Model organisms such as mice represent an attractive system for the characterization of modifying loci affecting the expression of mutant phenotypes. The existence of inbred strains permits the generation of progeny in which the parental origin of alleles is defined. The development of dense maps of highly polymorphic and easily testable genetic markers, as well as computer algorithms for correlating genotype distribution with quantitative trait data, has made the genetic localization of modifier loci feasible. However, despite the theoretical promise of quantitative trait locus (QTL) analysis, the identification of the molecular basis of modifying loci has remained difficult. One likely basis for this is that the modifying loci may not have simple additive effects, but rather may show complex epistatic interactions. Even when these can be localized in a genetic mapping experiment, they may be obscured when isolated in a different genetic background by congenic analysis, which is a commonly used strategy.

The juvenile cystic kidneys (jck) mutation is a model of recessive PKD (1). The position of this mutation was mapped using an intercross of C57BL/6J (B6) carrying jck and DBA/2J (D2) mice. In the F2 progeny, it was noted that the size of the polycystic kidneys found in age-matched affected mice was quite variable compared with that found in the parental line (Ref. 4; Fig. 1). This suggested that one or more modifying loci affecting severity had been introduced from the D2 background. Initial genetic analysis revealed nonrandom cosegregation with disease phenotype for two loci: one B6 locus on chromosome 1 and one D2 locus on chromosome 10. QTL analysis using the Mapmaker/ QTL program confirmed that inheritance of a B6 locus on chromosome 1 correlated significantly with increased kidney size; this accounted for 74% of the variance in affected F2 progeny and appeared to have its effect as a recessive locus.

The characterization of the jck mutation and the modifying loci affecting its expression has potential utility for understanding human PKD. For positional cloning, recombination analysis using multiple crosses is considered to be advisable because different mouse strains may have different meiotic recombination “hot spots.” As such, testing even large numbers of mice in a single cross may only identify limited numbers of recombination breakpoints, and it is consequently desirable to test more moderate numbers of mice using multiple different strain combinations (11). For this reason, we performed an intercross of B6 mice carrying jck and FVB wild-type mice. The FVB strain, which is commonly used for transgenic analysis, is frequently polymorphic compared with B6, and we have reported...
on the polymorphism frequency of over 390 microsatellite markers (B). In addition, the large litter size obtained facilitates an efficient genetic analysis.

Although the goal of this cross was to further narrow the genetic interval containing jck, we report here that it has also served to confirm the localization of a modifying locus on chromosome 1. In addition, analysis of congenic mice suggests that the severe disease seen in F2 progeny of a B6 × D2 cross is due to the effects of two chromosome 1 loci that are genetically linked. These results demonstrate some of the factors that complicate the identification of genes which have modifying affects and suggest an appropriate strategy for their genetic analysis.

METHODS

Mice and phenotype characterization. B6 jck−/− and jck/+ mice are maintained in our mouse colony. FVB and D2 mice were obtained from The Jackson Laboratory. For the B6 × FVB cross, B6 jck/jck and FVB mice were mated, and the F1 progeny were intercrossed to generate F2 mice. These were killed between 6 and 7 wk of age and scored for the presence of abnormal kidneys, which were removed, weighed, and fixed in 10% formalin (American Histology Reagent). Tail tissue was reserved for DNA extraction. Kidneys that appeared abnormal but were not obviously polycystic were analyzed by histopathology with the help of Dr. Robert Cardiff (Univ. of California, Davis).

To generate congenic lines, (B6 × D2)F1 jck/+ mice were crossed with D2 mice, and jck/+ heterozygous progeny were chosen that were homozygous D2 for all loci on chromosome 10 and were heterozygous B6/D2 for all loci on chromosome 1. This was done so that the alleles of the region on chromosome 10 that plays a role in modifying disease severity were fixed as D2 and would not need to be further tested. These D2.B6 N2 mice were mated again to wild-type D2 mice, and jck/+ heterozygous mice were identified. These D2.B6 N3 mice were typed for 11 microsatellite markers distributed along chromosome 1, and mice recombinant along this chromosome were selected and crossed. Affected mice were analyzed at 6–7 wk of age as described above. Figure 3 illustrates the recombinant haplotypes that were analyzed.

