Development of Overt Proteinuria in the Munich Wistar Frömter Rat Is Suppressed by Replacement of Chromosome 6 in a Consomic Rat Strain

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In a cross between the Munich Wistar Frömter (MWF) rat and spontaneously hypertensive rats (SHR), a major quantitative trait locus (QTL) was identified on rat chromosome 6 (RNO6) that demonstrated the strongest linkage to albuminuria among several QTL identified. The QTL represented the only locus that is linked to both early-onset albuminuria and increased renal interstitial fibrosis in adult animals. A consomic MWF-6SHR strain in which chromosome 6 from SHR was introgressed into the MWF background therefore was generated to test the relevance of this QTL. Phenotype analysis at 8 wk of age revealed that early onset of albuminuria in MWF with a 55-fold elevation of urinary albumin excretion compared with SHR ($P < 0.0001$) was completely abolished in MWF-6SHR. Time-course analysis until week 24 demonstrated only a moderate increase of urinary albumin excretion in MWF-6SHR, whereas MWF reached levels in the nephrotic range ($16.6 \pm 3.5$ versus $162.6 \pm 16.0$ mg/24 h; $P < 0.0001$). At this age, analysis of glomerulosclerosis, tubulointerstitial damage, renal interstitial fibrosis, and renal collagen III mRNA expression revealed a significant improvement of all parameters in MWF-6SHR compared with MWF ($P < 0.05$). At 32 wk, MWF but not MWF-6SHR demonstrated overt proteinuria ($354.6 \pm 37.6$ versus $48.8 \pm 13.2$; $P < 0.0001$), whereas serum urea, cholesterol, and triglyceride concentrations were lower and creatinine clearance was higher in MWF-6SHR compared with MWF ($P < 0.05$). Therefore, although albuminuria in MWF is determined by a complex interplay of several QTL, our data demonstrate that genetic exchange of one locus on RNO6 leads to marked suppression of early-onset albuminuria and renal damage in MWF.

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A n elevated urinary albumin excretion (UAE) rate is a predictor for the development of chronic nephropathy, and a moderate increase of UAE in the range of microalbuminuria represents an independent risk factor for cardiovascular events in the general population, in arterial hypertension, and particularly in patients with diabetes or documented cardiovascular disease (1–4). Albuminuria and proteinuria are complex phenotypes that are influenced by both environmental and genetic factors (5–7). Elevated UAE rates represent a hallmark of diabetic nephropathy (7), and previous genetic segregation analysis of UAE in families with type 2 diabetes demonstrated that levels of UAE are determined by mixture of probably recessive genes with large and small effect in individuals both with and without diabetes (8,9). More recently, genetic analyses in extended families confirmed that similar genes contribute to the heritability of UAE in family members with and without diabetes (7). Moreover, several quantitative trait loci (QTL) have been identified by genomewide linkage analyses for UAE in diabetes, hypertension, and the general population (6,7,10).

Previous studies in hypertensive genetic rat models have shown that elevated UAE levels are also influenced by several QTL in the Fawn-hooded hypertensive (FHH) rat (11,12), Dahl salt-sensitive rat (SS) (13,14), Munich Wistar Frömter rat (MWF) (5,15), and Sabra rat (16). Overall, it turned out that increased UAE levels that are observed in these models are influenced by multiple UAE QTL, whereas single-locus effects on UAE were modest and required the homozygous state of susceptibility alleles, as a result of a recessive mode of inheritance (15). Therefore, in experimental crosses with these strains, it could be shown that a considerable increase in UAE levels required the synergistic interaction among several UAE QTL (5,12–15). In our cross-breeding and linkage studies involving the MWF rat, we identified multiple potential QTL with suggestive or significant linkage to UAE (5,15) and proposed that a QTL that was identified on rat chromosome (RNO) 6 could play a major role. This QTL was identified in two independent crosses in-
volving either Lewis rats (5) or spontaneously hypertensive rats (SHR) (15) as reference strains with low UAE. Moreover, in the mapping study using SHR as a contrasting model, the homozygous MWF genotype at this QTL on RNO6 demonstrated the strongest linkage to UAE and was the only QTL linked to both the onset of albuminuria in young animals and morphologic changes in the kidney (e.g., increased renal interstitial fibrosis [RIF]), in adult animals (15). We therefore set out to test the potential functional relevance of this QTL for early manifestation of elevated UAE and development of overt proteinuria in MWF by generating a consomic MWF-6^SHR strain in which RNO6 from SHR was introgressed into the isogenic background of MWF.

