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

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4 **Running Head:** Ureteritis-hydronephrosis in MRL congenic mice

5
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27 **Abstract**

28 Inbred MRL/MpJ mice show several unique phenotypes in tissue regeneration processes and the
29 urogenital and immune systems. Clarifying the genetic and molecular bases of these phenotypes requires
30 the analysis of their genetic susceptibility locus. Herein, hydronephrosis development was incidentally
31 observed in MRL/MpJ-derived chromosome 11 (*D11Mit21-212*)-carrying C57BL/6N-based congenic
32 mice, which developed bilateral or unilateral hydronephrosis in both males and females with 23.5% and
33 12.5% prevalence, respectively. Histopathologically, papillary malformations of the transitional
34 epithelium in the pelvic-ureteric junction seemed to constrict the ureter luminal entrance.
35 Characteristically, eosinophilic crystals were observed in the lumen of diseased ureters. These ureters
36 were surrounded by infiltrating cells mainly composed of numerous CD3⁺ T-cells and B220⁺ B-cells.
37 Furthermore, several Iba-1⁺ macrophages, Gr-1⁺ granulocytes, mast cells, and chitinase 3-like 3/Ym1
38 (an important inflammatory lectin)-positive cells were detected. Eosinophils also accumulated to these
39 lesions in diseased ureters. Some B6.MRL-(*D11Mit21-D11Mit212*) mice had duplicated ureters. We
40 determined >100 single nucleotide variants between C57BL/6N- and MRL/MpJ-type chromosome 11
41 congenic regions, which were associated with nonsynonymous substitution, frameshift, or stopgain of
42 coding proteins. In conclusion, B6.MRL-(*D11Mit21-D11Mit212*) mice spontaneously developed
43 hydronephrosis due to obstructive uropathy with inflammation. Thus, this mouse line would be useful
44 for molecular pathological analysis of obstructive uropathy in experimental medicine.

45 **Introduction**

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

55 These phenotypes were closely associated with the genetic background of MRL/MpJ mice, and we
56 identified several susceptibility loci on their chromosomes (Chrs). From the genomic analysis, we
57 revealed that the phenotype-related susceptibility loci in MRL/MpJ mice, and multiple loci on Chrs. 3, 4,
58 6, 11, and 14 interacted, resulting in the development of ovarian cysts [10]. Two loci on Chr. 8 were
59 identified as susceptibility loci associated with the appearance of numerous ovarian mast cells in the
60 neonatal period [11]. Furthermore, we demonstrated that the appearance of testicular oocytes is
61 regulated by the genetic factors on Chrs. 15 and Y [12], and two significant quantitative trait loci (QTL)
62 were located on Chrs. 1 and 11 for the heat shock resistance of spermatocytes in MRL/MpJ mice [13].

63 We previously generated C57BL/6N-based congenic mice carrying the telomeric region of
64 MRL/MpJ-type Chr.1 (*D1Mit202-D1Mit403*) containing the susceptibility loci for testis- or autoimmune
65 disease-associated phenotypes, and demonstrated that this congenic mice, named
66 B6.MRL-(*D1Mit202-D1Mit403*), exhibited increased apoptosis of meiotic spermatocytes as well as
67 severe autoimmune glomerulonephritis [14]. From the analysis of B6.MRL-(*D1Mit202-D1Mit403*) mice,
68 exonuclease 1 on Chr. 1 was identified as a candidate gene of increased apoptosis of meiotic
69 spermatocytes, and Fc receptor genes and interferon activated genes were considered as candidate genes
70 for the development of glomerulonephritis in MRL/MpJ mice [15, 16]. We also examined the
71 histopathological changes resulting from experimental cryptorchidism in the testes of the
72 C57BL/6N-based congenic mice carrying the MRL/MpJ-derived loci responsible for
73 heat-shock-resistant spermatocyte [5]. From the results of the congenic mice, we demonstrated that

74 MRL/MpJ-derived loci on Chrs. 1 and 11 regulated testicular heat sensitivity [5]. Thus, the congenic
75 strain is a powerful tool to examine the pathological roles of phenotype-related susceptibility loci in
76 inbred mice. All MRL/MpJ-derived susceptibility loci identified in our previous study were associated
77 with immune or urogenital system-related phenotypes. Therefore, we speculated that congenic mice
78 carrying these loci would show immune or urogenital system-related phenotypes. To examine the
79 genetic function of the identified loci in MRL/MpJ-derived Chrs. and to discover candidate genes
80 associated with immune or urogenital system-related phenotypes, we created several congenic strains
81 carrying the genetic loci derived from MRL/MpJ [5, 14].

