TRS Translational and Regulatory Sciences

Translat Regulat Sci. 2(1): 30–35, 2020; doi: 10.33611/trs.2_30

SHORT COMMUNICATION

Experimental Animal

Long terminal repeat insertion in *Kit* causes unilateral renal agenesis in rats

Shiori ARA[1], Ayako MIYAZAKI[1], Hieu HOANG TRUNG[1], Mayuko YOKOE[2], Yuki NAKAGAWA[2,3], Takehito KANEKO[2,3] and Takashi KURAMOTO[1,2]*

[1] Laboratory of Animal Nutrition, Department of Animal Science, Faculty of Agriculture, Tokyo University of Agriculture, 1737 Funako, Atsugi, Kanagawa 243-0034, Japan
[2] Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Yoshidakonoe-cho, Sakyo-ku, Kyoto 606-8501, Japan
[3] Department of Chemistry and Biological Sciences, Faculty of Science and Engineering, Iwate University, 4-3-5 Ueda, Morioka, Iwate 020-8551, Japan

Abstract. Human unilateral renal agenesis (URA) is a urinary malformation characterized by congenital absence of one kidney. The inbred rat strain ACI is a known animal model for studying URA. Recently, a single locus responsible for URA, designated renal agenesis 1 (*Renag1*), has been mapped to a 379-kb interval that contains a single gene, *Kit*, which encodes c-kit that plays important roles in stem cell survival, migration, and differentiation. Within the *Renag1* interval, an insertion of a long terminal repeat (LTR) sequence has been found as a major variation specific for the ACI genome, and the LTR has been strongly suggested to be a causative factor of URA. Here, we removed the LTR from the ACI *Kit* gene by the CRISPR/Cas9 system and examined whether these gene-modified rats exhibited URA. Three gene-modified ACI strains were developed that lacked the LTR and flanking sequences of different lengths. Out of a total of 125 gene-modified rats observed, none of the rats exhibited URA. Besides URA, abdominal white spotting, Irish, has also been mapped to the *Renag1* locus. We also observed that the gene-modified ACI rats did not exhibit Irish spotting. Thus, we concluded that the LTR was causative of both URA and Irish spotting in ACI rats. This study suggests that the *Kit* signaling pathway plays an important role in kidney development and that ACI rats would be a promising animal model for regenerative medicine therapy of kidney diseases.
Key words: ACI rats, CRISPR/Cas, *Kit*, long terminal repeat, unilateral renal agenesis

Highlights

Human unilateral renal agenesis (URA) is a urinary malformation characterized by congenital absence of one kidney. The inbred rat strain ACI is a known animal model for URA, and a long terminal repeat (LTR) sequence within the ACI *Kit* gene has been suggested to be a causative variant of URA. We removed the LTR from the ACI *Kit* gene by the CRISPR/Cas9 system and found that gene-modified ACI rats were rescued from URA. These findings indicated that the LTR caused URA in ACI rats and suggested that the *Kit* signaling pathway plays an important role in kidney development.

Introduction

Unilateral renal agenesis (URA) is a form of renal agenesis characterized by the complete absence of one kidney, accompanied by an absent ureter. URA is a relatively common congenital urinary malformation, giving the incidence of 1 in ~2000 [1, 2]. Patients with URA are usually asymptomatic; however, it is becoming clear that the solitary kidney increases the risk of chronic kidney diseases and hypertension [3]. The etiology of URA remains unclear, but genetic factors have been thought to be largely involved.

Kidney development depends on a series of sequential and reciprocal inductive interactions between epithelial cells and mesenchyme [4]. The ureteric bud that arises from the wolffian (mesonephric) duct stimulates the metanephric blastema at an early embryonic stage. This process initiates kidney organogenesis and leads to formation of the kidney. Therefore, problems with formation of the wolffian duct and ureteric bud, or degeneration of the ureteric bud, result in subsequent renal abnormalities including agenesis.

So far, the most important genes identified to be involved in human murine kidney development are *RET*, *GDNF*, *WT1*, *EYA1*, and *PAX2* [5]. Indeed, mutations of these genes have been identified in some URA families [6, 7]. Mice carrying mutations of *Ret*, *Gdnf*, and *Eya1* exhibited the URA phenotype [6, 8, 9]. However, majority of the causes of URA are still unknown. For example, next generation sequencing analysis of rare variants in 208 candidate genes has recently suggested that the genetic architecture of URA can be more complex than previously suggested [10]. Thus,

*Correspondence to: Kuramoto, T.: tk206782@nodai.ac.jp
Received: Jan. 20, 2020; Accepted: Jan. 28, 2020

identification of causative genes of URA is required to understand the genetic components involved in URA. To identify such genes, animal models exhibiting congenital kidney malformation have been useful.

