Geno-transcriptomic dissection of proteinuria in the uninephrectomized rat uncovers a molecular complexity with sexual dimorphism

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Proteinuria is a phenotypic trait that results from and is often considered a surrogate of renal disease. Multiple types of injury to the kidneys can cause proteinuria, and the pathophysiology of proteinuria is not necessarily uniform in the various forms of renal diseases. Within each disease entity, glomerular and/or tubular and/or vascular pathologies can culminate in proteinuria (1, 4, 10, 11, 14, 37, 55, 73). The search for the mechanisms underlying proteinuria has been ongoing for several decades in a number of experimental models, each simulating a different renal disease or injury. As a consequence of these efforts, multiple quantitative trait loci (QTLs) have been identified in different strains, and at least 70 genes have been implicated as contributing to proteinuria (http://www.rgd.mcw.edu). Despite these efforts, the pathogenic mechanisms underlying proteinuria in the mammalian organism remain incompletely understood and require continuing investigation.

We previously initiated the investigation of the pathophysiology of proteinuria in the uninephrectomized Sabra rat (SBH/y strain) in which proteinuria develops spontaneously, as an expression of focal and segmental glomerulosclerosis (69, 72). Importantly, renal disease develops in SBH/y irrespective of salt loading or the development of hypertension (69). Our initial genomic investigation yielded several QTLs, but no candidate genes. Furthermore, it was limited to male rats only, rendering our investigation incomplete since sex differences have been reported in the phenotype of renal disease in humans (7, 56) as well as in rodent models (50, 56).

In this study we pursued the investigation of proteinuria in our model, aiming this time to include both male and female sexes and to achieve more definitive results by identifying candidate genes. To attain these aims, we integrated genomics with transcriptomics in males and females and tested the hypothesis that the pathophysiology of proteinuria is different between the sexes. The first genomic part of our study consisted of genetic mapping of QTLs, an approach that has been considered until recently a major tool to investigate the genetic basis of complex diseases, including proteinuria. This approach has indeed yielded a large number of disease-related QTLs, but has failed in most cases to identify definitive disease-related genes because often QTLs span over large chromosomal segments and incorporate a large number of genes. As there is a paucity of tools to identify which among the genes within the QTL are those that are involved in the pathophysiology of the disease under investigation, progression beyond QTL detection has been severely hampered. There have been attempts to advance a step beyond the QTL by constructing congenic strains, which, it was hoped, would effectively diminish the chromosomal span of the QTL and consequently reduce the number of candidate genes (47). The generation of congenic strains, however, is lengthy, laborious, costly, and fraught with pitfalls (73). Over the past decade it has become possible to...
integrate genomics with transcriptomics, which seeks to identify through high-throughput differential gene expression profiling genes that are “over-” or “underexpressed” between contrasting populations in organs of interest and that map within well-defined QTLs (44, 67). A working assumption in applying this strategy is that genes that are concurrently differentially expressed and that map to an identified disease-related QTL are likely to be involved in the pathophysiology of that disease.

In the present study, we applied the geno-transcriptomic strategy in a sex-specific manner. In the genomic arm of the study, we initially set out to identify sex-specific QTLs. We then strove to confirm their functional validity by applying the “chromosomal substitution” strategy, using consomic strains. In the transcriptomic arm of the study, we contrasted gene expression on a genomic scale in the kidneys of male and female uninephrectomized proteinuric SBH/y and nonproteinuric SBN/y. We integrated the genomic and transcriptomic strategies by identifying those genes that were concurrently differentially expressed between the strains within each sex and that mapped within the span of the proteinuria-related QTLs. This integrative approach led us to detect new sets of well-defined candidate genes for proteinuria within each sex.

METHODS

Animals

We carried out the study in the uninephrectomized Sabra rat model, which consists of two distinct strains, the SBH/y rat that develops spontaneous proteinuria to a significantly greater extent than the contrasting SBN/y strain (69). We obtained the animals from the colony at the Israeli Rat Genome Center (http://www.irgc.co.il). Animals were fed standard rat chow (Koffolk, Tel Aviv, Israel) and tap water ad libitum. Climate-controlled conditions were maintained, the temperature being set at 22–24°C. Regular timed diurnal light cycles were kept. We housed the animals in compliance with institutional regulations and in accordance with National Institutes of Health (NIH) principles of laboratory animal care (NIH Pub. No. 85-23, revised 1985) and the guidelines of the American Physiological Society for the care of laboratory animals. The study protocol was reviewed and approved by the Institutional Committee for Animal Welfare.

Research Strategy

Our strategy to identify proteinuria-related genes was based on integration of genetic mapping (genomic arm) with differential gene expression profiling (transcriptomic arm), as we previously described (67). Before engaging in the genomic and transcriptomic arms of the study, we established the phenotype of the parental SBH/y and SBN/y strains for reference.

Genomic Arm

Crossbreeding. Female SBH/y rats were crossbred with male SBN/y rats, generating F1 siblings that were mated brother to sister, in turn generating F2. F2 animals were weaned at age 1 mo, at which time their right kidney was surgically removed (right uninephrectomy) under anesthesia (ketamine-xylazine ip) through a small flank incision. Uninephrectomy was performed in the present study at the young age of 1 mo, as opposed to 2 mo in our previous study (72), accelerating the development of proteinuria and allowing us to shorten the duration of our studies to 4 mo in males and 5 mo in females, instead of 11–12 mo as before.

Phenotype. We focused in the present study on proteinuria as the central phenotype and as an early sign of glomerulosclerosis that precedes impairment of glomerular filtration, as we have previously demonstrated in this Sabra rat model (69). The phenotype consisted, therefore, of quantitative measurements of 24-h urinary protein excretion collected in metabolic cages. We calculated the amount of protein excreted from urinary protein concentration and 24-h urinary excretion rate. Urinary (total) protein concentration was determined colorimetrically by the microprotein-PR method (Sigma Diagnostics).

We studied proteinuria at baseline (before uninephrectomy) and at 2-wk intervals after uninephrectomy until termination of the studies. We studied male rats for 3 mo after uninephrectomy and females for one additional month, until 4 mo after uninephrectomy, at which time the level of proteinuria, which tended to lag behind, achieved levels similar to those in males. We euthanized the animals at the end of the studies, withdrew blood from the aorta, and extracted organs of relevance.

