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Study of genetic background-dependent diversity of renal failure caused
by the tensin2 gene deficiency in the mouse

(マウスのテンシン2遺伝子欠損に起因する腎障害における遺伝学的背景の
影響に関する研究)

Hayato Sasaki

PREFACE

Chronic kidney disease (CKD) is a public-health problem characterized as either kidney damage or kidney function decline for a long time, regardless of disease type or cause, and is increasingly common in the world irrespective of developed or developing countries. Patients with CKD are at high risk for developing end-stage renal disease (ESRD), requiring costly renal replacement therapy or renal transplantation. In addition, CKD is strongly linked to important diseases, such as diabetes, hypertension and cardiovascular disease (Couser *et al.*, 2011). Faced with these outcomes of CKD, clinical research efforts have been focused on preventing or delaying the progression of CKD for the last decades. Unfortunately, fundamental therapy is not available at present, and the major present therapies for CKD are palliative care and removing the risk factors such as hypertension, hyperglycemia and hyperlipidemia. Considering renal structure and function to filtrate blood flows continuously, it is easy to imagine that renal glomerular damage by abnormal blood pressure and components is a potential risk factor for CKD progression. Proteinuria is intimately involved in the dysfunction of glomerular visceral epithelial cell (podocyte) or its intercellular junction forming filtration slit (slit diaphragm), and glomerulosclerosis (GS) sequentially starts with decrease in podocytes (podocytopenia). GS is one of the most common forms of CKD, and mutations in a number of podocyte-specific genes (*NPHS1*, *NPHS2*, *CD2AP*, *ACTN4*, *TRPC6*, *PLCE1*, *MYH9*) responsible for GS have been identified in humans (Wiggins, 2007). Therefore, podocyte loss is a common determining factor for progression toward many types of kidney disease, resulting in CKD (Pavenstädt *et al.*, 2003; Warsow *et al.*, 2013). However, the molecular mechanisms of CKD progression are still unclear. The onset, progression and the severity of CKD can be strongly influenced by genetic backgrounds. For example, ESRD incidence rates are higher in blacks, Asians and Hispanics compared with whites (Derose *et al.*, 2013). These racial disparities indicate the presence of the modifier genes that prevent or accelerate the progression of CKD. Elucidating the reasons for these differences in diverse genetic backgrounds could help unraveling the mechanisms for the progression of CKD and developing novel therapies for it, regardless of predisposing factors.

This study approaches CKD from these aspects using ICGN mouse, one of the spontaneous CKD model mice. The null mutation of the *tensin2* gene (*Tns2*) is known as the major causative factor for renal failure in ICGN mice, and the function of *Tns2* in kidney

and the mechanism by which Tns2-deficiency leads to renal failure are unknown (Cho *et al.*, 2006). Also, the severity of the renal failure caused by Tns2-deficiency is strongly influenced by murine genetic backgrounds (Nishino *et al.*, 2010, 2012a; Uchio-Yamada *et al.*, 2013). This genetic background-dependent diversity indicates the presence of the modifier genes that prevent renal failure induced by Tns2-deficiency. Hence, comparisons of the genetic background-dependent differences in susceptibility to Tns2-deficiency could help identifying the modifier genes, and unraveling the function of Tns2 in kidney and the mechanism by which Tns2-deficiency leads to renal failure.

ABBREVIATIONS

129T	129 ^{+Ter} /SvJcl
129S1	129/Sv- ^{+P+Tyr-c} +Mgf ^{Sl-J} /+
129T- <i>Tns2</i> ^{nph}	129.ICGN- <i>Tns2</i> ^{nph}
129T2	129T2/SvEmsJ
129X1	129/SvJ
ACTN4	Actinin, alpha 4
B6	C57BL/6J
B6- <i>Tns2</i> ^{nph}	B6.ICGN- <i>Tns2</i> ^{nph}
Btla	B and T lymphocyte associated
BUN	Blood urea nitrogen
C1	Protein kinase C conserved region 1
CAS	Crk-associated substrate
<i>Cat</i>	Catalase
CBB	Coomassie brilliant blue
CHCM	Cell hemoglobin concentration mean
Chr	Chromosome
CD2AP	CD2-associated protein
cDNA	Complementary deoxyribonucleic acid
CKD	Chronic kidney disease
cM	Centimorgan
D2	DBA/2J
D2- <i>Tns2</i> ^{nph}	D2.ICGN- <i>Tns2</i> ^{nph}
DAB	3,3-diaminobenzidine
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
ESRD	End-stage renal disease
FAK	Focal adhesion kinase
FVB	FVB/NJ
FVB- <i>Tns2</i> ^{nph}	FVB.ICGN- <i>Tns2</i> ^{nph}
GCRMA	GC robust multi-array average
GEO	Gene Expression Omnibus

<i>Greml</i>	Gremlin 1
GBM	Glomerular basement membrane
GS	Glomerulosclerosis
H2-Q5	Histocompatibility 2, Q region locus 5
Hb	Hemoglobin concentration
HIV	Human immunodeficiency virus
HIVAN	HIV-associated nephropathy
HRP	Horseradish peroxidase
Ht	Hematocrit
ICCs	Interclass correlation coefficients
ICGN(-)	ICGN.B6- <i>Tns2</i> (+/+)
ICGN-2 ^{B6}	ICGN-Chr2 ^{B6}
IL-1F6	Interleukin 1 family, member 6
ILK	Integrin-linked kinase
Kif26a	Kinesin family member 26A
<i>Lcn2</i>	Lipocalin 2
LOD	Logarithm of odds
LRS	Likelihood ratio statistic
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MGI	Mouse Genome Informatics
MHC	Major histocompatibility complex
MPD	Mouse Phenome Database
mRNA	Messenger ribonucleic acid
MYH9	Myosin, heavy chain 9, non-muscle
<i>Nes</i>	Nestin
<i>nph</i>	Nephrosis
NPHS1	Nephrosis 1 (nephrin)
NPHS2	Nephrosis 2 (podocin)
nsSNP	Nonsynonymous single-nucleotide polymorphism
PAS	Periodic acid-Schiff
PCR	Polymerase chain reaction

PFA	Paraformaldehyde
PI3	Phosphoinositide 3
PLCE1	Phospholipase C, epsilon 1
PTB	Phosphotyrosine-binding
qPCR	Quantitative polymerase chain reaction
QTL	Quantitative trait loci
RBC	Red blood cell count
RNA	Ribonucleic acid
RT	Room temperature
SDS	Sodium dodecyl sulfate
<i>Synpo</i>	Synaptopodin
TRPC6	Transient receptor potential cation channel, subfamily C, member 6
SH2	Src homology 2
Sh3bp1	SH3 domain-binding protein 1
SNP	Single-nucleotide polymorphism
Src	Rous sarcoma oncogene
Tenc1	Tensin like C1 domain-containing phosphatase
<i>Ter</i>	Teratoma
<i>Themis</i>	Thymocyte selection associated
TNS1	Tensin1
TNS2	Tensin2
<i>Tns2^{nph}</i>	Tensin2, nephrosis
TNS3	Tensin3
TNS4	Tensin4
WT1	Wilms tumor 1

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Chapter 1

Quantitative trait loci on chromosome 2 for resistance to the congenital nephropathy in tensin2-deficient mice

The original paper of this chapter will be submitted for publication, and thus can not be shown at the time the thesis has been submitted to Hokkaido University.

