Intrauterine Low-Protein Diet Exacerbates Abnormal Development of the Urinary System in Gen1-Mutant Mice

Minghui Yu\textsuperscript{a} Yaxin Li\textsuperscript{a} Lihong Tan\textsuperscript{a} Jing Chen\textsuperscript{a} Yihui Zhai\textsuperscript{a} Jia Rao\textsuperscript{a}
Xiaoyan Fang\textsuperscript{a} Jialu Liu\textsuperscript{a} Jiaojia Liu\textsuperscript{a} Xiaohui Wu\textsuperscript{b} Hong Xu\textsuperscript{a} Qian Shen\textsuperscript{a}

\textsuperscript{a}Department of Nephrology, Children’s Hospital of Fudan University, Shanghai Kidney Development and Pediatric Kidney Disease Research Center, Shanghai, China; \textsuperscript{b}State Key Laboratory of Genetic Engineering and National Center for International Research of Development and Disease, Institute of Developmental Biology and Molecular Medicine, Collaborative Innovation Center of Genetics and Development, School of Life Sciences, Fudan University, Shanghai, China

Keywords
Intrauterine low-protein diet · Intrauterine growth retardation · Congenital anomalies of the kidney and urinary tract · Gen1

Abstract
Background: Gen1 mutation can cause various phenotypes of congenital anomaly of the kidney and urinary tract (CA-KUT). An intrauterine low-protein isocaloric diet can also cause CAKUT phenotypes in offspring. However, single factors such as gene mutation or abnormal environmental factor during pregnancy can only explain part of the pathogenesis of CAKUT. Objectives: A low-protein isocaloric diet was fed to Gen1-mutant mice throughout pregnancy to establish a Gen1-mutant mouse model exposed to a low-protein isocaloric intrauterine environment. The mice were divided into 4 groups: normal (22%) protein diet (ND) + wild-type mice (CON group), ND + Gen1\textsuperscript{PB/+} mice (Gen1 PB/+ group), low (6%)-protein isocaloric diet (LD) + wild-type mice (LD group), and the LD + Gen1\textsuperscript{PB/+} groups. Methods: The experimental design included observing proportion and distribution of CAKUT phenotypes of neonatal mice; evaluating the location of UBs on E11.5, and length of the common nephric duct (CND); isolating embryonic kidneys on E11.5 from the Gen1\textsuperscript{PB/+} group and culturing embryonic kidneys in medium containing 10% serum or serum-free medium to observe the branching of UBs; and detecting the p-PLCγ, p-Akt, and p-ERK1/2 in UBs and CND on E11.5, as well as the apoptosis and proliferation of tissues by immunofluorescence staining. Results: We found that the incidence of CAKUT in offspring of Gen1\textsuperscript{PB/+} mice under an intrauterine low-protein isocaloric diet environment was significantly increased, and a duplicated collecting system was the dominant phenotype of CA-KUT. During the early stage of metanephric development, ectopic protrusion of UBs may appear and lower locations of UBs in Gen1\textsuperscript{PB/+} mice under an intrauterine low-protein isocaloric diet environment and the number of UB branches in the serum-free culture condition significantly decreased. Further examination revealed that p-PLCγ signaling and tissue apoptosis were abnormal in UBs and the CND at the early stage of kidney development. Conclusions: The aforementioned findings suggest that an intrauterine low-protein isocaloric diet can aggravate the occurrence of CAKUT in Gen1-mutant mice, which might affect key steps in the metamorphosis of UBs and CND.
nephric development, such as the protrusion of UBs, which might be related to mediate UBs and CND apoptosis through p-PLCγ signaling.

© 2021 The Author(s).
Published by S. Karger AG, Basel

Introduction

Congenital anomaly of the kidney and urinary tract (CAKUT) is a common congenital malformation and is the leading cause of chronic kidney disease and end-stage renal disease in children [1, 2]. It can account for 20–30% of fetal malformations in prenatal diagnoses, and the incidence of CAKUT is approximately 3–6 per 1,000 live births [3, 4].

