Abstract—The inherited nephron deficit and progressive albuminuria development observed in hypertensive Munich Wistar Frömter (MWF) rats are influenced by quantitative trait loci on rat chromosome (RNO) 6 and RNO8. Previous studies in young MWF rats suggested that the nephron deficit represents a cause for glomerular hypertrophy preceding onset of albuminuria at 8 weeks and demonstrated a simultaneous induction of the podocyte stress marker desmin and podoplanin loss in podocytes. Here we investigated the separate genetic influence of RNO6 and RNO8 on early glomerular changes and subsequent albuminuria in single-consomic MWF rats in which RNO6 (MWF-6SHR) and RNO8 (MWF-8SHR) were replaced by the respective spontaneously hypertensive rat (SHR) chromosome. Furthermore, we tested the role of synergistic effects between both chromosomes in a double-consomic MWF-6SHR8SHR strain. Increased glomerular, extramasangial desmin expressions at 6 and albuminuria at 8 weeks were significantly reduced in single- and double-consomics (P<0.05 versus MWF, respectively). MWF-6SHR8SHR rats demonstrated the lowest desmin expression and glomerular volume (P<0.05 versus MWF, MWF-6SHR, and MWF-8SHR, respectively), indicating synergistic effects between RNO6 and RNO8. A significant and similar loss of podoplanin was only seen in MWF and MWF-6SHR rats but not in MWF-8SHR and MWF-6SHR8SHR rats (P<0.02, respectively); this refutes a mandatory coupling of desmin induction and podoplanin loss in podocytes preceding albuminuria and reveals a genetic link between RNO8 and loss of podoplanin protein. Long-term follow up in MWF-6SHR8SHR rats demonstrates the relevance of the absence of glomerular changes in young animals, because double-consomics demonstrate a complete suppression of progressive albuminuria and kidney damage compared with MWF rats despite similar blood pressures. (Hypertension. 2011;58:219-224.) ● Online Data Supplement

Key Words: genetics ● albuminuria ● glomerular damage ● podocyte ● consomic rat

Albuminuria is an important independent predictor for progression of both renal and cardiovascular disease and also of mortality risk in patients with hypertensive diabetes mellitus and even in the general population.1,2 Previous reports have shown that the genetic predisposition for development of albuminuria is complex and multifactorial.3-5 The Munich Wistar Frömter (MWF) rat is a suitable animal model to investigate the genetic and molecular mechanisms related to early development of albuminuria. MWF rats demonstrate an inherited nephron deficit and mild hypertension and develop at young age spontaneous albuminuria followed by progressive proteinuria in aging animals.6,9 In previous studies we have shown that increased urinary albumin excretion (UAE) in MWF rats is largely determined by quantitative trait loci on rat chromosome (RNO) 6 and RNO8, respectively.10,11 In these studies we used the spontaneously hypertensive rat (SHR) as a contrasting reference strain with low-grade UAE12 and transferred in separate experiments either the entire chromosome 6 or 8 from SHRs into the MWF genetic background. The resulting consomic rat strains, that is, MWF-6SHR and MWF-8SHR, demonstrated both a marked suppression of early albuminuria development compared with the MWF strain, thus providing evidence for the functional relevance of genes on RNO6 and RNO8 for albuminuria, respectively.10,11

Ijpeelaar et al13 showed previously that onset of albuminuria in young MWF animals is already preceded by glomerular hypertrophy and coincided with focal and segmental loss of podoplanin and de novo expression of desmin in affected podocytes when compared with SHRs.13 The genes coding for desmin and podoplanin map to RNO9 and RNO5 and can, therefore, be ruled out as positional candidate genes for the 2 important albuminuria quantitative trait loci on RNO6 and
RN08. Notwithstanding, the changes of these proteins may be crucially involved in the mechanisms leading to albuminuria in MWF rats, which are influenced by genes on RN06 and RN08, respectively. The aim of the current study was, therefore, to test whether the expression of podoplanin and desmin is affected by exchange of RN06 or RN08, respectively.

Thus, we tested whether differences exist between consomic MWF-6SHR and MWF-8SHR strains in the glomerular expression changes of these proteins and/or early hypertrophy development in comparison with MWF rats.