SUMMARY

ICGN mouse is a CKD model that is characterized histologically by GS and tubulointerstitial fibrosis, and clinically by proteinuria and anemia, which are common symptoms and pathological changes associated with a variety of kidney diseases. Previously, a QTL analysis identified a deletion mutation of the *Tns2* (*Tns2^{nph}*) as the causative gene for proteinuria in ICGN mice. Interestingly, the congenic strain carrying the *Tns2^{nph}* mutation on a B6 genetic background exhibited milder phenotypes than did ICGN mice, indicating the presence of several modifier genes controlling the disease phenotype. In the present study, to identify the resistant/susceptible loci for CKD progression in *Tns2*-deficient mice, a QTL analysis was performed using backcross progenies from susceptible ICGN and resistant B6 mice. The QTL analysis identified a significant locus on Chr 2 (LOD = 5.36; 31 cM) and two suggestive loci on Chrs 10 (LOD = 2.27; 64 cM) and X (LOD = 2.65; 67 cM) with linkage to the severity of tubulointerstitial injury. One significant locus on Chr 13 (LOD = 3.49; approximately 14 cM) and one suggestive locus on Chr 2 (LOD = 2.41; 51 cM) were identified as QTLs for the severity of GS. A suggestive locus in BUN was also detected in the same Chr 2 region (LOD = 2.34; 51 cM). A locus on Chr 2 (36 cM) was significantly linked with Hb (LOD = 4.47) and Ht (LOD = 3.58). Four novel epistatic loci controlling either Hb or tubulointerstitial injury were discovered. Then, the ICGN consomic mice introgressed Chr 2 (2.23-88.99 cM) from the B6 mouse exhibited milder CKD phenotypes than did ICGN mice. These results suggest the existence of the CKD-resistant/susceptible loci on Chr 2. However, for the primordial prevention of renal failure induced by *Tns2^{nph}*, the resistant effects of Chr 2 from the B6 mouse was insufficient as compared to those of the B6 genetic background itself, suggesting that there are other loci to confer an immediate resistance to *Tns2*-deficiency outside of the introduced Chr 2 region (2.23-88.99 cM).

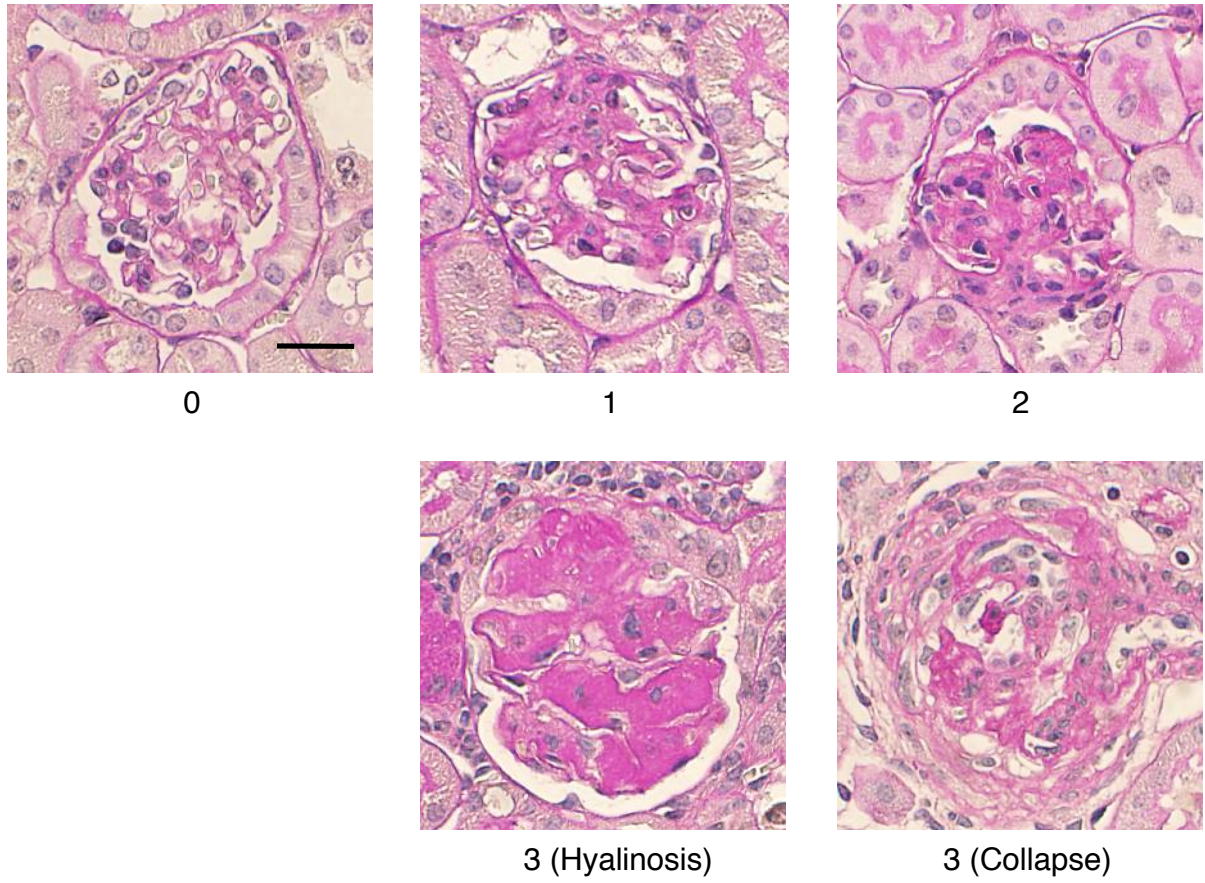


Figure 1. Representative examples of glomerular damage score. Bar indicates 25 μm.

