Deficiency of the tensin2 gene in the ICGN mouse, an animal model for congenital nephrotic syndrome

A-Ri Cho,[1,2] Kozue Uchio-Yamada,[3] Takeshi Torigai,[1,4] Tomomi Miyamoto,[1] Ichiro Miyoshi,[1] Junichiro Matsuda,[3] Tsutomu Kurosawa,[5] Yasuhiro Kon,[6] Atsushi Asano,[2] Nobuya Sasaki,[2] and Takashi Agui[1,2,]

[1]Center for Experimental Animal Science, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Aichi, Japan. [2] Laboratory of Experimental Animal Science, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan. [3]Department of Veterinary Sciences, National Institute of Infectious Diseases, Tokyo, Japan. [4]Department of Neurosurgery, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Aichi, Japan. [5] The Institute of Experimental Animal Science, Osaka University Medical School, Osaka, Japan. [6]Laboratory of Anatomy, Department of Biomedical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Hokkaido, Japan

Corresponding to: Takashi Agui; E-mail: agui@vetmed.hokudai.ac.jp

Abstract

The ICGN mouse is a model for the nephrotic syndrome (NS) showing proteinuria, hyperlipidemia, and edema. In this study, we attempted to identify gene(s) responsible for the NS. By analyzing albuminuria in 160 (ICGN x MSM)F_1 x ICGN backcross progenies, it is elucidated that the NS in the ICGN mouse is caused by polygene. We, then, performed a quantitative trait locus (QTL) analysis and detected a QTL showing a very high LOD score peak in the telomeric region of Chr 15. By analyzing nucleotide sequence of 22 genes located close to the QTL, we found that the tensin2 gene of the ICGN mouse possessed an 8-nucleotide deletion mutation in exon 18, leading to a frameshift and giving rise to a terminal codon at a premature position. Analyses of *in situ* hybridization and immunohistochemistry revealed that tensin2 was expressed in podocytes and tubular epithelial cells in normal mice but not in the ICGN mouse. These data raise the possibility that a mutation of the tensin2 gene is responsible for NS of the ICGN mouse and tensin2 is a prerequisite for the normal kidney function.

Nephrotic syndrome (NS) is among the most common kidney diseases in children and also occurs frequently in adults. In this disease, the filtration barrier of the kidney is altered allowing massive amounts of protein to be lost from serum, leading to low oncotic pressure in serum and leakage of fluid from the bloodstream into the surrounding tissues as edema. These clinical symptoms correlate with dramatic structural alterations in glomerular epithelial cells (podocytes), including retraction and effacement (spreading) of the distal foot processes of podocytes overlying the glomerular basement membrane (GBM), disruption and redistribution of the dense concentrations of actin within these processes, and in some areas detachment of podocytes from the GBM (Smoyer et al. 1998, Somlo and Mundel 2000, Ryan and Karnovsky 1975, Caulfield et al. 1976)

ICGN is a strain of mouse with hereditary NS. This strain was derived from an outbred ICR colony at the National Institute of Health (Japan) in 1986 (Ogura et al. 1989). Affected ICGN mice show podocyte damage, albuminuria, and edema. The NS of the ICGN mouse was reported to be controlled by a single recessive locus, *nep*, when a preliminary genetic analysis was performed using albuminuria as a symptom of the NS (Kurosawa et al. 1993). The *nep* locus was, further, mapped to Chr 15 by a preliminary genetic analysis, in which the *nep* locus was treated as a single recessive locus by omitting ambiguous data that showed a

faint albuminuria (Okamoto et al. 2001). Histological examinations demonstrated that glomerular lesions consisted of a marked thickening of the GBM, multilaminar splitting of the lamina densa of the GBM, and fusion of the epithelial foot processes (Ogura et al. 1991). Cellular and extracellular matrix abnormalities have been also described in the ICGN mouse kidney. These changes include: (a) excessive accumulation of extracellular matrix components such as type I, III, and IV collagens, laminin, fibronectin and tenastin (Uchio et al. 1999), (b) decreased matrix metalloproteinase activity (Uchio et al. 2000), and (c) abnormal expression of integrin ∂1, ∂2, and ∂5 subunits in glomeruli (Uchio-Yamada et al. 2001). Thus, the ICGN mouse is thought to be a good animal model for NS as well as chronic renal disease (Mizuno et al. 1998). Therefore, we attempted to find the gene(s) responsible for NS.

An efficient method for the identification of the gene(s) responsible for inheritable diseases in animal models is chromosomal mapping of their loci. Thus, we performed a quantitative trait locus (QTL) analysis and succeeded mapping a main QTL responsible for NS to Chr 15. We finally found that the tensin2 gene is mutated with a frameshift by sequencing several genes located in this region. The tensin2 gene was reported as *Tenc1*, tensin like C1 domain-containing phosphatase, in Mouse Genome Informatics

(http://www.informatics.jax.org/). We propose a gene symbol, *Tns2*, for Tensin2/*Tenc1* in this paper as unifying gene symbols for tensin family. Here, we show the identification of a mutation of the *Tns2* gene as a presumptive cause for NS in the ICGN mouse and discuss the role of *Tns2* in kidney function.

Materials and methods

Animals. Animal breeding rooms were maintained at 23 ± 2℃ and 50 ± 10% relative humidity with a 12-hour light-dark cycle (lights on from 8:00 a.m. to 8:00 p.m.). Research was conducted according to the *Guidelines for the Care and Use of Laboratory Animals* of both Nagoya City University Medical School and Graduate School of Veterinary Medicine, Hokkaido University. The experimental protocol was approved by the Institutional Animal Care and Use Committee of both Nagoya City University Medical School and Graduate School of Veterinary Medicine, Hokkaido University.

Estimation of the level of albuminuria. The urine was collected and analyzed with SDS-PAGE. Gels were stained with Coomassie brilliant blue R-250 and analyzed with NIH image software. The intensity of the albumin band at 66.1 kD in urine samples was estimated as arbitrary unit for the albuminuria with bovine serum albumin (BSA) as the standard.