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

90

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.

120 *Immunostaining*

121 For paraffin sections, immunohistochemistry for B220, CD3, Gr-1, Iba-1, and Ki67 was performed
122 to detect the B-cells, pan T-cells, granulocytes, macrophages, and proliferating cells, respectively. The
123 chitinase-like 3 (Chi3l3/Ym1) was also examined according to a previous study [17]. Details of the
124 staining conditions and primary antibodies are listed in Table 1. In brief, the sections were
125 deparaffinized, heated, and incubated with primary and secondary antibodies. For double staining, Alexa
126 Fluor-conjugated antibodies were used as secondary antibodies. For immunohistochemistry, the color
127 was developed by incubating the sections in a 3,3'-diaminobenzidine tetrahydrochloride-H₂O₂ solution.
128 These stained sections were visualized with BZ-X710 all-in-one fluorescence microscope (Keyence,
129 Osaka, Japan).

130

131 *Deep sequencing*

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

139

140 *Statistical analysis*

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

143

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.

173 3F). At the border between normal and abnormal transitional epithelium, the former showed a wrinkled
174 surface but the latter showed hexagonal features (Fig. 3G-I).

175

176 *Histopathology of the ureter in B6.MRL-(D11Mit21-D11Mit212) mice*

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

195

196 *Incidental duplicated ureters in B6.MRL-(D11Mit21-D11Mit212) mice*

197 We found duplicated ureters in two of all the examined B6.MRL-(D11Mit21-D11Mit212) mice
198 (Fig. 5). These ureters ran parallel to each other (Fig. 5A and B), had a common serosa (Fig. 5B), and
199 one of them showed stenosis features containing cell debris (Fig. 5A-C). These cell debris were
200 composed of dropped epithelial cells and crystals (Fig. 5D). Several mast cells, showing metachromasia
201 in toluidine blue stain, were also observed around the ureters (Fig. 5D).

202

203 *SNV between C57BL/6N- and MRL/MpJ-type genome on congenic region in*
204 *B6.MRL-(D11Mit21-D11Mit212) mice*

205 We compared the SNV between C57BL/6N- and MRL/MpJ-type genomes on the congenic region
206 in B6.MRL-(*D11Mit21-D11Mit212*) mice by Whole Exome sequencing. As a result, over 100 of
207 nonsynonymous single nucleotide variants (SNVs) and 12 variants associated with frameshift or
208 stopgain such as the olfactory receptor family, FAT atypical cadherin 2 (*Fat2*), butyrophilin-like 10
209 (*Btnl10*), obscurin, cytoskeletal calmodulin and titin-interacting RhoGEF (*Obscn*), IBA57 homolog,
210 iron-sulfur cluster assembly (*Iba57*), myosin XV (*Myo15*), and tripartite motif-containing 16 (*Trim16*),
211 were detected in MRL/MpJ-type Chr. 11 when compared to C57BL/6N (Table 1).

212

Discussion and Conclusion

213

214 Pelvic-ureteric junction obstruction is a common cause of human hydronephrosis and results from
215 the narrowing of the renal pelvis and ureter [19, 20]. In experimental medicine, the hydronephrosis
216 model is usually created by an ureteric obstruction operation, and few studies reported hydronephrosis in
217 specific-gene modification models such as the promyelocytic leukemia (*PML*)/retinoic acid receptor
218 alpha (*RARA*), a chimeric gene of *PML* and *RARA* knock-in mice, and interleukin (*IL9*)-overexpressing
219 mice [18, 21]. Because the spontaneous model of hydronephrosis due to obstructive uropathy without
220 gene modification is scarce, the details of its pathogenesis are still unclear.

