Induction of albuminuria and kidney damage in SHR by transfer of chromosome 8 from Munich Wistar Frömter rats

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Schulz A, Schütten-Faber S, van Es N, Unland J, Schulte L, Kossmehl P, de Heer E, Kreutz R. Induction of albuminuria and kidney damage in SHR by transfer of chromosome 8 from Munich Wistar Frömter rats. Physiol Genomics 44: 110–116, 2012. First published November 22, 2011; doi:10.1152/physiolgenomics.00123.2011.—Inbred Munich Wistar Frömter [MWF/FubRkb (RGD:724569), MWF] rats develop progressive albuminuria with age that is under polygenic influence. We previously identified a major albuminuria quantitative trait locus (QTL) on rat chromosome (RNO)8 in MWF. To test the independent role of QTL(s) for albuminuria development on RNO8, we generated a consomic SHR-Chr 8MWF/Rkb (SHR-8MWF) strain by transferring RNO8 from MWF into the albuminuria-resistant background of the spontaneously hypertensive rat [SHR/FubRkb (RGD:631696; SHR)]. Young male MWF, SHR, and SHR-8MWF were sham-operated or unilaterally nephrectomized (Nx) at 6 wk and followed up to 24 wk of age, respectively. Systolic blood pressure was significantly lower in SHR-8MWF Sham compared with Sham SHR (~19.4 mmHg, P = 0.03) at 24 wk. In contrast, transfer of MWF-RNO8 into SHR induced a significant elevation of urinary albumin excretion (UAE) between weeks 12 and 24 in SHR-8MWF compared with SHR Sham animals (P < 0.001, respectively). Nx resulted in a significant increase in UAE in both strains during follow-up (P < 0.001, respectively), with significant higher values in SHR-8MWF compared with SHR (P < 0.005, respectively). Renal structural changes as determined by glomerulosclerosis (GSI) and tubulointerstitial damage index (TDI) were significantly higher in consomic animals either at Sham (TDI) or Nx (GSI) conditions (P < 0.05, respectively). These data confirm the independent role of MWF QTL(s) on RNO8 for both albuminuria and structural kidney damage. Moreover, this study shows for the first time the induction of albuminuria by transferring one or more albuminuria QTL into a resistant recipient background in a consomic rat strain.

ALBUMINURIA IS AN IMPORTANT polygenic quantitative trait indicating renal target organ damage and increased cardiovascular risk in humans (2, 3, 8). Inbred Munich Wistar Frömter (MWF/FubRkb, referred to as MWF in this publication) rats develop spontaneous and progressive albuminuria with age that is under polygenic influence (19, 22). We previously demonstrated that although albuminuria in MWF is determined by an interaction between multiple quantitative trait loci (QTL), separate genetic exchange of two major loci on rat chromosome 6 (RNO6) or 8 (RNO8) leads to a marked suppression of early-onset albuminuria in these animals (18, 20, 23). Furthermore, we dissected early glomerular alterations before the onset of albuminuria in MWF rats and documented a genetic link between genes on RNO8 and reduction of podoplanin protein in podocytes preceding albuminuria (9, 25). Recently we showed that the combined effect of replacement of both RNO6 and RNO8 in double-consomic animals causes a complete elimination of the severe albuminuria phenotype observed in 24 wk old MWF animals (25). These findings were accompanied by a normalization of structural glomerular changes in these animals. Taken together, these results verified impressive synergistic effects of RNO6 and RNO8 QTL for albuminuria and kidney damage in MWF rats that were not attributable to blood pressure effects (25). These investigations were done in single- and double-consomic rat strains in which single replacement of either RNO6 [MWF-Chr 6SHR/Rkb (RGD:1641831)] or RNO8 [MWF-Chr 8SHR/Rkb (RGD:2312609), referred to as MWF-8SR in this publication] or double replacement of both chromosomes [MWF-Chr 6SHR Chr 8SHR/Rkb (RGD:1641831)] was achieved by introgressing these chromosomes from albuminuria-resistant spontaneously hypertensive rats (SHR/FubRkb, referred to as SHR in this publication) into the sensitive isogenic background of MWF (18, 20, 23). In contrast to this common protocol in which a QTL is replaced in the disease strain by alleles of the resistant strain (15), here in a reciprocal approach we generated a new consomic SHR-8MWF strain by transferring RNO8 from MWF into the albuminuria-resistant SHR strain (21). Thus, we tested whether QTL on RNO8 are capable of inducing the albuminuria phenotype in SHR rats after isolation from the permissive disease background in MWF.

