ONLINE SUPPLEMENTAL MATERIAL

SUPPLEMENTAL MATERIAL AND METHODS

Animals and Study Design

All experiments were approved by the Institutional Animal Care and Use Committee at either the Medical College of Wisconsin (MCW) or the University of Mississippi Medical Center (UMMC). The Dahl salt-sensitive (S) and the spontaneously hypertensive rat (SHR) inbred strains are maintained at our institutional animal facility.

Study 1: Fine-Mapping using Recombinant Progeny Testing (RPT) and Congenic Strains. Recombinant Progeny Testing (RPT) was used to fine-map the proteinuria locus as done previously[1]. New recombinant animals for RPT were generated from previously described animals [S.SHR(2)X4 or 20] [1] using two backcross populations, F1[S X SHR(2)X4 or 20] X S (n=151). The new recombinant families are denoted as S.SHR(2)X39, 40, 41, 42, 43, and 44 (**Fig. S2, Table S1**). At week 8, proteinuria was determined after 24-hour urine collection (Lab Products, Seaford, Delaware) in each recombinant family (X39, n=67; X40, n=38; X41, n=37; X42, n=37; X43, n=21; X44, n=23). The influence of each recombinant region (i.e. SHR genotype) was determined by subtracting average proteinuria of recombinant rats (SHR-like) from proteinuria of non-recombinant (S-like) littermates. A negative value indicates that rats containing SHR donor region (recombinant rats) exhibited lower proteinuria than non-recombinant rats, and when statistically significant indicates that the genes or genetic variants that influence proteinuria are with the SHR donated region.

To allow for comprehensive phenotyping and analysis of the refined genomic interval, the S.SHR(2)X39 recombinant family was used to generate a congenic strain that fixed the transferred genomic interval homozygous SHR/SHR (**Fig. 1**). The S.SHR(2)X47 was generated

from a new recombination that occurred while developing the S.SHR(2)X39 strain. These congenic strains were utilized for **Studies 2-6**.

Study 2: Proteinuria, Blood Pressure and Renal Hemodynamic Parameters. At 4 weeks of age, groups of age matched male S, S.SHR(2)X39, and SHR animals (n=6-8 per group) were weaned onto low-salt diet (0.3% NaCl; TD7034; Harlan Teklad, Madison, WI). 24-hr urine collections were performed at week 6, 9, and 12 for determination of proteinuria[2]. At week 12, mean arterial pressure (MAP), renal blood flow (RBF) and glomerular filtration rate (GFR) were measured in each group. Briefly, rats were anesthetized with ketamine (30 mg/kg, i.m.) and Inactin (100 mg/kg, i.p.) and placed on a heating table to maintain body temperature at 37^{o}C. Cannulas were placed into the femoral artery for measurement of MAP and femoral vein for i.v. infusion of 2% BSA in a 0.9% NaCl solution at a rate of 100 μL/min. A catheter was inserted into the left ureter for the collection of urine and FITC-labeled inulin (2 mg/mL, Sigma, St. Louis, MO) which was added to the i.v. infusion solution for the measurement of GFR. RBF was measured using an ultrasound flow probe (Transonic System, Ithaca, NY) on the renal artery. After a 30 minute equilibration period, urine and plasma samples were collected for a 30-minute period. At the end of each experiment, the left kidney was removed and weighed and the concentration of FITC-inulin in urine and plasma samples was determined.

Study 3: Time Course Measurement of Proteinuria and Telemetry Blood Pressure. At 4 weeks of age, groups of age matched male S and S.SHR(2) X47 rats (n=16 per group) were weaned to a low-salt diet (0.3% NaCl). Animals were studied from week 8 to 20. Blood pressure was measured by using a telemetry system (Data Sciences International, St. Paul, MN). At 7 weeks of age, animals were anesthetized using 2-3% isoflurane gas and a transmitter was surgically implanted in S (n=8) and S.SHR(2)X47 (n=10) animals. The probe body was placed

subcutaneously in the flank and the probe catheter was inserted into the femoral artery and the tip of the probe was advanced to the lower abdominal aorta as done previously[3]. Readings for each BP parameter was collected for a 24-hour period (5 minute time intervals for 10 seconds) at 4 week intervals. For collection of urine, rats were kept in metabolism cages (Lab Products, Seaford, Delaware) for 24 hours with free access to water[1, 4]. The animals were subsequently euthanized and heart and kidney weights were measured. The left kidney was processed for histological analysis.

Study 4: Glomerular Permeability and Number. The reflection coefficient of albumin (σalb was determined in isolated glomeruli from S and S.SHR(2)X39 raised on low-salt diet (week 12) using modifications of the Savin technique[5]. Rats were anesthetized with isoflurane and a catheter was inserted into the femoral vein for the infusion of a high molecular weight FITC-labeled dextran (75mg/kg) which remains in the glomerular capillaries and is not filtered. After 5 min, glomeruli were isolated using the sieving method in Hank's buffer solution containing 6% bovine serum albumin (BSA) and then transferred to 80 μL fast-exchange perfusion chamber mounted on the stage of an inverted microscope (TS100, Nikon Inc., Melville, NY). The FITC-labeled dextran localized in the glomeruli was imaged with the InCyt IM1 imaging system (Intracellular Imaging, Cincinnati, OH) using an excitation filter of 475 nm and an emission filter of 530 nm. σAlb was determined by measuring the changes in fluorescence in each glomeruli after lowering the concentration of BSA in the bath from 6 to 4%. σalb was calculated as % change of fluorescent intensity divided expected % change in glomerular volume relative to the 66% decrease in oncotic pressure). These experiments were performed using 4-5 rats per strain on a minimum of 20 glomeruli per rat (n=80-100 total glomeruli per group).

The determination of glomeruli number was performed using direct maceration/counting method[6]. Glomeruli were harvested from S and S.SHRX39 at two time points (week 6 and week 12). Kidneys were minced into fine cubes and the fragments were incubated in 5 ml of 6 M HCl at 37°C for 1.5 hours. The tissue was homogenized through repeated pipetting, and 45 ml of PBS was added. The homogenate was incubated overnight at 4°C at which time glomeruli were counted in triplicate from 1 ml aliquots using light microscopy. Total glomerular number per kidney was extrapolated mathematically.

Study 5: Renal Function and Survival Curve. At 4 weeks of age, groups of age matched male S and S.SHR(2)X39 animals (n=9 per group) were weaned to a normal rodent chow (0.7% NaCl; Purina 5010) to exacerbate hypertension and progression of injury compared to low-salt diet.

Proteinuria was determined at week 8 and 12. At week 12, the 24-hour urine collection was followed by blood collection via the tail for determination of creatinine and calculation of creatinine clearance (CrCl). Animals were subsequently euthanized and heart and kidney weights were measured. The left kidney was removed and cortex and medulla were dissected and snap frozen in liquid nitrogen for gene expression studies (e.g. isolation RNA) and western blot analysis. The right kidney was placed in buffered formalin for histological examination. An additional group of animals were used to study survival. Male S, S.SHR(2)X39, and SHR rats (n=12 rats per group) were maintained on Purina 5010 diet until the rats died. Animals were examined twice daily and were euthanized if they displayed signs of distress (e.g. shaking, lethargy, persistent lateral recumbency, or respiratory distress).

RBF autoregulation was evaluated in a separate group of animals raised on normal rodent diet (n=7-8 per group). Animals were anesthetized using isoflurane for placement of catheter into the femoral artery. The catheter was tunneled subcutaneously under the skin and emerged at the base

of the neck. After 24-hours, MAP was measured in conscious freely moving rat for 10 minutes. Subsequently, animals were anesthetized with ketamine (30 mg/kg) and Inactin (50 mg/kg) and maintained at 37°C. A catheter was placed into the femoral vein for infusion of 2% BSA in a 0.9% NaCl solution at a rate of 100 μL/min. The left kidney was exposed and the left ureter was cannulated for collection of urine. Clamps were placed around the abdominal aorta and ligatures placed around the celiac and mesenteric arteries so that renal perfusion pressure (RPP) could be varied by adjusting peripheral resistance[7]. After a 15-min equilibration period, RBF was measured (Transonic flow probe) as RPP was varied from 140 to 80 mmHg in steps of 10 mmHg. The kidney was perfused at each level of RPP for 3 min. RBF autoregulatory indexes (AI) over the range of pressures from 100 to 140 mmHg were calculated by the method of Semple and De Wardener (31). An AI of 0 indicates perfect autoregulation of RBF; an AI of 1 is characteristic of a circulation with a fixed vascular resistance. An autoregulatory index >1 is indicative of a compliant system in which vascular resistance decreases as RPP increases.

Study 6: Pharmacological inhibition of Rho-Rock pathway. At 4 weeks of age, groups of age matched male S, S.SHR(2)X39, and SHR animals (n=11 per group) were weaned onto low-salt diet (0.3% NaCl). At week 8, animals in each group were randomly assigned to either control (n=5 per strain) or fasudil treatment (n=6 per strain). Animal were provided fasudil (LC Laboratories, MA) in drinking water from week 8 to week 12. Drinking volume was assessed every 2-3 days and the concentration of fasudil was adjusted to achieve a dose of ~20 mg/kg/day. Proteinuria was determined at week 8, 10, and 12 after 24-hour urine collection. Subsequently, animals were euthanized and heart and kidney weights were measured.