PCR-based genotyping. Genomic DNA was prepared from tail and liver tissue according to standard techniques. Mit microsatellite markers polymorphic between B6 and FVB were identified by parental control DNA. PCR primers were purchased from Research Genetics and kinase-labeled using [32P]ATPs. The PCR fragments were amplified using recommended protocols (2) and analyzed on a 6% polyacrylamide denaturing gel.

Statistical analysis. Kidney weight distributions were analyzed using Excel 5.0. Genotype data were correlated with kidney weight trait data using Map Manager QT version b26 (http://mcbio.med.buffalo.edu/mapmgr.html). Permutation tests were done in 1-cM steps for 500 permutations. The threshold values of the permutation test, which are labeled “suggestive,” “significant,” and “highly significant,” are taken from the guidelines of Lander and Kruglyak (7) and correspond to the 37th, 95th, and 99.9th percentiles, respectively.

RESULTS

In an intercross between B6 and FVB/N (FVB) mice, kidney sizes of homozygous jck F2 progeny were recorded, and significant variation in disease was found compared with that seen in an inbred B6 background (mean 1.04 g, range 0.36–2.89 g, variance 0.29 g2, n = 97; Fig. 1). To test whether the same loci previously found to affect PKD severity contributed to disease variation in this population, a QTL analysis was done using genetic markers distributed on chromosomes 1 and 10, and the results were analyzed using Map Manager QT. For comparison, we reanalyzed our previous data using the same program. Both data sets were analyzed using a permutation test to establish the levels of suggestive, significant, and highly significant linkage according to the criterion recommended by Lander and Kruglyak (7). This analysis supports the presence on chromosome 1 of a QTL affecting PKD severity in the B6 × FVB cross with a maximum logarithm of odds ratio (LOD) value of 4.1 near D1Mit30 inferred by interval analysis (Fig. 2). Similar to the results in the B6 × D2 intercross, it is the B6 allele that is associated with the severe disease phenotype, and model testing supports that this effect is recessive. The results also support the localization of a QTL on chromosome 10 near D10Mit20, with a maximum inferred LOD score of 3.5.

Fig. 1. Scattergram of paired-kidney weight distributions. B6, distribution in affected inbred C57BL/6J (B6) mice (from Ref. 4; n = 18); F2 (D2), distribution in affected F2 progeny of a cross between C57BL/6J jck−/− and DBA/2 mice (from Ref. 4; n = 105); F2 (FVB), distribution in affected F2 progeny of a cross between C57BL/6J jck+/+ and FVB/N mice (n = 96); cong, distribution in affected progeny of a series of chromosome 1 incipient congenic lines (n = 47); A, distribution in affected progeny of a D2.B6(D1Mit76–D1Mit155) incipient congenic line (n = 22); B, distribution in affected progeny of a D2.B6(D1Mit76–D1Mit30) incipient congenic line (n = 8).
One common method to localize a gene that has as its effect a quantitative change is to generate congenic strains, in which a portion of a specific chromosome is introduced onto a different genetic background. These mice are tested for quantitative differences in a phenotype, and the locus of interest is assigned to a specific genetic interval defined by the congenic analysis. This approach seemed appropriate for the characterization of modifying loci on chromosome 1, in which B6-derived alleles are associated with increased disease severity, because the effect of this locus is large (contributing to >70% of the observed variance), which suggested that it could be readily scored when tested in a congenic background. In addition, because the jck mutation itself occurred in a B6 background, it is technically straightforward to identify jck/+ heterozygotes in D2 congenic mice by testing a pair of markers known to flank the mutation.