We recorded additional phenotypes reflecting or related to renal function (plasma creatinine, urea, and electrolytes, urinary creatinine and electrolytes, urine volume) for reference only, as well as body and kidney weights.

Genotype determination. For genotype determination, we extracted genomic DNA from the liver by salt precipitation, followed by cleaning with phenol-chloroform, as previously described (68). We assessed DNA purity and quantity by spectrophotometry. We determined the genotype of each animal by PCR amplification of genomic DNA with marker (microsatellite) specific primers, as we previously described (69).

We scanned the entire rat genome for linkage with our phenotypes, using microsatellite markers that we previously found to be polymorphic between SBH/y and SBN/y. We targeted markers spaced 10–20 cm apart on each chromosome. When we detected a QTL, we increased the density of the markers within that chromosome. The microsatellite markers were custom synthesized by Genosys (Sigma) with primer sequences provided by the Rat Genome Database (RGD) (http://www.rgd.mcw.edu).

Data analyses. For analysis of the genomic part of the study, we applied the paradigm we previously described (69), using linkage and cosegregation analyses, and requiring both to be positive for definite identification of a QTL.

Linkage analysis. For linkage, we used the MultiQTL software package, version 2.6 (www.multiqtl.com). In brief, we initially screened the entire genome for genetic linkage, using single trait analysis (STA). We analyzed each time period (in months after uninephrectomy) separately, the periodic measurements of urinary protein excretion representing renal injury as a “single trait.” We determined statistical significance (P value) of individual QTLs by permutation testing (PT). When applicable, we proceeded with bootstrap analysis (BA), which provided us with the power of QTL detection, the chromosomal position of the peak logarithm of odds (LOD) score, the confidence intervals of the QTL span, and the percentage of the phenotypic variation accounted for by the QTL. When we detected linkage at consecutive time points, we applied the multienvironment analysis (MENVA) option, which incorporates different time points after intervention (uninephrectomy) as separate “environments.” We utilized the default unrestricted model but strove at each step of the analysis to fit the QTL to the simplest and statistically justified model (dominant, recessive, or additive effect).

Cosegregation analysis. We compared the level of proteinuria in F2 with the SBH/y (H) or SBN/y (N) alleles by one-way analysis of variance (ANOVA), which allowed us to independently confirm the presence of the QTL and determine the effect of the genotype on the selected phenotype.

Consomic strains. To provide evidence for the functional role of the QTLs, we constructed consomic lines for the major QTLs that we detected in the genome scan, as we previously described (69). In brief, we crossed bred homozygous SBH/y (HH) with SBN/y (NN) rats,
backcrossed F1 heterozygotes (HN) to the parental SBH/y or SBN/y strain (BC1), selected heterozygotes at loci of interest within the targeted chromosome, and backcrossed again to SBH/y or SBN/y for at least eight times. At the end of backcrossing, we crossed two heterozygotes and confirmed fixation of the allele of interest in resulting homozygotes on the background of the other strain by performing a global genome scan. We designated the resulting consomic strains by the order of RECIPIENT.CHROMOSOMEDONOR (http://pga.mcw.edu/pga/jsp/components/genomics/genomics.jsp).

We thus constructed new consomic strains for RNO2 and 20 and determined their phenotype. In addition, we determined the phenotype of male and female consomic strains that we had constructed for another unrelated project, in which we introgressed RNO1, 17, and X from SBN/y onto the genomic background of SBH/y (70, 71).

Transcriptomic Arm

Study groups. We studied whole kidney gene expression in four groups of rats: SBH/y with two kidneys, SBH/y after uninephrectomy, SBN/y with two kidneys, and SBN/y after uninephrectomy. The study of the four groups was designed to allow us to determine separately the effects on gene expression of strain (SBH/y vs. SBN/y) and uninephrectomy (2 kidneys vs. 1 kidney) and the combination of the two (1-kidney SBH/y vs. all other groups). To determine the effect of sex per se on gene expression, we studied male and female animals separately. We thus studied eight groups, using ANOVA and clustering applications.

Using six or seven animals per group, we investigated gene expression in the kidneys of animals at the same time point (age) at which we detected the QTLs by genetic mapping. In males, we studied the kidneys at age 4 mo, 3 mo after uninephrectomy. In females, we studied the animals at age 5 mo, 4 mo after uninephrectomy, at which time proteinuria, which develops at a slower rate than in males, achieved the level found in males that were 1 mo younger.

Tissue and RNA preparation. For tissue extraction, we rapidly removed the entire left kidney from animals under light ether anesthesia, snap-froze it in liquid nitrogen, and stored it at −80°C until RNA extraction. We refrained from dissecting out select parts of the kidney, such as glomeruli and tubules, or separating cortex from medulla, as the procedure could affect gene expression.

We extracted total RNA from each animal independently, using the RNeasy mini-kit (Qiagen). We processed the tissue through to oligonucleotide microarray hybridization, as previously described (3, 67). In brief, we prepared cDNA from RNA using T7-(dT)24 primer, transcribed in vitro, and labeled with biotin with the IVT Labeling Kit, resulting in labeled cRNA. We hybridized fragmented cRNA to the microarray overnight. We used the Affymetrix GeneChip Rat Genome 230 2.0 Array, with 31,000 probe sets. After overnight hybridization, we wash-stained the array with the Affymetrix Fluidics station and laser scanned it, collecting the data with the GeneChip Scanner 3000 7G. We analyzed the pixel intensity level with GeneChip Operating Software (GCOS) software (Affymetrix) and calculated an absolute expression level for each transcript. We assessed the quality of the chips and of the hybridization experiments as we previously described (3). We used RMA-Express 0.2 software for quantile RMA normalization (5).

Data analyses. We analyzed the data within each sex (males and females) separately. We compared the level of gene expression between the four groups by two-way ANOVA, looking for a strain effect, the effect of uninephrectomy, and the combined effect of strain and uninephrectomy. When we detected differences in expression within each set, we proceeded with cluster analysis, identifying transcripts that were differentially or commonly regulated in the respective
groups. This analysis allowed us to identify within each strain genes that were differentially expressed between strains (SBH/y vs. SBN/y), because of nephrectomy (uninephrectomized vs. nonuninephrectomized animals), and specifically related to proteinuria (uninephrectomized SBH/y vs. all other animals).