The ACI rat spontaneously exhibits URA and associated urogenital anomalies at an incidence of approximately 10–15%. In addition to URA, ACI rats generally exhibit the absence of accessory reproductive organs, such as the uterine horn, vas deferens, and epididymis ipsilateral to the missing kidney [11]. URA and associated urogenital anomalies are inherited as an incompletely dominant trait with incomplete penetrance [12]. Besides URA, ACI rats exhibited abdominal white spotting, or Irish spotting.

A locus responsible for URA has been mapped to the rat chromosome 14 and designated renal agenesis 1 (*Renag1*) [12]. Genetic linkage analysis and haplotype mapping using congenic strains localized *Renag1* to a 379 kb interval that contained a single protein coding gene, *Kit* [13]. Within the *Renag1* interval, a long terminal repeat (LTR) sequence has been strongly suggested to be causative of the Irish spotting [14]. Interestingly, a congenic strain which harbored the ACI allele at *Renag1* on the genetic background of BN strain exhibited both URA and Irish spotting, which suggested the causal variants locate to the *Renag1* interval. Thus, we hypothesized that the LTR was causative of both URA and Irish spotting. In the present study, to prove our hypothesis, we removed the LTR from the ACI *Kit* gene by the CRISPR/Cas9 system and examined whether or not the gene-modified rats exhibited URA and Irish spotting.

Materials and Methods

Animals

ACI/NKyo rats were obtained from the National BioResource Project for the Rat (Kyoto, Japan) [15]. Jcl:Wistar rats were obtained from CLEA Japan, Inc. (Tokyo, Japan). All animals were housed in plastic cages with free access to drinking water and basal diet, under controlled conditions of humidity (50 ± 10%), lighting (12 hr light/dark cycle) and temperature (25 ± 2°C). All animal experiments were approved by the Animal Research Committees of Kyoto University, Iwate University, and Tokyo University of Agriculture and were conducted according to their regulations on animal experimentation.

Genome editing

Genome editing by CRISPR/Cas was performed as described previously [16]. Two crRNA target sequences (5′-TTTGTAAGTATGCAGCTGAG-3′ and 5′- AGCAGCTAGTACCTCTACACT-3′) were chemically synthetized. Pronuclear-stage embryos of ACI/NKyo rats were produced by natural mating. The oviducts of female rats with vaginal plugs were removed after euthanasia by CO_2 and cervical dislocation, and oocytes were flushed out from the ampullae with culture medium. Cas9 protein, chemically synthesized custom crRNA, tracrRNA (Integrated DNA Technologies, Inc., Coralville, IA, USA), and single-stranded oligo DNA nucleotides (ssODNs) were microinjected into intact rat embryos. Embryos that developed to the two-cell stage after the microinjection were transferred into the oviducts of pseudopregnant female Jcl:Wistar rats that were anesthetized using isoflurane.

Genotyping

Genomic DNA was isolated from tail or ear tips by KAPA Express Extract Kit (Merck KGaA, Darmstadt, Germany). PCR was performed to find founder rats using a pair of primers; 5′-CGAAGCAGGCATTAGGTAAGA-3′ and 5′-TGCGGACTCTTCTTCAAGGT-3′. To identify regions of genomic deletion induced by the CRISPR/Cas9 system, we used the following primers: Kit-int1-05; 5′-TAGACTCCAGGCCACAGACA-3′, Kit-Irish-ACI-4; 5′- TGCGGACTCTTCTTCAAGGT-3′, Kit-int1-10; 5′- AGAGATGGGCTCACCAAATG-3′, and Kit-int1-12; 5′- CCCCACTCCCGAGAACTT-3′.

Phenotyping

Rats were euthanized under deep anesthesia using isoflurane and examined for the presence of urogenital anomalies, such as agenesis and hydronephrosis. Anomalies of the accessory reproductive organs and abdominal white spotting were also examined.

