Quantitative Trait Loci Associated with Susceptibility to Bladder and Kidney Infections Induced by *Escherichia coli* in Female C3H/HeJ Mice

Walter J. Hopkins,1 Johny Elkahwaji,4 Christina Kendziorski,2 Amy R. Moser,3 Paulette M. Briggs,1 and Kaleigh A. Suhs1

Departments of 1Urology, 2Biostatistics and Medical Informatics, and 4Human Oncology, University of Wisconsin School of Medicine and Public Health, Madison; 3Department of Surgery, University of Nebraska Medical Center, Omaha

**Background.** The C3H/HeJ mouse strain develops severe bladder and kidney infections after receiving intravesical inoculation with uropathogenic *Escherichia coli*. This susceptibility is genetically determined, but the specific genes involved have not been completely defined. The objective of the present study was to use quantitative trait locus (QTL) mapping to identify chromosomal sites associated with susceptibility to infection in C3H/HeJ mice.

**Methods.** Female mice from a backcross of C3H/HeJ and (BALB/c x C3H/HeJ)F1 mice were inoculated with *E. coli*, and the number of *E. coli* colony-forming units present in the bladder and kidneys was quantified 10 days later. Genomic DNA was scanned using microsatellite markers to localize chromosomal segments derived from parental strains. Statistical analyses associated infection phenotypes with chromosomal sites.

**Results.** A highly significant QTL for susceptibility to bladder infection was identified on chromosome 4, and C3H/HeJ alleles at this locus interacted with BALB/c alleles on chromosome 19 to increase the severity of infection. A significant QTL on chromosome 6 was associated with severe kidney infections.

**Conclusions.** Increased susceptibility to *E. coli* bladder and kidney infections in female C3H/HeJ mice is associated with specific chromosomal sites located near genes contributing to host resistance to infection. The results demonstrate the multigenic nature of susceptibility to urinary tract infections.

It has been estimated that urinary tract infections (UTIs) occur in up to 50% of all women in their lifetimes, and 10%–15% of women experience recurrent infections that require ongoing medical care and may lead to pyelonephritis and renal scarring [1–4]. The exact reason for the increased susceptibility to recurrent infection among women prone to develop UTIs is largely unknown. A genetic predisposition is suggested by observations that recurrent UTIs occur in a limited segment of the female population and are seen more often within some families [5, 6].

An evaluation of candidate genes in women with recurrent UTIs did not detect disproportionate frequencies of major histocompatibility complex or red blood cell antigen phenotypes [7]; however, 2 more-recent reports noted a genetic basis for asymptomatic bacteriuria in children [8] and susceptibility to acute pyelonephritis [9]. Predisposition to asymptomatic bacteriuria was associated with reduced expression of Toll-like receptor 4 (TLR4) on neutrophils, and the development of acute pyelonephritis was associated with polymorphisms and mutations in the CXCR1 gene. Although these findings have identified correlations between individual genes and infections occurring in different organs, it is likely that, overall, increased susceptibility to UTIs is complex and multigenic.

Animal models of UTI have been valuable in defining host genetic factors contributing to both host resistance and increased susceptibility to infection. In particular, mouse models have shown that genetic background strongly influences the ability of an animal to resolve induced *Escherichia coli* bladder and kidney infections [10]. Female mice from the BALB/c, DBA, C3H/HeN,
and C57BL/6 inbred strains effectively clear bladder and kidney infections within 2 weeks after inoculation with uropathogenic E. coli and are resistant to kidney infections. In contrast, C3H/HeJ and C3H/HeOuJ mice develop bladder infections that are initially comparable in intensity to those that develop in resistant mouse strains but cannot be resolved over a 2-week period. These 2 susceptible mouse strains also develop severe, persistent kidney infections. Because each inbred strain is genetically distinct, the differential bladder and kidney infection phenotypes observed in the strains must be attributed to differences in the unique genetic background of each strain.