Moreover, we generated a double-consomic MWF-6SHR8SHR strain in which both chromosomes had been replaced in the MWF background to analyze the potential of synergistic effects between genes on RN06 and RN08 in relation to UAE development and the early changes of glomeruli preceding onset of albuminuria.

Methods

Animals and Experimental Design

Parental male MWF/Rkb, SHR/Rkb (laboratory code Rkb, http://dels.nas.edu/iar/) and the single-consomic strains MWF-6SHR and MWF-8SHR were obtained from our colonies at the Charité–Universitätsmedizin Berlin. The double-consomic strain MWF-6SHR8SHR was generated from our single-consomic MWF-6SHR10 and MWF-8SHR11 strains by sequential marker–assisted backcrossing (supplementary Methods, available in the online Data Supplement at http://hyper.ahajournals.org). The whole RN06 and RN08 were introgressed from SHRs into the isogenic MWF background as described previously.10 In a first step, an F1 population was generated between one male MWF-6SHR rat and female MWF-8SHR rats. To fix RN06 and RN08 from SHRs into the MWF background, 24 intercrosses were performed between male and female heterozygous offspring, with the highest number of homozygous SHR markers of RN06 and RN08. In the established double-consomic MWF-6SHR8SHR strain, the purity was confirmed by total genome screen analysis with 240 microsatellite markers.

Rats were grouped under conditions of regular 12-hour diurnal cycles using an automated light switching device and climate-controlled conditions at a room temperature of 22°C. The rats were fed a normal diet containing 0.2% NaCl and had free access to food and water. All of the animal experiments were approved by a government committee in accordance with national animal protection guidelines (Landesamt für Gesundheit und Soziales, Berlin, Germany).

Study 1

The aim in study 1 was to characterize the early glomerular changes in young consomic rats in relation to the original MWF strain. We demonstrated previously that the increase in UAE in MWF rats occurs in young animals at 8 weeks of age, whereas this finding is preceded by earlier glomerular changes in MWF animals between 4 and 6 weeks of age.1,10 We selected young MWF, consomic MWF-6SHR, MWF-8SHR, and MWF-6SHR8SHR rats as well as SHRs for determination of early glomerular changes at 6 weeks (n=5 to 10, each), that is, before the known onset of albuminuria in MWF rats.1,13 UAE was determined subsequently at 8 weeks in these strains (n=10 to 22, each), respectively. In addition, total nephron number and arterial blood pressure in young animals were evaluated.

Study 2

Time-course analysis of UAE and renal damage for the single-consomic strains in relation to parental MWF have been reported previously.11,14 The goal in study 2 was, therefore, to analyze the synergistic effects of replacement of both chromosomes on progressive albuminuria development and renal damage in old double-consomic MWF-6SHR8SHR rats. To this end we analyzed UAE and glomerular structural damage by histology analysis in 24-week–old MWF-6SHR8SHR rats in comparison with MWF and SHR strains, respectively. In addition, to evaluate tubular kidney damage, we measured gene expression of kidney injury molecule 1 (Kim-1, GenBank accession No. ENSRNOG00000007243) as a well-established molecular marker reflecting tubular injury.15

Laboratory Methods

Laboratory methods are presented in the online Data Supplement.

Statistical Analyses

Results are expressed as mean±SEM. Comparison between the different strains was performed by 1-way ANOVA followed by Bonferroni adjustment and unpaired Student t test.

Results

Study 1: Analysis of Glomerular Phenotypes in Young Consomic Animals

In all of the consomic strains, the increased UAE observed in young MWF animals at 8 weeks of age was clearly suppressed (P<0.0001, respectively; Figure 1). However, UAE levels were still somewhat higher in consomic animals compared with SHRs (P<0.04, respectively), which demonstrated a mean UAE rate of 0.3±0.1 mg/24 hours (Figure 1). Double-consomic MWF-6SHR8SHR and single-consomic MWF-6SHR rats exhibited similarly UAE rates (0.5±0.02 and 0.7±0.1 mg/24 hours) and significantly lower levels compared with MWF-8SHR animals (2.6±0.6 mg/24 hours, P<0.05, respectively; Figure 1).