Results

Development of gene-modified ACI strains

We transferred 63 microinjected embryos to pseudopregnant rats and obtained 11 offspring. Among these, three offspring had a genomic deletion of the expected size (Δ685). One had a smaller deletion than expected. Five offspring exhibited no PCR products. We selected 3 offspring as founder rats: one having the expected genomic deletion (Δ685) and the others exhibiting no PCR products. We expected that the latter had larger deletions than expected. After crossing with ACI/NKyo rats, we selected rats homozygous for deletions and established three gene-modified ACI strains: ACI-*Kit*^{em1}/Kyo, ACI-*Kit*^{em2}/Kyo, and ACI-*Kit*^{em3}/Kyo.

Genome analysis of the gene-modified ACI strains

To identify the deletion size of ACI-*Kit*^{em2}/Kyo and ACI-*Kit*^{em3}/Kyo rats, we performed sequence analyses of their genomes. We designed four primer sets on the flanking sequences of the LTR to amplify their genomes (Fig. 1A). We obtained PCR products from ACI-*Kit*^{em2}/Kyo and ACI-*Kit*^{em3}/Kyo genomes when using these primer sets (a and d) (Fig. 1A and 1B). Sequencing of the PCR products revealed that ACI-*Kit*^{em2}/Kyo had an 848-base pair (bp) deletion and ACI-*Kit*^{em3}/Kyo had a 1,389-bp deletion (Fig. 2).

No URA phenotype in the gene-modified ACI strains

We then checked whether or not three gene-modified ACI strains exhibited URA and found that offspring from every line exhibited normal kidney and normal accessory reproductive organs (Fig. 3, Table 1). In addition, they also exhibited no Irish spotting on their belly (Fig. 3). These findings indicated that the loss of the LTR rescued ACI rats from URA and Irish spotting and suggested strongly that the LTR was causative of the URA and Irish spotting in ACI rats.