We have been particularly interested in the genetic basis of increased susceptibility to bladder and kidney infections due to E. coli in C3H/HeJ and C3H/HeOuJ mice. Mice from the C3H/HeJ strain carry a mutation in the Tlr4 gene that makes them unresponsive to the biological effects of bacterial lipopolysaccharide (LPS) [11], and the increased susceptibility of C3H/HeJ mice to E. coli UTIs has been attributed to the absence of TLR4-mediated inflammatory responses [12–14]. This model, however, does not account for the observation that C3H/HeOuJ mice, which have a normal Tlr4 gene and LPS responsiveness, develop E. coli bladder and kidney infections that are equivalent in severity and duration to those observed in C3H/HeJ mice. Possible explanations for these results are that (1) genes other than Tlr4 play a significant role in susceptibility to UTIs or (2) a gene closely linked to, and segregating with, Tlr4 decreases host resistance to UTI. Although there is a clear association between Tlr4 deficiency and the development of severe E. coli bladder and kidney infections in C3H/HeJ mice, a model based on multiple genes is more consistent with other infectious disease models and with the infection data for C3H/HeOuJ mice [15]. Thus, the primary objective of the current study was to identify in C3H/HeJ mice the chromosomal sites associated with increased susceptibility to induced E. coli UTIs.

One approach to identifying genes associated with a specific disease is to evaluate the effects of candidate genes on 1 or more phenotypes related to that disease. Although this strategy has been successfully applied in demonstrating a genetic predisposition to some infectious diseases, it is unlikely that all genes contributing to a phenotype can be determined a priori. In addition, statistical analyses must be adequately corrected for multiple comparisons before any positive results can be viewed with certainty [16]. A preferable, unbiased method of identifying genetic loci contributing to susceptibility to infection is genetic linkage analysis in which a correlation can be determined between a disease phenotype and genotypes at numerous chromosomal sites [17–19]. Quantitative trait locus (QTL) mapping is an established method by which to map the genetic loci that play a significant role in predisposition to and defense against infectious diseases in animal models [20–25].

The QTL mapping approach is well-suited for exploration of the genetic basis of susceptibility to E. coli bladder and kidney infections in C3H/HeJ mice, for several reasons. First, a previous study of the inheritance of susceptibility to UTIs in C3H/HeJ mice demonstrated that susceptibility to bladder and kidney infections was associated with genetically distinct, recessive traits and that multiple genes likely contributed to the infection phenotypes [26]. Second, the intensities of bladder and kidney infection observed in susceptible and resistant mouse strains are quantitative phenotypes with widely separated numerical values. Third, in statistical models, results of QTL mapping can be used to discover the interacting loci that contribute to susceptibility to infection. Thus, in this disease model, QTL mapping has the potential to identify both the main effect and the interacting loci associated with E. coli bladder and kidney infections in C3H/HeJ mice, as well as to provide insight into novel genetic pathways not previously suspected of playing a role in disease susceptibility.

The genetic linkage analysis conducted in the present study revealed that a highly significant QTL associated with susceptibility to E. coli bladder infection is located on chromosome 4 near the Tlr4 locus. This QTL interacts with another locus on chromosome 19 to increase the severity of bladder infections. A single, significant QTL associated with susceptibility to E. coli kidney infection was located on chromosome 6 and did not appear to interact with other loci. These results demonstrate the multigenic and complex nature of susceptibility to UTIs in C3H/HeJ mice.

MATERIALS AND METHODS

Animals. Male BALB/c mice were purchased from Harlan Sprague Dawley, and female C3H/HeJ mice were supplied by Jackson Laboratories. The female mice used in the present study were bred in our animal facility (at the University of Wisconsin School of Medicine and Public Health) from a backcross of C3H/HeJ females with (BALB/c × C3H/HeJ)F1 males. Animals were housed in accordance with the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care, and all protocols were approved by the University of Wisconsin Animal Care and Use Committee.