At 6 weeks of age, desmin protein was seen in glomeruli of MWF rats, where it was found to be focally and segmentally expressed in podocytes (Figure 2A). In MWF-6SHR and MWF-8SHR rats, a small amount of protein was found in mesangial cells and podocytes. In contrast, almost no desmin expression was detectable in podocytes of double-consomic MWF-6SHR8SHR animals (Figure 2A).

The quantitative analysis of the percentage of glomeruli with increased desmin expression is depicted in Figure 2B. This analysis revealed that desmin expression was significantly lower in both MWF-6SHR and MWF-8SHR rats compared with MWF rats (P<0.01). However, both single-consomic animals demonstrated desmin expression levels that were still significantly higher compared with MWF-
6SHR8SHR rats, in which overall expression of desmin was hardly detectable and, thus, similar to SHRs in which extramesangial desmin expression was absent (Figure S1, available in the online Data Supplement).

Both MWF-8SHR and double-consomic MWF-6SHR8SHR rats demonstrated a normal podoplanin expression pattern in young animals at 6 weeks of age (Figure 3A), similar to SHRs (Figure S1). In contrast, MWF and MWF-6SHR rats exhibited a marked reduction in podoplanin expression with a focal and segmental loss of expression in glomeruli (Figure 3A). In the affected segments of the glomerulus, podoplanin expression was absent in podocytes (Figure 3A). The corresponding analysis of the percentage of glomeruli demonstrating podoplanin expression loss in podocytes revealed indeed that the loss of podoplanin expression was significantly lower in MWF-8SHR and MWF-6SHR8SHR strains ($P<0.01$ vs other strains; $P<0.05$ vs MWF-6SHR and MWF-8SHR, respectively).

MWF and MWF-8SHR demonstrate a similar reduced nephron number compared with SHRs ($P<0.001$, respectively; Table), whereas this deficit was abolished in single-consomic MWF-6SHR rats, as reported.14 In the double-consomic MWF-6SHR8SHR strain, mean nephron number was still somewhat lower and not statistically different from SHRs but significantly higher compared with MWF and MWF-8SHR rats ($P<0.05$). The analysis of glomerular surface area revealed the largest glomeruli in young MWF and MWF-6SHR rats without significant differences between the 2 strains (Table). Glomeruli of MWF-8SHR, MWF-6SHR8SHR, and SHRs tended to be smaller compared with MWF animals. However, only double-consomic MWF-6SHR8SHR animals demonstrated a significant reduction in glomerular surface area versus MWF rats and also in comparison with the other single-consomic strains ($P<0.05$, respectively; Table).

Direct blood pressure measurements in the group of young adult MWF animals demonstrated a mean arterial pressure of

![Figure 2. Desmin expression in Munich Wistar Frömter rats (MWF), spontaneously hypertensive rats (SHR), single-consomic MWF-6SHR, MWF-8SHR, and double-consomic MWF-6SHR8SHR rats at 6 weeks of age. A, Representative photographs of desmin protein staining in glomeruli. MWF rats demonstrated focal and segmental expression of desmin protein. In contrast, MWF-6SHR and MWF-8SHR rats exhibited a low expression level of desmin protein, whereas in MWF-6SHR8SHR rats almost no desmin expression was observed. B, Percentage of glomeruli exhibiting desmin expression. *$P<0.01$ vs other strains; #$P<0.05$ vs MWF-6SHR and MWF-8SHR, respectively.](image)

![Figure 3. Podoplanin expression in Munich Wistar Frömter rats (MWF), spontaneously hypertensive rats (SHR), single-consomic MWF-6SHR, MWF-8SHR, and double-consomic MWF-6SHR8SHR rats at 6 weeks of age. A, Representative photographs of podoplanin protein staining in glomeruli. Both MWF-8SHR and MWF-6SHR8SHR rats exhibited a diffuse podoplanin expression pattern in their glomeruli, whereas MWF and MWF-6SHR rats showed a marked focal and segmental loss of podoplanin expression. B, Percentage of glomeruli exhibiting segmental or total loss of podoplanin protein expression by podocytes. *$P<0.05$ vs MWF-8SHR and MWF-6SHR8SHR rats.](image)
125.7±5.1 mm Hg (n=6) and no significant difference in comparison with consomic MWF-6SHR (131.4±3.1 mm Hg; n=9), MWF-8SHR (120.2±3.8 mm Hg; n=10), or MWF-6SHR8SHR (125.6±2.0 mm Hg; n=11) strains, respectively.

