Role of Kidneys in Sex Differences in Angiotensin II–Induced Hypertension

Lei Wang,* Ximing Wang,* Helena Y. Qu, Shan Jiang, Jie Zhang, Liying Fu, Jacentha Buggs, Bo Pang, Jin Wei, Ruisheng Liu

Abstract—The significance of kidneys in regulation of sodium and water balance and hemodynamics has been demonstrated both in patients and animal models. In the present study, we tested our hypothesis that kidneys play an essential role in control of sex differences in angiotensin II (Ang II)–dependent hypertension. Kidney transplantations (KTXs) were performed between male (M) and female (F) C57BL/6 mice (donor→recipient: F→F, M→M, F→M, and M→F). Radiotelemetry transmitters were implanted for measurement of mean arterial pressure during the infusion of Ang II (600 ng·kg\(^{-1}\)·min\(^{-1}\)). Gene expressions and inflammatory responses in the transplanted grafts were assessed. We found that same-sex KTX mice still exhibited sex differences in Ang II–dependent hypertension (31.3±0.8 mm Hg in M→M versus 12.2±0.6 mm Hg in F→F), which were reduced between males and females when they received kidneys of the opposite sex (32.9±1 mm Hg in M→F versus 22.3±0.7 mm Hg in F→M). The sex differences in gene expressions, including AT1R (angiotensin II receptor, type 1), AT2R, ET-1 (endothelin-1), ETA (endothelin receptor type A), NHE3 (sodium–hydrogen exchanger 3), α-ENaC (α-epithelial sodium channel), and γ-ENaC, were unaltered in same-sex KTXs and much lessened in cross-sex KTXs. In addition, the cross-sex KTXs exhibited more robust inflammatory responses reflected by higher expression of IL-6 (interleukin 6), TNFα (tumor necrosis factor α), and KC (keratinocyte-derived chemokine) than same-sex KTX. Our results indicate that kidneys play an essential role in sex differences of Ang II–dependent hypertension. KTX of male kidneys to females augmented the blood pressure response, whereas KTX of female kidneys to males attenuated the blood pressure response. The host’s extrarenal systems modulate expressions of many genes and inflammatory response, which may also contribute to the sex differences in blood pressure regulation. (Hypertension. 2017;70:00-00. DOI: 10.1161/HYPERTENSIONAHA.117.10052.) ● Online Data Supplement

Key Words: arterial pressure ■ mice kidney transplantation ■ sex characteristics

Hypertension affects ≈1 in 3 US adults and is the leading risk factor for cardiovascular diseases.\(^1\)\(^–\)\(^3\) Multiple regulatory systems, such as the vasculature, the heart, the central nervous system, and the kidneys, have been demonstrated to participate in the regulation of the blood pressure (BP).\(^4\) Among them, the kidneys have been demonstrated to play a vital role in the development of hypertension in both humans and experimental animal models.\(^5\)\(^–\)\(^6\) Kidneys transplanted from hypertensive donors increase BP in normotensive recipients, whereas kidneys transplanted from normotensive donors lower BP in hypertensive recipients both in clinical\(^7\)\(^–\)\(^10\) and experimental renal transplantation studies.\(^11\)\(^–\)\(^14\)

Epidemiological studies indicate that the prevalence of hypertension is greater in men than premenopausal women regardless of race, ethnicity, or country of origin.\(^15\)\(^–\)\(^17\) Women are protected from more cardiovascular events compared with men until menopause. Sex differences in BP are also observed in experimental hypertensive models.\(^18\)\(^–\)\(^19\) The effects of sex hormones, such as estrogen and testosterone, on the sex differences in BP regulation have been extensively studied in recent years.\(^20\)\(^–\)\(^23\) However, whether kidneys play an important role in control of the sex differences in BP has not been clarified.

In the present study, we tested our hypothesis that kidneys play an essential role in the sex differences in Ang II–dependent hypertension. The sex differences pattern in high BP will be altered between males (M) and females (F) when they receive kidneys of opposite sex. To test our hypothesis, we performed kidney transplantation (KTX) between male and female mice, induced hypertension, and measured gene expressions and inflammatory responses in the transplanted grafts. Our results indicate that although the kidneys play an essential role in the control of the sex differences in hypertension, the extrarenal mechanisms also contribute to the sex differences in BP regulation.

