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Quantitative Trait Loci for Urinary Albumin in Crosses Between C57BL/6J and A/J Inbred Mice in the Presence and Absence of *Apoe*

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ABSTRACT

We investigated the effect of apolipoprotein E (Apoe) on albuminuria in the males of two independent F_2 intercrosses between C57BL/6J and A/J mice, using wild-type inbred strains in the first cross and B6-*Apoe*$^{-/-}$ animals in the second cross. In the first cross, we identified three quantitative trait loci (QTL): chromosome (Chr) 2 [LOD 3.5, peak at 70 cM, confidence interval (C.I.) 28–88 cM]; Chr 9 (LOD 2.0, peak 5 cM, C.I. 5–25 cM); and Chr 19 (LOD 1.9, peak 49 cM, C.I. 23–54 cM). The Chr 2 and Chr 19 QTL were concordant with previously found QTL for renal damage in rat and human. The Chr 9 QTL was concordant with a locus found in rat. The second cross, testing only *Apoe*$^{-/-}$ progeny, did not identify any of these loci, but detected two other loci on Chr 4 (LOD 3.2, peak 54 cM, C.I. 29–73 cM) and Chr 6 (LOD 2.6, peak 33 cM, C.I. 11–61 cM), one of which was concordant with a QTL found in rat. The dependence of QTL detection on the presence of *Apoe* and the concordance of these QTL with rat and human kidney disease QTL suggest that *Apoe* plays a role in renal damage.

CHRONIC kidney disease is a growing medical problem caused by various environmental and genetic factors. Identifying genes underlying common forms of kidney disease in humans has proven difficult, expensive, and time consuming. However, quantitative trait loci (QTL) for several complex traits are concordant among mice, rats, and humans, suggesting that genetic findings from animal models are relevant to human disease (Stoll *et al.* 2000; Sugiyama *et al.* 2001; Wang and Paigen 2002a,b). This has been supported by the discovery of human disease genes from candidates identified in mouse (Korstanje *et al.* 2004a; Hillebrandt *et al.* 2005). With respect to chronic kidney disease, QTL analysis using mice is likely to contribute new findings in the near future (Korstanje and DiPetrillo 2004).

The gene encoding apolipoprotein E (*APOE*) has been implicated in chronic kidney disease. Several human association studies have shown an association of *APOE* with renal damage parameters in different groups of patients (Werle *et al.* 1998; Araki *et al.* 2000; Joss *et al.* 2005). Additionally, we found an association between serum APOE levels and albuminuria in the general population (R. Korstanje, unpublished data). Recently, a direct, lipid independent role for APOE in the kidney and involvement in renal disease has been suggested. Chen *et al.* (2001) showed that APOE regulates mesangial cell proliferation in a dose-dependent fashion. APOE has an antiproliferative effect specific for mesangial cells (not endothelial cells) through the induction of matrix heparin sulfate proteoglycan (HSPG) (Chen *et al.* 2001). Thus, varying APOE levels or genetic variation in the APOE receptors involved in this mechanism could be expected to have an important effect on renal function and disease.

Studies of kidney disease involving *Apoe*$^{-/-}$ mice have led to contrasting results. In the study by Wen *et al.* (2002), *Apoe*$^{-/-}$ mice developed spontaneous glomerular lesions with macrophage accumulation, commonly with foam cell appearance, deposition of extracellular matrix, glomerular hyperplasia, and foci of mesangiolysis associated with capillary microaneurysms. On the other hand, in studies using uninephrectomy (UNX) and subtotal nephrectomy (SNX) on wild-type and *Apoe*$^{-/-}$ mice, Buzello *et al.* (2004) did not observe a difference in renal or glomerular injury after reduction of renal mass.

