Genetic Identification of Two Major Modifier Loci of Polycystic Kidney Disease Progression in *pcy* Mice

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Abstract

Unlike the uniform disease progression in inbred animals, polycystic kidney disease (PKD) progression within human families can be highly variable. This may be due to environmental or genetic factors or both. To determine if PKD severity can be influenced by modifier genes, we carried out an intercross between DBA/2-*pcy*/*pcy* and *Mus m. castaneous* involving 3,105 6-wk-old F2 mice. Large differences in PKD severity were observed in this cross. In addition, 23/800 phenotypically normal mice were *pcy*/*pcy* genotypically. These results demonstrated that PKD progression in *pcy*/*pcy* mice is a quantitative trait that is strongly modulated by modifier genes. Whole genome quantitative trait loci mapping of 114 selected *pcy*/*pcy* mice (68 with the mild PKD and 46 with severe PKD) identified two loci, MOP1 and MOP2 that strongly modulate PKD progression. MOP1 (max LOD score = 10.3 at D4Mit111) and MOP2 (max LOD score = 13.8 at D16Mit1) accounted for 36.7 and 46.8% of the phenotypic variance, respectively. Two-factor ANOVA of the phenotypes and genotypes of all 673 *pcy*/*pcy* mice from our cross indicated that MOP1 and MOP2 alleles regulate PKD progression in a complex additive manner. Characterization of these novel modifying loci may provide additional insights into the pathogenesis of polycystic kidney diseases. (*J. Clin. Invest.* 1997. 100:1934–1940.) Key words: quantitative trait • renal failure • renal cyst • apoptosis • gene mapping

Introduction

The polycystic kidney diseases (PKD)[1] are a group of disorders characterized by the bilateral presence of a large number of fluid-filled cysts throughout grossly enlarged kidneys (1). The progression of polycystic kidney disease is accompanied by gradual enlargement of cysts, apoptotic loss of renal tissue, and fibrosis of the renal interstitium (2, 3). Although the kidney is usually the most severely affected organ, PKD is a systemic disorder and extrarenal manifestations are common (1). Polycystic kidney diseases can be acquired or inherited in autosomal dominant (ADPKD) or autosomal recessive (ARPKD) forms. ADPKD occurring in 1 out of 800 individuals, is the most common, dominantly inherited kidney disease of humans while ARPKD occurs at a frequency of 1 in 10,000 individuals. Clinically, the polycystic kidney diseases represent a major cause of chronic renal failure in humans and accounts for $\sim$ 10% of all patients requiring chronic dialysis or renal transplantation. Currently, five million people worldwide are affected with this disorder. Except for dialysis and transplantation, which are palliative, no curative treatment exists.

Polycystic kidney diseases are genetically heterogeneous. Dominant mutations in at least three genes and recessive mutation in at least one locus are known to result in polycystic kidney disease in human. Mutations in the 55-kb PKD1 gene, located on human chromosome 16p13.3 are responsible for $>$ 80% of all polycystic kidney disease (4). The PKD1 gene encodes a 14-kb transcript and its gene product polycystin-1 is a large transmembrane protein with unknown function (5, 6). Polycystin-1 mRNA is expressed in many tissues including the kidney. A second polycystic kidney disease gene on human chromosome 4, PKD2 coding for a 5-kb polycystin-2 mRNA that shows a 24% sequence homology to polycystin-1 at the predicted protein sequence level has also been identified (7). The location of the third PKD gene has not been established. A locus responsible for ARPKD has been mapped to human chromosome 6 (8). Despite two of the human polycystic kidney disease genes have been identified and characterized, the direct biological defects mediated by these polycystic kidney disease mutations remain unknown.

The rate of disease progression and development of renal failure in different individuals with polycystic kidneys can be highly variable. For example, it is well known that $>$ 80% of all polycystic kidney disease families have mutated PKD1 but only $\sim$ 45% of all PKD patients will progress to renal failure by 60 yr of age (1). Differences in progression of polycystic kidney disease among different pedigrees may be due to allelic variants, modifying genes or environmental factors, or combinations of these factors.

Large differences in the age of onset among individuals sharing the same PKD1 mutation has recently been described. For example, in two pedigrees with different PKD1 mutations, 10- and 16-yr differences in renal survival have been reported for affected individuals sharing the same PKD1 mutation (9). More recently, Peral et al. (10) reported that a man with typical adult onset PKD shared the same Tyr3818Stop PKD1 mutation with both of his dizygotic twins. Interestingly, only one of the twins manifested severe polycystic kidney disease at birth while the other twin has the mutation but showed no evidence of renal cysts at birth. Since affected members within each of the pedigrees have inherited the same mutations, heterogeneity in the disease phenotype in these pedigrees may be the result of modifier genes or environmental effects or both.

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1. *Abbreviations used in this paper: cast*, castaneous; K/B, kidney weight to body weight; LOD, logarithm of odds; PKD, polycystic kidney disease; QTL, quantitative trait loci.

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Systematic searches for genetic modifiers of polycystic kidney disease progression in human patients can be complicated by uncertainties in the roles of allelic variants and environment effects on the progression of polycystic kidney disease. These limitations can be minimized by the use of defined animal models of congenital polycystic kidney disease where both the genetic and environmental variables can be controlled experimentally. We therefore carried out a systematic study of the genetics of the modulation of the progression of polycystic kidney disease in a well-characterized murine model of slow progressing polycystic kidney disease.