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Methods

All procedures and experiments were approved by the Institutional Animal Care and Use Committee at the University of South Florida College of Medicine. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) except as indicated.

Detailed methods and associated references are available in the online-only Data Supplement.

Statistical Analysis

All data were presented as mean values±SE. Mean arterial pressure (MAP) and heart rate (HR) curves between groups were compared using 2-way ANOVA with Tukey post hoc tests as appropriate. The MAP response curves within animals were compared using Student paired t test. The mRNA expressions of all the factors were compared among multiple groups using 1-way ANOVA with Tukey post hoc tests. A P value of <0.05 was considered to be statistically significant. Statistical analysis was performed with GraphPad Prism, version 6.0h (GraphPad Software).

Results

Graft Function Assessment Post-KTX

After KTX, the mice were allowed to recover for 4 weeks. Plasma creatinine concentration, glomerular filtration rate (n=7; Figure S1A and S1B in the online-only Data Supplement), and plasma testosterone and estradiol levels were all in normal range (n=7; Figure S2A and S2B).

Changes in MAP and HR in Response to Ang II Infusion

To determine sex differences in Ang II–induced hypertension, we measured the MAP with radiotelemetry system. The basal MAP was similar and within normal range in all groups of animals (Figure 1A; Figure S3A and S3B).

The MAP started to increase from about the fourth day after Ang II infusion. The maximum increase in MAP was 31.3±0.8 mmHg in M→M and 12.2±0.6 mmHg in F→F in the same-sex–KTX groups (P<0.01 Ang II infusion versus basal; n=7; Figure 1A). In cross-sex–KTX groups, the Ang II–induced maximum increase in MAP was 22.3±0.7 mmHg in F→M, which was significantly lower than M→M group but higher than F→F group. The maximum increase in MAP in M→F group was 32.9±1 mmHg, which was significantly higher than F→F group but similar to the M→M group. The results indicate that a female kidney in a male mouse attenuated the BP response, whereas a male kidney in a female mouse had the same response as in a male mouse. The sex differences in MAP in response to Ang II infusion were present in the same-sex–KTX groups but attenuated in cross-sex–KTX mice (P<0.01; n=7; Figure 1B).

Females showed higher basal HRs than males in the same-sex–KTX groups (613±11 bpm for F→F mice and 532±13 bpm for M→M mice). After cross-sex KTX, the HRs of female mice with implanted male kidneys (M→F) remained unchanged compared with F→F mice. However, the HRs increased 40 bpm for male mice with implanted female kidneys (F→M) compared with M→M mice (P<0.01; n=7; Figure 2A and 2B). Ang II infusion did not significantly change the HRs for either male or female mice.

Receptors and Sodium Cotransporters

To test whether the recipient’s internal environment modulates gene expression of the transplanted grafts, we measured the expression levels of genes related to vascular contractility and the sodium reabsorption after 2 weeks of Ang II infusion (n=7; Table S1). For all statistical comparisons, we focused on the comparison of the mRNA expressions of the donor kidneys between cross-sex recipients and the same-sex recipients (M→M versus F→F and F→F versus M→F), which were emphasized in the inset graphs in figures.

Angiotensin II Receptors

The mRNA levels of AT1R and AT2R (angiotensin II receptor, type 1 and 2) for both males and females in the same-sex–KTX groups did not change significantly compared with their respective controls. AT1R mRNA was 2-fold higher in males than females for both same-sex–KTX and control groups (P<0.01 M→M versus F→F and female control [FC] versus male control [MC]; n=7; Figure 3A). In the cross-sex–KTX groups, M→F mice showed a decrease in the expression level of AT1R mRNA compared with M→M mice. However, transplanted grafts from female donors to male recipients (F→M) resulted in a 50% increase of AT1R mRNA compared with F→F mice. Thus, the differences in the expression of AT1R between male and female grafts were attenuated after cross-sex KTX (P<0.05; n=7; Figure 3A).
AT_2R mRNA expression levels were higher in female than male mice in same-sex–KTX and control groups \((P<0.01\text{ }M\rightarrow M\text{ versus } F\rightarrow F\text{ and } FC\text{ versus } MC;\ n=7;\ Figure 3B)\). In cross-sex–KTX groups, the differences of AT_2R levels between male and female grafts did not change much.