221 In this study, we newly discovered the spontaneous development of hydronephrosis in congenic
222 mice carrying MRL/MpJ-type Chr.11 (*D11Mit21-212*) and C57BL/6N-type genetic background. From
223 the histopathological features, stenosis of the pelvic-ureteric junction due to papillary malformations of
224 the transitional epithelium seemed to contribute to the development of hydronephrosis. Interestingly,
225 their histopathological features were similar to the hydronephrosis observed in *PML/PARA* knock-in
226 mice and *IL9*-overexpressing mice [18, 21] as well as the F2 generation between C57BL/6 and DBA/2
227 mice as reported in our previous study [17]. IL-9 is a regulator of Th2 effector cytokines, and
228 *IL9*-overexpressing mice developed T-cell lymphoma [21]. *PML/RARA* induces myeloproliferative
229 syndrome, and *PML/RARA* knock-in mice developed acute myeloid leukemia [18]. Although (C57BL/6
230 × DBA/2) F2 mice [17] as well as B6.MRL-(*D11Mit21-D11Mit212*) mice did not show any systemic
231 immune disorders, both mice commonly manifested lymphocyte infiltrations in the diseased ureters. In
232 fact, the spleen weights increased in B6.MRL-(*D11Mit21-D11Mit212*) mice developing hydronephrosis,
233 but representative lesions associated with autoimmune disease such as glomerulonephritis were not
234 observed.

235 Characteristically, large granular leukocytes and eosinophils infiltrated the ureters of
236 B6.MRL-(*D11Mit21-D11Mit212*) mice. These cells were also found in *IL9*-overexpressing mice,
237 *PML/PARA* knock-in mice, and (C57BL/6 × DBA/2) F2 mice [17, 18, 21]. Compared to the incidence of
238 hydronephrosis in (C57BL/6 × DBA/2) F2 mice (5–7%) [21], B6.MRL-(*D11Mit21-D11Mit212*) mice
239 showed a higher incidence of hydronephrosis (23.5% in males and 12.5% in females). Although there
240 are several differences in the pathological features among hydronephrotic models, genetic factors

241 derived from MRL/MpJ-type Chr. 11 or C57BL/6N-type genome and/or their interactions contribute to
242 the development of hydronephrosis in B6.MRL-(*D11Mit21-D11Mit212*) mice, in particular due to the
243 alteration of immune conditions in the urinary system. Furthermore, because the development of
244 hydronephrosis was not observed in the F1 generation between C57BL/6 mice and DBA/2 mice in our
245 previous study [21], homogenous genetic factors may have crucial roles in the development of
246 hydronephrosis.

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

262 A previous study reported the spontaneous development of ureteric obstructions caused by
263 polyploid adenomas locating from the renal pelvis to the ureter with severe cell infiltration and the
264 appearance of Chil3l3/Ym1-positive crystals in acute myeloid leukemia of *PML/PARA* knock-in mice
265 [18]. Furthermore, in the hydronephrotic ureters of (C57BL/6 × DBA/2) F2 mice, comprehensive gene
266 expression analysis revealed that the factors regulating ureteric local inflammation, the *Chil3l3* gene in
267 particular, showed ectopic and remarkably elevated expression [17]. Interestingly, Chil3l3/Ym1 was also
268 detected in the cytoplasm of infiltrating cells, apical portion of transitional epithelial cells, and cell
269 debris or crystal structures of ureter lumen in B6.MRL-(*D11Mit21-D11Mit212*) mice. Chil3l3/Ym1 is an

270 endogenous lectin, widely distributed in normal mammalian bodies, and expressed transiently in early
271 myeloid precursor cells of hematopoietic tissues: initially in the yolk sac and subsequently in the fetal
272 liver, spleen, and bone marrow [18, 28, 29-30]. In addition, Chil3l3/Ym1 promotes Th2 cytokine
273 expression in allergic responses, suggesting an important role of Chil3l3/Ym1 in hematopoiesis as well
274 as inflammation [31]. Therefore, the appearance of Chil3l3/Ym1 expressing cells might reflect altered
275 local immune conditions in the ureters of B6.MRL-(*D11Mit21-D11Mit212*) mice.