For hierarchical cluster analysis, we used Cluster version 2.11 (15), applying mean-centering and normalization of genes and arrays before average linkage clustering with uncentered correlation. According to the expression pattern observed by hierarchical clustering, we subdivided the probe sets into 10 subclusters by k-means clustering. We performed the k-means cluster analysis with Cluster version 3.0 and Euclidean distance measurement (k = 10, runs = 100) to observe treatment and strain effects independent from existing sex effects or without sex-specific normalization to observe the complete range of expression pattern differences.

Validation of microarray gene expression data. To verify the validity of our microarray results, we performed real-time RT-PCR assays (TaqMan) for select genes. We compared mRNA levels in kidneys of SBH/y that had undergone nephrectomy with SBH/y control animals with two kidneys, SBN/y that had undergone uninephrectomy, and SBN/y control animals with two kidneys.

For RT-PCR, we reverse-transcribed DNA-free total RNA (2 μg) with oligo(dT) primers (GIBCO-BRL), SuperScript II reverse transcriptase (GIBCO-BRL), and dNTP (Boehringer Mannheim) in 40 μl of reaction buffer (GIBCO-BRL). We designed primers and probes with Primer Express 2.0 (Applied Biosystems) and placed them in nonpolymorphic regions of the respective genes, together with GAPDH as control. We used TaqMan 2× PCR Master Mix (Applied Biosystems) and a cDNA equivalent of 25 ng (Tubb5 and Psmb8) or 50 ng (C2 and Ubd) of the initial RNA template in each PCR reaction. We studied each sample in quintuplicate. For TaqMan analysis, we used the Applied Biosystems 7900HT system. We normalized expression levels to GAPDH, using the 2−ΔΔCT method (CT = threshold cycle).

Integration of Transcriptomics with Genetic Mapping

We integrated transcriptomics with genomics by seeking the genes that were differentially expressed and mapped within the defined boundaries [95% confidence interval (CI)] of the respective proteinuria-related QTLs within each sex. We identified the chromosomal position of the differentially expressed genes in relation to their respective QTLs by using web-based resources, including Affymetrix GeneChip NetAffx Analysis Center database analysis tools (29) (https://www.affy-metrix.com/analysis/netaffx/quick-query.affx), RGD (http://www.rgd.mcw.edu), NCBI Rat Genome Resources (http://www.ncbi.nlm.nih.gov/genome/guide/rat), TIGR Rat Gene Index (http://www.tigr.org/tdgscripts/tdg/index.cgi?species=rat), and Gene Cards version 3 (http://www.genecards.org/). We identified those genes that satisfied the two integration criteria (differential expression and mapping within a proteinuria-related QTL) and labeled them as high-priority candidate genes for proteinuria in the Sabra rat model.

RESULTS

Genomic Arm

Phenotype. The amount of protein excreted in the urine by parental SBH/y and SBN/y, F1, and F2 is shown in Fig. 1, A (males) and B (females). We found significant differences between male and female SBH/y and SBN/y.

PARENTAL STRAINS. In males, we detected proteinuria as of the first month after uninephrectomy in both SBH/y and SBN/y, the amount increasing steadily thereafter, as shown in Fig. 1A. Protein excretion by SBH/y was higher than by SBN/y at all time points. In females, SBH/y also had proteinuria as of the first month after uninephrectomy, increasing thereafter, as shown in Fig. 1B; the degree of proteinuria, however, was milder than in males at all time points. In female SBN/y, the amount of proteinuria was minimal throughout the study period. Since at 4 mo the amount of proteinuria in female SBH/y was less than in male SBH/y, we prolonged the study period in females from 4 to 5 mo so as to achieve in both sexes a similar level of proteinuria upon study termination. The amount of

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<td>RNOX</td>
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</table>

The level of proteinuria was measured in males (M) up to 3 mo and in females (F) up to 4 mo after uninephrectomy. Each cell represents the statistical significance of linkage for RNO1–20 and RNOX at each time point, calculated by 10,000 permutations and shown for each chromosome and at each time point for males (on left) and females (on right): —, of no statistical significance; M or F in italics, approaching statistical significance; *P < 0.05, **P < 0.01; /, not determined.
proteinuria in females at 5 mo increased to levels similar to those found in male SBH/y at 4 mo.

F1. The amount of protein excreted by both males and females was at all time points in the lower range, similar to the amount of protein excreted by SBN/y of the respective sex, as shown in Fig. 1, A and B. These findings suggest a recessive mode of inheritance of proteinuria in males and females.

F2. The amount of proteinuria in both sexes ranged from that found in parental SBH/y to that in SBN/y, as anticipated in an F2 population and as shown in Fig. 1, C and D.

Genome scan. We initially screened the entire rat genome for genetic linkage with our primary end point, 24-h urinary protein excretion, before nephrectomy at age 1 mo and at 2-wk intervals thereafter, until age 4 mo in males and 5 mo in females. We detected linkage in males on RNO2, 6, 9, and 20 and in females on RNO11, 13 and 20, as shown in Table 1. We found additional linkage on RNO5, but as it was transient at 1.5 and 2.5 mo after uninephrectomy and disappeared thereafter, we did not pursue this locus any further. We did not detect significant linkage \( P < 0.05 \) by permutation testing) on any of the other autosomes or on RNOX.

We selected those chromosomes in which we found linkage for proteinuria during the primary screening for more in-depth linkage and cosegregation analyses, the results of which are provided below.

RNO2. MALES. We detected by linkage analysis a QTL on RNO2 with a LOD score that approached or achieved statistical significance between ages 3 and 4 mo. At 3.5 mo, PT revealed significant linkage \( P = 0.039 \), and BA placed the maximum LOD score at position 80.5 ± 15.2 cM (71% power of detection at the \( P = 0.01 \) level), between microsatellite markers D2Mgh10 and D2Mgh12, with a span of 59.4 cM (95% CI) and contributing 16.8% to the phenotypic variation of the trait (PVT). After we incorporated the proteinuria data from 3, 3.5, and 4 mo into MENVA (3 environments) the QTL increased in significance \( P < 0.001 \), but the peak position of the LOD score remained unchanged at 79.0 ± 14.1 cM (89% power of detection at the \( P = 0.001 \) level), and its span was minimally narrowed to 55.3 cM (95% CI), as shown in Fig. 2A. Cosegregation analysis confirmed the presence of the QTL, as shown with a representative marker D2Mgh10 at 3.5 mo of age in Table 2. The H allele was associated with increased proteinuria, and the pattern was consistent with a recessive mode of inheritance.

