Hydronephrosis with ureteritis developed in C57BL/6N mice carrying the congenic region derived from MRL/MpJ-type chromosome 11

Running Head: Ureteritis-hydronephrosis in MRL congenic mice

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Abstract

Inbred MRL/MpJ mice show several unique phenotypes in tissue regeneration processes and the urogenital and immune systems. Clarifying the genetic and molecular bases of these phenotypes requires the analysis of their genetic susceptibility locus. Herein, hydronephrosis development was incidentally observed in MRL/MpJ-derived chromosome 11 (D11Mit21-212)-carrying C57BL/6N-based congenic mice, which developed bilateral or unilateral hydronephrosis in both males and females with 23.5% and 12.5% prevalence, respectively. Histopathologically, papillary malformations of the transitional epithelium in the pelvic-ureteric junction seemed to constrict the ureter luminal entrance. Characteristically, eosinophilic crystals were observed in the lumen of diseased ureters. These ureters were surrounded by infiltrating cells mainly composed of numerous CD3+ T-cells and B220+ B-cells. Furthermore, several Iba-1+ macrophages, Gr-1+ granulocytes, mast cells, and chitinase 3-like 3/Ym1 (an important inflammatory lectin)-positive cells were detected. Eosinophils also accumulated to these lesions in diseased ureters. Some B6.MRL-(D11Mit21-D11Mit212) mice had duplicated ureters. We determined >100 single nucleotide variants between C57BL/6N- and MRL/MpJ-type chromosome 11 congenic regions, which were associated with nonsynonymous substitution, frameshift, or stopgain of coding proteins. In conclusion, B6.MRL-(D11Mit21-D11Mit212) mice spontaneously developed hydronephrosis due to obstructive uropathy with inflammation. Thus, this mouse line would be useful for molecular pathological analysis of obstructive uropathy in experimental medicine.
Introduction

MRL/MpJ mice originate from C57BL/6J, C3H/HeDi, AKR/J, and LG/J strains. In particular, the mutant strain MRL/MpJ-\textit{Fas}^{bbr/lpr} is a representative model for autoimmune diseases [1]. In addition to autoimmune diseases, MRL/MpJ mice show some unique phenotypes related to wound healing such as accelerated ear punch closure and cardiomyocyte regeneration [2, 3]. We previously reported further unique characteristics in the urogenital organs of MRL/MpJ mice: i.e., increased apoptosis of meiotic spermatocytes [4], heat shock resistance of spermatocytes [5], existence of testicular oocytes in newborn males [6], development of ovarian cysts originating from the rete ovarii [7], appearance of numerous ovarian mast cells in neonatal females [8], and unique features of renal tissue repair after experimental kidney injuries [9].

These phenotypes were closely associated with the genetic background of MRL/MpJ mice, and we identified several susceptibility loci on their chromosomes (Chrs). From the genomic analysis, we revealed that the phenotype-related susceptibility loci in MRL/MpJ mice, and multiple loci on Chrs. 3, 4, 6, 11, and 14 interacted, resulting in the development of ovarian cysts [10]. Two loci on Chr. 8 were identified as susceptibility loci associated with the appearance of numerous ovarian mast cells in the neonatal period [11]. Furthermore, we demonstrated that the appearance of testicular oocytes is regulated by the genetic factors on Chrs. 15 and Y [12], and two significant quantitative trait loci (QTL) were located on Chrs. 1 and 11 for the heat shock resistance of spermatocytes in MRL/MpJ mice [13].