QTL analysis. Male ICGN mice were mated with female MSM mice, and the F_1 females were backcrossed to ICGN males. A total of 160 backcross progenies were generated and genotyped with respect to 127 microsatellite markers showing polymorphisms between the ICGN and MSM mice. QTL analysis was performed with arbitrary units for the albuminuria

as quantitative traits using the QTL software, Map Manager QTb2168k.

Reverse transcription (RT)-PCR and sequence analysis of the Tns2 gene. Total RNA was extracted from kidneys of 6-week-old male C57BL/6J, ICR, and ICGN mice using TRIZOL reagent (Invitrogen Corp.), and complementary (c)DNA templates were generated by RT using ReverTra Ace (TOYOBO). Oligonucleotide primers covering the entire coding region of the *Tns2* gene were designed based on the reported mouse *Tns2* cDNA sequence (GeneBank accession no. NM−153533). PCR was performed using the following primers: T-1F: 5'-AGAGGAAGGAGAGACAGGC-3'; T-1R: 5'-TGCTGTAGTGCATATAAGC-3'; T-2F: 5'-AAAGCCATGGAGACGTGGC-3'; T-2R: 5'-AGCAGGCTCTGTCGTGTTG-3'; T-3F: 5'-TTTCCAAGCCTCAGTGGAG-3'; T-3R: 5'-GCTGGACGGTAGCCAAAGT-3'; T-4F: 5'-TGGAGAAGAGACGCCTCTG-3'; T-4R: 5'-CAGACAGCTCTGAAGAAGC-3'; T-5F: 5'-TGGAGGGATGGCTCCAGTGG-3'; T-5R: 5'-CTGGACAAACTTGACATTGC-3'; T-6F: 5'-CGTCGCCACCAGCCAGAAG-3'; T-6R: 5'-CTTTCACAGGGGCCAGGCC-3'. PCR products were purified and directly sequenced with a set of suitable primer pairs or else cloned with pGEM-T Easy Vector Systems (Promega) and then sequenced. Sequencing was performed with an ABI (Applied

Biosystems) BigDye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer's directions.

Northern blot analysis. Electrophoresis, transfer, and hybridization were performed using standard methods. Total RNAs prepared from kidneys of ICR and ICGN mice were hybridized with a ^{32}P-labeled cDNA probe of the *Tns2* gene (nt567-nt1280) to examine messenger (m)RNA expression in the kidney.

In situ hybridization. For synthesizing the *Tns2*-antisense riboprobe, a 122-bp PCR product (nt47-nt168) was subcloned with pGEM-T Easy Vector Systems (Promega), digested with *Pst*I, and transcribed in the presence of Digoxigenin (DIG) RNA Labeling Mix (Roche) with T7 or sp6 RNA polymerase.

In situ hybridization using the DIG-labeled probes was performed as described previously (Butler et al. 2001). Briefly, paraffin-embedded tissue sections with a thickness of 5 μm were deparaffinized in xylene, rehydrated in an ethanol series, and washed for 5 min in phosphate-buffered saline (PBS). Slides were incubated overnight with the antisense or sense probe at 53 ℃ in a humidified chamber. Signals were detected with alkaline

phosphatase-conjugated anti-DIG antibody with freshly prepared substrate (nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolylphosphate) (Boehringer Mannheim). The reaction was stopped by soaking the slides in 10 mM Tris-HCl (pH 8.0) and 1 mM ethylenediaminetetraacetic acid (EDTA).

Anti-Tns2 antibody. A rabbit polyclonal antibody against Tns2 was produced by using a synthetic peptide MKPRKAEPHSFREK+C, corresponding to the N-terminal region specific to Tns2, whose sequence was not present in other tensins. Anti-Tns2-specific antibody was purified by affinity chromatography with the standard protocol.

Western blot analysis. Kidney tissues were homogenized with lysis buffer consisting of 150 mM NaCl, 50 mM Tris–HCl (pH 8.0), 1 mM phenylmethanesulphonyl fluoride (PMSF), and 1 μg/ml leupeptin. The protein concentration was determined with a Bradford Assay reagent (Sigma). Proteins were separated by SDS-PAGE, transferred to polyvinylidene difluoride (PVDF) membranes (Millipore Corp.), and the Tns2 protein was detected with a specific antibody using the standard methods.

Immunohistochemistry. Kidney tissues were fixed with formalin and embedded in

paraffin. Sections with a thickness of 3 μm were processed according to a routine histologic procedure. After rehydration, antigen was retrieved by autoclaving the sections for 5 min in 2 x standard saline citrate (SSC). Sections were then incubated with 3% H_2O_2 in water for 10 min to inhibit endogenous peroxidase activity and heated by using a pressure cooker in 0.01M citrate buffer (pH 6.0) at 120°C for 5 min. For immunohistochemistry with double staining, sections were first incubated with anti-Wilms' tumor antigen-1 (WT-1), a marker for the podocytes (Santa Cruz), Envision-horseradish peroxidase rabbit (Dako), and treated with diaminobenzidine (Dako). Then, the sections were incubated with anti-Tns2, Envision-Alkaline Phosphatase (Dako), and New Fuchsin (Dako). After washing with water, the sections were counterstained with hematoxylin and mounted with Entellan (Merck).

Results

Detection of albuminuria. We produced 160 progenies of (ICGN x MSM)F_1 x ICGN backcrosses. The urine samples collected were analyzed with SDS-PAGE to estimate the albumin contents. The protein fraction with 66.1 kD was ascertained to be an albumin fraction. The albumin band was identified in ICGN mice but not in MSM and F_1 mice (Fig. 1A). Backcross progenies showed various degrees of albuminuria and did not segregate into the ICGN and MSM types (Figs. 1B and 1C), suggesting that albuminuria was caused by multiple genetic loci.

