

Tensin2-deficient mice on FVB/N background develop severe glomerular disease

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ABSTRACT. Tensin2 (*Tns2*) is an essential component for the maintenance of glomerular basement membrane (GBM) structures. *Tns2*-deficient mice were previously shown to develop mild glomerular injury on a DBA/2 background, but not on a C57BL/6J or a 129/SvJ background, suggesting that glomerular injury by the deletion of *Tns2* was strongly dependent on the genetic background. To further understand the mechanisms for the onset and the progression of glomerular injury by the deletion of *Tns2*, we generated *Tns2*-deficient mice on an FVB/N (FVB) strain, which is highly sensitive to glomerular disease. *Tns2*-deficient mice on FVB (FVBGN) developed severe nephrotic syndrome, and female FVBGN mice died within 8 weeks. Ultrastructural analysis revealed that FVBGN mice exhibited severe glomerular defects with mesangial process invasion of glomerular capillary tufts, lamination and thickening of the GBM and subsequent podocyte foot process effacement soon after birth. Aberrant laminin components containing $\alpha 1$, $\alpha 2$ and $\beta 1$ chains, which are normally expressed in the mesangium, accumulated in the GBM of FVBGN, suggesting that these components originated from mesangial cells that invaded glomerular capillary tufts. Compared to *Tns2*-deficient mice on the other backgrounds in previous reports, FVBGN mice developed earlier onset of glomerular defects and rapid progression of renal failure. Thus, this study further extended our understanding of the possible genetic background effect on the deterioration of nephrotic syndrome by *Tns2* deficiency.

KEY WORDS: FVB/N, glomerular basement membrane (GBM), ICR-derived glomerulonephritis (ICGN) mouse, nephrotic syndrome, tensin2 (*Tns2*, *Tenc1*)

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Tensin2 (*Tns2*, also known as *Tenc1*), an integrin-associated focal adhesion molecule, is widely expressed in mouse tissues [6, 16, 20]. It is primarily expressed in podocytes within the glomerulus and contributes to the maintenance of glomerular basement membrane (GBM) structures [7, 20, 25]. A mouse strain carrying a mutated Tensin2 gene (*Tns2*), the ICR-derived glomerulonephritis (ICGN) strain, was shown to develop severe nephrotic syndrome [7, 23]. Previous studies reported that *Tns2*-deficient mice developed mild nephrotic syndrome on a DBA/2 (D2) background, but not on a C57BL/6J (B6) or a 129/SvJ background [21, 22, 25]. The initial manifestation of *Tns2*-deficient mice on a D2 background (D2.ICGN-*Tns2*^{nph}, D2GN) is an abnormality in the GBM, i.e. the abnormal accumulation of laminin-111, an immature GBM component [25]. Focal adhesion kinase (FAK) activation in podocytes and the effacement of podocyte foot processes have also been reported

following GBM abnormalities in D2GN mice [25]. These glomerular changes resemble those observed in Alport and CD151 knockout mice [4, 8, 9, 25, 27].

Genetic backgrounds are considered to influence the development and progression of kidney diseases. The B6 strain is known to be relatively resistant to kidney diseases [3, 4, 12, 13, 24]. In contrast, the FVB/N (FVB) strain is highly sensitive to glomerular diseases including HIV-nephropathy, diabetic nephropathy and others [4, 5, 12]. In the present study, we generated *Tns2*-deficient mice on an FVB strain in order to better understand the role of *Tns2* in glomeruli, and how the genetic background influences the progression of kidney diseases.

MATERIALS AND METHODS

Animals: ICGN mice maintained at the National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), and FVB mice obtained from Clea Japan (Tokyo, Japan) were used in this study. The congenic strain carrying the *Tns2* mutation of the ICGN strain was made by generating an F1 hybrid between ICGN and FVB, followed by 15 generations of backcrossing to FVB. Genotyping was performed as previously described [25]. *Tns2*^{nph} heterozygous congenic mice were then intercrossed to generate *Tns2*^{nph} homozygous mice (FVB.ICGN-*Tns2*^{nph},

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FVBGN). Littermate wild-type mice (FVB) were used as controls. Male and female FVB and FVBGN mice, aged 1, 3 and 5 weeks, were used for examinations. All animals were housed in autoclaved cages and were given a standard diet (CMF; Oriental Yeast, Tokyo, Japan) and tap water *ad libitum* in an air-conditioned room ($23 \pm 1^\circ\text{C}$) under controlled lighting conditions (12-hr light/12-hr dark). All animal experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committees of NIBIOHN.

Measurements in serum and urine: Spot urine was collected from 3 and 5-week-old mice before sacrifice, and urine samples of 1-week-old mice were collected from bladder under Isoflurane anesthesia (Wako, Osaka, Japan). Mice were anesthetized with somnopenyl (Kyoritsu Seiyaku, Tokyo, Japan), and blood was collected by cardiac puncture. Serum total protein, albumin, creatinine, blood urea nitrogen (BUN) and total cholesterol levels were measured on an automatic analyzer (Fuji DRI-CHEM FDC7000, Fujifilm, Tokyo, Japan). Urine albumin levels were analyzed by SDS-PAGE, followed by Coomassie Brilliant blue staining. Bovine serum albumin (BSA) was used as the standard.