The aim of the current study is to investigate whether presence or absence of APOE would have an effect on the genetic susceptibility to albuminuria in mice. To this purpose we performed two independent F_2 intercrosses between C57BL/6J mice, which do not develop albuminuria, and A/J mice, which do develop albuminuria (Qi *et al.* 2005). In the first cross only wild-type inbred

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strains B6 and A/J were used; in the second cross B6.*Apoe*$^{-/-}$ animals were crossed to A/J mice and only the *Apoe*$^{-/-}$ F_2 animals were analyzed. We expected that the combination of *Apoe* deficiency and alleles from the susceptible A/J strain would allow us to map loci involved in the difference in susceptibility to kidney disease between B6 and A/J mice, as well as the effect of APOE on these alleles.

MATERIALS AND METHODS

Mice and phenotype characterization: A/J, C57BL/6J (B6), and B6-129P2-Apoe^{tm1Unc}/J (B6-Apoe$^{-/-}$) mice, which were backcrossed at least 12 times to B6 and are now at N12F13, were obtained from The Jackson Laboratory (Bar Harbor, ME). A/J females were mated to B6 (cross 1) or B6-Apoe$^{-/-}$ (cross 2) males to produce the F_1 progeny; F_1 mice were intercrossed to produce 381 male F_2 (cross 1) and 145 male F_2-Apoe$^{-/-}$ (cross 2) progeny. Mice were housed in a climate-controlled facility with a 14-hour:10-hour light–dark cycle with free access to food and water throughout the experiment. After weaning, mice were maintained on a chow diet (Old Guilford 234A, Guilford, CT). Spot urine samples were taken between 8 and 10 weeks, and albumin and creatinine concentrations were measured on a Beckman Synchron CX5 chemistry analyzer. Actual mouse albumin concentrations were calculated by linear regression from a standard curve generated with mouse albumin standards (Kamiya Biomedical, Seattle) (Grindle *et al.* 2006). All experiments were approved by The Jackson Laboratory's Animal Care and Use Committee.

Genotyping: DNA was isolated as described previously (Korstanje *et al.* 2004b). Each F_2 animal was genotyped using 98 SNPs (cross 1) or 140 SNPs (cross 2) polymorphic between A/J and B6 covering the whole genome (supplemental Tables 1 and 2). Genotyping was performed by KBiosciences (Hoddesdon, UK) using the Amplifluor chemistry (Serologicals, Norcross, GA).

QTL mapping and statistics: QTL analysis and genome scans were carried out using the scanone and bayesint functions of the R/qtl package (Broman *et al.* 2003). Urinary albumin presents a highly skewed, two-part distribution in which many individuals have a score of zero (Figure 1). Broman (2003) studied the problem of mapping such phenotypes and concluded that the nonparametric procedure (Kruglyak and Lander 1995) provides the most reliable analysis. Thus, we used the nonparametric method to analyze urinary albumin as a binary trait (Alb = 0 *vs.* Alb > 0). Significance of QTL LOD scores was assessed using 1000 permutations of the phenotypic data (Churchill and Doerge 1994). Significant QTL are reported at the genomewide adjusted 0.05 level (LOD 2.97 in cross 1 and LOD 3.35 in cross 2) and suggestive QTL at genomewide 0.63 level (LOD 1.93 in cross 1 and LOD 2.01 in cross 2) (Lander and Kruglyak 1995). Bayesian credible intervals for particular chromosomes are computed and reported as confidence intervals. The 10^{LOD} is taken, rescaled to have area 1, and followed by calculating the connected interval with density above threshold having coverage matching the target probability at 0.96. The nonparametric analysis presents some limitations. It is difficult to estimate effect sizes of the QTL, multiple QTL modeling is not available, and adjustment for covariates such as sex cannot be made. Nonetheless, it is justified in the case of such an extreme phenotype distribution. Due to the extreme skew in the distribution of albumin levels, the pairscans, to test for epistasis, were highly unstable. A number of transformations were tried but did not lead to reliable results. The mode of inheritance was determined by performing an ANOVA on the mean values for the three genotypes.