CAKUT often has the characteristics of familial aggregation and is congenital. CAKUT may be a part of the phenotype of syndromes caused by single-gene mutation, and the CAKUT phenotype has been seen in a single-gene-mutation animal model. Therefore, some scholars have speculated that CAKUT can be caused by single-gene mutations. However, the most common mutations of CAKUT can only explain 14–20% of CAKUT, while copy number variation can explain 16% of CAKUT. In addition, a retrospective analysis with a large population size also found some adverse factors during pregnancy that were associated with CAKUT, such as an intrauterine low-protein diet [5], intrauterine hypoxia [6], and intrauterine hyperglycemia [7]. It is believed that the pathogenic factors of CAKUT include gene mutations and environmental factors [8–10], but gene mutation or environmental factors themselves cannot fully explain the pathogenic mechanism of CAKUT [11, 12]. In animal models of gene mutations, the differences in the CAKUT phenotype are also very large, which further suggests that the interaction between genes and environment and that between genes and genes may partially explain the pathogenesis of CAKUT [13].

Our group has used a low (6%)-protein isocaloric diet to successfully construct a mouse model of intrauterine growth retardation and confirmed that the intrauterine low-protein isocaloric diet causes an increase in the number of CAKUT mice in the offspring [5]. These offspring have multiple phenotypes of CAKUT, such as duplex kidney and hydronephrosis. In addition, to explore the interaction between genes and environment, a 6% protein isocaloric diet has been used to successfully construct an intrauterine low-protein isocaloric diet roundabout guidance receptor 2 (Robo2)-mutant mice [14]. The study confirmed that the intrauterine low-protein isocaloric diet increased the incidence of CAKUT in offspring of Robo2-mutant mice, accompanied by abnormally expressed key signaling molecules in metanephric development. These findings suggest that an adverse intrauterine environment can increase the incidence of gene mutation to a certain extent, resulting in a significantly increased risk of CAKUT in offspring. Does an intrauterine adverse environment increase the incidence of CAKUT in the offspring of mice with other gene mutation?

Genl (Holliday junction 5′ flap endonuclease) is a nuclease that is responsible for the opening of Holliday junctions during DNA homologous recombination [15, 16]. Our research group insert the piggyBac (PB) transposon into the second intron of Genl to suppress Genl expression, and GenlPB/PP and GenlPB/+ mice were obtained. We also found, for the first time, that GenlPB/PP and GenlPB/+ mice had multiple CAKUT phenotypes [17–19]. Does an intrauterine adverse environment increase the incidence of CAKUT in the offspring of mice with Genl gene mutation?

Therefore, this study aimed to establish an intrauterine low-protein isocaloric diet-fed Genl-mutant mouse model to observe postnatal urinary system malformations and abnormalities throughout the early stage of kidney development to determine whether the intrauterine low-protein isocaloric diet would increase the incidence of CAKUT in Genl-mutant mice, as well as to explore its underlying mechanism.

Materials and Methods

Intrauterine Low-Protein Diet Genl-Mutant Mouse Model

In Gen1-mutant FVB/Nj mice, PB was inserted into the second intron of the Genl gene. Hoxb7/myr-Venus mice expressing a fluorescent protein were donated by Dr. Costantini (Department of Genetics and Development, Columbia University, USA). GenlPB/+ mice were hybridized with Hoxb7/myr-Venus mice to obtain Genl-heterozygous-mutant mice (GenlPB/+; Hoxb7/myr-Venus, abbreviated as GenlPB/+; Hoxb7) and wild-type mice (Genl+/+; Hoxb7/myr-Venus, abbreviated as Genl+/+; Hoxb7) in which epithelial cells of the urinary system were labeled with fluorescent protein. The genotype was identified as previously described [17–19]. The experimental mice were bred in a specific pathogen-free-grade animal room. The protocol was approved by the welfare and use of experimental animals issued by the Animal Care and Use Committee of the Institute of Developmental Biology and Molecular Medicine at Fudan University (Permit number: SYXK [hu]2020-0011). And, 6- to 8-week-old mice with the same genetic type were housed in the same cage at a female-to-male ratio of 3:1–4:1. The female mice were observed for pregnancy at 8 a.m./p.m. The earliest time when a vaginal plug was observed was recorded as embryonic day (E) 0.5. After the vaginal plug was observed, the female mice were randomly divided into the experimental group and the
CON group. The experimental group was given 6% low-protein isocaloric diet (Double Lion Experimental Animal Feed Technology Co., Ltd.) throughout pregnancy [5, 14], and the CON group was given the normal 22% protein diet (Suzhou Su-Hang Science and Technology Equipment Co., Ltd.) throughout pregnancy until natural birth. Both groups of pregnant mice had free access to food and water. Starting from E16.5, the pregnant mice were observed at 8 a.m./p.m. every day to see whether pups had been delivered. The earliest time when the newborn pups were observed was marked as postnatal day 0.5 (day P0.5).