Histological and ultrastructural analyses: Formalin-fixed kidney samples from 3 and 5-week-old mice were dehydrated through a graded ethanol series and embedded in Histosec (Merck, Darmstadt, Germany) for histology. In the conventional pathological evaluation, kidney sections ($3 \mu\text{m}$ thick) were stained with periodic acid Schiff (PAS). Regarding transmission electron microscopy (TEM), blocks of 1-mm renal cortex cubes from 1, 3 and 5-week-old mice were fixed in 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 hr, washed with phosphate buffer and post-fixed in 2% osmic acid solution (EM grade, Merck) for 1 hr at 4°C . After dehydration through ascending grades of ethanol, they were embedded in Epon resin (TAAB, Berks, U.K.). Ultra-thin sections (60 nm thick) were cut using a diamond knife (DiATOME, Biel, Switzerland) on an EM UC6 ultramicrotome (Leica, Wetzlar, Germany), mounted on copper grids and double stained with saturated uranyl acetate solution and Sato's lead staining solution containing lead citrate, lead nitrate and lead acetate. They were observed with a Hitachi H-7650 electron microscope (Hitachi, Tokyo, Japan) operating at an accelerating voltage of 80 kV.

Determination of podocyte number: Paraffin sections ($3 \mu\text{m}$ thick) were immunostained for WT-1. Briefly, the sections were treated by autoclaving in 0.01 M citric acid buffer (pH 6.0) at 121°C for 5 min, and then incubated with anti-WT-1 rabbit polyclonal antibody diluted with 3% skim milk and 1% BSA in phosphate buffered saline (PBS, pH 7.4) at 1:300 (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). After being incubated, the sections were incubated with horseradish peroxidase (HRP)-labeled Envision for rabbits (DAKO, Glostrup, Denmark). Sections were then treated with 3,3'-diaminobenzidine tetrahydrochloride (DAKO). WT-1 positive cells from 20 glomeruli were counted from five individual mice.

Immunofluorescence: The kidneys were rapidly frozen

in dry ice-cooled isopentane. Fresh frozen sections ($5 \mu\text{m}$ thick) were fixed with cold acetone (-80°C), air-dried, rehydrated in PBS and incubated with each primary antibody. The primary antibodies used were as follows: anti-laminin $\alpha 1$ rat monoclonal and anti-fibronectin rabbit polyclonal antibodies at 1:100 (Millipore, Billerica, MA, U.S.A.), anti-laminin $\alpha 2$, $\beta 1$ and $\gamma 1$ rat monoclonal and anti-CD151 rabbit polyclonal antibodies at 1:200 (Santa Cruz), anti-laminin $\alpha 5$ and $\beta 2$ rabbit polyclonal antibodies (Sigma, St. Louis, MO, U.S.A.), anti- $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\alpha 5$ chains of collagen IV rat monoclonal antibodies at 1:100 (Chondrex, Redmond, WA, U.S.A.), anti-phospho-FAK rabbit polyclonal antibody at 1:100 (Abcam, Cambridge, U.K.) and anti-integrin $\alpha 8$ goat polyclonal antibody at 1:200 (R&D Systems, Minneapolis, MN, U.S.A.). Slides were then washed with PBS and incubated with secondary antibodies conjugated with Alexa Fluor[®] 488 or 594 at 1:200 (Invitrogen, Carlsbad, CA, U.S.A.). All dilutions were made in 1% BSA in PBS. Sections were examined with a microscope (Axioplan2; Carl Zeiss Vision, Munich, Germany).

Statistical analysis: Distributed data were expressed as mean values \pm SD ($n=5$ in each group) and assessed for significance by Student's *t*-test using Microsoft Excel on a Macintosh computer. Differences at a probability of $P<0.05$ were considered significant.

RESULTS

Clinical features: We produced a congenic strain by introducing the *Tns2* mutation of the ICGN strain into the FVB strain (FVBGN). FVBGN mice appeared to be normal at birth, but the female mice died unexpectedly at around 6 weeks of age and the male mice at around 8 weeks of age (Fig. 1A). Because slight edema developed from 4 weeks of age in FVBGN mice, the mice seemed to suffer from end stage renal failure. From this point, the Institutional Animal Care and Use Committees of NIBIOHN allowed us to keep FVBGN mice only up to 5 weeks of age. A urine analysis revealed that FVBGN mice presented with proteinuria as early as postnatal 1 week and continued to progress with age in both genders (Fig. 1B). As shown in Tables 1 and 2, renal hypertrophy, hypoalbuminemia and hypercholesterolemia were detected in FVBGN mice.

