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Evidence that two phenotypically distinct mouse PKD mutations, *bpk* and *jcpk*, are allelic

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Evidence that two phenotypically distinct mouse PKD mutations, *bpk* and *jcpk*, are allelic. Numerous mouse models of polycystic kidney disease (PKD) have been described. All of these diseases are transmitted as single recessive traits and in most, the phenotypic severity is influenced by the genetic background. However, based on their genetic map positions, none of these loci appears to be allelic and none are candidate modifier loci for any other mouse PKD mutation. Previously, we have described the mouse *bpk* mutation, a model that closely resembles human autosomal recessive polycystic kidney disease. We now report that the *bpk* mutation maps to a 1.6 cM interval on mouse Chromosome 10, and that the renal cystic disease severity in our intersubspecific intercross progeny is influenced by the genetic background. Interestingly, *bpk* co-localizes with *jcpk*, a phenotypically-distinct PKD mutation, and complementation testing indicates that the *bpk* and *jcpk* mutations are allelic. These data imply that distinct PKD phenotypes can result from different mutations within a single gene. In addition, based on its map position, the *bpk* locus is a candidate genetic modifier for *jck*, a third phenotypically-distinct PKD mutation.

The formation of renal cysts characterizes several inherited human disorders. These diseases, which are transmitted as single Mendelian traits, include: (1) autosomal dominant polycystic kidney disease (ADPKD), (2) autosomal recessive polycystic kidney disease (ARPKD), (3) juvenile nephronophthisis-medullary cystic disease complex (JN-MCD), and (4) several multiple malformation syndromes [1]. Taken together, these disorders cause significant morbidity and mortality in both adults and children. For example, ADPKD causes 10 to 12% of adult end-stage renal disease (ESRD) [2], while in ARPKD, the majority of affected children die in the perinatal period [3]. Surviving ARPKD patients, combined with children who have JN, comprise 6% of all pediatric ESRD patients [4].

Among these inherited disorders, the phenotypic features and temporal onset of renal cyst formation vary widely. This phenotypic variability appears to be the result of several factors, including: different mutant genes; different mutations within the same gene; and different genetic backgrounds in which the mutant gene is expressed. Linkage analyses have identified at least three distinct loci for ADPKD [5-8]: a single locus for ARPKD on chromosome 6p [9, 10]; at least two separate loci for JN [11, 12]; and single discrete loci for several of the malformation syndromes [13-15]. Given the number of PKD mutations, multiple genes are involved in renal cystogenesis. However, to date, only the PKD1 locus has been identified and sequenced [16-18]. None of the other genes have been cloned. No putative human genetic modifier has been mapped and the molecular pathogenesis of renal cyst formation in human PKD remains undefined.

As a complementary research model, the mouse provides several experimental systems to study the pathogenesis of polycystic kidney disease. Numerous mouse cystic kidney mutations have been described [19-25]. While each mouse mutation is transmitted as a single recessive trait, the mutant phenotypes resemble the full range of human PKD. Three mutations, *cpk*, *bpk*, and the insertional mutation, *Tg737Rpw*, phenotypically resemble human ARPKD [19, 20, 26]. In contrast, the renal lesions in the *pcy*, *jck*, and *jcpk* mouse models correspond more closely to that evident in human ADPKD. As in human PKD, genetic background affects the disease phenotype in most of these mouse PKD models [20-22, 27, 28]. Recently, modifier genes have been mapped to Chromosome (Chr) 1 and Chr 10 for *jck*, Chr 4 and Chr 16 for *pcy* as well as for *cpk* [22, 29, 30]. Therefore, in a pattern analogous to human disease, multiple genes appear to be involved in renal cystogenesis in the mouse. It is tempting to speculate that at least some of these genes operate in common molecular pathways. However, this hypothesis has been difficult to address since none of the previously mapped mutations are allelic, and based on map positions, none are candidate modifier loci for any of the other mouse PKD mutations.

Previously we have described the mouse *bpk* mutation that arose spontaneously in the BALB/c strain [19]. In this model, dual renal tubular and biliary epithelial abnormalities are present in a pattern that closely resembles human ARPKD. We now report the localization of the *bpk* mutation on mouse Chr 10, and document that the severity of the renal cystic disease in our intersubspecific intercross progeny is clearly influenced by genetic background. Because this mutation maps close to the *jcpk*, a phenotypically-distinct PKD mutation, we performed a complementation test. Our data demonstrate that the *bpk* and *jcpk* mutations are allelic. Interestingly, the phenotype in the *bpk/jcpk* compound heterozygotes closely resembles that of *bpk/bpk* homozygotes. Finally, we note that based on its map position, the

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bpk locus is a candidate modifying gene for *jck*, a third phenotypically-distinct PKD mutation.

Methods

Mice and phenotype characterization

The *bpk* mutation arose spontaneously in the BALB/c inbred strain and has been transmitted as a stable recessive trait for over 40 intercrossed generations [19]. The mice used in these studies were from a sub-colony that has been maintained at the University of Alabama at Birmingham for four generations.

The *jcpk* mutation was induced by chlorambucil mutagenesis of (101 x C3H/HeJ)F2 mice. Males were then mated to normal females of the Tester strain. The F1 pups were intercrossed twice and the *jcpk* phenotype was first observed in the resulting progeny. The *jcpk* colony has been maintained by backcrossing test-proven +/*jcpk* males to normal C57BL/6J females [24]. The *jcpk* mice used in these studies were from this colony maintained at the Wadsworth Center (Albany, NY, USA).