Histological Assessment

Kidney tissue was fixed in zinc formalin and embedded in paraffin, cut into 4-μm sections and stained with hematoxylin and eosin (H&E) and/or Masson's trichrome. At least two central longitudinal sections from each kidney were examined in a blinded fashion. Glomerular injury was assessed using a semi-quantitative scoring system in 20 randomly selected images (H&E at 40X) as previously described[2, 8]. Tubulointerstitial injury was determined by evaluation of slides stained with Masson's trichrome to quantify the area of tissue fibrosis (blue) versus unstained region of image. Morphometric analysis was used to evaluate glomerular area (um^2) and vessel wall thickening (vessel media, um^2) by measuring the outer circumference of the vessel minus the inner circumference of the lumen (20 random images at 40X per rat)[8].

Immunohistochemistry was performed on unstained sections, deparaffinized with xylene and ethanol, and incubated with proteinase K for antigen retrieval. Endogenous biotin and peroxidase activity was blocked by incubation with avidin and biotin, and hydrogen peroxide, respectively. Primary anti-α-SMA (Santa Cruz Biotech) or anti-CD68 (Serotec, Inc) were used, followed by incubation with horse anti-mouse secondary antibody (Vectastain ABC kits; Vector Laboratory, Burlingame, CA) and 0.02% H_2O_2 + 0.1% diaminobenzidine tetrahydrochloride (DAB). The slides were lightly counterstained with aniline blue dye and photographed under the microscope. Images were captured using Nikon 55i microscope with DS-Fi1 5-Meg Color C digital camera (Nikon, Melville, NY) and analyzed using Nis-Elements image analysis software (version 3.03, Nikon Instruments Inc., Melville, NY).

Molecular Methods

Genotyping and Sequencing. Genomic DNA was obtained by tail biopsy or liver for development of congenic strains or sequencing and prepared using Wizard SV 96 Genomic DNA kit (Promega, San Luis Obispo, CA). Genotyping was done using a fluorescent-based approach

on a Beckman Coulter CEQ8000 XL capillary sequencer as described in detail previously[1]. The coding region of genes (highlighted region **Table S4**) was sequenced from cDNA, except for *Hapln2* and *Paqr6* which were sequenced from genomic DNA. Primers were designed from known databases (www.ncbi.nlm.nih.gov or www.ensembl.org) (**Table S2**) to amplify the full-transcript. RNA was extracted using Trizol® reagent (Invitrogen, Carlsbad, CA) and purified using Mini RNeasy kit (Qiagen,Valencia, CA) according to manufacturer's protocol. cDNA was prepared by reverse transcription using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). PCR products were cloned into a TOPO TA sequencing vector (Invitrogen, Carlsbad, CA) and transformed into TOP10 E. Coli. Plasmid DNA was isolated using the Purelink™ Quick96 Plasmid Kit (Invitrogen, Carlsbad, CA), evaluated for quality and quantity and sequencing using standard automated fluorescence-based DNA sequencing. Sequencing reads were assessed for quality and aligned to the BN reference sequence using the DNASTAR's Lasergene v7.2 software package. All SNP and insertion-deletions (INDEL) were visually confirmed in the trace files. All identified variants were verified by direct sequencing from genomic DNA isolated from at least two rats per strain (S or SHR).

Real-Time PCR and Multiplex Gene Expression. RNA was extracted using Trizol® reagent as described above. cDNA was obtained by reverse transcription using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA). Gene expression was evaluated using the using SYBR-green dye chemistry on a Stratagene MX3000p Real-Time PCR machine or Bio-Rad CFX96 (minimum n=6 each strain) and using Beckman Coulter GeXP platform. For RT-PCR, gene expression between the congenic strain and its control were evaluated using the comparative method (ΔΔCt) using GAPDH and/or β-Actin as "housekeeping" control genes (**Table S6**). For the GeXP platform, multiplex RT-PCR involved

two stages: (1) reverse transcription and amplification from RNA (GeXP Start Kit protocol) using chimeric primers, in this case directed at all 26 genes within the QTL (including GAPDH and β-Actin as control genes). PCR primers were designed to yield PCR products 4 to 7 bp apart, ranging from 137 to 338 bp (**Table S7**) using GeXP Express Profiler Primer Design Module (Beckman, Fullerton, CA); and (2) amplification using a single pair of universal primers as used by others[9, 10]. The products obtained from multiplex amplification were analyzed using the Beckman GeXP Platform. The peak location represents the gene identity, and the peak area represents gene expression level. Data was analyzed using the eXpress Analysis Module of the GeXP Genetic Analysis System.

Western blot analysis. Tissue homogenates were prepared in RIPA lysis buffer (Santa Cruz Biotechnology) from the kidney cortex isolated from S and S.SHR(2)X39 under **Study 2.** Protease and phosphatase inhibitors were included in the lysis buffer. Protein concentrations were determined using Bio-Rad protein assay kit and normalized to 10 μg/μl. A total of 50 μg of each sample was separated by electrophoresis on a 4-12% gradient gel and transferred to a PVDF membrane. The membranes were blocked with 1X TBST containing 2.5% non-fat milk (Bio-Rad) and probed with primary antibody, including Arhgef11 (Life Span Bioscience; LS-B1801), RhoA (Santa Cruz Biotechnology; SC-418), Rock1 (SC-17794), Limk1 (SC-8387), Cofilin (SC-8442), or p Cofilin (SC-21867-R). The membranes were washed and then incubated with HRP-conjugated secondary goat anti-mouse, goat anti-rabbit, or donkey anti-goat and then washed again. A working solution of the Pierce ECL Substrate (Thermo Scientific) was prepared according to the manufacturers' instructions and added to membranes. The membranes were removed from the substrates and placed in plastic sheet protectors and imaged using a ChemiDoc

XRS+ System (Bio-Rad). Equal loading of protein was confirmed by GAPDH (Millipore; MAB374) on same blot probed with Rho-Rock signaling protein.

Population studies: Candidate Gene Association Resource (CARe) Consortium.

The candidate region was evaluated in humans using data evaluated in the Candidate gene Association Resource (CARe) consortium. The CARe consortium evaluated the association of renal function measures in approximately 23,000 individuals of European descent[11] using the IBC SNP array, a 50K SNP genotyping array of candidate genes and pathways related to cardiovascular, inflammatory and metabolic phenotypes[12]. Briefly, individuals were participants of six population-based studies: the Atherosclerosis Risk in Communities (ARIC) study, the Coronary Artery Risk Development in Young Adults (CARDIA), the Cardiovascular Health Study (CHS), the Framingham Heart Study, and the Multi-Ethnic Study of Atherosclerosis (MESA). Serum creatinine was calibrated to NHANES to account for between-laboratory variation as previously described[13]. eGFR was estimated using the Modification of Diet in Renal Disease (MDRD) equation[14]. We also evaluated associations with albuminuria using urine albumin to creatinine ratio (UACR, mg/g). Genotyping quality control was conducted centrally and is described elsewhere[11]. Study-specific principal components were estimated using EIGENSTRAT[15]. We identified 22 SNPs within the region of interest.

Study-specific residuals from linear regression models of natural log-transformed eGFR or UACR were adjusted for age, sex and study site (if needed). The residuals were regressed into genotypes using additive genetic models while adjusting for 10 principal components. A linear regression approach was used that modeled the conditional probability distribution of the trait given genotype (SNPs) dosage. Models were fitted using the least

squares approach (parametric model). Relatedness (family data) was accounted for using linear mixed effect (LME) models. Meta-analyses of study-specific results were then performed using an inverse variance weighted fixed effect model (implemented in METAL, at www.sph.umich.edu/csg/abecasis/Metal/). Significant findings are reported using Bonferroni adjustments for 22 SNP tested ($p<0.002$).

The SNP selection was based on the target region based on the fine-mapping and experimental finding for the rat studies. We selected 22 genotyped SNPs that passed quality control and mapped homologous region in human genes of interest or their intergenic regions. (ARHGEF11, PEAR1, SH2D2A, and NES). The most significantly associated SNP, rs7534418, is an intronic variant of the neurotrophic tyrosine kinase, receptor, type 1 gene. This SNP is in strong LD ($r2=0.73$, $D'=0.87$ in CEU 1000 Genome Pilot Data) with a non-synonymous variant in the SH2D2A gene, rs926103 (not available in our data and predicted benign in Polyphen), and in moderate LD with rs2182761, an intronic variant of SH2D2A ($r2=0.63$, D' 0.86). We now include this information in the result section.