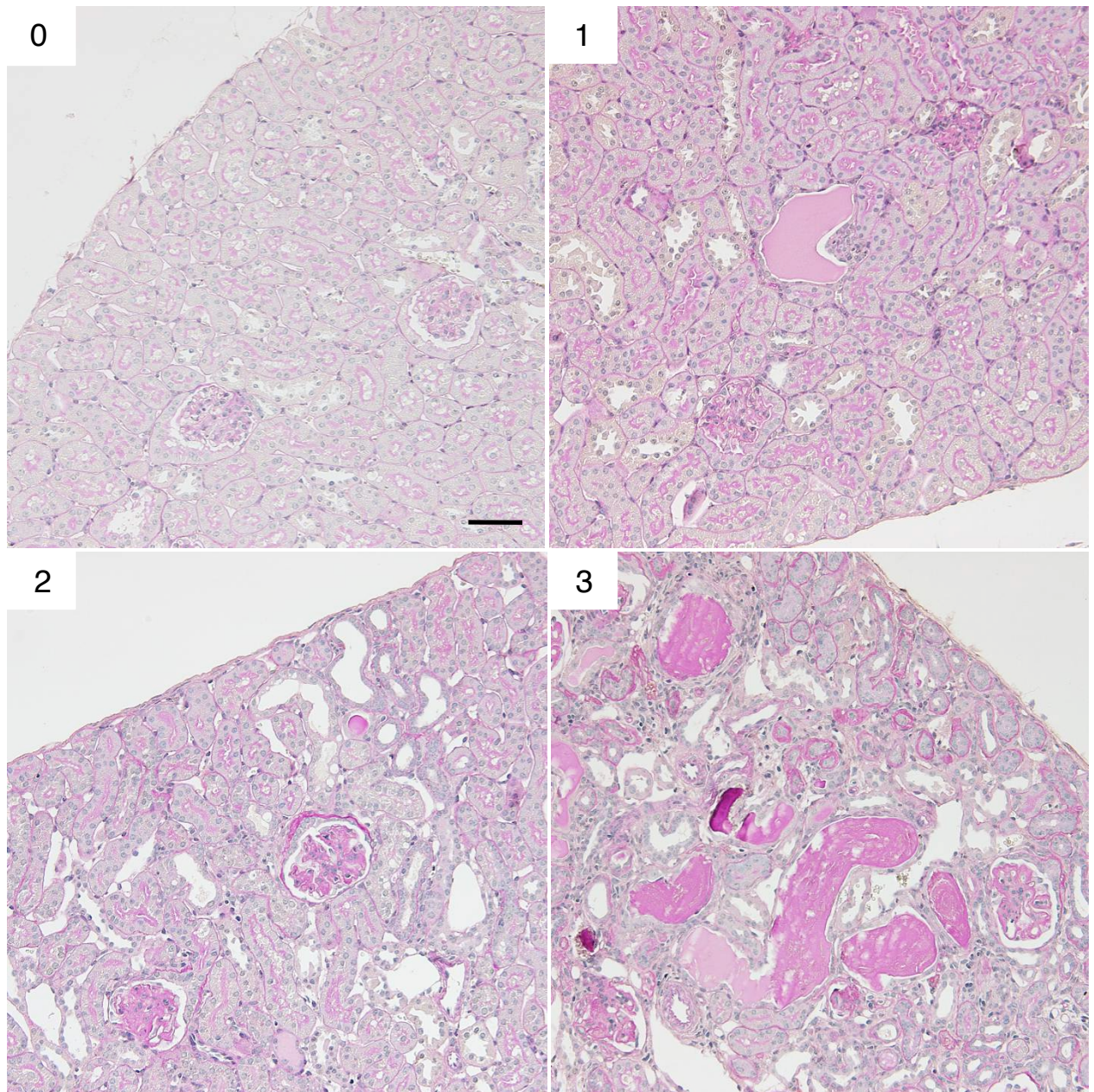
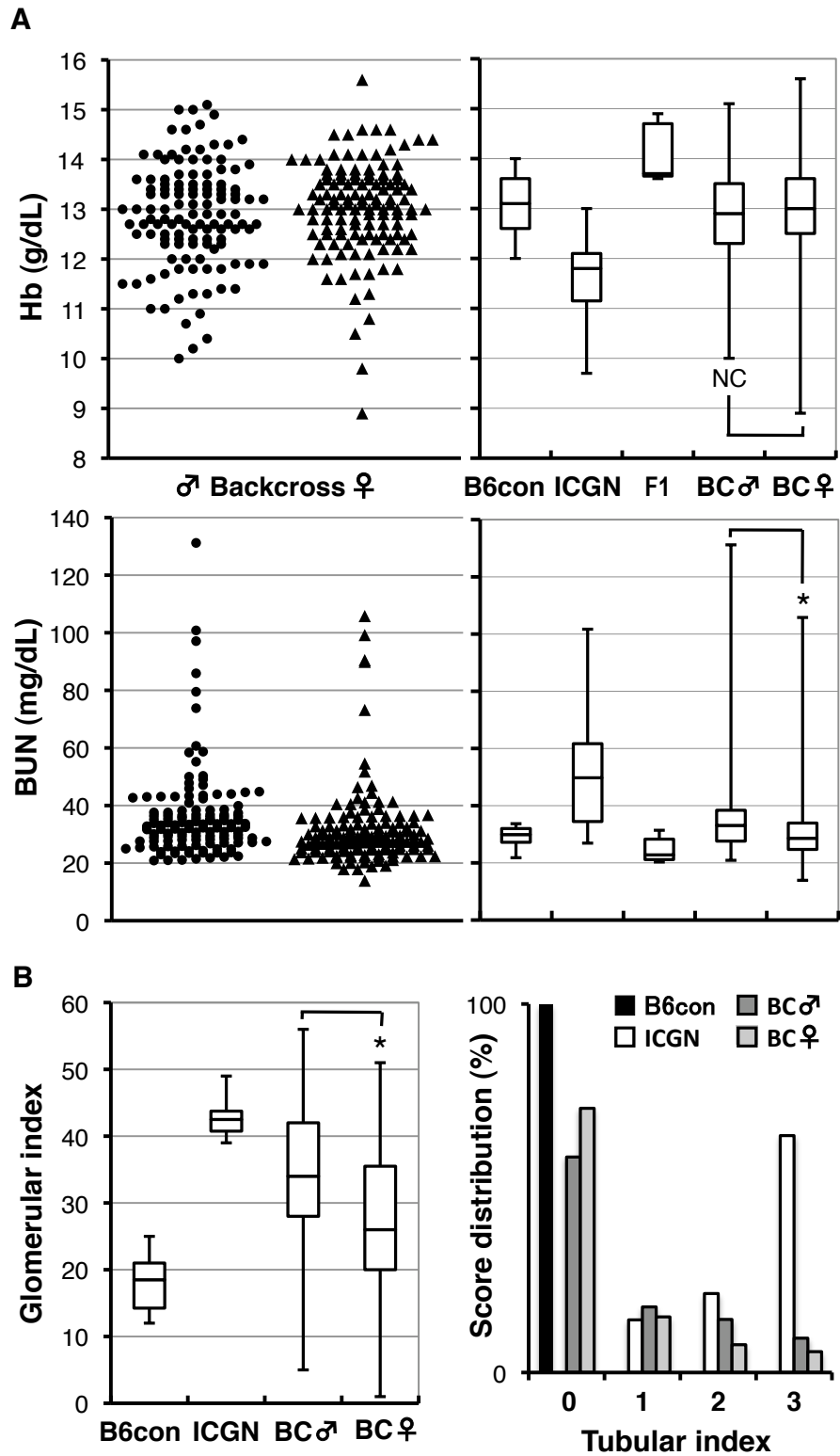


Figure 2. Representative examples of tubular damage score. Bar indicates 50 μm .