In 1986, Takahashi et al. (11) reported a spontaneous occurrence of a recessive form of polycystic kidney disease in the KK strain of diabetic mouse. To isolate the mutation from the diabetic background, the mutation was bred into the DBA/2 background and a congenic strain, DBA/2-*pcy/pcy*, was developed (11). Genetic linkage analysis showed that the *pcy* mutation is located on mouse chromosome 9 (12).

Kidney maldevelopment and progression of polycystic kidney disease in inbred DBA/2-*pcy/pcy* mice has been characterized in detail (11, 12). Noticeably larger than normal kidneys are found in 8-wk-old animals (0.65±0.04 vs. 0.38±0.02 grams). The polycystic kidneys progressively enlarge, reaching almost 2 grams in kidney weight compared with ~ 0.7 grams for normal mature kidneys, at 18 wk (11, 12). The range of reported total kidney weight to body weight ratio (K/B ratio) in endstage mice is between 4 and 10% (2–5% for comparing to the single kidney weight to total body weight used in this report). The mean age of death is ~ 25 wk for affected females and 32 wk for affected males. Within animals of the same sex, no significant variation in the disease progression and the onset of kidney failure was observed (11, 12). Histologically, microscopic cysts can be seen in both the cortex and medulla in kidneys of newborn mice. Cysts enlarge with age and eventually severely distort the entire kidney in adult animals. Hallmark features of cystic changes in human polycystic kidney epithelia such as renal tubular apoptosis, cellular hyperplasia, and abnormal basement membrane are observed at both the light and electron microscopic level (11, 12). The accumulation of fluids in cysts also indicates abnormality in fluid transport in *pcy* cystic epithelia.

To systematically study the effect of differences in genetic background on the progression of polycystic kidney disease in *pcy* mice, we performed an extensive intersubspecific cross of DBA/2-*pcy/pcy* mice (obtained from Dr. H. Takahashi, Fujita Health University, Toyoake, Japan, via Dr. Jared Grantham, University of Kansas Medical Center, Kansas City, KS) with an inbred strain of *Musculus mus castaneous* mice (Cast/Ei from the Jackson Laboratories, Bar Harbor, ME). The *castaneous* strain of mouse was chosen because of its one to two million years of evolutionary and genetic divergence from most strains of laboratory mice.

Methods

Animals. Our colony of DBA/2 *pcy/pcy* polycystic mice was established by mating a single *pcy/pcy* male obtained from Dr. Jared Grantham (Kansas City, KS) with female DBA/2 mice from the Jackson Laboratories. The colony is maintained in the DBA/2 background by backcrossing phenotypically normal DBA/2-*pcy*/+ females with polycystic DBA/2-*pcy/pcy* males. Homozygous *pcy/pcy* mice are identified by palpation of enlarged kidneys at 8 wk of age. Mice were backcrossed to DBA/2 for nine additional generations before the intersubspecific crosses with *Mus m. castaneous* was carried out. All *pcy* × *cast* F2 mice were harvested at 6 wk of age. At the time of harvest, the body weight and the left kidney weight of each individual were recorded. Both kidneys of each F2 mice were also fixed and processed for histology. All mice are maintained under specific pathogen free (SPF) conditions to minimize environmental variables.

Genotypic analysis. Genomic DNA were prepared from the spleen of each animal according to the standard proteinase K/SDS protocol (13). A set of 150 microsatellite markers spanning the entire mouse genome at ~ 10-cm intervals chosen from the MIT mouse genome map (14) were used for genotype analysis. All markers were chosen to have polymorphisms of between 10 and 25 bp. Primers were purchased from Research Genetics (Huntsville, AL) and PCR were performed as described (15). PCR products were separated on 5% denaturing polyacrylamide DNA sequencing gels, and autoradiographs of the gel were used for genotype determination.

Linkage and data analysis. All data were analyzed by the Mapmaker/EXP 3.0 (16) and the Mapmaker/QTL 1.1 (17) computer programs. For additional characterization of quantitative trait loci (QTL), the free, dominant, recessive, and additive genetics models in Mapmaker/QTL were used to determine the most likely mode of inheritance. The "fixed QTL" functions of Mapmaker/QTL were used to determine the individual contribution of each QTL to the quantitative trait. Statistical analyses were carried out using the StatView program from Abacus Concepts, Inc. (Berkeley, CA). Two-factor ANOVA tests of the phenotypic effect of the various combinations of MOP1 and MOP2 alleles were calculated using the SuperANOVA program from Abacus Concepts, Inc.

Results

Phenotypic heterogeneity in pcy × cast F2 mice. Since *pcy* is a recessive mutation, 25% of the F2 mice in our cross were expected to develop polycystic kidney disease. However, even after careful histological examination of the kidneys of all 3,105 F2 mice in our cross, only 21% (651/3,105) of the mice were found to be polycystic at 6 wk of age. The deviation of this observed number of polycystic mice from the Mendelian ratio is highly significant ($\chi^2 = 17.78$, $P = 0.000025$). Within the 651 6-wk-old mice histologically confirmed to have polycystic kidneys, we observed a dramatic range of kidney sizes in affected animals (Figs. 1 and 2). It can be seen in Figs. 1 and 2 that the progression of polycystic kidney disease in polycystic *pcy* × *cast* F2 mice can be greatly suppressed (Fig. 1, *b* and *c*) or greatly accelerated (Fig. 1, *f* and *g*). The ratio of right kidney weight to total body weight (K/B ratio) ranged from 0.65 to 18% (mean = 2.55±2.69%). This is significantly different from the K/B ratio of 1.2–1.5% in 6-wk-old polycystic mice in our colony of inbred DBA/2-*pcy/pcy* mice.