SUPPLEMENTAL REFERENCES

1. Garrett MR, Gunning WT, Radecki T, Richard A. Dissection of a genetic locus influencing renal function in the rat and its concordance with kidney disease loci on human chromosome 1q21. *Physiol Genomics*. 2007;30:322-334.
2. Packard M, Saad Y, Gunning WT, Gupta S, Shapiro J, Garrett MR. Investigating the effect of genetic background on proteinuria and renal injury using two hypertensive strains. *Am J Physiol Renal Physiol*. 2009;296:F839-846.
3. Joe B, Garrett MR, Dene H, Rapp JP. Substitution mapping of a blood pressure quantitative trait locus to a 2.73 mb region on rat chromosome 1. *J Hypertens*. 2003;21:2077-2084.
4. Garrett MR, Joe B, Yerga-Woolwine S. Genetic linkage of urinary albumin excretion in dahl salt-sensitive rats: Influence of dietary salt and confirmation using congenic strains. *Physiol Genomics*. 2006;25:39-49.
5. Savin VJ, Sharma R, Lovell HB, Welling DJ. Measurement of albumin reflection coefficient with isolated rat glomeruli. *J Am Soc Nephrol*. 1992;3:1260-1269.
6. Kaufman JM, Hardy R, Hayslett JP. Age-dependent characteristics of compensatory renal growth. *Kidney Int*. 1975;8:21-26.
7. Lopez B, Ryan RP, Moreno C, Sarkis A, Lazar J, Provoost AP, Jacob HJ, Roman RJ. Identification of a qtl on chromosome 1 for impaired autoregulation of rbf in fawn-hooded hypertensive rats. *Am J Physiol Renal Physiol*. 2006;290:F1213-1221.
8. Solberg Woods LC, Stelloh C, Regner KR, Schwabe T, Eisenhauer J, Garrett MR. Heterogeneous stock rats: A new model to study the genetics of renal phenotypes. *Am J Physiol Renal Physiol*. 2010;298:F1484-1491.

9. Boyer JD, Robinson TM, Kutzler MA, Vansant G, Hokey DA, Kumar S, Parkinson R, Wu L, Sidhu MK, Pavlakis GN, Felber BK, Brown C, Silvera P, Lewis MG, Monforte J, Waldmann TA, Eldridge J, Weiner DB. Protection against simian/human immunodeficiency virus (shiv) 89.6p in macaques after coimmunization with shiv antigen and il-15 plasmid. *Proc Natl Acad Sci U S A*. 2007;104:18648-18653.

10. Chen QR, Vansant G, Oades K, Pickering M, Wei JS, Song YK, Monforte J, Khan J. Diagnosis of the small round blue cell tumors using multiplex polymerase chain reaction. *J Mol Diagn*. 2007;9:80-88.

11. Musunuru K, Lettre G, Young T, Farlow DN, Pirruccello JP, Ejebe KG, Keating BJ, Yang Q, Chen MH, Lapchyk N, Crenshaw A, Ziaugra L, Rachupka A, Benjamin EJ, Cupples LA, Fornage M, Fox ER, Heckbert SR, Hirschhorn JN, Newton-Cheh C, Nizzari MM, Paltoo DN, Papanicolaou GJ, Patel SR, Psaty BM, Rader DJ, Redline S, Rich SS, Rotter JI, Taylor HA, Jr., Tracy RP, Vasan RS, Wilson JG, Kathiresan S, Fabsitz RR, Boerwinkle E, Gabriel SB. Candidate gene association resource (care): Design, methods, and proof of concept. *Circ Cardiovasc Genet*. 2010;3:267-275.

12. Keating BJ, Tischfield S, Murray SS, Bhangale T, Price TS, Glessner JT, Galver L, Barrett JC, Grant SF, Farlow DN, Chandrupatla HR, Hansen M, Ajmal S, Papanicolaou GJ, Guo Y, Li M, Derohannessian S, de Bakker PI, Bailey SD, Montpetit A, Edmondson AC, Taylor K, Gai X, Wang SS, Fornage M, Shaikh T, Groop L, Boehnke M, Hall AS, Hattersley AT, Frackelton E, Patterson N, Chiang CW, Kim CE, Fabsitz RR, Ouwehand W, Price AL, Munroe P, Caulfield M, Drake T, Boerwinkle E, Reich D, Whitehead AS, Cappola TP, Samani NJ, Lusis AJ, Schadt E, Wilson JG, Koenig W, McCarthy MI, Kathiresan S, Gabriel SB, Hakonarson H, Anand SS, Reilly M, Engert JC, Nickerson

DA, Rader DJ, Hirschhorn JN, Fitzgerald GA. Concept, design and implementation of a cardiovascular gene-centric 50 k snp array for large-scale genomic association studies. *PLoS One*. 2008;3:e3583.

13. Coresh J, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, Levey AS. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *American journal of kidney diseases : the official journal of the National Kidney Foundation*. 2002;39:920-929.

14. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: A new prediction equation. Modification of diet in renal disease study group. *Ann Intern Med*. 1999;130:461-470.

15. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904-909.

16. Garrett MR, Dene H, Rapp JP. Time-course genetic analysis of albuminuria in dahl salt-sensitive rats on low-salt diet. *J Am Soc Nephrol*. 2003;14:1175-1187.

SUPPLEMENTAL TABLES

Table S1- Localization of Proteinuria QTL by Recombinant Progeny Test (RPT)

Strain	S/SHR Genotype Proteinuria; n	S/S Genotype Proteinuria; n	Proteinuria Effect (mg/24hours) (recombinant- non-recombinant progeny)	p_value
S.SHR(2)X39	50.90±2.83 (33)	71.86±2.80(34)	-20.96	0.008
S.SHR(2)X40	56.85±3.28 (16)	55.80±3.96 (22)	1.05	0.620
S.SHR(2)X41	67.2±3.73 (20)	68.1±4.05 (17)	-0.9	0.873
S.SHR(2)X42	78.9.±5.65 (16)	72.5±4.92 (21)	6.4	0.403
S.SHR(2)X43	56.0±6.61 (9)	82.7.±5.07 (12)	-26.7	0.020
S.SHR(2)X44	62.1.±5.61 (11)	58.0±5.85 (12)	4.1	0.650

Table S2- Primers used to amply gene transcript for cloning- only those with coding sequence differences (Table 1)

Name	Forward (-F)	Reverse (-R)
Arhgef11-1	TTCCTCCTGTCCTGAATCACTCTC	CCGTGGCATCTTTCAAGGG
Arhgef11-2	ACCATCTCCAGTGTCTTGTCTGGT	AAGCCATGGAGACTTGGGCTAACA
Bcan-1	GGAAGAAAGGGGGTTTTGTG	ATTTCCACCTGGTTTTGCTG
Bcan-2	GGAGGGGACCTCACAAGTTC	ACTGCCTGTGCTCTTCCCTA
Bcan-3	ATGATCCCATTGCTTCTGTCC	CTAGAGACTGGAGGGAGGTGTCAAC
C1orf85-F1	CAGGAGGAGAGGCAGGAGTA	CAGATCACAGCACTGGCACT
C1orf85-F2	AGGATCAGCAGGAAGGGAGT	GCAGCCAACAGTCACTCTCA
Insrr-F1	TAGAAGTCTGAAAGCCCAGGAGGT	ACAGGCTGTCCCTGTGAGTTTGTT
Insrr-F2	AGAGAAGTCGGTGGCGTAAACCAA	GTGCTGAGCTAATTCTTGACCCGT
Iqgap3-1	TCAGGTTCATGGGAAGGGCTGAAA	AGCTGTGTACAATGGGTGGAAGGA
Kpp-1	AAAGGATCAGCAGGAAGGGAGTCA	GCCCAGCAGCACTCACACATTAAA
Kpp-2	GAAGCGGTCCCTTTAAGAACTGGT	CGCCACACTGCACATCCATACAAA
Mrpl24-1	GAAAGTTGGTGGAAGGCAAA	TGGAGCTGAGGACTGAACCT
Mrpl24-2		CTGACAGTCTGAAGCCACCA
Paqr6-1	CTGAAGACCCCCAGAGACTG	GCCTGGTACCTGTGTCCACT
Paqr6-2	GAGGACCTCTGAATGGATGC	
Rhbg-1	GGGCTAGTCCTTGCCTACCT	AGTCACCTAGGGCTGGCTTT
Rhbg-2	TTGGGGAAGTTCACACATCA	GGAGTAACCAATGGCAGGAG
sema4a-1	GACAGACAGTGCGGAACAGA	GGAGGAGGAAGAGGTCAAGG
sema4a-2	ATCTGCACACTGGTGACTGC	TTTCAGGGTCCTGGAGAATG
smg5-1	TGCTGATTCCGGTAACTTCC	CGAGTGAGTCTGCATGTGGT
smg5-2	ATTTTTCGCGAGACTTGGTG	TCCAAGCACTTCCCTCACTT
Pear1-1	CTTCCTGCTTGGATCTTTGC	CAGGCACTCTCCCATACCAT
Pear1-2	ATGCCACTCTGTCCCCTCCTC	TCAGCGGTCCTGGCGCCGGGA

Table S3- Haplotype analysis using panel of inbred with susceptibility or resistance to kidney injury