TABLE 1

Renal parameters of male parental and F_2 animals from both crosses

	N	Albumin/creatinine (mg/g ± SD)
B6	4	12.5 ± 7.2
B6-*Apoe*$^{-/-}$	18	11.6 ± 4.4
A/J	4	118.2 ± 66.4
F_2 (wild type)	381	8.9 ± 35.1
F_2-*Apoe*$^{-/-}$	145	13.2 ± 2.4

RESULTS

Renal parameters in parental and F_2 animals: Table 1 shows the values for the male parental and F_2 animals. In each group, the males were more susceptible to albuminuria than the females, which had almost no detectable albuminuria (data not shown) and therefore were not analyzed in the F_2. In inbred B6 mice, the presence or absence of *Apoe* did not affect albuminuria.

Nonparametric analyses of albuminuria: Because most F_2 animals did not have detectable urinary albumin concentrations, the trait was not normally distributed (Figure 1). Therefore, we used the presence or

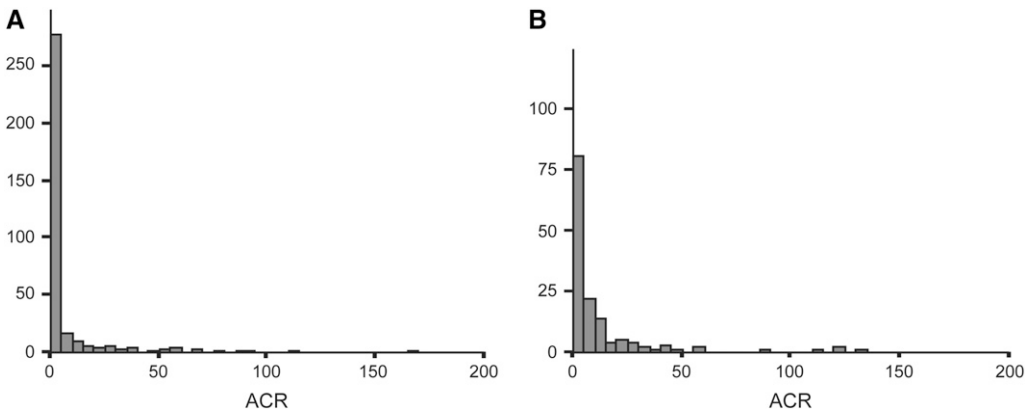

Figure 1.—Distribution of the albumin/creatinine ratio (ACR) in cross 1 (A) and cross 2 (B).

TABLE 2

QTL identified for single gene genomewide scans of the two crosses

Cross	Chr[a]	Peak (cM)	95% C.I. (cM)	Locus name	LOD[b]	Susc. allele, (inheritance)[c]	Nearest marker
1	2	70	28–88	*Albq1*	3.5	A (rec)	02-139603599-M
	9	5	5–25	*Albq2*	2.0	Het	09-013396060-N
	19	24	12–27	*Albq3*	2.1	A and B6	19-059089086-M
2	4	54	29–73	*Albq4*	3.2	A (dom)	04-107204545-N
	6	29	11–61		2.6	A (rec)	06-076285738-M

[a] Chromosome.
[b] Significance of QTL LOD scores was assessed using 1000 permutations of the phenotypic data (Churchill and Doerge 1994). Significant QTL are reported at the genomewide adjusted 0.05 level (LOD 2.97 in cross 1 and LOD 3.35 in cross 2) and suggestive QTL at genomewide 0.63 level (LOD 1.93 in cross 1 and LOD 2.01 in cross 2) (Lander and Kruglyak 1995).
[c] Susceptible allele and likely mode of inheritance: rec, recessive; dom, dominant.