For both mutations, individuals heterozygous for the mutant allele were identified in each generation by test-crossing phenotypically normal individuals to known heterozygotes.

CAST/Ei mice, an inbred strain derived from the wild mouse species *Mus musculus castaneus* (CAST), were obtained from The Jackson Laboratory (Bar Harbor, ME, USA).

To map the *bpk* mutation genetically, BALB/c-+/*bpk* heterozygotes were bred to CAST/Ei mice, and F1 progeny heterozygous for the *bpk* mutation, identified by test crossing, were intercrossed to generate F2 mice. Initially, offspring affected with cystic kidney disease died between 14 and 21 days after birth, so subsequent F2 litters were sacrificed 14 days after birth. The F2 pups were dissected and examined visually for the presence of enlarged cystic kidneys, gallbladder distension and abnormal biliary tracts.

To assess the severity of the renal cystic disease, we initially analyzed kidney weight alone, kidney weight as a function of body wt, and kidney length alone as well as the kidney length as a fraction of crown-rump length (K/C-R ratio). While in each data set, the variance in the F2 *bpk/bpk* pup parameters was greater than for the BALB *bpk/bpk* pups, the K/C-R ratio was the most convenient and reproducible index of disease severity. For all progeny, gross renal and biliary abnormalities were confirmed by histopathologic analysis. Whole kidneys and livers were removed and fixed in 70% alcoholic formalin (10% formalin in 70% ethanol). Paraffin sections were stained with hematoxylin and eosin and examined microscopically.

Complementation testing was performed at the Wadsworth Center. Three breeding pairs of test-proven BALB/c-+/*bpk* and +/*jcpk* heterozygotes were crossed. Progeny were sacrificed 11 to 17 days after birth and scored as above for visible and histopathological evidence of cystic kidney disease and biliary abnormalities.

PCR-based genotyping

To type progeny for inheritance of alleles of anonymous DNA microsatellite markers, liver genomic DNA was prepared according to standard protocols. Microsatellite markers whose BALB/c and CAST/Ei alleles differed in size by at least 6 base pairs (bp) and which mapped to proximal and distal ends of each chromosome (chromosome end-mapping study), to 10 cM intervals spaced along Chromosome 10 (low-resolution linkage mapping study), or within 3 M of *D10Mit199* (high-resolution linkage mapping study), were chosen from the on-line Whitehead/MIT database (accessible at http://www-genome.wi.mit.edu/; described by Dietrich et al [31]). PCR primer pairs for these markers were purchased from Research Genetics, Inc. (Huntsville, AL, USA).

Forward primers were end-labeled with γ32P-adenosine triphosphate, and PCR amplification was performed as described by Dietrich et al [31]. Amplified fragments were analyzed on denaturing 6% polyacrylamide gels.

Analysis of genetic data

Genotype data obtained by analyzing 42 affected F2 progeny for microsatellite markers known to map to the ends of each autosome were subjected to end-mapping analysis exactly as described by Iakoubova, Dushkin and Beier [22]. To construct low- and high-resolution linkage maps, individual chromosomal haplotypes were inferred from F2 genotypic data as described previously [32], and markers were ordered so as to minimize the numbers of crossover events needed to account for the inferred haplotypes.

Results

(BALB/c-+/bpk X CAST)F1 intercross

In our (BALB/c-+/*bpk* X CAST)F1 intercross, F1 +/*bpk* hybrids were identified by progeny testing. While we have not formally evaluated our F1 pups, they have exhibited no manifestations of disease and both F1 males and females breed in a robust fashion.

Of the 530 F2 progeny generated to date, 124 (23.4%) have recessive PKD. The number of F2 *bpk/bpk* pups is consistent with that expected for the Mendelian inheritance of a single recessive trait. Among the F2 progeny, the affected pups were significantly runted shortly after birth as compared with normal litter mates. They developed prominent abdominal distension by 9 to 15 days of age and rapid progression to death by 21 days. In comparison, abdominal distension is not detected in the BALB/c-*bpk/bpk* pups until after two weeks of age and these pups typically survive to 4 to 5 weeks of age [19]. Gross inspection of the F2 pups at necropsy at 14 days revealed markedly enlarged kidneys with a cystic pattern reminiscent of BALB/c-*bpk/bpk* mice at 24 to 28 days of age. The K/C-R was normally distributed in both the F2 (Fig. 1) and parental populations (data not shown). The mean K/C-R was 0.36 ± 0.8 for the F2 pups as compared with 0.35 ± 0.2 for the BALB/c-*bpk/bpk* homozygotes ($N = 12$). An F test of these variances confirmed that they are statistically different ($P < 0.001$). In addition, the liver parenchyma in these F2 affected pups was pale and marked gallbladder dilation was common. Preliminary examination of 10 F2 *bpk/bpk* livers suggested that as with the renal lesion, the severity of the biliary lesion is variable in the intercross pups (data not shown). Direct visual inspection failed to reveal evidence of other visceral, cranial or skeletal abnormalities.