As can be seen in Fig. 3, ~ 30% of all 6-wk-old polycystic *pcy* × *cast* F2 mice have single kidney K/B ratio > 2%. In these animals the disease is clearly accelerated above that observed in our inbred DBA/2-*pcy/pcy* mice. Approximately 15% of all 6-wk-old polycystic *pcy* × *cast* F2 mice have single kidney K/B ratio > 5%. The highest single kidney K/B ratio observed for 8-mos-old endstage *pcy* mice in our inbred DBA/2-*pcy*/*pcy* colony is ~ 5%. The kidneys of these severely affected animals are illustrated in Fig. 1, *f* and *g* and Fig. 2, *f* and *g*. Clearly, in a large proportion (25–30%) of polycystic *pcy* × *cast* F2 mice, the progression of PKD is not only accelerated but the disease severity is also greatly increased.

Qualitatively, the distribution of the K/B ratio is continuous and not presented in discrete steps (Fig. 3). Since the pro-

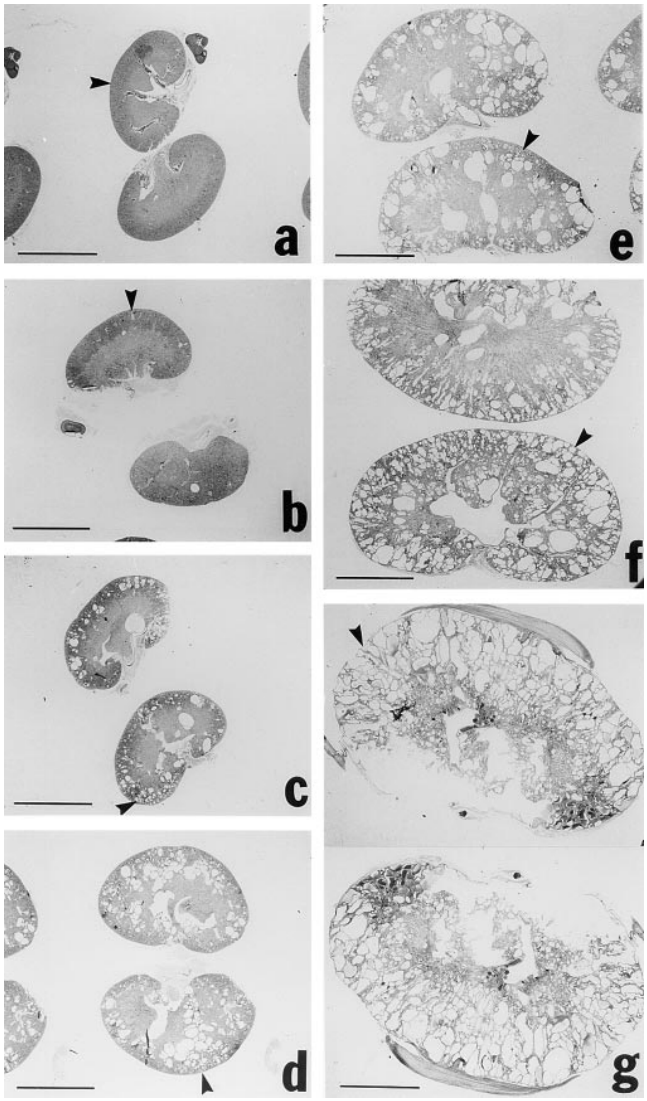

Figure 1. Spectrum of polycystic kidney disease progression in 6-wk-old DBA/2 × *castaneous* F2 *pcy/pcy* mice. The right kidney of each of the 3,035 DBA/2-*pcy/pcy* × *castaneous* F2 mice were harvested at 6 wk of age and processed for histology to document the extent of polycystic kidney disease involvement. An example of *pcy/pcy* kidney in which polycystic kidney disease is completely suppressed is shown in *a.* 6-wk-old *pcy/pcy* kidneys in which polycystic kidney disease is suppressed are illustrated in *b* and *c. pcy/pcy* kidneys in which polycystic kidney disease is similar to that observed in 6-wk-old inbred DBA/2 mice are illustrated in *d* and *e.* 6-wk-old *pcy/pcy* kidneys in which the progression of polycystic kidney disease has been accelerated are illustrated in *f* and *g.* The arrowheads point to areas shown magnified in Fig. 2. The bar represents 0.5 cm.

gression of PKD in our inbred DBA/2-*pcy*/*pcy* mice is uniform, and that both the DBA/2-*pcy*/*pcy* and the *castaneous* mice are inbred, the presence of a large variation of the polycystic phenotype in F2 animals indicated that the progression of the polycystic kidney disease, as measured by the K/B ratio, is a quantitative trait that is subjected to strong modulation by modifier genes. Contributions from environmental factors to the observed variation in disease progression can be ruled out because (*i*) all the mice were raised under identical conditions and (*ii*) polycystic mice within the same litter have been measured to have K/B ratio ranging from 1.8 to 13%. To define the genetics underlying this dramatic variation in the polycystic kidney phenotype, we performed a whole genome search for the presence of QTL or modifier genes that modulate polycystic kidney disease progression.