Ensembl ID (ENSRNO)	Position (Chr. 2)	Allele	Susceptible to Kidney Injury/ Elevated Proteinuria					Resistance to Kidney Injury/ Low Proteinuria							
			SS/Jr	MWF /Hsd	BUF/ Sim	MNS	FHH /Eur	SHR/ Mol	WKY/ NCrl	ACI/ Nkyo	F344/ Han	LOU /CHan	MHS	LEW/ Crl	BN/ SsNSlc
SNP2786638	178561587	T/C	CC	TT	TT	TT	CC	TT	TT	CC	CC	CC		TT	TT
SNP2786639	178561788	G/A	AA	AA	GG	GG	AA	GG	GG	AA	AA	AA	GG	GG	GG
SNP2786640	178724625	G/A	AA	AA	GG	GG	AA	GG	GG	AA	AA	AA		GG	GG
SNP2786641	178815064	T/C	CC	TT	TT	TT	CC	TT	TT	CC	CC	CC		TT	TT
SNP2786642	178871056	C/T	TT	CC	CC		TT	CC	CC	TT	TT	TT	CC	CC	CC
SNP2786643	179011247	G/A	GG	GG	GG	GG	GG	AA	AA	GG	GG	GG		GG	GG
SNP2786646	179506095	C/T	TT	TT	TT	TT	TT	CC	CC	TT	TT	TT		TT	CC
SNP2786647	179508457	A/G	GG	AA	AA	AA	GG	AA	AA	GG	GG	GG	AA	AA	
SNP2786648	179645084	C/G	GG	CC	GG	GG	CC	CC	CC	CC	CC	CC		GG	CC
SNP2786652	**179892073**	**C/T**	**CC**	**CC**	**CC**	**CC**	**TT**	**TT**	**TT**	**TT**	**TT**	**TT**	**TT**	**CC**	**CC**
SNP2786655	180264633	C/T	CC	TT	CC	CC	TT	CC	CC	TT	CC	TT	CC	CC	CC
SNP2786656	180441222	C/T	CC	CC	CC	CC	CC	TT	TT	CC	CC	CC		CC	CC
SNP2786657	180481614	C/A	CC	CC	CC	CC	CC	AA	AA	CC	CC	CC		CC	CC
SNP2786660	180513367	T/A	AA	AA	AA	AA	AA		TT	AA	AA	AA	AA	AA	

analyzed on 05/10/12, http://snplotyper.mcw.edu/analyses/2913/edit

Haplotype across a panel of inbred strains (http://snplotyper.mcw.edu/analyses/2913/edit). Inbred strains were classified as high proteinuria and/or susceptibility to kidney injury or low proteinuria and/or susceptibility to kidney injury. Only SNPs in the region that are different between S and SHR are shown. There was a strong concordance between the strain classifications and the allelic variant (C or T) at **ENSRNOSNP2786652**. No other SNP in the region exhibited this clear pattern. The S, MWF, BUF, and MNS (strains demonstrating high proteinuria and those that have been used in several linkage analysis studies as susceptible strains) all possess the C allele, whereas the SHR, WHY, ACI, F344, LOU, and MHS (demonstrating low proteinuria) possess the T allele. The three instances of disagreement (FHH, LEW, and BN) can be explained based on the results of FHH and ACI (Brown et al, Nat Genet, 1996) and MWF and LEW (Schulz et al, J Am Soc Nephrol, 2002) linkage analyses or congenic strain analysis [S and S.BN(2); www.pga.mcw.edu] for proteinuria. No QTL for proteinuria was observed in either linkage study, consistent with the same variant being present (FHH=ACI= T allele; MWF=LEW=C allele) and no significant difference in proteinuria was observed in the congenic, consistent with the finding that the same variant is present for S and BN. It is important to note that this SNP is in itself is likely not causative, but closely linked to the genetic variant(s) that are causative.

Table S4- Annotated genes the smallest congenic strain [S.SHR(2)X47], introgressed SHR segment from D2Rat127-D2Rat230

Gene #	Ensembl Gene ID	Ensembl Transcript ID	Associated Gene Name	Gene Start (bp)	Gene End (bp)	Description
	D2Rat127			177914620		
1	ENSRNOG00000024215	ENSRNOT00000037507	D4A935_RAT	177915826	177916946	Uncharacterized protein
2	ENSRNOG00000024208	ENSRNOT00000046286	LOC365835	177944739	177952063	Uncharacterized protein
3	ENSRNOG00000011752	ENSRNOT00000015802	Sh3d19	178045383	178081565	Similar to SH3 domain protein D19
4	ENSRNOG00000011893	ENSRNOT00000016329	Rps3a	178092354	178096722	40S ribosomal protein S3a40S ribosomal protein S3b
5	ENSRNOG00000023453	ENSRNOT00000023418	F1LZG6_RAT (LRBA)	178257271	178786598	lipopolysaccharide-responsive and beige-like anchor protein
6	ENSRNOG00000017461	ENSRNOT00000023482	D3ZC03_RAT	178470040	178471228	Enolase
7	ENSRNOG00000031398	ENSRNOT00000049461	Mab21l2	178523415	178524494	protein mab-21-like 2
8	ENSRNOG00000016550	ENSRNOT00000057062	Dclk2	178793765	178923600	Serine/threonine-protein kinase DCLK2
9	ENSRNOG00000037617	ENSRNOT00000057059	F1LZB3_RAT	179009201	179010060	Uncharacterized protein
10	ENSRNOG00000016451	ENSRNOT00000022150	Cd1d1	179011165	179014672	Antigen-presenting glycoprotein CD1d
11	ENSRNOG00000042212	ENSRNOT00000068041	F1M679_RAT	179043169	179044178	Uncharacterized protein
12	ENSRNOG00000029933	ENSRNOT00000051275	F1M8Z1_RAT	179088369	179227738	Uncharacterized protein
13	ENSRNOG00000016408	ENSRNOT00000031743	Kirrel	179114757	179169185	Kin of IRRE-like protein 1
	D2Rat131			179252555		
14	ENSRNOG00000016164	ENSRNOT00000021938	Fcrls	179283609	179294259	Fc receptor-like S, scavenger receptor
15	ENSRNOG00000016146	ENSRNOT00000021604	LOC365839	179320926	179322275	similar to elongation protein 4 homolog
16	ENSRNOG00000023068	ENSRNOT00000057022	Cd5l	179383719	179394731	CD5 antigen-like

17	ENSRNOG00000034230	ENSRNOT00000052347	Fcrl1	179416714	179422922	Uncharacterized protein
18	ENSRNOG00000043414	ENSRNOT00000046778	F1LX58_RAT	179482220	179486101	Uncharacterized protein
19	ENSRNOG00000030593	ENSRNOT00000050486	F1M3A1_RAT	179486911	179498198	Uncharacterized protein
20	ENSRNOG00000043095	ENSRNOT00000021577	Etv3	179567146	179576683	ETS translocation variant 3
21	ENSRNOG00000015533	ENSRNOT00000020819	F1LV61_RAT	179629568	179630812	Uncharacterized protein
	ENSRNOSNP2786648- no association between high and low proteinuria strains (Table S3)			179645084		
22	**ENSRNOG00000015026**	**ENSRNOT00000065755**	**ARHGB_RAT**	**179676236**	**179796785**	**Rho guanine nucleotide exchange factor 11**
23	**ENSRNOG00000014937**	**ENSRNOT00000020147**	**RGD1309453 (Lrcc)**	**179796567**	**179809219**	**Putative uncharacterized protein RGD1309453**
24	**ENSRNOG00000014243**	**ENSRNOT00000019502**	**Pear1**	**179809435**	**179829207**	**platelet endothelial aggregation receptor 1**
25	**ENSRNOG00000013953**	**ENSRNOT00000018961**	**Ntrk1**	**179838740**	**179855545**	**High affinity nerve growth factor receptor**
26	**ENSRNOG00000013344**	**ENSRNOT00000018638**	**Insrr**	**179857189**	**179876572**	**Insulin receptor-related protein**
	ENSRNOSNP2786652- association between high and low proteinuria strains (Table S3)			**179892073**		
27	**ENSRNOG00000013294**	**ENSRNOT00000017798**	**Sh2d2a**	**179916108**	**179924623**	**SH2 domain-containing protein 2A**
28	**ENSRNOG00000012933**	**ENSRNOT00000017735**	**Prcc**	**179931934**	**179957221**	**papillary renal cell carcinoma (translocation-associated)**
29	**ENSRNOG00000012836**	**ENSRNOT00000017234**	**HDGF_RAT**	**179981617**	**179989406**	**Hepatoma-derived growth factor**
30	**ENSRNOG00000022234**	**ENSRNOT00000035271**	**mrpl24**	**179995713**	**180001725**	**39S ribosomal protein L24, mitochondrial**
31	**ENSRNOG00000011645**	**ENSRNOT00000015793**	**RGD1311265**	**180001178**	**180009775**	**Protein RRNAD1**
32	**ENSRNOG00000011402**	**ENSRNOT00000015471**	**Isg20l2**	**180009276**	**180018761**	**Interferon-stimulated 20 kDa exonuclease-like 2**
33	**ENSRNOG00000022101**	**ENSRNOT00000025183**	**Crabp2**	**180029801**	**180034174**	**Cellular retinoic acid-**