Materials and Methods

Animals
Male MWF/Rkb and SHR/Rkb rats were obtained from our colonies (laboratory code Rkb) at the Charité, Campus Benjamin Franklin, Germany. 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 that contained 0.2% NaCl and had free access to food and water.

Experimental Groups
To test the influence of chromosome 6 transfer from SHR on early onset of UAE and the subsequent development of proteinuria in MWF, we first performed in accordance with our previous linkage studies (5,15) time-course analysis for UAE at 8, 14, and 24 wk of age, comparing MWF (n = 18) with MWF-6^SHR (n = 19) during time-course analysis. In addition, because the absolute amount of UAE (in the range of 1 mg/24 h) and variation over time are low in the parental SHR donor strain, a somewhat smaller group of SHR (n = 10) also were included for comparison. After completion of UAE analysis, systemic BP (SBP) was measured at 24 wk of age and all rats subsequently were killed at the end of wk 24 for further analysis.

In addition, to test whether the transfer of RNO6 from SHR into MWF protects against the development of overt proteinuria and has an impact on secondary changes related to nephritic-range proteinuria, we compared UAE and renal function parameters between proteinuric MWF and consomic MWF-6^SHR at 32 wk of age (n = 9 to 10). Finally, because previous studies in the MWF strain demonstrated a lower number of nephrons compared with Wistar rats (17,18), the total number of glomeruli was compared in all three strains. However, in this analysis, to exclude an influence of secondary changes as a result of a loss of damaged glomeruli during the determination of glomeruli number in adult rats, we used an additional set of young rats at 4 wk of age (n = 7, respectively).

Development of the Consomic Strain
The consomic strain was derived from our MWF/Rkb and SHR/Rkb colonies. To develop the consomic strain, we crossed the MWF strain with the SHR strain in accordance with our linkage results (15) and introgressed the whole RNO6 from SHR into the MWF background (19). In a first step, an F1 population was generated between one male MWF rat and female SHR. Male heterozygous offsprings were backcrossed with female MWF to conserve the Y chromosome from MWF. In each of seven backcross generations in total, the male breeders with the highest number of homozygous markers of the MWF background and concomitant heterozygous for RNO6 were crossed with four MWF female rats, respectively, by sequential marker-assisted backcrossing (19). To fix RNO6 from SHR into the MWF background, we then performed several intercrosses between backcross male and female rats that were homozygous for all MWF chromosomes except RNO6 and heterozygous for RNO6. Finally, the purity of the consomic MWF-6^SHR strain was confirmed by total genome screen analysis with 240 microsatellite markers. In the MWF-6^SHR colony, no genetic contaminations of SHR in the background genome could be detected. Genotyping was performed by standard methods as previously reported (5).

Phenotyping
For urine analysis, rats were placed in metabolic cages for 2 d. The first day was used for adaptation, and urine was collected for the last 24 h for biochemistry analysis. UAE was measured with a rat-specific ELISA technique as described previously (20). In addition to UAE, the urinary excretion of low molecular weight (LMW) proteins was analyzed as previously reported (21). In brief, 4 μg of total urinary protein together with 3 μl of Precision Plus Protein Standard (Bio-Rad, Hercules, CA) and 2 μg of rat serum albumin (Sigma, St. Louis, MO) as a control were resolved on a 10% SDS-PAGE. The gels then were stained with Coomassie blue and photographed using different exposure times to ensure unsaturated band intensities. The negatives with the optimal exposure subsequently were scanned under standard conditions. The digital analysis of the resulting images was done with ImageJ (http://rsb.info.nih.gov/ij/) using the gel analysis features.

