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# Genetic analysis of albuminuria in collaborative cross and multiple mouse intercross populations

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[1]*Novartis Institutes for BioMedical Research, East Hanover, New Jersey;* [2]*Novartis Pharmaceuticals Corporation, East Hanover, New Jersey;* [3]*The Jackson Laboratory, Bar Harbor, Maine;* [4]*Oak Ridge National Laboratory, Oak Ridge, Tennessee*

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**Thaisz J, Tsaih S, Feng M, Philip VM, Zhang Y, Yanas L, Sheehan S, Xu L, Miller DR, Paigen B, Chesler EJ, Churchill GA, DiPetrillo K.** Genetic analysis of albuminuria in collaborative cross and multiple mouse intercross populations. *Am J Physiol Renal Physiol* 303: F972–F981, 2012. First published August 1, 2012; doi:10.1152/ajprenal.00690.2011.—Albuminuria is an important marker of nephropathy that increases the risk of progressive renal and chronic cardiovascular diseases. The genetic basis of kidney disease is well-established in humans and rodent models, but the causal genes remain to be identified. We applied several genetic strategies to map and refine genetic loci affecting albuminuria in mice and translated the findings to human kidney disease. First, we measured albuminuria in mice from 33 inbred strains, used the data for haplotype association mapping (HAM), and detected 10 genomic regions associated with albuminuria. Second, we performed eight $F_2$ intercrosses between genetically diverse strains to identify six loci underlying albuminuria, each of which was concordant to kidney disease loci in humans. Third, we used the Oak Ridge National Laboratory incipient Collaborative Cross subpopulation to detect an additional novel quantitative trait loci (QTL) underlying albuminuria. We also performed a ninth intercross, between genetically similar strains, that substantially narrowed an albuminuria QTL on Chromosome 17 to a region containing four known genes. Finally, we measured renal gene expression in inbred mice to detect pathways highly correlated with albuminuria. Expression analysis also identified *Glcci1*, a gene known to affect podocyte structure and function in zebrafish, as a strong candidate gene for the albuminuria QTL on Chromosome 6. Overall, these findings greatly enhance our understanding of the genetic basis of albuminuria in mice and may guide future studies into the genetic basis of kidney disease in humans.

haplotype association mapping; quantitative trait locus

DIABETIC NEPHROPATHY (DN) is a substantial medical burden worldwide and the leading cause of end-stage renal disease (ESRD). The cost of treating ESRD in the United States alone is estimated to exceed $30 billion annually (42), and DN contributes additional medical costs during earlier stages of kidney disease and by increasing the risk of myocardial infarction, stroke, atherosclerosis, heart failure, and all-cause mortality (26, 33, 38, 39, 43). Current therapies can slow the progression to ESRD associated with DN but not nearly to the rate of normal, age-related renal function decline.

Albuminuria is a clinical sign of nephropathy that significantly increases the risk of progressive kidney disease (13, 34), as well as cardiovascular disease and death (34), in diabetic patients. Moreover, pharmacological reduction of albuminuria lowers the risk of ESRD (16) and cardiovascular outcomes (12) in patients with DN. One estimate indicated that the lowered risk of ESRD alone with such treatment could reduce medical costs and increase survival of patients with DN (6). Therefore, identifying novel therapeutic targets to further reduce albuminuria in diabetic patients could provide substantial medical and economic benefits.

The genetic basis of DN in humans is well established and the causal genes underlying DN may represent novel drug targets. Many genomic regions containing genes affecting human DN [i.e., quantitative trait loci (QTL)] have been identified [see Bowden (3) for review], but it remains difficult to map and prove causal genes underlying polygenic diseases in humans. Because QTL identified in rodent models of kidney disease often correspond to human nephropathy QTL (27), rodents provide useful models to identify QTL that can predict or narrow kidney disease QTL in humans (19). Therefore, we applied the genetic resources available in mice to identify QTL underlying albuminuria using 2,900 mice derived from 9 intercross populations plus 190 mice from the Oak Ridge National Laboratory Collaborative Cross [pre-CC (10)].

## METHODS

*Phenotyping inbred mice for the strain survey of albuminuria.* Male mice from 33 inbred strains were obtained from The Jackson Laboratory (Bar Harbor, ME). Spot urine samples were collected from 10-wk-old mice daily in the morning for 1 wk. Urine samples from each mouse were pooled, and urinary albumin and creatinine concentrations were measured at The Jackson Laboratory using a Beckman Synchron CX5 Clinical Chemistry Autoanalyzer. A series of mouse albumin standards (Kamiya Biomedical, Seattle, WA) was quantified with each set of urine samples, and the final urinary albumin concentration in each sample was calculated by linear regression from the mouse albumin standard curve (20). These studies were approved by the Animal Care and Use Committee of The Jackson Laboratory.

*Haplotype association mapping.* Haplotype association mapping (HAM) analysis was performed using a free efficient mixed-model association (EMMA) add-on package in R [http://mouse.cs.ucla.edu/emma (25)]; we chose EMMA because it accounts for population structure better than other methods to reduce false-positive rates. Strain mean albuminuria data for 32 classical inbred strains was log transformed and combined with genotype data for 623,124 single nucleotide polymorphisms (SNPs) derived using the Mouse Diversity Genotyping Array (see Ref. 44; available at http://cgd.jax.org//tools/diversityarray.shtml). The association of each SNP with albuminuria was individually calculated as log10($P$ value), and $1 \times 10^{-6}$ was used as the significance threshold.

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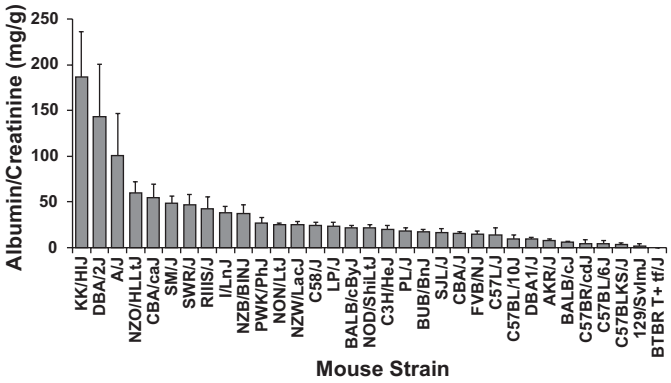


Fig. 1. Albuminuria in mice from 33 inbred strains. Bars represent urinary albumin-to-creatinine ratios in mice from each strain given as means ± SE ($n$ = 6–24 mice/group). KK/HIJ, DBA/2J, and A/J mice are significantly different ($P < 0.05$ by Tukey's Honestly Significantly Differences test) from all other mice tested.