Renal histopathology: We performed PAS staining at different ages in order to assess structural alterations in the kidneys of FVBGN mice. In the glomeruli of FVB mice, we could not detect any abnormalities at 3 and 5 weeks of age (Fig. 2A). In contrast, thickening of the glomerular basement membrane (GBM) and enlargement of the mesangial region were observed in the glomeruli of 3-week-old FVBGN mice (Fig. 2A). The decreased number of podocytes was also observed in FVBGN mice (Fig. 2B). In addition to glomerular defects, tubulointerstitial injury occurred at 5 weeks of age in the kidneys of FVBGN mice (Fig. 2A). In order to further elucidate glomerular morphological changes in FVBGN mice, we performed an ultrastructural analysis using TEM. In the glomeruli of FVB mice, a single layer of lamina densa sandwiched between lamina rara interna and

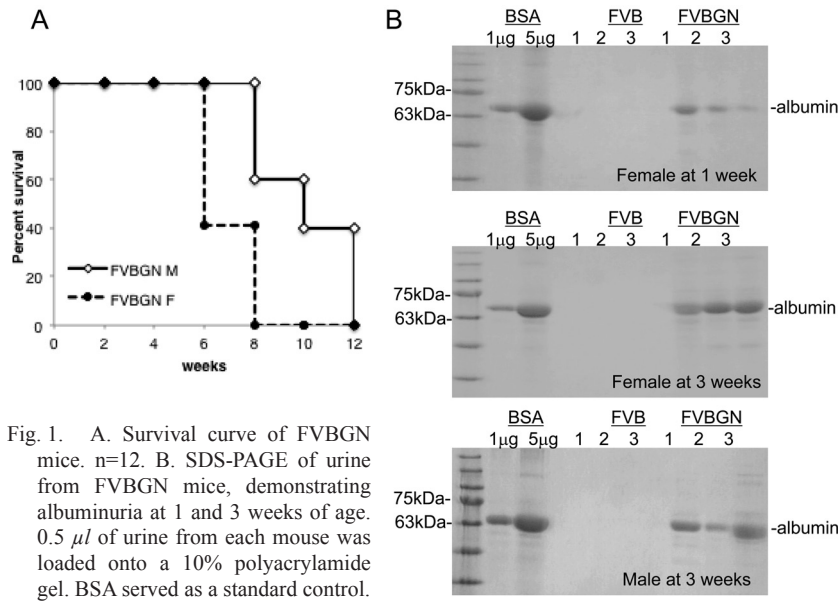


Fig. 1. A. Survival curve of FVBGN mice. n=12. B. SDS-PAGE of urine from FVBGN mice, demonstrating albuminuria at 1 and 3 weeks of age. 0.5 µl of urine from each mouse was loaded onto a 10% polyacrylamide gel. BSA served as a standard control.

Table 1. Body weight and relative kidney weight in the mice used

	3 w			
	FVB		FVBGN	
	Male	Female	Male	Female
Body weight (g)	15.34 ± 3.43	12.23 ± 0.42	14.88 ± 2.68	12.22 ± 0.26
Kidney weight/body weight (%)	1.37 ± 0.02	1.41 ± 0.06	1.75 ± 0.23 ^{a)}	1.64 ± 0.09 ^{a)}
	5 w			
	FVB		FVBGN	
	Male	Female	Male	Female
Body weight (g)	21.23 ± 0.74	18.83 ± 0.74	19.34 ± 1.00	18.54 ± 1.22
Kidney weight/body weight (%)	1.46 ± 0.09	1.27 ± 0.04	2.06 ± 0.23 ^{a)}	1.71 ± 0.14 ^{a)}

a) $P < 0.05$ between age- and sex-matched FVB and FVBGN mice.

Table 2. Serum biochemical features in FVB and FVBGN mice

	3 w			
	FVB		FVBGN	
	Male	Female	Male	Female
Total protein (g/dl)	4.42 ± 0.29	4.36 ± 0.27	3.36 ± 0.51 ^{a)}	3.74 ± 0.21 ^{a)}
Albumin (g/dl)	2.06 ± 0.18	1.76 ± 0.17	1.20 ± 0.25 ^{a)}	1.38 ± 0.28
Creatinine (mg/dl)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
BUN (mg/dl)	15.24 ± 2.87	16.08 ± 2.86	19.8 ± 6.77	17.66 ± 4.32
Total cholesterol (mg/dl)	106.2 ± 12.75	108.0 ± 14.71	113.6 ± 20.03	139.2 ± 13.59 ^{a)}
	5 w			
	FVB		FVBGN	
	Male	Female	Male	Female
Total protein (g/dl)	4.78 ± 0.11	4.83 ± 0.13	3.78 ± 0.15 ^{a)}	3.72 ± 0.40 ^{a)}
Albumin (g/dl)	2.42 ± 0.13	2.56 ± 0.15	1.68 ± 0.16 ^{a)}	1.70 ± 0.25 ^{a)}
Creatinine (mg/dl)	0.10 ± 0.00	0.10 ± 0.00	0.12 ± 0.04	0.12 ± 0.04
BUN (mg/dl)	22.78 ± 1.14	16.41 ± 1.98	32.37 ± 16.05	31.04 ± 21.98
Total cholesterol (mg/dl)	139.4 ± 8.73	119.7 ± 8.99	149.8 ± 10.92	140.6 ± 12.78 ^{a)}

a) $P < 0.05$ between age- and sex-matched FVB and FVBGN mice.