						binding protein 2
34	**ENSRNOG00000018681**	**ENSRNOT00000025314**	**NEST_RAT**	**180052034**	**180060546**	**Nestin**
35	**ENSRNOG00000018798**	**ENSRNOT00000025496**	**PGCB_RAT**	**180067867**	**180080947**	**Brevican core proteinBrevican core protein isoform 1Brevican core protein isoform 2**
36	**ENSRNOG00000018870**	**ENSRNOT00000025624**	**Hapln2**	**180104688**	**180110115**	**Hyaluronan and proteoglycan link protein 2**
37	**ENSRNOG00000018969**	**ENSRNOT00000056923**	**Gpatch4**	**180123437**	**180132223**	**G patch domain-containing protein 4**
38	**ENSRNOG00000019201**	**ENSRNOT00000025986**	**Apoa1bp**	**180132505**	**180134553**	**apolipoprotein A-I-binding protein**
39	**ENSRNOG00000028765**	**ENSRNOT00000050476**	**LOC100365656 (Ttc24)**	**180138738**	**180144068**	**Uncharacterized protein**
40	**ENSRNOG00000027894**	**ENSRNOT00000038589**	**Iqgap3**	**180155679**	**180197918**	**ras GTPase-activating-like protein IQGAP3**
41	**ENSRNOG00000031778**	**ENSRNOT00000066642**	**Mef2d**	**180221078**	**180246916**	**Myocyte-specific enhancer factor 2D**
	ENSRNOSNP2786655-no association between high and low proteinuria strains (Table S3)			180264633		
42	ENSRNOG00000019412	ENSRNOT00000026396	Rhbg	180320788	180333050	Ammonium transporter Rh type B
43	ENSRNOG00000037552	ENSRNOT00000056898	RGD1304953	180371102	180380656	similar to SSTK-interacting protein
44	ENSRNOG00000019090	ENSRNOT00000025824	Cct3	180381044	180434151	T-complex protein 1 subunit gamma]
45	ENSRNOG00000019281	ENSRNOT00000026153	RGD1303130 (Kpp)	180438070	180441659	Lysosomal protein NCU-G1
46	ENSRNOG00000019414	ENSRNOT00000026414	Tmem79	180442064	180446842	Transmembrane protein 79
47	ENSRNOG00000019590	ENSRNOT00000026514	Smg5	180448815	180474674	Uncharacterized protein
48	ENSRNOG00000026059	ENSRNOT00000036143	Paqr6	180478303	180481707	progestin and adipoQ

						receptor family member 6
49	ENSRNOG00000019607	ENSRNOT00000026530	Bglap	180482313	180483290	Osteocalcin
50	ENSRNOG00000019620	ENSRNOT00000035383	Pmf1	180491955	180511936	polyamine-modulated factor 1
51	ENSRNOG00000025269	ENSRNOT00000068360	Slc25a44	180512111	180526810	solute carrier family 25 member 44
52	ENSRNOG00000019737	ENSRNOT00000056862	Sema4a	180552412	180570359	semaphorin-4A
53	ENSRNOG00000019638	ENSRNOT00000026705	Lmna	180595724	180616354	Prelamin-A/CLamin-A/C
54	ENSRNOG00000029812	ENSRNOT00000052079	Mex3a	180651103	180652134	Uncharacterized protein
55	ENSRNOG00000023640	ENSRNOT00000032355	Rab25	180657125	180663222	ras-related protein Rab-25
	D2Rat230			*180666055*		

There are 55 genes annotated in the smallest congenic strain [S.SHR(2)X47], with introgressed SHR segment from D2Rat127-D2Rat230. The coding regions of gene #14-55 were sequenced. Important microsatellite and SNP markers are shown in italics. The region shown in bold is based on refinement of the QTL based on congenic strains, haplotype analysis, and comparative mapping (**Fig. 4**). 52 of these genes are orthologous in humans and 55 in mouse

Table S5- GeXP Multiplex Gene Expression

Genes	Curve Fit (R2)	Ratio S/C-1	Ratio S/C-2	Average	p_value
RatApoa1bp	0.999	1.10	1.02	0.92	0.53
RatC1orf92 (Lrcc)	1.000	0.70	0.70	0.51	0.03
RatCct3	0.999	1.17	1.23	0.93	0.20
RatCrabp2	1.000	1.45	1.57	1.09	0.03
RatGpatch4	1.000	1.43	1.54	1.09	0.00
RatHdgf	1.000	1.07	1.10	0.96	0.73
RatInsrr	1.000	0.82	0.77	0.64	0.19
RatIqgap3	1.000	1.24	1.45	1.05	0.12
RatIsg20I2	1.000	1.01	0.85	0.94	0.95
RatKpp	1.000	0.74	0.64	0.53	0.07
RatLmna	0.999	1.02	1.01	0.96	0.88
RatMef2d	1.000	0.99	1.00	0.97	0.93
RatMex3a	0.997	0.99	1.25	1.05	0.91
RatMrpl24	1.000	0.86	0.87	0.69	0.28
RatNeph1	1.000	1.16	1.08	0.91	0.25
RatNes	1.000	0.98	0.96	0.94	0.91
RatNtrk1	0.989	0.77	0.81	0.56	0.13
RatPear1	1.000	1.90	1.85	1.87	0.03
RatPrcc	1.000	0.80	0.73	0.61	0.09
RatRab25	0.999	0.87	0.78	0.73	0.51
RatRhbg	1.000	0.83	0.62	0.62	0.25
RatSema4a	0.999	1.24	1.36	1.00	0.15
RatSh2d2a	0.994	1.47	2.02	1.30	0.04
RatSlc25a44	1.000	0.90	0.74	0.74	0.57
RatSmg5	1.000	0.98	1.01	0.96	0.90
RatTmem79	1.000	0.85	0.76	0.69	0.38

Genes in bold demonstrated differential expression at $p<0.05$

Table S6- Real-Time PCR primers

EnsembleID	Gene Transcript/Exon	Primer-Forward	Primer-Reverse
ENSRNOT00000049112	Arhgef11_E37-38_GE	CTACTCCCTGGTGGTGGTGT	GCTGGCATGCTGACATAGAA
ENSRNOT00000019502	Pear1_E21-22_GE	CCAGGCAGCCTCTTCATCT	GTGATGGAGGATGCCGTACT
ENSRNOT00000018638	Insrr_E21-22_GE	GTCATGGACGGTGGAGTTCT	ATCCAGGATGTGGACGAAAG
ENSRNOT00000017798	Sh2d2a_E6-7_GE	AGGCCCCAGGACATAAAGAG	AGACGGGGTTAATGGGTAGG
ENSRNOT00000017735	Prcc_E7-8_GE	CGGAGGAAACACCAGATCAC	CCATATTTGGCTTGGGTCTG
ENSRNOT00000035271	Mrpl24_E5-6_GE	ATGGCCTCCATCACATCTTC	ATCCCCAAACCTGAATTTCC
ENSRNOT00000065211	Hdgf_E3-4_GE	GTGGGAGATCGAGAACAACC	CTGCATTGCCCTTCTTGTC
ENSRNOT00000025183	Crabp2_E3-4_GE	CAAAATGGTGTGCGAACAGA	ACAACGTCGTCTGCTGTCAT
ENSRNOT00000025314	Nes_E3-4_GE	CTCTGCTGGAGGCTGAGAAC	GGGAAGGGAAGGATGTGG
ENSRNOT00000056923	Gpatch4_E6-7_GE	GACTGATGAGATGCTACTCAAAGC	TTTTGGGAGGGCTCACTGT
ENSRNOT00000025986	Apoa1bp_E5-6_GE	CGCGACACCTCAAACTTTTT	TTTCACCAAGGAAAGGGATG

Table S7- GeXP Multiplex Primers

Gene #	Gene Name	Product Size	Forward Primer	Reverse Primer	Product size with Universal Tags
1	Neph1	99	CTCACCTGGACCAAGAAGGAT	GCAGGTATAGGTGCCAGCAT	136
2	C1orf92	112	CCTGGGAACAAGGTCCTCTT	TGGACTTGGAGACCTGAACC	149
3	Pear1	118	CTTGCCAAAATGGAGGTGTT	GTACAGTTGGGTCCGTGGAA	155
4	Ntrk1	125	GGCCTCTCCCTACAGGACTT	GAGCCCTGAAGCTTTTGTGT	162
5	Insrr	131	CCCTGTGTGAGAACCACCTT	CATTCACTGTGGCAGCACTC	168
6	Sh2d2a	138	GGTGAACTGTCATTGCAAGC	TTGGGACTGCAGTAGCCTCT	175
7	Prcc	145	TCCCAAGCCAAAGAAGAGAA	CAAGGCAGACAAACCAGTCC	182
8	Hdgf	151	CCCAAAGACCTCTTCCCTTA	CTGCGCAGCTCTTTTTCTG	188
9	Mrpl24	157	TCTGTGGAGACATGGTGGAA	ATCATGGTCCCTCGGTGAT	194
10	Isg20l2	163	GCCACATGGTTAATGCTACC	AGGAGCGGTATTTGGGAAGT	200
11	Crabp2	169	GCAGACTGTGGATGGGAGAC	TACAACGTCGTCTGCTGTCA	206
12	Nest	175	TAAGTTCCAGCTGGCTGTGG	AGGTGTCTGCAACCGAGAGT	212
13	Gpatch4	194	ACTCTGGCAAGGATGGAGTG	CAGAATCTTTGGGGGTATGG	231
14	Apoa1bp	200	GACATCCCTTTCCTTGGTGA	CCCTTCTCTACATCCCATCC	237
15	Iqgap3	206	AGGTGTGCTTGAAGGAGGAG	TACCGCAGAAAGCCAGAAGT	243
16	Mef2d	212	TGGGGAGGAAAAAGATTCAG	CTCCGTGTACTTGAGCAGCA	249
17	Rhbg	218	CAGATCCCAGCTGGAGAAGA	CACTGACAAGGGCTGACAAG	255
18	Cct3	224	AGACATCATCCGGACCTGTT	CCACAGACAGCATTTCTCCA	261
19	Kpp	230	CTCCTGCTGTCTCCTGATCC	CCTCGAAAGATGGCACTCA	267
20	Tmem79	236	GTCTGTGCAGCTCTTCATCCT	GCTCCACCACGAACATGTAA	273
21	Smg5	242	CGGCTAGACCTCATCCTTTG	GTCCTGTAGGCACATTCCAAG	279
22	Slc25a44	254	GCAAGAGCCAGACAGAAGGT	GGTAGAGGCCAGTCACTCCA	291
23	Sema4a	260	ACAGATGGGAAGGGTCAAAG	AAGTCAAACTCGCTGGCTGT	297
24	Lmna	266	GTGCGTGAGGAGTTCAAGGA	AGCCTGTTCTCAGCATCCAC	303
25	Mex3a	273	GTGCAGTCATCGATTGGTCA	GCGGTAGGGCACTCTTACAC	310
26	Rab25	279	CAATGAATTCAGCCACGACA	CTTTTGTTCCCCACAAGCAT	316