*Breeding, phenotyping, and genotyping $F_2$ populations.* Mice from 15 inbred strains were purchased from The Jackson Laboratory and bred at Novartis Pharmaceuticals to generate nine populations of male $F_2$ mice for QTL analysis. Blood pressure was measured in $F_2$ mice at 8 wk of age [QTL results for this phenotype are published (18)] and then spot urine samples were collected at 10 wk of age and analyzed with mouse albumin standards using a Roche Hitachi 917 Clinical Chemistry Autoanalyzer. DNA isolated from each $F_2$ mouse was genotyped by KBiosciences (Herts, UK) as described previously (18). These studies were approved by the Novartis Animal Care and Use Committee.

*QTL analyses using $F_2$ populations.* Because the distribution of albuminuria was skewed in all $F_2$ populations, main-effect QTL linked to urinary albumin concentrations were identified using nonparametric genomewide scans (5, 29). Logarithm of the odds ratio (LOD) scores were calculated at 2-cM steps across the genome and compared with significance thresholds computed by permutation analysis (see Ref. 11; genomewide adjusted $P = 0.10$, suggestive; $P = 0.05$, significant). Confidence intervals were calculated based on Bayesian probability.

*Breeding, phenotyping, and genotyping collaborative cross mice.* Collaborative cross (CC) mice were bred at Oak Ridge National Laboratory as previously described (10). Spot urine samples were collected from 190 10-wk-old male mice, frozen, shipped to Novartis Pharmaceuticals, and analyzed with mouse albumin standards using a Roche Hitachi 917 Clinical Chemistry Autoanalyzer. Multiple generations of CC mice from $G_2$:$F_5$–$F_8$ were included in the phenotype population, but only the $G_2$:$F_7$ generation was genotyped using 13,000 SNPs that differentiate the eight founder haplotypes at >1,200 loci (10). Therefore, genotypes for mice in the $G_2$:$F_5$, $G_2$:$F_6$, and $G_2$:$F_8$ generations were inferred from $G_2$:$F_7$ parents or siblings that were genotyped directly; because the population is 80% inbred, it is possible to determine haplotype of origin with certainty at most locations for most mice using this approach.

*Mapping with the CC.* Urinary albumin concentrations were log transformed to account for the skewed distribution, and linkage analysis was performed by fitting a genomewide additive model to the trait data using R/Happy [version 2.1 (31)]. Genomewide significance thresholds were computed from 1,000 additive model permutations ($P = 0.10$, suggestive; $P = 0.05$, significant), and confidence intervals were calculated as the 1-LOD drop interval around the peak marker.

*Gene expression and pathway analysis.* Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and purified with Qiagen RNeasy separation columns (Qiagen, Hilden, Germany). For microarray analysis, first-strand cDNA was synthesized and hybridized to Affymetrix GeneChip Mouse Genome 430v 2.0 arrays (Affymetrix, Santa Clara, CA). Statistical analysis of the microarray data was performed within the R statistical environment (36) using bioinformatics packages from Bioconductor (22). The raw signals from CEL files were normalized and summarized into probe-set level intensities using a Robust Multichip Average method (23). Affymetrix MAS5 present/absent calls were calculated, and probe sets with "present" calls in fewer than 50% of samples within all strains were removed from further statistical analyses. The moderated $t$-test, implemented in the *limma* package (40), was applied to determine if the differential expression of each probe set between any strain pair was significant. $P$ values were further adjusted using the Benjamini and Hochberg (1) multiple-testing procedures for false discovery rate control.

The urine albumin concentration values were log2 transformed (constant number 1 was added before transformation) to ensure that the data fit an approximately normal distribution. Data from the entire collection of 26 strains (129S1/SvImJ, A/J, AKR/J, BALB/cByJ, BALB/cJ, BTBR-T+tf/J, BUB/BnJ, C3H/HeJ, C57BL/6J, C57BR/cdJ, C58/J, CBA/caJ, CBA/J, DBA/2J, FVB/NJ, I/LnJ, KK/HIJ, NOD/LtJ, NON/LtJ, NZO/HILtJ, NZW/LacJ, PL/J, RIIIS/J, SJL/J, SM/J, and SWR/J) were used to calculate Pearson correlations between the transformed urinary albumin level and all the probe sets that passed filtering. The functional enrichment of genes highly correlated with albuminuria (defined as >0.6 or ≤0.6) were performed using the Database for Annotation, Visualization, and Integrated Discovery website [DAVID; http://david.abcc.ncifcrf.gov/ (21)].

*Statistics.* Tukey's Honestly Significant Differences test was used to test the significance of unplanned pairwise comparisons among the 33 inbred strains. Values for groups sorted by genotype are presented as means ± SE and were compared by ANOVA followed by Bonferroni posttest using SigmaStat. $P < 0.05$ was considered significant.

## RESULTS

*Survey of albuminuria in inbred mice.* To evaluate albuminuria among inbred mice and identify strains useful for QTL analysis, we measured urinary albumin and creatinine concentrations in mice from 33 inbred strains. We found a wide variation in albumin-to-creatinine ratios (ACR) between mice from different inbred strains, from BTBR T+ tf/J mice with no detectable albuminuria to KK/HIJ mice with ACR near 200 mg/g (Fig. 1).

*Haplotype association mapping.* The first approach we used to identify genomic loci affecting albuminuria in mice was HAM by EMMA. Genotype data collected with the Mouse Diversity Genotyping Array for many inbred mouse strains is freely

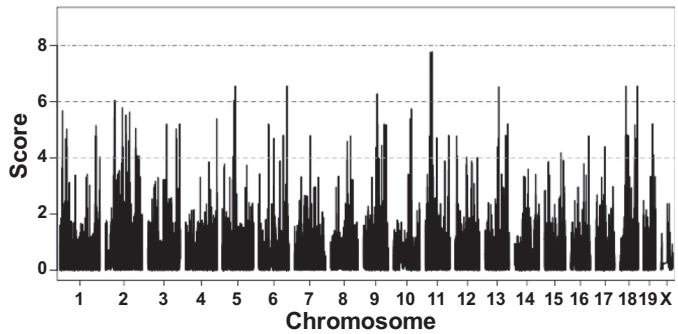


Fig. 2. Haplotype association mapping (HAM) using classical inbred strains. Albuminuria data from 32 classical inbred strains were combined with genotype data at 623,124 single nucleotide polymorphisms (SNPs) for HAM analysis; Manhattan plot shows genomic regions associated with albuminuria. Score represents log10($P$ value).

