ウェブサイト公開用

（様式 10）
（海外特別研究員事業）
令和　3 年　4 月　30 日

海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

採 用 年 度　平成 31 年度 (2019 年度)
受 付 番 号　201960537
氏　　名　渡辺博文

（氏名は必ず自署すること）

海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：　ヴァージニア大学　（米国）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
レニン細胞の核メカノトランスダクション機構の探索と恒常性維持機能の解明

3. 派遣期間：平成　31　年　4　月　1　日　～　令和　3　年　3　月　31　日

4. 受入機関名及び部局名
受入機関名：　University of Virginia School of Medicine
部局名：　Department of Pediatrics, Child Health Research Center

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

REPORT

Grant Title: Nuclear mechanotransduction of renin cells in homeostasis
Hirofumi Watanabe, M.D., Ph.D.

[Aim. 1] Test the hypothesis that the changes in perfusion pressure sensed by renin cells and/or its descendants result in unique and specific changes in chromatin architecture.

We have established our *in vivo* high and low perfusion pressure model. We perform the surgery of the aortic coarctation (AoCo) between the roots of the right and left renal arteries. In this model, the right and left kidneys receive high and low perfusion pressure, respectively, resulting after 72 hours in 10 mmHg differential between upper and lower body blood pressure. This results in a marked difference in renin expression between the right and left kidneys (Figure 1).