SBP was measured at 24 wk of age by a noninvasive tail-cuff method in awake rats using a computer-assisted oscillatory detection device (TSE, Bad Homburg, Germany) as described previously (20). These measurements involved two training sessions on 2 d followed by up to 18 (minimum 12) recordings in awake rats on 3 consecutive days performed as previously reported (20). Subsequently, rats were killed at 24 wk, and blood was drawn from the aorta for the determination of serum creatinine, urea, cholesterol, and triglycerides with standard methods. Both kidneys and the heart were excised. The body, total kidney, and heart weights were determined. For light microscopy evaluation, a midcoronal section of the left kidney was fixed and embedded in paraffin for histology studies (5,22). The 3-μm sections of the kidneys were stained with the periodic acid-Schiff technique for the determination of glomerulosclerosis and tubulointerstitial damage indices by semiquantitative scoring methods as reported (5,22). RIF was determined after staining of sections with Sirius red following previous recommendations (23). In addition, we determined the numbers of both surface glomeruli with direct contact to the surface of the kidney and the total number of all glomeruli present in the renal cortex corticis zone but without direct surface contact (i.e., superficial glomeruli) in three midcortical sections for each rat as reported (5).

Determination of Glomerular Number
After perfusion fixation with 4% phosphate-buffered formaldehyde, one kidney was used for estimating glomerular number in 4-wk-old rats. The kidney was dehydrated in graded ethanol and embedded in glycolmethacrylate (Technovit 7100; Heraeus Kulzer, Wehrheim, Germany). Using a Microm HM 355 microtome, each kidney was cut exhaustively in 20-μm-thick sections. Every 30th section and its adjacent section (nine to 11 section pairs) were selected using systematic, uniformly random sampling (24). The sampled section pairs were mounted on one slide and stained with periodic acid-Schiff and Mayer’s hematoxylin. Counting was performed using an Olympus BX-50 microscope at a magnification of ×113 with an automated Mahrzäuber Multi Control 2000 specimen stage (Märzhäuser, Wetzlar-Steindorf, Germany) and a fast digital camera (Pixelink PL-A686C) connected to a...
computer (Dell Optiplex GX110) with newCAST software (Visiopharm, Hørsholm, Denmark) to superimpose the counting frame. Glomeruli were counted in six consecutive section pairs starting with the third because of the problem of artificial edges in the first two sections and the last sections. Therefore, a sampling fraction \( Ps/Pf \) was introduced: \( Ps \) is the number of points that hit all kidney tissue, and \( Pf \) is the number of points that hit only kidney tissue that was used for glomerular counting.

The number of glomeruli was estimated by the physical fractionator (25). The glomeruli were counted if they were present inside the two-dimensional unbiased counting frame in one section (the sampling frame) but not in the adjacent section plane (the look-up section) and vice versa. On average, 187 glomeruli \( \left( 2Q \right) \) were counted per kidney. The total number of glomeruli per kidney \( N_{\text{glom}} \) was calculated using the following formula:

\[
N_{\text{glom}} = \frac{L}{2SF} \cdot \frac{1}{\text{ASF}} \cdot \frac{P_s}{P_f} \cdot \sum \frac{Q}{2^n}
\]

The factor \( \frac{1}{2} \) was introduced because glomeruli were counted both ways in the disector.

Area sampling fraction \( \text{ASF} \) was calculated as the counting frame area \( [A(\text{frame})] \) divided by the step lengths in the x and y direction \( (dx \cdot dy) \) of the counting frame. The error variance of this technique that is used for counting glomeruli was estimated to be \( 8\% \) (24).