Table 1. *HAM peaks for albuminuria*

| Chr | SNP ID | HAM Peak, Mb | Interval, Mb | *P* Value |
|---|---|---|---|---|
| 2 | rs28320970 | 47.14 | 47.06–48.06 | $8.91 \times 10^{-7}$ |
| 5 | rs38597735 | 64.72 | 58.28–58.35 | $2.87 \times 10^{-7}$ |
| | | | 63.97–65.05 | |
| 6 | rs30265037 | 138.44 | 138.42–139.19 | $2.87 \times 10^{-7}$ |
| 9 | rs30223940 | 71.32 | 69.97–71.38 | $5.46 \times 10^{-7}$ |
| 11 | rs26886616 | 36.20 | 27.28–28.71 | $1.77 \times 10^{-8}$ |
| | | | 35.99–36.45 | |
| 13 | rs29552670 | 69.86 | 69.86–70.01 | $2.87 \times 10^{-7}$ |
| 18 | rs31600034 | 32.02 | 32.00–32.04 | $2.87 \times 10^{-7}$ |
| 18 | rs38179874 | 86.76 | 86.73–86.76 | $2.87 \times 10^{-7}$ |

Chr, chromosome; SNP, single nucleotide polymorphism; ID, identification; HAM, haplotype association mapping. Mb positions are based on NCBI mouse genome build 37.

available from the Center for Genome Dynamics at The Jackson Laboratory, so we combined this genotype data with the albuminuria data from the inbred strain survey for HAM analysis. Because HAM is dependent on conservation of ancestral alleles among inbred strains, we included only data from the 32 classical inbred strains (excluding PWK) in the HAM analysis. Using a threshold of $1 \times 10^{-6}$, we identified 10 genomic regions on 8 chromosomes (Chr) that were associated with albuminuria across inbred mice (Fig. 2 and Table 1). Each HAM region was <1.5 Mb and contained only a few genes.

*QTL analyses of albuminuria in $F_2$ populations.* To further map the loci underlying the variation in albuminuria between inbred mice, we generated eight $F_2$ populations derived from 12 inbred strains selected for genetic and phenotypic diversity; ACR was significantly different between seven of these eight strain pairs (Table 2). Because cross direction can substantially affect albuminuria, we generated all $F_1$ mice for each cross in the same direction and intercrossed them to produce the $F_2$ progeny, meaning that maternal, imprinting, and mitochondrial effects were fixed within each $F_2$ population. Albuminuria was not normally distributed in any of the $F_2$ populations (data not shown), so we used nonparametric QTL analyses to detect main-effect QTL underlying albuminuria (Fig. 3, *A–H*) because this method is not adversely affected by a skewed trait distribution. The (AKR × NZW)$F_2$ and (FVB × RIIIS)$F_2$ populations, which had the smallest ACR differences between the parental strains (Table 2), failed to identify any QTL significantly linked to albuminuria. Linkage analyses detected one significant main-effect QTL in each of the remaining $F_2$ populations (peak locations, confidence intervals, allele effects, LOD scores, and modes of inheritance are summarized in Table 3).

*Chromosome 4.* The (SJL × RIIIS)$F_2$ population identified a significant albuminuria QTL on Chr 4 (Fig. 4*A*). At this locus, RIIIS mice contributed a recessive allele for albuminuria (Fig. 4*B*). The concordant region of the human genome, Chr 9q22, was previously linked to albuminuria in a study of >3,600 American Indians (32). This region of the human genome has also been linked to nephropathy in patients with type II diabetes (4).

*Chromosome 6.* Proximal Chr 6 was significantly linked to albuminuria in the (BTBR × SWR)$F_2$ cross (Fig. 4*C*), where SWR mice contributed a recessive allele for albuminuria (Fig. 4*D*). This QTL in mice is concordant with an albuminuria QTL on human Chr 7q21 detected in European Americans as part of the Family Investigation of Nephropathy and Diabetes study (24).

*Chromosome 7.* The (PL × CBA)$F_2$ intercross detected a significant QTL underlying albuminuria in the middle of Chr 7 (Fig. 4*E*). $F_2$ mice that inherited two PL alleles at this locus displayed higher ACR than heterozygotes or CBA homozygotes (Fig. 4*F*), indicating a recessive PL albuminuria allele at this QTL. The concordant region of the human genome on Chr 16p12 has been linked to ESRD, with proteinuria, in African American families (4). According to the rat genome database (www.rgd.mcw.edu), the corresponding region of rat Chr 1 has also been linked to urinary albumin excretion, with QTL detected in three separate populations: *UAE1* in (MWF × LEW)$F_2$ rats, *UAE5* in (SS × SHR)$F_2$ rats, and *Rf2* in (FHH × ACI) × FHH $N_2$ rats.

*Chromosome 13.* The middle portion of Chr 13 was significantly linked to albuminuria in the (C3H × KK)$F_2$ intercross (Fig. 5*A*). At this locus, KK mice contributed an additive allele for high albuminuria (Fig. 5*B*). This mouse QTL is concordant to two separate regions of the human genome that have been linked to albuminuria: Chr 5q14 is linked to albuminuria in families with type II diabetes (28) and Chr 9q22 is linked to albuminuria in American Indians (32).

*Chromosome 17.* The strongest QTL linked to albuminuria in these eight intercrosses was detected on proximal Chr 17 in

Table 2. *Characteristics of $F_2$ populations for albuminuria QTL analysis*

| Grandmaternal Strain | Grandpaternal Strain | *n* | No. of Markers | ACR Difference* | *P* Value† |
|---|---|---|---|---|---|
| | | *Genetically diverse strain pairs* | | | |
| AKR/J (AKR) | NZW/LacJ (NZW) | 333 | 94 | 17 | <0.001 |
| FVB/NJ (FVB) | RIIIS/J (RIII) | 252 | 90 | 27 | 0.019 |
| SJL/J (SJL) | RIIIS/J (RIII) | 336 | 91 | 26 | 0.045 |
| BTBR T+ tf (BTBR) | SWR/J (SWR) | 328 | 93 | 47 | 0.001 |
| PL/J (PL) | CBA/J (CBA) | 324 | 90 | 37 | 0.024 |
| C3H/HeJ (C3H) | KK/HIJ (KK) | 335 | 91 | 167 | 0.006 |
| 129S1/SvImJ (129) | A/J (A) | 333 | 91 | 99 | 0.056 |
| 129S1/SvImJ (129) | DBA/2J (D2) | 319 | 90 | 141 | 0.034 |
| | | *Genetically similar strain pair* | | | |
| DBA/1J (D1) | DBA/2J (D2) | 340 | 33 | 133 | 0.003 |

*n*, No. of experments. QTL, quantitative trait loci; ACR, albumin-to-creatinine ratio. Strain abbreviations used throughout the manuscript are shown in parentheses. *Difference in ACR between the strains (mg/g). †*P* value for ACR difference between inbred strains.