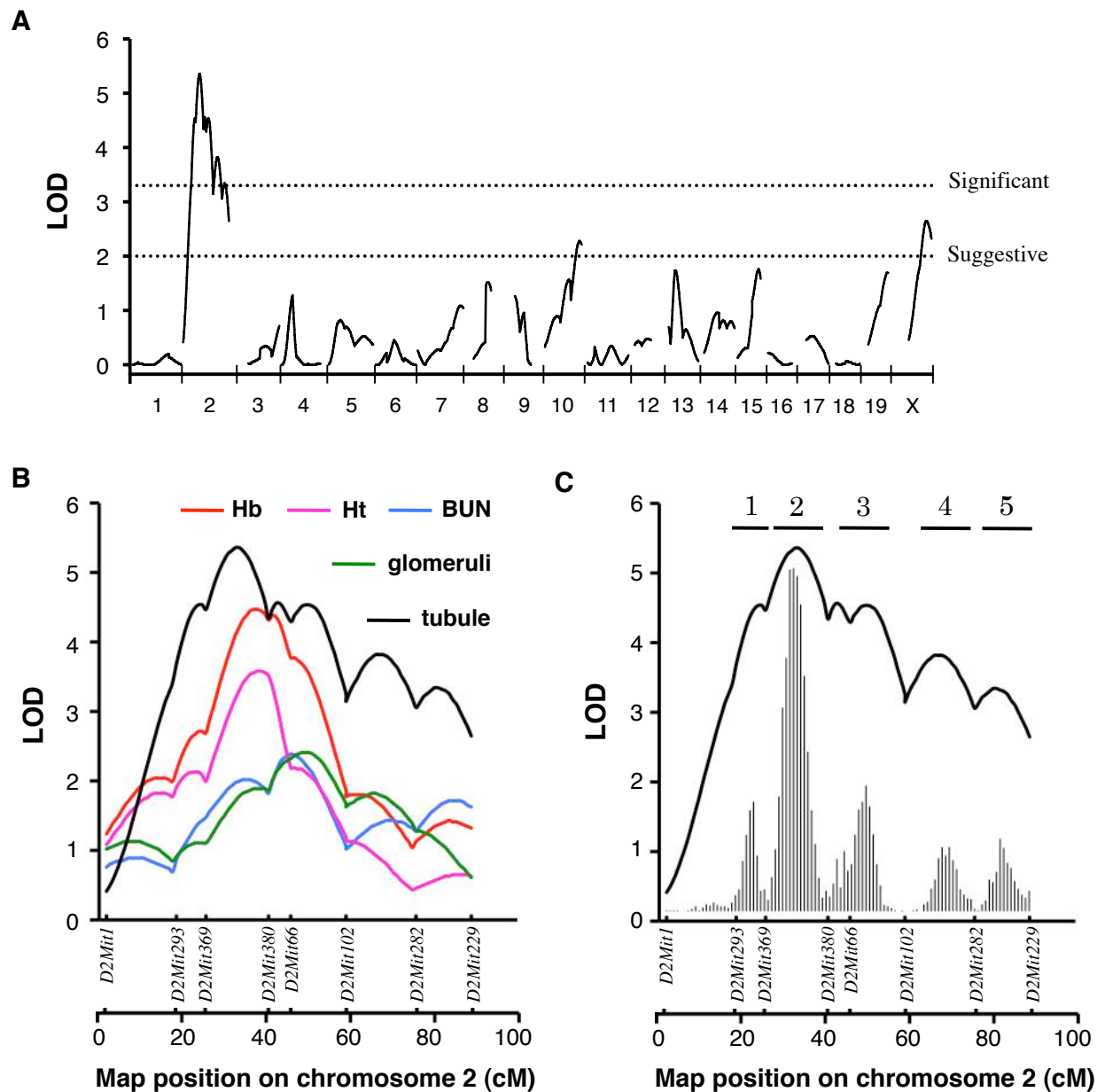


Figure 5. Linkage of nephropathy to chromosome 2. (A) Genome-wide linkage analysis of the tubular index. (B) LOD plots show the linkage of nephropathic traits to Chr 2. Hb (red line) and tubular index (black line) yielded significant LOD scores. The approximate 95% confidential intervals: 19.6-56.4 cM (tubular index), 26-73 cM (glomerular index), 26-55 cM (Hb), 23-59 cM (Ht), 27-72 cM (BUN). (C) LOD curve and bootstrap histogram of the QTL on Chr 2 for tubular index. The histogram appears to have five peaks.