Whole genome search for genetic modifiers of PKD progression. For QTL mapping, individuals with phenotypes more than one standard deviation from the mean (33% of the total population) contribute ~ 81% of the total linkage information while progenies with phenotypes more than two standard deviations (~ 5% of the total population) from the mean can contribute about 28% of the total linkage information

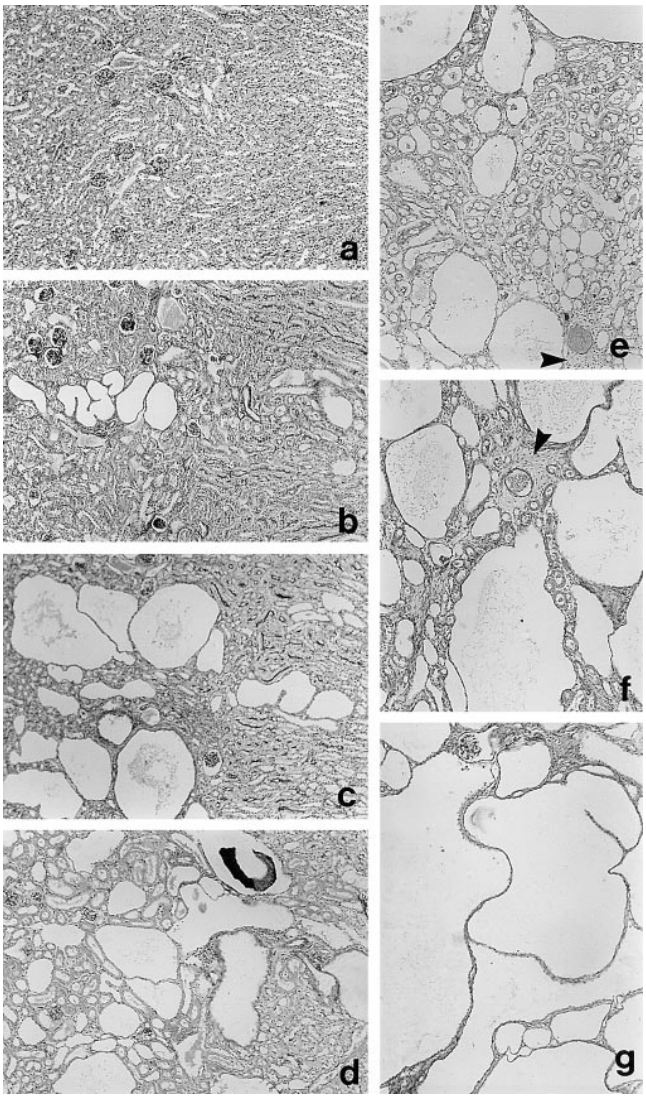

Figure 2. Histological variations in polycystic kidney disease involvement in 6-wk-old DBA/2 × *castaneous* F2 *pcy/pcy* mice. The histology of cystic involvement in the areas pointed out in each of the kidneys in Fig. 1, *a–g* are shown magnified in *a–g*, respectively. It can be seen that the progressive overall enlargement of polycystic kidneys are paralleled by increases in microscopic cyst size (*a–g*), increased presence of apoptotic (atrophic) tubules (*e* and *f*), and progressive disappearance of normal tubules (*a–g*). The beginning of interstitial fibrosis can be seen to develop in samples *e* and *f.* Areas of interstitial fibrosis are indicated by arrowheads.

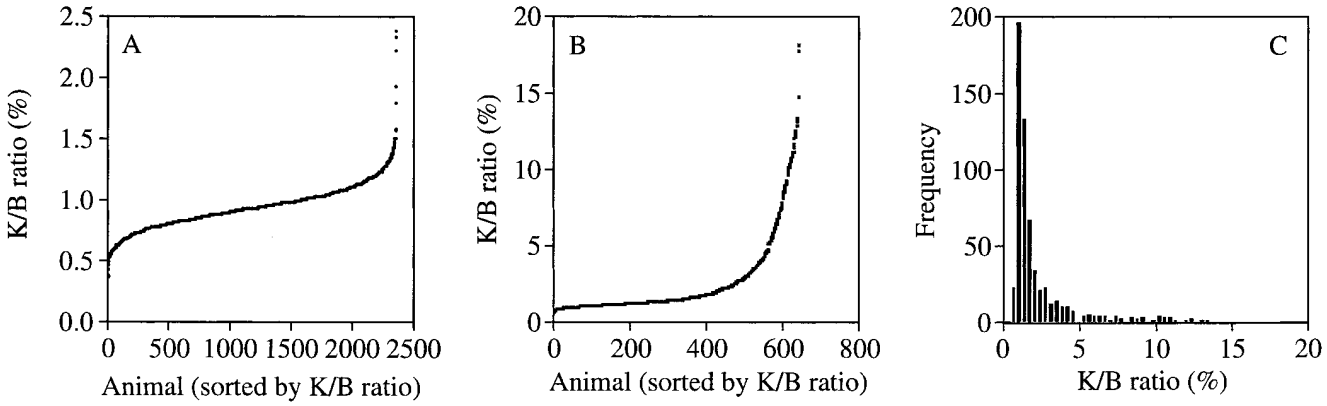

Figure 3. Range and distribution of left kidney weight to body weight ratio (*K/B ratio*) in 6-wk-old DBA/2 × *castaneous* F2 *pcy/pcy* mice. The range of K/B ratio in normal 6 wk old kidneys (judged to be cyst free by histology; $n = 2,357$; mean = 0.72±0.08%) are shown in *A*. The range of K/B ratio in 6-wk-old polycystic kidneys (judged to be cystic by histology; $n = 651$; mean = 2.52±2.69%) are shown in *B*. The distribution of the K/B ratio in 6-wk-old polycystic kidneys in F2 mice is shown in *C*. Due to complete suppression of the development of polycystic kidney disease in ~ 100 *pcy/pcy* mice, the K/B ratio of 6-wk-old polycystic kidneys is not distributed symmetrically.