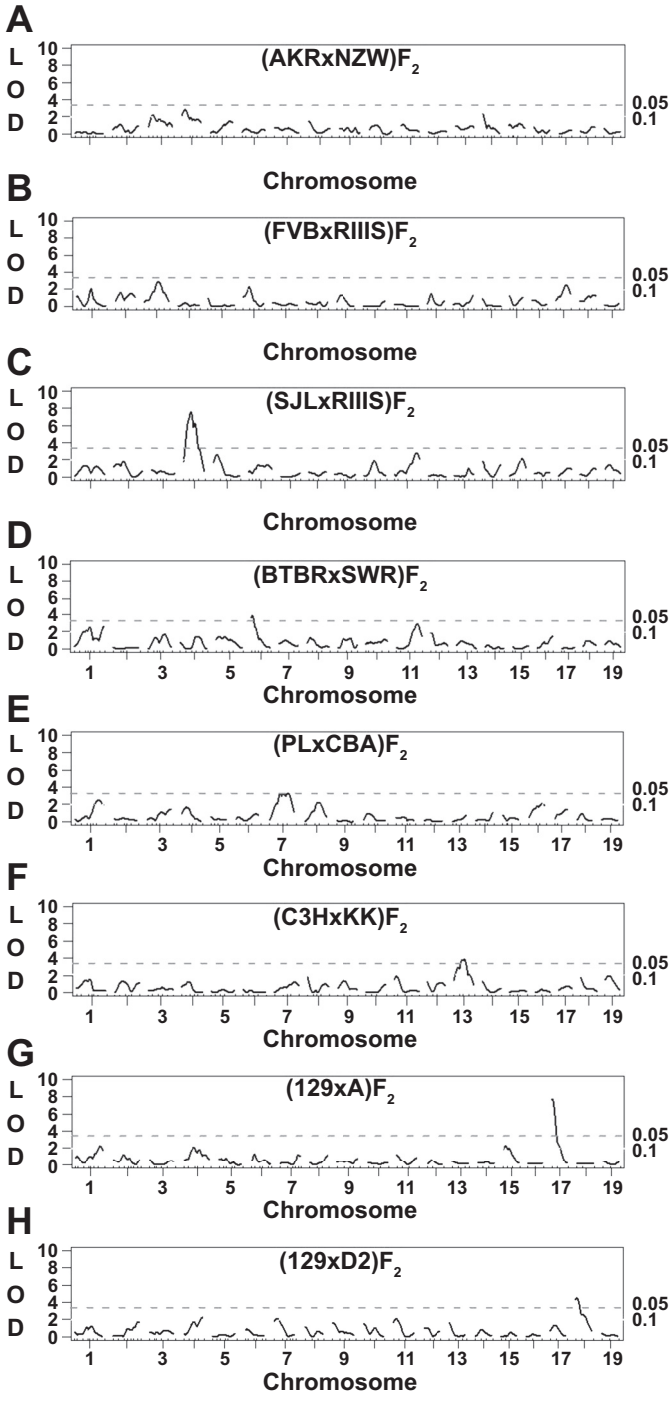


Fig. 3. Nonparametric genomewide scans for albuminuria quantitative trait loci (QTL) in 8 intercross populations. Suggestive ($P < 0.10$) and significant ($P < 0.05$) logarithm of the odds ratios (LOD) scores, as determined by permutation testing, are shown as dotted lines.

the (129 × A)$F_2$ population (Fig. 5*C*). $F_2$ mice that inherited two A alleles at this locus displayed higher ACR than heterozygotes or 129 homozygotes (Fig. 5*D*), indicating a recessive A allele for albuminuria at this locus. This region of the mouse genome corresponds to a rat Chr 17 interval that was linked to increased urinary albumin excretion in (MWF × LEW)$F_2$ rats (*UAE4*; www.rgd.mcw.edu). Portions of this genomic region in mice are concordant to human Chr 6p21, which falls within the 1-LOD interval of the Chr 6p QTL linked to albuminuria in American Indians by Mottl et al. (32). Human Chr 6p21 is also modestly linked to albuminuria in an analysis of extended families by Krolewski et al. (28). The Chr 17 QTL is also concordant to human Chr 6q26, which is associated with estimated glomerular filtration rate (7).

*Chromosome 18.* The final albuminuria QTL detected in our analyses of eight intercrosses between genetically diverse mouse strains was on Chr 18 (Fig. 5*E*). In the (129 × D2)$F_2$ population, this QTL was inherited as a recessive albuminuria allele from D2 mice (Fig. 5*F*). This region of the mouse genome is concordant with human Chr 18q12, which is suggestively linked to albuminuria in American Indians (32).

*QTL analysis of albuminuria in the CC.* In addition to the mouse intercross populations, we also examined the genetic basis of albuminuria in pre-CC mice. We detected a significant QTL underlying albuminuria on proximal Chr 10 in the pre-CC population (Fig. 6, *A* and *B*). Several trait values were censored because they were below the limits of detection; therefore, we confirmed the mapping result by applying proportional hazard regression to the six markers within the 1-LOD interval around the peak marker and detected two markers significantly linked to albuminuria in this population (Fig. 6*C*). Because the pre-CC population originated from eight wild-derived and classical inbred mouse strains, we used diagnostic haplotypes to determine which strains contributed albuminuria alleles at this QTL. Pre-CC mice carrying haplotypes from WSB/EiJ (WSB) and A at this locus displayed significantly higher albuminuria than mice carrying haplotypes from the other founder strains (Fig. 6*C*).

*Analysis of albuminuria linkage to Chr 17 in (D1 × D2)$F_2$ mice.* Because D1 and D2 mice are nearly 95% genetically identical, QTL detected between these mice are likely to have narrow confidence intervals lying within the small regions of genetic diversity. D1 and D2 are identical by descent over most of the QTL intervals detected in the intercross and pre-CC populations, but they differ at two distinct regions on Chr 17, including the region on proximal Chr 17 harboring the albuminuria QTL detected in the (129 × A)$F_2$ population. Thus, we generated an intercross population of 340 (D1 × D2)$F_2$ mice, quantified urinary ACR, and genotyped the mice at three SNPs within these two regions on Chr 17. All three SNPs were significantly linked to albuminuria (Fig. 7, *A–C*), indicating the presence of two albuminuria QTL on Chr 17 in the (D1 × D2)$F_2$ population. Whereas the proximal QTL in (129 × A)$F_2$ mice extended from 0 to 23 Mb, the interval in (D1 × D2)$F_2$ mice ranged from 0 to only 4.5 Mb, (Fig. 7*D*), a significant refinement of the QTL to an interval containing only a few genes.

