cyst area (mm²)</td>
<td>0.119 ± 0.006</td>
</tr>
</tbody>
</table>

Values and are expressed as mean ± SEM. Preliminary analysis identified no sex effect and so male/female data are pooled. N = 5 (2-3 males/females) per group except for the WKY-LPK:Pr/Pn group where n = 6 (3 male and 3 female). (*) Significantly different to all other groups, p < 0.05.
Impact of maternal environment on PKD

**Figure Legends**

*Figure 1.*

Peanut agglutinin (PNA) staining of wildtype Lewis (A, a) and Lewis Polycystic Kidney (LPK) (B, b) kidneys from animals at 12 weeks of age, illustrating renal morphology and identification of glomeruli (a, b) used for estimation of nephron number. The marked cystic phenotype of the LPK is evident, with cysts distributed throughout the renal parenchyma. No cysts were evident around individual glomeruli (d) in the LPK. Scale bar (A, B) represents 1000 µM and scale bars in (a, b) inset represents 50 µM.
Impact of maternal environment on PKD

Figure 2.

Body weight growth profiles from 6 to 16 weeks separated into male (panel A) and female (panel B) outcomes. Data points represent mean ± SEM. (*) indicates significantly different to all other cohorts across the data set as a whole. In the male LPK, this difference in body weight relative to all other groups was statistically significant at 14, 15 and 16 weeks while in the female LPK it was evident at 6 weeks and 15 weeks. Further, the male LPK aged 6 weeks weighed significantly less than that of the WKY-LPK:Pr/Pn group. p < 0.05. N values are as per Table 1.
Impact of maternal environment on PKD

Figure 3.

Systolic blood pressure (SBP) from 8 to 16 weeks. Data are combined male and female results represented as means ± SEM. # indicates significantly different to LPK:Pr/Pn & WKY-LPK:Pr/Pn across the data set as a whole. At 16 weeks, the LPK control animals were significantly different to all other groups including the LPK:Pn at that age point. p < 0.05. N values are as per Table 1.
Impact of maternal environment on PKD

Figure 4.

Changes in urinary protein to creatinine ratio (UPC) from 8 to 16 weeks separated into male (panel A) and female (panel B) outcomes. Data points represent mean ± SEM. (†) indicates significant difference from WKY-LPK:Pr/Pn across the data set as a whole and (‡) significantly different to LPK:Pr/Pn across the data set as a whole. At age 12 weeks, the LPK:Pn were significantly different to all other groups at that age point. p < 0.05. N values are as per Table 1.
Impact of maternal environment on PKD

*Figure 5.*

Proportion kidney weight to body weight (KW/BW; Panel A) and heart weight to body weight (HW/BW Panel B) ratio in male and female animals. Data expressed as means ± SEM in animals at age 16 weeks. (*) Indicates significantly different to other groups as indicated, p < 0.05. N = 3 male/3 female per group except for the LPK:Pn female group where n= 5. LPK: Lewis polycystic kidney, LPK:Pn (improved postnatal environment), LPK:Pr/Pn (improved prenatal and postnatal environment), WKY-LPK:Pr/Pn (improved prenatal and postnatal environment on Wistar Kyoto genetic background).
Impact of maternal environment on PKD

Figure 6.

Individual data points for male and female animals illustrating relationship between plasma urea (A) and creatinine (B) and body weight at age 16 weeks.
Impact of maternal environment on PKD

*Figure 7.*

Estimated nephron count and representative images of cystic kidneys in animals at 16 weeks of age. Panel A represents combined male and female nephron count data and is expressed as means ± SEM. (*) Indicates significantly different to all other groups with p < 0.05. N values are as per Table 4. Lower panels are illustrative images of kidneys from animals from each of the different maternal environment groups: B: LPK (control Lewis polycystic kidney), C: LPK:Pn (improved postnatal environment), D: LPK:Pr/Pn (improved prenatal and postnatal environment) and E: WKY-LPK:Pr/Pn (improved prenatal and postnatal environment on Wistar Kyoto genetic background). Scale bar in panel E represents 1000 μM. *