(18). Selectively typing these individuals can greatly reduce the number of samples that has to be analyzed (18) to detect modifier genes. Thus, for an initial screen for QTL that control the progression of polycystic kidney disease in *pcy/pcy* mice, DNA from 46 F2 mice from our *pcy* × *cast* intercross were selected for genotyping with a set of 150 markers that span the mouse genome at approximately 10-cm intervals. The first 23 of the selected mice have the most severe polycystic kidney disease. Their K/B ratio ranged from 7.34 to 18.06%. The other 23 of the selected mice have the mildest measurable polycystic kidney disease. These mice have K/B ratio ranging from 0.65 to 0.92%. Whole genome genotype data from these 46 mice were first used to construct a mouse genome map using the Mapmaker computer program. The resulting map (not shown) is in good agreement with the published mouse genome map. The genotype data were further analyzed with the Mapmaker/QTL computer program to identify QTLs that contribute to the modulation of the progression of the polycystic phenotype in these 46 *pcy/pcy* mice. The corresponding K/B ratio for each of these 46 mice was used as the quantitative trait in the analysis.

Results of these analysis for each of the 20 mouse chromosomes are shown in Fig. 4. It can be seen that two QTL with significant influence on the progression of PKD in *pcy/pcy* mice can be identified. These were named MOP1 and MOP2 for modifier of polycystic kidney locus 1 and 2. MOP1 and MOP2 are located on mouse chromosome 4 and 16, respectively. The logarithm of odds (LOD) score supporting the existence of MOP1 near the D4Mit111 marker was 4.5. The LOD score supporting the existence of MOP2 near the D16Mit1 marker was 5.6. In addition, there were no regions with LOD score of > 2.0 to suggest the presence of additional QTLs elsewhere in the mouse genome. Mapmaker/QTL analyses also indicated that the MOP1 locus can account for 36.5% of the observed phenotypic variance in the K/B ratio of *pcy/pcy* F2 mice and the MOP2 locus can explain 46.2% of the observed variance of the K/B ratio in polycystic *pcy/pcy* F2 mice.

Complete suppression of the polycystic kidney phenotype in pcy mice. Approximately 25% of the polycystic *pcy* × *cast* F2 mice have K/B ratio and gross kidney morphology indistinguishable from normal mice. The kidneys of these animals, illustrated in Fig. 1, *b* and *c* and Fig. 2, *b* and *c* were found to be cystic only after histological examination of their kidneys. The development of polycystic kidney disease in these mice is significantly suppressed. As discussed above, only 651 rather than the expected 750 of the 3,105 *pcy* x *cast* F2 mice were found to be polycystic histologically. Based on Mendelian ratio, up to 100 of the F2 mice showed absolutely no detectable signs of PKD at 6 wk of age.

If these 100 completely protected *pcy/pcy* mice are mixed in with the 2,455 phenotypically normal *cast* × *pcy* F2 mice, we would expect to find ~ 30 mice to be homozygous for the DBA/2 allele of the *pcy* locus if we genotype 800 of these 2,455 *cast* × *pcy* F2 mice. If all of the 2,455 normal F2 mice are true normals because they are genotypically normal, we should not find any animals homozygous for the DBA/2 allele of the *pcy* locus. To distinguish between these two alternatives, we prepared genomic DNA from 800 out of the 2,455 phenotypically normal mice and genotyped all 800 samples for their *pcy* locus using a marker closely linked to the *pcy* locus as a probe. D9Mit77 is a marker that we found to have no recombination with the *pcy* mutation out of $> 1,300$ (2×651) meiosis examined. Among the 800 samples genotyped, we found 23 individuals with histologically normal kidneys and are homozygous for the DBA allele of D9Mit77. Unlike the kidneys of DBA/2-*pcy/pcy* mice where microscopic cysts can be detected at birth, no cysts were found in the kidneys of these mice at 6 wk of age (Fig. 1 *a* and Fig. 2 *a*). These results confirmed that the polycystic kidney phenotype in *cast* × *pcy* F2 mice can be completely suppressed by the combined actions of modifying genes.

To characterize the genetic basis of the suppression of polycystic kidney disease in these animals, whole genome genotyping and QTL analysis were carried out with 22 of these 23 mice. These additional genotype and phenotype data are combined with our original data from the first 46 samples and analyzed with the Mapmaker/QTL program. By including data from these 22 additional animals, the maximum LOD score for MOP1 increased from 4.5 to 9.6 and the maximum LOD score for MOP2 increased from 5.4 to 11.4, respectively, while the maximum LOD score supporting the presence of addition QTLs for the rest of the genome remained < 2.0.