QTL analysis. Since albuminuria in ICGN mice appeared to be controlled by multiple genetic loci, mapping was performed by QTL analysis. The analysis was carried out with 127 microsatellite markers that span the mouse genome at average intervals of 10~20 cM. A significant QTL was identified in the telomeric region of Chr 15 and the LOD score peak was located near the *D15Mit42* locus (55.5 cM). No other significant QTLs were detected. Based on the mouse genome database, *Aqp2*, *Aqp6*, and *Itga5* were attractive candidate genes, since they locate near the *D15Mit42* locus and are expressed in the kidney with particular functions. Although nucleotide substitutions were found in all three genes between the ICGN and MSM

mice or a normal strain such as ICR or C57BL/6J mice, they were all silent mutations. Then, we utilized these nucleotide polymorphisms and remade the genetic map. We subsequently detected 2 backcross progenies that showed recombination between the *Itga5* and *D15Mit42* loci. The renewal LOD score of the peak in our final version of the genetic map was 26.1 at the *Itga5* locus of the telomeric region of Chr 15 (Fig. 2). We, then, investigated the mouse genome database again and selected 22 genes located between the *D15Mit42* and *Itga5* loci to narrow the region where a gene responsible for NS would locate (Fig. 3A). To detect the single nucleotide polymorphism, sequencing the coding regions of these genes were performed. We subsequently found that the *Tns2* gene showed a mutation in the ICGN mouse. The *Tns2* cDNA extracted from the ICGN mouse kidney revealed a deletion of 8 nucleotides (nt1477-nt1484) in exon 18 of the ICGN *Tns2* gene (Fig. 3C). It is expected that this mutation causes a frameshift, leading to a terminal codon at a premature position, nt1538 (Fig. 3D). We examined nucleotide sequence of the *Tns2* gene obtained from other mouse strains such as ICR, C57BL/6J, BALB/c, C3H/HeN, AKR/N, DBA/2J, CBA/J, and MSM/Ms. However, no strain is found the same mutation in the *Tns2* gene, suggesting that the 8-nucleotide deletion is not a polymorphism.

Detection of the Tns2 mRNA and protein. The expression of *Tns2* mRNA in various tissues of normal mice was examined by RT-PCR. *Tns2* mRNA was ubiquitously expressed in all organs examined. The intensity of the bands for RT-PCR in the ICGN mouse was low in all organs compared to that of the ICR mouse kidney (Fig. 4A). We further analyzed the expression of *Tns2* gene in the ICGN mouse kidney. The 4.1-kb transcript was detected in kidney from 7-month-old ICR and ICGN mice, but the band's intensity was extremely low in the ICGN mouse, confirming a much less of the *Tns2* transcript in the ICGN mouse kidney (Fig. 4B). The decrease or loss of the *Tns2* transcript in the ICGN mouse seems to be due to nonsense-mediated mRNA decay (Mendell and Dietz 2001). Next, the Tns2 protein in the kidney was detected by Western blotting. Blotting with anti-Tns2 antibody revealed a positive signal on the 173-kDa band in the kidney of ICR but not of ICGN mice (Fig. 4C). Further, the band for a premature Tns2 protein with an expected size of 60 kDa was not detected in the ICGN mouse kidney (data not shown).

Identification of the cells expressing Tns2. In order to consider the role of Tns2, localization of Tns2 in the kidney was examined in normal mice. We performed *in situ* hybridization of the *Tns2* mRNA in the kidney of ICR mice using a DIG-labeled probe. *In*

situ hybridization yielded a strong signal in podocyte-like cells of the glomeruli and epithelial cells of the tubules (Fig. 5A). *Tns2* mRNA was undetectable in the ICGN mouse kidney section (data not shown). To confirm that the *Tns2*-expressing cells in the glomeruli are podocytes, a marker for the podocytes, WT-1 (Sanden et al. 2003) was stained with a specific antibody in the same slide. The nuclei of the cells positive for *Tns2* in the glomeruli were also stained with anti-WT-1 antibody (Fig. 5B), confirming that they were podocytes. The immunohistochemical double-staining also revealed that positive signals for Tns2 were detected in podocytes, parietal epithelial cells in the glomeruli and the tubular epithelial cells of the ICR but not of the ICGN mouse kidney (Fig. 5C). Thus, we concluded that Tns2 is expressed in podocytes and tubular epithelial cells in the normal kidney but not in the ICGN mouse kidney.

Discussion

The kidney glomerulus is a highly specialized structure that ensures selective ultrafiltration of plasma so that essential proteins are retained in the blood. The common denominator in a variety of human and experimental glomerular diseases, including diabetes mellitus, focal segmental glomerulosclerosis, and systemic lupus erythematosus, is glomerular dysfunction, involving a massive loss of protein in the urine (proteinuria) (Somlo and Mundel 2000, Kerjaschki 2001). These diseases are characterized by numerous morphological changes in podocytes, including retraction (effacement) of podocyte foot processes and frequently detachment of podocytes from the underlying GBM. The foot process effacement is a process that correlates closely with the development of proteinuria both in human disease and in experimental models. The detachment of effaced foot processes from the GBM is generally considered the most severe structural manifestation of NS (Smoyer et al. 1998, Somlo and Mundel 2000, Ryan and Karnovsky 1975, Caulfield et al. 1976, Reiser et al. 2002). Kim et al (2001) showed that repeated injections of puromycin aminonucleoside also augmented podocyte loss and that the regions devoid of podocytes developed glomerulosclerosis. In this report, the glomerulosclerosis was only initiated when podocyte numbers decreased by 10 to 20%. Therefore, recent studies have focused on the causes underlying podocytopenia. The

etiology of podocytopenia includes apoptosis, detachment, and the inability or a lack of podocytes to proliferate (Mundel and Shankland 2002). The discovery and mutational analysis of several podocyte proteins, including NEPHRIN (Kestila et al. 1998), CD2AP (Shih et al. 1999), ACTN-4 (∂-actinin-4) (Kaplan et al. 2000), NPHS2 (podocin) (Boute et al. 2000), and NEPH1 (Donoviel et al. 2001) has revealed the pathogenesis of proteinuria and the critical role of the podocyte and the slit-diaphragm in maintaining the function of the glomerular filtration barrier.