SUPPLEMENTAL FIGURES AND LEGEND

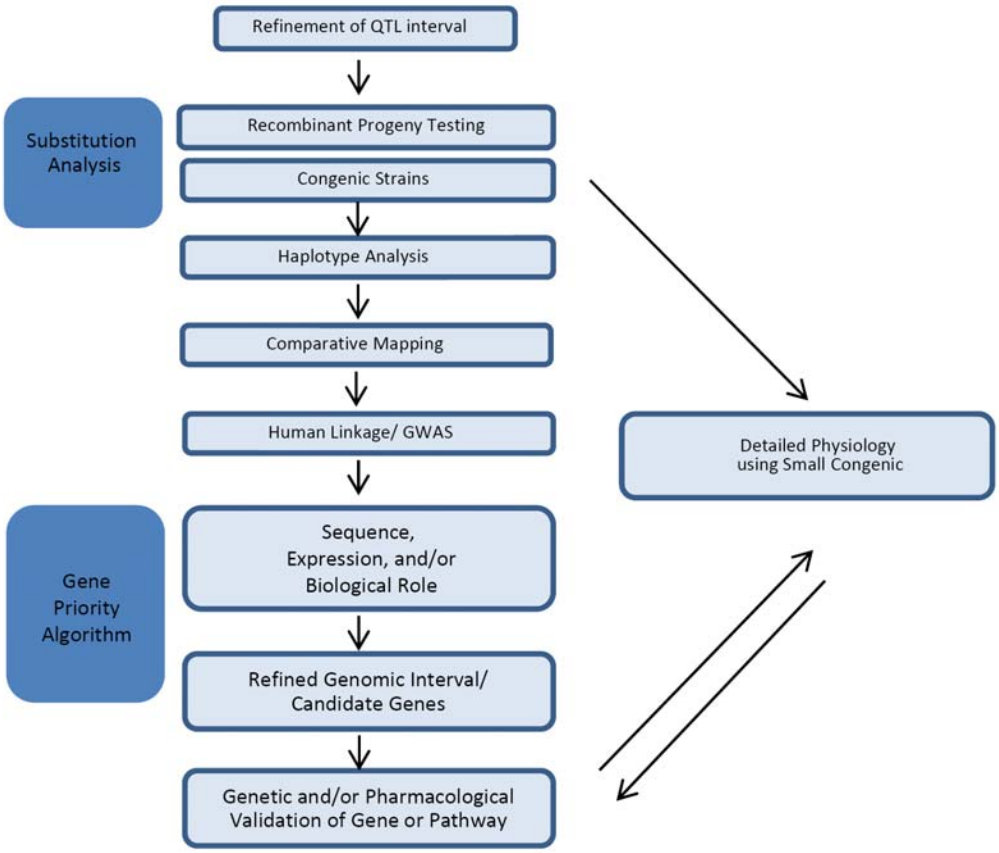


Figure S1: Schematic diagram of the approach used to narrow gene/ genetic variants associated with kidney injury on rat chromosome 2. The predominant method was substitution analysis using recombinant progeny testing (RPT) and/or congenic strain analysis. The use of other methods, such as haplotype analysis, comparative mapping, and linkage or genome-wide association studies (GWAS) in humans was used to further refine the likely location of the QTL. Once the number of genes were narrowed to a manageable number (~20-30), sequencing, gene expression, and bioinformatics analysis was performed to identify strong candidate genes that can be further studied *in vivo* by genetic modification (e.g., gene knockout) or by pharmacological agents.

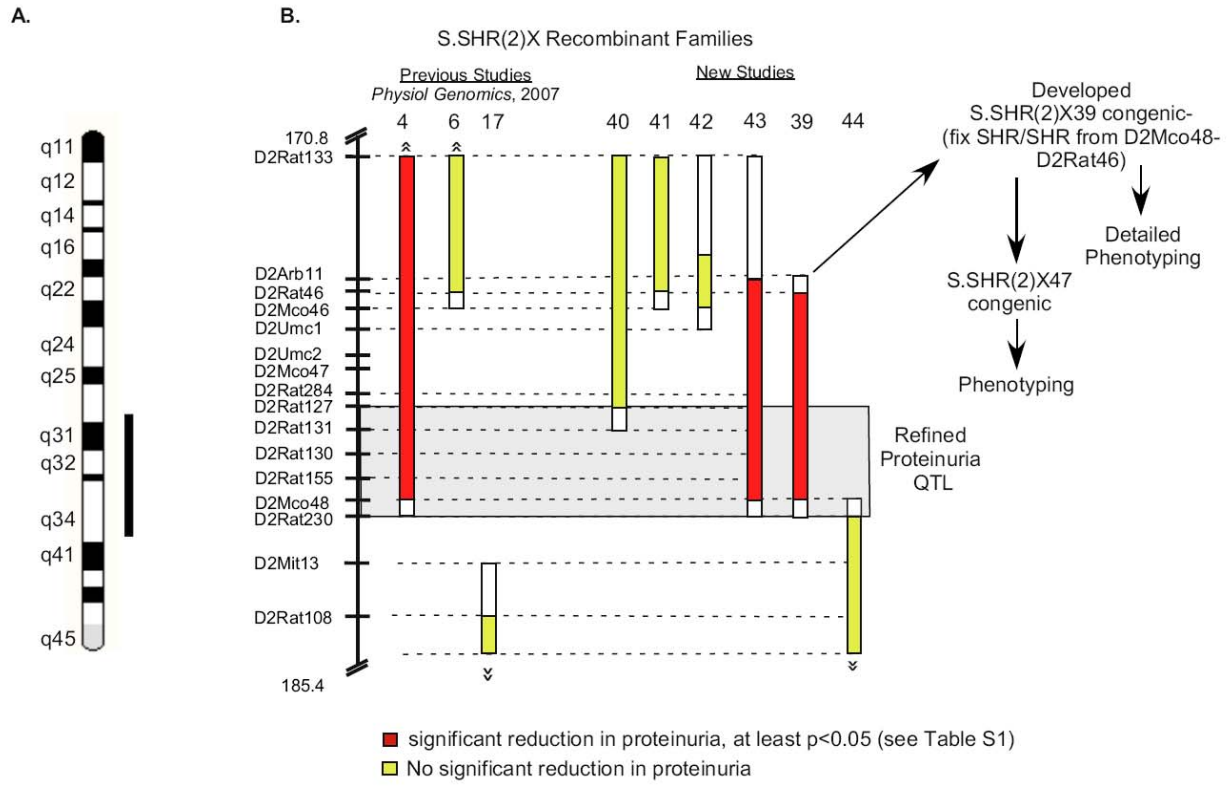


Figure S2: Fine-mapping of the chromosome 2 genomic interval associated with kidney injury in the Dahl S. (**A**) Ideogram showing the 95% confidence interval (CI) of proteinuria quantitative trait loci (QTL) from original linkage analysis (solid black bar)[16]. (**B**) Recombinant progeny testing (RPT) was employed to refine the genomic region containing the genetic variants linked to kidney injury. The basis of RPT is to identify recombinant animals (e.g. different donor SHR regions), to propagate these recombinant animals by backcross to S, then to measure the phenotypes of the progeny. The influence of each recombinant region (i.e. SHR genotype) was determined by subtracting average proteinuria of recombinant rats (SHR-like) from proteinuria of non-recombinant (S-like) littermates. Red bars denote that recombinant animals (SHR-like) had significantly ($p < 0.05$) lower proteinuria compared to non-recombinant (S-like) littermates. Yellow bars denote there was not a significant difference in proteinuria between recombinant and non-recombinant littermates. The proteinuria effect and number of

animals tested from each recombinant family are shown in **Table S1**. Based on RPT, the QTL interval was narrowed to genomic region defined to D2Rat131-D2Rat230 (~2.75 Mb). The S.SHR(2)X39 recombinant family was used to generate a congenic strain that fixed the transferred genomic interval homozygous SHR/SHR (**Fig. 1**). The S.SHR(2)X47 was generated from a new recombination that occurred while developing the S.SHR(2)X39 strain. These fixed congenic strain were phenotyped for other measures of kidney injury and function (**Studies 2-6**).