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

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

295 To identify the SNV between the C57BL/6N- and MRL/MpJ-type genome on the congenic region
296 in B6.MRL-(*D11Mit21-D11Mit212*) mice, we performed Whole Exome sequencing and identified over
297 100 nonsynonymous SNVs. Interestingly, 12 variants associated with frameshift or stopgain such as the

298 *Olf* family, *Fat2*, *Btnl10*, *Obscn*, *Iba57*, *Myo15*, and *Trim16* were identified between MRL/MpJ-type
299 Chr. 11 and C57BL/6N. However, there are no report about the relationship between these genes and the
300 urinary tract system. Mice carrying the null-mutated Forkhead box C1 (*Foxc1*) gene frequently develop
301 congenital anomalies of the kidney and urinary tract such as duplicated ureters [34], but this gene is
302 coded on Chr. 13. The coding genes of RARA, PML, and IL-9, hydronephrosis-associated molecules,
303 were not located on Chr. 11 (*D11Mit21-212*). Thus, the SNV identified in this study might be useful to
304 elucidate the novel pathogenesis associated with the immune system and the development of urinary
305 tract system in future studies. In addition, we should consider the contribution of the C57BL/6N-genetic
306 background, because mice carrying the C57BL/6N-type genetic background have been shown to
307 develop eosinophilic macrophage pneumonia with the appearance of eosinophilic crystals [35, 36].
308 Large eosinophilic crystals were also observed in the lumen of ureters in
309 B6.MRL-(*D11Mit21-D11Mit212*) mice, and similar crystals composed of endogenous lectin were
310 observed in several diseases with severe eosinophil infiltration such as parasitosis and asthma [37].

311 In conclusion, B6.MRL-(*D11Mit21-D11Mit212*) mice spontaneously developed hydronephrosis
312 due to obstructive uropathy with inflammation. Therefore, this animal model would be useful for the
313 molecular pathological analysis of obstructive uropathy and developmental anomaly of the urinary
314 organs.

315

316

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318

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321

322

Declaration of Interest

323 This manuscript has not been published or presented elsewhere in part or in entirety, and is not under
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427 blood in vitro. *Biomarkers* 15: 646–654.

428

429

Figure Legends

430 **Fig. 1. Chr. 11 of B6.MRL-(*D11Mit21-D11Mit212*) mice.**

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

435

436 **Fig. 2. Hydronephrosis in B6.MRL-(*D11Mit21-D11Mit212*) mice.**

437 (A, B) Gross anatomical features of hydronephrosis in B6.MRL-(*D11Mit21-D11Mit212*) mice.
438 Unilateral hydronephrosis showing urine retention to the renal pelvis are observed. Arrows indicate
439 ureter hypertrophy in proximal portions.

440 (C) Relative increase of weights in the hydronephrotic kidneys of B6.MRL-(*D11Mit21-D11Mit212*)
441 mice. Values = mean \pm S.E., $n > 4$.

442 (D) The incidence of hydronephrosis in B6.MRL-(*D11Mit21-D11Mit212*) mice. The number of analyzed
443 mice is described in the graphs. N.D.: not detected.

444 (E) Ratio of spleen weight to body weight in B6.MRL-(*D11Mit21-D11Mit212*) mice with or without the
445 development of hydronephrosis. Male and female individuals are included at 11–28 weeks of age.
446 Values = mean \pm S.E. *: significant difference with normal group.

447

448 **Fig. 3. Histopathology of hydronephrosis in B6.MRL-(*D11Mit21-D11Mit212*) mice**

449 (A-C) Histopathological features of the kidney and ureter (UR) in hydronephrotic
450 B6.MRL-(*D11Mit21-D11Mit212*) mice assessed by hematoxylin and eosin staining. Thinning renal
451 cortex (CO) and enlarged renal pelvis (RP) are observed (A and B). Papillary malformations of the
452 transitional epithelium (arrows) are observed in the pelvic-ureteric junction (C). Numerous cell
453 infiltrations are also observed around the mucosa of the proximal ureter (C, left side).

454 (D-I) Surface structures of the lumens in RP and UR of B6.MRL-(*D11Mit21-D11Mit212*) mice assessed
455 by SEM. Smooth transition of RP mucosa to UR mucosa (D). AT: adipose tissue. Kid: kidney. The renal

456 papilla is shortened (arrow) and RP is enlarged (E). Papillary malformations of the transitional
457 epithelium constricted the entrance of the ureter lumen (F, asterisk). Border between normal (dagger)
458 and abnormal (asterisk) transitional epithelium (G). In these positions, the former shows a wrinkled
459 surface (arrows), but the latter shows hexagonal features (arrowheads) (H and I).

460

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

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

481 (L) Histopathological features of the renal cortex in hydronephrotic B6.MRL-(*D11Mit21-D11Mit212*)
482 mice at 20 weeks of age assessed by periodic acid Schiff staining. There is no glomerular lesion (arrows).
483 Asterisks indicate dilated renal tubules.