We previously generated C57BL/6N-based congenic mice carrying the telomeric region of MRL/MpJ-type Chr.1 (\textit{D1Mit202-D1Mit403}) containing the susceptibility loci for testis- or autoimmune disease-associated phenotypes, and demonstrated that this congenic mice, named B6.MRL-(\textit{D1Mit202-D1Mit403}), exhibited increased apoptosis of meiotic spermatocytes as well as severe autoimmune glomerulonephritis [14]. From the analysis of B6.MRL-(\textit{D1Mit202-D1Mit403}) mice, exonuclease 1 on Chr. 1 was identified as a candidate gene of increased apoptosis of meiotic spermatocytes, and Fc receptor genes and interferon activated genes were considered as candidate genes for the development of glomerulonephritis in MRL/MpJ mice [15, 16]. We also examined the histopathological changes resulting from experimental cryptorchidism in the testes of the C57BL/6N-based congenic mice carrying the MRL/MpJ-derived loci responsible for heat-shock-resistant spermatocyte [5]. From the results of the congenic mice, we demonstrated that
MRL/MpJ-derived loci on Chr. 1 and 11 regulated testicular heat sensitivity [5]. Thus, the congenic strain is a powerful tool to examine the pathological roles of phenotype-related susceptibility loci in inbred mice. All MRL/MpJ-derived susceptibility loci identified in our previous study were associated with immune or urogenital system-related phenotypes. Therefore, we speculated that congenic mice carrying these loci would show immune or urogenital system-related phenotypes. To examine the genetic function of the identified loci in MRL/MpJ-derived Chrs. and to discover candidate genes associated with immune or urogenital system-related phenotypes, we created several congenic strains carrying the genetic loci derived from MRL/MpJ [5, 14].

In this study, we report the development of hydronephrosis incidentally observed in C57BL/6N-based congenic mice carrying the MRL/MpJ-derived Chr. 11. These mice showed ureter abnormalities causing hydronephrosis due to obstructive uropathy with constant probability, and some mice presented with duplicated ureters. Furthermore, we identified more than 100 single nucleotide polymorphisms between C57BL/6N- and MRL/MpJ-type genome on the Chr. 11 congenic region, and they were associated with nonsynonymous substitution, frameshift, or stopgain of coding proteins. Thus, our congenic mice would be useful for the molecular pathological analysis of obstructive uropathy and developmental anomaly of the urinary organs in the field of experimental medicine.
Methods

Ethics statement

Animal experimentation was approved by the Institutional Animal Care and Use Committee, which was convened at the Graduate School of Veterinary Medicine, Hokkaido University (approval No. 13-0032). The investigators adhered to the Guide for the Care and Use of Laboratory Animals of Hokkaido University, Graduate School of Veterinary Medicine (approved by the Association for the Assessment and Accreditation of Laboratory Animal Care International).

C57BL/6N-based congenic mice carrying the MRL/MpJ-derived Chr. 11

Congenic mice were created as described previously from crossing C57BL/6N and MRL/MpJ mice purchased from Japan SLC, Inc. (Hamamatsu, Japan) [5]. Genomic DNA was prepared from the tail of each animal as described previously [14]. Briefly, these samples were incubated in lysis buffer and proteinase K, and then treated with two-phenol extraction. Genomic DNA was purified by ethanol precipitation, and congenic regions were examined by genotyping based on genome polymerase chain reaction for the microsatellite markers \(D11Mit62\), \(D11Mit21\), \(D11Mit320\), \(D11Mit212\), \(D11Mit288\), \(D11Mit199\), and \(D11Mit48\) (Fig. 1). The amplified samples were electrophoresed with 2% agarose gel and photographed under an ultraviolet lamp. The map positions of the microsatellite loci were based on information from the Mouse Genome Database (MGD) of The Jackson Laboratory (www.informatics.jax.org/).

Histopathological analysis

All mice were euthanized under deep anesthesia by exsanguination from the carotid arteries, and the spleens, kidneys, and ureters were immediately collected. Each tissue sample was fixed in 4% paraformaldehyde at 4°C for histopathological analysis. From fixed tissues, paraffin-embedded sections of kidneys and ureters were stained with hematoxylin-eosin, Masson’s trichrome, or periodic acid Schiff. A part of the ureter was fixed in 2.5% glutaraldehyde and 2% paraformaldehyde, dehydrated in a graded alcohol series, and embedded in Quetol 812. Semi-thin sections (500 nm) were stained with toluidine blue.
Immunostaining