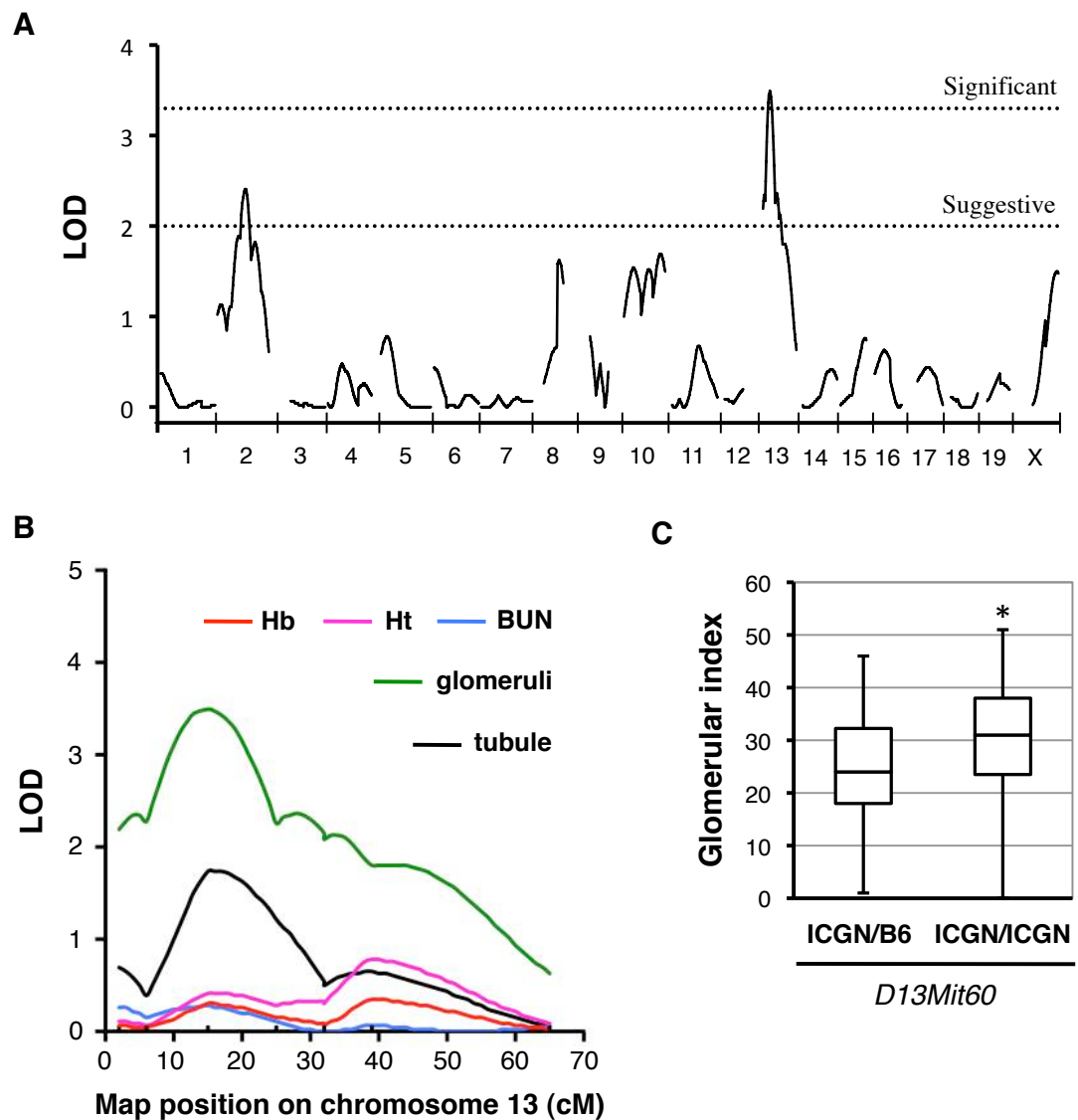


Figure 6. LOD score curves on chromosome 13. (A) Genome-wide linkage analysis of the glomerular index. (B) A significant QTL for glomerular index was detected on Chr 13. The approximate 95% confidential intervals: 0-31.5 cM. (C) Glomerular index at the peak LOD score (*D13Mit60*). The resistance phenotype associated with ICGN/B6 genotype ($P < 0.001$, Mann-Whitney's U test).

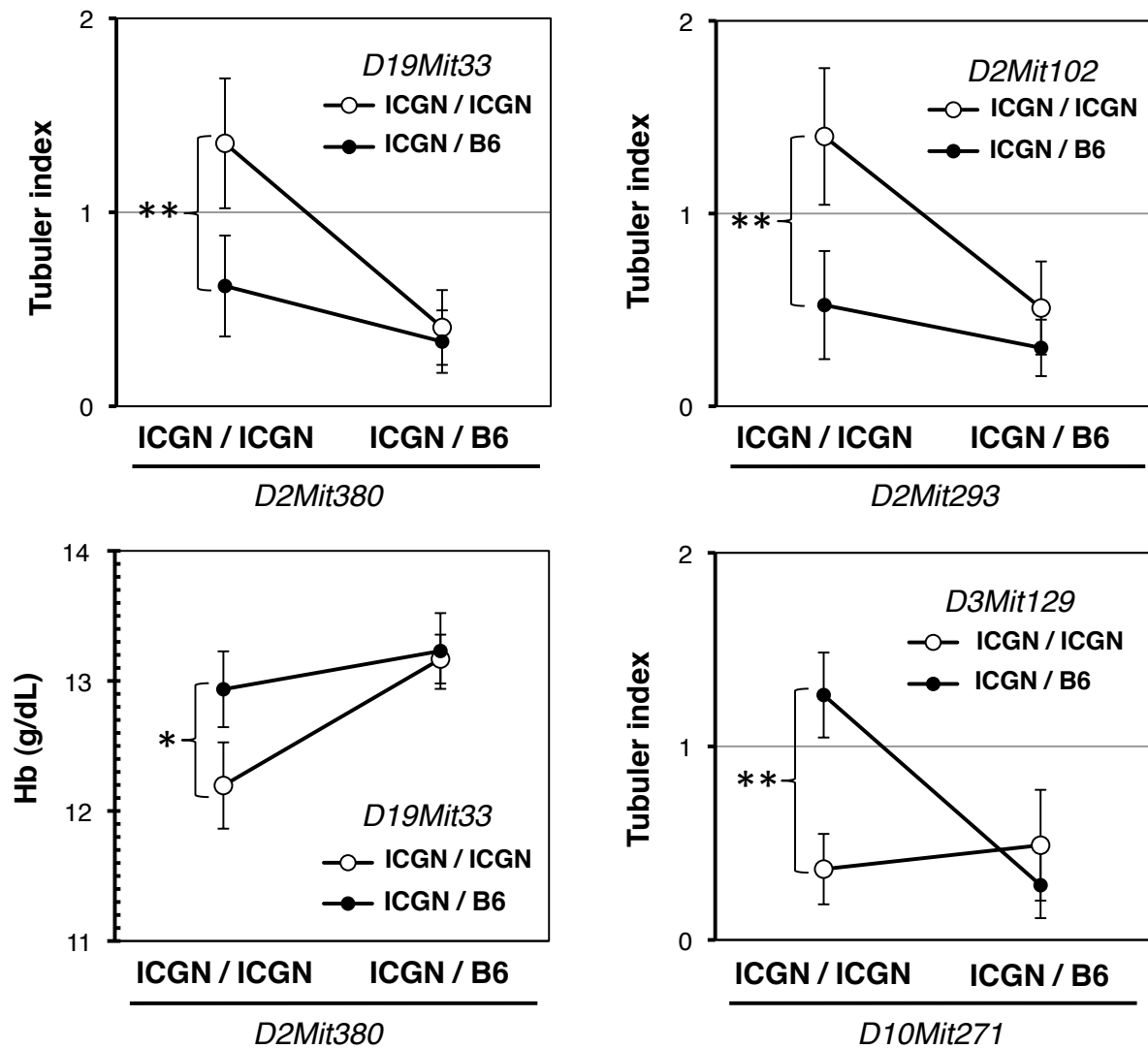


Figure 7. Epistasis associated with QTLs. Epistatic interactions were detected associated with the markers on Chr 2 and 10. Open circles and closed circles represent homozygous and heterozygous variants for the tested markers, respectively. Asterisks signify significant differences between the two genotypes (**, $P < 0.001$; *, $P < 0.01$; Mann-Whitney's U test).