Study 2: Phenotypes in Old Double-Consomic MWF-6SHR8SHR Animals

UAE analysis in older animals showed that the progressive increase in UAE observed in MWF rats between 8 and 24 weeks of age was completely abolished in double-consomic MWF-6SHR8SHR rats (P<0.0001; Figure 4A). Interestingly, double-consomic animals showed UAE levels that were even lower than those observed in albuminuria-resistant SHRs (0.8±0.1 versus 2.3±0.3 mg/24 hours; P<0.0001; Figure 4A). Moreover, old double-consomic MWF-6SHR8SHR animals exhibited only mild glomerular structural changes (Figure 4B). Thus, the glomerulosclerosis damage index (scored from 0 to 4) was significantly lower in MWF-6SHR8SHR than in MWF rats and also numerically, although not significantly, lower than in SHRs (Figure 4B). In addition, Kim-1 mRNA expression was clearly downregulated in double-consomic MWF-6SHR8SHR and SHR rats compared with MWF rats (P<0.0001, respectively; Figure 4C). Interestingly, old MWF-6SHR8SHR and MWF rats demonstrated similar mean arterial blood pressures (139.7±4.0 and 136.4±2.2 mm Hg) that were significantly lower compared with SHRs (172.1±2.6 mm Hg; P<0.0001).

Discussion

The exact time window of albuminuria onset in individual patients with diabetes mellitus or hypertension is difficult to identify, and to obtain renal tissues from patients with repetitive biopsies would be, for ethical reasons, not feasible. Thus, to identify the early molecular glomerular changes preceding the onset of albuminuria at the tissue level appears impossible in humans. In contrast, a recent study in the MWF rat model demonstrated the importance and feasibility of the experimental approach to obtain new insights into the sequence of events leading to the development of spontaneous albuminuria.13

We demonstrated that reduction of the nephron number in MWF animals and the consecutive increase in single glomerular filtration rate demonstrated previously in this strain16 associates with early adaptation of the glomerulus, which includes the development of glomerular hypertrophy in young MWF animals at 4 weeks of age.13 Moreover, preceding the onset of significant albuminuria at 6 weeks of age, it was shown that glomerular hypertrophy in MWF animals occurred with a simultaneous de novo induction of desmin and a loss of podoplanin in a focal and segmental pattern in affected podocytes.13 Taken together, these data suggested a functional link between desmin and podoplanin as a concerted early response of podocytes to glomerular hyperfiltration preceding the development of albuminuria.17

Our current analysis, however, sheds new light on this issue and refutes a mandatory coupling between desmin induction and podoplanin loss, because we show dissociation between the changes of both proteins in consomic strains. Hence, the 2 MWF-6SHR and MWF-8SHR single-consomic strains demonstrated a similar reduction of desmin expression compared with MWF animals, but only MWF-8SHR animals exhibited the expected reduction in podoplanin loss. In contrast, podoplanin loss was still high and similar to MWF in the MWF-6SHR strain. Thus, despite the fact that desmin expression and subsequently UAE were both significantly decreased in MWF-6SHR, podoplanin loss still occurred in this strain. Our data, therefore, indicate that the loss of podoplanin expression in podocytes of the MWF strain is genetically linked to the presence of RNO8 from MWF rats, because replacement of RNO8 in the corresponding consomic MWF-8SHR strain and in double-consomic MWF-6SHR8SHR rats abolished podoplanin loss, whereas the presence of the MWF chromosome in MWF-6SHR rats was associated with podoplanin loss similar to that in MWF rats. The genes that are responsible for the strong effects on albuminuria on RNO6 and RNO8 in the MWF strain are currently not characterized, and we aim to identify those by ongoing congenic substitution mapping experiments.18 Nevertheless, current genomic analysis reveals that the gene encoding podoplanin, that is, Pdpn, maps to rat chromosome 5, indicating that the effect of RNO8 on podoplanin loss identified in the current report is not directly caused by allelic variation of Pdpn but possibly attributable to another genetic mechanism involving a yet-to-be-identified genetic variant on RNO8. In Dahl/salt-sensitive rats we previously found a similar early, segmental disappearance of podoplanin protein, whereas the mRNA level for this gene was even increased.19 This points to a translational defect of podoplanin by modifying genes encoded by a quantitative trait loci on RNO8 from MWF rats. However, podoplanin loss is not sufficient to account for the albuminuria in MWF rats, because the MWF-6SHR rats showed as much podoplanin loss as the MWF strain but significantly less albuminuria.