RNA Extraction and cDNA Synthesis

RNA was isolated from kidneys by the TRIzol reagent (Invitrogen, Karlsruhe, Germany) according to the manufacturer’s instructions and was resuspended in DEPC-treated \( \text{H}_2\text{O} \). First-strand cDNA synthesis was carried out on 2 \( \mu \text{g} \) of total RNA in a 20-\( \mu \text{l} \) reaction using the First Strand cDNA Synthesis Kit (Fermentas Life Sciences, St. Leon-Rot, Germany) following the manufacturer’s recommendations.

Collagen III mRNA Expression Analysis

To quantify mRNA expression of collagen III in kidney, we used the real-time quantitative reverse transcriptase (TaqMan) PCR method. Appropriate primers and fluorogenic probes were designed with the Primer Express software. The ABI PRISM 7000 SDS instrument in conjunction with the ABI TaqMan Universal Master Mix (Applied Biosystems, Darmstadt, Germany) was used to perform the assays. The reaction volume was 25 \( \mu \text{l} \) with a final concentration of 900 nM for the primers and 200 nM for the cDNA probes. PCR conditions were used as recommended by the manufacturer. The fluorogenic probes were synthesized by TIB Molbiol (Berlin, Germany), and the primers were obtained from Proligo (Paris, France; Table 1).

Relative quantification was done using the standard curve method. For each gene, a PCR fragment that contained the sequence of the TaqMan system was generated. Seven serial 1:10 dilutions of this fragment served as a standard curve that was assayed together with the corresponding unknown samples on each plate. Every sample was measured in triplicate. To normalize our expression data, we used porphobilinogen deaminase as a housekeeping gene (GenBank accession no. X06827) (26).

Statistical Analyses

Statistical analysis was performed using one-way ANOVA followed by Bonferroni adjustment and by nonparametric Mann-Whitney U test. Data are means ± SEM, and \( P < 0.05 \) was considered significant.

Results

The evaluation of early-onset albuminuria in MWF at 8 wk of age demonstrated a marked 55-fold increase in MWF compared with SHR \( (18.1 \pm 2.0 \text{ versus } 0.3 \pm 0.1 \text{ mg/24 h}; P < 0.0001; \) Figure 1). This increase was completely abolished in MWF-6\text{SHR}, which showed similar UAE levels of 0.7 ± 0.1 mg/24 h compared with SHR. To analyze the gene dosage effect of the SHR allele on the early onset of UAE at 8 wk, we studied one group of F1 hybrid rats \( (n = 8) \) that were derived from MWF and consomic MWF-6\text{SHR}. In this analysis, a significant increase in UAE to 5.7 ± 1.7 mg/24 h was found in F1 rats in comparison with SHR and MWF-6\text{SHR} \( (P < 0.05; \) Figure 1). The F1 rats, however, demonstrated UAE levels that were significantly below the values that were observed in parental MWF \( (P < 0.05; \) Figure 1).

Further time-course analysis of UAE between weeks 8 and 24 revealed a further marked increase of UAE in MWF, particularly between weeks 14 and 18. In this time period, MWF reached UAE levels at the nephrotic range of approximately 160 mg/24 h. In aging SHR, UAE remained low at 24 wk \( (2.3 \pm 1.1 \text{ mg/24 h}) \), whereas only a moderate increase in UAE levels was observed in MWF-6\text{SHR} between weeks 14 \( (2.9 \pm 3.5 \text{ mg/24 h}) \) and 24 \( (16.6 \pm 15.2 \text{ mg/24 h}; \) Figure 2). Overall, in comparison with parental MWF, UAE was markedly suppressed in consomic MWF-6\text{SHR} at all time points investigated \( (P < 0.0001) \). The analysis of urinary excretion of LMW proteins in SHR revealed that the amount of excreted LMW proteins is larger than UAE (albumin/LMW protein ratio 0.41 ± 0.04; Figure 2). In contrast, the high protein excretion in MWF is attributable largely to albumin, whereas the contribution of LMW proteins to total protein excretion is only minor (albumin/LMW ratio 9.50 ± 0.55; \( P < 0.001 \) versus SHR). Consomic MWF-6\text{SHR} dem-