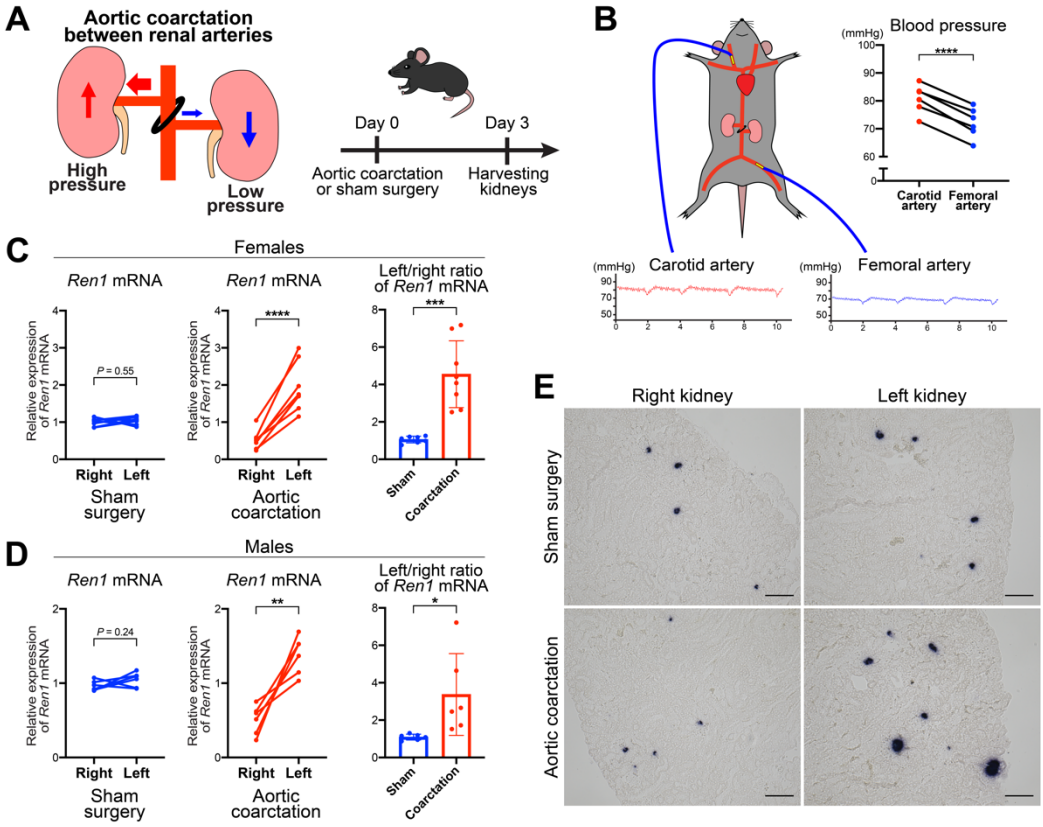

Figure 1. Changes in perfusion pressure induce modifications in *Ren1* expression. A, Schematic of the aortic coarctation (AoCo) surgical model. The ligation between the roots of the right and left renal arteries results in an increase in perfusion pressure in the right kidney and a decrease in the left kidney. Kidneys were harvested 72 hours after the AoCo or sham surgeries. **B,** Simultaneous blood pressure (BP) measurement under anesthesia from the carotid and femoral arteries of mice with AoCo. The BP was significantly different between upper arteries (carotid arteries) and lower arteries (femoral arteries) of AoCo (n=6, paired t-test). **C,** Changes in *Ren1* expression in kidneys subjected to AoCo in female mice by quantitative reverse transcription PCR (qRT-PCR) showed a significant difference in *Ren1* mRNA between the right and left renal cortices from mice with AoCo (n=8, paired t-test), whereas *Ren1* mRNA was not different between right and left renal cortices from mice with sham surgeries (n=7, paired t-test). With AoCo, the left/right ratio of *Ren1* mRNA was significantly higher than the one of sham surgeries (Student's t-test). **D,** Changes in *Ren1* expression in kidneys subjected to AoCo in male mice by qRT-PCR showed a significant difference in *Ren1* mRNA between the right and left renal cortices from mice with AoCo (n=6, paired t-test), whereas *Ren1* mRNA was not different between right and left renal cortices from mice with sham surgeries (n=6, paired t-test). With AoCo, the left/right ratio of *Ren1* mRNA was significantly higher than the one of sham surgeries (Student's t-test). **E,** *In situ* hybridization for *Ren1* mRNA in the kidneys. The intensity and extension of the *Ren1* mRNA signals at the juxtaglomerular areas were not different between the right and left kidneys from mice with sham surgery. With AoCo, signals in right kidneys were decreased, and signals in left kidneys were significantly increased, compared to sham surgeries. Scale bar, 100 µm. All data are reported as means ± standard deviation. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

To investigate the transcriptional profile changes in the renin cells occurring in response to perfusion pressure changes, we conducted transcriptome analysis. We performed the AoCo surgeries with *Ren1^{c}-YFP* mice. These mice express YFP under the control of 5 Kb of the 5' regulatory region of the renin gene. After 72 hours of AoCo, we isolated YFP-positive cells with fluorescence-activated cell sorting and performed RNA-seq.

After the differential gene expression analysis, we found 108 genes that are highly expressed in the renin cells from the left kidneys and 94 genes that are highly expressed in the cells from the right kidneys (Figure 2).

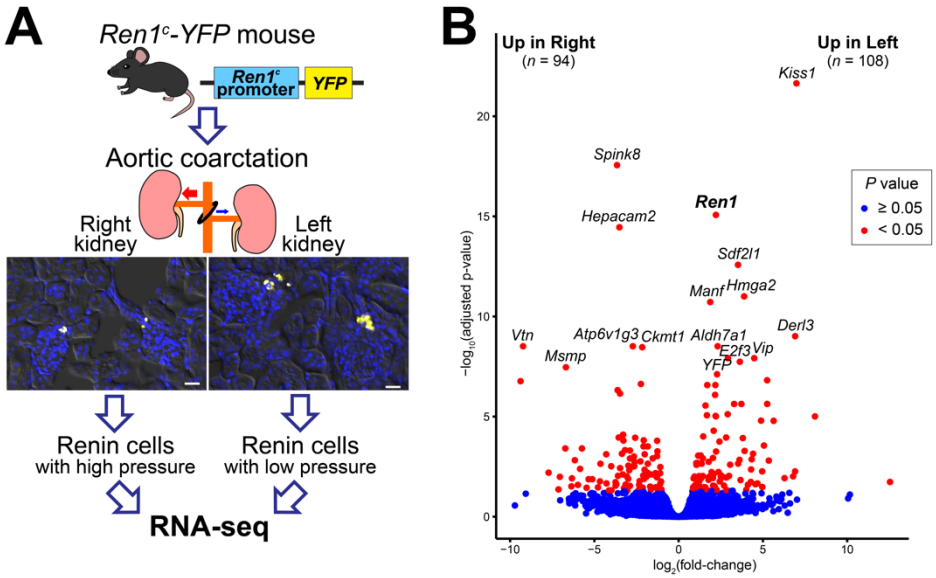

Figure 2. Renin cells change their transcription programs in response to changes in perfusion pressure. A, RNA-seq of renin cells from the kidneys subjected to aortic coarctation (AoCo) surgery. YFP-positive cells from *Ren1^{c}-YFP* mice were isolated using fluorescence-activated cell sorting. Each kidney cortex was processed separately. Scale bar, 20 μm. **B,** Volcano plot of RNA-seq analysis. The 202 differentially expressed genes between renin cells in the right kidneys and left kidneys were shown in red.