The previously observed de novo induction of desmin, a well-established marker of damaged or stressed podocytes,20 in young MWF animals with glomerular hypertrophy was in

<table>
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<th>Table.</th>
<th>Nephron No. and Glomerular Surface in Young MWF, SHR, and MWF-6SHR8SHR Rats</th>
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<tr>
<td>Phenotype</td>
<td>MWF</td>
</tr>
<tr>
<td>Nephron No., n</td>
<td>27027.9±1322.2*</td>
</tr>
<tr>
<td>Glomerular surface, μm²</td>
<td>6145.9±217.0*</td>
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Data have been reported previously for MWF,15 MWF-6SHR,15 MWF-8SHR,11,13 and SHR.10 MWF indicates Munich Wistar Fröntner rats; SHR, spontaneously hypertensive rats.

*P<0.05 vs SHR.
†P<0.05 vs MWF and MWF-6SHR.
‡P<0.05 vs MWF and MWF-8SHR.
§P<0.05 vs MWF, MWF-6SHR, and MWF-8SHR.
only factor influencing extramesangial desmin expression. Moreover, the current findings indicate that the inherited nephron deficit is not necessary for the development of glomerular hypertrophy, because the MWF-6\(^6\)SHR rats exhibited glomerular hypertrophy in the apparent absence of any nephron deficit.\(^{10}\) In addition, our previous studies demonstrated that the nephron deficit is not sufficient to fully account for the progressive development of albuminuria, because the single-consomic MWF-8\(^8\)SHR rat was found to be characterized by markedly suppressed albuminuria despite the presence of a nephron deficit similar to that observed in the MWF strain.\(^{11}\) The current findings demonstrate that glomerular hypertrophy is also not sufficient to account for the albuminuria observed in the MWF strain, because MWF-6\(^6\)SHR animals were characterized by relatively little albuminuria despite clear glomerular hypertrophy.

In study 1 of this report, we performed direct blood pressure measurements in awake rats and detected no significant differences in mean arterial blood pressure between MWF animals and consomic MWF-6\(^6\)SHR, MWF-8\(^8\)SHR, or MWF-6\(^6\)SHR8\(^8\)SHR strains, respectively. Particularly, the MWF and double-consomic strains exhibited identical mean arterial blood pressure values, suggesting that blood pressure differences do not play a major role for the differences in albuminuria and glomerular phenotypes observed between the strains. However, because blood pressure readings were obtained during the day and in young adult animals at the age of 12 weeks, we cannot rule out blood pressure differences between strains that occurred during the night or in younger animals during the onset of albuminuria. Taken together, our findings indicate that the nephron deficit is not necessary for the development of glomerular hypertrophy and that the nephron deficit, glomerular hypertrophy, or podoplanin loss is not sufficient to fully account for the albuminuria observed in the MWF strain.

Thus, although the concept that hyperfiltration and hypertrophy play a pivotal role in the development of podocyte damage and subsequent albuminuria development is well established,\(^{17,22}\) it cannot solely account for the early molecular events preceding albuminuria in the MWF model. These early mechanisms are, however, critically determined by genetic mechanism on RNO6 and RNO8. Although podoplanin loss is determined by a genetic locus only on RNO8, both chromosones exhibit a synergistic effect on desmin induction in podocytes and glomerular hypertrophy. Hence, young double-consomic animals with exchange of both chromosomes showed the smallest glomerular surface area and a nullification of desmin expression in podocytes.