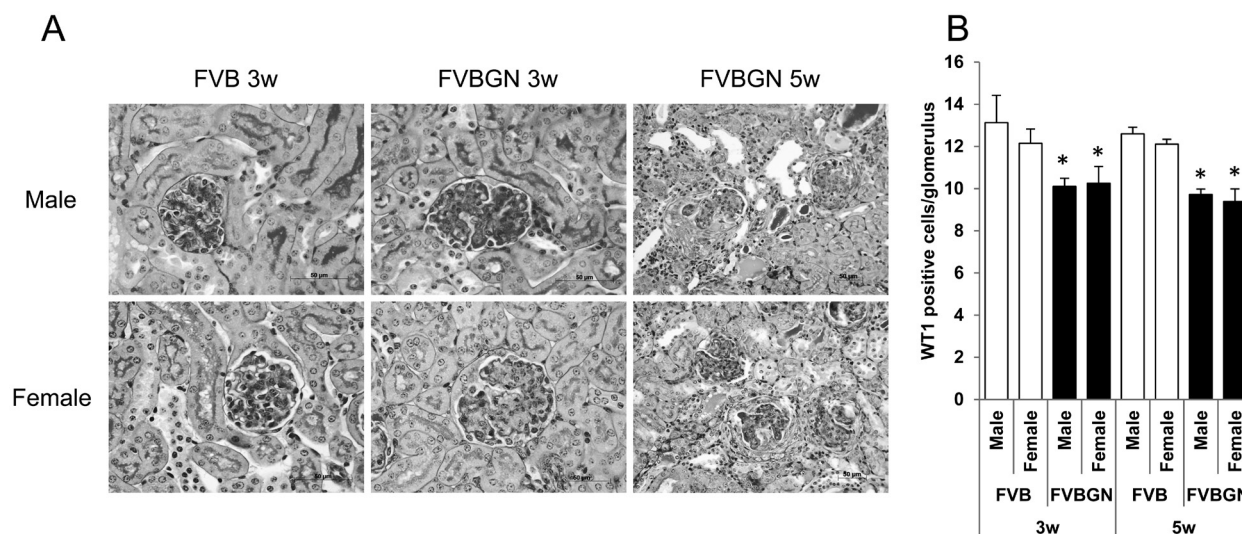


Fig. 2. A. Representative light microscopy images (PAS staining) of kidneys from FVB and FVBGN mice at 3 and 5 weeks of age. No glomerular defects were observed at 3 weeks of age in FVB mice. In the glomeruli of 3-week-old FVBGN mice, thickening of the GBM and enlargement of the mesangial region were observed. In the kidneys of 5-week-old FVBGN mice, tubular casts and accumulation of infiltrated cells in tubulointerstitium were observed in addition to severe glomerular defects. Scale bars=50 μ m. B. Podocyte number per glomerulus was estimated by counting nuclei positive for WT1 staining. The numbers of WT-1 positive cells in FVBGN mice were significantly lower than in those FVB mice. *: $P < 0.05$ between age- and sex- matched FVB and FVBGN mice.

lamina rara externa in the GBM was observed (Fig. 3A and 3B). Developing foot processes were partially detected in 1-week-old FVB mice, since new glomeruli continue to develop up to approximately 2 weeks after birth (Fig. 3A). Mature podocyte foot processes were clearly observed in glomeruli of 3-week-old FVB mice (Fig. 3B). In contrast, focal thickening and thinning of the GBM, the lamination of lamina densa and many small granules in the GBM were observed at 1 week of age in FVBGN mice (Fig. 3C–E). In addition, mesangial process invasion of glomerular capillary tufts and the effacement of podocyte foot processes were observed in FVBGN mice (Fig. 3C). Since we observed normal podocyte foot processes and slit diaphragms in small areas in the glomeruli of FVBGN mice (Fig. 3E), GBM thickening may have preceded podocyte abnormalities. The degree of morphological changes in the GBM and podocytes progressively increased with age (Fig. 3F–H). On occasion, podocyte endoplasmic reticulum distension was observed at 5 week of age in FVBGN mice. There were no gender differences in the morphological changes of the GBM and podocytes.