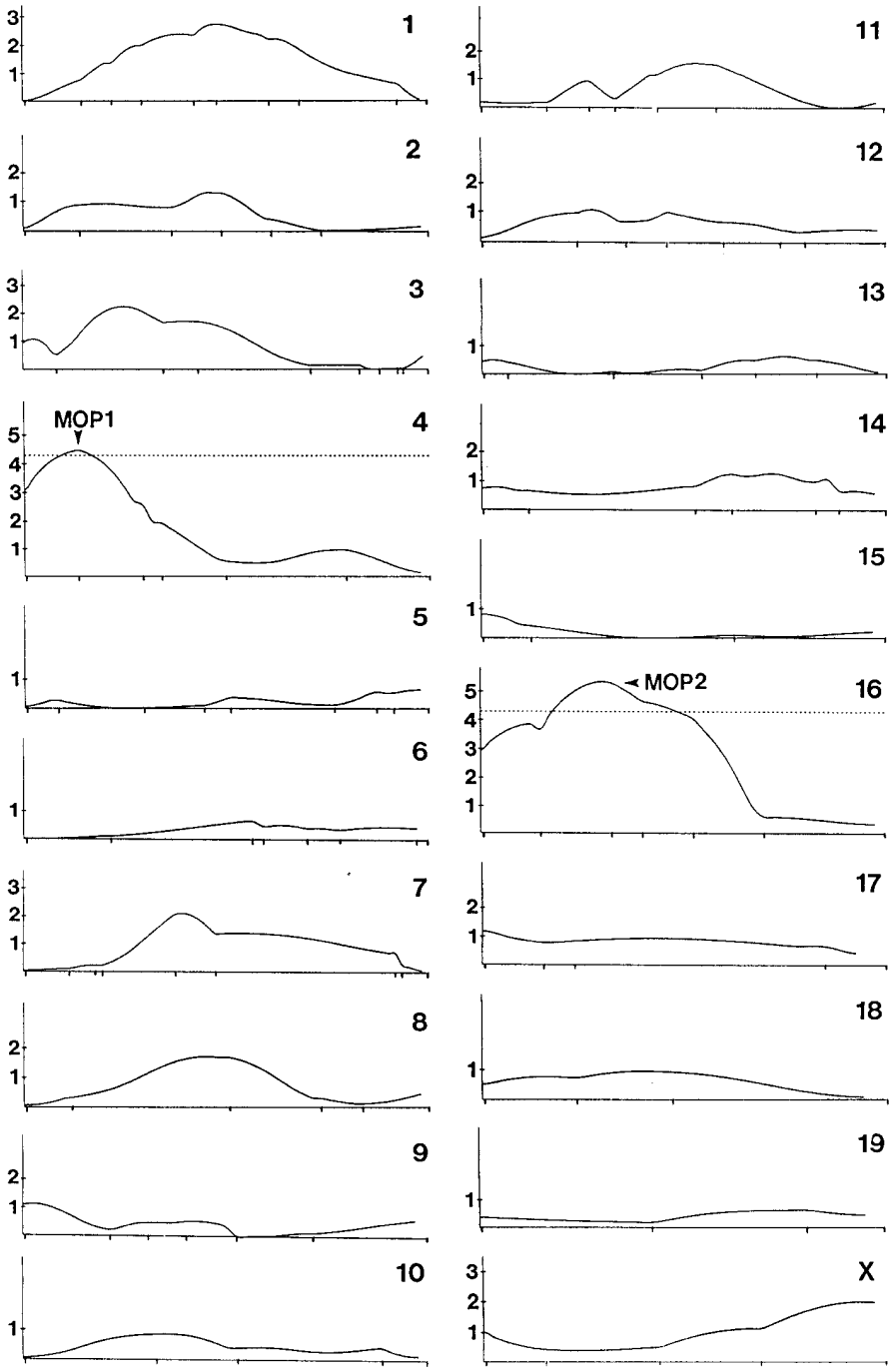

Figure 4. Plot of maximum LOD score for the presence of modifier loci affecting the progression of polycystic kidney disease in *pcy/pcy* mouse. The maximum LOD score calculated by MapMaker/QTL from the combined genotype and phenotype data from 23 most severe and 23 most mildly affected mice are shown for chromosome 1–19 and chromosome x. LOD score are plotted on the Y-axis. The centromere of each chromosome is placed at the origin and distance from the centromere is plotted on the X-axis. LOD score of 4.3 (*dash line*) represent significance at the $P = 0.05$ level. Microsatellite markers (*markers on the X-axis*) assayed in this analysis are D1Mit4, 122, 18, 127, 45, 91, 101, 110, 291, 17; D2Mit232, 294, 89, 92, 188, 136, 139, 111; D3Mit149, 46, 206, 173, 98, 49, 106, 249, 84, 32; D4Mit93, 111, 44, 79, 245, 246, 12, 68, 131; D5Mit180, 3, 77, 5, 155, 177, 95, 98, 62; D6Mit89, 46, 241, 128, 230, 37, 13, 200; D7Mit75, 245, 77, 162, 97, 108, 151, 109, 177; D8Mit158, 65, 128, 110, 55, 245; D9Mit60, 2, 95, 103, 108, 24, 120; D10Mit168, 45, 186, 117, 136; D11Mit1, 31, 21, 54, 14, 18, 10; D12Mit37, 59, 54, 17, 51, 27, 16, 18; D13Mit44, 61, 11, 12, 74, 31, 35; D14Mit40, 51, 28, 34, 73, 38, 131; D15Mit175, 55, 72, 108; D16Mit8, 74, 1, 38, 147, 14, 77, 49, 190, 20; D17Mit11, 10, 6, 18, 73; D18Mit30, 12, 105, 40; D19Mit31, 30, 90, 37; DXMit27, 57, 45, 64, 15. The maximum LOD score for MOP1 on chromosome 4 is 4.46 and the maximum LOD score for MOP2 on chromosome 16 is 5.36.