FEMALES. We did not detect significant linkage on RNO2 in females.

RNO6. MALES. We detected significant genetic linkage between 1.5 and 4 mo. At 3.5 mo, we identified a QTL \( P = 0.004 \) with a peak LOD score (86.3% power of detection, \( P = 0.05 \)) at location 28.9 ± 9.1 cM, in the vicinity of the microsatellite marker D6Mgh5, with a span of 35.7 cM (95% CI) and contributing 10.7% to the PVT. When we incorporated the data from 1.5, 2, 2.5, 3, 3.5, and 4 mo into MENVA (6 environments) the QTL became highly significant \( P < 0.0001 \),
leaving the location of the peak LOD score at 30.0 ± 3.0 cM (99.9% power of detection, \( P = 0.001 \)) but reducing the QTL span to 11.7 cM with demarcation between \( D6Rat25 \) and \( D6Rat165 \), as shown in Fig. 2B. Co segregation analysis confirmed the presence of the QTL, as shown with a representative marker \( D6Mgh5 \) at age 3.5 mo in Table 2. However, the N allele was associated with increased proteinuria and the H allele with decreased proteinuria, suggesting a “protective” QTL. The pattern was consistent with a recessive mode of inheritance.

**FEMALES.** We did not detect significant linkage on RNO6 in females.

**RNO9. MALES.** We detected linkage between ages 2 and 4 mo. At 4 mo, the QTL was significant (\( P = 0.0095 \)) and the peak LOD score (80.6% power of detection, \( P = 0.05 \)) was at location 63.8 ± 13.1 cM next to \( D9Mgh2 \), with a span of 41.3 cM (95% CI) and contributing 15.9% to the PVT. Incorporating the data from 2, 2.5, 3, 3.5, and 4 mo into MENVA (5 environments) increased the significance of the QTL (\( P < 0.0001 \)), positioned the peak LOD score at 65.6 ± 1.9 cM (94.7% power of detection, \( P = 0.001 \)) next to \( D9Mgh2 \), and reduced the span to 7.6 cM, as shown in Fig. 2C. Co segregation analysis confirmed the QTL, as shown with the representative marker \( D9Mgh2 \) at age 4 mo, as shown in Table 2. The N allele was here too associated with increased proteinuria, and the pattern was consistent with a recessive mode of inheritance.

**FEMALES.** We did not detect significant linkage on RNO9 in females.

**RNO11. MALES.** We did not detect significant linkage on RNO11 in males.

**FEMALES.** We detected significant linkage between ages 1.5 and 5 mo. At 4 mo, the QTL was significant (\( P = 0.001 \)), the peak LOD score (80% power of detection, \( P = 0.01 \)) was at 24.7 ± 7.0 cM in the vicinity of \( D11Rat38 \), and the span was 27.4 cM (95% CI), contributing 16.8% to the PVT. After incorporation of the data from 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mo into MENVA (8 environments) the significance of the QTL increased (\( P = 0.0002 \)), placing the peak LOD score at 18.2 ± 1.1 cM (99.7% power of detection, \( P = 0.001 \)) and markedly reducing the span to 4.4 cM, as shown in Fig. 3A. Co segregation analysis confirmed the presence of the QTL, as shown with the representative marker \( D11Rat38 \) at age 5 mo in Table 2. The H allele was associated with increased proteinuria, and the pattern was consistent with a recessive mode of inheritance.

**RNO13. MALES.** We did not detect significant linkage on RNO13 in males.

**FEMALES.** We detected a QTL with a LOD score that approached or achieved statistical significance between 3 and 5 mo. At 4 mo, the QTL was significant (\( P = 0.008 \)), with the peak LOD score at position 27.0 ± 12.2 cM (84.4% power of detection at the \( P = 0.05 \) level) in the vicinity of \( D13Rat85 \) and a span of 48 cM (95% CI), contributing 14.5% to the PVT. With incorporation of the data from 3, 3.5, 4, 4.5, and 5 mo into MENVA (5 environments) the QTL was highly significant (\( P < 0.0001 \)), moving the peak LOD score to position 33.0 ± 10.7 cM (99.1% power of detection at the \( P = 0.001 \) level) and reducing the QTL span only mildly to 42 cM, as demarcated by \( D13Mgh4 \) and \( D13Rat57 \) and as shown in Fig. 3B. Co segregation analysis confirmed the presence of the QTL, as shown with the representative marker \( D13Rat85 \) at age 4 mo in Table 2. The H allele was associated with increased proteinuria, and the pattern was consistent with a recessive mode of inheritance.

**RNO20. MALES.** We detected linkage between ages 2.5 and 4 mo. At 4 mo the QTL was significant (\( P = 0.0004 \)), placing the peak LOD score at position 3.6 ± 8.6 cM (89.4% power of detection, \( P = 0.01 \) level) next to microsatellite marker \( D20Rat32 \) with a QTL span of 20.4 cM (95% CI) and contributing to 14.6% of the PVT. With incorporation of the data from 2.5, 3, 3.5, and 4 mo into MENVA (4 environments) the QTL became highly significant (\( P < 0.0001 \)), moving the peak LOD score to position 2.4 ± 5.0 cM and reducing the QTL span to 12.1 cM (95% CI), as demarcated by \( D20Rat41 \) and \( D20Rat5 \) and as shown in Fig. 4A. The results of cosegregation analysis were consistent with the presence of a QTL, as shown with the representative marker \( D20Rat32 \) at age 4 mo in Table 2. The H allele was associated with increased proteinuria, and the mode of inheritance was consistent with a recessive pattern.