MATERIALS AND METHODS

Animals. All rats were obtained from our MWF/FubRkb (RGD:724569) and SHR/FubRkb (RGD:631696) colonies (laboratory code Rkb, http://delns.nas.edu/illar/) at the Charité - Universitätsmedizin Berlin, Germany. The consomic strain SHR-8MWF was generated by sequential marker-assisted backcrossing by introgressing the whole RNO8 from MWF into the isolated SHR background as described previously (23). The purity of the consomic SHR-8MWF strain was confirmed by analysis of 240 microsatellite markers (19). Rats were grouped under conditions of regular 12 h 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 animal experiments were approved by a government committee in accordance with national animal protection guidelines [Landesamt für Gesundheit und Soziales (LAGeSo) Berlin, Germany].

Experimental groups. One set of male SHR and consomic SHR-8MWF animals was sham-operated (n = 9–12, respectively) between 6 and 24 wk of age. We expected that the change in albuminuria induced by transfer of RNO8 into the SHR background could be rather mild in consomic rats studied without further experimental
manipulation. Thus, to aggravate systolic blood pressure (SBP) and the susceptibility for renal damage (24) we studied one additional set of animals of each strain (n = 15–18, respectively) after unilateral nephrectomy (Nx). Surgical procedures were performed during anesthesia with ketamine/xylazine (87 and 13 mg/kg body wt, respectively) at 6 wk of age, respectively. A retroperitoneal incision was performed, and for the Nx group the right kidney was rapidly excised.

**Phenotyping.** Time-course analysis for urinary albumin excretion (UAE) at 12, 18, and 24 wk of age was performed in accordance with our previous studies (21, 23). SBP was determined by a tail-cuff method in awake animals at 24 wk of age on a computer-assisted oscillatory detection device (TSE, Bad Homburg, Germany) (12). In brief, training sessions were carried out for 2 days at each occasion and followed by measurements on three consecutive days. Each session included three sets of two measurements, so that a minimum of 12 and a maximum of 18 measurements were used for the determination of the SBP of each rat.

**Laboratory measurements.** For albumin analysis animals were placed in metabolic cages for 2 days. The first day was used for adaptation, and urine was collected for the last 24 h for determination of UAE by a rat-specific ELISA technique (12). Blood, heart, and urine was collected for the last 24 h for determination of the SBP of each rat.

**Glomerular histology and immunohistochemical analysis.** Structural kidney injury was analyzed by glomerular damage index (GSI) and tubulointerstitial damage index (TDI) as previously described (23).

In addition, to evaluate tubular kidney damage we measured gene expression of hepatitis A virus cellular receptor 1 (Havcr1, kidney injury molecule 1), GenBank accession number: NM_173149 as a well-established molecular marker reflecting tubular injury (11). Quantitative gene expression analysis of Havcr1 was performed by PCR analysis using the following primers: Havcr1 forward ATTGTTGCCGAGTGGAGAT and Havcr1 reverse TGTGGTTGTGGGTCTTGTAGT. At 24 wk of age rats (n = 8–12, 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 reagent (Invitrogen, Karlsruhe, Germany), according to the manufacturer’s instructions, and was resuspended in 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 Havcr1 we employed a real-time quantitative reverse transcriptase (TaqMan) PCR method using the standard curve method. To normalize our expression data, Hmbs (hydroxymethylbilane synthase) was used as a housekeeping gene (GenBank accession no. X06827) (20).

To evaluate glomerular damage we performed immunohistochemistry staining for the podocyte protein podoplanin in kidneys of animals at 24 wk of age as previously described (9). In brief, 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 reduction of podoplanin in podocytes in one or more segments. We scored 30 glomeruli per section.

**Statistical analysis.** Data are presented as means ± SE. Differences between experimental groups were analyzed by ANOVA with post hoc Bonferroni adjustments, and the Mann-Whitney-U-test. A probability of P < 0.05 was considered to be statistically significant.