<table>
<thead>
<tr>
<th>Gene</th>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen III</td>
<td>Coll3-F</td>
<td>TGTTAACGCAAAATAGAGATGTCCTATCAG</td>
</tr>
<tr>
<td></td>
<td>Coll3-R</td>
<td>CATCTTGACGCTTTGTTAGG</td>
</tr>
<tr>
<td></td>
<td>Coll3 probe</td>
<td>FAM-TCCTGACACCCTGAACTCAAGACCG---TAMRA</td>
</tr>
<tr>
<td>PBGD</td>
<td>PBGD-F</td>
<td>TGAAATGTTACCCCTGCGATTA</td>
</tr>
<tr>
<td></td>
<td>PBGD-R</td>
<td>TCCATTTAGAGTGGGAGATTCAAGA</td>
</tr>
<tr>
<td></td>
<td>PBGD probe</td>
<td>FAM-TCGAAATGTTAGCTATGGCAGCATCAAGA</td>
</tr>
</tbody>
</table>

\( ^a \)PBGD, porphobilinogen deaminase gene.
onstrated an almost equal distribution of both protein species; the increased albumin to LMW protein ratio observed in MWF clearly is suppressed in this strain (albumin/LMW protein ratio 1.59/H11006 0.42; P/H11021 0.01 versus MWF and SHR, respectively; Figure 2).

At 24 wk of age, no significant difference in body weight was observed among strains (Table 2). Both absolute and relative kidney weight in relation to body weight were significantly lower in MWF compared with SHR (Table 2). SBP differed significantly among strains (P < 0.0001) at 24 wk of age. Both MWF and consomic MWF-6SHR exhibited lower SBP compared with SHR, whereas SBP was lower in MWF compared with consomic rats (P = 0.034; Table 2). The significant differences observed in absolute and relative heart weight among the three strains mirrored the differences that were seen in SBP (Table 2). No significant difference in serum creatinine and creatinine clearance was found among strains, whereas serum urea differed significantly among strains (Table 2). MWF showed the highest and MWF-6SHR exhibited intermediate serum urea concentrations (Table 2). Both serum triglycerides and cholesterol concentrations were significantly higher in MWF and MWF-6SHR compared with SHR (P/H11021 0.001; Table 2). A significant reduction in cholesterol levels but not in triglycerides was seen in MWF-6SHR compared with MWF (Table 2) at the age of 24 wk.

The determination of total glomerular number per kidney in young rats at 4 wk of age revealed overall a significant difference between strains (P < 0.005) with a significantly lower number (approximately −30%) in MWF compared with SHR (27,000 ± 3500 versus 37,000 ± 5900; P = 0.006; Figure 3). Glomerular number in consomic MWF-6SHR (34,600 ± 5700) was significantly higher compared with MWF (P = 0.04) and not statistically different from SHR (Figure 3).

The results that were obtained for renal histology analysis in adult animals at 24 wk of age are summarized in Table 3. The number of surface glomeruli (i.e., with direct contact to the surface) but not the total number of superficial glomeruli was significantly reduced in the MWF-6SHR strain compared with MWF. Glomerulosclerosis index and tubulointerstitial damage index were elevated significantly in MWF compared with SHR and significantly reduced in MWF-6SHR compared with MWF (Table 3). Quantitative analysis of RIF revealed a similar pattern with a significant reduction of fibrosis in the MWF-6SHR consomic strain (Table 3; Figure 4A). In addition, the increased collagen III mRNA expression that was observed in MWF compared with SHR was normalized in MWF-6SHR (Figure 4B).