**FEMALES.** We detected linkage between ages 1.5 and 5 mo. At 4.5 mo the QTL was significant (\( P = 0.002 \)), placing the peak LOD score at position 7.3 ± 5.2 cM (72.4% power of detection, \( P = 0.010 \)) next to \( D20Rat67 \) and with a span of 17.5 cM. Incorporating the data from ages 1.5, 2, 2.5, 3, 3.5, 4, 4.5, and 5 mo (8 environments) into MENVA increased the level of significance of the QTL (\( P < 0.0001 \)), leaving the peak at 7.7 ± 5.1 cM, without diminishing the QTL span, which remained at 17.7 cM (95% CI), demarcated by \( D20Rat41 \) and \( D20Rat64 \) and thus overlapping with the QTL detected in males, as shown in Fig. 4B. The results of cosegregation analysis were consistent with the presence of the QTL, as shown with the representative marker \( D20Rat67 \) at age 5 mo in

### Table 2. Results of cosegregation analysis in F2 males and females

<table>
<thead>
<tr>
<th>Sex</th>
<th>RNO</th>
<th>Locus</th>
<th>Pheno</th>
<th>NT</th>
<th>HN</th>
<th>F</th>
<th>P</th>
<th>HH vs. NN</th>
<th>HH vs. HN</th>
<th>NN vs. HN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2</td>
<td>D2Mgh10</td>
<td>UP 3.5 mo</td>
<td>102 ± 14</td>
<td>72 ± 6</td>
<td>63 ± 9</td>
<td>4.404</td>
<td>0.014</td>
<td>0.008</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>D6Mgh5</td>
<td>UP 3.5 mo</td>
<td>54 ± 8</td>
<td>68 ± 6</td>
<td>98 ± 11</td>
<td>6.080</td>
<td>0.003</td>
<td>0.002</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>D9Mgh2</td>
<td>UP 4 mo</td>
<td>91 ± 11</td>
<td>72 ± 6</td>
<td>126 ± 17</td>
<td>7.100</td>
<td>0.001</td>
<td>0.029</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>D20Rat32</td>
<td>UP 4 mo</td>
<td>130 ± 15</td>
<td>79 ± 7</td>
<td>64 ± 9</td>
<td>10.851</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<tr>
<td>Female</td>
<td>11</td>
<td>D11Rat38</td>
<td>UP 5 mo</td>
<td>63 ± 14</td>
<td>22 ± 4</td>
<td>25 ± 7</td>
<td>7.685</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>D13Rat85</td>
<td>UP 4 mo</td>
<td>32 ± 7</td>
<td>18 ± 4</td>
<td>14 ± 2</td>
<td>5.777</td>
<td>0.005</td>
<td>0.030</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>D20Rat67</td>
<td>UP 5 mo</td>
<td>58 ± 15</td>
<td>28 ± 9</td>
<td>22 ± 3</td>
<td>5.624</td>
<td>0.005</td>
<td>0.020</td>
<td>0.001</td>
</tr>
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</table>

The genotype is shown as NN for homozygous for the SBN/y N allele, HH for homozygous for the SBN/y H allele, and HN for heterozygous. The phenotype is urinary protein (UP) excretion at the ages indicated. We compared average protein excretion of the 3 genotypes for each time point by one-way ANOVA; \( F \) and \( P \) values are provided. Post hoc analysis was performed by the least significant difference (LSD test); \( P \) values are provided for between-group analyses. NS, not significant.
Table 2. The H allele was associated with increased proteinuria, and the mode of inheritance was consistent with a recessive pattern.

**QTLs for additional phenotypes.** QTLs for plasma creatinine, urea, and electrolytes, urinary creatinine and electrolytes, urine volume, as well as body, heart, and kidney weight and plasma lipid profile are provided as supplemental data (Supplemental Table S1) for reference only and were not further processed or analyzed.1 Interestingly, sexual dimorphism was apparent in all of these phenotypes as well.

1 Supplemental Material for this article is available online at the Journal website.

**Chromosomal transfer.** We generated de novo consomic strains for RNO2 and RNO20 and studied their phenotype. In addition, we studied the phenotype of consomic strains for RNO1, 17, and X, which were available to us from previous studies.

RNO2. Introgression of RNO2 from SBN/y onto the background of SBH/y in males (SBH/y.2SBN/y) markedly reduced the level of proteinuria, thereby providing functional confirmation for the presence of a proteinuria-related QTL on RNO2 in males, as shown in Fig. 5. Even though we did not detect a QTL on RNO2 in females, we studied the phenotype of female consomic SBH/y.2SBN/y as well, as these animals were available to us as a by-product of generating the male consomics. We found that the level of proteinuria in female SBH/y.2SBN/y was markedly lower than in parental female SBH/y, as shown in Fig. 5. These results suggest the presence of proteinuria-

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Fig. 3. LOD score tracing for linkage of a locus on RNO11 (A) and 13 (B) with urinary protein excretion in female rats by MENVA. Horizontal bar represents the 95% CI span of the QTL; inverted triangle indicates the location of the peak LOD score.

Fig. 4. LOD score tracing for linkage of a locus on RNO20 with urinary protein excretion in male (A) and female (B) rats by MENVA. Horizontal bar represents the 95% CI span of the QTL; inverted triangle indicates the location of the peak LOD score.
related gene(s) on RNO2 in females as well, despite the absence of a detectable QTL by linkage analysis.

**RNO20.** Introgression of RNO20 from SBN/y onto the background of SBH/y in males (SBH/y.20SBN/y) markedly reduced the level of proteinuria, thereby providing functional confirmation of the proteinuria-related QTL on RNO20, as shown in Fig. 5. In females, the level of proteinuria in SBH/y.20SBN/y was also reduced, as shown in Fig. 5, thereby providing functional confirmation of the proteinuria-related QTL in females as well.

**RNO1.** Introgression of RNO1 from SBN/y onto the genomic background of SBH/y (SBH/y.1SBN/y) resulted in a significant reduction in the level of proteinuria in both male and female consomics, compared with the parental SBH/y strain, as shown in Fig. 6, A and B, respectively. These findings provide functional evidence for the presence of a proteinuria-related QTL on RNO1, which had not been detected by linkage analysis.

**RNO17.** Introgression of RNO17 from SBN/y onto the genomic background of SBH/y (SBH/y.17SBN/y) also resulted in a significant reduction in the level of proteinuria in male but not female consomics compared with the parental SBH/y strain, as shown in Fig. 6, A and B. These findings provide functional evidence for the presence of a proteinuria-related QTL on RNO17 in males, which had not been detected in the present linkage analysis.

**RNOX.** Introgression of RNOX from SBN/y onto the genomic background of SBH/y (SBH/y.XSBN/y) had no significant effect on proteinuria in either sex, as shown in Fig. 6, thus rendering the RNOX consomic an important negative control.