All glomeruli of ICGN mice were affected by 14 days after birth. Electron microscopy showed the fusion and loss of epithelial cell foot processes in severely affected glomeruli (Ogura et al. 1994, Ogura et al. 1995). In this paper, we have identified *Tns2* as a candidate gene responsible for NS in the ICGN mouse, in which 8 nucleotides are deleted from exon 18, resulting in a frameshift. The results of both the Northern and Western blot analyses showed a loss of expression of Tns2 in the kidney of the ICGN mouse.

Tensin is an actin-binding protein that is concentrated in some submembranous cytoskeletal focal contacts (Weight et al. 1992). In addition to its three actin-binding domains, tensin protein contains a Src homology (SH) 2 domain that mediates protein-protein contacts and is shared by a variety of signal transduction molecules. It has recently been reported that

three molecules, Tns2, Tns3, and Tns4, show extensive homology to Tns1. The most conserved areas are in the N- and C-terminal regions (Lo 2004). Lo et al (1997) generated *Tns1*-null mice to explore the role of Tns1. These mice appeared normal and healthy for several months, but eventually developed cystic kidneys. Progressive cyst formation led to kidney degeneration and death from renal failure. On the other hand, the ICGN mouse shows a very different pathological phenotype in the kidney from the *Tns1*-null mouse. Thus, we attempted to examine the localization of Tns2 in the kidney tissue by *in situ* hybridization and immunohistochemistry. Both histological examinations revealed that Tns2 is present in glomerular podocytes and tubular epithelial cells (Fig. 5). In contrast, Tns1 is concentrated in glomerular mesangium cells and basal aspects of the tubular cells (Lo et al. 1997, Takahara et al. 2004). The difference in the distribution of the Tns1 and Tns2 proteins, namely the difference in the glomeruli, may account for the different phenotypes in null mice for each tensin family protein.

We cannot yet fully explain how the *Tns2* mutation leads to morphological changes of the glomerulus and proteinuria in the ICGN mouse. However, we can speculate based on the conserved Tns2 domain and subcellular localization. First, mobility is dependent on cell adhesion. Tns2 is localized to the sites of focal adhesions and expression of Tns2 is able to

promote cell migration on fibronectin in a cell migration assay (Chen et al. 2002). Also, Chen et al (2003) showed that the SH2 domain of C-terminal region is critical for Tns1-mediated cell migration. The SH2 domain of the Tns2 is supposed to play the same role. Therefore, without Tns2, cell adhesion might be weakened in the kidney, leading to an alteration in the adhesive properties of cells and subsequently to the disruption of cell-cell and cell-GBM interactions, detachment of podocytes, proteinuria, and glomerulosclerosis. Second, podocyte foot processes are known to contain high concentrations of actin. F-actin localizes to the submembranous regions of all three membrane domains of podocytes (Drenckhahn and Franke 1988). The membrane at the foot processes' soles is attached to this actin meshwork *via* dystroglycan and utrophin or *via* integrin, talin, paxillin, and vinculin. This actin fiber formation is a highly dynamic process that is governed by continuous assembly and disruption of filaments. The submembranous actin cytoskeleton is important for maintaining the infrastructure of the membrane domains and for the stability of the podocyte's shape (Kerjaschki 2001). This critical role is highlighted by the finding that supression of the actin bundling protein ACTN-4 (Michaud et al. 2003) or actin binding protein-CD2AP (Shih et al. 1999) by genetic engineering in mice causes podocyte damage. Tns2 is an actin-binding protein and speculated to be a mediator that facilitates the recruitment of actin filaments

(Chen et al. 2002). Immunofluorescence microscopy with fluorescence-tagged Tns2 revealed a subcellular localization at the end of actin stress fibers and colocalization with vinculin at focal adhesions (Chen et al. 2002). Therefore, the absence of Tns2 has a direct or indirect effect on the actin cytoskeleton of podocytes. These hypotheses require further experimental investigation. However, the results presented here suggest that morphological changes to podocytes and proteinuria result from a loss-of-function of the adhesion molecule Tns2.

In this study, the NS of the ICGN mouse was attributed to a mutation of the *Tns2* gene. This may be inconsistent with the result that the NS of the ICGN mouse is controlled by polygenes, since the individual level of the albuminuria in backcross progenies could not be segregated into two groups (Fig. 1C). However, in QTL analysis, we detected only one significant QTL located in the telomeric region of Chr 15 with a very high LOD score. This suggests that the NS of the ICGN mouse is mostly caused by a mutation of the *Tns2* gene with minimal modifications by unknown genetic factors not detected in the QTL analysis this time. This is consistent with the interpretation derived from a preliminary genetic analysis that the NS of the ICGN mouse is controlled by a single recessive locus (Kurosawa et al. 1993, Okamoto et al. 2001).

In addition, the results of RT-PCR showed that the *Tns2* mRNA is ubiquitously present

in various tissues (Fig. 4A). However, these organs in the ICGN mouse do not show detectable anomalies even in the terminal stage (Ogura et al. 1989). There may be other molecules in these tissues that can compensate for the absence of Tns2. Tns2 has a domain that shows high homology to the N- and C-terminal regions of Tns1. However, the C1 (protein kinase C conserved region 1) domain is unique to Tns2 and central regions are highly divergent in the tensin family (Lo 2004). The central region of Tns2 is very rich in proline residues, which provides potential binding sites for interacting modules such as SH3 and WW domains (Chen et al. 2002). Thus, the C1 domain and central region may contribute to regulate the signal pathway that determines unique biological roles not substituted by Tns1 and other molecules in the kidney. Therefore, it is important to determine in future which signal pathways Tns2 participates in for the maintenance or disruption of the interaction between podocytes and GBM.

In conclusion, it has been elucidated from this study that the *Tns2* gene in the ICGN mouse, an animal model for NS, possesses an 8-bp nucleotide deletion, leading to a frameshift that produces a terminal codon at a premature position. This result raises the possibility that a deficiency of Tns2 in the kidney is responsible for NS in the ICGN mouse, although the full confirmation has to wait for further investigations such as transgenic rescue or production of

Tns2-knockout mice. A deficiency of the Tns2 may be applicable to the cause of some patients with familial or sporadic NS.