Determination of infection phenotype. Bladder and kidney infections were induced in 154 female backcross mice by intravesical inoculation with uropathogenic E. coli [27, 28]. Escherichia coli strain 1677 was grown from frozen stock in tryptose broth (Difco Laboratories), washed with PBS by centrifugation, and resuspended to a concentration of $2 \times 10^{10}$ E. coli/mL. Mice were deprived of water for 1 h and had urine expressed from their bladders immediately before inoculation. Ten microliters of bacterial inoculum were instilled into the bladder by urethral catheterization while the animal was anesthetized with isoflurane, resulting in a dose of $2 \times 10^{8}$ E. coli/mouse. The animals were allowed to recover from the anesthesia and were given free access to water 1 h later.
Mice were euthanized 10 days after inoculation, to assess the intensities of bladder and kidney infections. After the organs were removed, weighed, and homogenized in sterile PBS, the homogenates were serially plated onto Levine’s eosin-methylene blue agar (Difco Laboratories). The number of E. coli colony-forming units on each plate was determined after incubation overnight at 37°C. The total number of colony-forming units in each bladder and pair of kidneys was normalized by weight, as has been done elsewhere [26], and was used as the quantitative phenotype for bladder (BLCFU) and kidney (KDCFU) infections, respectively.

**Genotype determination.** Genomic DNA was prepared from the spleen of each backcross mouse by use of the Puregene Tissue Kit (Gentra) and was used in polymerase chain reactions (PCRs) to determine the parental genotype at DNA microsatellite markers spaced at a minimum distance of 20 cM on each chromosome. For the present study, the 19 autosomal chromosomes were evaluated. The X chromosome was not genotyped, because the backcross mice were bred from a female C3H/HeJ parent and a male F1 mouse, which would not have recombinations in the X chromosome, making backcross females homozygous for C3H/HeJ alleles and not informative.

The PCRs used microsatellite primer pairs (obtained from Invitrogen or Integrated DNA Technologies) that previously had been tested to ensure size polymorphisms in the products generated from each parental DNA. Cycling conditions were 96°C for 2 min and 30 cycles each at 94°C for 45 s, at 57°C for 45 s, and at 72°C for 60 s, followed by a final extension at 72°C for 7 min. Size polymorphisms in PCR products were determined by agarose gel electrophoresis.

**Statistical analyses.** The phenotype distributions were skewed toward higher values, so log-transformed phenotypes were considered. The log transformation attenuated the effect of outliers on the mean but did not result in a phenotype distribution that approximated a normal distribution for the bladder or kidney phenotypes. The effects of square- or cube-root transformations were also tested, and they did not result in an approximately normal distribution of phenotypes. For this reason, traditional mapping approaches that assume normal distribution of phenotypes could not be used. Instead, we used the nonparametric (NP) mapping approach developed by Kruglyak and Lander [29] and implemented in R/qtl software (version 1.06-43; available at: http://www.biostat.jhsph.edu/~kbroman/qtl) [30] (denoted as “R/qtl-NP”) to calculate the log_{10} value of the odds (LOD) scores for associations between BLCFU or KDCFU phenotypes and specific chromosomal sites. Permutation tests were performed (10,000 permutations/phenotype) to determine thresholds for suggestive (P < .05) and significant (P < .01) linkage at the genome level [31].

To assess the presence of interactions among QTLs, we first considered a model selection procedure, as detailed by Lan et al. [32]. This procedure identifies potential interactions among significant QTLs and also allows for the possibility that significant QTLs interact with other genome regions that do not show significant main effects. The procedure first identifies putative QTLs by use of a LOD score profile. In the initial step, we do not require that putative QTLs be statistically significant; they are defined as QTLs with a maximum LOD score (1 QTL per chromosome) when LOD scores were calculated using R/qtl-NP. We then consider all possible models, allowing for additive effects among the putative QTL and pairwise interactions. The Bayesian information criterion (BIC) is used to score each model [33]. The BIC balances the goodness of the model fit with the number of model parameters. The model with the best (i.e., lowest) BIC is then identified. We note that this procedure requires that some parametric specification be made. We conducted the analysis twice, once under the assumption of a Gaussian-distributed phenotype and then again under the assumption of a Poisson-distributed phenotype. The results were robust to these assumptions, and P values associated with the Gaussian model are reported.