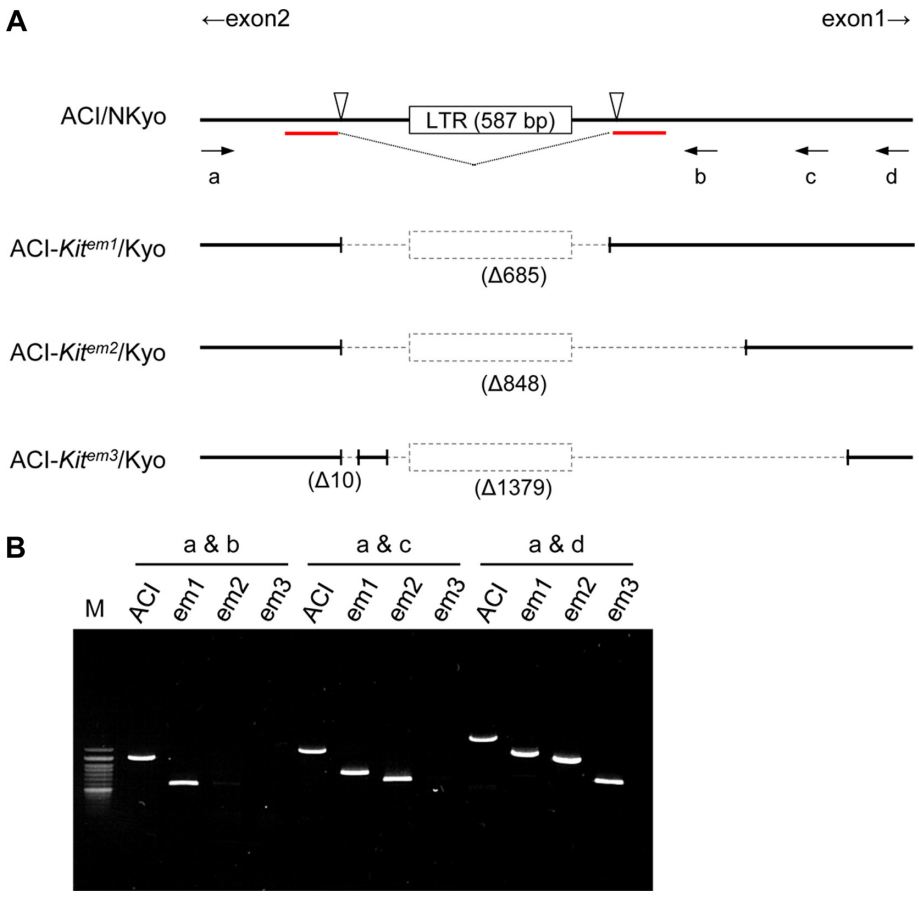

Fig. 1. Genomic organization of gene-modified ACI rats. (A) Upper: Schematic illustration of intron 1 of the *Kit* gene of ACI rats. The *Kit* gene locates along the minus strand of the rat genome (Rnor_6.0 assembly). The open box represents long terminal repeat (LTR) of the rat endogenous retrovirus sequence [14]. Two CRISPR/Cas cleavage sites on 5′ and 3′ flanking sequences of the LTR are indicated by open arrowheads. The red lines indicate 80 base pair (bp) single-stranded oligo DNA nucleotide (ssODN) that were designed to connect CRISPR/Cas cleavage sites and remove the LTR. Primers to detect deletion in each line are indicated by arrows. Lower: Schematic illustrations of genome organizations of gene-modified rat lines. Deleted regions are indicated by dashed lines. ACI-*Kit*^{em1}/Kyo carried a 685 bp deletion. ACI-*Kit*^{em2}/Kyo carried an 848 bp deletion. ACI-*Kit*^{em3}/Kyo carried a 1,389 bp deletion which consisted of 10 bp and 1,379 bp deletions. (B) PCR products amplified with primer pairs (a & b, a & c, and a & d) from ACI and gene-modified rat (em1, em2, and em3) genomes. M: 50 bp ladder molecular weight marker segregated on the Tris-borate-EDTA-buffered 1.0% agarose gel.

Discussion

Our genome editing experiment demonstrated that 10 (91%) of 11 offspring from the pseudopregnant rats harbored genome alterations, which confirmed the high efficiency of introducing Cas9 protein and guide RNAs into the rat embryos [16]. We also found three (30%) offspring harbored the expected deletion allele, which also confirmed the high accuracy and efficiency of oligonucleotide-mediated gene modification in rats [17].

Provided that 10–15% of ACI rats exhibit URA [12], 13 to 19 gene-modified ACI rats were expected to exhibit URA when we examined a total of 125 gene-modified ACI rats in the present study. We, however, failed to find URA in them (Table 1) and every rat exhibited normal kidney and urinary ducts, and normal accessory reproductive organs (Fig. 3). Thus, it is very likely that the gene-modified ACI rats were rescued from URA. Genomes of the gene-modified ACI rats lacked not only the LTR sequence but also the flanking sequences with different lengths (Fig. 1). This finding indicated that the flanking sequences were not necessary to induce URA. Instead, the insertion of the LTR was essential to provoke URA in ACI rats. LTRs are known to contain promoter activity and contribute to the transcriptional regulation of certain human and murine genes [18, 19]. Together, we concluded that the LTR insertion in the *Kit* gene was causative of URA in ACI rats.

In contrast to URA, the abdominal white spotting, Irish spotting, in the ACI rats is a fully penetrant phenotype. We found that the Irish spotting was rescued in all gene-modified ACI rats we examined (Fig. 3). The causative mutation of the Irish spotting has been thought to be the insertion of the LTR [14] and the Irish was mapped to the *Renag1* locus [13]. Thus, we concluded that the LTR insertion in the *Kit* gene was also causative of the Irish spotting in ACI rats.