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Figure legends

Fig. 1. Measurement of albuminuria in the ICGN and backcross mice. (A) SDS-PAGE analysis of urine from ICGN, MSM/Ms, and F_1 mice. Two microliters of urine from each mouse and the albumin standard were loaded on a 12% polyacrylamide gel. Massive proteinuria, mainly involving albumin, was observed in ICGN but not in MSM/Ms and F_1 mice. Closed and open arrows indicate the alubumin and murine urinary protein fractions, respectively. (B) SDS-PAGE analysis of urine from (MSM/Ms x ICGN)F_1 x ICGN backcrosses progenies. Data represent samples showing mild, moderate, and severe proteinuria. An arrow indicates the albumin fraction. (C) Distribution of albuminuria levels of 160 (MSM/Ms x ICGN)F_1 x ICGN backcross progenies. Data represent the mean value and standard error of three independent urine samples collected from the same mouse every 3 days to exclude diurnal variation. The data obtained from individuals are arranged according to the mean value.

Fig. 2. The result of QTL analysis on Chr 15. The data were analyzed using the free regression model. The numbers on the right of the line indicate the genetic distance (cM) between each two loci. The number on the peak indicates the LOD score that was calculated by dividing the likelihood ratio statistics (LRS, 120) by 4.60.

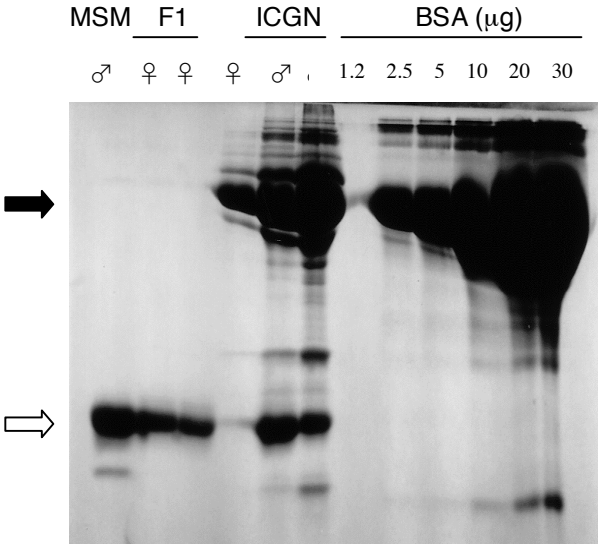
Fig. 3. Identification of a mutation of the *Tns2* gene. (A) Physical map of the interval between the *D15Mit42* and *Itga5* loci. All genes examined their nucleotide sequences and their directions of transcription are shown. (B) Exon-intron structure of the *Tns2* gene. Exons are shown as vertical bars. (C) DNA sequence of part of *Tns2* exon 18. Left, normal mice (C57BL/6J and ICR); Right, ICGN mouse. Eight nucleotides are deleted in the ICGN mouse. Since anti-sense sequences are shown in both figures, the sense sequence of the 8- deleted nucleotides (CACCTACT) is shown above the figure. (D) Schematic representation of the predicted structure of Tns2 protein of the wild type (WT, upper) and ICGN type (lower). An arrow indicates the position of a mutation.

Fig. 4. Expression of Tns2 in the control and ICGN mice. (A) RT-PCR of *Tns2* mRNAs from various tissues of 2-month-old ICR and ICGN mice. (B) Northern blot of *Tns2* mRNA from kidney of 7-month-old ICR and ICGN mice. (C) Western blot of Tns2 in the kidney homogenates of 15-week-old normal ICR and ICGN mice. An arrow indicates the 173-kDa band that corresponds to the Tns2 protein.

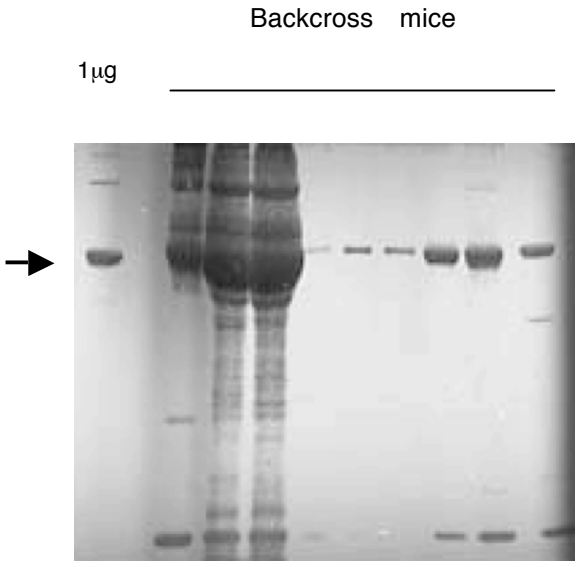
Fig. 5. Histological examination of Tns2 in the kidney tissue sections. (A) *In situ* hybridization using an antisense (left) or sense (right) probe for *Tns2* mRNA in kidney of 3.5-month-old ICR mouse. Blue shows a positive reaction. Scale bars indicate 10 μm. (B)

Immunohistochemistry of WT-1 antigen, a marker of the podocytes. A tissue section used in (A) was autoclaved and incubated with rabbit anti-WT-1 antibody followed by visualizing with peroxidase enzymatic staining. Brown shows a positive reaction. Scale bar indicates 10 μm. (C) Immunohistochemistry with double staining of anti-WT-1 (brown) and anti-Tns2 (red) antibodies. Kidney tissue sections were prepared from 3.5-month-old ICR (left) and ICGN (right) mice. Scale bars indicate 10 μm.