**Transcriptomic Arm**

To determine whether differences in gene expression were due to strain, uninephrectomy, or the combination of both, we studied the kidneys of SBH/y or SBN/y with and without uninephrectomy. To ascertain the adequacy of the animals for the gene expression studies, we investigated their proteinuric phenotype.

**Phenotype.** In males, we studied four groups of animals aged 5 mo: SBH/y with two kidneys (n = 6) and mild levels of proteinuria (45 ± 7 mg/24 h), SBH/y 4 mo after uninephrectomy (n = 6) with high levels of proteinuria (212 ± 23 mg/24 h), SBN/y with two kidneys (n = 5) and very mild proteinuria (29 ± 1 mg/24 h), and SBN/y 4 mo after uninephrectomy (n = 6) with mild to moderate proteinuria (78 ± 12 mg/24 h).

In females, we studied four groups as well, but at the age of 6 mo (at which time the level of proteinuria in SBH/y approached that in 5-mo-old males): SBH/y with two kidneys (n = 7) and no significant proteinuria (8 ± 1 mg/24 h), SBH/y 5 mo after uninephrectomy (n = 7) with high levels of proteinuria (202 ± 24 mg/24 h), SBN/y with two kidneys (n = 7) and no proteinuria (7 ± 1 mg/24 h), and SBN/y 5 mo after uninephrectomy (n = 7) with no proteinuria (7 ± 3 mg/24 h).

The levels of proteinuria found in these animals were as we previously reported (69, 72) and clearly separated the uninephrectomized SBH/y from the other three groups.

**Differentially expressed genes.** Of 31,099 probe sets on the Affymetrix GeneChip Rat Genome 230 2.0 Array, 54% (range 50–58) were expressed in the kidneys of male SBH/y or SBN/y among any of the four groups studied. The data sets have been deposited at the NCBI Gene Expression Omnibus (GEO) database repository and are identified as accession no. GSE14666.

Among the expressed genes, the number of genes that were differentially expressed between any of the groups varied by the level of statistical significance. Taking $P < 0.001$ as the cutoff level for strain, treatment (uninephrectomy), or combined effects and uniting the results of the two-way variance analyses, the number of differentially expressed transcripts was 4,278 in males and 6,724 in females, as shown in Table 3.
In females, we also identified three subsets consisting of transcripts that were differentially expressed (either up- or downregulated) due to 1) strain differences (SBN/y vs. SBH/y, clusters 3, 4, 5, 6, 9, and 10; n = 4,453), 2) treatment (uninephrectomy vs. 2 kidneys, clusters 2 and 8; n = 797), and 3) combined effect of strain and treatment (clusters 1 and 7; n = 1,474).

Integration of Genomic and Transcriptomic Arms

The genomic arm of the study led to the detection and chromosomal localization and demarcation of proteinuria-related QTLs in males on RNO2, 6, 9, and 20 and in females on RNO11, 13, and 20. The total number of genes that have been annotated to these QTLs is shown in Table 5 (NCBI, http://www.ncbi.nlm.nih.gov).

Since our aim was to detect those differentially expressed transcripts that were biologically related to proteinuria, we focused on the clusters that contrasted SBH/y after uninephrectomy (which developed the most significant degree of proteinuria) with the other three groups (which developed proteinuria to a significantly lesser extent or not at all). Specifically, we sought within these clusters those differentially expressed transcripts that mapped within the proteinuria-related QTLs. In males we focused, therefore, on clusters 1 and 6, while in females we focused on clusters 7 and 1. We proceeded to search for the genes within the relevant clusters that mapped within the respective QTLs. In males, we focused on the proteinuria-related QTLs on RNO2 demarcated by the microsatellite markers D2Mgh10 and D2Mgh12 (span 55.3 cM, 95% CI), on RNO6 demarcated by D6Rat25 and D6Rat165 (span 11.7 cM), on RNO9 demarcated by D9Rat153 and D9Mit5 (span 7.6 cM), and on RNO20 demarcated by D20Rat41 and D20Rat5 (span 12.1 cM). In females, we focused on the proteinuria-related QTLs on RNO11 in the vicinity of microsatellite marker D11Rat38 (span 4.4 cM), on RNO13 demarcated by D13Mgh4 and D13Rat57 (span 42 cM), and on RNO20 demarcated by D20Rat41 and D20Rat64 (span 17.7 cM).

Among the transcripts that were differentially expressed with \( P < 0.001 \) as the cutoff level for strain, treatment (uninephrectomy), or combined effects, we selected the sub-group that answered the threshold criterion of \( P < 0.05 \) for differential expression of the combined effect of strain and uninephrectomy by two-way ANOVA. In males we found a total of 24 transcripts for proteinuria (21 known genes), 22 of which were upregulated and 2 downregulated, as shown in Table 6; in females we found 30 such transcripts (26 known genes), 17 of which were upregulated and 13 downregulated, as shown in Table 7.

We reasoned that candidate genes that were common to both males and females would be of particular interest. Since the QTL on RNO20 was the only one that overlapped between the two sexes, we searched for transcripts that were commonly differentially expressed and that mapped to that QTL in males and females. We found four such genes: Tubb5, C2, Ubd, and Psmb8.

We verified our results from the microarray experiments with real-time PCR for the four transcripts (Tubb5, C2, Ubd, and Psmb8) that were common to males and females. We found concordance in the trend of fold change when the microarray results were compared with RT-PCR, as shown in Fig. 8.

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**Cluster analysis.** We addressed the relevance of the differentially expressed genes within each sex to the effects of strain, treatment to which the animals were subjected (uninephrectomy), or the combination of both (interaction of strain and uninephrectomy), as shown in Fig. 7 and Table 4.

In males, we identified three subsets of transcripts that were differentially expressed (either up- or downregulated) predominantly in one of the following: 1) strain differences (SBN/y vs. SBH/y, clusters 2, 3, 4, 8, 9, and 10; n = 3,191), 2) treatment (uninephrectomy vs. 2 kidneys, clusters 5 and 7; n = 356), and 3) combined effect of strain and treatment (clusters 1 and 6; n = 731).