*Renal gene expression, pathway analysis, and candidate gene identification.* To better understand the molecular pathways underlying albuminuria in inbred mice and to identify candidate genes for further evaluation, we used microarray analysis to evaluate renal gene expression in kidneys from mice from 26 inbred strains. Renal gene expression and urinary albumin/creatinine data for mice from the 26 strains were used to calculate Pearson correlations. The subset of genes highly correlated with albuminuria ($>0.6$ or $\leq 0.6$) were evaluated for functional enrichment using the DAVID website (http://david.abcc.ncifcrf.gov/). Pathways for cell adhesion and cell projection organization were among the top pathways negatively

Table 3. *Summary of significant main-effect QTL linked to albuminuria*

| Chr | QTL Name | Population | Peak, cM | 95% CI, cM | 95% CI, Mb | LOD | High Allele | ACR Effect* | Mode of Inheritance | Human QTL |
|---|---|---|---|---|---|---|---|---|---|---|
| 4 | *Albq12* | SJL × RIIIS | 26.1 | 16.1–32.1 | 34.0–58.4 | 7.61 | RIIIS | 16.5 | Rec | 9q22 (32) |
| 6 | *Albq13* | BTBR × SWR | 6.8 | 2.8–12.8 | 6.8–32.0 | 3.82 | SWR | 34.3 | Rec | 7q21 (24) |
| 7 | Albq14 | PL × CBA | 64.4 | 34.4–74.4 | 70.0–138.7 | 3.32 | PL | 48.3 | Rec | 16p12 (4) |
| 13 | *Albq15* | C3H × KK | 36.7 | 14.7–44.7 | 37.4–84.8 | 3.86 | KK | 44.2 | Add | 9q22 (32), 5q14 (28) |
| 17 | *Albq16* | 129 × A | 5.5 | 0–11.5 | 0–23.3 | 7.71 | A | 78.8 | Rec | 6p21 (32), 6q26 (7) |
| 18 | *Albq17* | 129 × D2 | 9.7 | 0–17.7 | 0–30.9 | 4.51 | D2 | 20.2 | Rec | 18q12 (32) |
| 10 | *Albq18* | preCC | 4.7 | 3.0–5.1 | 8.7–13.5 | 9.0 | WSB | n/a | n/a | |

CI, confidence interval; cM, centimorgan; Mb, megabase; LOD, logarithm of the odds ratio; Rec, recessive; Add, additive; n/a, not availalbe. Reference for each human QTL is indicated in parentheses. *ACR effect is the maximum ACR difference between the three genotypes at the QTL expressed as mg albumin/g creatinine.

correlated with albuminuria, whereas pathways affecting mitochondrial structure and function were among the pathways positively correlated with albuminuria in mice from these 26 strains (Table 4).

We also evaluated whether genes located in each QTL interval exhibited expression differences between the mouse strains used to generate the $F_2$ population that detected the QTL [full results in Supplemental Information (Supplemental data for this article may be found on the *American Journal of Physiology Renal Physiology* website)]. For example, we tested probe sets representing 77 genes within *Albq13* on mouse Chr 6 and found that 11 genes showed