**Postnatal Gross Phenotype Analysis**
After the newborn mice in the 4 groups were euthanized using CO₂, the urinary tract was dissected through a midline incision in the anterior abdomen. The position, morphology, and number of kidneys; the morphology and number of ureters; and the morphology of the bladder of the newborn pups were observed under a fluorescence microscope.

**Embryonic Kidney Phenotype Analysis**
Pregnant mice at E11.5 were anesthetized using CO₂. Dissection was performed layer by layer, the uteri were isolated, the embryos were removed, and the fetal membranes were retained for genotype identification. Genotypes were determined as previously described [17–19]. The embryonic kidneys were photographed and observed for the number and location of ureteric buds (UBs), common nephric duct (CND) length, and number of UB branches under a fluorescence stereomicroscope.

**Embryonic Kidney Culture**
The E11.5 embryonic kidneys from the Gen1 PB/+ group were isolated and cultured in the absence of serum [20, 21] or in the presence of 10% fetal bovine serum with Dulbecco’s modified Eagle’s medium/Nutrient Mixture F-12 (DMEM/F-12) (Gibco) in Transwell for 72 h. The culture condition was 5% CO₂ at 37°C. Pictures were taken every 24 h under a fluorescence microscope (Leica).

**Immunofluorescence Staining**
The E11.5 embryonic kidneys from mice were isolated and cultured in formaldehyde overnight. After washing with 0.3% Triton X-100, tissues were incubated in a shaker at 4°C overnight in 5% donkey serum to block nonspecific antigen binding. The tissues were incubated with anti-caspase-3 antibody (CST, #9662, 1:400), anti-Phh3 antibody (CST, #3377, 1:800), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200), phosphorylated (p-)Akt antibody (CST, #9272, 1:200).
lated extracellular signal-regulated kinase 1/2 (p-ERK1/2) antibody (CST, #4695, 1:200), and phospho-PLCγ (p-PLCγ) antibody (R & D systems, #MAB74542, 1:100) in a shaker at 4°C overnight. The tissues were washed with PBS and incubated with Cy5-donkey anti-rabbit antibody (Jackson, 1:1,000). DAPI (1:1,000) was used to stain the nuclei. Five embryonic kidneys from each group were used. The samples were mounted with anti-fade reagent. Images were taken under a confocal microscope. NIH ImageJ software was used for data analysis.

Statistical Analysis

All data were analyzed by GraphPad7 and SPSS. All results were expressed as means ± standard error of the mean. p > 0.05 was not significantly different, while p < 0.05 was considered significantly different. An unpaired t-test was used for data analysis between groups.

Results

Successful Construction of Intrauterine Low-Protein Isocaloric Diet Gen1-Mutant Mouse Model

Gen1<sup>PB</sup>+; Hoxb7 pregnant mice were given a low-protein isocaloric diet throughout pregnancy to successfully construct an intrauterine low-protein isocaloric diet Gen1-mutant mice model (LD + Gen1<sup>PB</sup>+/+). The LD + Gen1<sup>PB</sup>+/+ neonates weighed significantly less than the Gen1<sup>PB</sup>+/+ neonates (0.9526 ± 0.1174 g vs. 1.277 ± 0.121 g, p < 0.0001) (shown in Fig. 1a). The number of neonatal mice at birth in the LD + Gen1<sup>PB</sup>+/+ group was lower than that in the Gen1<sup>PB</sup>+/+ group (4 [3, 7] vs. 8 [8, 9], p = 0.0065) (shown in Fig. 1b). The intrauterine low-protein isocalo-

Fig. 2. Analysis of new born mice phenotypes in 4 groups at P 0.5d. a LD + Gen1<sup>PB</sup>+/+ group (LD + Gen1<sup>PB</sup>+/+). b LD group (LD). c Gen1<sup>PB</sup>+/+ group (Gen1<sup>PB</sup>+/+). d Gen1<sup>−/−</sup> group (CON).
ric diet did not have a significant effect on the pregnancy rate, gestation period, crown-rump length on E11.5, the number of E11.5 embryos, the number of mice at birth, or the gestation period in Gen1PB/+ mice (shown in Fig. 1c–f).