![Fig. 1](http://physiolgenomics.physiology.org/doi/abs/10.1152/physiolgenomics.00123.2011)

**Table 1. Overall characteristics of parental male SHR and SHR-8MWF at 24 wk of age**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Group</th>
<th>SHR</th>
<th>SHR-8MWF</th>
<th>P-ANOVA SHR vs. SHR-8MWF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>Sham</td>
<td>329.2 ± 8.2</td>
<td>345.0 ± 7.8</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>Nx</td>
<td>330.4 ± 6.0</td>
<td>336.5 ± 6.7</td>
<td>0.52</td>
</tr>
<tr>
<td>Right KW, g</td>
<td>Sham</td>
<td>1.07 ± 0.05</td>
<td>1.16 ± 0.04</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Nx</td>
<td>1.63 ± 0.05</td>
<td>1.59 ± 0.04</td>
<td>0.50</td>
</tr>
<tr>
<td>Right KW/BW, mg/g</td>
<td>Sham</td>
<td>3.25 ± 0.09</td>
<td>3.36 ± 0.08</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>Nx</td>
<td>5.02 ± 0.16</td>
<td>4.71 ± 0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Cystatin C, mg/l</td>
<td>Sham</td>
<td>0.05 ± 0.003</td>
<td>0.05 ± 0.002</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>Nx</td>
<td>0.07 ± 0.003</td>
<td>0.07 ± 0.003</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Phenotype values are means ± SE. BW, body weight; KW, kidney weight; SHR, spontaneously hypertensive rat; MWF, Munich Wistar Frömter.
RESULTS

Body weight, kidney weight, and cystatin C in plasma. Body weight showed no significant differences between strains under Sham and Nx conditions and was not affected by Nx in either strain (Table 1). Absolute kidney weights and the kidney weight to body weight ratios were not significantly different between strains, while Nx resulted in a significant increase in both parameters in SHR and consomic animals compared with the corresponding Sham groups (\( P < 0.0001 \), respectively, Table 1). Cystatin C plasma concentrations were higher in Nx animals compared with Sham in both strains (\( P < 0.05 \), respectively; Table 1), while no significant differences between the two strains were detectable both under Sham and Nx conditions (Table 1).

UAE. Transfer of MWF-RNO8 into the SHR background induced a significant increase in UAE in sham-operated SHR-8MWF between 12 and 24 wk of age compared with SHR Sham at all time points investigated (\( P < 0.0001 \), respectively, Fig. 1A). At 12 and 24 wk of age UAE levels were significantly three- to eightfold higher in SHR-8MWF Sham compared with age-matched SHR Sham (1.3 ± 0.2 vs. 0.4 ± 0.1 and 10.8 ± 2.1 vs. 1.3 ± 0.3 mg/24 h; \( P < 0.0001 \), respectively), although the UAE levels remained relatively low in consomic animals with two kidneys (Fig. 1A). Unilateral nephrectomy resulted in a significant increase in UAE in both strains at all time points investigated (\( P < 0.0001 \), respectively, Fig. 1B), while the significant strain differences with higher UAE levels in consomic rats were maintained (\( P < 0.002 \), respectively, Fig. 1B). Thus, a substantial increase in albuminuria to 37.2 ± 4.9 mg/24 h was observed only after Nx in consomic animals at 24 wk of age.

Renal histology analysis. In sham-treated consomic animals obtained at 24 wk of age GSI was slightly, but not significantly, higher with SHR rats (\( P = 0.055 \), Fig. 2A). Nx treatment of SHR rats had no significant influence on GSI compared with the corresponding Sham group. In contrast, SHR-8MWF animals exhibited a significant elevation of GSI compared with the consomic Sham response to Nx (\( P = 0.03 \), Fig. 2A). Under Sham conditions transfer of MWF-RNO8 leads to significantly higher TDI values in consomic SHR-8MWF compared with SHR (\( P = 0.01 \), Fig. 2B). In response to Nx TDI were significantly higher in both strains compared with the corresponding Sham groups (\( P < 0.05 \), respectively, Fig. 2B), while no significant strain difference was observed anymore. The renal tubular injury marker (mRNA) Havcr1 paralleled the findings obtained for TDI with higher (mRNA) Havcr1 expression in consomic Sham animals (\( P = 0.02 \)) and a significant increase of (mRNA) Havcr1 in response to Nx in both strains (\( P < 0.05 \), respectively; Fig. 2C). Examples of the histopathology findings are presented in Fig. 3.