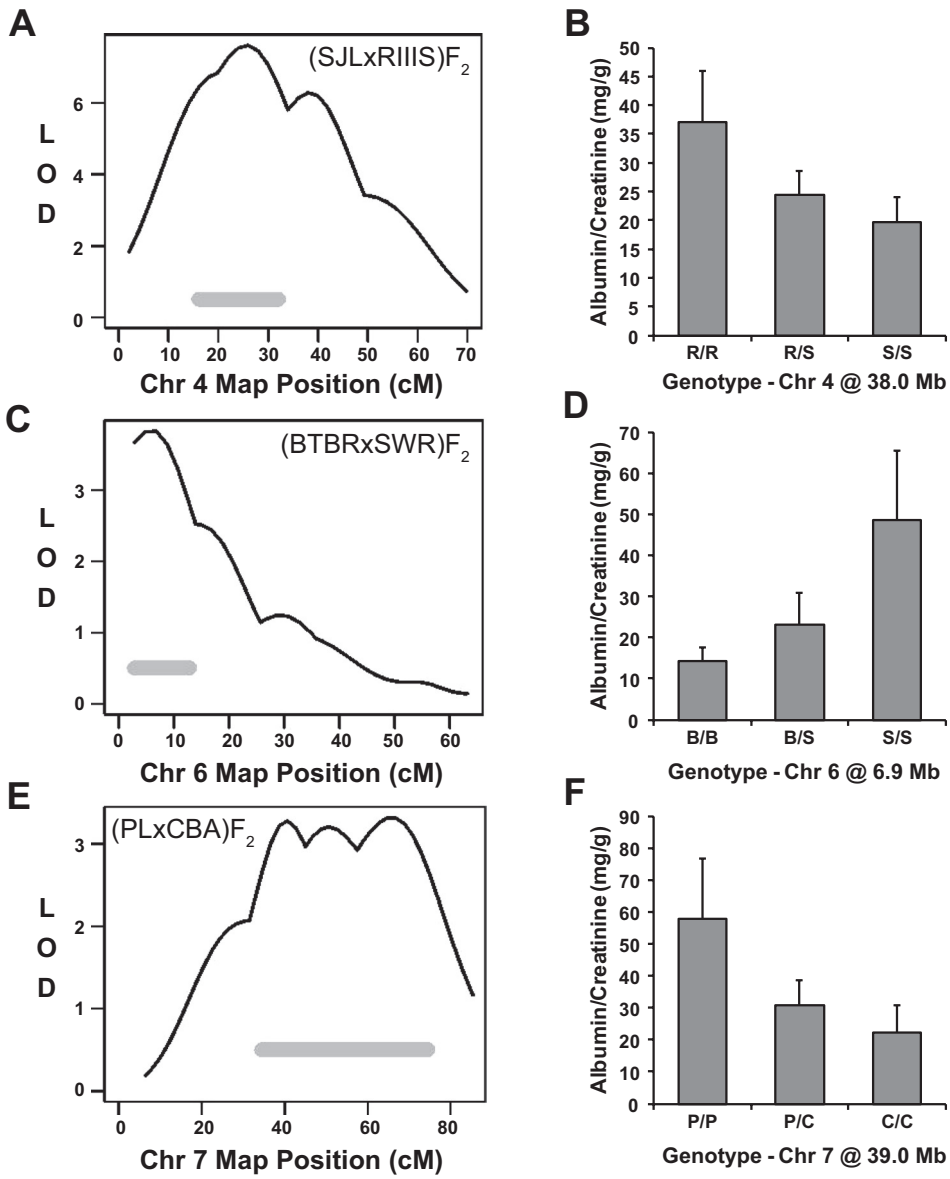


Fig. 4. Mapping of albuminuria QTL on Chromosomes (Chr) 4, 6, and 7. Mapping and allelic effect plots for albuminuria QTL in the (SJL × RIII)$F_2$ (*A* and *B*), (BTBR × SWR)$F_2$ (*C* and *D*), and (PL × CBA)$F_2$ (*E* and *F*) populations. Albumin-to-creatinine ratios are expressed as means ± SE. The solid horizontal gray bars represent the Bayesian confidence intervals.

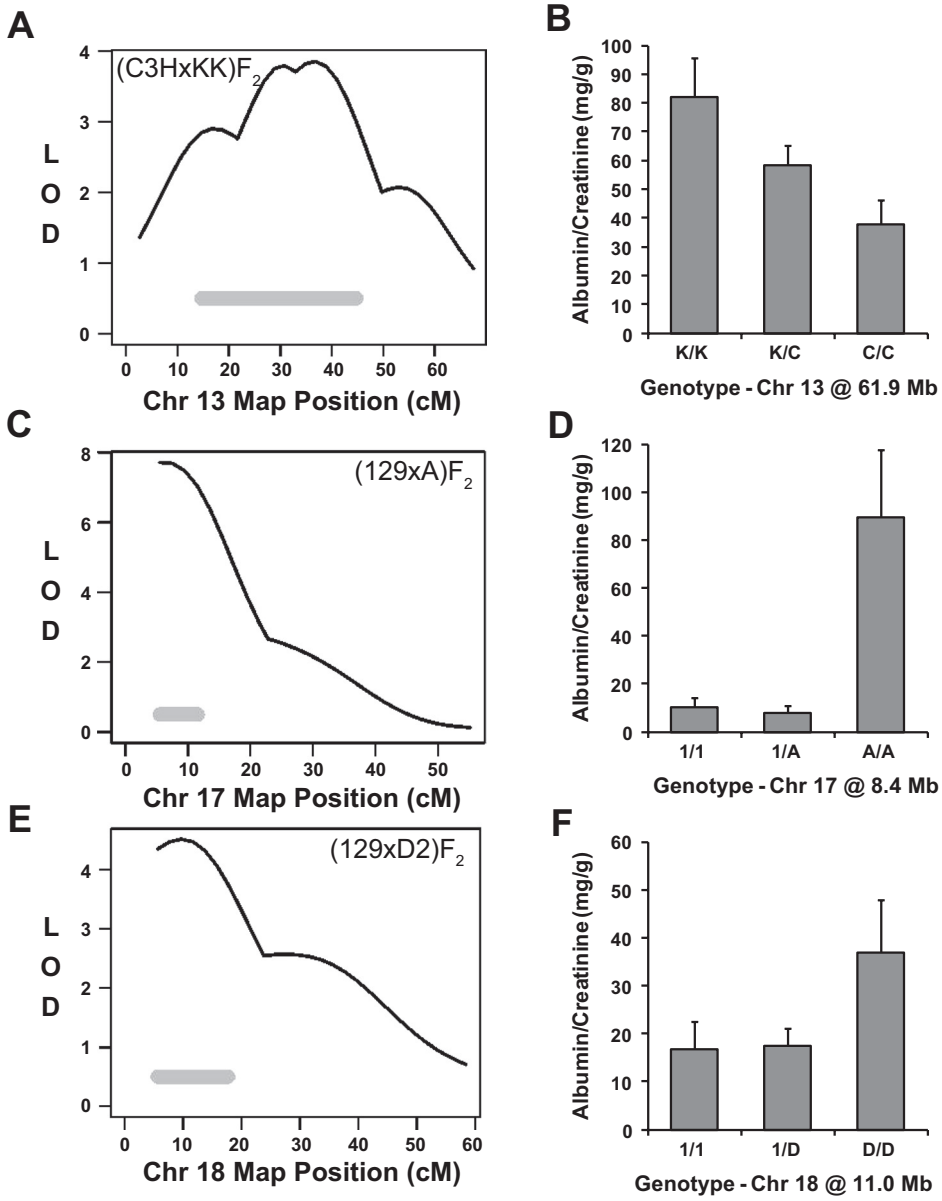


Fig. 5. Mapping of albuminuria QTL on Chromosomes 13, 17, and 18. Mapping and allelic effect plots for albuminuria QTL in the (C3H × KK)$F_2$ (*A* and *B*), (129 × A)$F_2$ (*C* and *D*), and (129 × D2)$F_2$ (*E* and *F*) populations. Albumin-to-creatinine ratios are expressed as means ± SE. The solid horizontal gray bars represent the Bayesian confidence intervals.

significant expression differences between BTBR and SWR mice. One of these 11 genes, *Glcci1* (glucocorticoid-induced transcript 1), is known to affect podocyte structure and proteinuria (35).

## DISCUSSION

The overall goal of this study was to investigate the genetic basis of albuminuria in mice, and we employed several experimental strategies to accomplish this. First, we measured urinary ACR in mice from 33 inbred strains and used the strain survey data for HAM. Second, we designed eight experimental QTL crosses between genetically diverse mice to identify novel QTL underlying albuminuria; we also performed one cross with closely related DBA/1 and DBA/2 mice to refine some of the QTL intervals. Finally, we mapped albuminuria in pre-CC mice derived from inbreeding the progeny of an eight-way intercross of inbred mice. Each of these experimental strategies detected genomic regions that contribute to albuminuria, but we found surprisingly little overlap between the results from the different methods, which limited our power to narrow the intervals using bioinformatics methods (15).

All but one of the strain pairs used to produce the $F_2$ populations had a significant difference in ACR (Table 2), although the two crosses with the smallest ACR differences failed to detect any significant QTL affecting albuminuria. Each of the remaining intercrosses identified one QTL underlying albuminuria, nearly all of which are novel QTL affecting this phenotype. None of these albuminuria QTL overlapped the blood pressure QTL detected within the same population (18), suggesting that the genetic factors regulating blood pressure are independent from those affecting albuminuria.