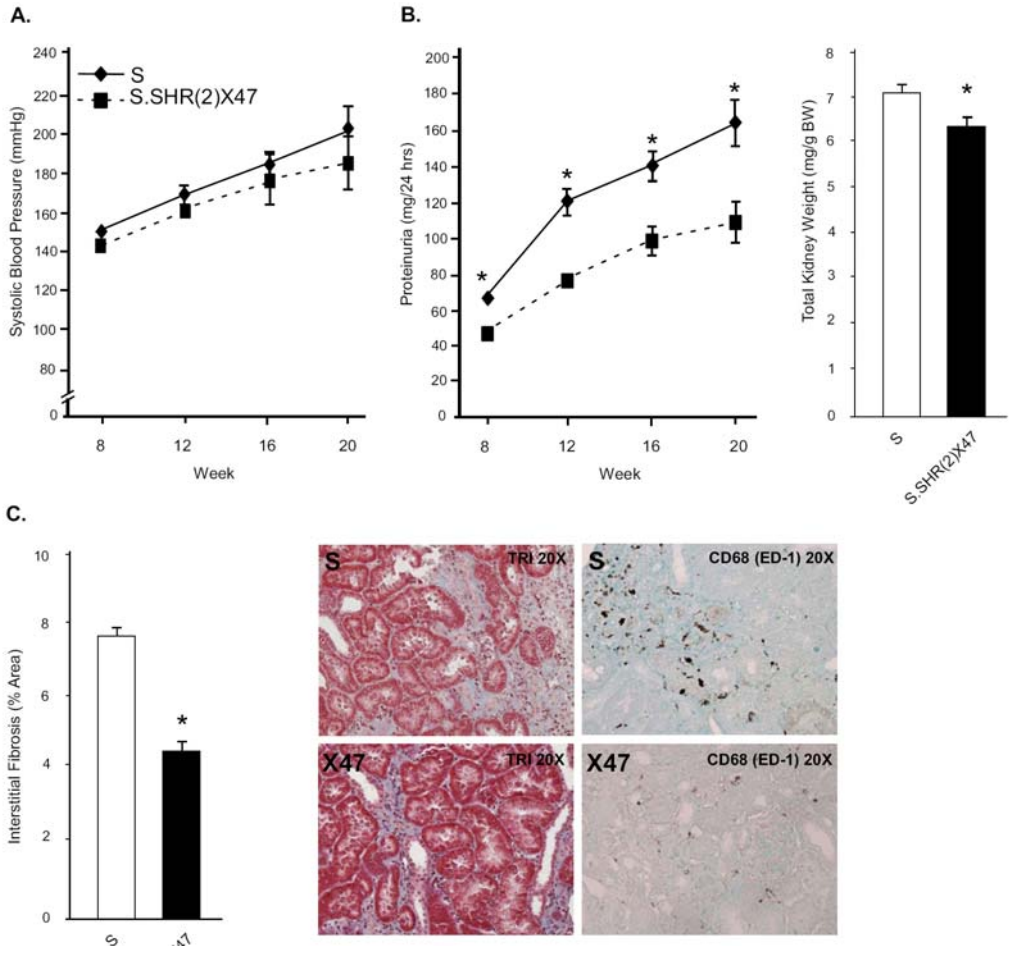


Figure S3: Time course assessment of blood pressure, proteinuria, and kidney injury in S and S.SHR(2)X47 from week 8 to 20. (**A**) Systolic blood pressure measured by telemetry (n=10 per group). (**B**) Proteinuria and kidney weight (n= 12 per group). (**C**) Morphometric analysis of tubulointerstitial fibrosis, representative images of Masson's Trichrome and immunohistochemical straining of CD-68 (ED-1; macrophage). The S.SHR(2)X47 congenic exhibited significantly less proteinuria, which is supported by the decreased tubulointerstitial injury (fibrosis and less macrophage infiltration). This data is constant with the larger congenic for which detailed phenotyping was performed.*, $p<0.05$.

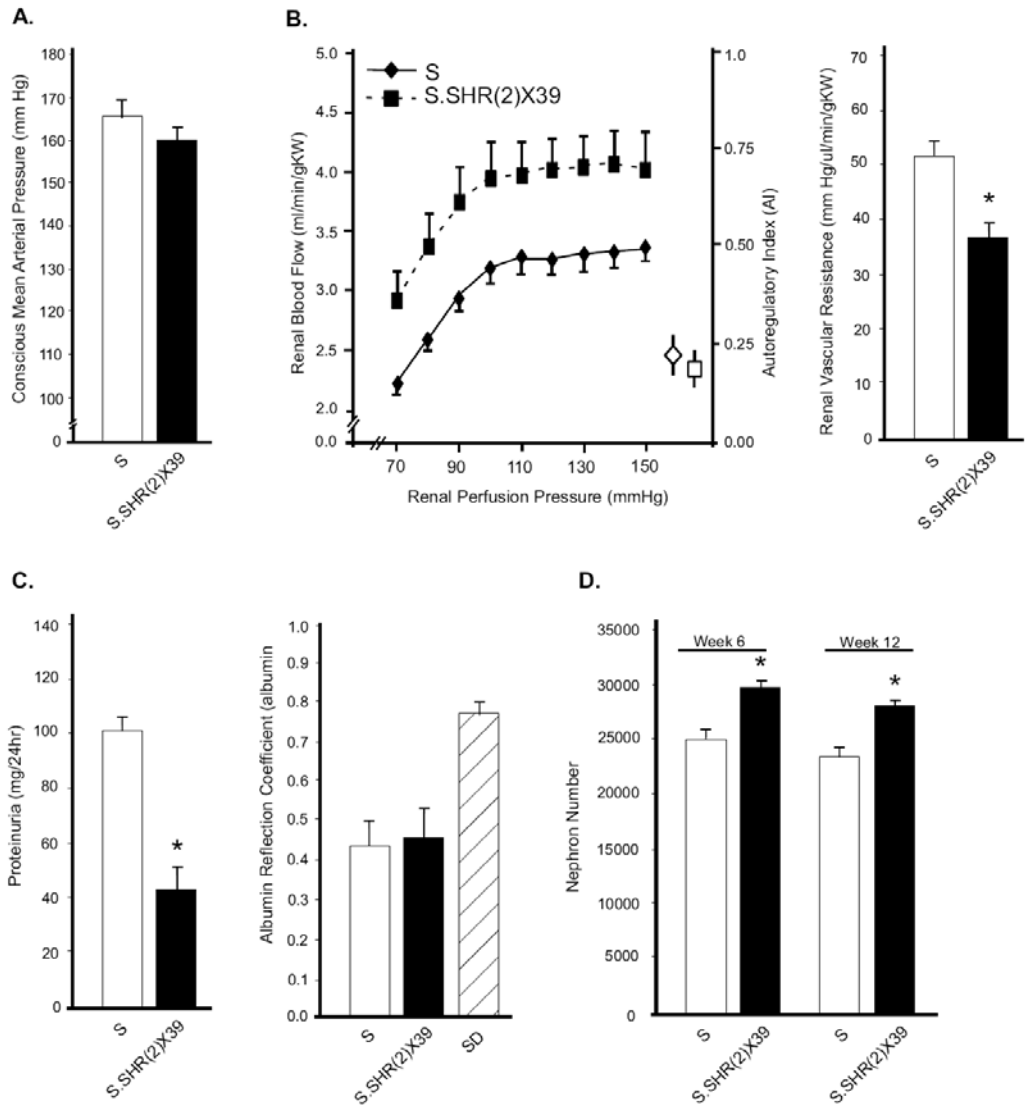
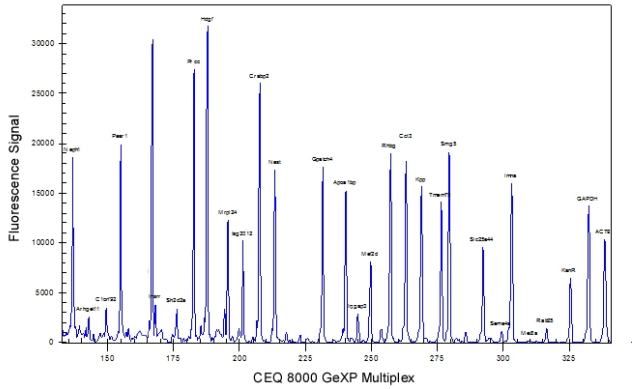


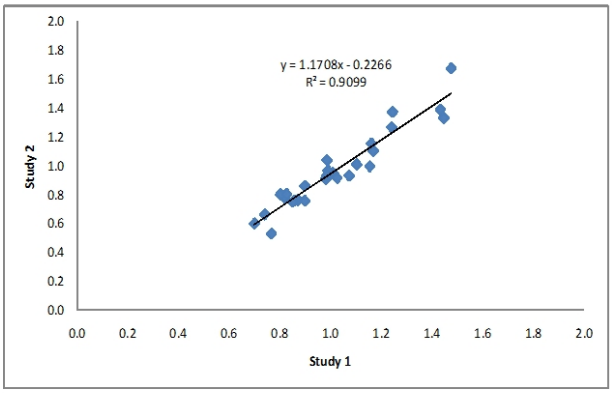
Figure S4: Blood pressure, autoregulation of renal blood flow (RBF) and glomerular number and permeability between S and S.SHR(2)X39 on normal rodent diet (0.7% NaCl, Purina 5010). (**A**) Conscious mean arterial pressure (MAP). (**B**) RBF autoregulation (left y-axis) and autoregulatory index (right y-axis) and RVR (renal vascular resistance). Both groups effectively autoregulate RBF as renal profusion pressure is increased (AI ~ 0). (**C**) Proteinuria and glomerular permeability. Proteinuria was evaluated in groups of S and S.SHR(2)X39 animals

before glomeruli were isolated for permeability assay. There is a distinct difference in proteinuria between groups, but no difference in the reflection coefficient of albumin. This suggests that the detected difference in proteinuria is not likely due to the degree of filtered protein. (**D**) Nephron/glomeruli number. Measurements were made at week 12 (n=8 per group), except for nephron number which was also evaluated week 6.*, $p<0.05$.