**RESULTS**

Main effect QTLs for susceptibility to bladder and kidney infection. A genome scan of backcross mice was conducted using DNA microsatellite size polymorphisms to identify the chromosomal segments derived from each parental strain. The statistical analyses described in Materials and Methods were used to associate bladder and kidney phenotypes with the marker genotype for chromosomes 1 through 19. Figure 1 shows the logarithm (base 10) of odds (LOD) scores calculated by interval mapping performed using markers at a density of at least 20 cM on each chromosome. A single, highly significant QTL located at 29.0 cM on chromosome 4 had a LOD score of 4.91 (P < .001). This QTL has been named “Becis1” to denote bladder E. coli infection susceptibility. The LOD scores for all other markers were <1.00.

A single, statistically significant QTL for the kidney infection phenotype was identified at 17.0 cM on chromosome 6, by means of a similar statistical analysis (figure 2). This QTL had a LOD score of 3.27 (P < .01). We refer to this locus as “Kecis1,” to denote kidney E. coli infection susceptibility. The second highest QTL (not on chromosome 6) was identified at 5.0 cM on chromosome 4, and its LOD score was 2.10 (P = .15).

Interacting QTLs for susceptibility to bladder and kidney infections. Some of the investigators who performed the present study previously investigated the genetics of susceptibility to bladder and kidney infections due to E. coli, and they noted that each of these recessive traits was determined by >1 gene [26]. The traditional mapping approach used in the present study identified one highly significant QTL for susceptibility to bladder infection due to E. coli and one QTL for susceptibility to kidney infection due to E. coli; however, the analysis did not
consider the possibility of interacting loci contributing to the infection phenotypes. As a second analysis, we used the BIC approach to evaluate models of interactions between the main effect QTLs and other loci.

The BIC procedure confirmed a model for bladder infection susceptibility where the main effect was associated with C3H/HeJ alleles near marker D4Mit84. In addition, the procedure identified a model for bladder infection susceptibility in which there was an interaction between marker D4Mit84 located at 37.7 cM, the marker closest to Becis1, and marker D19Mit69 located at 6.0 cM on chromosome 19. We refer to this latter locus as “I-Becis1.” The P values in the identified regression model for Becis1, I-Becis1, and the interaction term are /H11021, .06, and .006, respectively. As detailed in Materials and Methods, the BIC approach can identify genomic regions that are not significant on their own but show significant interactions. That was the case for I-Becis1. The data in table 1 demonstrate the effect of the interaction of Becis1 and I-Becis1 on the intensity of E. coli bladder infection in backcross mice.

As shown in table 1, when backcross mice were heterozygous for both BALB/c and C3H/HeJ alleles near microsatellite markers D4Mit84 (Becis1) and D19Mit69 (I-Becis1), their mean (log-transformed) BLCFU phenotype was 3.23 cfu. The mean BLCFU phenotype increased to 4.81 cfu when D4Mit84 remained heterozygous and the D19Mit69 marker was homozygous for C3H/HeJ alleles, indicating an increase in susceptibility resulting from the effects of C3H/HeJ alleles. These infection intensities were lower than those observed for mice homozygous for C3H/HeJ alleles at both D4Mit84 and D19Mit69 (BLCFU phenotype, 5.16 cfu).

There was no demonstrable effect on the phenotype when the D4Mit84 marker was changed from heterozygous for C3H/HeJ and BALB/c alleles (mean BLCFU phenotype, 4.81 cfu) to homozygous for C3H/HeJ alleles (mean BLCFU phenotype, 5.16 cfu), whereas the D19Mit69 locus remained homozygous for C3H/HeJ alleles. There was, however, a large change in the mean BLCFU phenotype when the D19Mit69 marker was changed from homozygous for C3H/HeJ alleles to heterozygous for both BALB/c and C3H/HeJ alleles and the D4Mit84 marker remained homozygous for C3H/HeJ alleles (the mean BLCFU phenotype increased from 5.16 to 6.67 cfu, respectively). The implication of these results is that the levels of bladder infection seen in C3H/HeJ mice can be further increased by the addition of BALB/c alleles at the I-Becis1 locus on chromosome 19.

**DISCUSSION**

The objective of the present study was to perform genetic linkage analysis of a cross between C3H/HeJ and BALB/c mice to localize genes associated with the increased susceptibility of C3H/HeJ
female mice to induced E. coli bladder and kidney infections. Our previous analysis of the bladder and kidney infection phenotypes of a backcross between (BALB/c × C3H/HeJ)F1 mice and C3H/HeJ mice demonstrated that susceptibility to E. coli infection of either organ was multigenic; however, that study could not identify the specific genetic loci involved. We have now identified specific QTLs linked to severe E. coli bladder and kidney infections in female C3H/HeJ mice.