Previous studies in aging animals at 24 weeks of age demonstrated already that progressive albuminuria development in MWF rats with albuminuria levels $>150$ mg/24 hours is clearly suppressed in MWF-6\(^6\)SHR (to $\approx 16$ mg/24 hours)\(^{10}\) and MWF-8\(^8\)SHR (to $\approx 20$ mg/24 hours) rats.\(^{11}\) Thus, both strains showed UAE levels that were still significantly elevated compared with SHR animals that exhibited albuminuria levels in the range of 2 mg/24 hours. In this regard, the current data are striking, because double-consomic animals show UAE rates that are even significantly lower compared with SHRs (Figure 4A). This documents the importance of synergistic effects conferred by the genetic loci on RNO6 and

**Figure 4. Urinary albumin excretion (UAE) in A, glomerulosclerosis index (GSI) in B, and kidney injury molecule 1 (Kim-1) mRNA expression in C of Munich Wistar Frömter rats (MWF), spontaneously hypertensive rats (SHR), and double-consomic MWF-6\(^6\)SHR8\(^8\)SHR rats at 24 weeks of age. *P<0.0001 vs other strains, respectively.**
RN08 for albuminuria development. As reported previously, the structural glomerular damage in old MWF animals at 24 weeks as determined by glomerulosclerosis index was only partially reduced in single-consomic MWF-6\textsuperscript{SHR} animals\textsuperscript{10} and not affected by transfer of chromosome 8 in MWF-8\textsuperscript{SHR} animals.\textsuperscript{11} In contrast, transfer of both chromosomes in double-consomic animals as reported here leads to a complete reduction of glomerular structural damage and tubular damage as reflected by Kim-1 expression.\textsuperscript{12} This occurred on the background of similar blood pressures compared with MWF, indicating an interaction between genes on RN06 and RN08 influencing the development of renal damage as well.

Perspectives
In our study we dissected the early glomerular changes preceding the onset of albuminuria by using the MWF rat model and 3 consomic rat strains derived from MWF rats. Our results demonstrate that the early glomerular alterations involving de novo expression of desmin and loss of podoplanin in podocytes are associated with mechanistic pathways on rat chromosomes 6 and 8 that are involved in albuminuria. The data refute a mandatory coupling between desmin induction and podoplanin loss and unravel a genetic link between genes on rat chromosome 8 and loss of podoplanin protein in podocytes.

We showed that the simultaneous paucity of early glomerular changes including absence of glomerular volume expansion, desmin induction, and loss of podoplanin in podocytes in young double-consomic animals, associates subsequently with a complete elimination of the progressive albuminuria and kidney damage in aging rats as compared with MWF rats. Our findings shed new light on the early glomerular mechanisms governing albuminuria development and subsequent progressive renal damage. This provides the basis for future research aiming at early prevention of chronic kidney disease.

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We acknowledge the contributions of Sabine Wunderlich and Claudia Plum for excellent laboratory assistance, as well as Bettina Bublath and Christiane Priebsch for excellent support in animal breeding.

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Disclosures
None.

References
Elimination of Severe Albuminuria in Aging Hypertensive Rats by Exchange of 2 Chromosomes in Double-Consomic Rats

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ELIMINATION OF SEVERE ALBUMINURIA IN AGING HYPERTENSIVE RATS BY EXCHANGE OF TWO CHROMOSOMES IN DOUBLE-CONSOMIC RATS

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Supplemental Methods

Animals and experimental design
For our consomic studies we first generated the single-consomic strains MWF-6\textsuperscript{SHR} and MWF-8\textsuperscript{SHR} by analysing a panel of 240 microsatellite markers, respectively\textsuperscript{1,2}. For breeding of the double-consomic strain MWF-6\textsuperscript{SHR8SHR} we used the same set of 240 microsatellite markers. The interval between the polymorphic markers was on average about 10 centMorgan. All chromosomes except the Y-chromosome were covered. Genotypes were generated by PCR from 50 ng of genomic DNA in a final reaction volume of 10 µl, containing 100 nM each primer, 200 µM dNTPs, 1.5 mM MgCl\textsubscript{2}, 10x PCR buffer (Rapidozym, Germany), and 0.4 U/µl of Taq DNA polymerase. The forward primer was labelled with \([\gamma^{32P}]ATP\) by T4 polynucleotide kinase. PCR products were processed and subsequently analyzed by autoradiography after polyacrylamide gel electrophoresis.

We tested the purity of our double-consomic strain in 22 animals (8 males and 14 females). All tested animals showed only homozygosity for SHR alleles only on chromosome 6 and 8, respectively, and on the other chromosomes exclusively homozygosity for MWF alleles. We selected 3 males and 5 females to establish a colony of the double-consomic strain at our facility. All investigated animals of the current studies were taken from this colony.