A


MSM
F1
ICGN
BSA (μg)
♂ ♀ ♀ ♀ ♂
1.2 2.5 5 10 20 30

B


Backcross mice
1μg

C

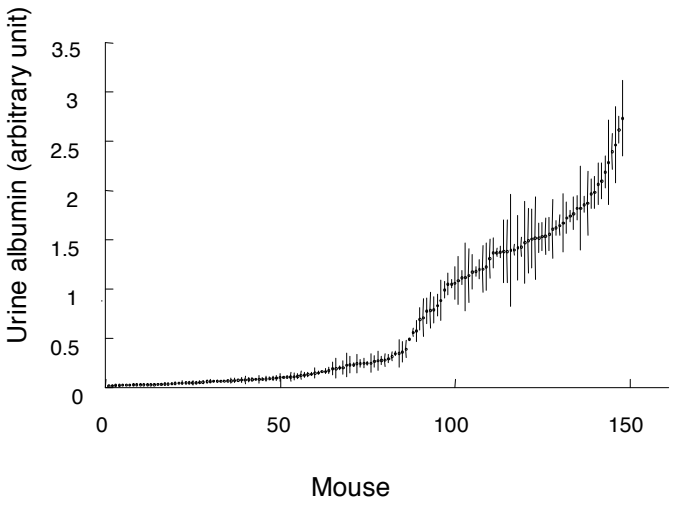

Urine albumin (arbitrary unit)
3.5
3
2.5
2
1.5
1
0.5
0
0
50
100
150
Mouse

Figure 1

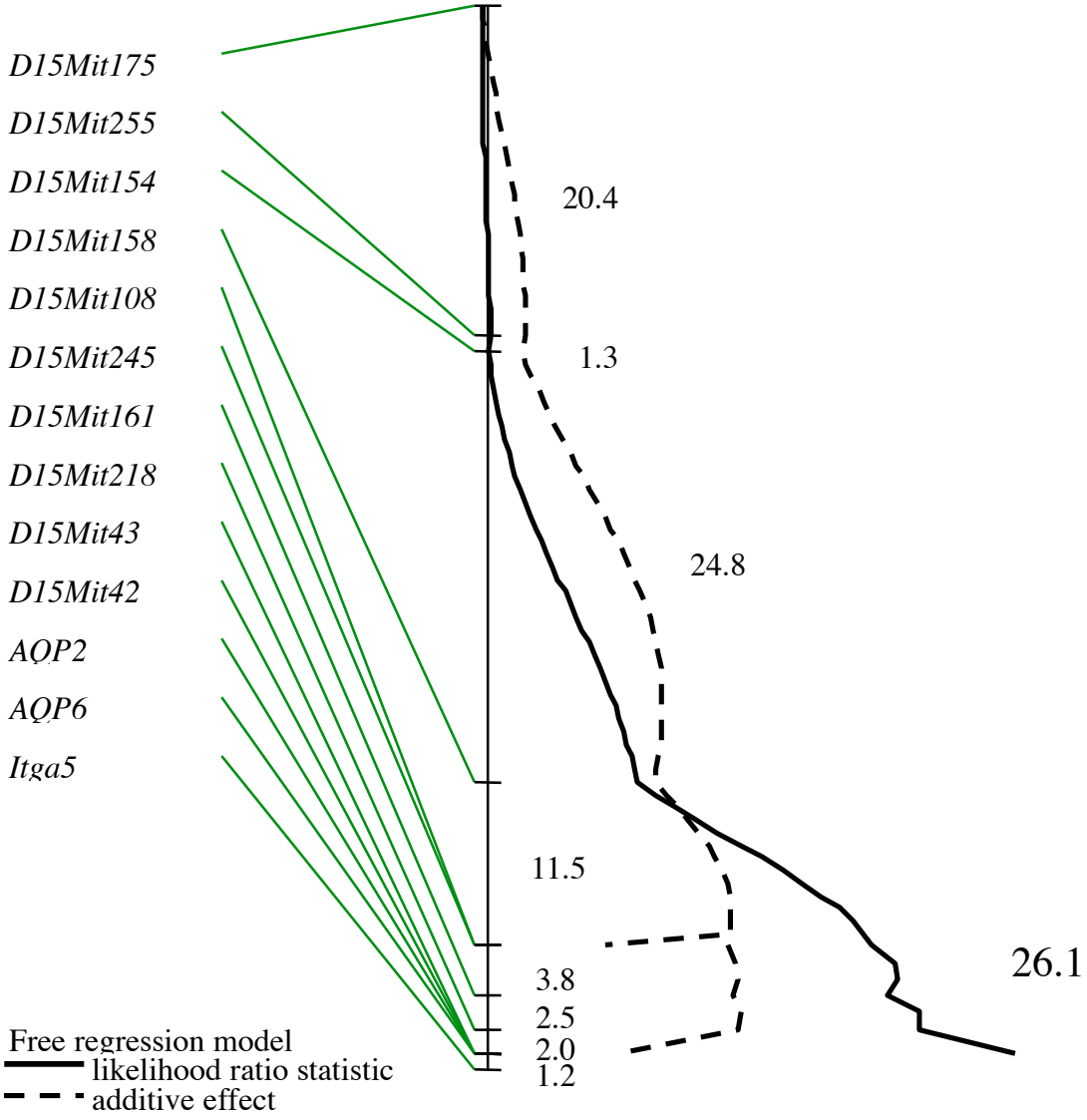

D15Mit175
D15Mit255
D15Mit154
D15Mit158
D15Mit108
D15Mit245
D15Mit161
D15Mit218
D15Mit43
D15Mit42
AQP2
AQP6
Itga5
20.4
1.3
24.8
11.5
3.8
2.5
2.0
1.2
26.1
Free regression model
likelihood ratio statistic
additive effect