For paraffin sections, immunohistochemistry for B220, CD3, Gr-1, Iba-1, and Ki67 was performed to detect the B-cells, pan T-cells, granulocytes, macrophages, and proliferating cells, respectively. The chitinase-like 3 (Chi3l3/Ym1) was also examined according to a previous study [17]. Details of the staining conditions and primary antibodies are listed in Table 1. In brief, the sections were deparaffinized, heated, and incubated with primary and secondary antibodies. For double staining, Alexa Fluor-conjugated antibodies were used as secondary antibodies. For immunohistochemistry, the color was developed by incubating the sections in a 3,3'-diaminobenzidine tetrahydrochloride-H$_2$O$_2$ solution. These stained sections were visualized with BZ-X710 all-in-one fluorescence microscope (Keyence, Osaka, Japan).

Deep sequencing

Kidney samples from C57BL/6N and MRL/MpJ mice were collected, and genomic DNA was isolated with DNeasy kit (Qiagen, Valencia, CA, USA). Exome-capture was performed using Sureselect XT Mouse All Exon kit (Agilent Technologies, Santa Clara, CA, USA). Whole Exome sequencing was performed with HiSeq2000 (Illumina, San Diego, CA, USA). UCSC mm10 (http://genome.ucsc.edu/) was used as the reference genome for alignment. The reads were mapped by BWA (version 0.5.9) (http://bio-bwa.sourceforge.net/). SNVs and small insertions/deletions were identified using SAMtools (ver.0.1.18) (http://samtools.sourceforge.net/).

Statistical analysis

The results are expressed as mean ± standard error (S.E.). Significant differences between 2 groups were analyzed by Mann-Whitney $U$-test with $P < 0.05$. 
**Results**

*C57BL/6N-based congenic mice carrying the MRL/MpJ-derived Chr. 11*

Figure 1 shows the results of genotyping on Chr. 11 of C57BL/6N, congenic strain, and MRL/MpJ by using genomic DNA and microsatellite markers. In the congenic mice, *D11Mit21-212* (25.94 cM-54.34 cM; Chr. 11: 44,174,948–88,808,902 bp) were MRL/MpJ-type homozygous and the other regions were C57BL/6N-type homozygous. The mice were named B6.MRL-(*D11Mit21-D11Mit212*).

**Incidence of hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice**

In the necropsy, B6.MRL-(*D11Mit21-D11Mit212*) mice developed bilateral or unilateral hydronephrosis showing urine retention to the renal pelvis (Fig. 2A and B). There was no apparent gross alteration in the other organs. The hydronephrotic kidney was approximately 2 folds heavier than healthy kidney (Fig. 2C). The incidence of hydronephrosis was higher in males (23.5%) compared to that of females (12.5%) at 11–15 weeks of ages, and these differences remained in males (25.0%) and females (13.0%) until 20–28 weeks of ages (Fig. 2D). The total incidence of hydronephrosis was higher in males (24.1%) compared to that in females (12.8%) (Fig. 2D). In males, the left kidney showed a slightly higher incidence rate than the right kidney, and bilateral hydronephrosis was found in several individuals (Fig. 2D). In females, a similar tendency was observed, but no bilateral hydronephrosis was observed in the mice examined (Fig. 2D). The ratio of spleen weight to body weight, an index of systemic immune condition, was significantly higher in animals developing hydronephrosis compared to healthy mice in B6.MRL-(*D11Mit21-D11Mit212*) mice (Fig. 2E).

**Histopathology of hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice**

Hydronephrotic B6.MRL-(*D11Mit21-D11Mit212*) mice showed thinning renal cortices and enlarged renal pelvises containing retained urine (Fig. 3A and B). Furthermore, the mice showed papillary malformations of the transitional epithelium in the pelvic-ureteric junctions (Fig. 3A and C). Numerous cell infiltrations were observed around the mucosa of the proximal ureters (Fig. 3C). The healthy kidney showed the distinct connection between the renal pelvis and the ureter lumen (Fig. 3D). On the other hand, the hydronephrotic kidney showed a shortened renal papilla (Fig. 3E) and a constricted entrance of the ureter lumen by papillary malformations of the transitional epithelium (Fig.
3F). At the border between normal and abnormal transitional epithelium, the former showed a wrinkled surface but the latter showed hexagonal features (Fig. 3G-I).