Increased Incidence of CAKUT in Neonatal Mice of the LD + Gen1PB/+ Group

The proportion of neonatal mice with CAKUT in the LD + Gen1PB/+ group was slightly higher than that in the Gen1PB/+ group (37.74% [20/53] vs. 25.69% [28/109], $p = 0.0620$), while the proportion of duplicated collecting systems was significantly higher (28.30% [15/53] vs. 13.76%

Fig. 3. Analysis of new born mice phenotypes in the LD + Gen1PB/+ group. b, d, f Visualized by Hoxb7/myr-Venus expression. a Solitary kidney. b Normal kidney. c Unilateral duplicated collecting system. d Unilateral duplicated collecting system. e Unilateral duplicated collecting system with hydronephrosis and megaureter. f Unilateral duplicated collecting system with hydronephrosis and megaureter. White arrowheads point to the normal Ur, red arrowheads point to duplicated Ki, or double Urs, or hydronephrosis, or megaureter. Bl, bladder; Ki, kidney; Ur, ureter.
Intrauterine Low-Protein Diet Increases
CAKUT Phenotype in Gen1-Mutant Mice

Intrauterine Low-Protein Diet Increases CAKUT Phenotype in Gen1-Mutant Mice

The proportion of neonatal mice with CAKUT in the LD + Gen1PB/+ group was significantly higher than that in the LD group (37.74% [20/53] vs. 18.03% [11/61], p = 0.0180), and the incidence of duplicated collecting systems was also significantly higher (28.30% [15/53] vs. 11.48% [7/61], p = 0.0170) (shown in Fig. 2, 3).

Increased Duplicated Budding of UBs, Lower UB Location, and Fewer UB Branches

Duplicated budding on E11.5 was significantly more in the LD + Gen1PB/+ group than the Gen1PB/+, LD, and CON groups (respectively, 39.13% [18/46] vs. 19.70% [13/66], p = 0.0240; 39.13% [18/46] vs. 5.41% [2/37], p < 0.0001; and 39.13% [18/46] vs. 0.91% [1/110], p < 0.0001) (shown in Fig. 4a). Culturing the E11.5 embryonic kidneys of the duplicated budding for 72 h in vitro, the ectopic budding could branch stepwise (shown in Fig. 4b). Compared with the location of UB protrusions in the Gen1PB/+ group, the LD + Gen1PB/+ group was lower, and the length of CND in the LD + Gen1PB/+ group was longer (respectively, 293.9 ± 85.29 µm vs. 185.6 ± 81.29 µm, p = 0.0004; 293.9 ± 85.29 µm vs. 227.6 ± 52.84 µm, p = 0.0046; and 293.9 ± 85.29 µm vs. 205 ± 40.14 µm, p < 0.0001) (shown in Fig. 4c).

Fig. 4. Comparison of the number and location of UB in 4 groups. 

a Analysis at E11.5 shows different types of ectopic UBs and normal UB in LD + Gen1PB/+ group embryos (visualized by Hoxb7/myr-Venus expression). b Culturing the ectopic UBs of E11.5 LD + Gen1PB/+ embryonic kidney under 10% serum condition for 72 h. The nephric duct is shown in lateral view (dorsal to the right).