[Aim. 2] Test the hypothesis that integrin β1 is a part of the mechanosensor that controls renin cell identity via SE formation.

To test whether integrin β1 plays a role in renin cells in response to changes in perfusion pressure, we used a conditional knockout mouse model of integrin β1 in cells of the renin lineage (*Itgb1cKO*), which we recently generated. We performed AoCo surgeries on *Itgb1cKO* mice at 4 weeks of age. When subjected to AoCo, the decrease in *Ren1* mRNA expression in the right kidneys and the increase in *Ren1* mRNA in the left kidneys were significantly blunted in *Itgb1cKO* mice when compared to controls. *In situ* hybridization for *Ren1* mRNA showed that *Ren1* expression did not change in response to the AoCo in the *Itgb1cKO* mice when compared to control or sham groups (Figure 3).

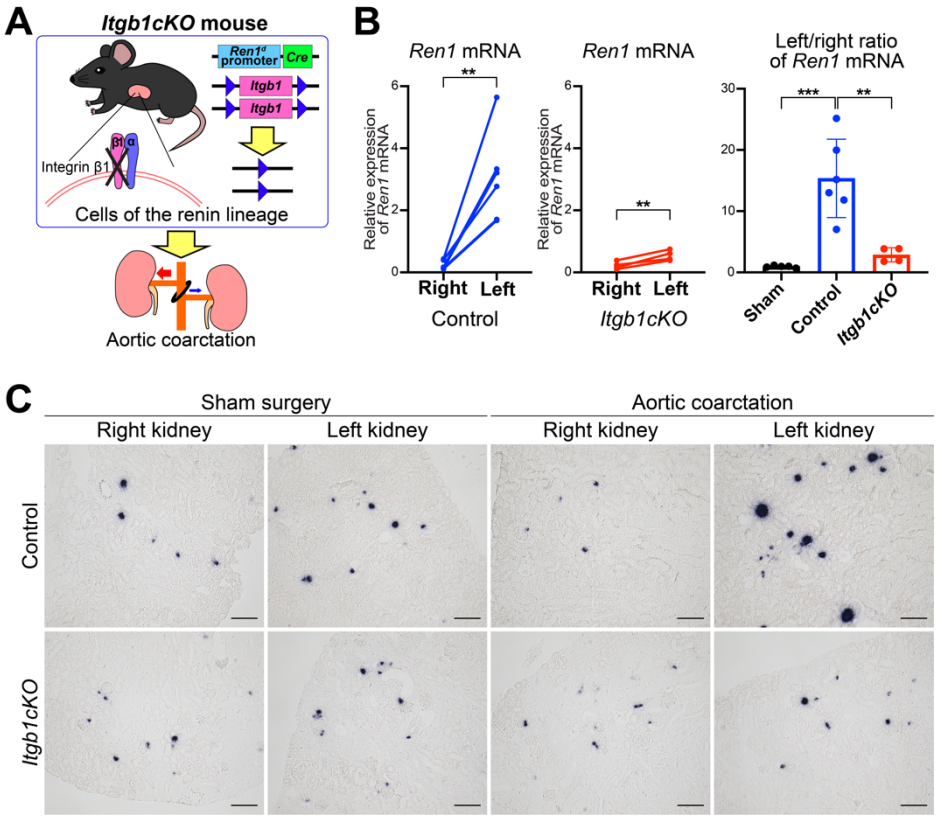

Figure 3. *Itgb1* gene knockout in cells of the renin lineage inhibited the response to changes in perfusion pressure. A, Mice with conditional deletion of the *Itgb1* gene in cells of the renin lineage (*Itgb1cKO*) and control mice were subjected to aortic coarctation (AoCo). **B,** *Itgb1cKO* mice showed impaired responses in *Ren1* expression to changes in perfusion pressure. Quantitative reverse transcription PCR showed significant differences in *Ren1* mRNA between the right and left renal cortices from the control mice (n=6, paired t-test) and from the *Itgb1cKO* mice (n=4, paired t-test), respectively. The left/right ratio of *Ren1* mRNA of *Itgb1cKO* mice was significantly lower than the one of control animals (one-way ANOVA followed by Tukey's multiple comparison test). **C,** *in situ* hybridization for *Ren1* mRNA in the kidneys from *Itgb1cKO* mice subjected to the AoCo. For Itgb1cKO mice, the decrease in *Ren1* mRNA in the right kidneys and the increase in that in the left kidneys were milder than those in control mice, respectively. Scale bar, 100 μm. All data are reported as means ± standard deviation. $^{**}P<0.01$, $^{***}P<0.001$.