Laboratory methods

Urinary albumin excretion (UAE)
Animals were placed in metabolic cages for 2 days. The first day was used for adaptation and urine was collected for the last 24h for UAE analysis measured with a rat-specific ELISA technique\textsuperscript{3}.

Glomerular histology and immunohistochemical analysis
In kidney of young animals at 6 weeks of age stainings were performed for desmin and podoplanin as previously described\textsuperscript{4}. Briefly, paraffin sections were dewaxed and endogenous peroxidase was blocked. Sections were incubated with primary antibodies, followed by incubation with peroxidase-labeled secondary antibody. Sections were counterstained with hematoxylin. Staining for podoplanin was analyzed by counting the percentage of glomeruli that showed loss of podoplanin in podocytes in one or more segments. For desmin the percentage of glomeruli that showed extra-mesangial expression of desmin were counted. 30 glomeruli per section were scored. Ten randomly chosen regions of the outer glomerular cortex were photographed at 200x magnification with a Zeiss Axioplan microscope equipped with a Sony DXC-950P 3CCD color camera (Sony, Tokyo, Japan). The surface area of all glomeruli in the photographs was measured using Image-J 1.34 software (National Institutes of Health, http://rsb.info.nih.gov/ij). From these measurements, the mean glomerular surface was calculated as described previously\textsuperscript{5}.

Structural glomerular changes in old MWF, SHR and double-conomic animals were analyzed at 24 weeks of age by determination of glomerular sclerosis index (GSI)\textsuperscript{1}.

Realtime PCR analysis for Kim-1
Quantitative gene expression analysis of Kim-1 was performed by PCR analysis using the following primers: Kim-1\textsuperscript{forward} ATTGTGGCCGAGTGAGAT and Kim-1\textsuperscript{reverse} TGTGTTGTTGAGTCTTGAGT. At 24 weeks of age rats (n=7-8, each strain) were killed and the left kidney was excised and snap frozen in liquid nitrogen for subsequent expression analysis. RNA was isolated by the TRIzol\textsuperscript{®} reagent (Invitrogen, Karlsruhe, Germany), according to the manufacturer’s instructions, and was resuspended in DEPC (diethyl pyrocarbonate)-treated water. First-strand cDNA synthesis was carried out on 2 µg of total RNA in a 20 µl reaction using the First Strand cDNA Synthesis Kit (Fermentas Life Sciences,
St. Leon-Rot, Germany), following the manufacturer’s recommendations. To quantify mRNA expression of Kim-1 we employed a real-time quantitative reverse transcriptase (TaqMan) PCR method using the standard curve method. To normalize our expression data, PBGD (porphobilinogen deaminase) was used as a housekeeping gene (GeneBank® accession no. X06827) 6.

**Total nephron number determination**

Total glomerular number per kidney was analyzed in an independent set of young male consomic MWF-6\(^{SHR}\)\(^{8SHR}\) (n=7) at 4 weeks of age by using the stereoscopic physical fractionator method 7. The data were compared to previously reported and in replication experiments confirmed and compared to previously reported nephron numbers of kidneys from SHR, MWF, single-consomic MWF-6\(^{SHR}\) and MWF-8\(^{SHR}\) animals 1, 2.

**Blood pressure measurements**

Direct blood pressure measurements by indwelling arterial catheters were performed in MWF and consomic animals at 12 and 24 weeks of age as reported 8. Catheters were connected to a pressure transducer system (ADInstruments, Spechbach, Germany) and 3 repetitive blood pressure recordings were obtained in awake animals on 2 consecutive days, respectively; data were averaged to obtain individual blood pressure values for each rat.
Supplemental Reference List


**Supplemental Figure**

**Figure S1.** Desmin and podoplanin expression in spontaneously hypertensive rats (SHR) and double-consomic MWF-6$_{SHR}$8$_{SHR}$ rats at 6 weeks of age. Panel A, representative photographs of desmin protein staining in glomeruli. Both SHR and MWF-6$_{SHR}$8$_{SHR}$ exhibited a similar small amount of desmin expression in their glomeruli. Panel B, representative photographs of podoplanin protein staining in glomeruli. Both SHR and MWF-6$_{SHR}$8$_{SHR}$ exhibited a similar amount of podoplanin expressed in a similar pattern.