White arrow line points to the normal UB. Red arrow line points to ectopic UBs. c Analysis at E11.5 shows a lower located in the LD + Gen1PB/+ group, red dashed lines represent length of the CND; each value is expressed as mean ± SE. CND, common nephric duct; ND, nephric duct; UB, ureteric bud.
After culturing the E11.5 embryonic kidneys of the Gen1 PB/+ group for 24, 48, and 72 h in vitro, the protrusion and branching of UBs were observed. After culturing for 24 h, the number of UB branches was significantly decreased (BP: \( p = 0.0309 \)) in serum-free culture compared to culture with medium containing 10% serum. After culturing for 48 h, the number of UB branches and UB tips was significantly decreased in serum-free culture compared to 10% serum culture (BP: \( p = 0.0016 \); UB tips: \( p = 0.0015 \)). After culturing for 72 h, the number of UB branches and UB tips was more significantly decreased in serum-free culture compared to 10% serum culture (BP: \( p = 0.0044 \); UB tips: \( p = 0.0017 \)) (shown in Fig. 5).

**Reduced Activity of p-PLCγ and Increased Apoptosis during the Protrusion of UBs in the LD + Gen1 PB/+ Group**

Immunofluorescence was used to detect the levels of p-PLCγ, p-Akt, and p-ERK1/2 in the UBs and the CND on E11.5 in the LD + Gen1 PB/+, Gen1 PB/+, and LD groups. The results showed that p-PLCγ was downregulated in the LD + Gen1 PB/+ group compared to the Gen1 PB/+ group and the LD group (UB: \( p = 0.0385, p < 0.0001 \); CND: \( p = 0.0482, p = 0.0064 \), respectively) (shown in Fig. 6). p-Akt and p-ERK1/2 did not show significant differences between the LD + Gen1 PB/+ group and the Gen1 PB/+ group.

The activation of the p-PLCγ signal is associated with tissue cell proliferation and apoptosis. Therefore, immunofluorescence was used to detect the apoptosis and proliferation of UBs and the CND on E11.5 in the LD + Gen1 PB/+, Gen1 PB/+, and LD groups. UB apoptosis was higher in the LD + Gen1 PB/+ group (\( p = 0.0377 \)) than in the Gen1 PB/+ group. CND apoptosis was higher in the LD + Gen1 PB/+ group than in the Gen1 PB/+ and LD groups (\( p = 0.0037; p = 0.0040 \), respectively) (shown in Fig. 7). There was no significant difference in cell proliferation between the LD + Gen1 PB/+ group and the Gen1 PB/+ group.

**Discussion/Conclusion**

In this study, a low (6%)-protein isocaloric diet was provided throughout pregnancy to successfully construct an intrauterine low-protein isocaloric diet Gen1 PB/+ neonatal mouse model. It was found that under environmental stimulation with an intrauterine low-protein isocaloric diet, the incidence of CAKUT was increased in offspring of Gen1-mutant mice, and a duplicated collecting system was the dominant CAKUT phenotype. In the early stage of metanephric development, ectopic protrusion of UBs may appear and lower locations of UBs. The intrauterine low-protein isocaloric diet environment was simulated in vitro using serum-free culture medium [20, 21]. The culture experiments with Gen1 PB/+ embryonic kidneys showed that the number of UB branches in the serum-free culture was significantly decreased compared to when they were cultured in 10% serum medium. Further examination revealed that p-PLCγ signaling and tis-
Intrauterine Low-Protein Diet Increases CAKUT Phenotype in Gen1-Mutant Mice

Kidney Dis 2021;7:482–493
DOI: 10.1159/000516942

Fig. 6. Detect the expression levels of p-Plcγ in UB and CND tissues at E11.5 embryonic kidney by immunofluorescence. a–c Detect the p-Plcγ in 4 groups at E11.5. Each value is expressed as mean ± SE. CND, common nephric duct; ND, nephric duct; UB, ureteric bud. Five embryonic kidneys from each group were used.
Fig. 7. Detect the apoptosis in UB and CND tissues at E11.5 embryonic kidney by immunofluorescence. a–c Detect the apoptosis in groups. Each value is expressed as mean ± SE. CND, common nephric duct; ND, nephric duct; UB, ureteric bud. Five embryonic kidneys from each group were used.
Intrauterine Low-Protein Diet Increases CAKUT Phenotype in Gen1-Mutant Mice