Histopathology of the ureter in B6.MRL-(D11Mit21-D11Mit212) mice
Eosinophilic crystals were observed in the lumen of the diseased ureter surrounded by mononuclear cells, and eosinophilic materials were observed in the cytoplasm of transitional epithelial cells (Fig. 4A and B). Furthermore, mononuclear cells with non-segmented nuclei and eosinophilic granules as well as eosinophils infiltrated underneath the transitional epithelium (Fig. 4B). Gland-like structures containing dead mononuclear cells, granulocytes, dropped transitional epithelial cells, and eosinophilic crystals were observed outside the muscular layer (Fig. 4C). Severe cell infiltrations from the lamina propria to the adventitia and these lesions were also noted (Fig. 4C). The Chi3l3/Ym1 protein, a lectin overexpressed in the ureter of hydronephrosis mice [17] and associated with inflammation, transitional epithelium adenoma, and the formation of eosinophilic crystals [18] was observed in the cytoplasm of infiltrating cells, apical portion of transitional epithelial cells, and cell debris or crystal structures of the ureter lumen (Fig. 4D). In the pelvic-ureteral junction of hydronephrotic mice, Ki67-positive proliferative cells were scarcely observed in the ureteral transitional epithelium (Fig. 4E), but were abundant in the central position of cell infiltration lesions (Fig. 4F). The distal ureter also showed an increase in the interstitium and cell infiltrations from the lamina propria to the adventitia (Fig. 4G). The cell infiltration was composed of numerous CD3-positive T-cells, B220-positive B-cells, several Gr-1-positive granulocytes, and Iba-1-positive macrophages (Fig. 4H and I). In addition, numerous eosinophil infiltrations were observed in some regions (Fig. 4J). Along the whole length of the diseased ureters, fibrotic features were observed from the lamina propria to the adventitia (Fig. 4K).

Incidental duplicated ureters in B6.MRL-(D11Mit21-D11Mit212) mice
We found duplicated ureters in two of all the examined B6.MRL-(D11Mit21-D11Mit212) mice (Fig. 5). These ureters ran parallel to each other (Fig. 5A and B), had a common serosa (Fig. 5B), and one of them showed stenosis features containing cell debris (Fig. 5A-C). These cell debris were composed of dropped epithelial cells and crystals (Fig. 5D). Several mast cells, showing metachromasia in toluidine blue stain, were also observed around the ureters (Fig. 5D).
We compared the SNV between C57BL/6N- and MRL/MpJ-type genomes on the congenic region in B6.MRL-(D11Mit21-D11Mit212) mice. As a result, over 100 of nonsynonymous single nucleotide variants (SNVs) and 12 variants associated with frameshift or stopgain such as the olfactory receptor family, FAT atypical cadherin 2 (Fat2), butyrophilin-like 10 (Btnl10), obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF (Obscn), IBA57 homolog, iron-sulfur cluster assembly (Iba57), myosin XV (Myo15), and tripartite motif-containing 16 (Trim16), were detected in MRL/MpJ-type Chr. 11 when compared to C57BL/6N (Table 1).
Pelvic-ureteric junction obstruction is a common cause of human hydronephrosis and results from the narrowing of the renal pelvis and ureter [19, 20]. In experimental medicine, the hydronephrosis model is usually created by an ureteric obstruction operation, and few studies reported hydronephrosis in specific-gene modification models such as the promyelocytic leukemia (PML)/retinoic acid receptor alpha (RARA), a chimeric gene of PML and RARA knock-in mice, and interleukin (Il9)-overexpressing mice [18, 21]. Because the spontaneous model of hydronephrosis due to obstructive uropathy without gene modification is scarce, the details of its pathogenesis are still unclear.