**ET System**

The level of ET-1 (endothelin-1) mRNA for both males and females in the same-sex–KTX groups did not change significantly compared with their respective controls. ET-1 mRNA levels in male kidneys were \#8-folds higher than that in female kidneys, \((P<0.01\text{ } M\rightarrow M\text{ versus } F\rightarrow F\text{ and } FC\text{ versus } MC;\ n=7;\ Figure 3C)\). In the cross-sex–KTX groups, the ET-1 mRNA level in the kidneys of M→F mildly decreased compared with M→M mice and showed a 5-fold increase in the kidneys of F→M compared with F→F mice \((P<0.01\text{ } F\rightarrow M\text{ versus } F\rightarrow F;\ n=7;\ Figure 3C)\). After cross-sex KTX, the sex differences of ET-1 mRNA expression were dramatically mitigated \((P<0.01\text{ } M\rightarrow M\text{ versus } F\rightarrow F\text{ and } M\rightarrow F\text{ versus } F\rightarrow F;\ n=7;\ Figure 3C)\).

Male mice exhibited almost 2-fold higher of ET_\alpha mRNA expression than females in both control and same-sex–KTX groups \((P<0.01\text{ } FC\text{ versus } MC\text{ and } M\rightarrow M\text{ versus } F\rightarrow F;\ n=7;\ Figure 3D)\). After cross-sex KTX, the abundance of the ET_\alpha mRNA was decreased in grafts of M→F group compared with M→M mice. The sex differences were attenuated in ET_\alpha mRNA expression in cross-sex–KTX groups in female recipients. No significant change in the expression of ET_\alpha mRNA was found in F→M group compared with F→F mice \((P<0.05;\ n=7;\ Figure 3D)\), and there was no change of the sex differences in the ETA (endothelin receptor type A) mRNA expression in cross-sex–KTX groups in male recipients. The expression levels of ET_\alpha did not differ in all groups \((n=7;\ Figure 3E)\).

**Sodium Transporters**

Expressions of sodium transporter mRNAs were measured in whole kidney after 2 weeks of Ang II infusion. Figure 4 summarized the observed decreases in NHE3 (sodium–hydrogen exchanger 3) and ENaC (epithelial sodium channel) mRNA expression and unaltered mRNA expression in NKCC2 and NCC between transplanted grafts and their respective controls.

Male mice exhibited \#33\%, \#45\%, and \#40\% higher mRNA expression of NHE3 and 2 subunits of ENaC, \(\alpha\)-ENaC, and \(\gamma\)-ENaC, respectively, than female mice in the control and same-sex–KTX groups \((P<0.01\text{ } M\rightarrow M\text{ versus } F\rightarrow F\text{ and } FC\text{ versus } MC;\ n=7;\ Figure 4A, 4D, and 4F)\). Reductions of \#22\%, \#20\%, and \#24\% in NHE3, \(\alpha\)-ENaC, and \(\gamma\)-ENaC mRNAs were detected, respectively, in M→F mice compared with M→M mice. However, the expression of the NHE3, \(\alpha\)-ENaC, and \(\gamma\)-ENaC mRNA increased \#10\%, \#50\%, and \#70\%, respectively, in F→M mice compared with F→F mice. Thus, the sex differences in the expression of NHE3, \(\alpha\)-ENaC, and \(\gamma\)-ENaC mRNA were lessened post-cross-sex KTX compared with same-sex KTX \((P<0.05\text{ } F\rightarrow M\text{ versus } M\rightarrow M\text{ and } M\rightarrow F\text{ versus } F\rightarrow F;\ n=7;\ Figure 4A, 4D, and 4F)\).