Analysis of podoplanin protein expression in glomeruli of SHR Sham animals demonstrated only a few (4.2%) glomeruli showing a focal and segmental reduction of expression (Fig. 4). In contrast, SHR-8MWF Sham exhibited a significantly 3.6-fold higher number of glomeruli with reduction of podoplanin protein (\( P < 0.004 \), Fig. 4). Reduction of podoplanin expression was greater in response to Nx in both strains, while no significant differences between SHR Nx and SHR-8MWF Nx were observed (Fig. 4).

Blood pressure and left ventricular hypertrophy. Indirect blood pressure measurements at 24 wk of age under Sham conditions revealed significantly lower systolic arterial blood pressure levels in consomic SHR-8MWF compared with SHR rats (\( P = 0.03 \), Fig. 5A). SBP was not affected by Nx in SHR.
animals, while consomic SHR-8\textsuperscript{MWF} demonstrated a significant increase in SBP ($P = 0.04$, Fig. 5A).

Determination of relative left ventricular weights as an index of left ventricular hypertrophy revealed significantly lower values in Sham and Nx-treated SHR-8\textsuperscript{MWF} rats compared with the corresponding SHR groups ($P < 0.001$, respectively, Fig. 5B). Nx had no effect on relative left ventricular weights in either strain (Fig. 5B).

**DISCUSSION**

In previous studies we confirmed that one or more albuminuria QTL on RNO6 and RNO8, respectively, play an important role for progressive albuminuria development in the MWF rat model (18, 22, 23, 25). In these studies RNO6 and/or RNO8 from the reference strain SHR with low-grade albuminuria were transferred into the sensitive isogenic background of MWF, thereby causing a decrease or even normalization of UAE in the resulting consomic animals (18, 20, 23, 25). In the current work we demonstrate for the first time that the reciprocal approach, by transferring the MWF RNO8-QTL into the albuminuria-resistant SHR background, is directly capable of inducing an albuminuria phenotype. This notion is based on the result that under Sham-operated conditions consomic animals developed a significant increase in albuminuria compared with SHR. Thus, this result confirms the important and independent influence of albuminuria QTL on RNO8.

In contrast, the transfer of the other important albuminuria QTL on MWF-RNO6 into the isolated SHR background failed to induce an albuminuria phenotype either under normal Sham conditions or in response to a 50% nephron reduction by Nx in the SHR-6\textsuperscript{MWF} rat strain (21). This observed lack of albuminuria development pointed to critical synergistic effects between MWF-RNO6 and further MWF-QTL or protective effects of SHR alleles due to a complete neutralization of albuminuria-inducing loci of the RNO6-QTL. Thus, RNO8 QTL but not albuminuria QTL on RNO6 are capable of inducing albuminuria independently from MWF alleles on other chromosomes.

On the other hand we cannot exclude the possibility that the resistance of the SHR strain to developing significant albuminuria despite the hypertension might be conferred at least in part by protective genetic factors inherited in this strain, which could be located on RNO8. This rationale would be in agreement with the albuminuria findings observed in both the previously characterized MWF-8\textsuperscript{SHR} (18) and the currently analyzed SHR-8\textsuperscript{MWF} animals. Thus, transfer of a protective SHR locus from RNO8 into the MWF background could explain the demonstrated reduction of albuminuria in consomic animals (18), while replacement of the protective locus in the SHR strain by transfer of the MWF chromosome could explain the induction of albuminuria in consomic SHR-8\textsuperscript{MWF} reported

![Fig. 3. Histopathology of the kidney in Sham-operated animals (SHR in A and SHR-8MWF in C) and Nx animals (SHR in B and SHR-8MWF in D) at 24 wk of age. Paraffin sections were stained with periodic acid-Schiff reagent. Only mild glomerular and tubular damage was observed in Sham-operated SHR animals (A). More pronounced glomerulosclerosis and tubular changes were observed in consomic animals particularly after Nx (D), including tubular dilatation and cast formation (arrow).](image)