Genetic mapping of bpk

To expedite the process of assigning *bpk* to a chromosome, we applied the end-mapping strategy of Iakoubova et al [22] to 42 affected F2 progeny of the (BALB/c-+/*bpk* X CAST)F1 intercross. In effect, by typing only markers at the ends of each chromosome, we generated a series of "chromosomal haplotypes" for each mutant mouse. Among the progeny of an intercross, a proportion of the F2 pups will inherit chromosomes that are apparently non-recombinant, that is, the alleles of markers along

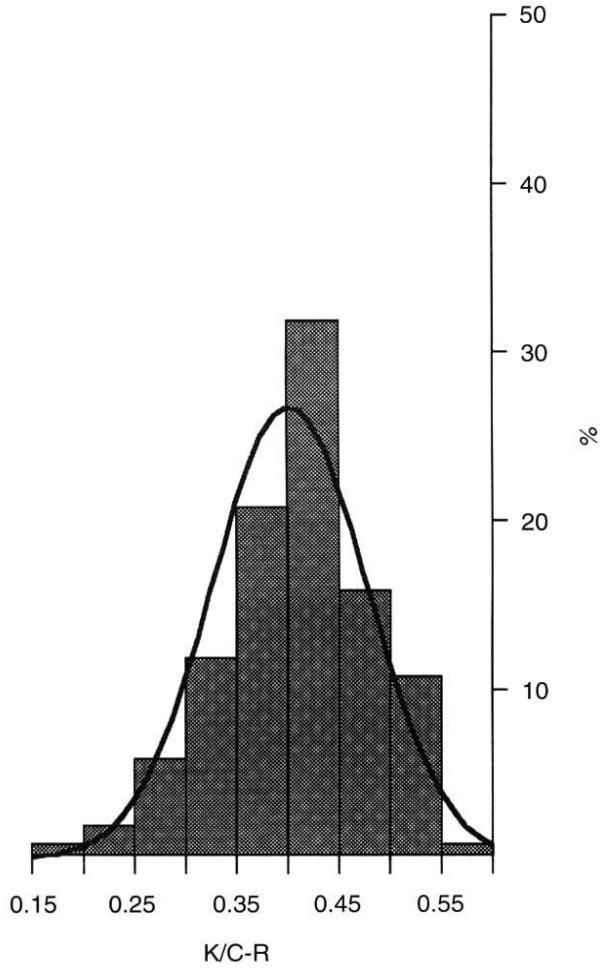

Fig. 1. *The distribution of the kidney lesion severity in the F2 bpk/bpk pups.* Necropsy at 14 days revealed massively enlarged kidneys in each F2 *bpk/bpk* pup. In order to quantitatively assess the severity of the kidney lesion, the ratio of kidney length to crown-rump length (K/C-R) was measured in each pup. This ratio corrected the renal size for the degree of linear growth retardation and was highly reproducible. Therefore, among the possible parameters to assess the severity of renal disease, the K/C-R was the most reliable. While the mean K/C-R in the F2 pups (0.36 ± 0.08; data presented as the mean ± SD) was quite similar to that in BALB/c-*bpk/bpk* pups (0.35 ± 0.02; data not shown), the variance in the F2 K/C-R was fourfold larger. An F test of these variances confirms that they are statistically different ($P < 0.001$). These data are consistent with the presence of modifying loci in the CAST genetic background which accelerate the progression of the renal cystic disease severity in the F2 intercross pups. The relatively large variance in the F2 K/C-R suggests that relatively few modifying loci are involved [36].

these chromosomes will correspond to a single parental strain, in this case either BALB/c or CAST. In the analysis of a recessive mutation like *bpk*, only non-recombinant chromosomes (NR) that are inherited from the disease-bearing strain (BALB/c) can carry the mutation. For non-linked chromosomes, there is a random distribution of NR chromosomes for each parental strain. In contrast, for a linked chromosome, that is, the one carrying the *bpk* mutation, there will be a paucity of CAST NR chromosomes. Therefore, this strategy provides an efficient method to exclude chromosomes that are unlikely to carry the mutation and to identify a cohort of candidate chromosomes for more detailed analysis using standard recombinational mapping techniques.

For two chromosomes, Chr 10 and 18, a significant paucity of apparently non-recombinant CAST haplotypes (NR_{CAST}) was observed. For Chr 10, 2 NR_{CAST} were observed versus 19.6 expected among 68 informative chromosomes, χ^2 (1 d.f.) = 21.77, $P < 0.0001$, whereas for Chr 18, 6 NR_{CAST} were observed versus 20.2 expected among 70 informative chromosomes, χ^2 (1 d.f.) = 11.46, $P = 0.0007$. These data are consistent the hypothesis that either of these chromosomes harbors the *bpk* gene. Because the paucity of NR_{CAST} was particularly dramatic in the case of Chr 10, we first typed the 42 affected F2 progeny for a series of anonymous DNA microsatellite markers known to be spaced at ~10 cM intervals along this chromosome. As shown in Figure 2, these data position *bpk* within a 3.6 cM interval centered on *D10Mit199*. Additional F2 pups (total: 124 pups/248 meioses) were then typed with the immediately flanking markers and recombinational mapping was used to further refine the localization. These data, summarized in Figure 3, establish that the *bpk* locus lies within a 1.6 cM interval flanked by *D10Mit115* and *D10Mit173/Mit199/Mit200*.