To determine if additional modifying loci can be identified by increasing the sample size of F2 mice analyzed, we carried out selective genotyping with 46 additional polycystic F2 mice. 23 of these mice were chosen because they were the second 23 mice with the mildest disease and the other 23 mice were chosen because they were the second 23 mice with the most severe disease. Genotype and phenotype data from these 46 additional animals were combined with data from the first 68 mice genotyped and analyzed with Mapmaker/QTL. Using the combined data from all 114 animals, the maximum LOD score for MOP1 increased to 10.3 and the maximum LOD score for MOP2 increased to 13.8, respectively. However, the amount of phenotypic variance explained by MOP1 and MOP2 remained at 36.7 and 46.8%, respectively. In addition, the LOD scores supporting the presence of addition QTLs for the rest of the genome also remained < 2.0. The LOD score plots for MOP1 and MOP2 calculated using the free genetics, dominant, recessive, and additive models in Mapmaker/QTL are shown in Fig. 5. These results also showed that within each locus, the *castaneous* and the DBA alleles of the MOP1 locus and the *castaneous* and the DBA alleles of the MOP2 locus acted in an additive mode to regulate the progression of the polycystic kidney phenotype in *pcy/pcy* mice.

Based on the intervals calculated to have LOD scores of

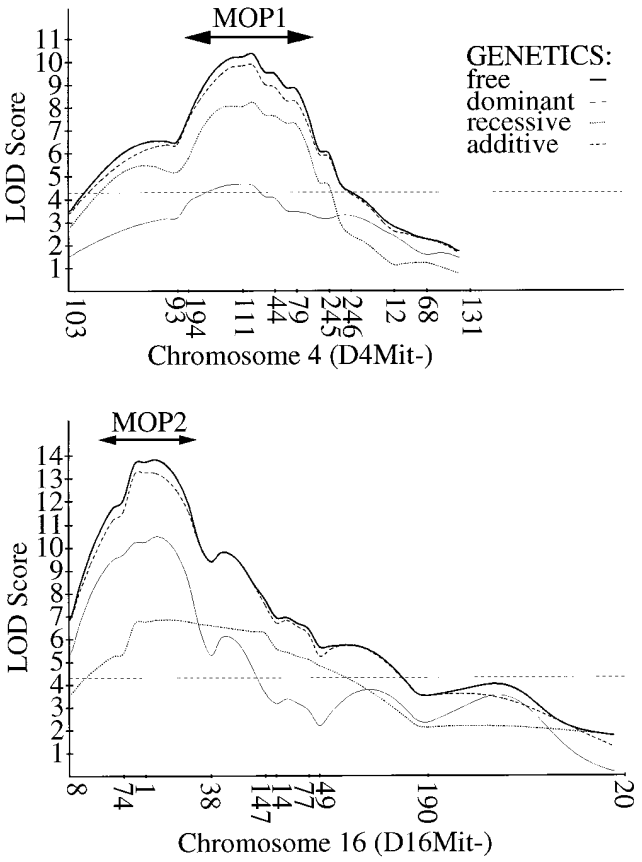

Figure 5. Plot of maximum LOD score for MOP1 and MOP2. The LOD scores for MOP1 and MOP2 obtained by using Mapmaker/QTL and selective genotyping of 114 *pcy/pcy* F2 mice chosen from the mildest ($n = 68$) and the most severely affected animals ($n = 46$) are shown in *A* and *B* respectively. The maximum LOD score for MOP1 was 10.3 and the maximum LOD score for MOP2 was 13.8. The LOD score calculated by Mapmaker/QTL assuming dominant, recessive, and additive genetics for MOP1 and MOP2 are also shown in each plot. The dotted line at LOD = 4.3 represent the 95% significance threshold for whole genome QTL analyses in F2 intercrosses.

three logs less than the maximum LOD scores, the LOD score plots in Fig. 5 indicated that MOP1 is located within the $\sim$ 15-cM interval between D4Mit194 and D4Mit245 while MOP2 is located within the $\sim$ 15-cM interval between D16Mit74 and D16Mit38.

The relatively small increase in maximum LOD scores for MOP1 and MOP2 by increasing the sample size from 68 to 114 illustrated the power of selective genotyping. In addition, it can be inferred that the majority of the information contents of the entire cross were analyzed by studying the 114 chosen animals.

To determine the phenotypic effects of the interactions of MOP1 and MOP2 loci on the progression of the polycystic kidney phenotype, we determined the correlation of the quantitative phenotype (K/B ratio) to the individual genotypes of D4Mit111 (MOP1) and D16Mit1 (MOP2) in all 673 (651+22) *pcy/pcy* F2 mice in our cross using two-factor ANOVA. Results of the analyses of the effect of the genetic status of MOP1 and MOP2 on the polycystic kidney phenotype in *pcy/pcy* mice in our *pcy* $\times$ *cast* F2 intercross are shown graphically in Fig. 6.

The phenotypic effects of genetic interactions between MOP1 and MOP2 loci can be summarized as follows. As predicted by the fact that the disease severity in inbred DBA/2-*pcy/pcy* mice is intermediate between the extremes observed in our *pcy* $\times$ *cast* F2 intercross, *pcy/pcy* mice that are homozygous DBA for both MOP1 and MOP2 showed an intermediate polycystic kidney disease phenotype. Except in mice that were also $MOP2^{DBA/DBA}$, $MOP1^{DBA/DBA}$ mice developed severe polycystic disease. In contrast, independent of their MOP2 status, all $MOP1^{cast/cast}$ mice had mild polycystic kidney disease. The polycystic kidney phenotype in $MOP1^{DBA/cast}$ animals was either mild, intermediate or severe and was dependent on their MOP2 status.

Except in $MOP1^{cast/cast}$ mice where the polycystic disease phenotype is always strongly suppressed, homozygosity for the $MOP2^{cast}$ allele is exclusively found in mice with severe disease while homozygosity in $MOP2^{DBA}$ is found only in mice with mild or intermediate polycystic kidney disease. The progression of polycystic kidney disease in animal heterozygous for MOP2 can be either mild, intermediate, or severe and is dependent on the MOP1 status.