![Fig. 4. Percentage of glomeruli exhibiting reduction of podoplanin protein expression by podocytes of Sham-treated (white bars) and Nx (black bars) SHR and consomic SHR-8\textsuperscript{MWF} rats at 24 wk of age. *$P = 0.003$ compared with SHR of the same treatment. #$P = 0.003$ compared with Sham group of the same strain.](image)
emphasize the important and independent influence of the congeneric strains in the development of renal damage and locus in MWF. Taken together, these findings clearly highlight pressure-increasing locus in SHR or blood pressure-decreasing support the conclusion that RNO8 harbors at least one blood pressure-lowering effect. Consequently, these findings strongly of MWF-RNO8 into the SHR background, a significant blood local approach of the current study we found, by introgression indicating that RNO8 carries a blood pressure locus. In the reciprocation that RNO8 of SHR carries an here. Determining whether or not RNO8 of SHR carries an albuminuria-resistance locus or RNO8 from MWF carries an albuminuria-susceptibility locus can be made only when the underlying molecular mechanisms for the albuminuria phenotypes have been identified. Nevertheless, the findings obtained in SHR-6MWF do not support an albuminuria-resistance locus on RNO6 of SHR, since replacement of this chromosome by the MWF chromosome had no effect on albuminuria (21).

Importantly, the induction of increased albuminuria in SHR-8MWF is not attributable to worsening of hypertension, since these animals showed even lower blood pressures compared with SHR. In a previous study (18) transfer of RNO8 from SHR into the MWF background resulted in higher blood pressures in MWF-8SHR compared with MWF animals, indicating that RNO8 carries a blood pressure locus. In the reciprocal approach of the current study we found, by introgression of MWF-RNO8 into the SHR background, a significant blood pressure-lowering effect. Consequently, these findings strongly support the conclusion that RNO8 harbors at least one blood pressure-increasing locus in SHR or blood pressure-decreasing locus in MWF. Taken together, these findings clearly highlight the importance of the genetic background in conomic and congeneric strains in the development of renal damage and emphasize the important and independent influence of the RNO8-QTL in the development of progressive albuminuria in MWF rats.

In the current study, transfer of MWF-RNO8 into SHR induced not only albuminuria, but also structural kidney damage. Thus, in consomic SHR-8MWF glomerular and tubulointerstitial damage was significantly enhanced. However, glomerular damage tended to be higher in consomic animals under Sham conditions but was significantly increased only in response to Nx in these animals compared with SHR. Interestingly, tubulointerstitial damage and (mRNA) Haverl expression were significantly enhanced in SHR-8MWF Sham compared with SHR Sham. This points to the involvement of tubular changes in albuminuria development in MWF, either as a consequence of albuminuria or potentially as a causative albuminuria-promoting factor (1).

We have previously shown that onset of albuminuria in young MWF animals at 6 wk of age coincided with focal and segmental reduction of podoplanin protein in podocytes. We hypothesized that segmental reduction of this 43-kDa glycoprotein leads to structural changes in podocytes and to a dysfunctional glomerular filtration barrier within the affected segments (9). More recently, we demonstrated a genetic link between RNO8 and reduction of podoplanin protein in MWF-8SHR consomic animals, because the podoplanin reduction observed in MWF was fully abolished (25). The current finding of a significant increased glomerular podoplanin reduction in consomic animals under Sham conditions is therefore of interest, because it indicates the presence of podocyte damage in these animals even without nephron number reduction. Moreover, it confirms an independent genetic link between QTL on RNO8 and podoplanin expression in podocytes that is mediated by yet unknown trans mechanisms, because the gene encoding podoplanin maps on rat chromosome 5. In response to unilateral Nx podoplanin reduction was enhanced in both strains, although with statistical significance only in the SHR strain compared with Sham. Moreover, no significant strain difference was detected after Nx, suggesting that compensatory mechanisms due to the 50% nephron reduction, e.g., hyperfiltration, override the genetically induced podoplanin reduction observed in consomic animals under Sham conditions.

The power of genome-wide association studies to identify genetic loci for complex multifactorial diseases such as chronic kidney disease (1, 4–7, 10, 13, 14) has been recently demonstrated. So far none of the implicated genomic regions in humans (8, 13, 16, 17) overlap with albuminuria QTL on RNO8 (22).

Nevertheless, the dissection of the molecular genetic basis of albuminuria development in animal models such as MWF is important in generating new insights into disease mechanism and pathways that are important for a better understanding of complex diseases. Moreover, as recently reinforced, the bioinformatic integration of genetic data obtained from animal models and from humans may be helpful by developing a systematic and global perspective on the genetics of common human disease (26).

Taken together our results demonstrate the independent role of MWF QTL on RNO8 for both albuminuria and structural kidney damage. Moreover, this study shows for the first time the induction of albuminuria by transferring one or more
albuminuric QTL into a resistant recipient background in a consomic rat strain.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