[Aim. 3] Test the hypothesis that Lamin A regulates chromatin remodeling and the formation of SEs in renin cells in response to changes in perfusion pressure.

To investigate the role of lamin A/C in renin cells, we generated mice with conditional deletion of the *Lmna* gene in cells of the renin lineage (*LmnacKO*) by crossing *Ren1*^{dCre} mice and *Lmna* floxed mice. We confirmed the deletion of the *Lmna* gene using fluorescence ISH for *Lmna* mRNA and *Ren1* mRNA and immunohistochemistry and immunofluorescence staining for lamin A/C and renin. *Lmna* mRNA and lamin A/C were clearly observed in renin expressing cells at JG areas in the control mice, whereas *LmnacKO* mice did not show signals in renin expressing cells. *LmnacKO* mice were born at the expected mendelian ratio, developed normally, and showed no alterations in renal function, renin expression, or kidney morphology under basal conditions. To test how renin cells lacking lamin A/C react to changes in perfusion pressure, we performed AoCo on *LmnacKO* mice. AoCo induced significant differences in *Ren1* mRNA between the right and left renal cortices in both control and *LmnacKO* mice. The expression changes shown by the left/right ratio of *Ren1* mRNA were significantly lower in *LmnacKO* mice than those of control mice (in both female and male mice). ISH showed that the decrease in *Ren1* mRNA at JG areas in the right kidneys and the increase in the left kidneys were milder in the *LmnacKO* mice than those of the control mice, respectively. Taken together, *LmnacKO* mice showed a diminished ability to respond to changes in perfusion pressure resulting from AoCo, underscoring the role of lamin A/C in transcriptional regulation of the renin gene in response to changes in perfusion pressure (Figure 4).