As described in Methods, a total of nine D2.B6 incipient congenic lines representing seven haplotypes were generated, and heterozygous jck/+ mice were identified. (Note that these mice have not been serially backcrossed for sufficient generations to be considered true congenics. However, in our initial studies, we found no regions of the genome other than chromosomes 1 and 10 to show an association with PKD severity and considered it likely that these incipient congenic lines would be informative for localizing the chromosome 1 modifying gene.) These were crossed with D2.B6 jck/+ mice carrying a large heterozygous congenic region from D1Mit20 (16 cM) to D1Mit155 (116 cM). Thus, for each incipient congenic line, 25% of affected progeny should be homozygous for B6 alleles in the congenic region and should show the severe disease phenotype. Contrary to this expectation, the distribution of kidney size does not differ significantly from that of the B6 inbred mice (Fig. 1). Furthermore, QTL analysis of markers in the congenic lines (analyzed across the entire set) shows no significant association with kidney size (data not shown).

As discussed below, one possible explanation for these results is that the severe disease phenotype is caused by two linked loci, which have been separated in the congenic lines that were generated. This hypothesis can be tested by creating a congenic line containing an interval of B6-derived alleles over the large region implicated in the original (B6 × D2)F2 analysis. An N3 D2 jck/+ mouse heterozygous for the region between D1Mit76 (33 cM) and D1Mit155 (116 cM) was identified (Fig. 3), and an additional mating was performed to make the congenic interval homozygous. Heterozygous jck/+ mice from this line were intercrossed, and the size distribution of affected kidneys was measured. As shown in Fig. 1, the distribution of kidney size is considerably skewed to larger sizes (mean 2.12 g, variance 0.78 g², range 0.92–3.62; n = 22). This result, especially in conjunction with the previous congenic analysis, strongly supports the model that two linked loci on chromosome 1 are required for the severe PKD phenotype. Additional support for the model is provided by another congenic line generated over a smaller interval [from D1Mit76 (33 cM) to D1Mit30 (73 cM); Fig. 3] using the same strategy. This line, which encompasses the peak of the single locus identified in

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the B6
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FVB analysis, does not show nearly the same skewing of kidney size as the large congenic (mean 1.24 g, variance 0.05 g², range 1.00–1.76; n = 8). The difference of the means for these two cohorts is highly significant (P < 0.0003), supporting the hypothesis that a large B6 region on chromosome 1 is required for the severe disease phenotype in B6
3
D2 mice.

DISCUSSION

We have previously demonstrated a highly significant effect of a locus on chromosome 1 on the progression of PKD in the jck mutant mouse, when tested using a B6 × D2 intercross. In this study we demonstrate that this effect can be found in a cross between B6 and FVB mice as well. This result is notable for several reasons. Most importantly, in both cases the severe disease phenotype is associated with inheritance of the B6 allele. We have previously suggested that, because the PKD phenotype in jck mice is not severe in the original B6 background on which the mutation occurred (Fig. 1), the severe disease occurs as a result of an interaction between a B6 allele of a locus on chromosome 1 and a D2 allele of a locus localized elsewhere in the genome. Our original data suggested that this interacting locus may be on chromosome 10 (4). This study also supports the localization of a modifying locus on chromosome 10 that is significantly associated with severe PKD, although whether this locus has its effect as a result of an interaction is not proven.

The highly significant effect of the chromosome 1 locus suggested that analysis using congenic strains could localize the modifier to a small genetic interval that might be amenable to a positional cloning strategy. However, when a series of incipient congenic lines generated for this purpose was tested, no mice in the entire set demonstrated the severe disease phenotype. Although the modest numbers of mice analyzed for each congenic line in this experiment preclude definitive interpretation, one possibility suggested by a variety of evidence is that the severe disease phenotype in the (B6 × D2)F2 population requires the contribution of two linked markers on chromosome 1, and these loci have been separated in the congenic analysis. First, there is evidence for a highly significant association with severe kidney disease for a region of chromosome 1 spanning 60 cM, which is remarkable even acknowledging that QTL analysis generally does not permit precise localization. Additionally, there is evidence for two peaks on this distribution, both in the original analysis.