These results, coupled with the fact that *bpk* is transmitted as a single recessive trait in both the BALB/c strain and our (BALB/c-+/*bpk* X CAST)F1 intercross, confirm that the *bpk* disease-susceptibility locus maps to Chr 10. Therefore, no further mapping analyses were performed for Chr 18.

Allele testing with +/jcpk heterozygotes

As shown in Figure 3, the *bpk* locus co-localized with the previously assigned map position for the *jcpk* mutation. To test whether these mutations were allelic, test-proven +/*bpk* and +/*jcpk* heterozygotes were crossed. Of the 19 F1 pups, six (31%) developed abdominal distension by 7 to 10 days of age. In all six affected pups, necropsy at 11 to 17 days revealed markedly enlarged kidneys that were studded with small opalescent cysts. The liver parenchyma in these F1 affected pups was pale and marked gallbladder dilatation was evident. Direct visual inspection failed to reveal the presence of other visceral, cranial or skeletal abnormalities. A detailed histopathologic survey will be required to assess the severity of the kidney and biliary lesions and to evaluate other organs at a microscopic level for associated abnormalities. These analyses are in progress.

Given that both *bpk* and *jcpk* are recessive mutations, the expression of a mutant phenotype in approximately one quarter of the F1 offspring indicates that these mutations are allelic. However, we did explore the alternative possibility that on the BALB/c background, the *jcpk* gene acts in a dominant fashion with variable penetrance. Specifically, we crossed test-proven +/*jcpk* heterozygotes and wild-type BALB/c mice. No affected F1 pups were detected ($N = 12$). Moreover, we have no evidence of dominant early onset PKD caused by either of the *bpk* or *jcpk* genes on any other genetic background, including CAST. Therefore, given the co-localization of these genes on Chr 10 and the presence of affected pups in a standard allelism test, we believe that the simplest hypothesis is that these mutations are allelic.

Comparative histopathology

In *bpk/bpk* homozygotes the renal cysts are radially arrayed from the cortex to the medulla, in a pattern typical of cystic collecting ducts (Fig. 4A). The predominance of collecting duct

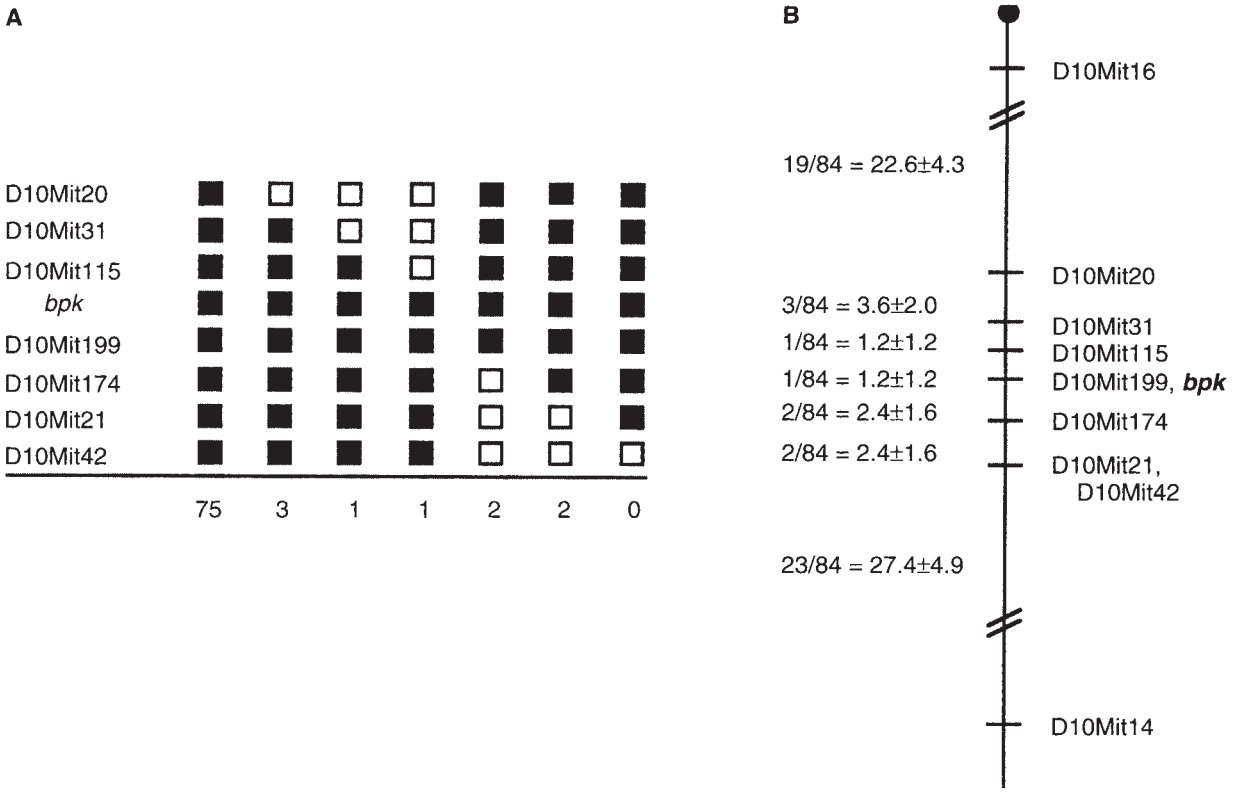

Fig. 2. *Genetic localization of bpk on Chromosome 10.* (**A**) Haplotype distribution among 84 test chromosomes from the (BALB/c-*bpk*/+ X CAST)F1 intercross. For each locus, solid boxes represent the inheritance of BALB/c-derived alleles from the mutant chromosome, and open boxes represent the inheritance of CAST-derived alleles. The number of chromosomes for each haplotype is shown below the columns. (**B**) Genetic map of Chr 10 constructed from the haplotype distribution in (A). The map distances, in cM, were calculated from the recombination frequency observed for each interval and are expressed with standard errors.