NKCC2 mRNA in females \((F\rightarrow F)\) was \#5\% higher than in males \((M\rightarrow M)\) in same-sex–KTX groups \((P<0.01\text{ } M\rightarrow M\text{ versus } F\rightarrow F\text{ and } FC\text{ versus } MC;\ n=7;\ Figure 4B)\). In cross-sex–KTX groups, the mRNA level of NKCC2 in the female grafts did not change after transplanted into male recipients compared with that in female recipients. The mRNA level of NKCC2 in the male grafts in the female recipients increased to the level of female grafts in female recipients. Sex differences in NKCC2 mRNA between male and female grafts did not change in the male recipients. However, sex differences in NKCC2 mRNA between male and female grafts diminished in the female recipients. No significant sex differences in the mRNAs of NCC and \(\beta\)-ENaC have been detected in either same-sex or cross-sex–KTX groups \((n=7;\ Figure 4C\text{ and } 4E)\).

**Inflammatory Response**

Several typical inflammatory markers, including IL-6 (interleukin 6), KC (keratinocyte–derived chemokine), TGF\(\beta\) (transforming growth factor \(\beta\)), and TNF\(\alpha\) (tumor necrosis factor \(\alpha\)) were measured after Ang II infusion after KTX. Both same-sex and cross-sex–KTX groups exhibited an enhanced inflammatory response compared with control groups. Males exhibited higher levels of IL-6, KC, and TNF\(\alpha\) than females in the same-sex–KTX groups. Cross-sex KTX further stimulated the inflammation response for both males and females. In particular, the IL-6 levels were raised \#4-folds in the cross-sex–KTX groups for both male and female kidneys when they were transplanted to opposite sex recipients. There were significant sex differences in the IL-6 levels in the cross-sex–KTX groups \((P<0.01\text{ } F\rightarrow M\text{ versus } M\rightarrow M\text{ and } M\rightarrow F\text{ versus } F\rightarrow F;\ n=7;\ Figure 5A)\). No significant sex differences were found in the expression of KC and TGF\(\beta\) mRNA in cross-sex–KTX groups.
Histology

Kidney histology was assessed with light microscopy. The kidney grafts in all groups demonstrated minor tubular injuries with focal intratubular hyaline casts. The tubular injuries were more severe in the M→F cohort compared with all other cohorts. No significant interstitial fibrosis or vascular abnormalities were evident in the kidney grafts (n=7; Figure 6).

Body and Kidney Weight

After KTX, the body weight of all the mice dropped ≈10% in the first 3 days and gradually recovered in 2 weeks. At the end of the experiment, the body weight of all the groups was comparable with the mice without any operation for both sexes, respectively. The kidney weight of the M→M mice was about 32% heavier than that of F→F mice; however, the kidney weight between the M→F and the F→M
transplanted mice was similar \( (P<0.05 \text{ versus other groups; } n=7; \text{ Table S2}) \).

**Discussion**

The present study examined the role of the kidneys in sex differences in Ang II–dependent hypertension by same- and cross-sex KTX in C57BL/6 mice. The sex differences in Ang II–induced hypertension still existed after same-sex KTX. The sex differences in hypertension were attenuated after cross-sex KTX, which were accompanied by lessened or diminished sex differences in mRNA levels of AT1, AT2, AT1/AT2 ratio, ET-1, ETA, NHE3, and α and γ units of ENaC in the transplanted grafts. In addition, cross-sex KTX enhanced inflammatory response in the transplanted kidneys, reflected by significant

![Graph showing relative renal gene expression of sodium transporters](image_url)
increases in mRNA levels of IL-6, TNFα, and KC. These results indicated that kidneys play an essential role in sex differences in Ang II–dependent hypertension. Meanwhile, the host’s extrarenal environment may contribute to the sex differences in BP by modulating gene expressions and inflammatory responses in the transplanted grafts.