Further follow-up of a subgroup of MWF and consomic
Table 2. Overall characteristics of parental MWF, SHR, and consomic MWF-6\(^{\text{SHR}}\) rats at 24 wk of age\(^a\)

| Characteristic                              | SHR  
| (n = 10) | MWF-6\(^{\text{SHR}}\)  
| (n = 19) | MWF  
<table>
<thead>
<tr>
<th>(n = 18)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>377.5 ± 4.4</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>175.3 ± 2.8</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>2.23 ± 0.02</td>
</tr>
<tr>
<td>Kidney weight/body weight (mg/g)</td>
<td>5.90 ± 0.05</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>1.11 ± 0.01</td>
</tr>
<tr>
<td>Heart weight/body weight (mg/g)</td>
<td>2.93 ± 0.03</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>35.7 ± 1.1</td>
</tr>
<tr>
<td>Serum urea (mmol/L)</td>
<td>7.07 ± 0.13</td>
</tr>
<tr>
<td>Crea Cl (ml/min per 100 g)</td>
<td>0.52 ± 0.08</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.70 ± 0.04</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>1.58 ± 0.05</td>
</tr>
</tbody>
</table>

\(^a\)Crea Cl, creatinine clearance; MWF, Munich Wistar Frömter rat; SHR, spontaneously hypertensive rat.

**Discussion**

In our previous cross-breeding study between MWF and SHR, we mapped a QTL on RNO6 that was linked to albuminuria in young animals at 8 wk of age and to both elevated UAE and increased RIF in adult backcross animals at 24 wk of age (15). We therefore proposed that this QTL could be important not only for the manifestation of early onset albuminuria but also for the development of overt proteinuria and decline in renal function. An important subsequent step to test this hypothesis is the generation of consomic strains in which an entire chromosome is introgressed into the isogenic background of another inbred strain using marker-assisted selection (27). Therefore, the generation of MWF-6\(^{\text{SHR}}\) was of particular importance for testing the functional relevance of the QTL on RNO6, because we had shown that UAE in MWF probably is influenced by up to 11 different QTL (5,15). Here, we can demonstrate that the genetic exchange of one QTL has a striking effect on the development of overt proteinuria in MWF. It has been well established that MWF develop overt proteinuria with age with progressive glomerular injury and proteinuric renal disease. Moreover, this strain represents a valuable model for functional analysis of glomerular damage and repair mechanisms and intervention studies in progressive renal failure (28–33). In agreement with these studies, we confirmed in this study that MWF from our colony reached UAE levels in the nephrotic range of approximately 150 mg/24 h (34) between 14 and 24 wk followed by a further more than two-fold progression of UAE in week 32. In contrast to our previous results in younger animals (5,15) and in agreement with other reports (17,21), we determined in this set of experiments lower SBP...
Table 3. Renal histology phenotypes in parental MWF, SHR, and MWF-6<sup>SHR</sup> at 24 wk of age

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHR (n = 10)</th>
<th>MWF-6&lt;sup&gt;SHR&lt;/sup&gt; (n = 19)</th>
<th>MWF (n = 18)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td></td>
<td>Overall</td>
<td>MWF versus SHR</td>
<td>MWF versus MWF-6&lt;sup&gt;SHR&lt;/sup&gt;</td>
</tr>
<tr>
<td>Superficial glomeruli (n)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 0.1</td>
<td>15.2 ± 1.1</td>
<td>16.7 ± 1.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Surface glomeruli (n)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0 ± 0.0</td>
<td>1.6 ± 0.4</td>
<td>3.8 ± 0.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glomerulosclerosis index (grade 0 to 3)</td>
<td>0.9 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tubulointerstitial damage index (grade 0 to 3)</td>
<td>0.04 ± 0.01</td>
<td>0.11 ± 0.03</td>
<td>0.38 ± 0.05</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Renal interstitial fibrosis (%)</td>
<td>3.1 ± 0.2</td>
<td>5.4 ± 0.3</td>
<td>7.5 ± 0.5</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Number of superficial and surface glomeruli per section as averaged from analysis of three midcoronal sections of the kidney as previously reported (5).