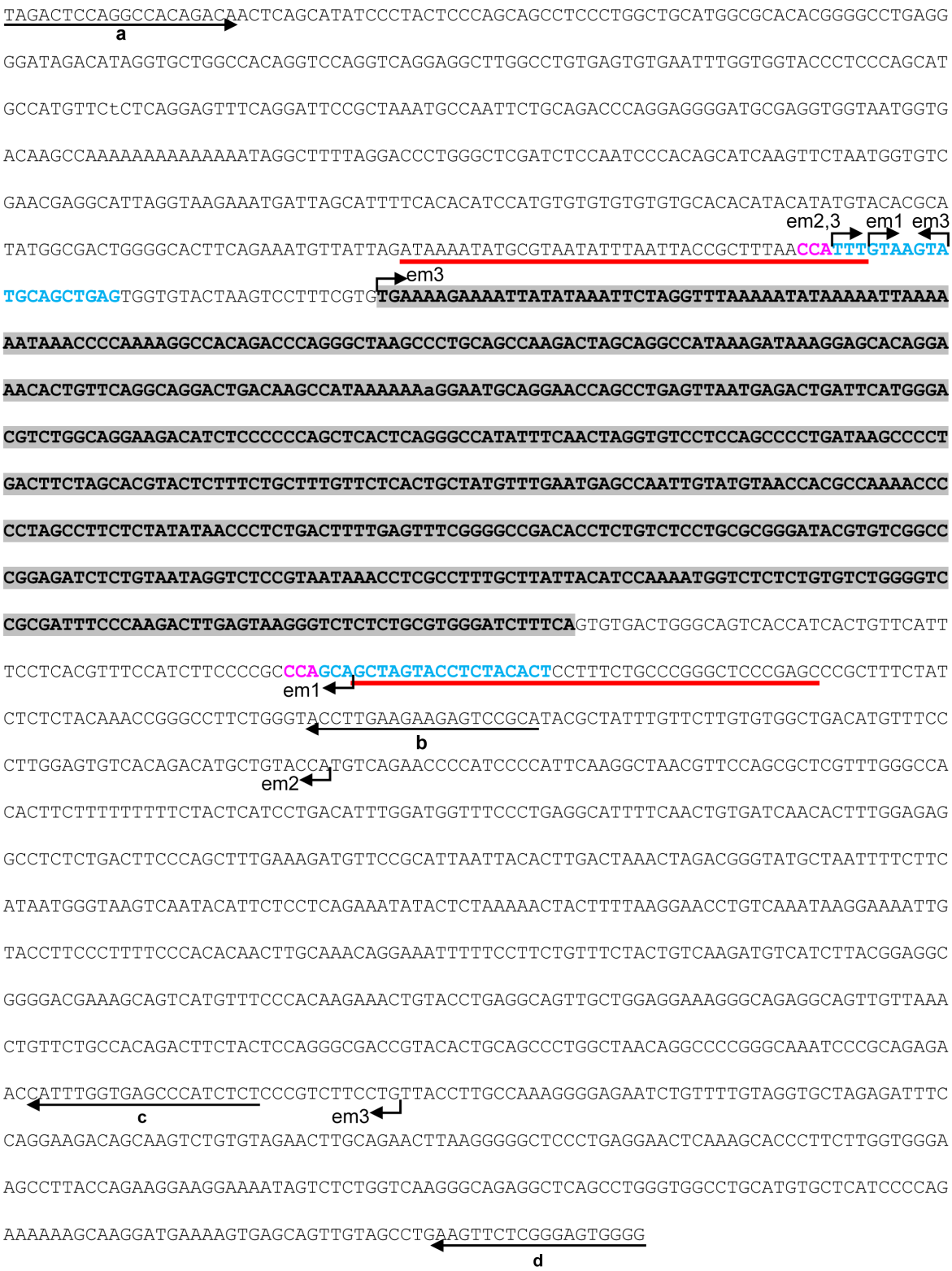

Fig. 2. Positions of deletions in gene-modified ACI rat strains. The long terminal repeat (LTR) sequence is highlighted in grey. Target sequences of guide RNAs and protospacer adjacent motifs (PAMs) are indicated by blue and magenta, respectively. The ssODN (80 bp) used to connect the flanking sequences cleaved by Cas9 nuclease is indicated by red horizontal bars. Primers used to detect deletions are underlined. Cleavage sites observed in gene-modified rat strains (em1, em2, and em3) are indicated by arrows. Names of primers are as follows: a, Kit-int1-05; b, Kit-Irish-ACI-4; c, Kit-int1-10; d, Kit-int1-12.