involvement has previously been confirmed by lectin-binding studies [19]. In our analyses, we noted occasional cystic dilatation of Bowman's spaces, involving 6% of the glomeruli. In contrast, in *jcpk/jcpk* homozygotes, the kidney lesion is remarkable for dilatation of Bowman's space in 95% of the glomeruli, as well as cysts in both proximal and distal tubular elements (Fig. 4E). In the *bpk/jcpk* compound heterozygote, the cysts are arrayed in a radial fashion, very similar to the cystic pattern seen in *bpk/bpk* kidneys (Fig. 4C). Dilation of Bowman's space was evident in 17% of the glomeruli and normal proximal tubular elements were seen. Thus, in the compound heterozygote, the renal cystic phenotype appears to more closely resemble that manifest in *bpk/bpk* homozygotes.

Previous analysis of the portal tracts from both the *bpk/bpk* and *jcpk/jcpk* homozygotes revealed evidence for biliary dysgenesis [19, 24]. Similarly, in the six *bpk/jcpk* compound heterozygotes, the portal tracts are expanded with multiple irregularly shaped and variably dilated bile ducts. These tortuous ducts are generally lined with a hyperplastic epithelium. The comparative biliary histopathology of these mutants is shown in Figure 5.

Discussion

Using the chromosomal exclusion method recently described by Iakoubova et al, we have rapidly and efficiently mapped the mouse *bpk* mutation to a well-defined interval on Chr 10. In the course of these mapping studies, we noted that the renal cystic phenotype was generally more severe and more variable in the affected F2 intercross pups than in affected pups from the BALB/c parental strain. Preliminary analyses suggest that the severity of the biliary lesion in our affected F2 pups is also variable. These data implicate the presence of modifying gene(s) in the CAST genetic background that accelerate the progression of the renal cystic disease in the F2 intercross pups. In addition, the end-analysis genetic mapping of *bpk/bpk* F2 progeny demonstrated a significant paucity of Chr 18 with apparently non-recombinant CAST/Ei haplotypes (NR_{CAST}). A subsequent review of the disease severity in these initial 42 pups revealed that 12 of the 42 had mild kidney disease as assessed by a K/C-R ratio of < 0.3. Interestingly, the majority of the informative chromosomes from this mildly affected cohort were (NR_{BALB}) while the informative chromosomes from the other 30 pups were evenly distributed as NR_{CAST} and NR_{BALB}. This result raises the possibility that a modifier of the *bpk* phenotype maps to Chr 18, and that the BALB/c allele attenuates disease severity. Taken together, these data are consistent with previous reports that genetic background influences the renal disease phenotype of the *cpk*, *pcy* and *jck* mutations [21, 22, 28]. Further phenotypic and genetic analysis of additional F2 progeny from this cross will be needed to resolve this issue and specifically, to better characterize the role of genes on Chr 18 in this cystic kidney disease model.

Our mapping data position the *bpk* locus within the same discrete interval as the phenotypically distinct *jcpk* mutation. As a somewhat unexpected finding, we have determined that despite

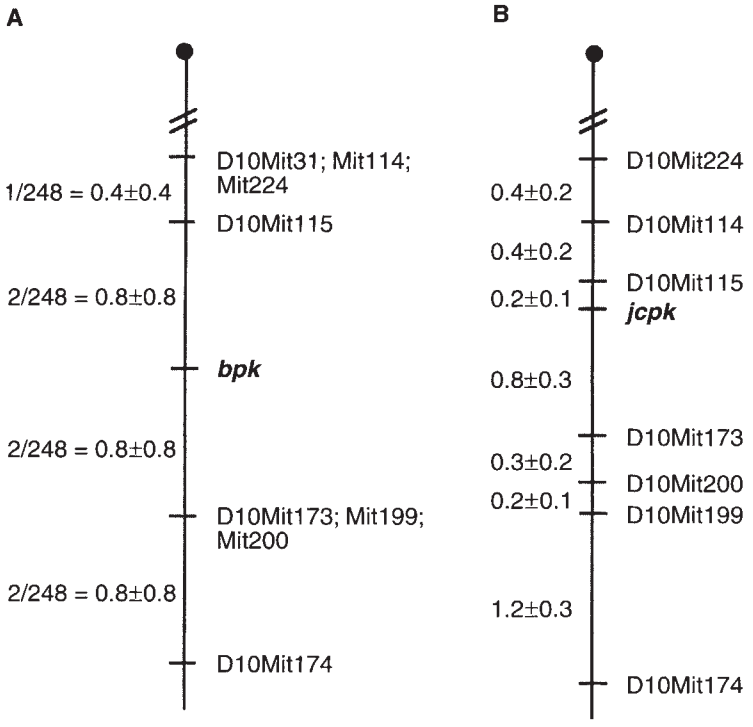

Fig. 3. *Refined genetic localization of the bpk and jcpk mutations on Chromosome 10.* (**A**) The refined genetic maps for the *bpk* mutation and (**B**) the *jcpk* mutation (E. Bryda, unpublished data). For *bpk*, the map distances, in cM, were calculated from the recombination frequency observed for each interval and are expressed with standard errors.