Sex differences in BP are observed both in humans and experimental animal models with hypertension. However, the underlying mechanisms for the sex differences in BP have not been completely elucidated. The renal renin–angiotensin–aldosterone system plays an essential role in the long-term control of arterial pressure. Chronic Ang II infusion is a widely used method for experimental hypertension. In this study, we performed KTX between same sex and cross sexes mice and induced hypertension by chronic infusion of subpressor dose of Ang II to test the significance of the kidneys in sex differences in BP regulation. Mice with same-sex KTX showed similar basal MAP compared with nontransplanted mice for both males and females, demonstrating that the transplant procedure and nephrectomy did not significantly affect BP. We observed that same-sex KTX did not change the sex differences in Ang II–induced hypertension compared with control groups. This result confirmed the previous finding that in response to Ang II infusion, males exhibit greater responses compared with females. Interestingly, we found that the cross-sex KTX attenuated the sex differences in MAP in response to the chronic infusion of Ang II. The MAP in the group of M→F was similar to that in the group of M→M. The presence of a kidney from a male donor in female recipient restored the magnitude of the hypertension to the levels similar to those observed in male mice. However, the MAP in the group of F→M was significantly higher than that in the group of F→F but still significantly lower than that in the mice with male donors (M→M and M→F groups). Abundant evidence indicated that manipulation of sex steroid hormones through gonadectomy alters the course of hypertension. Here, we uncovered the impact of the sex steroid hormones on BP under physiological conditions. These data may indicate that although the kidneys play an important role in control of sex differences in BP, other factors also contribute to the regulation of BP after cross-sex KTX.
Multiple mechanisms have been reported to contribute to the sex differences in hypertension, including vascular contractility and tubular sodium reabsorption controlled by the renin–angiotensin system, endothelin system, sodium transporters, such as NHE3, NKCC2, NCC, and ENaC, and the immune system. Previously, we demonstrated the changes in gene expression and renal function after cross-sex KTX in normotensive animals. In the present study, we investigated the sex differences in gene expression and renal function after cross-sex KTX in normotensive animals.39 In the present study, we compared the sex differences of inflammatory response to Ang II–induced hypertension by measuring the renal expression of IL-6, TNF-α, TGFβ, and KC after same-sex and cross-sex KTXs. Cross-sex KTX increased the inflammatory response to Ang II in both males and females, especially in the expression levels of IL-6 compared with same-sex KTXs. These data suggest that alterations in the extrarenal environment enhanced inflammatory responses, which may be significant in long-term graft function and could have potential translational significance in human KTXs. It should be noted that minor histocompatibility antigen rejection, in which female recipients reject grafts from the male donors because of incompatibility with the male H-Y antigen, may be one of the factors that induce higher inflammatory response in M→F group compared with other paired groups. The enhanced inflammatory response in M→F group may partially contribute to the high response of the MAP to the infusion of Ang II. Our results are in agreement with the findings from several clinical trials, in which female recipients from male donors exhibited a higher rate of graft failure.50,51 However, other clinical trials did not find the correlation between M→F group and graft function.52 These inconsistent observations may reflect the complexities in human KTX. It should be pointed out that implanted transmitters may enhance inflammatory response in the present study.

Sex steroids have been demonstrated to play an important role in modulating physiological factors in control of sex differences in BP. Ovariectomy, orchietomy, central blockade, or total knockout of estrogen or androgen receptors and administration of steroid have been applied to study the sex differences in hypertension.55,56 Although these studies have provided valuable information and advanced our understanding of sex differences in many areas, all these strategies do not accurately mimic the physiological conditions. Here, we took advantage of cross-sex KTXs to provide a real opposite sex environment for the transplanted grafts and investigated the role of the kidneys in sex differences in hypertension. We think that this approach would provide an additional tool in...
studying the significance of the kidneys in sex differences in both physiological and pathological situations.

Perspectives

The present study investigated the sex differences in Ang II–induced hypertension and assessed the expression of main components of the renin–angiotensin system, endothelin system, and tubular sodium transporters in same-sex and cross-sex–KTX mice. We demonstrated that cross-sex KTX decreased sex differences in Ang II–induced hypertension compared with same-sex–KTX and control groups. These findings indicate that although the kidneys played an important role in determining the sex differences in BP, the hosts’ extrarenal environment also contributed to the sex differences in the regulation of renal hemodynamics by modulating the gene expressions in the transplanted grafts. Apparently enhanced inflammatory response in the grafts of cross-sex–KTX groups may influence the sex differences in hypertension and could have potential translational significance in the graft function in human KTXs. The results of the present study also revealed the complexity of the mechanisms involved in regulation of the gene expression, renal function, and hemodynamics after KTX. Our findings may also provide some useful information in precision medicine based on sex for prevention and treatment of hypertension, as well as in improvement in graft function in KTXs.