Figure 2

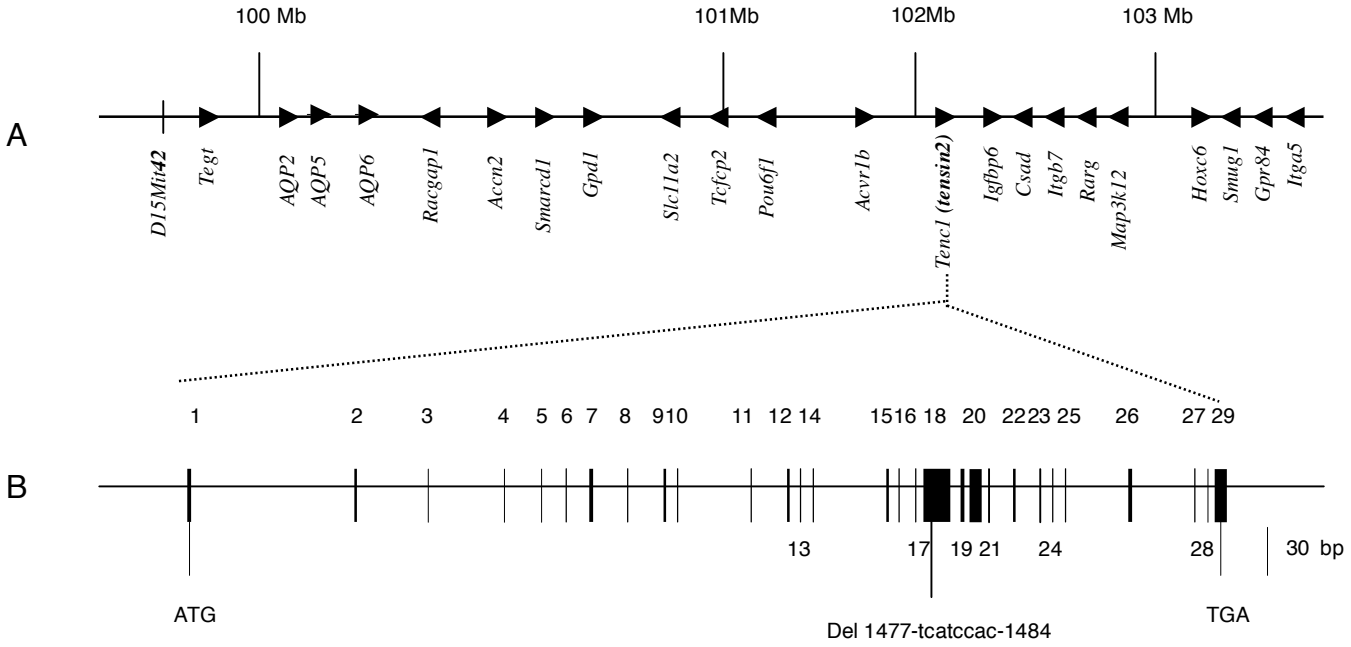


Figure 3, Part A

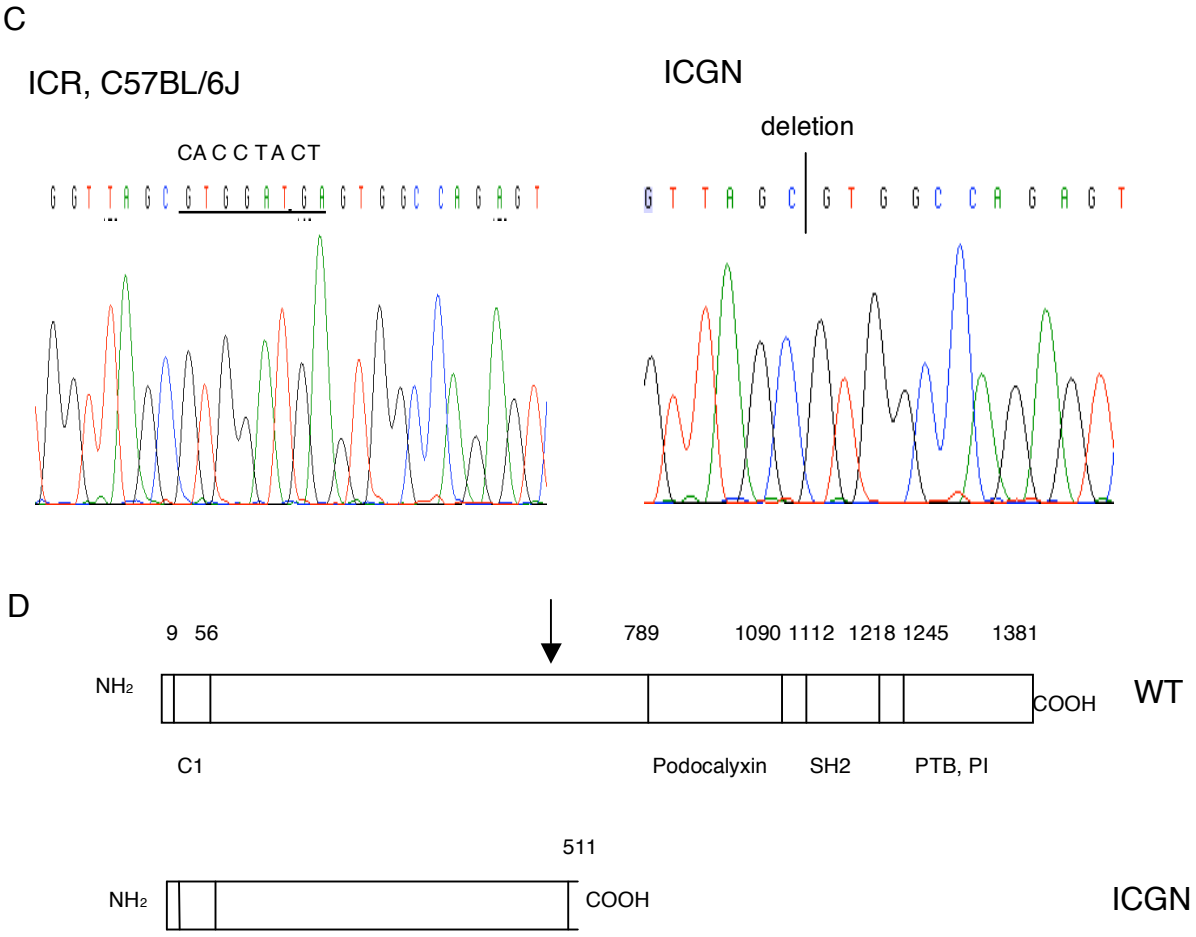
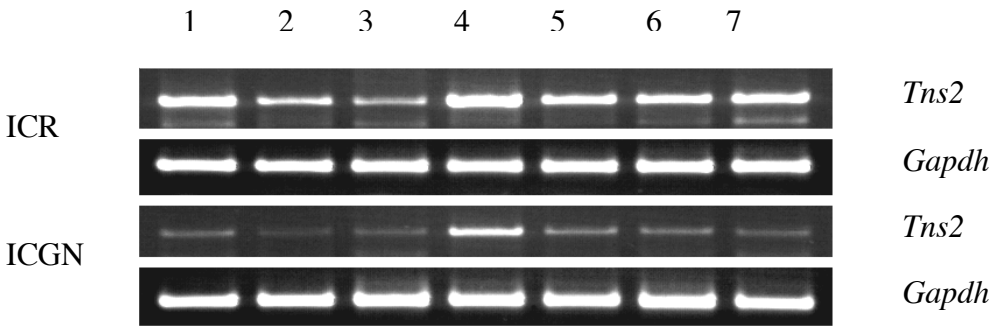