their distinctly different PKD phenotypes, the *bpk* and *jcpk* mutations appear to disrupt the same gene. However, given the origin of each mutation, this conclusion requires some qualification. The *bpk* mutation arose spontaneously in an inbred laboratory strain, and thus it likely represents a point mutation or a sequence alteration involving few base pairs. In contrast, the *jcpk* mutation was induced by the chemical mutagen, chlorambucil [24]. Since all known chlorambucil mutations involve deletions or chromosomal rearrangements, it is very likely that *jcpk* involves a significantly large genomic alteration [33]. At this point, we cannot determine whether the *jcpk* phenotype results from disruptions in the same single locus as *bpk* or whether it involves two closely linked genes, one of which is *bpk*. The latter possibility has important precedence in PKD research because such a contiguous gene syndrome provided the major breakthrough in the positional cloning effort to identify the PKD1 locus [34]. These two possibilities will only be distinguished by identifying the candidate gene(s) and establishing the specific mutations by sequence analysis. Currently, efforts to isolate the disease-susceptibility gene(s) at the *bpk/jcpk* locus using positional cloning strategies are underway in our laboratories.

The Chr 10 map position of the *bpk/jcpk* locus also coincides with the map position of the principal modifying gene for the *jck* mutation, a third phenotypically distinct PKD model [22] (D. Beier, personal communication). Therefore, based on these genetic mapping data, the gene identified by the *bpk* and *jcpk* mutations is also a candidate PKD modifying gene. Our data combined with the *jck* data suggest that there are at least four alleles at this putative modifying locus. The first is an allele carried by C57BL/6J mice that appears to act as a wild-type allele and is not associated with any defect. The second is a mild loss-of-function allele, exemplified by the DBA/2 form of the gene. Homozygotes for this allele do not manifest any kidney defect, but when this allele is expressed together with mutations at the unlinked *jck* locus, the cystic kidney lesion is exacerbated [22]. The third is the *bpk* allele, a stronger loss-of-function allele, which in the homozygous state causes a recessive polycystic kidney phenotype. The fourth is *jcpk*, which given its origin may be a null allele. In homozygotes, *jcpk* causes particularly severe cystic kidney disease, whereas aged heterozygotes develop a late-onset glomerulocystic disease phenotype that is not fully penetrant. Using specific breeding strategies, the potential interaction of the *bpk/jcpk* locus with *jck* is currently being investigated.

These findings have several important implications for PKD research. First, the *bpk* model provides the reagents to identify both a PKD-susceptibility gene as well as additional loci that modify the phenotypic expression of this disease gene. Given the histopathologic findings in *bpk/bpk* homozygotes from both the BALB/c parental strain and our intercross, it is likely that these genes operate in a molecular pathway that is important in both renal cystogenesis as well as in the terminal differentiation of renal tubular and biliary epithelia. The isolation of this PKD-susceptibility gene and its genetic modifiers, and ultimately examination of their interactions, should help elucidate the molecular mechanisms involved in disease pathogenesis as well as in normal renal and biliary development.

Finally, our findings have potential ramifications for the conceptual understanding of PKD. In the current nosology, human PKD is generally classified as either ADPKD or ARPKD, based in part on genetic transmission and in part, on histopathologic features. Implicit in this formulation is the hypothesis that the molecular pathogenesis of human ADPKD and ARPKD involve distinct sets of genes. Indeed, most mouse PKD mutations are characterized and discussed as pathologic models for either human ADPKD or ARPKD.