values in the MWF strain and significant higher SBP in SHR. It is interesting that SBP was significantly increased, albeit moderately, in MWF-6<sup>SHR</sup> compared with MWF, suggesting that RNO6 from SHR contains a BP-increasing QTL that was not detected in the original linkage study (15). The latter notion should be viewed with caution until this finding is evaluated in further studies that involve the characterization of congenic lines. Currently, it should not be dismissed, however, that an important conclusion can be derived from this finding, namely that the protective effect on the development of proteinuria that was observed in the consomic strain cannot be attributed to a decrease in SBP in this strain.

Nevertheless, in consomic MWF-6<sup>SHR</sup>, the development of nephrotic-range proteinuria clearly was suppressed and analysis of structural changes at 24 wk showed that glomerular and tubulointerstitial damage as well as RIF and collagen III expres-

sion were reduced significantly in consomic rats. Subsequently, the decline of renal function and progression of dyslipidemia that were observed in aging 32-wk-old MWF also were improved significantly in consomic rats.

The suppressive effect on UAE that was conferred by introduction of RNO6 from SHR occurred very early in MWF-6<sup>SHR</sup> at 8 wk of age, when consomic and SHR were indistinguishable with regard to UAE, demonstrating levels below the 1-mg/24 h range. The subsequent comparison between MWF-6<sup>SHR</sup> and the contrasting low UAE strain SHR showed a continuous but modest increase of UAE in MWF-6<sup>SHR</sup> that became significant at week 18 (Figure 2). Therefore, the development of elevated UAE is delayed significantly and in magnitude clearly suppressed in the consomic strain. In addition, the comparison between UAE and LMW protein excretion at week 24 by gel electrophoresis in the three strains revealed that the increased urinary albumin to LMW protein ratio of MWF clearly was suppressed in the consomic strain, thus confirming the reversal of albuminuria in these rats.

It is interesting that the evaluation of early-onset albuminuria in young F1 hybrid rats that were derived from MWF and consomic MWF-6<sup>SHR</sup> revealed a gene dosage effect on UAE. These F1 hybrids carry a heterozygous genotype on RNO6 (i.e., they carry one allele from SHR and one from MWF). Young F1 rats exhibited a significant increase of UAE compared with SHR and MWF-6<sup>SHR</sup>, they demonstrated approximately one third of UAE levels that were observed in MWF. This finding indicates that one SHR allele impairs the increase of UAE, whereas two alleles are required to suppress fully the development of early-onset albuminuria in MWF-6<sup>SHR</sup>.

In backcrossed rats in our QTL mapping study, we observed that the MWF allele of the QTL on RNO6 also was linked to the presence of increased numbers of superficial and surface glomeruli (15), which represent additional traits that are inherited in the MWF rat (35) together with a significant reduction in glomerular number (17,18). Although we could confirm a reduced number of glomeruli with direct contact to the surface in MWF-6<sup>SHR</sup>, the total number of superficial glomeruli in the outer cortex zone was not affected significantly. It is interesting,
however, that transfer from RNO6 into the MWF background resulted in a significant increase in total glomerular number per kidney in consomic MWF-6<sup>SHR</sup> compared with MWF. These findings point to the possibility that a causative gene on RNO6 is linked to early-onset albuminuria, and reduced glomerular number in the MWF strain. Alternatively, the selective breeding of the MWF strain for enrichment in surface glomeruli (35) may have selected and captured by chance this important UAE QTL on RNO6. Our comparison of renal structural damage between the consomic strain and SHR indicated that glomerulosclerosis and RIF were reduced only partially in MWF-6<sup>SHR</sup>, whereas tubulointerstitial damage was similar in MWF-6<sup>SHR</sup> and SHR. However, despite the low and normal UAE levels that were observed in SHR, secondary renal changes as a result of hypertension in the SHR strain may hamper this comparison. Therefore, it also is of further interest to compare the protective effect on renal structural damage between consomic or subsequently congeneric strains with a normotensive reference strain.