In this study, we newly discovered the spontaneous development of hydronephrosis in congenic mice carrying MRL/MpJ-type Chr.11 (D11Mit21-212) and C57BL/6N-type genetic background. From the histopathological features, stenosis of the pelvic-ureteric junction due to papillary malformations of the transitional epithelium seemed to contribute to the development of hydronephrosis. Interestingly, their histopathological features were similar to the hydronephrosis observed in PML/PARA knock-in mice and Il9-overexpressing mice [18, 21] as well as the F2 generation between C57BL/6 and DBA/2 mice as reported in our previous study [17]. IL-9 is a regulator of Th2 effector cytokines, and Il9-overexpressing mice developed T-cell lymphoma [21]. PML/RARA induces myeloproliferative syndrome, and PML/RARA knock-in mice developed acute myeloid leukemia [18]. Although (C57BL/6 × DBA/2) F2 mice [17] as well as B6.MRL-(D11Mit21-D11Mit212) mice did not show any systemic immune disorders, both mice commonly manifested lymphocyte infiltrations in the diseased ureters. In fact, the spleen weights increased in B6.MRL-(D11Mit21-D11Mit212) mice developing hydronephrosis, but representative lesions associated with autoimmune disease such as glomerulonephritis were not observed.

Characteristically, large granular leukocytes and eosinophils infiltrated the ureters of B6.MRL-(D11Mit21-D11Mit212) mice. These cells were also found in Il9-overexpressing mice, PML/PARA knock-in mice, and (C57BL/6 × DBA/2) F2 mice [17, 18, 21]. Compared to the incidence of hydronephrosis in (C57BL/6 × DBA/2) F2 mice (5–7%) [21], B6.MRL-(D11Mit21-D11Mit212) mice showed a higher incidence of hydronephrosis (23.5% in males and 12.5% in females). Although there are several differences in the pathological features among hydronephrotic models, genetic factors
derived from MRL/MpJ-type Chr. 11 or C57BL/6N-type genome and/or their interactions contribute to
the development of hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice, in particular due to the
alteration of immune conditions in the urinary system. Furthermore, because the development of
hydronephrosis was not observed in the F1 generation between C57BL/6 mice and DBA/2 mice in our
previous study [21], homogenous genetic factors may have crucial roles in the development of
hydronephrosis.

Urinary tract obstruction could be caused by ureteral stones, urothelial tumors, and infectious or
idiopathic inflammation in humans. The pathological features of the ureters in
B6.MRL-(D11Mit21-D11Mit212) mice partially overlapped with human idiopathic ureteritis diagnosed
as inflammatory pseudotumor (IPT) of the ureter, idiopathic segmental ureteritis (ISU), idiopathic
retroperitoneal fibrosis (IRF) involving the ureters, or eosinophilic ureteritis [19, 22-25]. The latter 2
diseases are accompanied by peritoneal fibrosis and systemic immunological changes such as atopy,
respectively [19, 22, 23], but these symptoms were not observed in the diseased
B6.MRL-(D11Mit21-D11Mit212) mice. Similar to the B6.MRL-(D11Mit21-D11Mit212) ureters, the
ureters in IPT and ISU show infiltrations of lymphoplasma cells and eosinophils as well as sclerotic
fibrosis [24, 25] and some cases of IPT show ureteric malformations [25]. Furthermore, male infants
show a greater prevalence of hydronephrosis compared to females in humans [26, 27], and this
sex-related tendency resembles the hydronephrosis observed in the B6.MRL-(D11Mit21-D11Mit212)
mice. This is an important consideration because obstructive uropathies are diagnosed in aging human
populations. However, no age-related tendency was observed in the development of hydronephrosis in
the B6.MRL-(D11Mit21-D11Mit212) mice (Fig. 1D).