Increased susceptibility to bladder infections caused by uropathogenic E. coli is associated with a single, strong QTL ("Be-cis1") that is located at 29.0 cM on chromosome 4. At this time, it is not possible to specify exactly which gene in the vicinity of Becis1 directly promotes intense, protracted colonization of the bladder, because the markers used for screening were spaced at ~20-cM intervals along the chromosome. Nevertheless, it is noteworthy that the Tlr4 gene is located at 33.0 cM on chromosome 4.

**Figure 2.** Genome scan of female backcross mice for chromosomal sites associated with susceptibility to kidney infection. A total of 154 mice from a backcross between C3H/HeJ and (BALB/c × C3H/HeJ)F1 mice were inoculated with Escherichia coli strain 1677 and were assayed for E. coli colony-forming units in the kidneys 10 days later. Logarithm (base 10) of the odds (LOD) scores are shown, with thresholds for statistical significance denoted by dashed lines.

**Table 1.** Bladder infection intensities in mice with different combinations of BALB/c and C3H/HeJ alleles at the quantitative trait loci (QTLs) Becis1 and I-Becis1

<table>
<thead>
<tr>
<th>Marker D19Mit69&lt;sup&gt;a&lt;/sup&gt;</th>
<th>C3H/C3H&lt;sup&gt;b&lt;/sup&gt;</th>
<th>BALB/C3H&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3H/C3H&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.16 ± 0.44 [174.2] (n = 45)</td>
<td>4.81 ± 0.54 [122.7] (n = 41)</td>
</tr>
<tr>
<td>BALB/C3H&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.67 ± 0.69 [788.4] (n = 35)</td>
<td>3.23 ± 0.38 [25.2] (n = 31)</td>
</tr>
</tbody>
</table>

**NOTE.** Mean log<sub>e</sub>-transformed bladder infection phenotype expressed as the total no. of colony-forming units ± SE (antilog of the transformed mean value) (no. of animals in the group). A total of 152 of a possible 154 animals were analyzed, because 2 animals were missing genotypes at ≥1 of the markers considered.

<sup>a</sup> I-Becis1 is the QTL near marker D19Mit69 located at 6.0 cM on chromosome 19.

<sup>b</sup> Becis1 is the QTL for bladder E. coli infection susceptibility located at 29.0 cM on chromosome 4.

<sup>c</sup> Mice with only C3H/HeJ alleles at the D4Mit84 or D19Mit69 loci.

<sup>d</sup> Mice with both BALB/c and C3H/HeJ alleles at the D4Mit84 or D19Mit69 loci.
some 4 and may be a candidate for Becis1 within the limitations of the analysis performed here. Mice from the C3H/HeJ strain carry a point mutation in the Tlr4 gene that makes them unresponsive to the biological effects of E. coli LPS [11–13], and the lowered resistance of these mice to an E. coli UTI has been attributed to their inability to develop local inflammatory responses initiated by the interaction of LPS with TLR4 receptors on bladder epithelial cells [14]. Although TLR4 plays a central role in this model of host resistance to infection, we also demonstrated that female C3H/HeOuJ mice are highly susceptible to both bladder and kidney infections caused by E. coli, even though the C3H/HeOuJ mice have a normal Tlr4 gene and are responsive to LPS from E. coli and other gram-negative bacteria [15].

Because the C3H/HeOuJ and C3H/HeJ mouse strains were derived from a common ancestor but likely diverged genetically while being bred separately for several decades, it is conceivable that alleles of 1 or more genes closely linked to the Tlr4 locus in both strains, rather than Tlr4 alone, may be responsible for the severe bladder infections induced by E. coli. The importance of Tlr4 versus another gene, or genes, on chromosome 4 could potentially be determined by analyzing additional animals bred from the current cross, screening a similar cross using C3H/HeOuJ mice, or sequencing chromosome 4 from Becis1 through Tlr4 to identify nucleotide variations.