The correct protrusion of UBs and the process of stepwise branching are regulated by a complex signaling network in which the glial cell-derived neurotrophic factor / rearranged during transfection (RET) signal is critical [22, 23]. RET is a transmembrane receptor tyrosine kinase. RET activation can participate in the regulation of the normal development of tissues and organs, and its abnormal expression can lead to the occurrence of many congenital diseases, such as CAKUT [24]. Akt, PLCγ, ERK1/2 mitogen-activated protein kinase, and SRC are the main signaling molecules in the cytoplasm that are activated after RET activation [24]. These molecules participate in the regulation of cell proliferation, apoptosis, differentiation, and migration and maintain normal tissue development. Abnormal proliferation and apoptosis can affect UB protrusion and cause different phenotypes of CAKUT [25]. A low-protein diet can cause abnormal expression of key molecules in kidney development in Robo2-mutant mice and mainly affect key steps in meta-

In the previous studies, our team preliminarily explored the interaction between genes and environment by constructing a Robo2-mutant mouse model in an adverse intrauterine environment and found that under the stimulation of a low-protein diet [14], the incidence of CAKUT in the offspring of Robo2-mutant mice was significantly increased, and the phenotype was dominated by a duplicated collecting system, accompanied by the duplicated protrusion of UBs at an early stage, lower UB protrusions, and fewer UB branches. These findings suggested that an intrauterine adverse environment could increase the impact of Robo2 gene mutations to a certain extent, resulting in a significant increase in the risk of CAKUT in the offspring. To find out whether an adverse intrauterine environment increases the incidence of CAKUT in the offspring of mice with other gene mutations, this study constructed a Gen1-mutant mouse model in an adverse intrauterine environment. Under low-protein dietary stimulation, the proportion of CAKUT mice in the offspring of Gen1-mutant mice was increased, and the CAKUT phenotype was mainly dominated by duplex kidney, accompanied by duplicated protrusion of UBs at an early stage, lower UB protrusions, and fewer UB branches. These findings suggest that a low-protein diet could lead to an increased risk of CAKUT in the offspring of Gen1-mutated mice. These results further indicate that a low-protein diet, as an environmental stimulus, could significantly increase the risk of CAKUT in offspring of mice with different gene mutants.

The correct protrusion of UBs and the process of stepwise branching are regulated by a complex signaling network in which the glial cell-derived neurotrophic factor / rearranged during transfection (RET) signal is critical [22, 23]. RET is a transmembrane receptor tyrosine kinase. RET activation can participate in the regulation of the normal development of tissues and organs, and its abnormal expression can lead to the occurrence of many congenital diseases, such as CAKUT [24]. Akt, PLCγ, ERK1/2 mitogen-activated protein kinase, and SRC are the main signaling molecules in the cytoplasm that are activated after RET activation [24]. These molecules participate in the regulation of cell proliferation, apoptosis, differentiation, and migration and maintain normal tissue development. Abnormal proliferation and apoptosis can affect UB protrusion and cause different phenotypes of CAKUT [25]. A low-protein diet can cause abnormal expression of key molecules in kidney development in Robo2-mutant mice and mainly affect key steps in meta-

Intrauterine Low-Protein Diet Increases CAKUT Phenotype in Gen1-Mutant Mice

DOI: 10.1159/000516942

Kidney Dis 2021;7:482–493

481
nephric development, such as UB protrusion, and mediate UB tissue apoptosis through Akt/Creb3 signaling [14]. How can low-protein diets as environmental stimuli significantly increase the risk of CAKUT in the offspring of Gen1-mutant mice? Is the mechanism similar to that in Robo2-mutant mice? We measured the activation of the main signaling molecules in the cytoplasm downstream of RET-p-PLCγ, p-Akt, and p-ERK1/2 in UBs and the CND on E11.5, as well as the changes in tissue proliferation and apoptosis, in the LD + Gen1PB/+ group and LD groups, the LD + Gen1PB/+ group had lower p-PLCγ activity and increased apoptosis. During the development of the urinary system, different tyrosine docking sites of RET have different functions [26]. Mice with a mutation at RETY1015 (docking site for PLCγ) show duplex ureter, megaureter, and other CAKUT phenotypes [27], which are similar to the CAKUT phenotypes in offspring of the intrauterine low-protein diet fed Gen1 mutation group in this study. These findings indicate that a low-protein diet, as an environmental stimulus, can mediate RET downstream signaling and aggravate CAKUT in Gen1-mutant mice by influencing tissue apoptosis.