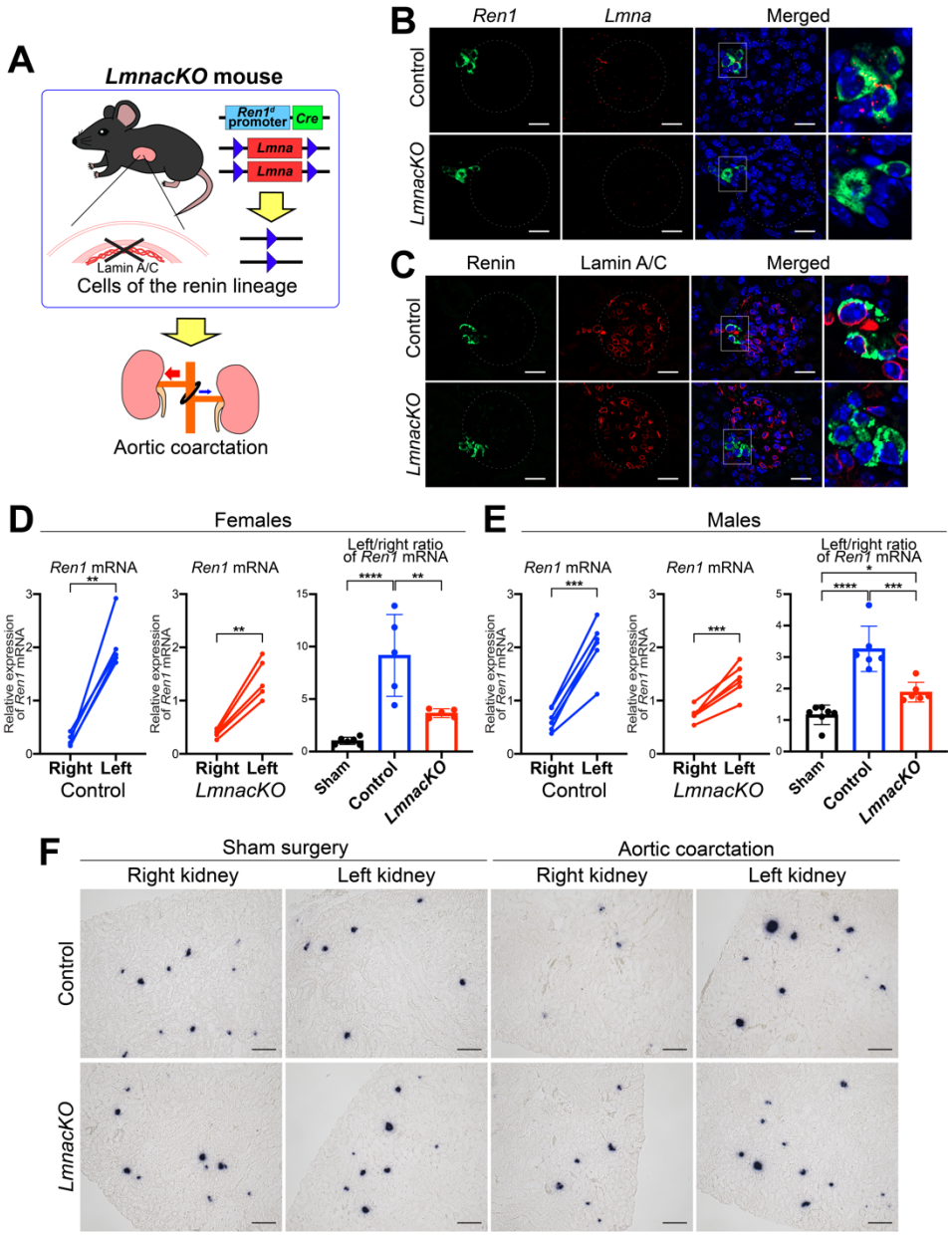

Figure 4. *Lmna* gene knockout in cells of the renin lineage inhibited the response to changes in perfusion pressure. A, Mice with conditional deletion of the *Lmna* gene in cells of the renin lineage (*LmnacKO*) and their controls were subjected to aortic coarctation (AoCo). **B,** Fluorescence *in situ* hybridization (ISH) for *Ren1* mRNA (green), *Lmna* mRNA (red), and DAPI (blue). *Lmna* was highly expressed in the renin-expressing cells in the control mice. There was no *Lmna* expression in renin-expressing cells in *LmnacKO* mice. Dashed circles indicate glomeruli. Scale bar, 20 μm. **C,** Immunofluorescence staining for renin (green), lamin A/C (red), and Hoechst (blue). Lamin A/C was detected in the nuclei of renin cells in control mice and absent in the nuclei of renin cells in *LmnacKO* mice while preserved in non-renin cells. Dashed circles indicate glomeruli. Scale bar, 20 μm. *LmnacKO* showed impaired responses of *Ren1* expression to changes in perfusion pressure in both female (**D**) and male mice (**E**). Quantitative reverse transcription PCR showed significant differences in *Ren1* mRNA between the right and left renal cortices from control mice (n=5 for females and n=6 for males, paired t-test) and from *LmnacKO* mice (n=5 for females and n=6 for males, paired t-test). The left/right ratio of *Ren1* mRNA from *LmnacKO* mice was significantly lower than the one from control animals (one-way ANOVA followed by Tukey's multiple comparison test). **F,** ISH for *Ren1* mRNA in kidneys from *LmnacKO* mice subjected to the AoCo. In *LmnacKO* mice, the decrease in *Ren1* mRNA in the right kidneys and the increase in the left kidneys were milder than those from the control mice, respectively. Scale bar, 100 μm. All data are reported as means ± standard deviation. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

We report the results including above in the following paper, which was accepted in the journal *Circulation Research*.

Watanabe H, Belyea BC, Paxton RL, Li M, Dzamba BJ, DeSimone DW, Gomez RA, Sequeira-Lopez MLS. The Renin Cell Baroreceptor, a Nuclear Mechanotransducer Central for Homeostasis. *Circ Res.* 2021. *in press.*

Other projects the JSPS fellow performed –Study of β1-Integrin in renin cells–

Renin cells and juxtaglomerular cells are crucial for blood pressure and fluid-electrolyte homeostasis. Renin cells receive constant cell-to-cell, mechanical, and neurohumoral stimulation that maintain their identity and function. Integrins are the largest family of cell adhesion molecules that mediate cell-to-cell and cell-to-matrix interactions. To test the hypothesis that cell-matrix interactions are fundamental not only to maintain the identity and function of juxtaglomerular cells but also to keep them alive, we deleted β1-integrin *in vivo* in cells of the renin lineage. In mutant mice, renin cells died by apoptosis, resulting in decreased circulating renin, hypotension, severe renal-vascular abnormalities, and renal failure (Figure 5, 6).