484

485 **Fig. 5. Duplicated ureters in B6.MRL-(*D11Mit21-D11Mit212*) mice.**

486 Histopathological features of duplicated ureters are incidentally observed in some
487 B6.MRL-(*D11Mit21-D11Mit212*) mice. Hematoxylin and eosin staining (A). Toluidine blue staining
488 (B-D). Duplicated ureters run parallel to each other (A). Duplicated ureters have common serosa (B).
489 One of the ureters shows stenosis features containing cell debris (A, left side; B, upper one; C, lower
490 one). These cell debris are composed of dropped epithelial cells and crystals (C and D). Several mast
491 cells showing metachromasia are observed around the ureters (D, arrow). Lu: lumen.

492

493

494 **Table 1. Antibodies, working dilutions, and methods for antigen retrieval.**

Antibody	Source	Dilution	Antigen retrieval	Heating condition
Rabbit anti-Ki67		1:150	10 mM Citrate buffer (pH 6.0)	105°C, 20 min
Rat anti-Chi313/Ym1	R and D system (Minneapolis, MN, USA)	1:400	20 mM Tris-HCl (pH 9.0)	105°C, 20 min
Rabbit anti-Iba1	Wako (Osaka, Japan)	1:1000	20 mM Tris-HCl (pH 9.0)	37°C, 5 min
Rat anti-Gr1	R and D system	1:400	0.1% pepsin/ 0.2 N HCl	37°C, 5 min
Rat anti-B220	Cedarlane (Ontario, Canada)	1:800	20 mM Tris-HCl (pH 9.0)	105°C, 20 min
Rabbit anti-CD3	Nichirei (Tokyo, Japan)	1:200	0.1% pepsin/ 0.2 N HCl	105°C, 20 min

495

496

497

498 **Table 2. Summary of SNV determined by Whole Exome sequencing between C57BL/6N and**
499 **MRL/MpJ in Chr. 11 congenic regions.**