Defects in the GBM assembly in FVBGN mice: To further investigate glomerular defects, we first analyzed the GBM assembly and maturation in FVBGN mice by immunofluorescence analysis. $\alpha 1$ – $\alpha 5$ chains of collagen IV expressed with normal patterns in the GBM of FVB and FVBGN mice (data not shown). Laminin-521, which is a component in the mature glomeruli, was also observed with normal patterns in the GBM of FVB and FVBGN mice (data not shown). These data suggest that the GBM maturation normally occurs in FVBGN mice. Subsequently, we detected laminin $\alpha 1$, $\alpha 2$,

$\beta 1$ and fibronectin, which normally express in the mesangium of the normal matured glomeruli but are absent in the GBM. Laminin-211 and fibronectin had already begun to be deposited in the GBM at 1 week of age, while the expression of laminin-111 was detected in the GBM of FVBGN mice at 3 weeks of age (Fig. 4). These abnormal accumulations were similarly detected in both genders. Further, we performed immunofluorescence for phospho-FAK, associated with retraction of the foot processes and leading to effacement. The expression of phospho-FAK was barely detectable in the podocytes of FVBGN mice at 1 week of age and increased with age in both genders (Fig. 4). Finally, to determine whether the abnormal GBM components originate from invaded mesangial cell process, a dual immunofluorescence analysis using a mesangial marker, integrin $\alpha 8$ and laminin $\alpha 2$, was performed. Figure 5 demonstrated that integrin $\alpha 8$ and laminin $\alpha 2$ colocalized in the GBM and spread into the glomerular capillary loops in FVBGN mice.

DISCUSSION

Tns2 is a cytoplasmic phosphoprotein that is localized to integrin-mediated focal adhesions [6, 16, 20]. In the kidneys, Tns2 is mainly expressed in the podocytes [7, 20]. Previous studies revealed that *Tns2*-deficient mice developed nephrotic syndrome in a strain-specific manner [21, 22, 25]. Since Tns2 is known to stabilize integrin adhesive contacts, its loss may lead to defects in podocyte integrin $\alpha 3\beta 1$ -GBM binding intensity. To further elucidate the functions of Tns2 in the glomeruli and the mechanisms for the onset and the progression of glomerular injury by the deletion of *Tns2*, we

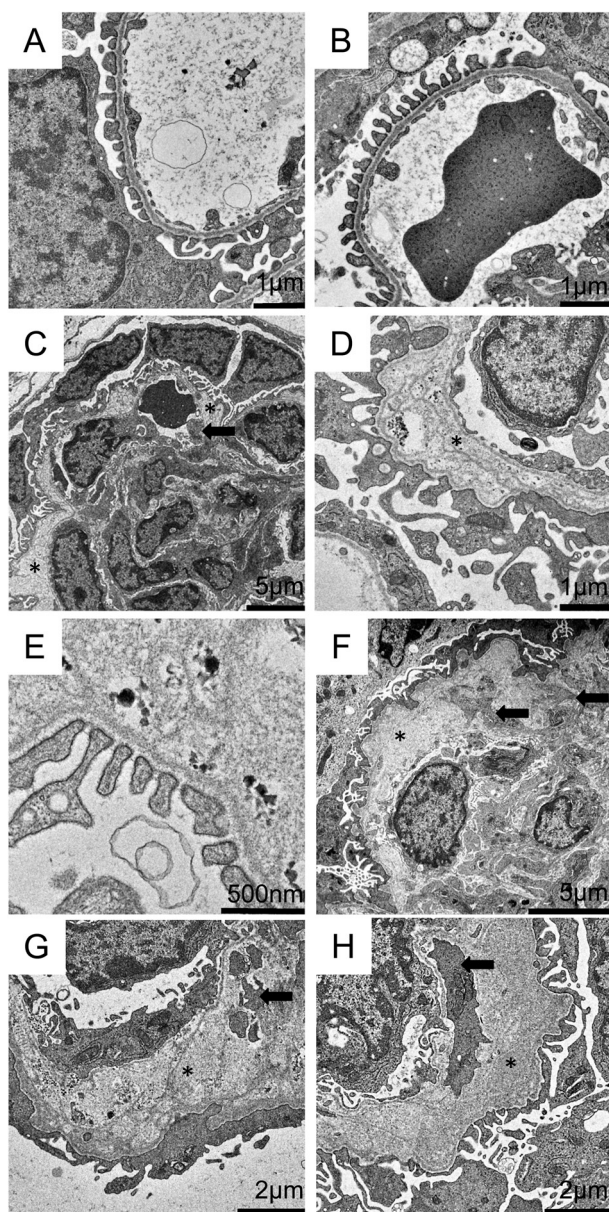


Fig. 3. A TEM analysis of FVB and FVBGN mice. Three layers of the GBM, consisting of the lamina densa, lamina rara interna and lamina rara externa were observed in FVB mice (A and B). Immature foot processes were partially found in 1-week-old FVB mice (A). The lamination of lamina densa with lumpy thickening GBM (*) was observed as early as 1-week-old FVBGN (C-E). In addition, the effacement of podocyte foot processes, many small granules in the GBM and mesangial process invasion of the glomerular capillary tufts (arrows) were noted. A: male FVB at 1 week of age. B: male FVB at 3 weeks of age. C-E: male FVBGN at 1 week of age. F: male FVBGN at 3 weeks of age. G: male FVBGN at 5 weeks of age. H: female at 5 weeks of age.

newly produced *Tns2*-deficient mice on an FVB background, which is highly sensitive to glomerular diseases.