ADPKD is generally considered to be a systemic disorder with

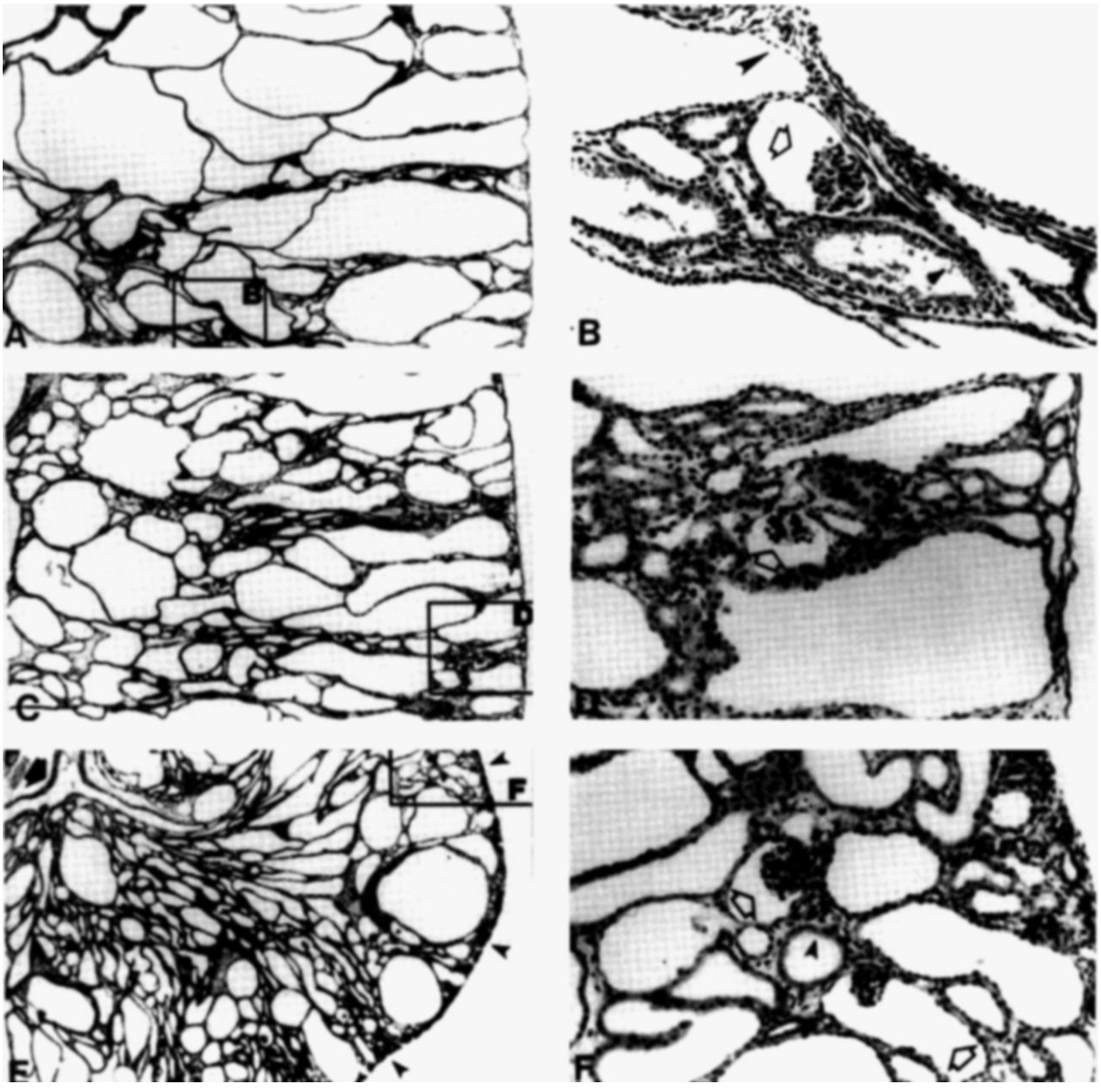

Fig. 4. *Comparative renal histopathology in bpk/bpk pups, F1 bpk/jcpk pups, and jcpk/jcpk pups.* **A.** The renal histopathology of a 28-day old, BALB/c-*bpk/bpk* pup. The cystic dilatations are arrayed perpendicular to the renal capsule in a pattern most consistent with collecting duct involvement. Occasional cystic dilatations of Bowman's space are evident (H&E ×100). **B.** The area of detail from panel A. While the majority of cystic tubules in the *bpk/bpk* kidney are lined by a flattened epithelium (large arrow), occasionally the cyst-lining epithelia exhibit a brush border (small arrow), suggestive of proximal tubular origin. In 6% (6/100) of the glomeruli, Bowman's space is dilated (open arrow; H&E ×600). **C.** The cystic kidneys from a 14-day old, F1 *bpk/jcpk* compound heterozygote. The histopathology closely resembles that of *bpk/bpk* homozygotes. The predominant lesion involves radially arrayed cystic tubules and occasional cystic dilations of Bowman's space are evident (H&E ×100). **D.** The area of detail from panel C. While the majority of glomeruli are normal, Bowman's space is dilated (open arrow) in 17% (17/100) (H&E ×600). **E.** The renal histopathology in a seven days old, C57BL/6J- *jcpk/jcpk* pup. In contrast to the cystic lesions evident in both *bpk/bpk* homozygotes and F1 *jcpk/bpk* heterozygotes, in the *jcpk/jcpk* kidney extensive cystic dilation involves Bowman's space, as well as virtually all tubular elements from the renal pelvis (large arrow) to the cortex. The terminal phase of normal nephrogenesis is evident in the subcapsular region (small arrows) (H&E ×100). **F.** The area of detail from panel E. At higher magnification, the dilated tubules appear to be of heterogeneous origin. Some tubules are lined by cuboidal epithelia with an associated brush border, suggesting proximal tubular origin (small arrow). The radially arrayed tubules are lined with a flattened epithelia consistent with a distal tubular origin. The most striking feature of these cystic kidneys is the dilatation of Bowman's space (open arrow) in 95% (95/100) of the glomeruli (H&E ×600).

abnormalities occurring in several epithelial organs (kidney, liver, pancreas, lung) as well as the heart and the vasculature. In the kidney, cysts can arise anywhere along the nephron length, from the glomerulus to the collecting duct [2]. In comparison, ARPKD is a malformation complex that involves only the kidney and liver. The predominant renal cystic lesion is confined to the collecting ducts [3]. However, careful clinical review reveals that there can be phenotypic overlap between ADPKD and ARPKD. For example, in a subset of ADPKD families, affected children can present in the neonatal period with massively enlarged kidneys that are