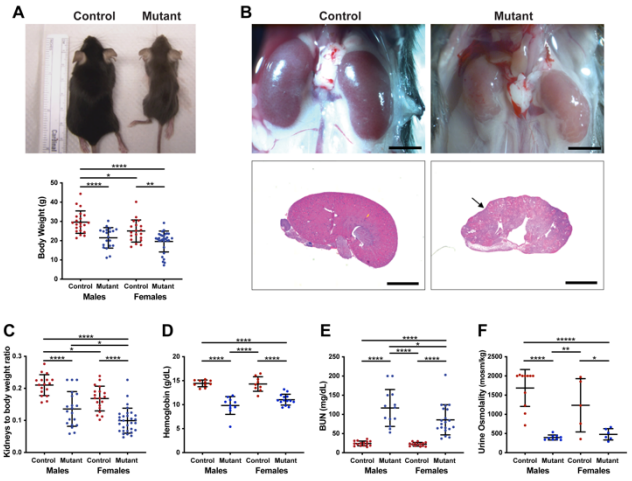

Figure 5. ***Itgb1*** **mutant mice had failure to thrive, small fibrotic kidneys, hydronephrosis, anemia, renal failure, and hyposthenuria.** Mutant mice had smaller body size and significantly lower body weight (**A**). Kidneys of mutant mice showed pale and irregular surface (scale bars=2 mm) and by hematoxylin and eosin staining cortical concavities, poor corticomedullary differentiation, and hydronephrosis (scale bars=1 mm; **B**). Mutants had significantly lower kidney weight/body weight (**C**), lower hemoglobin levels (**D**), higher blood urea nitrogen (BUN) levels (**E**), and lower urine osmolality (**F**). One-way ANOVA with Tukey multiple comparison post test. Only significant differences are displayed: *P<0.05, **P<0.01, ****P<0.0001.

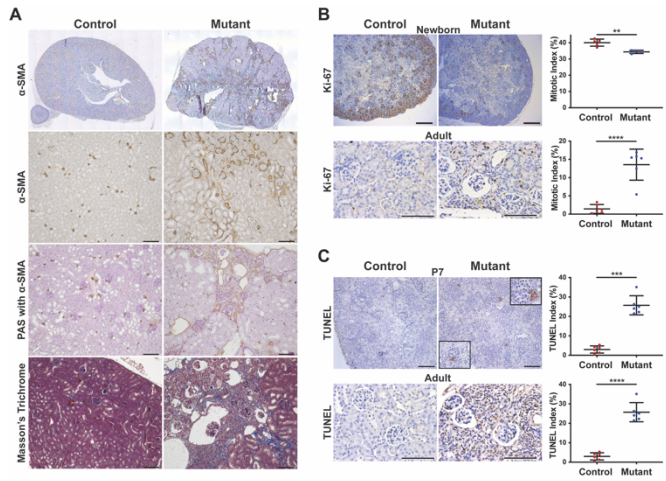

Figure 6. Lack of β1-integrin in renin cells leads to renal fibrosis with increased apoptosis. Immunostaining for α-SMA (alpha smooth muscle actin) (brown) showing at lower magnification the overall fibrotic bands extending from the cortex to the medulla in the mutant kidney. At higher magnification, α-SMA was present in the walls of arterioles and arteries in the control kidney, while mutants showed excessive peritubular, periglomerular, and interstitial deposition of α-SMA. Periodic Acid Schiff (PAS) combined with α-SMA immunohistochemistry showed normal tubules, glomeruli, and α-SMA distribution in control kidneys but fewer arterioles, dilated tubules, and fibrocystic glomeruli in mutants. Masson trichrome staining showed, in mutants, excessive collagen deposition in the interstitium, indicating diffuse interstitial fibrosis (**A**). Immunostaining for Ki-67 showed positive areas of proliferation in the nephrogenic zone of newborn control kidneys and a marked decrease in mutants, as well as the mitotic index. In adults, both immunostaining for Ki-67 and the mitotic index showed few proliferative cells in control kidneys and diffuse proliferation in mutants (**B**). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays in postnatal day 7 (P7) kidneys showed excessive apoptosis in mutants, especially in the classical juxtaglomerular areas (insets). Adult mutant kidneys also had significantly increased apoptosis (**C**). Scale bars=100 μm. Student *t* test, **P<0.01, ***P<0.001,****P<0.0001.

Results indicate that cell-to-cell and cell-to-matrix interactions via β1-integrin is essential for juxtaglomerular cells survival, suggesting that the juxtaglomerular niche is crucial not only for the tight regulation of renin release but also for juxtaglomerular cell survival—a sine qua non condition to maintain homeostasis.

We have reported these findings in the following paper.

Mohamed TH, **Watanabe H**, Kaur R, Belyea BC, Walker PD, Gomez RA, Sequeira-Lopez MLS. Renin-Expressing Cells Require β1-Integrin for Survival and for Development and Maintenance of the Renal Vasculature. *Hypertension*. 2020 Aug;76(2):458-467.