In our previous studies of bladder infection phenotypes of backcross mice, the Wright-Castle analysis concluded that multiple genes contributed to the high-susceptibility phenotype [26]. The current results provide experimental evidence supporting this prediction. Statistical modeling of the main effect QTL (Becis1) with the use of the nearest microsatellite marker (D4Mit84) on chromosome 4 revealed an interacting QTL (I-Becis1) near marker D19Mit69 on chromosome 19. Interestingly, the greatest increase in bladder infection intensity was seen in backcross mice that were homozygous for C3H/HeJ alleles at D4Mit84 and heterozygous for C3H/HeJ and BALB/c alleles at D19Mit69. One implication of these results is that the severity of bladder infection can be increased by the addition of BALB/c alleles at I-Becis1, suggesting the presence in BALB/c mice of previously unrecognized susceptibility-associated alleles that are observed only in combination with the C3H/HeJ alleles on chromosome 4.

In contrast to the results for QTLs associated with severe E. coli bladder infection, increased susceptibility to E. coli kidney infections has thus far been associated with a single QTL in backcross mice (Kecis1), which is located at 17.0 cM on chromosome 6. This QTL on chromosome 6 is located near genes coding for variable regions of the β chain of the T cell antigen receptor at 20.5 cM. By analyzing phenotype and genotype data from additional mice bred by the current backcross mating scheme and by increasing the marker density around Kecis1, it may be possible to determine whether Kecis1 resides among the T cell receptor genes. If this is the case, there would be a strong indication that host defense against E. coli kidney infection is largely dependent on T cell recognition of antigens on uropathogenic E. coli and subsequent T cell responses to those antigens. Additional studies could investigate whether C3H/HeJ mice are potentially immunodeficient in their responses to specific E. coli antigens, as well as assess the relative importance of their CD4+ and CD8+ T cell responses to E. coli. It can also be noted that genes for immunoglobulin κ chain variable regions are located at 30.0 cM on chromosome 6; however, the current scan did not place any QTLs in this region, even with a microsatellite marker located at 34.0 cM. Thus, host resistance to ascending E. coli kidney infection appears to be more dependent on T cell participation in immune responses than on antibody production by B cells.

The multigenic nature of increased susceptibility to E. coli kidney infections in C3H/HeJ mice was inferred from a previous study by Hopkins et al. [26], but statistical modeling of the current data did not detect any QTLs interacting with Kecis1. There were, however, distinct LOD score peaks below the level of statistical significance on chromosomes 1, 4, and 9. It is interesting to note that Tlr5−/− mice have increased susceptibility to E. coli UTI [34] and that the antimicrobial peptide cathelicidin plays a role in resistance to UTI in humans and mice [35]. The TLR5 and cathelicidin genes are located on mouse chromosomes 1 and 9, at 98.0 cM and 61.0 cM, respectively, in the vicinity of the LOD score peaks for these chromosomes. For kidney infections, the increased LOD score on chromosome 4 was noted at ~10 cM, which is not close to Becis1 or Tlr4. Whether alleles at any of these chromosomal sites significantly contribute to increased susceptibility to kidney infection could be determined by analysis of additional animals bred according to the current backcross mating scheme.

We can draw several important conclusions from the current mapping study. One of the most significant conclusion is that QTL analysis has been successful in localizing chromosomal sites where the allelic differences in genes present in C3H/HeJ and BALB/c mice strongly affect susceptibility to E. coli bladder and kidney infections. These 2 distinct QTLs are located on different chromosomes, indicating that susceptibility is complex and is determined by polymorphisms in alleles of multiple host genes. Furthermore, we now have strong evidence that host factors affecting E. coli colonization and defense mechanisms are clearly different for the bladder and kidney. This finding may have been anticipated, because the bladder is subject to mucosal colonization by bacteria and is protected by mucosal defense mechanisms, whereas the kidney is a more vascularized organ for which systemic immune responses may be more effective. On the basis of these results, it will be possible to propose and test new hypotheses for genetic predisposition and host resistance/susceptibility to bladder and kidney infection in both animal models and patients susceptible to UTI.
References