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Disclosures

None.

References

What Is New?
- This study investigated the role of kidneys in sex differences in blood pressure by same-sex and cross-sex kidney transplantation in mice.

What Is Relevant?
- We demonstrated novel information in understanding the underlying physiological mechanisms of sex differences in blood pressure regulation, which may provide new approaches in precision medicine based on sex in prevention and treatment of hypertension.

Summary
Kidneys play an essential role in determining sex differences in blood pressure. The hosts’ extrarenal environment also contributes to the sex differences in the regulation of renal hemodynamics by modulating the gene expressions and inflammations in the transplanted grafts.
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The Role of Kidneys in Sex Differences in Ang II-Induced Hypertension
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METHODS

Animals

Male C57BL/6J mice, aged 10-12 weeks (22–25g), and female mice, aged 13-14 weeks (19-22g) were obtained from a vendor (Jackson Lab, Indianapolis, IN). The mice were randomly divided into 4 groups based on donor→recipient sex combinations of male (M) and female (F) mice: M→M, F→F, M→F and F→M. Male and female mice underwent unilateral nephrectomy of the right kidney were used as male (MC) and female (FC) controls.

Kidney Transplantation (KTX)

KTXs between same-sex and cross-sex mice were completed in five steps: donor nephrectomy, recipient preparation, kidney implantation, ureteral implantation and contralateral nephrectomy. The new, simple, and rapid knotless technique for vessel anastomosis was used in the transplant procedure with some slight modifications.1,2

1. Donor nephrectomy

The donor mice were anesthetized via inhalation with isoflurane (2% in air; flow 200 ml/min). A midline abdominal incision was made to fully expose the left kidney, aorta and inferior vena cava (IVC). The left ureter was isolated and cut close to the bladder. The aortic region between the left and right renal arteries was mobilized. The infrarenal IVC and aorta were separated, and a piece of 8.0 silk suture was tied loosely around the aorta. The aorta below the right renal artery, distal aorta, and IVC were successively cross-clamped with two 5 mm microvascular Yasargil clamps. The left renal vein was transected at the vena cava. One ml of cold saline was perfused through the aorta, followed by tightening the ligature around the aorta. The aorta was cut below the ligature and below the proximal clamp. The kidney and associated vessels were completely isolated and stored in saline at 4°C. The entire donor preparation was finished in 10 min. The donor mouse was then euthanized by cervical dislocation under anesthesia.

2. Recipient preparation

The recipient mice were anesthetized as described above. A midline abdominal incision was made, and the bowel was moved to the right abdomen. The left ureter was cauterized and cut. The renal artery and vein were ligated, and the left kidney was nephrectomized. We then mobilized and fully dissected the aorta and vena cava inferior to the renal artery and vein. A section of aorta and IVC was dissected and then cross-clamped with two microvascular clamps. An aortotomy was generated by cutting an elliptical patch of approximately 1 mm. The IVC was cut longitudinally with an elliptical patch of approximately 1.5 mm similar to the arteriotomy. The blood clots were thoroughly flushed out from the vessels with saline. The recipient preparation was finished in 8 min.

3. Recipient kidney implantation

The donor’s kidney was transferred from the ice into the right flank of the recipient mouse. The arterial anastomosis was performed in an end-to-side manner between the donor and recipient aorta with 10-0 Ethilon sutures. Two stay sutures were first tied at the proximal and distal apices of the recipient’s arteriotomy and the donor’s aortic cuff. The
left wall of the anastomosis was sewn continuously with two-three stitches. We turned over the donor kidney graft in the left flank of the recipient and repeated the previous procedure. A length of about 2-3 mm of the suture was left free at the pole of the arteriotomy. The venous anastomosis was achieved the same way as the arterial anastomosis. The arterial anastomosis and the venous anastomoses were finished in 20 min. The inferior and superior clamps were released sequentially to reperfuse the transplanted kidney.