Chr	Markers	cM (MGI)		Chr	Markers	cM (MGI)		Chr	Markers	cM (MGI)	
1	<i>D1Mit1002</i>	4.94	1, 2	5	<i>D5Mit122</i>	89.28	2	13	<i>D13Mit17</i>	7.73	1, 2
1	<i>D1Mit123</i>	17.63	2	6	<i>D6Mit86</i>	1.81	1, 2	13	<i>D13Mit60</i>	14.44	1, 2
1	<i>D1Mit212</i>	18.55	1	6	<i>D6Mit159</i>	12.36	2	13	<i>D13Mit94</i>	24.5	1, 2
1	<i>D1Mit213</i>	22.88	2	6	<i>D6Mit274</i>	23.7	1, 2	13	<i>D13Mit66</i>	34.54	1, 2
1	<i>D1Mit282</i>	37.02	2	6	<i>D6Mit3</i>	34.81	1, 2	13	<i>D13Mit68</i>	41.38	1, 2
1	<i>D1Mit131</i>	39.16	1	6	<i>D6Mit194</i>	62.9	1, 2	13	<i>D13Mit262</i>	63.93	1, 2
1	<i>D1Mit191</i>	52.66	2	6	<i>D6Mit201</i>	77.7	1, 2	14	<i>D14Mit49</i>	7.08	1, 2
1	<i>D1Mit14</i>	67.71	1, 2	7	<i>D7Mit178</i>	2.02	1, 2	14	<i>D14Mit121</i>	25.36	1, 2
1	<i>D1Mit355</i>	80.36	2	7	<i>D7Mit117</i>	17.26	1, 2	14	<i>D14Mit225</i>	39.46	1, 2
1	<i>D1Mit291</i>	88.97	2	7	<i>D7Mit82</i>	32.76	1, 2	14	<i>D14Mit266</i>	64.86	1, 2
1	<i>D1Mit511</i>	97.3	1, 2	7	<i>Tyr</i> (coat color)	49.01	1, 2	15	<i>D15Mit175</i>	3.96	1, 2
2	<i>D2Mit1</i>	2.23	1, 2	7	<i>D7Mit222</i>	59.13	1, 2	15	<i>D15Mit255</i>	16.41	1
2	<i>D2Mit293</i>	17.24	1, 2	7	<i>D7Mit105</i>	70.29	1, 2	15	<i>D15Mit152</i>	17.41	2
2	<i>D2Mit369</i>	24.51	1, 2	7	<i>D7Mit189</i>	88.85-89.02	1	15	<i>D15Mit270</i>	27.16	2
2	<i>D2Mit91</i>	39.24	2	8	<i>D8Mit124</i>	7.59	2	15	<i>D15Mit156</i>	32.19	1
2	<i>D2Mit380</i>	40.89	1	8	<i>D8Mit4</i>	18.89	1, 2	15	<i>D15Mit71</i>	37.8	2
2	<i>D2Mit66</i>	49.45	1, 2	8	<i>D8Mit234</i>	39.33	1, 2	15	<i>D15Mit108</i>	47.79	1, 2
2	<i>D2Mit102</i>	57.65	1	8	<i>D8Mit211</i>	52	1, 2	15	<i>Tns2</i>	57.29	2
2	<i>D2Mit164</i>	60.69	2	9	<i>D9Mit2</i>	20.76	1, 2	16	<i>D16Mit182</i>	2.57	1, 2
2	<i>D2Mit282</i>	73.59	1, 2	9	<i>D9Mit49</i>	34.32	1, 2	16	<i>D16Mit166</i>	21.4	2
2	<i>D2Mit229</i>	88.99	1, 2	9	<i>D9Mit113</i>	46.4	1, 2	16	<i>D16Mit59</i>	26.86	1, 2
3	<i>D3Mit164</i>	2.01	2	9	<i>D9Mit355</i>	51.41	1, 2	16	<i>D16Mit76</i>	38.95	2
3	<i>D3Mit203</i>	10.82	2	9	<i>D9Mit212</i>	59.58	1	16	<i>D16Mit152</i>	48.23	1, 2
3	<i>D3Mit182</i>	21.73	1, 2	10	<i>D10Mit298</i>	2.78	2	16	<i>D16Mit106</i>	57.68	2
3	<i>D3Mit78</i>	48.81	1, 2	10	<i>D10Mit124</i>	9.75	1, 2	17	<i>D17Mit135</i>	15.8	1, 2
3	<i>D3Mit291</i>	63.05	1, 2	10	<i>D10Mit196</i>	32.28	1, 2	17	<i>D17Mit119</i>	38.15	2
3	<i>D3Mit129</i>	80.49	1, 2	10	<i>D10Mit42</i>	39.72	2	17	<i>D17Mit93</i>	45.2	1
4	<i>D4Mit149</i>	2.04	1	10	<i>D10Mit264</i>	45.66-46.68	1, 2	17	<i>D17Mit187</i>	50.17	2
4	<i>D4Mit235</i>	3.57	2	10	<i>D10Mit233</i>	61.58	2	17	<i>D17Mit221</i>	59.77	1, 2
4	<i>D4Mit172</i>	16.7	2	10	<i>D10Mit271</i>	72.31	1, 2	18	<i>D18Mit132</i>	11.92	1, 2
4	<i>D4Mit139</i>	29.65	1, 2	11	<i>D11Mit226</i>	5.64	1, 2	18	<i>D18Mit94</i>	19.97	1
4	<i>D4Mit27</i>	42.13	1, 2	11	<i>D11Mit230</i>	16.15	1, 2	18	<i>D18Mit53</i>	28.28	1, 2
4	<i>D4Mit31</i>	50.04	2	11	<i>D11Mit140</i>	32.13	1, 2	18	<i>D18Mit186</i>	45.63	1, 2
4	<i>D4Mit12</i>	57.76	1, 2	11	<i>D11Mit4</i>	41.87	1, 2	18	<i>D18Mit4</i>	57.33	1, 2
4	<i>D4Mit13</i>	75.67	1, 2	11	<i>D11Mit179</i>	54.6	1, 2	19	<i>D19Mit60</i>	13.9	1, 2
5	<i>D5Mit346</i>	2.62	1, 2	11	<i>D11Mit199</i>	65.48	1, 2	19	<i>D19Mit39</i>	23.68	2
5	<i>D5Mit352</i>	18.4	1, 2	11	<i>D11Mit333</i>	71.83	2	19	<i>D19Mit19</i>	34.08	1, 2
5	<i>D5Mit254</i>	30.56	2	11	<i>D11Mit48</i>	82.96	1, 2	19	<i>D19Mit33</i>	51.76	1, 2
5	<i>D5Mit201</i>	39.55	1, 2	12	<i>D12Mit209</i>	6.2	1, 2	X	<i>DXMit123</i>	3.19	2
5	<i>D5Mit338</i>	53.23	2	12	<i>D12Mit243</i>	15.71	1, 2	X	<i>DXMit73</i>	33.5	1, 2
5	<i>D5Mit367</i>	60.43	1	12	<i>D12Mit36</i>	26.43	2	X	<i>DXMit38</i>	57.4	1, 2
5	<i>D5Mit370</i>	65.23	2	12	<i>D12Mit214</i>	37.86	1, 2	X	<i>DXMit186</i>	76.75	1, 2
5	<i>D5Mit376</i>	86.87	1	13	<i>D13Mit205</i>	3.08	1, 2				