A previous study reported the spontaneous development of ureteric obstructions caused by
polyploid adenomas locating from the renal pelvis to the ureter with severe cell infiltration and the
appearance of Chil3l3/Ym1-positive crystals in acute myeloid leukemia of PML/para knock-in mice
[18]. Furthermore, in the hydronephrotic ureters of (C57BL/6 × DBA/2) F2 mice, comprehensive gene
expression analysis revealed that the factors regulating ureteric local inflammation, the Chil3l3 gene in
particular, showed ectopic and remarkably elevated expression [17]. Interestingly, Chil3l3/Ym1 was also
detected in the cytoplasm of infiltrating cells, apical portion of transitional epithelial cells, and cell
debris or crystal structures of ureter lumen in B6.MRL-(D11Mit21-D11Mit212) mice. Chil3l3/Ym1 is an
endogenous lectin, widely distributed in normal mammalian bodies, and expressed transiently in early myeloid precursor cells of hematopoietic tissues: initially in the yolk sac and subsequently in the fetal liver, spleen, and bone marrow [18, 28, 29-30]. In addition, Chil3l3/Ym1 promotes Th2 cytokine expression in allergic responses, suggesting an important role of Chil3l3/Ym1 in hematopoiesis as well as inflammation [31]. Therefore, the appearance of Chil3l3/Ym1 expressing cells might reflect altered local immune conditions in the ureters of B6.MRL-(D11Mit21-D11Mit212) mice.

Additionally, comprehensive gene expression analysis of the hydronephrotic ureters of (C57BL/6 × DBA/2) F2 mice revealed that the B-cell functions such as B-cell activation, the B-cell receptor signaling pathway, and B-cell proliferation were important for the pathogenesis in this disease based on gene ontology and expression analyses [17]. Furthermore, inflammatory mediators such as the family members of mast cell protease, matrix metalloproteinase, or chemokine (C-C motif) ligand were remarkably upregulated in the diseased ureters compared to that in the healthy ureters in hydronephrotic (C57BL/6 × DBA/2) F2 mice [17]. Numerous B-cell infiltrations were also observed in the ureters of B6.MRL-(D11Mit21-D11Mit212) mice developing hydronephrosis. Therefore, in future studies, the determination of specific cell subsets, in particular those of B-cells, with the analysis of inflammatory mediator producing capacities would be needed to better understand the pathogenesis of ureteritis-hydronephrosis developing mice.

The phenotype of heat shock resistance of the spermatocytes in MRL/MpJ is closely associated with Chr. 11 (D11Mit21-212) [5]. However, there seemed to be no significant association between this testicular phenotype and the hydronephrosis detected in B6.MRL-(D11Mit21-D11Mit212) mice. Interestingly, a few B6.MRL-(D11Mit21-D11Mit212) mice developed duplicated ureters as previously reported in humans and dogs [32, 33], and this malformation could cause hydronephrosis due to urinary tract obstruction. Therefore, the molecular pathogenesis of hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice might involve immune functions as well as urinary tract development.

To identify the SNV between the C57BL/6N- and MRL/MpJ-type genome on the congenic region in B6.MRL-(D11Mit21-D11Mit212) mice, we performed Whole Exome sequencing and identified over 100 nonsynonymous SNVs. Interestingly, 12 variants associated with frameshift or stopgain such as the
Olfr family, Fat2, Btn110, Obscn, Iba57, Myo15, and Trim16 were identified between MRL/MpJ-type Chr. 11 and C57BL/6N. However, there are no report about the relationship between these genes and the urinary tract system. Mice carrying the null-mutated Forkhead box C1 (Foxc1) gene frequently develop congenital anomalies of the kidney and urinary tract such as duplicated ureters [34], but this gene is coded on Chr. 13. The coding genes of RARA, PML, and IL-9, hydronephrosis-associated molecules, were not located on Chr. 11 (D11Mit21-212). Thus, the SNV identified in this study might be useful to elucidate the novel pathogenesis associated with the immune system and the development of urinary tract system in future studies. In addition, we should consider the contribution of the C57BL/6N-genetic background, because mice carrying the C57BL/6N-type genetic background have been shown to develop eosinophilic macrophage pneumonia with the appearance of eosinophilic crystals [35, 36]. Large eosinophilic crystals were also observed in the lumen of ureters in B6.MRL-(D11Mit21-D11Mit212) mice, and similar crystals composed of endogenous lectin were observed in several diseases with severe eosinophil infiltration such as parasitosis and asthma [37]. In conclusion, B6.MRL-(D11Mit21-D11Mit212) mice spontaneously developed hydronephrosis due to obstructive uropathy with inflammation. Therefore, this animal model would be useful for the molecular pathological analysis of obstructive uropathy and developmental anomaly of the urinary organs.
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Declaration of Interest