4. Recipient ureteral implantation

The ureter was isolated from surrounding fat and pulled by a forceps which punctured through the bladder. We gently pulled the forceps out of the bladder and clipped the end of the ureter with a microvascular clamp. The ureter was fixed to the exterior wall of the bladder dome using a 10-0 Ethilon suture. The ureteral implantation took about 10 min. Warm saline (0.4 ml) was administrated subcutaneously after the operation. The mice were placed in an incubator with the temperature at 32 °C until fully awake. Buprenorphine (0.2 mg/kg) was administrated intramuscularly on day 0 and day 1 post operation for pain. The mice was then returned to their regular housing with free access to food and water.

5. Contralateral Nephrectomy

Four days after transplantation, the mice were anesthetized as described above and the remaining contralateral native kidneys were removed.

Radio-telemetry transmitter implantation

The native kidney was removed 4 days after KTX and the mice were allowed to recover for 3 days. Radio-telemetry transmitters (PA-C10) were implanted subcutaneously in all groups of mice as we described previously. The mice were anesthetized via inhalation of isoflurane (2% in air; flow 200 mL/min). A small incision was made in the middle of the neck, and the catheter of telemetry transmitter (PA-C10) was inserted into the left carotid artery and advanced down to the aortic arch. The body of the transmitter was placed subcutaneously in the right ventral flank of the animal. The wound was closed, and the mice were allowed to recover for 10 days. Basal MAP was measured for 20 seconds every 2 min for 5 days.

Osmotic Minipump Implantation

After basal MAP measurement for 5 days, osmotic minipumps (Alzet Osmotic Pumps 1002) filled with a subpressor dose of Ang II (600ng/kg/min) were implanted into all animals as we described previously. The animals were anesthetized with isoflurane. A small incision was made in the mid-scapular area on the animal’s back. The subcutaneous tissue was separated to create a pouch in which the minipump was inserted and the wound was closed. The MAP was measured for 14 days from the second day after minipump implantation.

Plasma Creatinine (Pcr) Measurement
PCR concentrations were measured at 4 weeks after transplantation as described previously with HPLC-mass spectrometry at the O'Brien Center at the University of Alabama at Birmingham and were expressed as milligrams per deciliter (mg.dL⁻¹).

**Glomerular filtration rate (GFR) measurement in Conscious Animals**

GFR was measured in conscious mice 4 weeks after transplantation using a single bolus intravenous injection of Fluorescein isothiocyanate-sinistrin (FITC-sinistrin) (Fresenius Kabi Austria GmbH) as we described recently with a modification. The FITC-sinistrin solution (4 μl/g body weight) was injected via retro orbital sinus of the mice under light anesthesia with isoflurane. Blood was collected (≈ 5 μl/each) from a small tail nick at the end of the tail into a heparinized microcapillary tube at 3, 7, 10, 15, 35, 55, 75 and 90 min after injection. The blood samples were centrifuged and plasma fractions (2 μl/each) were collected. FITC-sinistrin fluorescent intensities of the plasma samples were measured using a plate reader (Cytation5, BioTek, VT). GFR was calculated using a two compartment model of two-phase exponential decay (GraphPad Prism, San Diego, CA) and was presented as microliters per minute (µl.min⁻¹).

**Real-time PCR**

At the end of the experiment, we measured the mRNA levels of genes that relate to the vascular contractility and sodium reabsorption in kidney homogenate as we previously described: angiotensin II receptors (AT1R and AT2R) and endothelin system (ET-1, ETA and ETB), Na+/H+ exchanger 3 (NHE3), Na+K-2Cl cotransporter2 (NKCC2), Na+-Cl- cotransporter (NCC) and Epithelial Na+ channels (ENaC). We also measured the mRNA expression levels of following inflammatory genes: Pro-inflammation cytokines tumor necrosis factor α (TNF-α), interleukin (IL)-6, transforming growth factor beta (TGFβ) and keratinocyte-derived chemokine (KC) mRNA. The total RNA was extracted from total kidney homogenates using TRIzol. One microgram of total RNA was digested with RNase-free DNase (Promega, WI, USA), the cDNAs were synthesized with a reverse transcription system using corresponding primer sets. β-actin was used as a housekeeping gene as the reference for internal standardization. The qRT-PCR primers were designed using the primer3Plus based on the sequences deposited in the GenBank (Table 1). After qualification of the cDNA template, Quantitative PCR analysis was performed using iQ SYBR Green Supermix (iTa SYRB, Bio-Rad, CA, USA) and CFX96 Real-Time Detection System (Chromo4, Bio-Rad, CA, USA) according to the manufacturer's protocol. Reaction conditions were set as follows: 95 °C for 1 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s. The reaction of each sample was performed in triplicate. Dissociation analysis was performed at the end of each PCR reaction to confirm the amplification specificity. After the PCR program, data were analyzed and quantified with the comparative Ct method (2−ΔΔCt) based on Ct values for complement genes and β-actin to calculate the relative mRNA expression level.