Other projects the JSPS fellow performed –Study of *Acsm2*–

Acyl-CoA synthetase medium-chain family member 2 (*Acsm2*) gene was first identified and cloned by our group as a kidney-specific "KS" gene. However, very little has been reported on *Acsm2*, and the expression pattern and function of this gene remain to be clarified.

We found that the *Acsm2* gene is specifically expressed in proximal tubules, and not in other tissues. The expression of *Acsm2* paralleled the structural and functional maturation of proximal tubular cells (Figure 7).

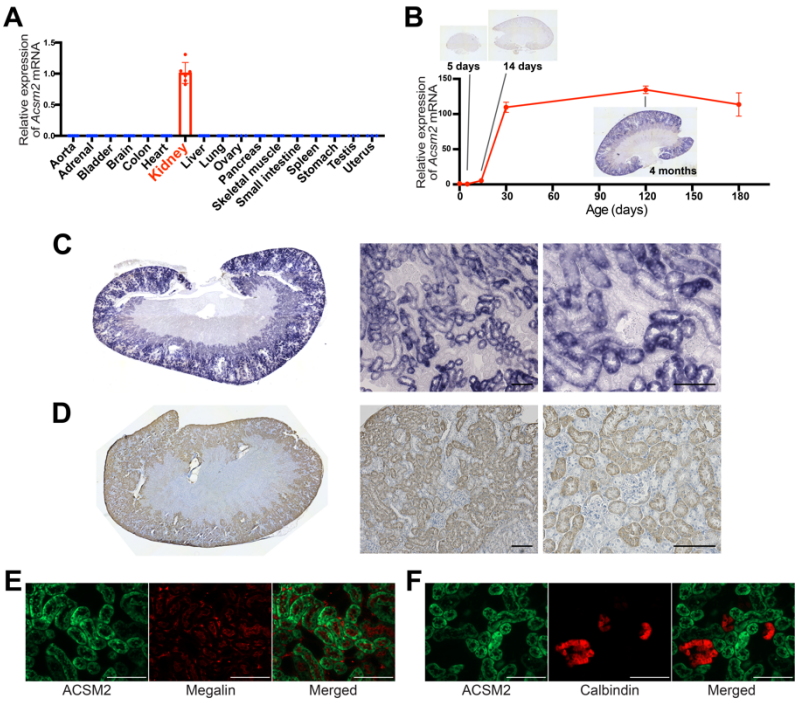

Figure 7. Expression of acyl-CoA synthetase medium-chain family member 2 (*Acsm2*) is restricted in proximal tubular cells in kidneys. A, Quantitative RT-PCR for *Acsm2* mRNA with multiple organs from adult wild-type C57/Bl6 mice showed the specific expression of *Acsm2* in the kidneys [*n*=3 for sex-specific organs, *n*=6 for other organs (from 3 male and 3 female mice)]. Data are presented as means ± SD. **B,** The expression level of *Acsm2* in the kidneys was very low at birth, increased with animal growth, and reached a plateau by 2 mo of age, which was shown with quantitative RT-PCR for *Acsm2* mRNA at multiple ages in male wild-type C57/Bl6 mice (*n*=6 at *postnatal day 0*, *n*=3 per group at other ages) and in situ hybridization. **C,** *In situ* hybridization showed the high expression of *Acsm2* in the kidneys from adult wild-type mice. Expression of *Acsm2* mRNA was observed in proximal tubular cells. Bars=100 μm. **D,** Immunohistochemistry for ACSM2 protein showed signals restricted in the tubular cells in adult wild-type mice. Bars=100 μm. **E,** Immunofluorescence staining showed that ACSM2 exists in megalin-positive proximal tubular cells. Bars=100 μm. **F,** Immunofluorescence staining showed that ACSM2 is not expressed in cells positive for calbindin-D-28K. Bars=100 μm.

We also found that following acute kidney injury, partial unilateral ureteral obstructions, and chronic kidney diseases, the *Acsm2* expression in the proximal tubules was decreased significantly (Figure 8).