FVBGN mice developed proteinuria soon after birth and

severe hypoalbuminemia at a young age. Female FVBGN mice died at earlier age than male mice, but the causes of the death were not defined. Several studies have demonstrated that the progression of most renal diseases is more rapid in male gender than female [19]. By contrast, female gender is more susceptible to autoimmune diseases, such as lupus nephritis [26]. The cause and mechanism of gender differences in renal disease development are largely unknown. Additional studies are needed to better ascertain the relationship between gender and renal disease in this strain.

The initial manifestation of FVBGN mice was thickened GBM and mesangial cell process invasion of glomerular capillary tufts, followed by the effacement of podocyte foot processes. GBM integrity and proper podocyte anchorage are required for a functional glomerular filter [18]. The GBM is a network formed by laminin, collagen IV, nidogen and negatively charged proteoglycans [18], and among the components, substitutions of laminin (the transition from laminin-111 to laminin-521) and collagen IV (the transition from $\alpha 1$ and $\alpha 2$ chains to $\alpha 3$, $\alpha 4$ and $\alpha 5$ chains) occur during glomerular maturation [18]. Both endothelial cells and podocytes produce laminin-111 and then laminin-521 [1]. By contrast, both cells produce $\alpha 1$ and $\alpha 2$ chains of collagen IV, and then, $\alpha 3$, $\alpha 4$ and $\alpha 5$ chains originate solely from podocytes [1]. Mutations in GBM components, such as Alport syndrome and Pierson's syndrome, are known to result in the loss of selective GBM permeability and glomerular structural damage [18]. In addition to mutations in GBM components, loss of CD151 leads to a specific defect of $\alpha 3\beta 1$ integrin-GBM adhesion and also results in similar GBM abnormalities [4]. These different mutations affecting structural integrity of the glomerular capillary tufts result in unnatural stresses on the cells in contact with tuft [27]. Abnormal biomechanical stresses on the glomerular capillary tufts activate a pro-migratory signaling cascade in mesangial cells and trigger mesangial process invasion of the glomerular capillary tufts [27]. In fact, these mesangial cell process invasion has been reported in Alport and CD151 knockout mice, and hypertension accelerates this event, suggesting that biomechanical stress influences glomerular cell defects [9, 27]. In this study, we observed mesangial cell process invasion in the glomerular capillary tufts of the FVBGN mice by TEM and immunofluorescence analysis using a mesangial cell marker, integrin $\alpha 8$. FVBGN strain has normal GBM collagen IV network and expressed CD151 on podocytes (data not shown), suggesting that biomechanical stress on the glomerular cells was elevated by deletion of *Tns2*. Since *Tns2* is known to stabilize integrin adhesive contacts, its loss may induce defects in the structural integrity of glomerular capillary tufts and unusual biomechanical stress on glomerular cells due to increased pressure at birth. The fetal kidneys are regulated by low blood pressure and low renal blood flow, however, after birth, the neonate kidneys are immediately subjected to a relatively higher blood pressure. Elevated biomechanical stress on the glomerular cells by deletion of *Tns2* probably contributed to mesangial cell process invasion in the glomerular capillary tufts of the FVBGN mice.