absence of detectable urinary albumin as a binary phenotype and performed nonparametric analyses to identify loci involved in the phenotype. An overview of the QTL found in both crosses is given in Table 2, which provides the QTL peak, 95% confidence interval, locus name, LOD score, allele conferring the high value, and nearest SNP marker. The QTL were named if they were significant (LOD 2.97 in cross 1 and LOD 3.35 in cross 2) or if they were suggestive (LOD 1.93 in cross 1 and LOD 2.01 in cross 2) and found previously as homologous QTL in rat crosses. In the analysis of cross 1 we detected three loci (Figure 2A). A significant locus was found on chromosome (Chr) 2 (LOD = 3.5), while two suggestive loci were found on Chr 9 (LOD = 2.0) and Chr 19 (LOD = 1.9). The allele-effect plots of the three loci (Figure 3) show a recessive high allele from A/J at the Chr 2 locus, while for both the Chr 9 and 19 loci the heterozygotes represent the respectively susceptible and resistant phenotype. We found weak statistical evidence for a second QTL on Chr 2 (1-QTL LOD = 3.46 and 2-QTL LOD = 4.95, difference = 1.49, nominal *P*-value for $\chi^2 = 0.03$). However, the tests for linked QTL were underpowered and, although we cannot provide strong evidence to support a second QTL, we cannot rule it out. In the analysis of cross 2 none of these three loci was detected. Instead, two suggestive loci on Chr 4 (LOD = 3.2) and Chr 6 (LOD = 2.6) were found (Figure 2B). For both loci the A/J allele represents the susceptible phenotype but the allele is dominant at the Chr 4 locus and recessive at the Chr 6 locus (Figure 4). We reduced the interval of *Albq1* by applying the bioinformatics strategy described by DiPetrillo *et al.* (2005). First we reduced the 95% C.I. by comparative mapping with the rat and human genome using albuminuria as the kidney disease phenotype in all three species (Figure 5). The locus is concordant with the rat *Renal failure 3* (*Rf3*) locus on rat Chr 3 described by Shiozawa *et al.* (2000) and a locus on 20p13 associated with albuminuria in

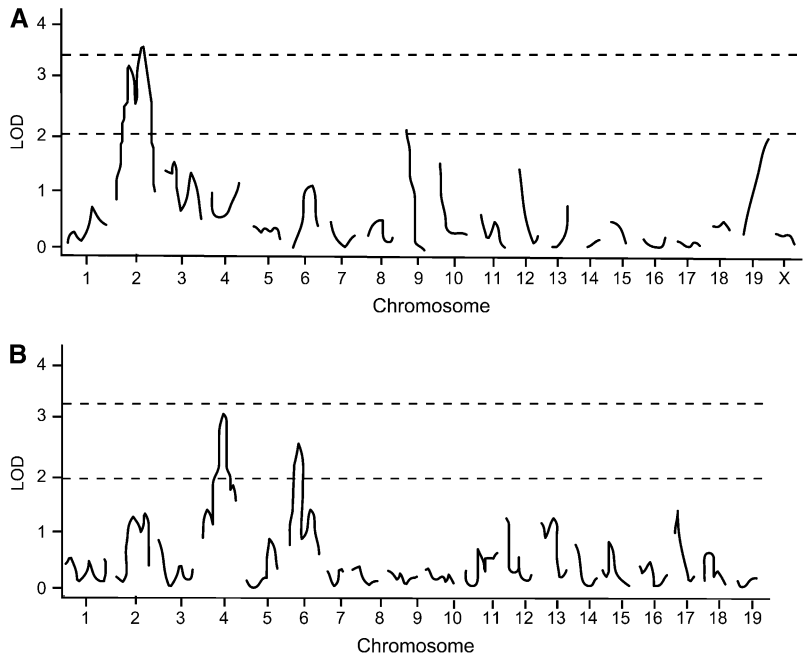

Figure 2.—Genomewide scans for albuminuria using nonparametric analysis for (A) cross 1 and (B) cross 2. The dotted line at the top depicts a significant LOD score ($P < 0.05$) and the dotted line at the bottom a suggestive LOD score ($P < 0.63$).