This manuscript has not been published or presented elsewhere in part or in entirety, and is not under consideration by another journal. All authors have approved the manuscript and agree with submission to this journal. There are no conflicts of interest to declare.


Figure Legends

Fig. 1. Chr. 11 of B6.MRL-(D11Mit21-D11Mit212) mice.
Genotyping genomic DNA using microsatellite markers (left panels). The polymorphisms between the strains are detected as size differences in the PCR products. Schematic representation (right panel). In B6.MRL-(D11Mit21-D11Mit212) mice, D11Mit62 (5.78 cM) and D11Mit288-48 (58.90–82.96 cM) were C57BL/6N types, and D11Mit21-320 (25.94–54.34 cM) was MRL/MpJ type.

Fig. 2. Hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice.
(A, B) Gross anatomical features of hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice. Unilateral hydronephrosis showing urine retention to the renal pelvis are observed. Arrows indicate ureter hypertrophy in proximal portions.
(C) Relative increase of weights in the hydronephrotic kidneys of B6.MRL-(D11Mit21-D11Mit212) mice. Values = mean ± S.E., n > 4.
(D) The incidence of hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice. The number of analyzed mice is described in the graphs. N.D.: not detected.
(E) Ratio of spleen weight to body weight in B6.MRL-(D11Mit21-D11Mit212) mice with or without the development of hydronephrosis. Male and female individuals are included at 11–28 weeks of age. Values = mean ± S.E. *: significant difference with normal group.

Fig. 3. Histopathology of hydronephrosis in B6.MRL-(D11Mit21-D11Mit212) mice.
(A–C) Histopathological features of the kidney and ureter (UR) in hydronephrotic B6.MRL-(D11Mit21-D11Mit212) mice assessed by hematoxylin and eosin staining. Thinning renal cortex (CO) and enlarged renal pelvis (RP) are observed (A and B). Papillary malformations of the transitional epithelium (arrows) are observed in the pelvic-ureteric junction (C). Numerous cell infiltrations are also observed around the mucosa of the proximal ureter (C, left side).
papilla is shortened (arrow) and RP is enlarged (E). Papillary malformations of the transitional epithelium constricted the entrance of the ureter lumen (F, asterisk). Border between normal (dagger) and abnormal (asterisk) transitional epithelium (G). In these positions, the former shows a wrinkled surface (arrows), but the latter shows hexagonal features (arrowheads) (H and I).

Fig. 4. Histopathology of the ureter in B6.MRL-(D11Mit21-D11Mit212) mice.