**Histology**

Histologic examination of all kidney grafts was performed at the end of the experiments by an experienced renal pathologist (L. Fu) blinded to the experimental procedures. Kidneys were flushed with 1x phosphate-buffered saline solution through left ventricle until they turned to grayish-white. Kidneys were then removed and dissected on
the longitudinal axis. For each kidney, half of the kidney sample was used for gene expression measurement and the other half of the kidney sample was preserved and fixed in 4% paraformaldehyde solution for at least 24 hrs and then embedded in paraffin wax, cut into 3µm sections and stained with Periodic Acid Schiff (PAS). Kidney injury was evaluated based on percentage of necrotic tubules analyzed as described previously.  

Reference List


### Table S1. Primers

<table>
<thead>
<tr>
<th></th>
<th>β-actin</th>
<th>AT1</th>
<th>AT2</th>
<th>ET-1</th>
<th>ETa</th>
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<th>βENaC</th>
<th>γENaC</th>
<th>IL-6</th>
<th>KC</th>
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<th>TNFα</th>
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<td>TTACCAGCAGCCGTCTTTT-3'</td>
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### Table S2. Body weight (BW) and kidney weight (KW)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>M→M</th>
<th>M→F</th>
<th>F→F</th>
<th>F→M</th>
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<tbody>
<tr>
<td>BW (g)</td>
<td>27.8±0.3</td>
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<td>22.1±0.3</td>
<td>25.4±0.5</td>
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<td>KW (g)</td>
<td>0.2954±0.03*</td>
<td>0.2353±0.02</td>
<td>0.1984±0.03</td>
<td>0.2206±0.04</td>
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</tbody>
</table>
*p<0.05 vs. other groups in KW

**Fig S1. Kidney graft functional assessment**

**A. plasma creatinine**

Plasma creatinine (mg/dL)

0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
0.18

M   M M   F    F    FF   MC FC

**B. GFR**

GFR (ul/min)

0
50
100
150
200

M   M M   F    F    FF   MC FC

Plasma creatinine and conscious GFR were measured 4 weeks after transplantation. **A**: All mice showed normal plasma creatinine values; **B**: Conscious GFR (comparison between donor kidneys). Male grafts showed higher GFR than female grafts.
(*\(p<0.01\) vs. FC; \#\(p<0.01\) vs. F→F and F→M; \%\(p<0.01\) vs. F→F; \(n=7\); by 1-way ANOVA). There were no differences among the same sex grafts for both males and females. (donor→recipient; M: male, F: female).

**Fig S2. Plasma sex steroid levels**

**A. Plasma testosterone levels**

![Plasma testosterone levels chart](chart)

**B. Plasma estradiol levels**

![Plasma estradiol levels chart](chart)

**A. Plasma testosterone levels (**\(p<0.01\) vs. female recipients); B. Plasma estradiol levels (**\(p<0.01\) vs. male recipients) (\(n=7\); donor→recipient; M: male, F: female)**
Fig S3. MAP

A. Basal MAP and MAP response to Ang II infusion for male mice

B. Basal MAP and MAP response to Ang II infusion for female mice
A. Same-sex KTX in males and male control group. (*p<0.01 vs. basal); B. Same-sex KTX in females and female control group. (*p<0.01 vs. basal) (n=7; by Student paired t-test; donor→recipient; M: male, F: female)