Fig. 3. Incipient congenic lines. The chromosome 1 genotypes of the parental jck/− mice tested are shown. Solid boxes are heterozygous B6/D2; open boxes are homozygous D2; shaded boxes correspond to region between recombinant markers, whose genotype is indeterminate. Marker names are listed at left; their map position on the chromosome is given at right. Nos. at top correspond to individual lines generated. Three lines (153, 149, 206) had the same haplotype. "A" represents the D2.B6(D1Mit76–D1Mit155) incipient congenic line. "B" represents the D2.B6(D1Mit76–D1Mit30) incipient congenic line. Nos. at bottom correspond to affected progeny obtained for each line.

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DISCUSSION

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using MapMaker QTL (4) and the reanalysis with Map Manager QT (Fig. 2, top). Of note is that in the analysis of the B6 × FVB cross, the region of significant linkage is much smaller, suggesting only a single chromosome 1 locus contributes to severe disease in this strain combination.

Additionally, we observe that the histopathological phenotypes of kidneys derived from the congenic strains were widely varying with respect to size and distribution of cysts, even in comparison to the F2 population (Fig. 4). Although the implications of this must be taken cautiously, it is at least consistent with the possibility that multiple loci are contributing to the cystic phenotype in the B6 × D2 strain combination. The evidence that affected kidneys of the same size show different histopathology suggests kidney weight alone is not the most sensitive assay for the PKD phenotype. However, because, in general, the cohort of mice with large kidneys shows more severe histopathology than an age-matched cohort with small kidneys, kidney weight is a valid measure of PKD severity.

The evidence that a modifying effect can be due to two linked loci has implications for how these studies should be pursued. This result is not unique, and similar observations have been made in the analysis of mouse models of nonobese diabetes (9) and epilepsy (3). These observations suggest that the most prudent strategy for the general localization of presumptive QTLs is to generate a consomic strain in which the entire chromosome carrying the putative locus is derived from the strain of interest. In this protocol, no presumptions are made about the specific position of the locus on the chromosome or whether a single locus has an effect. Once the consomic line has been characterized and the presence of a modifying effect is confirmed, it is then a relatively straightforward strategy to subdivide this line into smaller congenic intervals for the purpose of more specifically localizing a QTL. We have continued our analysis of the jck modifying locus with this in mind and are now generating a set of nested congenic lines derived from the large incipient congenic described above.

In addition to the loci we have described, modifying loci affecting the progression of PKD have been reported for other mouse models systems, including the pcy, cpk, bpk, and kat mutations (12) (13) (5). Although loci on chromosome 1 have been implicated in several of these studies, it is not clear how these relate to each other or to those reported here, inasmuch as the resolution of QTL studies is modest and the strain combinations tested are not identical.

Despite the technical advances that have facilitated identifying loci that affect quantitative traits, progress on the molecular characterization of these genes has been slow. At present, perhaps the only example is the identification of secretory type II phospholipase (Pla2g2a) as a strong candidate for the Mom1 (modifier of Min 1) locus, based on both genetic mapping and functional analysis. Mom1 affects the expression of mutations in the Apc gene, which cause intestinal neoplasia in a background-dependent manner. This characterization is clearly the exception, and the molecular basis of most genes that have quantitative effects is unknown. This is likely due to a number of factors, including the lengthy time period required for generating appropriate strains, as well as the low resolution of QTL genetic analysis. However, a variety of studies also suggest that modifying effects may be due to complex genetic interactions that are lost when individual contributing loci are isolated. The results presented here demonstrate that the characterization of modifying genes requires carefully designed genetic studies in which the possibility of unexpected outcomes should be anticipated.

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