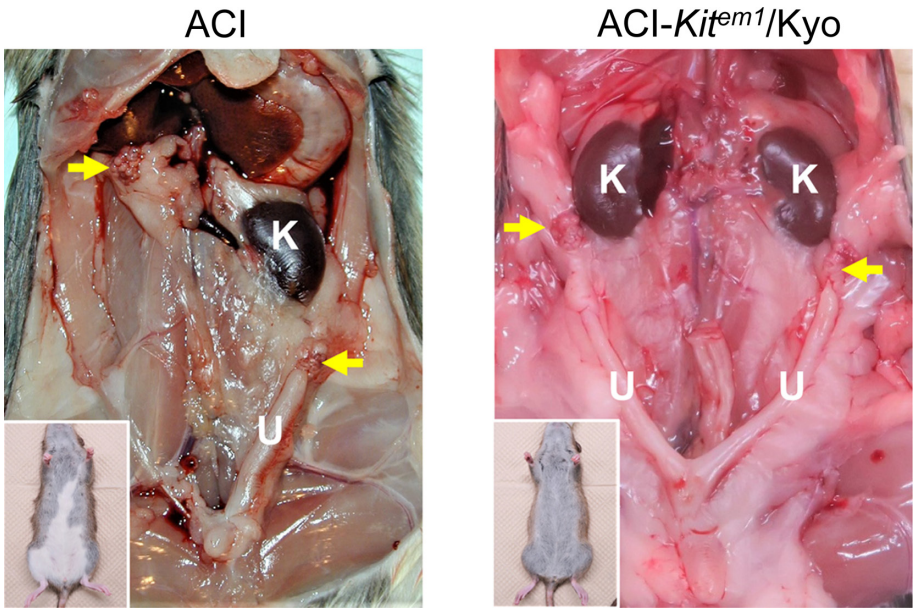

Fig. 3. Representative macroscopic observation of the gene-modified ACI rats. Left: an ACI rat exhibiting the agenesis of the right side of the kidney. Note that the ipsilateral uterus horn was also absent, although ovaries were intact on both sides (yellow arrows). Inset: gross appearance of the ventral side of the ACI rat. Abdominal white spotting (Irish) was observed. Right: an ACI-*Kit*^{em1}/Kyo rat exhibiting the normal kidneys on both sides. Note that uterus horns and ovaries (yellow arrows) on both sides were also normal. Inset: gross appearance of the ventral side of the ACI-*Kit*^{em1}/Kyo rat. No white spotting was observed. K: kidney, U: uterus horn.

Table 1. Incidence of unilateral renal agenesis (URA) in the gene-modified ACI rats

Strain name	No. of rats examined	No. of URA rats
ACI-Kit^{em1}/Kyo	53	0
ACI-Kit^{em2}/Kyo	46	0
ACI-Kit^{em3}/Kyo	26	0
Total	125	0

Kit encodes a transmembrane receptor tyrosine kinase (c-kit) that is activated by its cognate ligand KITL, also known as stem cell factor (SCF). The C-kit/SCF pathway is involved in transducing important signals in a variety of physiological and pathological processes related to cell survival, proliferation, migration, and differentiation [20]. Expression of c-kit is detected in differentiated cells, such as melanocytes, gametocytes, mast cells, and interstitial cells of Cajal. Additionally, c-kit positive cells have been described as a marker of stem cells in various organs, such as bone marrow, liver, and heart [21–23]. These c-kit positive cells have been thought to contribute to regenerating ability of hematopoietic, liver, and myocardial cells.

Recently, Rangel *et al.* have shown that neonatal kidney-derived c-kit positive cells have stem cell properties [24, 25]. These cells exhibited clonogenicity, self-renewal, and multipotentiality with differentiation capacity into mesoderm and ectoderm progeny. Additionally, these cells integrated into kidney compartments, such as tubules, vessels, and glomeruli, and contributed to functional and morphological improvement of the kidney from acute ischemia-reperfusion injury and chemically induced acute proteinuria in rats [24, 26].

Kit is expressed in the nephric duct of ACI rat embryos [13]. Failed development of the nephric duct has been suggested to give rise to URA in ACI rats [27]. Thus, it is likely that the survival, proliferation, migration, and differentiation of c-kit positive stem cells may be dysregulated during kidney development in ACI rats. In humans and mice, *KIT*/*Kit* genes have not been thought to be involved in URA. Thus, this study, to our knowledge, was the first to report that the *Kit* gene was causative of URA and involved in kidney development. Further investigation of URA patients or families may identify mutations in the *KIT* gene as causal variants of URA and contribute to developing of new diagnosis method for URA.

Conclusion

Our study demonstrated that the LTR in the *Kit* gene provoked URA and abdominal white spotting in ACI rats and suggested that the Kit signaling pathway may play an important role in kidney development. The ACI rat strain would be a promising animal model for regenerative medicine for the kidney.

Acknowledgement

This study was supported in part by JSPS KAKENHI Grant Number 17H03569 (T.K.).