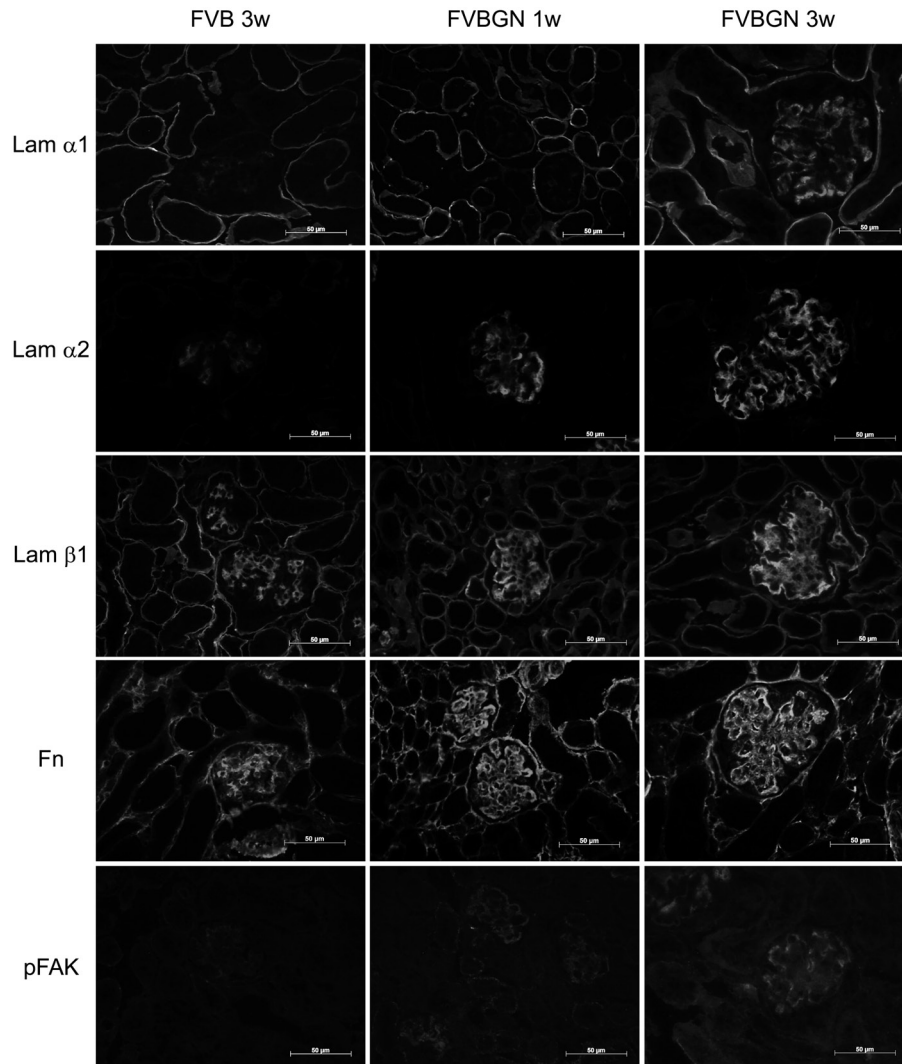


Fig. 4. Immunofluorescence study for laminin, fibronectin and phospho-FAK (pFAK) in female FVB and FVBGN mice at 1 week and 3 weeks of age. Laminin α 1 (Lam α 1), Laminin α 2 (Lam α 2), Laminin β 1 (Lam β 1) and fibronectin (Fn), which are expressed in the mesangium of normal matured glomeruli, but are absent in the GBM, were expressed in the GBM of FVBGN. The expression of pFAK was detected in podocytes of FVBGN mice. Scale bars=50 μ m.

The thickening and lamination of the GBM occurred simultaneously with mesangial cell process invasion of glomerular capillary tufts in FVBGN mice. These morphological changes were also similar to those in Alport and CD151 knockout mice. Previous studies reported that the deposition of laminin-211 occurred in the GBM of Alport and CD151 knockout mice [8, 9, 27]. Laminin-211 is normally found only in the mesangium and is not expressed in the GBM at any stage of embryonic development. Since mesangial cell process invasion has been reported in Alport and CD151 knockout mice, laminin-211 in the GBM is probably of mesangial cell origin [9, 27]. Furthermore, several other mesangial matrix proteins have been detected in the GBM of these mice, including laminin-111 and fibronectin [4, 8,

9, 13, 18]. Laminin-111 is also found in the immature GBM during development, but fibronectin is not expressed in the GBM at any stage of glomerular development. The abnormal GBM of these mice may have been elastic, resulting in elevated biomechanical stress on glomerular cells [9, 17, 27]. Especially, abnormal laminin-rich regions of the GBM might contribute to the permeability defect, progressive leakiness and proteinuria [2]. Further, the abnormal deposition of laminin-211, but not laminin-111, in the GBM induces FAK activation on podocytes, resulting in foot process effacement [9, 17]. In this study, we detected the accumulation of laminin-211 and fibronectin in the GBM at 1 week of age, and the deposit of laminin-111 and podocyte FAK activation at 3 weeks of age in FVBGN mice by immunofluorescence