In this study, we constructed a Gen1PB/+ mouse model with intrauterine growth retardation and found that compared with the Gen1PB/+ group and the LD group, the LD + Gen1PB/+ group more often had CAKUT phenotypes, mainly dominated by a duplicated collecting system. In addition, the LD + Gen1PB/+ group had more duplicated protrusions at E11.5 than the Gen1PB/+ and LD groups. The intrauterine low-protein diet and Gen1 mutation might influence the apoptosis of UBs and the CND through the PLCγ signaling pathway, leading to the abnormal development of the urinary system in the offspring of the Gen1-mutant mice. We can speculate that a low-protein diet could synergize with Gen1 mutation to increase the incidence of abnormal urinary system development in Gen1-mutant mice.

Statement of Ethics

The protocol was approved by the welfare and use of experimental animals issued by the Animal Care and Use Committee of the Institute of Developmental Biology and Molecular Medicine at Fudan University ( Permit number: SYXX [hu]2020-0011).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This work was supported by the National Natural Science Foundation of China (82070686, 81741033, and 81670609) and the National Children’s Medical Center “DengFeng” cross creative team (EK112520180201).

Author Contributions

X.W., H.X., and Q.S. conceived and designed research; M.Y. and Y.L. performed experiments; M.Y. analyzed data; J.C., Y.Z., J.R., and X.F. interpreted results of experiments; M.Y., L.T., and Y.L. prepared figures; M.Y. drafted the manuscript; Q.S. edited and revised the manuscript; and Q.S. approved final version of the manuscript.

References

1 Sanna-Cherchi S, Westland R, Ghiggeri GM, Gharavi AG. Genetic basis of human congenital anomalies of the kidney and urinary tract. J Clin Invest. 2018 Jan;128(1):4–15.
2 Renkema KY, Winyard PJ, Skovorodkin IN, Levtchenko E, Hindryckx A, Jeppiar C, et al. EUCAKUT consortium. Novel perspectives for investigating congenital anomalies of the kidney and urinary tract (CAKUT). Nephrol Dial Transplant. 2011 Dec;26(12):3843–51.
3 Queisser-Luft A, Stolz G, Wiesel A, Schlaefer K, Spranger J. Malformations in newborn: revised the manuscript; and Q.S. approved final version of the manuscript.

4 Hildebrandt F. Genetic kidney diseases. Lancet. 2010 Apr;375(9722):1287–95.
5 Yu M, Tan L, Chen J, Zhai Y, Wu X, Xu H, et al. Intrauterine low-protein diet disturbs metanephric gene expression and induces urinary tract developmental abnormalities in mice. Biochem Biophys Res Commun. 2019 Jun;513(3):732–9.
6 Wilkinson LJ, Neal CS, Singh RR, Sparrow DB, Kurniawan ND, Ju A, et al. Renal developmental defects resulting from in utero hypoxia are associated with suppression of ureteric β-catenin signaling. Kidney Int. 2015 May;87(5):975–83.
7 Hokke SN, Armitage JA, Puelles VG, Short KM, Jones L, Smyth IM, et al. Altered ureteric branching morphogenesis and nephron endowment in offspring of diabetic and insulin-treated pregnancy. PLoS One. 2013;8(3):e58243.
8 Vivante A, Kohl S, Hwang DY, Dwsorschak GC, Hildebrandt F. Single-gene causes of congenital anomalies of the kidney and urinary tract (CAKUT) in humans. Pediatr Nephrol. 2014 Apr;29(4):695–704.
9 van der Ven AT, Connaughton DM, Ityel H, Mann N, Nakayama M, Chen J, et al. Whole-exome sequencing identifies causative mutations in families with congenital anomalies of the kidney and urinary tract. J Am Soc Nephrol. 2018 Sep;29(9):2348–61.
Intrauterine Low-Protein Diet Increases CAKUT Phenotype in Gen1-Mutant Mice

10 Bekheirnia MR, Bekheirnia N, Bainbridge MN, Gu S, Coban Akdemir ZH, Gambin T, et al. Whole-exome sequencing in the molecular diagnosis of individuals with congenital anomalies of the kidney and urinary tract and identification of a new causative gene. Genet Med. 2017 Apr;19(4):412–20.