Table 1. Genotyping markers. B6 (aBC at these three loci for coat color) and ICGN (ABc). *Tyrc* (MGI:1855976) is located on Chr 7 (49 cM). Coat color (black or albino) was used for genotyping of this locus. 1, for the QTL analysis; 2, for the consomic analysis.

Chr	peak (cM)	nearest marker	LOD	phenotype	ICGN /ICGN ^a	B6 /ICGN ^a	resistance allele
2	31	<i>D2Mit369</i>	5.36	tubule	1.0	0.4	B6 ^{b**}
2	36	<i>D2Mit380</i>	4.47	Hb (g/dL)	12.6	13.2	B6 ^c
			3.58	Ht (%)	43.1	45.2	B6 ^c
2	51	<i>D2Mit66</i>	2.41	glomeruli	30.2	25.9	B6 ^{b**}
			2.34	BUN (mg/dL)	38.2	31.3	B6 ^c
10	64	<i>D10Mit271</i>	2.27	tubule	0.8	0.4	B6 ^{b*}
13	14	<i>D13Mit60</i>	3.49	glomeruli	30.4	24.9	B6 ^{b**}
X	67	<i>DXMit186</i>	2.65	tubule	0.8	0.4	B6 ^{b*}

Table 2. QTL identified for nephropathic traits.

a. Data resent the mean of the phenotype

b. Mann-Whitney's U test, **, P< 0.001; *, P< 0.01

c. Student's T test, P< 0.001

Chapter 2

Comparative analyses between resistant and susceptible murine strains to tensin2-deficiency

The original paper of this chapter will be submitted for publication, and thus can not be shown at the time the thesis has been submitted to Hokkaido University.

SUMMARY

Tns2 is thought to be a component of the cytoskeletal structures linking actin filaments with focal adhesions and plays a role as an intracellular signal transduction mediator through integrin in podocytes, and how it functions is unclear. Tns2-null mutation (*Tns2^{nph}*) leads to massive albuminuria following podocyte foot process effacement in ICGN mice, the origin of the mutation, and D2 mice, but not in B6 mice and 129T mice. Elucidating the reasons for these differences in diverse genetic backgrounds could help unraveling Tns2 function in podocytes. The author produced the congenic mouse in which *Tns2^{nph}* is introgressed into FVB background (FVB- *Tns2^{nph}*), and evaluated the progression of kidney disease. FVB-*Tns2^{nph}* mice developed albuminuria, renal fibrosis and renal anemia, like ICGN mice. In FVB-*Tns2^{nph}* mice, podocyte foot process effacement was observed with an electron microscope at as early as 4 weeks of age. This revealed that FVB strain is susceptible to Tns2-deficiency. Then, two resistant strains (B6 and 129T) and three susceptible strains (D2, FVB and ICGN or ICGN(-)) were subjected to comparative analyses to elucidate the reasons for the difference in susceptibility to Tns2-deficiency. Comparative analysis of glomerular gene expression identified *H2-Q5*, major MHC-I antigen canonical splice variant, that was expressed in the susceptible strains at level >2-fold lower than in the resistant strains. Comparative analysis of nsSNP revealed 4 candidate genes associated with *Tns2^{nph}*-induced nephropathy, *Kif26a*, *Nes*, *Btla* and *Sh3bp1*. Among them, mouse Kif26a missense variant T690I lies within a kinesin motor domain, and the corresponding amino acid residue of KIF26A is highly conserved among other animals including humans.

CONCLUSION

ICGN mouse is a spontaneous CKD model mouse with null mutation in *Tns2*. *Tns2* is a multidomain protein that binds to β -integrin cytoplasmic tails and tyrosine-phosphorylated proteins, and considered to mediate integrin-associated signaling cascades. Despite its ubiquitous expression and predicted function, *Tns2*-deficiency leads to only alterations of podocytes. Moreover, this pathology critically depends on genetic backgrounds. While the *Tns2*-deficient podocytes of the resistant murine strains remain intact, the susceptible murine strains with *Tns2*-deficiency, including ICGN mice, develop massive proteinuria, GS and tubulointerstitial fibrosis following the alteration of podocytes foot processes. These evidence suggest a novel podocyte-specific function of *Tns2* and the presence of the modifier genes determining the disease phenotype.

In Chapter 1 of this study, backcrossing the resistant B6 mice onto the susceptible ICGN mice, a genome-wide linkage analysis revealed that the resistance to renal failure induced by *Tns2*-deficiency maps to Chr 2. However, the replacement of Chr 2 in a consomic strain showed that the resistant loci on Chr 2 are insufficient to prevent the alteration of podocyte foot processes due to *Tns2*-deficiency, suggesting the presence of the other resistant loci outside of the introduced Chr 2 region. In Chapter 2 of this study, firstly, congenic analysis revealed that a FVB strain is susceptible to *Tns2*-deficiency as well as ICGN and D2 strains. Secondly, no prominent difference was detected in glomerular gene expression between the resistant and susceptible strains. Thirdly, searching for missense variants sorted according to resistant or susceptible strains in podocyte-related genes, *Kif26a*, *Nes*, *Btla* and *Sh3bp1* were identified as the candidate genes associated with *Tns2*^{np^h}-induced nephropathy by the prediction tools for functional effects of missense mutation. Each of the candidate genes lies outside of Chr 2 and considered possible to be involved in the formation and maintenance of the unique cytoskeleton of podocyte foot processes.