Finally, these results showed that MOP1 and MOP2 alleles interact with each other to modulate the progression of poly-

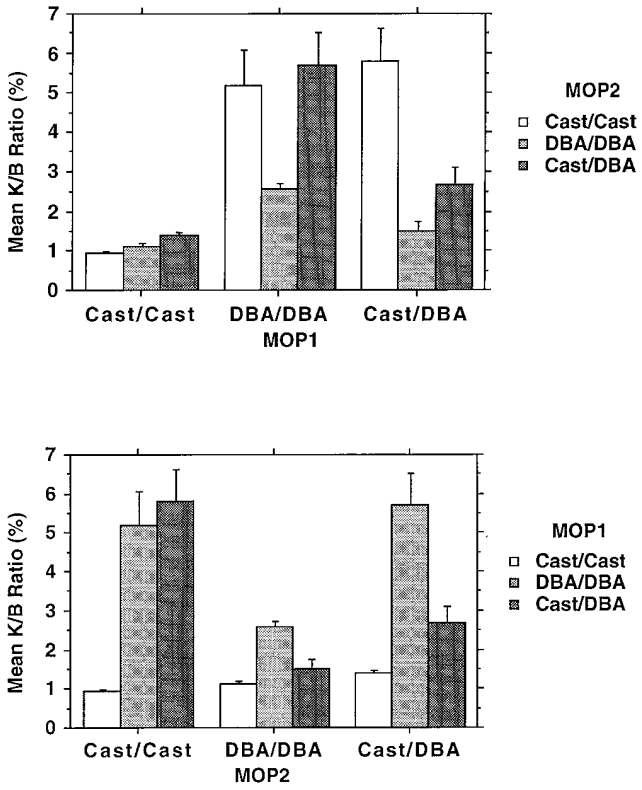

Figure 6. Two-factor ANOVA of the effects of MOP1 and MOP2 alleles on the polycystic kidney disease phenotype in *pcy/pcy* mice. The mean kidney weight to body weight ratio (*K/B ratio*) for each combination of the MOP1 and MOP2 genotypes is shown as a function of the MOP1 and MOP2 genotypes. Results were derived from two-factor ANOVA of the K/B ratio associated with the MOP1 (D4Mit111) and MOP2 (D16Mit1) status of 673 *pcy/pcy* F2 mice from a *pcy* $\times$ *cast* F2 intercross. The combinations of MOP1 and MOP2 loci are sorted by MOP1 and MOP2 status, respectively. Error bars represent standard errors of the means. Differences among each group are highly significant statistically ($P < 0.0001$).

Table I. Interaction of MOP1 with MOP2 and the Resultant Phenotype in pcy/pcy Mice

MOP1	MOP2	PKD progression
cast/cast	*cast/cast*	Slow
cast/cast	DBA/DBA	Slow
cast/cast	*cast*/DBA	Slow
DBA/DBA	*cast/cast*	Fast
DBA/DBA	*cast*/DBA	Fast
DBA/DBA	DBA/DBA	Intermediate
cast/DBA	*cast/cast*	Fast
cast/DBA	DBA/DBA	Slow
cast/DBA	*cast*/DBA	Intermediate

cast, *castaneous*.

cystic kidney disease. The phenotypic outcome of the interactions of the MOP1 and MOP2 alleles shown in Fig. 6 are summarized in Table I.

Discussion

A whole genome search has identified two major modifier loci that strongly modulate the progression of the polycystic kidney phenotype in *pcy/pcy* mice. The MOP1 locus maps to mouse chromosome 4 in a region that shows conserved synteny with the human chromosome region 9p22–q32. The MOP2 locus maps to mouse chromosome 16 in a region that shows conserved synteny with the human chromosomal region 3q13–28. Among the currently mapped or identified polycystic kidney disease predisposing mutations, only the *Cy* rat mutation is known to be located in the MOP1 genomic region (19, 20). Interestingly, the MOP1 locus also strongly modulates the progression of the polycystic kidney phenotype in *cpk/cpk* mice (21).

Significant strain differences in the expression of the polycystic kidney phenotype in *pcy/pcy* mice has been described in the literature. Nagao et al. reported that in the C57BL/6 background, the development of the polycystic kidney phenotype in *pcy/pcy* mice is greatly suppressed (22). Although a few microscopic cysts were observed in affected mice at birth, cystic involvement in kidneys of 30-wk-old mice are very limited both in number and in size. In addition, no significant changes in red blood cell count, platelet count, and hematocrit are observed between 30-wk-old cystic and 30-wk-old normal C57 mice, suggesting these mice are healthy. Whether the suppression of the progression of the *pcy/pcy* induced polycystic kidney disease in a B6 genetic background is due to MOP1 or MOP2 or other modifying locus is unknown.

The progression of polycystic kidney disease in humans is known to be variable. The identification of modifying loci associated with the *pcy* model of murine polycystic kidney disease suggests that modifying genes may also play a role in the progression of the human polycystic kidney diseases. In addition to elucidating the nature of the primary polycystic kidney disease mutations, defining the molecular nature of the genes underlying MOP1, MOP2, and the other modifying loci will provide novel information and insight into the pathogenesis of polycystic kidney disease. Understanding the molecular nature of these modifying genes may lead to novel prophylactics or therapeutics for the treatment of polycystic kidney diseases.

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