This study highlights the power of the CC for precisely mapping complex traits, even traits with highly skewed distri-

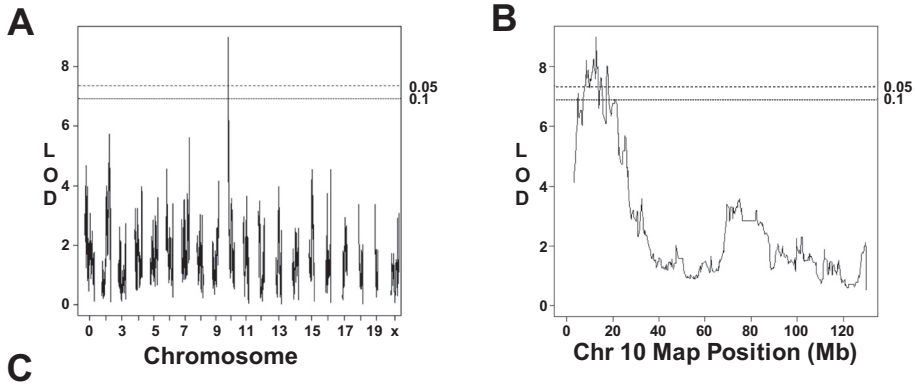


Fig. 6. Mapping of albuminuria QTL in precollaborative cross mice (pre-CC). *A*: genomewide scan for albuminuria QTL. Suggestive ($P < 0.10$) and significant ($P < 0.05$) LOD scores, as determined by permutation testing, are shown as dotted lines. *B*: fine mapping plot of Chr 10 QTL in the pre-CC population. *C*: allelic effects for the Chr 10 QTL were calculated using haplotypes diagnostic for each of the 8 founder strains at the SNPs significantly linked to albuminuria in the pre-CC population.

C

| SNP | cM | Mb | LOD | Strain *P* value | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 129 | A | B6 | CAST | NOD | WSB | NZO | PWK |
| rs49948081 | 3.01 | 8.74 | 8.21 | 0.498 | **0.012** | 0.0744 | 0.088 | 0.132 | **0.0009** | 0.108 | 0.473 |
| rs29334401 | 3.74 | 11.73 | 8.26 | 0.706 | **0.013** | 0.752 | 0.158 | 0.342 | **0.004** | 0.092 | 0.545 |

butions like albuminuria. Whereas the $F_2$ populations identified QTL with 20- to 70-Mb confidence intervals, the pre-CC population detected a 5-Mb QTL interval despite having only about half as many mice as the $F_2$ populations. Additionally, the eight-way cross design used to produce pre-CC mice provides genetic diversity beyond that of common inbred laboratory strains (37), and the pre-CC population detected an albuminuria QTL on Chr 10 that was not identified in any of the $F_2$ populations. Future studies using final CC strains or The Jackson Laboratory Diversity Outcross population derived by outcrossing CC mice should be well powered to detect narrow QTL affecting diverse phenotypes.

Linkage studies using rodent models can guide the genetic analysis of the corresponding disease in humans. For example, we previously identified a mouse blood pressure QTL on Chr 1 that was concordant to blood pressure QTL in both rats and humans (14). Chang et al. (8) further investigated this region using human linkage and association studies and found three genes significantly associated with blood pressure; these genetic associations were confirmed by Faruque et al. (17) using a separate human population. Similarly, albuminuria QTL identified in mice or rats are often concordant with human kidney disease QTL (19, 27), and we found that the region of the human genome corresponding to each albuminuria QTL

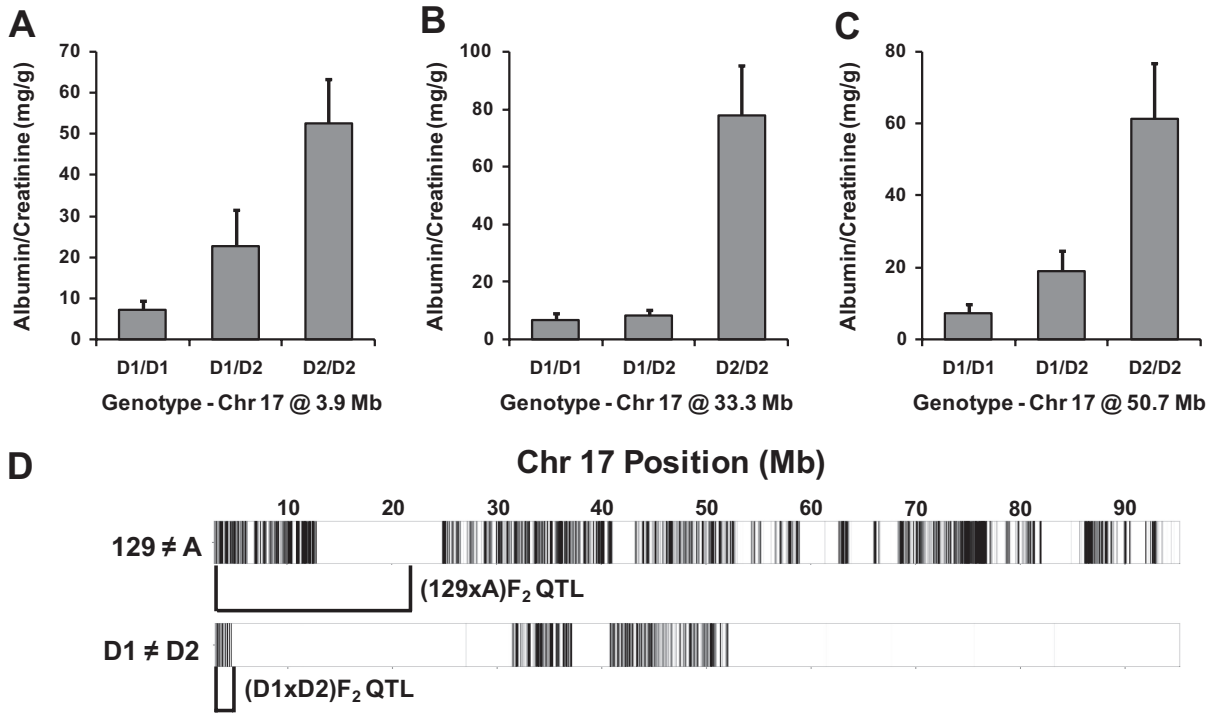


Fig. 7. (D1 × D2)$F_2$ population substantially narrows *Albq16*. Allelic effect plots for Chr 17 albuminuria QTL in the (D1 × D2)$F_2$ population (*A*, *B*, and *C*) are shown; albumin-to-creatinine ratios are expressed as means ± SE. *D*: comparison of Chr 17 SNPs (represented by vertical black lines) in the (D1 × D2)$F_2$ and (129 × A)$F_2$ populations refined the proximal Chr 17 QTL to a small interval containing 4 genes.