(A-K) Histopathological features of the ureters in hydronephrotic B6.MRL-(D11Mit21-D11Mit212) mice. Hematoxylin and eosin staining (A-C, G, and J). Immunohistochemistry (D-F), Immunofluorescence (H and I). Masson’s trichrome staining (K). Eosinophilic crystals and eosinophilic materials are observed in the lumen (Lu) of the diseased ureter surrounded by mononuclear cells (A, arrow) and in the cytoplasm of transitional epithelial cells (A, arrowheads). Eosinophilic materials are clearly observed in the cytoplasm of transitional epithelial cells (B). Mononuclear cells with non-segmented nuclei and eosinophilic granules (B, arrow) and eosinophils (B, arrowheads) infiltrated the transitional epithelium. Gland-like structures containing dead mononuclear cells, granulocytes, dropped transitional epithelial cells, and eosinophilic crystals are observed outside of the muscular layer (C, arrow). Severe cell infiltrations are noted (C) Chi3l3/Ym1-positive reactions are observed in the cytoplasm of infiltrating cells (D, arrows), apical portion of transitional epithelial cells (D), and cell debris or crystal structures (D, arrowheads and inset) of the ureter lumen. Ki67-positive proliferative cells are scarcely observed in the ureteral transitional epithelium (E), but are present in the central position of cell infiltration lesions (F, arrow). The distal ureter shows increased interstitium and cell infiltrations (G). The cell infiltrations are composed of numerous CD3-positive T-cells (H, red), B220-positive B-cells (I, green), several Gr-1-positive granulocytes (H, green, arrow), and lba-1-positive macrophages (I, red). Numerous eosinophil infiltrations are observed in some regions (J). Along the whole length of the diseased ureter, fibrotic features (blue) are observed from the lamina propria to the adventitia (K).

(L) Histopathological features of the renal cortex in hydronephrotic B6.MRL-(D11Mit21-D11Mit212) mice at 20 weeks of age assessed by periodic acid Schiff staining. There is no glomerular lesion (arrows). Asterisks indicate dilated renal tubules.
Histopathological features of duplicated ureters are incidentally observed in some B6.MRL-(D11Mit21-D11Mit212) mice. Hematoxylin and eosin staining (A). Toluidine blue staining (B-D). Duplicated ureters run parallel to each other (A). Duplicated ureters have common serosa (B). One of the ureters shows stenosis features containing cell debris (A, left side; B, upper one; C, lower one). These cell debris are composed of dropped epithelial cells and crystals (C and D). Several mast cells showing metachromasia are observed around the ureters (D, arrow). Lu: lumen.
Table 1. Antibodies, working dilutions, and methods for antigen retrieval.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Heating condition</th>
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<td>Rabbit anti-Ki67</td>
<td>R and D system</td>
<td>1:150</td>
<td>10 mM Citrate buffer (pH 6.0)</td>
<td>105°C, 20 min</td>
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<tr>
<td>Rat anti-Chi3l3/Ym1</td>
<td>R and D system (Minneapolis, MN, USA)</td>
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<td>20 mM Tris-HCl (pH 9.0)</td>
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Iba57  nonsynonymous_SNV2  59161555  59161555  T  C  hom  222
Gja2  nonsynonymous_SNV2  59176433  59176433  T  C  hom  199
Mrp355  nonsynonymous_SNV1  59204589  59204589  A  T  hom  203
Pres38  nonsynonymous_SNV2  59375551  59375551  G  T  hom  187
Jmjd4  nonsynonymous_SNV3  59450788  59450788  A  G  hom  154
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Zkscan17  nonsynonymous_SNV4  59487650  59487650  C  T  hom  222
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Olfr225  nonsynonymous_SNV7  59613209  59613209  A  G  hom  222
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Pemt  nonsynonymous_SNV1  60031784  60031784  T  C  hom  222
Rat1  nonsynonymous_SNV5  60185857  60185857  A  G  hom  212
Srebf1  nonsynonymous_SNV1  60200146  60200146  T  C  hom  222
Lrc48  nonsynonymous_SNV5  60364958  60364958  G  A  hom  191
Myo15  nonsynonymous_SNV7  60477716  60477716  A  G  hom  189
Flii  nonsynonymous_SNV2  60716732  60716732  T  C  hom  222
Mef2  nonsynonymous_SNV5  60730943  60730943  C  T  hom  222
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Tup2b3  nonsynonymous_SNV1  62881943  62881943  A  C  hom  154
Myb8  nonsynonymous_SNV1  62728653  62728653  A  G  hom  222
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SNV: single nucleotide variant. SNP: single nucleotide polymorphism. The letter after SNV shows the number of SNV in the coding region of each gene.