Gene	Change description	Start	End	C57BL/6N	MRL/MpJ	Homo/Hetero	SNP quality
Nonsynonymous substitution							
<i>Havcr2</i>	nonsynonymous_SNV6	46456267	46456267	A	G	hom	153
<i>Gm12169</i>	nonsynonymous_SNV2	46528416	46528416	C	G	hom	186
<i>Gm12171</i>	nonsynonymous_SNV1	46548446	46548446	C	T	hom	160
<i>BC053393</i>	nonsynonymous_SNV2	46577230	46577230	A	G	hom	222
<i>Havcr1</i>	nonsynonymous_SNV3	46752306	46752306	G	A	hom	222
<i>Timd4</i>	nonsynonymous_SNV2	46810846	46810846	C	T	hom	222
<i>Maml1</i>	nonsynonymous_SNV1	50263308	50263308	T	A	hom	59
<i>Csf2</i>	nonsynonymous_SNV1	54247598	54247598	C	A	hom	220
<i>Il3</i>	nonsynonymous_SNV4	54266977	54266977	T	C	hom	150
<i>4930404A10Rik</i>	nonsynonymous_SNV1	54370917	54370917	G	A	hom	212
<i>Tnip1</i>	nonsynonymous_SNV1	54940825	54940825	C	G	hom	140
<i>Anxa6</i>	nonsynonymous_SNV3	54983317	54983317	T	A	hom	214
<i>Fat2</i>	nonsynonymous_SNV2	55262499	55262499	G	A	hom	221
<i>Nmur2</i>	nonsynonymous_SNV1	56040278	56040278	T	C	hom	222
<i>Fam114a2</i>	nonsynonymous_SNV5	57484032	57484032	A	G	hom	167
<i>Mfap3</i>	nonsynonymous_SNV2	57528040	57528040	A	T	hom	198
<i>Galnt10</i>	nonsynonymous_SNV1	57765688	57765688	G	A	hom	222
<i>Larp1</i>	nonsynonymous_SNV1	58042340	58042340	G	A	hom	222
<i>Gemin5</i>	nonsynonymous_SNV6	58122289	58122289	C	G	hom	222
<i>Mrpl22</i>	nonsynonymous_SNV1	58171695	58171695	G	A	hom	222
<i>Gm12250</i>	nonsynonymous_SNV3	58187802	58187802	T	C	hom	222
<i>Igtp</i>	nonsynonymous_SNV4	58206343	58206343	T	G	hom	222
<i>Irgm2</i>	nonsynonymous_SNV9	58219513	58219513	T	A	hom	195
<i>Zfp692</i>	nonsynonymous_SNV2	58309033	58309033	G	T	hom	222
<i>Lypd8</i>	nonsynonymous_SNV6	58382775	58382775	T	C	hom	222
<i>1810065E05Rik</i>	nonsynonymous_SNV3	58422201	58422201	C	T	hom	170
<i>Gm12253</i>	nonsynonymous_SNV6	58434541	58434541	G	C	hom	222
<i>Olfir332</i>	nonsynonymous_SNV2	58490297	58490297	C	T	hom	222
<i>Olfir331</i>	nonsynonymous_SNV11	58501648	58501648	T	C	hom	222
<i>Olfir329-ps</i>	nonsynonymous_SNV1	58543446	58543446	G	T	hom	222
<i>Olfir328</i>	nonsynonymous_SNV3	58551561	58551561	T	C	hom	152
<i>Olfir224</i>	nonsynonymous_SNV2	58566562	58566562	G	A	hom	222
<i>Olfir325</i>	nonsynonymous_SNV4	58580931	58580931	T	C	hom	221
<i>Olfir324</i>	nonsynonymous_SNV4	58597518	58597518	A	C	hom	222
<i>2210407C18Rik</i>	nonsynonymous_SNV3	58608509	58608509	G	A	hom	32.3
<i>Olfir323</i>	nonsynonymous_SNV3	58625762	58625762	G	A	hom	156
<i>Trim58</i>	nonsynonymous_SNV3	58640858	58640858	C	A	hom	222
<i>Olfir322</i>	nonsynonymous_SNV3	58665691	58665691	T	C	hom	102
<i>Olfir320</i>	nonsynonymous_SNV8	58683932	58683932	G	T	hom	157
<i>Olfir319</i>	nonsynonymous_SNV2	58701958	58701958	A	C	hom	222
<i>Olfir318</i>	nonsynonymous_SNV2	58720458	58720458	G	A	het	107
<i>Olfir317</i>	nonsynonymous_SNV2	58732215	58732215	T	C	hom	199
<i>Olfir316</i>	nonsynonymous_SNV7	58757967	58757967	G	A	hom	183
<i>Olfir313</i>	nonsynonymous_SNV5	58817061	58817061	A	G	hom	222
<i>Olfir311</i>	nonsynonymous_SNV2	58841119	58841119	G	T	hom	222
<i>2810021J22Rik</i>	nonsynonymous_SNV6	58878844	58878844	A	G	hom	218