This study verified that the genetic loci on mouse Chr 2 contribute to the difference in the susceptibility to renal failure induced by *Tns2*-deficiency, and indicates that the other genetic loci outside of Chr 2 (2.23-88.99 cM) also contribute to the difference in the immediate susceptibility to *Tns2*-deficiency, and speculates that *Kif26a* is the first candidate gene for the latter contribution and the resistant effects are exhibited by underpinning the skeletal fragility of the podocyte foot process due to *Tns2*-deficiency. Further study is required to elucidate the podocyte-specific function of *Tns2* and identify the resistant genes.

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SUMMARY IN JAPANESE

慢性腎臓病（CKD）は腎障害や腎機能低下が長く続いている病態であり、疾患の種類・原因に関わらず、腎臓病の予後を診断する指標として重篤度が評価される。自覚症状はあまりなく、病態は通常、不可逆的に進行し、最終的に腎移植あるいは人工透析が必要となる末期腎不全に至る。末期腎不全患者は高齢化社会を背景に世界中で増えており、さらにCKDは糖尿病、高血圧、心疾患といった重要疾患と互いに危険因子の関係にある。これら重要疾患の予備軍と言えるCKDの予防および治療はヒト医療における重大な課題の一つであるが、現在の治療法は危険因子を取り除くことや対症療法によって病態の進行を遅らせるだけで、抜本的な治療法は未だ確立されていない。一方、CKDの発症時期や重篤度には人種差があることが知られている。

ICGNマウスはICR系マウスの突然変異体に由来し、加齢に伴って多くのCKDに認められる症状（蛋白尿、腎性貧血、浮腫）と病理的变化（糸球体上皮細胞足突起の異常、糸球体基底膜の肥厚、糸球体硬化症、腎臓の線維化）を呈し、最終的に末期腎不全となる。このような特徴からICGNマウスはCKDのモデルマウスとして有用であり、その主な原因遺伝子は腎臓において糸球体上皮細胞及び近位尿細管に発現しているテンシン2の欠失変異であることが明らかになっている。テンシン2はアクチン線維とインテグリンβ鎖に結合するマルチドメイン蛋白質であり、糸球体上皮細胞において細胞骨格の形成やインテグリンを介した細胞内シグナル伝達に関与している事が予想されているが、その機能は明らかでなく、その欠損（*nph*変異）によって糸球体上皮細胞が変性する機序は不明である。そして、この*nph*変異による腎症も他のいくつかの腎症や実験的腎症モデル同様に遺伝的背景を変えると重篤度が著しく軽減する場合がある。すなわち、*nph*変異による腎症に対して抵抗性のマウス系統と感受性のマウス系統が存在する。このような系統差が生まれる原因には、その発症機序に修飾遺伝子が関与していることが示唆され、それらの遺伝的背景を比較することは修飾遺伝子の同定、さらには糸球体上皮細胞におけるテンシン2の機能解明につながると考えられる。

そこでまず第1章では、修飾遺伝子座をマッピングするため、抵抗性であるC57BL/6J（B6）マウスと感受性であるICGNマウスのF₁個体をICGNマウスに戻し交配し、*nph*変異をホモに持つバッククロス個体群を用いて、腎性貧血、血液尿素窒素、糸球体傷害、尿細管間質傷害を量的形質にした全ゲノム連鎖解析を行った。その結果、複数の遺伝子座が重篤度に寄与していることが明らかとなり、第2染色体上に主要な修飾遺伝子座が存在することが示唆された。続いて、B6由来の第2染色体をICGNマウスに導

入したコンソミックマウスを作製し、腎臓病評価を行った。野生型のICGNマウスと比較してコンソミックマウスは、腎性貧血を示さず、尿細管及び間質傷害が著しく軽減、糸球体傷害（糸球体上皮細胞の変性と糸球体基底膜の肥厚）が軽度になり蛋白尿が有意に軽減していることから、第2染色体上に修飾遺伝子座が存在することが示された。しかし、抵抗性系統であるB6及び129^{+Ter}/SvJcl (129T) マウスに*nph*変異を導入したコンジェニックマウスにおいては、蛋白尿そのものが検出されず、糸球体の超微細構造はほぼ正常であると報告されていることから、ICGNマウスに導入された第2染色体の領域による抵抗性の効果だけでは不十分であり、*nph*変異に対して糸球体上皮細胞において直接的に効果を発揮する修飾遺伝子が他の領域に存在することが示唆された。また、コンソミックマウスにおいて軽減された蛋白尿は依然として重度でありながら、尿細管及び間質傷害は著しく軽減されていることから、第2染色体の領域による抵抗性の効果は主にCKDにおける尿細管および間質の線維化に対して働いていることが示唆された。

第2章ではFVB/NJ (FVB) マウスに*nph*変異を導入したコンジェニックマウスを作製及び解析し、FVBマウスが*nph*変異に対して感受性であることを明らかにした。続いて、抵抗性のマウス2系統 (B6及び129T) と感受性のマウス3系統 (DBA/2J及びFVB、ICGN) について比較解析を行った。まず、糸球体の遺伝子発現について、データベースから利用できるB6及びD2、FVBマウスの糸球体の遺伝子発現データと、独自に行ったB6及びICGNの非発症コントロール系統の糸球体の遺伝子発現データを用いて、抵抗性系統と感受性系統の間において発現量に差がある遺伝子を検索した。そして、リアルタイムポリメラーゼ連鎖反応によって129Tマウスを含めた5系統の糸球体で遺伝子発現を調べたところ、抵抗性系統と感受性系統の間で顕著に発現量の差が見られる遺伝子は検出されなかった。

次に糸球体上皮細胞に関連する遺伝子について、データベースの一塩基置換データと次世代シーケンサーを用いて、抵抗性系統と感受性系統によって分けられるミスセンス変異を検索したところ、15個の遺伝子が検出された。さらに配列相同性などによってアミノ酸置換の影響を予測するツールによって、4つの候補遺伝子が検出され、中でも細胞骨格の微小管に結合してこれを束ねる役割があるとされているKIF26Aの690番目のアミノ酸は微小管に結合するサイトに位置し、感受性のマウス系統を除き、ヒトを含めた他の動物でよく保存されていた。

本研究により、連鎖解析によって示唆されたB6マウス由来の第2染色体の領域が*nph*変異による腎症に対して抵抗性を示すことが証明された一方、糸球体上皮細胞において直接的に*nph*変異に対して抵抗性を示す修飾遺伝子が他の領域に存在すること

が示唆された。そしてミスセンス変異の比較により、Kif26a遺伝子とその第一候補として挙げられ、正常なKIF26Aは*nph*変異によって構造的に脆弱になった糸球体上皮細胞足突起を補強しているのではないかと推測された。本仮説を検証するため、さらなる研究が必要である。