Table 4. *Top pathways positively and negatively correlated with albuminuria in inbred mice*

| Term | Count | Percent | Fold Enrichment | *P* Value | Benjamini Hochberg |
|---|---|---|---|---|---|
| Negatively correlated | | | | | |
| GO:0007155 (cell adhesion) | 8 | 11.94 | 4.40 | $1.74 \times 10^{-3}$ | 0.44 |
| GO:0022610 (biological adhesion) | 8 | 11.94 | 4.40 | $1.76 \times 10^{-3}$ | 0.25 |
| GO:0016337 (cell-cell adhesion) | 5 | 7.46 | 6.54 | $6.44 \times 10^{-3}$ | 0.51 |
| GO:0030030 (cell projection organization) | 5 | 7.46 | 4.84 | $1.79 \times 10^{-2}$ | 0.77 |
| GO:0044421 (extracellular region part) | 7 | 10.45 | 2.41 | $6.27 \times 10^{-2}$ | 1.00 |
| Positively correlated | | | | | |
| GO:0006091 (generation of precursor metabolites and energy) | 12 | 17.91 | 14.20 | $3.55 \times 10^{-10}$ | $1.38 \times 10^{-7}$ |
| GO:0044429 (mitochondrial part) | 12 | 17.91 | 6.66 | $8.28 \times 10^{-7}$ | $1.04 \times 10^{-4}$ |
| GO:0006119 (oxidative phosphorylation) | 6 | 8.96 | 33.09 | $8.46 \times 10^{-7}$ | $1.64 \times 10^{-4}$ |
| GO:0005739 (mitochondrion) | 17 | 25.37 | 3.74 | $2.56 \times 10^{-6}$ | $1.61 \times 10^{-4}$ |
| GO:0031966 (mitochondrial membrane) | 10 | 14.93 | 7.90 | $2.84 \times 10^{-6}$ | $1.19 \times 10^{-4}$ |
| mmu00190 (oxidative phosphorylation) | 8 | 11.94 | 11.03 | $4.36 \times 10^{-6}$ | $2.05 \times 10^{-4}$ |
| GO:0005740 (mitochondrial envelope) | 10 | 14.93 | 7.44 | $4.66 \times 10^{-6}$ | $1.47 \times 10^{-4}$ |
| GO:0005743 (mitochondrial inner membrane) | 9 | 13.43 | 8.84 | $5.26 \times 10^{-6}$ | $1.33 \times 10^{-4}$ |
| GO:0015985 (energy-coupled proton transport, down electrochemical gradient) | 5 | 7.46 | 41.73 | $5.32 \times 10^{-6}$ | $6.88 \times 10^{-4}$ |
| GO:0015986 (ATP synthesis-coupled proton transport) | 5 | 7.46 | 41.73 | $5.32 \times 10^{-6}$ | $6.88 \times 10^{-4}$ |

identified in the intercross populations was linked to kidney disease in humans (Table 3). *Albq15* on Chr 13 is concordant to human kidney disease QTL on Chr 9q22 (32) and 5q14 (28); *AUH* on Chr 9 is significantly associated with human DN (30), and *SLC34A1* is significantly associated with incident kidney disease (2). On Chr 17, we identified an albuminuria QTL that is concordant with a locus on Chr 6p21 linked to albuminuria in humans (32). We separated this QTL (*Albq16*) interval into two loci using the $(D1 \times D2)F_2$ population; the distal QTL is concordant to a human region containing *GRIK2*, a gene associated with DN, and the proximal locus is concordant to a human locus linked to renal function (9). The proximal Chr 17 QTL contains only a few genes, including *Nox3*, which encodes NADPH oxidase 3. *Nox3* represents a strong candidate gene for this albuminuria QTL because inhibition of NADPH oxidase can ameliorate albuminuria and renal morphological changes in diabetic animals (41). Finally, the albuminuria QTL detected on Chr 10 in the pre-CC population (*Albq18*) contains the mouse ortholog of human *SASH1*, which is significantly associated with DN in humans (30). These findings suggest evolutionary conservation of kidney disease genes between rodents and humans.

We also identified candidate genes for these QTL by evaluating renal gene expression in mice from 26 inbred strains. Using correlation and enrichment analysis, we found that downregulation of cell adhesion genes as well as upregulation of pathways affecting mitochondrial structure and function were highly correlated with albuminuria across the 26 mouse strains. In addition to uncovering these general pathways correlated with albuminuria in mice, we also identified specific genes within each QTL interval that exhibited significant differences in renal expression. One intriguing candidate gene with a renal expression difference between BTBR and SWR mice is *Glcci1*, which is expressed in mouse and zebrafish mesangial cells and podocytes, including foot processes (35). Morpholino knock down of *Glcci1* expression in zebrafish induced podocyte effacement and proteinuria (35), indicating that reduced *Glcci1* expression can be a causal factor affecting renal structure. The authors also observed decreased glomerular *Glcci1* expression in mice with either adriamycin or lipopolysaccharide-induced nephropathy, as well as in *db/db* diabetic mice (35). These data implicate *Glcci1* as a strong candidate gene for *Albq13*.

The findings from our studies represent a substantial advance in our understanding of the genetic basis of albuminuria. Nearly all of the QTL detected in these populations are novel albuminuria QTL that are concordant with loci affecting kidney disease in humans, suggesting that future crosses may detect other new albuminuria QTL. We also used an additional mouse cross between genetically similar strains to refine the QTL intervals, which substantially narrowed the proximal Chr 17 QTL to a small region. Gene expression analysis identified several molecular pathways and candidate genes that could underlie the development of albuminuria. Future studies to fine map the remaining albuminuria QTL and identify the causal genes may elucidate novel pathways affecting kidney disease in both mice and humans.

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## AUTHOR CONTRIBUTIONS

Author contributions: J.T., M.F., L.Y., S.S., L.X., and K.J.D. performed experiments; J.T., Y.Z., G.A.C., and K.J.D. approved final version of manuscript; S.-W.T., V.P., Y.Z., G.A.C., and K.J.D. analyzed data; Y.Z., E.J.C., G.A.C., and K.J.D. interpreted results of experiments; L.X.,

D.R.M., E.J.C., G.A.C., and K.J.D. edited and revised manuscript; D.R.M., B.P., E.J.C., G.A.C., and K.J.D. conception and design of research; K.J.D. prepared figures; K.J.D. drafted manuscript.

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