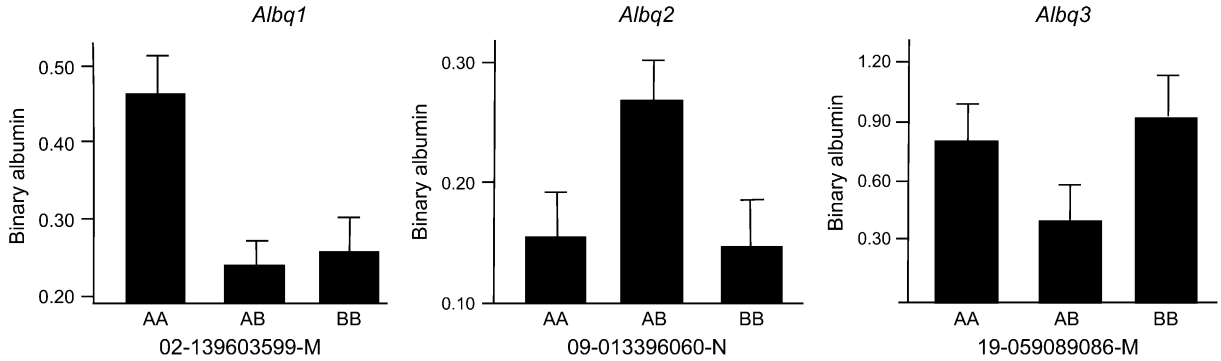

Figure 3.—Allele effects for the loci found in cross 1. Homozygosity for A/J alleles is represented by AA, homozygosity for C57BL/6J alleles by BB, and heterozygosity by AB. The SNP at which the allele effect is determined is shown under each graph.

humans (Imperatore *et al.* 1998). This approach reduced the 95% C.I. of the mouse locus from 124 Mb [containing 1570 genes according to the Ensembl database (release 42)], to 51 Mb (661 genes). Second, we used the Jackson Laboratory's SNP databases (http://www.jax.org/phenome) to compare B6 and A/J haplotypes. When we exclude the regions that are identical by descent between the two strains, we can reduce the interval to three small regions with different haplotypes, which are 2, 9, and 24 Mb, respectively. Together, these regions contain 343 genes according to the Ensembl database (release 42, December 2006). As a QTL for albuminuria was previously reported in a backcross between BALB/c and KK/Ta (Shike *et al.* 2005), we investigated the possiblity of further narrowing the region using SNP data on BALB/cByJ and KK/HiJ. Although this cross confirmed our mapping results, it did not improve resolution.

DISCUSSION

The gene encoding apolipoprotein E (*APOE*) has been implicated in chronic kidney disease. Therefore, the aim of this study was to investigate whether the presence or absence of APOE would have an effect on the genes involved in kidney damage in mice. To achieve this, we crossed two strains, B6 and A/J, which are known to be near the extremes of the urinary albumin spectrum for inbred strains (Qi *et al.* 2005; K. DiPetrillo, unpublished results). We used wild-type B6 and A/J mice, which have identical *Apoe* alleles according to their genome sequence, for the first cross, whereas we used the B6-*Apoe*$^{-/-}$ knockout mice for the second cross and selected only the homozygous knockout mice in the F_2 population.

Analysis of cross 1 between A/J and B6 mice identified one significant locus and two suggestive loci; in cross 2 between A/J and B6-*Apoe*$^{-/-}$ mice, these loci did not reach the suggestive level and two different loci were identified. The significant Chr 2 locus maps to the same region as the previously reported albuminuria QTL that was found in a KK/Ta × (BALB/c × KK/Ta) F_1 backcross (Shike *et al.* 2005) with a similar LOD score (3.5). In a review, we previously discussed concordance of renal damage loci between mouse, rat, and human (Korstanje and DiPetrillo 2004). Most of the loci found in the current crosses are concordant with rat loci for renal parameters (Table 3). The Chr 2 region is homologous with the distal part of rat Chr 3 containing QTL for kidney mass and renal function (Shiozawa