A.



B.



C.

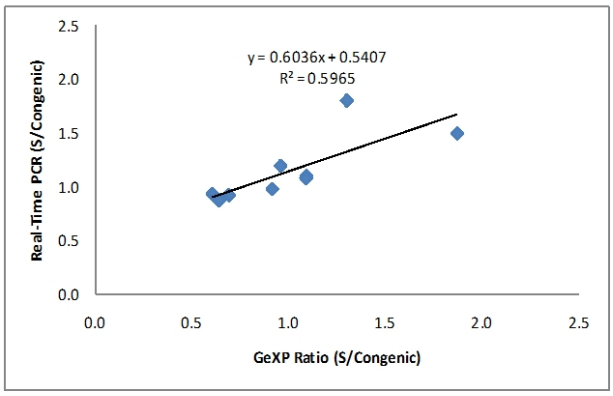


Figure S5: Multiplex gene expression profiling between S and S.SHR(2)X39 normal rodent diet (0.7% NaCl, Purina 5010) at week 12. (**A**) Representative multiplex gene expression chromatograph of all the genes in the genomic region using Beckman Coulter (GeXP) platform. The benefit to this approach is the ability measure gene expression patterns of genes within the

genomic locus simultaneously. (**B**) Scatter plot and regression of two independent multiplex studies which demonstrates high correlation ($r=0.95$, $p<0.0001$) between experiments. The average of two experiments (each sample run in triplicate) is presented in **Table S5.** (**C**) Scatter plot and regression of multiplex gene expression (GeXP) and follow-up using real-time PCR on a subset of genes **(Fig. 5**). The two methods are highly correlated ($r=0.77$, $p<0.001$).

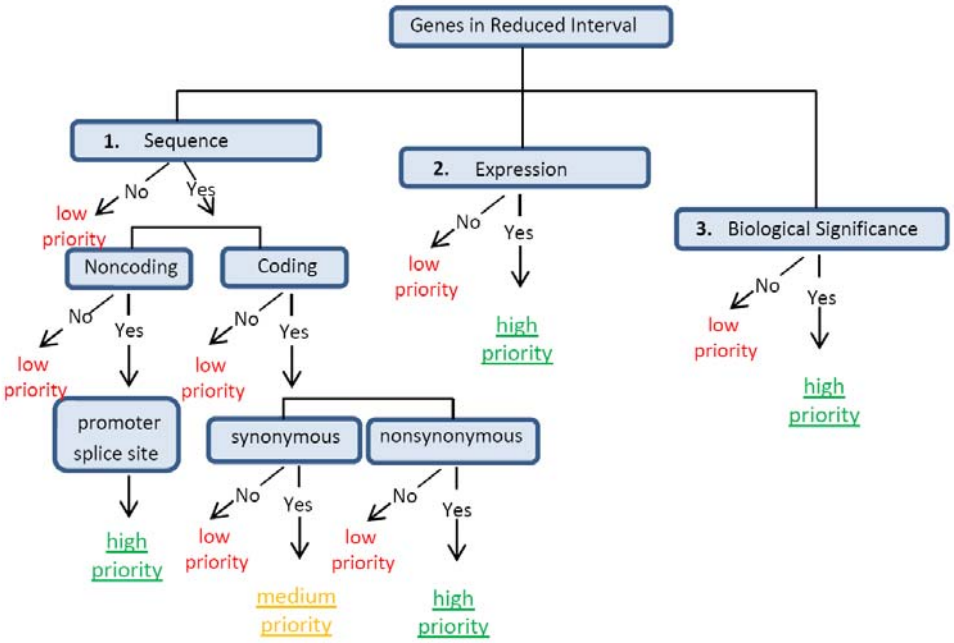


Figure S6: Decision tree used to establish top candidate genes based on three classifications: (1) nature and type of sequence variants; (2) differential expression; and (3) potential biological significance. Depending on the path (no or yes), genes were classified as low priority (shown in red), medium priority (shown in yellow), or high priority (shown in green). A gene that exhibited "high priority" designation in each classification is considered an important gene for additional study. Thus, a gene that is denoted as high priority for every classification (1. sequence variants, 2. expression, and 3. biological significance) is assumed to have highest probability to play a functional role, more so than other genes in the genomic interval designated as low or medium priority.

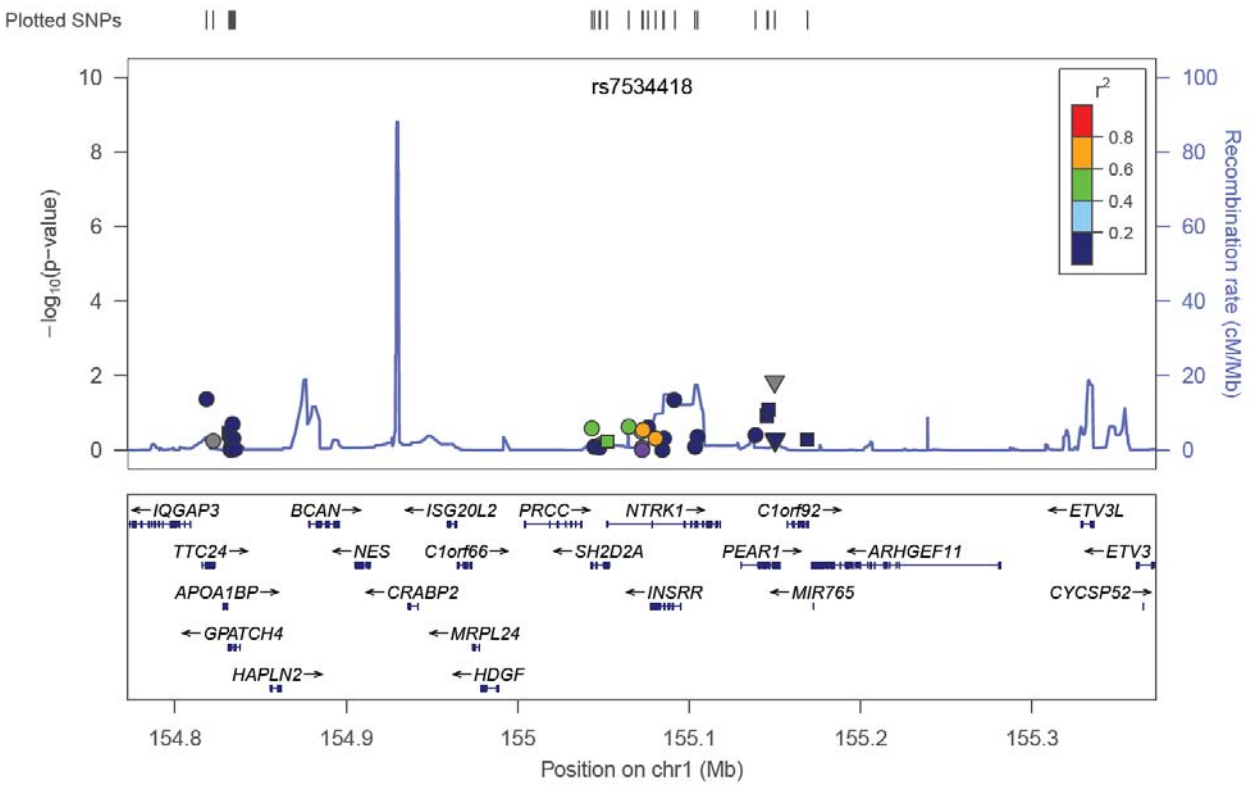


Figure S7: Regional plot for the candidate region associated with UACR in individuals. X-axis shows the genomic region and genes and the y-axis shows the -log10 p-values for associations with UACR. The panel also shows the recombination rate in the region estimated from HapMap CEU data and pairwise linkage disequilibrium (r^2) between SNPs in the region and rs7534418 (labeled in purple) estimated from the meta-analyses. The r^2 values are color coded according to the scale on each panel.