<i>Zfp39</i>	nonsynonymous_SNV7	58889867	58889867	C	T	hom	112
<i>Trim17</i>	nonsynonymous_SNV1	58970446	58970446	A	G	hom	221
<i>Obscn</i>	nonsynonymous_SNV54	59000884	59000884	C	T	hom	196
<i>Iba57</i>	nonsynonymous_SNV2	59161555	59161555	T	C	hom	222
<i>Gjc2</i>	nonsynonymous_SNV2	59176433	59176433	T	C	hom	199
<i>Mrp155</i>	nonsynonymous_SNV1	59204589	59204589	A	T	hom	203
<i>Prss38</i>	nonsynonymous_SNV2	59375551	59375551	G	T	hom	187
<i>Jmjd4</i>	nonsynonymous_SNV3	59450788	59450788	A	G	hom	154
<i>Zfp867</i>	nonsynonymous_SNV2	59463105	59463105	A	G	hom	33.3
<i>Zkscan17</i>	nonsynonymous_SNV4	59487650	59487650	C	T	hom	222
<i>Nlrp3</i>	nonsynonymous_SNV2	59555785	59555785	G	A	hom	222
<i>Olfir223</i>	nonsynonymous_SNV1	59589934	59589934	G	T	hom	219
<i>Olfir225</i>	nonsynonymous_SNV7	59613209	59613209	A	G	hom	222
<i>Mrip1</i>	nonsynonymous_SNV2	59749606	59749606	G	A	hom	222
<i>Pemt</i>	nonsynonymous_SNV1	60031784	60031784	T	C	hom	222
<i>Rai1</i>	nonsynonymous_SNV5	60185857	60185857	A	G	hom	212
<i>Srebfl</i>	nonsynonymous_SNV1	60200146	60200146	T	C	hom	222
<i>Lrrc48</i>	nonsynonymous_SNV5	60364958	60364958	G	A	hom	191
<i>Myo15</i>	nonsynonymous_SNV7	60477716	60477716	A	G	hom	189
<i>Flii</i>	nonsynonymous_SNV2	60716732	60716732	T	C	hom	222
<i>Mief2</i>	nonsynonymous_SNV3	60730943	60730943	C	T	hom	222
<i>Top3a</i>	nonsynonymous_SNV5	60742577	60742577	G	A	hom	193
<i>Smcr8</i>	nonsynonymous_SNV3	60779106	60779106	T	C	hom	222
<i>Kcnj12</i>	nonsynonymous_SNV3	61066752	61066752	T	C	hom	144
<i>Zfp287</i>	nonsynonymous_SNV2	62713807	62713807	C	T	hom	222
<i>Zfp286</i>	nonsynonymous_SNV4	62780692	62780692	T	C	hom	92
<i>Trim16</i>	nonsynonymous_SNV4	62820602	62820602	C	T	hom	222
<i>Fbxw10</i>	nonsynonymous_SNV1	62847292	62847292	G	C	hom	164
<i>Tvp23b</i>	nonsynonymous_SNV1	62881943	62881943	A	C	hom	154
<i>Myh8</i>	nonsynonymous_SNV1	67289653	67289653	A	G	hom	222
<i>Glp2r</i>	nonsynonymous_SNV8	67709568	67709568	C	A	hom	186
<i>Rnf222</i>	nonsynonymous_SNV1	68893019	68893019	A	T	hom	165
<i>Aurkb</i>	nonsynonymous_SNV1	69047870	69047870	T	C	hom	184
<i>Per1;Per1</i>	nonsynonymous_SNV1	69108510	69108510	C	T	hom	222
<i>Hes7</i>	nonsynonymous_SNV1	69122956	69122956	T	C	hom	33
<i>Aloxe3</i>	nonsynonymous_SNV1	69128675	69128675	A	G	hom	222
<i>Aloxe3</i>	nonsynonymous_SNV1	69134001	69134001	G	A	hom	211
<i>Chd3</i>	nonsynonymous_SNV1	69361451	69361451	A	G	hom	221
<i>Cyb5d1</i>	nonsynonymous_SNV1	69395202	69395202	A	G	hom	148
<i>Cyb5d1</i>	nonsynonymous_SNV1	69395272	69395272	C	A	hom	164
<i>Tmem102</i>	nonsynonymous_SNV1	69805101	69805101	G	C	hom	140
<i>Nlgn2</i>	nonsynonymous_SNV1	69828394	69828394	A	G	hom	187
<i>Kif1c</i>	nonsynonymous_SNV1	70724114	70724114	C	T	hom	172

Frameshift, stopgain

<i>Fat2</i>	frameshift_deletion	55308977	55308989	CACCCCAGTACCT	-	hom	214
<i>Olfir332</i>	stopgain_SNV	58489748	58489748	C	A	hom	82.1
<i>Olfir331</i>	nonframeshift_deletion	58502393	58502404	TGGTATGTGGAT	-	hom	55.5
<i>Btml10</i>	stopgain_SNV	58926877	58926877	G	T	hom	222
<i>Obscn</i>	nonframeshift_insertion	59000527	59000527	-	TGG	hom	214
<i>Iba57</i>	frameshift_insertion	59161506	59161506	-	GA	hom	214
<i>Olfir223</i>	stopgain_SNV	59589685	59589685	G	A	hom	215
<i>Olfir225</i>	frameshift_insertion	59613118	59613118	-	T	hom	214
<i>Olfir225</i>	stopgain_SNV	59613206	59613206	C	T	hom	222
<i>Olfir225</i>	frameshift_insertion	59613903	59613903	-	T	hom	214

<i>Myo15</i>	nonframeshift_deletion	60504278	60504280	TAG	-	hom	214
<i>Trim16</i>	nonframeshift_insertion	62820695	62820695	-	AGA	hom	214

SNV: single nucleotide variant. SNP: single nucleotide polymorphism. The letter after SNV shows the number of SNV in the coding region of each gene.

500