References

1. Westland, R., Schreuder, M. F., Ket, J. C. and van Wijk, J. A. 2013. Unilateral renal agenesis: a systematic review on associated anomalies and renal injury. *Nephrol. Dial. Transplant.* **28**: 1844–1855. [Medline] [CrossRef]
2. Laurichesse Delmas, H., Kohler, M., Doray, B., Lémery, D., Francannet, C., Quistrebert, J., Marie, C. and Perthus, I. 2017. Congenital unilateral renal agenesis: Prevalence, prenatal diagnosis, associated anomalies. Data from two birth-defect registries. *Birth Defects Res* **109**: 1204–1211. [Medline] [CrossRef]
3. Sanna-Cherchi, S., Ravani, P., Corbani, V., Parodi, S., Haupt, R., Piaggio, G., Innocenti, M. L., Somenzi, D., Trivelli, A., Caridi, G., Izzi, C., Scolari, F., Mattioli, G., Allegri, L. and Ghiggeri, G. M. 2009. Renal outcome in patients with congenital anomalies of the kidney and urinary tract. *Kidney Int.* **76**: 528–533. [Medline] [CrossRef]
4. Dressler, G. R. 2006. The cellular basis of kidney development. *Annu. Rev. Cell Dev. Biol.* **22**: 509–529. [Medline] [CrossRef]
5. Capone, V. P., Morello, W., Taroni, F. and Montini, G. 2017. Genetics of congenital anomalies of the kidney and urinary tract: The current state of play. *Int. J. Mol. Sci.* **18**: 796. [Medline] [CrossRef]
6. Negrisolo, S., Benetti, E., Centi, S., Della Vella, M., Ghirardo, G., Zanon, G. F., Murer, L. and Artifoni, L. 2011. PAX2 gene mutations in pediatric and young adult transplant recipients: kidney and urinary tract malformations without ocular anomalies. *Clin. Genet.* **80**: 581–585. [Medline] [CrossRef]
7. Skinner, M. A., Safford, S. D., Reeves, J. G., Jackson, M. E. and Freemerman, A. J. 2008. Renal aplasia in humans is associated with RET mutations. *Am. J. Hum. Genet.* **82**: 344–351. [Medline] [CrossRef]
8. Johnson, K. R., Cook, S. A., Erway, L. C., Matthews, A. N., Sanford, L. P., Paradies, N. E. and Friedman, R. A. 1999. Inner ear and kidney anomalies caused by IAP insertion in an intron of the *Eya1* gene in a mouse model of BOR syndrome. *Hum. Mol. Genet.* **8**: 645–653. [Medline] [CrossRef]
9. Moore, M. W., Klein, R. D., Fariñas, I., Sauer, H., Armanini, M., Phillips, H., Reichardt, L. F., Ryan, A. M., Carver-Moore, K. and Rosenthal, A. 1996. Renal and neuronal abnormalities in mice lacking GDNF. *Nature* **382**: 76–79. [Medline] [CrossRef]
10. Nicolaou, N., Pulit, S. L., Nijman, I. J., Monroe, G. R., Feitz, W. F., Schreuder, M. F., van Eerde, A. M., de Jong, T. P., Giltay, J. C., van der Zwaag, B., Havenith, M. R., Zwakenberg, S., van der Zanden, L. F., Poelmans, G., Cornelissen, E. A., Lilien, M. R., Franke, B., Roeleveld, N., van Rooij, I. A., Cuppen, E., Bongers, E. M., Giles, R. H., Knoers, N. V. and Renkema, K. Y. 2016. Prioritization and burden analysis of rare variants in 208 candidate genes suggest they do not play a major role in CAKUT. *Kidney Int.* **89**: 476–486. [Medline] [CrossRef]
11. Marshall, F. F., Garcia-Bunuel, R. and Beisel, D. S. 1978. Hydronephrosis, renal agenesis, and associated genitourinary anomalies in ACI rats. *Urology* **11**: 58–61. [Medline] [CrossRef]
12. Shull, J. D., Lachel, C. M., Strecker, T. E., Spady, T. J., Tochacek, M., Pennington, K. L., Murrin, C. R., Meza, J. L., Schaffer, B. S., Flood, L. A. and Gould, K. A. 2006. Genetic bases of renal agenesis in the ACI rat: mapping of *Renag1* to chromosome 14. *Mamm. Genome* **17**: 751–759. [Medline] [CrossRef]
13. Samanas, N. B., Commers, T. W., Dennison, K. L., Harenda, Q. E., Kurz, S. G., Lachel, C. M., Wavrin, K. L., Bowler, M., Nijman, I. J., Guryev, V., Cuppen, E., Hubner, N., Sullivan, R., Vezina, C. M. and Shull, J. D. 2015. Genetic etiology of renal agenesis: fine mapping of *Renag1* and identification of *Kit* as the candidate functional gene. *PLoS One* **10**: e0118147. [Medline] [CrossRef]