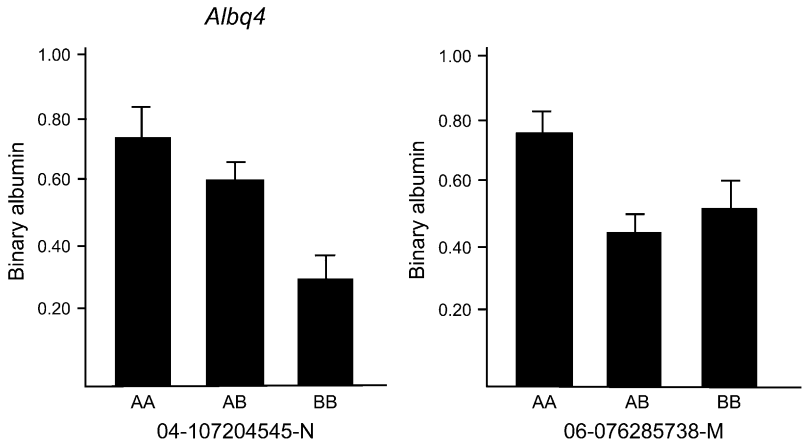

Figure 4.—Allele effects for the loci found in cross 2. Homozygosity for A/J alleles is represented by AA, homozygosity for C57BL/6J alleles by BB, and heterozygosity by AB. The SNP at which the allele effect is determined is shown under each graph.

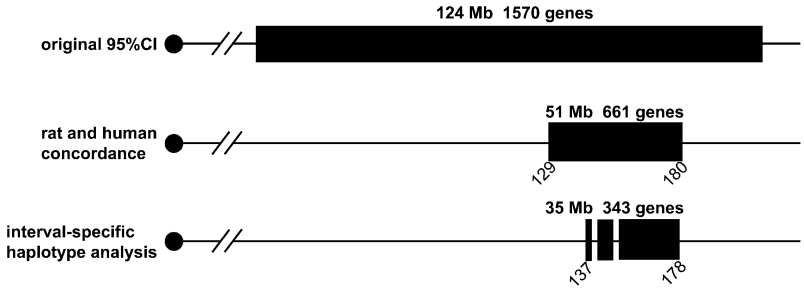

FIGURE 5.—Using comparative genomics and interval-specific haplotype analysis to narrow the chromosome 2 QTL. Comparing the C.I. of our cross with the interval on rat Chr 3 found in the (FHH × ACI) F_2 intercross and the human chromosome 20 interval narrowed the region to 51 Mb. Interval-specific haplotype analysis of this region, comparing SNPs between B6 and A, resulted in three regions that have different haplotypes between the strains and contain 343 genes according to the Ensembl database (release 42).

et al. 2000). The homologous human region (20p13) is also associated with albuminuria (IMPERATORE *et al.* 1998). Using this concordance to narrow the interval region by comparative mapping significantly reduces the number of candidate genes (Figure 5). The Chr 9 locus is homologous to a part of rat Chr 8 to which a QTL for urinary albumin excretion was mapped in three independent crosses (POYAN MEHR *et al.* 2003; SCHULZ *et al.* 2003). The QTL mapped to Chr 19 is concordant with the rat *Renal failure 1* (*Rf1*) locus on Chr 1, which was mapped in four independent rat crosses (BROWN *et al.* 1998; SHIOZAWA *et al.* 2000; GARRETT *et al.* 2003; SCHULZ *et al.* 2003) and is also concordant with a human locus (19q13) affecting renal disease (FREEDMAN *et al.* 2002) and creatinine clearance (HUNT *et al.* 2002). Finally, the Chr 4 region found in cross 2 is concordant with QTL for renal function (MORENO *et al.* 2003), kidney mass (UENO *et al.* 2003), and renin concentration (BILUSIC *et al.* 2004) mapped to the homologous region on rat Chr 5. Because we performed the QTL analysis on two independent crosses, there might be some confounders that could lead to differences in the analysis. For this study, confounders such as different mouse rooms (in the same building) and different seasonal effects could influence the results. However, we do not believe that the variability caused by these factors substantially affects the results. Evidence for this comes from comparing published QTL crosses for blood pressure in B × A populations (SUGIYAMA *et al.* 2001; WOO and KURTZ 2003). Although the independent crosses were conducted at different institutions under different dietary and environmental conditions—one cross used only males and the other used both sexes, the population sizes differed by more than fourfold, and one cross was a backcross while the other an intercross—the experiments produced similar results. The QTL with the highest LOD scores identified by WOO and KURTZ (2003) were also detected by SUGIYAMA *et al.* (2001), using one-quarter the population size. The differences between the crosses in our study were fewer than between these published crosses. Thus, we believe that the presence or absence of Apoe is the primary reason for the different QTL identified in cross 1 *vs.* cross 2, suggesting that the absence of APOE modifies the QTL involved in albuminuria. The strain that we used has been backcrossed 12 times, leaving a theoretical 0.05% of the 129 genome in the mice. Although chances are small, it would be possible for a 129 allele to contribute to the susceptibility (or resistance) to albuminuria. We do not have data of a cross between A/J and 129 to test this. However, we do have data from a cross between B6 and 129 for albuminuria, in which a QTL was found on Chr18 (S. SHEEHAN, unpublished results). This suggests that the only difference between B6 and 129 with respect to albuminuria is on Chr 18; we did not observe this QTL in our crosses (Figure 2).