TABLE 3. Differentially expressed transcripts with a $P$ value lower than given under one or more of the three conditions (strain, treatment or combined) in two-way ANOVA in males and in females

<table>
<thead>
<tr>
<th>Strain Effect</th>
<th>Treatment Effect</th>
<th>Combined Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>$P$ value</td>
<td>FDR</td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8,778</td>
<td>0.05</td>
<td>$1.77 \times 10^{-1}$</td>
</tr>
<tr>
<td>5,853</td>
<td>0.01</td>
<td>$5.31 \times 10^{-2}$</td>
</tr>
<tr>
<td>3,769</td>
<td>0.001</td>
<td>$8.24 \times 10^{-3}$</td>
</tr>
<tr>
<td>2,520</td>
<td>0.0001</td>
<td>$1.23 \times 10^{-3}$</td>
</tr>
<tr>
<td>1,742</td>
<td>0.00001</td>
<td>$1.78 \times 10^{-4}$</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11,915</td>
<td>0.05</td>
<td>$1.30 \times 10^{-1}$</td>
</tr>
<tr>
<td>8,473</td>
<td>0.01</td>
<td>$3.67 \times 10^{-2}$</td>
</tr>
<tr>
<td>5,800</td>
<td>0.001</td>
<td>$5.35 \times 10^{-3}$</td>
</tr>
<tr>
<td>4,188</td>
<td>0.0001</td>
<td>$7.43 \times 10^{-3}$</td>
</tr>
<tr>
<td>3,149</td>
<td>0.00001</td>
<td>$9.87 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

Values are numbers of differentially expressed transcripts with a $P$ value lower than given under 1 or more of the 3 conditions (strain, treatment or combined) in 2-way ANOVA in males and in females. FDR, false discovery rate.

DISCUSSION

In the present study, we pursued the pathophysiological dissection of proteinuria in a uninephrectomized rodent model. We demonstrated differences in the severity of proteinuria between the sexes, proteinuria developing in our model earlier and more profoundly in males than in females. These findings, which are consistent with similar reports in humans (7, 56) and in other rodent models of renal disease (50, 56), led us to investigate not only the pathophysiology of proteinuria but also the basis for the sexual dimorphism relating to this phenotype. To achieve our aims, we used an integrated geno-transcriptomic approach.

The main finding of the genomic arm of our study was the detection in males of two proteinuria-related QTLs on RNO2 and 20 and in females of three QTLs on RNO11, 13, and 20. We also detected two additional “protective” QTLs on RNO6.

Fig. 7. Heat map of the k-means clustering at 2-way ANOVA $P < 10^{-3}$ in males (left) and females (right). Clustering was performed with Euclidean distance measurement, $k = 10$ groups and 100 runs.
and 9 in males but none in females. The QTL on RNO20 was thus the only one that overlapped, the remaining QTLs being different between the sexes. The sexual dimorphism in the proteinuric phenotype was thus also expressed in the culprit QTLs.

The present cross in males was our second proteinuria-related cross in the Sabra rat model of proteinuria; in females, this was our first cross. In the first cross in males, we detected three proteinuria-related QTL on RNO2, 17, and 20 and one QTL on RNO3 that was protective of proteinuria (72). Our present study thus successfully reproduced the QTLs in males on RNO2 and 20, but could not reproduce the QTL on RNO17. We did not reproduce the “protective” QTL on RNO3 either, but detected two other “protective” QTLs on RNO6 and 9. Why do the results of the present cross in males differ from those of our previous cross, which we performed several years earlier? Both crosses were between SBH/y and SBN/y, which are not likely to have undergone a genetic drift during the time interval between the two crosses. A difference in the results could be attributed, at least in part, to dissimilarities in the methodology we used in preparing the animals for the two crosses. In the first cross, we performed uninephrectomy at age 2 mo and allowed proteinuria to develop thereafter for the next 8 mo. In the present study, we performed uninephrectomy at age 1 mo, immediately after weaning, which resulted in more severe proteinuria that developed earlier and at a faster rate than in the first cross, allowing us to shorten the duration of the study to 3 mo after uninephrectomy. Interestingly, the QTLs on RNO2 and 17 surfaced in the first cross only 7 mo after study, whereas in the present cross the QTLs on RNO2 and 20 surfaced much earlier, already at 2 and 1.5 mo after uninephrectomy. We cannot exclude the possibility that had we continued following the animals in the present cross for a longer period of time, we might have detected the QTL on RNO17 as well, perhaps as an indication of proteinuria that develops in the aging rat rather than proteinuria related to glomerulosclerosis.

How do the proteinuria-related QTLs we detected in males and females compare with those previously reported in the literature? Supplemental Table S2 provides an update of all the proteinuria-related QTLs reported so far in the rat. It is notable that most studies have been performed in male rats. In males, proteinuria-related QTLs have been reported previously on RNO1–4, 6–15, 17, 19, 20, and X, i.e., on most chromosomes. The QTLs we detected in the Sabra model on RNO2, 6, and 9 are concordant with proteinuria-related QTLs described by Garrett et al. (19), Poyan Mehr et al. (43), and Schulz et al. (51). Our QTL on RNO20, however, is novel and is distinct from those reported by Schulz et al. (52) and Shiozawa et al. (54) on the same chromosome. Data on proteinuria-related QTLs in females are scarce. QTLs have been previously reported on RNO2 and 11 (33). Our QTLs in females on RNO13 and 20 can thus be considered novel. Interestingly, even though a large number of proteinuria-related QTLs have been detected in the male rat, the findings are quite divergent between the strains. What are the possible explanations for such diverging results in the genetic basis of proteinuria between the various studies and strains? First, it has been shown repeatedly that crosses between different strains can yield different QTLs, thereby demonstrating the genetic and pathophysiological heterogeneity of complex traits such as proteinuria. Second, differences in phenotype can account for differ-