14. Kuramoto, T., Nakanishi, S., Ochiai, M., Nakagama, H., Voigt, B. and Serikawa, T. 2012. Origins of albino and hooded rats: implications from molecular genetic analysis across modern laboratory rat strains. *PLoS One* **7**: e43059. [Medline] [CrossRef]
15. Serikawa, T., Mashimo, T., Takizawa, A., Okajima, R., Maedomari, N., Kumafuji, K., Tagami, F., Neoda, Y., Otsuki, M., Nakanishi, S., Yamasaki, K., Voigt, B. and Kuramoto, T. 2009. National BioResource Project-Rat and related activities. *Exp. Anim.* **58**: 333–341. [Medline] [CrossRef]
16. Kaneko, T. 2017. Genome Editing of Rat. *Methods Mol. Biol.* **1630**: 101–108. [Medline] [CrossRef]
17. Yoshimi, K., Kaneko, T., Voigt, B. and Mashimo, T. 2014. Allele-specific genome editing and correction of disease-associated phenotypes in rats using the CRISPR-Cas platform. *Nat. Commun.* **5**: 4240. [Medline] [CrossRef]
18. Franke, V., Ganesh, S., Karlic, R., Malik, R., Pasulka, J., Horvat, F., Kuzman, M., Fulka, H., Cernohorska, M., Urbanova, J., Svobodova, E., Ma, J., Suzuki, Y., Aoki, F., Schultz, R. M., Vlahovicek, K. and Svoboda, P. 2017. Long terminal repeats power evolution of genes and gene expression programs in mammalian oocytes and zygotes. *Genome Res.* **27**: 1384–1394. [Medline] [CrossRef]
19. Dunn, C. A., Romanish, M. T., Gutierrez, L. E., van de Lagemaat, L. N. and Mager, D. L. 2006. Transcription of two human genes from a bidirectional endogenous retrovirus promoter. *Gene* **366**: 335–342. [Medline] [CrossRef]
20. Ashman, L. K. 1999. The biology of stem cell factor and its receptor C-kit. *Int. J. Biochem. Cell Biol.* **31**: 1037–1051. [Medline] [CrossRef]
21. Beltrami, A. P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., Leri, A., Kajstura, J., Nadal-Ginard, B. and Anversa, P. 2003. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* **114**: 763–776. [Medline] [CrossRef]
22. Crosby, H. A., Kelly, D. A. and Strain, A. J. 2001. Human hepatic stem-like cells isolated using c-kit or CD34 can differentiate into biliary epithelium. *Gastroenterology* **120**: 534–544. [Medline] [CrossRef]
23. Ogawa, M., Nishikawa, S., Yoshinaga, K., Hayashi, S., Kunisada, T., Nakao, J., Kina, T., Sudo, T., Kodama, H. and Nishikawa, S. 1993. Expression and function of c-Kit in fetal hemopoietic progenitor cells: transition from the early c-Kit-independent to the late c-Kit-dependent wave of hemopoiesis in the murine embryo. *Development* **117**: 1089–1098. [Medline]
24. Rangel, E. B., Gomes, S. A., Dulce, R. A., Premer, C., Rodrigues, C. O., Kanashiro-Takeuchi, R. M., Oskouei, B., Carvalho, D. A., Ruiz, P., Reiser, J. and Hare, J. M. 2013. C-kit$^+$ cells isolated from developing kidneys are a novel population of stem cells with regenerative potential. *Stem Cells* **31**: 1644–1656. [Medline] [CrossRef]
25. Gomes, S. A., Hare, J. M. and Rangel, E. B. 2018. Kidney-derived c-Kit$^+$ cells possess regenerative potential. *Stem Cells Transl. Med.* **7**: 317–324. [Medline] [CrossRef]
26. Rangel, E. B., Gomes, S. A., Kanashiro-Takeuchi, R., Saltzman, R. G., Wei, C., Ruiz, P., Reiser, J. and Hare, J. M. 2018. Kidney-derived c-kit$^+$ progenitor/stem cells contribute to podocyte recovery in a model of acute proteinuria. *Sci. Rep.* **8**: 14723. [Medline] [CrossRef]
27. Fujita, K., Fujita, H. M., Ohtawara, Y., Suzuki, K., Tajima, A. and Aso, Y. 1979. Hydronephrosis in ACI/N rats. *Lab. Anim.* **13**: 325–327. [Medline] [CrossRef]