APOE, a component of very low-density lipoprotein (VLDL) particles as they are secreted from the liver, is acquired by chylomicrons soon after their synthesis and secretion by the small intestine (MAHLEY and RALL 2000). APOE accomplishes its lipid transport and delivery function mainly through binding to several receptors like the LDL receptor (LDLR), VLDL receptor (VLDLR), and megalin (LRP2). This binding of lipoproteins to the receptors is enhanced by lipoprotein li-

TABLE 3

Concordance between the mouse urinary albumin QTL and QTL found for related phenotypes in rat and human

QTL	Chr	Peak (cM)	Concordance in rat	Concordance in human
Albq1	2	70	Chr 3 for UAE, UPE	Chr 20p13 for ACR
Albq2	9	5	Chr 8 for UAE, UPE	
Albq3	19	24	Chr 1 for UAE, UPE	Chr 10q23 for ESRD, CC
Albq4	4	54	Chr 5 for CC, KM	

UAE, urinary albumin excretion; UPE, urinary protein excretion; ACR, urinary albumin-to-creatinine ratio; ESRD, end-stage renal disease; CC, creatinine clearance; KM, kidney mass.

pase (LPL) (Stevenson *et al.* 2001). Both the receptors and LPL have been implicated previously in renal damage (Leheste *et al.* 1999; Sato *et al.* 2002). In addition to its possible role in the kidney through lipid metabolism, recent data show that APOE also has direct affects on mesangial cell proliferation and extracellular matrix expansion. Mesangial expansion is a key feature in the pathogenesis of renal diseases (Couser and Johnson 1994). Moreover, *Apoe* knockout mice show increased mesangial cell proliferation and matrix formation compared with wild-type mice or *Apob*-overexpressing mice, which have elevated plasma cholesterol and triglycerides. This suggests that lack of APOE, rather than hyperlipidemia, contributes to mesangial expansion (Chen *et al.* 2001). In line with these findings, *in vitro* experiments show that APOE inhibits mesangial cell proliferation and apoptosis induced by oxidized LDL. In addition, APOE induces the mesangial matrix heparin sulfate proteoglycan (Chen *et al.* 2001). Loss of anionic heparin sulfate proteoglycan in the basement membrane and mesangial matrix is associated with disruption of the filtration barrier (Tamsma *et al.* 1994).

Finding one significant QTL in the presence of *Apoe* and only two (different) suggestive QTL in the absence of *Apoe* suggests that the underlying gene of the Chr 2 QTL is dependent on *Apoe* to cause increased albumin/creatine ratio (ACR). When APOE is absent, this dependency gets lost and the Chr 2 locus no longer contributes to the difference in susceptibility. Instead, ACR is determined by other (*Apoe*-independent) loci.

In conclusion, we detected five loci using nonparametric analyses for albuminuria in a cross between A/J and either B6 or B6-*Apoe*$^{-/-}$ mice. However, the loci detected depend on the presence or absence of APOE. Only one of the loci (Chr 2) was significant, but all loci, except for the locus on Chr 6, are concordant with QTL for renal damage in rat, and two of the loci (Chr 2 and Chr 19) are concordant with associations found in human. The dependence on APOE suggests the involvement of this pathway in renal disease.

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