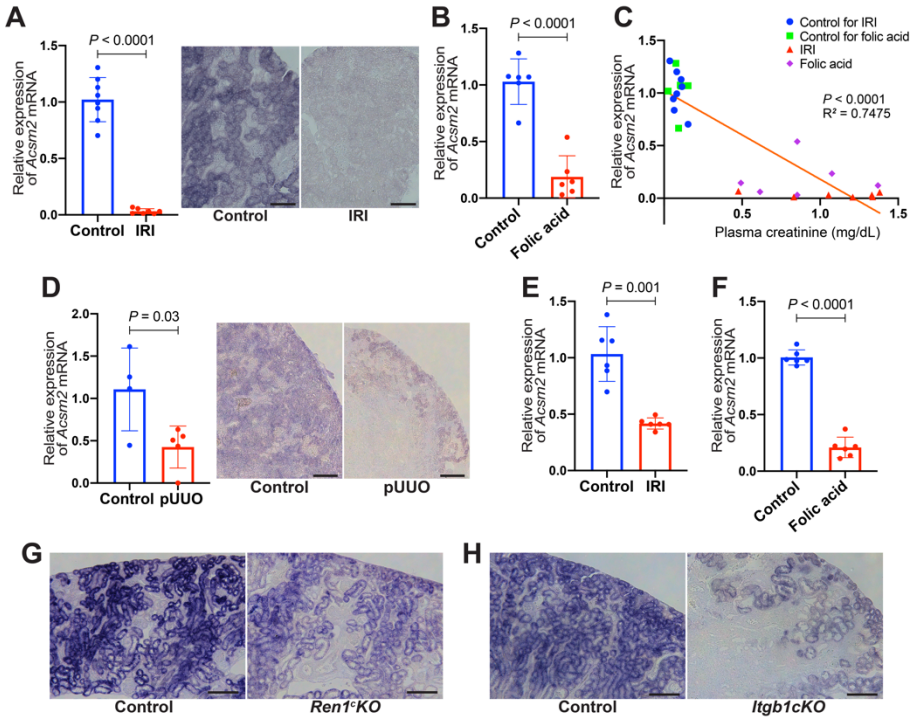

Figure 8. Acyl-CoA synthetase medium-chain family member 2 (*Acsm2*) expression decreases in multiple types of mouse kidney disease models. A, *Acsm2* gene expression was decreased in the kidneys with acute kidney injury (AKI) by bilateral kidney ischemia-reperfusion injury (IRI). Quantitative RT-PCR was performed (*n*=8 control; *n*=7 IRI). *In situ* hybridization showed lower expression of *Acsm2* in proximal tubular cells of kidneys subjected to bilateral IRI compared with control. Bars=100 μm. **B,** Quantitative RT-PCR showed a decrease in *Acsm2* mRNA expression in kidneys with AKI 24 h after injection of folic acid (*n*=6 each). **C,** There was a negative correlation between *Acsm2* expression in the kidney and plasma creatinine. The x-axis shows plasma creatinine levels (in mg/dL), whereas the y-axis shows relative expression of *Acsm2* mRNA. Each dot represents the values (creatinine, mRNA) obtained from each individual animal belonging to the AKI models and their controls. The line is the fitted correlation value. **D,** *Acsm2* mRNA expression by quantitative RT-PCR in kidneys was decreased following partial unilateral ureteral obstruction (pUUO; *n*=4 control, *n*=5 pUUO). *In situ* hybridization showed lower expression of *Acsm2* in tubular cells from kidneys subjected to pUUO. Bars=200 μm. **E,** Quantitative RT-PCR showed a decrease in *Acsm2* mRNA expression in the kidneys with chronic kidney disease, 14 days after unilateral IRI (*n*=6 each). **F,** Quantitative RT-PCR showed a decrease in *Acsm2* mRNA expression in another chronic kidney disease model at 14 days after injection of folic acid (*n*=6 each). **G,** Expression of *Acsm2* mRNA in proximal tubular cells of kidneys of *Ren1^c* gene knockout (*Ren1^{c}KO*) mice was lower than that of control. Bars=200 μm. **H,** Conditional knockout of the β1-integrin (*Itgb1*) gene in cells of kidneys from the renin progeny (*Itgb1cKO*) showed lower expression of *Acsm2* in proximal tubular cells compared with control. Bars=200 μm. All data are reported as means ± SD. Statistical comparison was by Student's t test.

These results indicate that the expression of *Acsm2* parallels the structural and functional maturation of proximal tubular cells. Downregulation of its expression in several models of kidney disease suggests that *Acms2* may serve as a novel marker of proximal tubular injury and/or dysfunction.

We have reported these findings in the following paper.

Watanabe H, Paxton RL, Tolerico MR, Nagalakshmi VK, Tanaka S, Okusa MD, Goto S, Narita I, Watanabe S, Sequeira-Lopez MLS, Gomez RA. Expression of Acsm2, a kidney-specific gene, parallels the function and maturation of proximal tubular cells. *Am J Physiol Renal Physiol.* 2020 Oct 1;319(4):F603-F611.