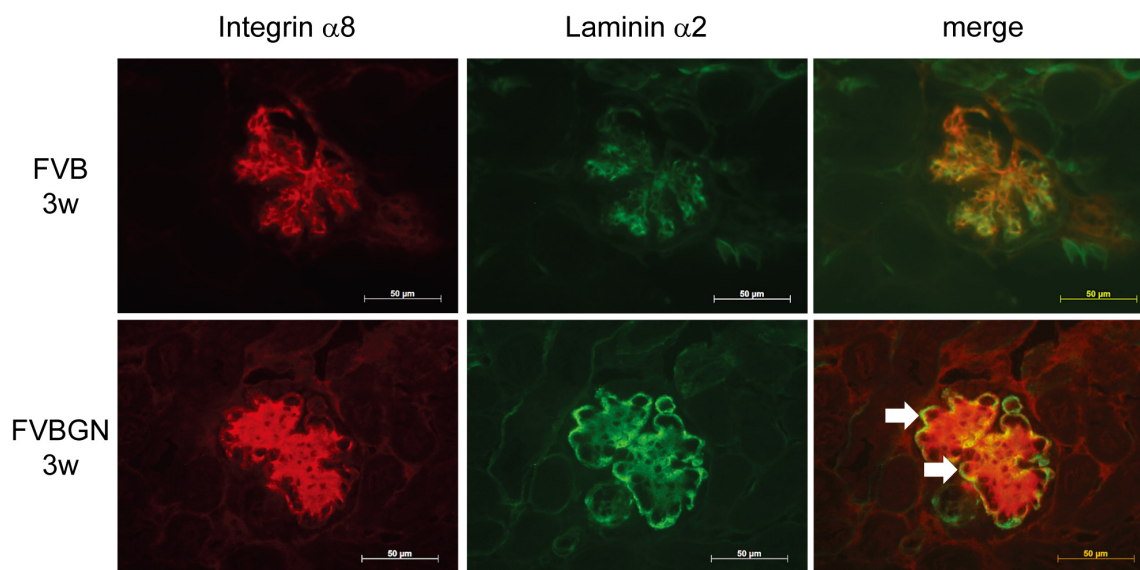


Fig. 5. Dual immunofluorescence study for a mesangial marker, integrin $\alpha 8$ and laminin $\alpha 2$ in 3-week-old female FVB and FVBGN mice. Mesangial process invasion of the glomerular capillary tufts was observed in FVBGN mice. Laminin $\alpha 2$ localized to the mesangium and the GBM in FVBGN mice. Arrows denote area of dual immunostaining in the glomerular capillary loop. Scale bars=50 μm .

analysis. Since the deposition of laminin-211 and fibronectin in the GBM preceded podocyte foot process effacement, the abnormal GBM, that was abnormally permeable and elastic, probably led to proteinuria and influenced podocyte behavior in FVBGN mice. Our data of TEM and immunofluorescence analysis raise the possibility that laminin-211 and fibronectin in the GBM are of mesangial cell origin and laminin-111 is of damaged podocyte origin. Additional studies are needed to identify the earliest events in the glomeruli and the origin of abnormal GBM components, and to understand the mechanisms regulating the synthesis of abnormal laminins.

In addition to its roles in cell adhesion, *Tns2* has been identified as a negative regulator of the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway and has been shown to regulate cell survival, proliferation and migration [10, 11]. PI3K/Akt also regulates the expression of genes involved in ECM metabolism; Akt is required to induce the synthesis of laminin and collagen IV and to repress the expression of matrix metalloproteinases (MMPs) [14, 15]. Both molecules are essential for maintaining the GBM structure; laminin and collagen IV are GBM components, and MMPs influence the proteolytic degradation of GBM. Further studies are needed in order to determine whether *Tns2*-mediated PI3K/Akt pathways are important for GBM and podocyte functions, and may provide insights into the mechanisms underlying glomerular injury.

Genetic backgrounds influence the development and progression of kidney diseases, e.g. diabetic nephropathy and lupus nephritis [5, 12, 13]. The B6 strain is known to be relatively resistant to kidney diseases [3, 12, 13, 21, 24]. For example, Alport mice exhibited more severe GBM damage on a 129/Sv than on a B6 background [3, 13], and CD151

knockout mice displayed GBM abnormalities on an FVB, but not on a B6 background [4]. The present study and previous findings revealed that *Tns2*-deficient mice displayed severe glomerular defects shortly after birth on FVB and milder defects on D2, but not on B6 or 129/Sv [21, 22, 25]. Our preliminary experiments revealed that *Tns2*-deficient mice on BALB/c strain also did not develop any glomerular defects. *Tns2*-deficient mice on D2 and FVB backgrounds developed similar glomerular morphological changes, i.e. thickened GBM, mesangial cell process invasion of glomerular capillary tufts and the effacement of podocyte foot processes, and subsequent tubulointerstitial inflammation, but the progression of these defects was more rapid on FVB than on D2 [25]. FVB strain is already known to be highly susceptible to glomerular diseases, but the loci that contribute to the onset and the progression of glomerular diseases are poorly understood. To understand how genetic background influences the onset, the rate of progression and the severity of glomerular diseases, *Tns2*-deficient strains on multiple genetic backgrounds may be possible to identify modifier loci through linkage analysis by crossing the different strains.

In conclusion, we here generated a novel *Tns2* mutant strain on the FVB strain and first demonstrated that deletion of *Tns2* led to mesangial process of invasion of glomerular capillary tufts and ectopic expression of laminin-211 and fibronectin in the GBM. The *Tns2* mutant strain on the FVB background may be used to understand the functions of *Tns2* in glomeruli and to map the modifiers affecting susceptibility to glomerular diseases by comparison with on the other multiple genetic backgrounds. The identification of these modifying loci may provide a mechanism for the pathogen-

esis of glomerular diseases and assist in the development of novel therapeutic approaches.

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