Figure 5. Phenotypes in parental MWF (□) and consomic MWF-6<sup>SHR</sup> (■) at 32 wk of age. (A) UAE. (B) Creatinine clearance (Crea Cl). (C) Urea concentration in serum. (D) Triglyceride concentration in serum. (E) Total cholesterol concentration in serum. *<i>P < 0.05</i>.
Comparative mapping analysis (36) between the 99 and 95% confidence intervals for placement of this QTL (Figure 3 of reference [15]) with the human genome maps this region to human chromosomes 14q23.1 to 14q31.3. This region contains 146 annotated genes, 46 of which are predicted or based on expressed sequence tag data and require further confirmation (Supplementary Table 1). Currently, we are not able to give any indication which gene(s) within the RNO6 region is (are) responsible for early-onset albuminuria. However, the development of MWF-6SHR validates this region and provides the rationale to develop further congenic rats that carry smaller intervals, thereby reducing the number of genes within the QTL for further investigation.

The homologous region in the human genome, 14q23.1 to q31.3, is different from UAE QTL that were identified recently on human chromosomes 5q, 7q, 8q, 12q, 19p, 21p, and 22q by genome-wide linkage analysis (6,7,10). This does not exclude, however, its potential clinical relevance considering that UAE in humans represents a highly complex phenotype that is influenced by multiple interactions between genetic susceptibilities and environmental factors. Strain-specific genetic differences in inbred animal models may reflect phenotypic subtypes of a more complex UAE phenotype that is observed in human populations, which exhibit greater genetic and phenotypic heterogeneity than the inbred lines that are used for QTL identification. The systematic analysis of consomic strains using the SS rat model as a recipient and the normotensive Brown-Norway rat as a donor strain within the Programs for Genomic Applications funded by the National Heart, Lung, and Blood Institute (data are available at http://pga.mcw.edu) demonstrated also a protective effect of RNO6 on the development of UAE in SS, whereas BP was not affected (http://pga.mcw.edu). In contrast, transfer of RNO6 from Brown-Norway rats into the FHH rat model of renal damage, which also is analyzed in this program, had no effect on either UAE or BP (http://pga.mcw.edu). Therefore, the identification of the RNO6 UAE QTL in the MWF and SS rat models may be indicative of the existence of a subtype-specific genetic predisposition, which has not yet been determined through linkage analysis in human populations because of genetic heterogeneity and the complexity of the UAE phenotype. The confirmation of the pivotal role of the RNO6 locus for the development of UAE in the MWF model may guide targeted analysis in humans by more advanced and powerful genetic techniques that involve single-nucleotide polymorphism and haplotype analyses in the corresponding region on human chromosome 14 in clinical studies. At the same time, the establishment of MWF-6SHR and the demonstration of its phenotypic characteristics provide the next essential step for the identification of the causative gene(s) by breeding congenic lines and further functional analysis. The power of using rat models to dissect the genetics of UAE and renal disease and to identify new targets was highlighted recently by the identification of Rab38 for elevated UAE in the FHH rat model (37) and Fcgr3 as a determinant of susceptibility to immunologically mediated glomerulonephritis in WKY rats (38).

Conclusion

From this study, we conclude that the removal of one important susceptibility locus for UAE from the complex interplay among several UAE QTL leads to a marked suppression of proteinuric renal disease in the MWF strain. We propose that the availability of consomic MWF-6SHR will complement the MWF strain as a valuable model for the isolation and characterization of putative candidate genes and the genetic dissection for disease pathways in proteinuric renal disease.

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Disclosures

None.

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