11 Madariaga L, Morinière V, Jeanpierre C, Bouvier R, Loget P, Martinovic J, et al. Severe prenatal renal anomalies associated with mutations in HNF1B or PAX2 genes. Clin J Am Soc Nephrol. 2013 Jul;8(7):1179–87.

12 Verbitsky M, Westland R, Perez A, Kyriluk K, Liu Q, Krithivasan P, et al. The copy number variation landscape of congenital anomalies of the kidney and urinary tract. Nat Genet. 2019 Jan;51(1):117–27.

13 Narlis M, Grote D, Gaitan Y, Boualia SK, Bouchard M. Pax2 and pax8 regulate branching morphogenesis and nephron differentiation in the developing kidney. J Am Soc Nephrol. 2007 Apr;18(4):1121–9.

14 Yu M, Tan L, Li Y, Chen J, Zhai Y, Rao J, et al. Intrauterine low-protein diet aggravates developmental abnormalities of the urinary system via the Akt/Creb3 pathway in Robo2 mutant mice. Am J Physiol Renal Physiol. 2020 Jan;318(1):F43–52.

15 Rass U, Compton SA, Matos J, Singleton MR, Ip SC, Blanco MG, et al. Mechanism of Holliday junction resolution by the human GEN1 protein. Genes Dev. 2010 Jul;24(14):1559–69.

16 Wang X, Wang H, Guo B, Zhang Y, Gong Y, Zhang C, et al. Gen1 and Em1 play redundant roles in DNA repair and meiotic recombination in mice. DNA Cell Biol. 2016 Oct;35(10):585–90.

17 Wang H, Zhang C, Wang X, Lian Y, Guo B, Han M, et al. Disruption of Gen1 causes congenital anomalies of the kidney and urinary tract in mice. Int J Biol Sci. 2018 Jan;14(1):10–20.

18 Zhang Y, Zhang X, Wang X, Wang H, Wu X, Xu H, et al. Gen1 modulates metanephric morphology through retinoic acid signaling. DNA Cell Biol. 2019 Mar;38(3):263–71.

19 Wang X, Wang H, Liu J, Gong Y, Zhang C, Fang F, et al. Gen1 mutation caused kidney hypoplasia and defective ureter-bladder connections in mice. Int J Biol Sci. 2020 Mar;16(9):1640–7.

20 Paroly SS, Wang F, Spraggion L, Merregaert J, Batourina E, Tycko B, et al. Stromal protein Ecm1 regulates ureteric bud patterning and branching. PLoS One. 2013 Dec;8(12):e84155.

21 Ekblom P, Miettinen A, Virtanen I, Wahlström T, Dawnay A, Saxen L. In vitro segregation of the metanephric nephron. Dev Biol. 1981 May;84(1):88–95.

22 Davis TK, Hoshi M, Jain S. To bud or not to bud: the RET perspective in CAKUT. Pediatr Nephrol. 2014 Apr;29(4):597–608.

23 Uy N, Reidy K. Developmental genetics and congenital anomalies of the kidney and urinary tract. J Pediatr Genet. 2016 Mar;5(1):51–60.

24 Jain S. The many faces of RET dysfunction in kidney. Organogenesis. 2009 Oct;5(4):177–90.

25 Hoshi M, Reginensi A, Joens MS, Fitzpatrick JAJ, McNeill H, Jain S. Reciprocal spatiotemporally controlled apoptosis regulates Wolffian duct cloaca fusion. J Am Soc Nephrol. 2018 Mar;29(3):775–83.

26 Jain S, Encinas M, Johnson EM Jr, Milbrandt J. Critical and distinct roles for key RET tyrosine docking sites in renal development. Genes Dev. 2006 Feb;20(3):321–33.

27 Jijiwa M, Fukuda T, Kawai K, Nakamura A, Kurokawa K, Murakumo Y, et al. A targeting mutation of tyrosine 1062 in Ret causes a marked decrease of enteric neurons and renal hypoplasia. Mol Cell Biol. 2004 Sep;24(18):8026–36.