Table 5. Integration of transcriptomics with genomics

<table>
<thead>
<tr>
<th>RNO</th>
<th>No. of Annotated Genes on Chromosome</th>
<th>No. of Differentially Expressed Transcripts</th>
<th>QTL Span, cM</th>
<th>No. of Annotated Transcripts Within QTL</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (M)</td>
<td>1,779</td>
<td>38 (2.1%)</td>
<td>55.3</td>
<td>318 (17.9%)</td>
</tr>
<tr>
<td>6 (M)</td>
<td>1,196</td>
<td>12 (1.0%)</td>
<td>11.7</td>
<td>56 (4.7%)</td>
</tr>
<tr>
<td>9 (M)</td>
<td>898</td>
<td>13 (1.4%)</td>
<td>7.6</td>
<td>26 (2.9%)</td>
</tr>
<tr>
<td>11 (F)</td>
<td>700</td>
<td>23 (3.3%)</td>
<td>4.4</td>
<td>17 (2.4%)</td>
</tr>
<tr>
<td>13 (F)</td>
<td>823</td>
<td>36 (4.4%)</td>
<td>42</td>
<td>167 (20.3%)</td>
</tr>
<tr>
<td>20 (M/F)</td>
<td>844</td>
<td>14 (1.7%)/18 (2.1%)</td>
<td>12.1/17.7</td>
<td>58 (6.9%)</td>
</tr>
</tbody>
</table>

QTL, quantitative trait locus.
ing QTLs. For example, some of the models in which proteinuria-related QTLs were detected were normotensive, while others were hypertensive, hypertension being one of the possible confounding factors. Another phenotypic feature of possible significance is the presence of one or two kidneys. In our model, we utilized the uninephrectomy model of proteinuria with reduced nephron number, whereas other studies have used the two-kidney model, often with a very low level of proteinuria. Third, the age at which animals are studied may have been another important confounder, as different studies have used animals of different ages. Linkage analysis at different time points (age or time after uninephrectomy) has been shown to alter the phenotype and that our findings could be nonspecific and nonindicative. We studied, therefore, the level of proteinuria in another consomic strain in which we introgressed RNOX from SBH/y onto the genetic background of SBH/y. As we found no significant effect on proteinuria, the SBH/y-X\textsuperscript{SNB}y consomic became an important negative control that reassured us of the veracity of our interpretation of the substitution mapping experiments.

The contribution of substitution mapping to the genetic dissection of proteinuria deserves comment. The generation of consomic strains in our study was primarily intended to confirm the functional significance of our proteinuria-related QTLs, which it did. The significance of substitution mapping appears, however, to extend well beyond confirmation of QTLs detected by linkage analysis or to QTL span reduction, as it led us to detect additional unsuspected chromosomes that are involved in the pathogenesis of proteinuria. Our study goes further to suggest that consomic/congenic strains may be even more sensitive than linkage or cosegregation analyses to direct our attention to genomic locations that harbor disease-related genes. Why do linkage or cosegregation analyses not detect QTLs that are detected by congeneric/consomic strains? One possible explanation is that substitution mapping identifies both physiological QTLs (pQTLs) and expression QTLs (eQTLs), whereas traditional genetic mapping, such as that performed in the present study, seeks primarily pQTLs. Other yet unknown explanations must also be considered when linkage analysis and substitution mapping yield such conflicting results. The importance of our findings, however, lies not in the conflicting results but in the fact that substitution mapping is evolving to be an important complementary strategy to genomewide scanning in search of disease-related genes.
The transcriptomic arm of the study led to the detection of hundreds of differentially expressed genes within each sex that we were able by cluster analysis to attribute to differences in strain (SBH/y vs. SBN/y), uninephrectomy (uninephrectomy vs. 2 indwelling kidneys), or the combination of both. We were also able to study males and females differentially and determine sex differences in gene expression. However, the abundance of expression data, although of interest, would not have led us to the proteinuria-related genes. We proceeded, therefore, by seeking the differentially expressed genes that were related to both strain and uninephrectomy, focusing on uninephrectomized SBH/y versus the other three groups (transcriptomic arm), and that concurrently mapped within the proteinuria-related QTLs (genomic arm), integrating the results within each sex. The important finding in the integration of genomics with transcriptomics was the identification of two novel and separate sets of genes for proteinuria in males (24 transcripts) and females (30 transcripts), only four of which (Tubb5, C2, Ubd, and Psmmb8) were shared by both sexes. Many of these genes have been previously associated with renal disease or proteinuria, 18 of 24 in males and 17 of 30 in females. The renal localization of these genes has been determined in some, but not all. Tubb5 has been localized to the glomerulus (22), C2 to the glomerular mesangium and proximal tubular epithelial cells (12, 59), Ubd to tubular epithelial cells (48), and Psmmb8 to the glomerulus (64). The relatively large number of candidate genes within each sex that we identified and that are allegedly related with the pathophysiology of proteinuria are associated with multiple molecular pathways, including immunity, inflammation, apoptosis, matrix deposition, cytoskeleton, mitochondria, and others (see Tables 6 and 7). No single molecular pathway or mechanism predominates in its association with proteinuria. Even when we focused on the small number of proteinuria-related candidate genes that are shared by both sexes, they appear to contribute to a variety of cellular functions, including cell structure, immunity, and apoptosis, none of which aligns into one major or predominant pathway. Thus, as predicted for other complex diseases, proteinuria appears to involve multiple genes, each likely to exert a small effect, with involvement of multiple pathophysiological pathways.

There are important limitations to the present study, as our strategy necessarily incorporates problems and limitations inherent to the model per se, to genetic mapping, and to differential gene expression profiling. With regard to the model, we investigated the pathophysiology of proteinuria in a rodent model after uninephrectomy, thus reducing important variables including strain specificity and reduced nephron number due to removal of one kidney. Attempts to extrapolate our findings to other models or to humans should thus be very cautious. With regard to the genomic component of the study, the hazard of false positive and false negative QTLs is always worrisome. The QTLs we detected on RNO2 and 20 are strengthened by the results of our studies using consomic and congenic strains. RNO6, 9, 11, and 13, on the other hand, are not validated by congenics and consomics at present. The likelihood that other QTLs are present on additional chromosomes, even though they
were not detected by linkage analysis in our study, cannot be precluded unless consomic strains are constructed for each chromosome separately and tested individually. The existence of additional proteinuria-related QTLs is in fact very likely, as demonstrated by our studies using consomic and congenic strains in which we introgressed RNO1 and 17 from SBN/y onto the background of SBH/y, resulting in a very significant reduction in the level of proteinuria.

Fig. 8. Validation experiments by microarray (top) and real-time PCR (bottom), showing the relative expression of the 4 candidate genes that were common to males and females, Tubb5, C2, Ubd, and Psmb8 in uninephrectomized SBH/y vs. SBH/y with 2 kidneys (1), SBN/y with 2 kidneys (2), and SBN/y with 1 kidney (3).

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