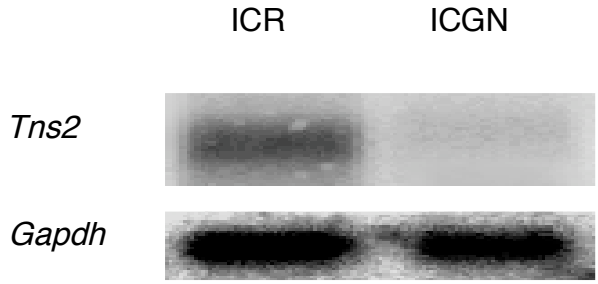
Figure 3, Part B

A



1. Heart, 2. Brain, 3. Spleen, 4. Lung, 5. Liver, 6. Muscle, 7. Kidney

B



C

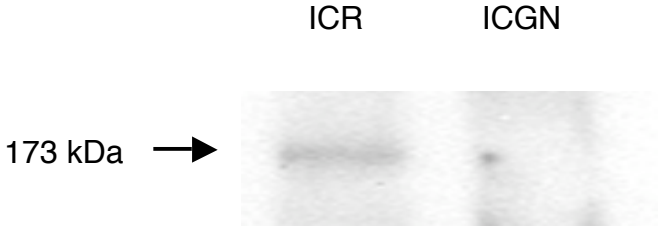
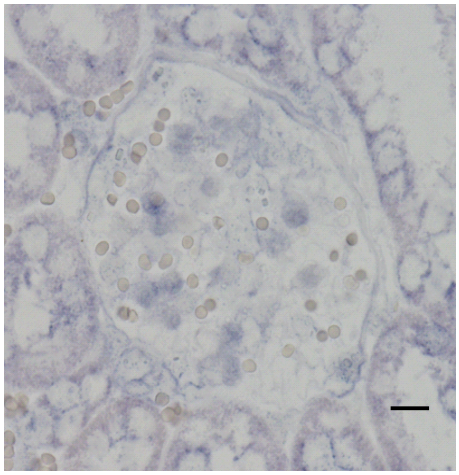
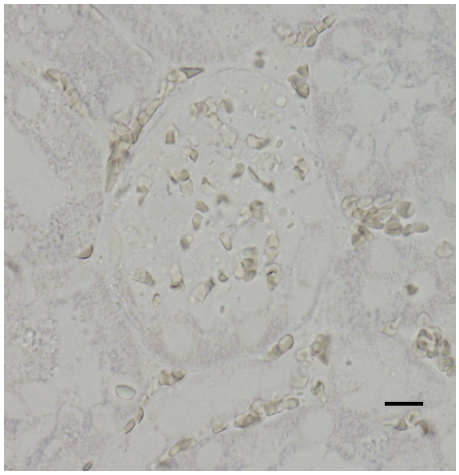


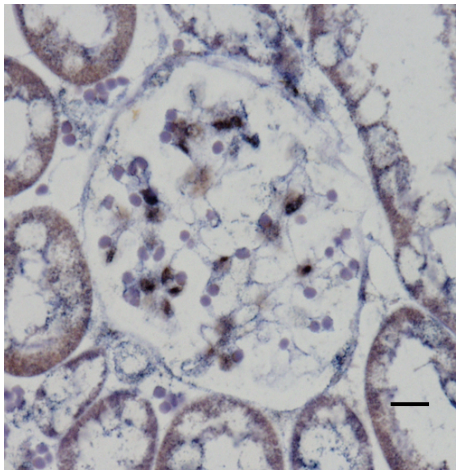
Figure 4

A

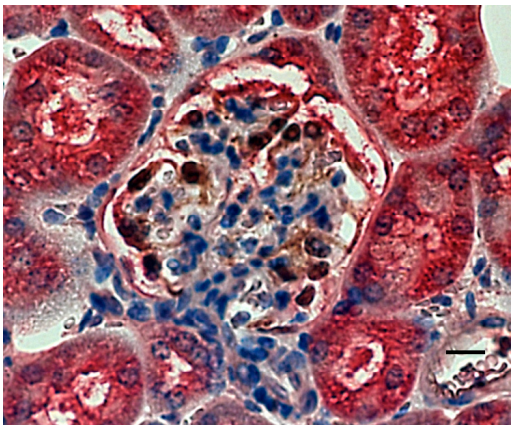




B



C



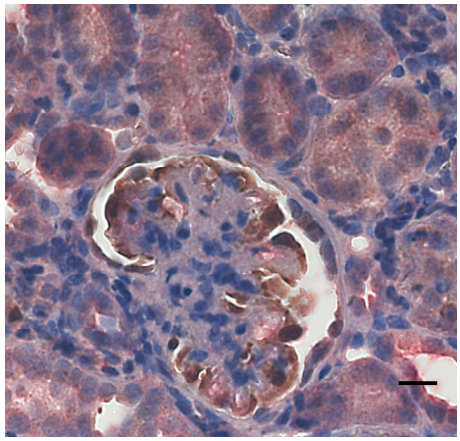


Figure 5