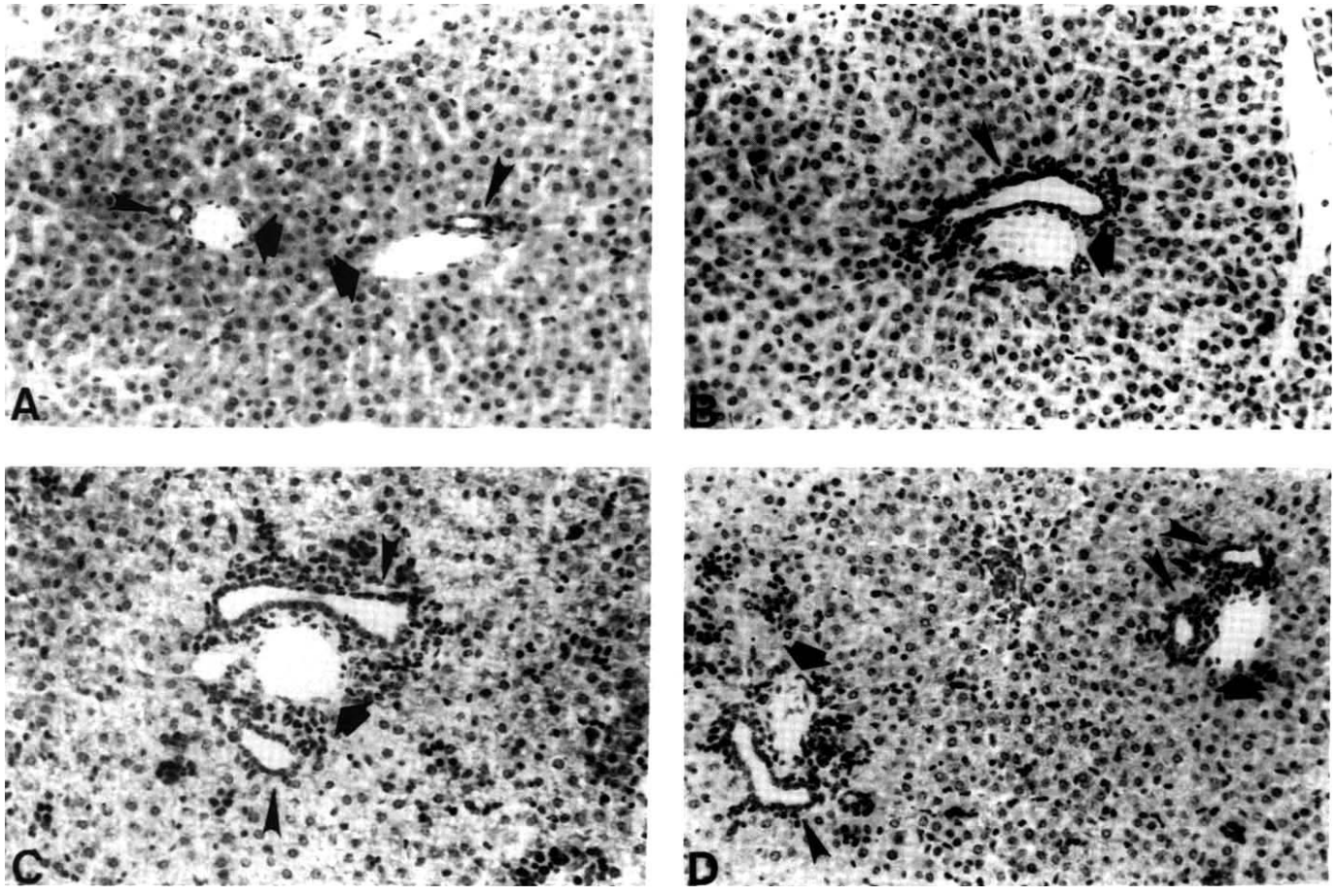

Fig. 5. *Comparative biliary histopathology.* **A.** The normal portal triad histology from a 14-day-old BALB/c pup. The portal vein is indicated by the large arrows and the associated bile ductules by the smaller arrows (H&E ×250). The mild portal vein dilation was interpreted as normal and presumed to be age-related since the same findings were evident in age-matched DBA/2J pups (data not shown). **B.** The biliary histopathology of a 28-day-old BALB/c-*bpk*/*bpk* pup. The bile ductule is curvilinear and lined by hyperplastic epithelia (H&E ×250). **C.** The portal triad from a 14-day-old F1 *bpk*/*jcpk* compound heterozygote. As in both the BALB/c-*bpk*/*bpk* and the C57BL/6J-*jcpk*/*jcpk* homozygotes, the bile ductules are branched and lined by hyperplastic epithelia. In addition, the portal triad is expanded in part by hematopoetic cells and in part by immature stromal cells (H&E ×250). **D.** The biliary histopathology of a seven days old, C57BL/6J-*jcpk*/*jcpk* pup. The branched bile ductules are lined by hyperplastic epithelia and the portal triad is expanded by both hematopoetic cells and immature stromal cells (H&E ×250).

reminiscent of ARPKD. Often these infants have a more virulent clinical course that is similar to ARPKD infants and characterized by the development of hypertension and renal insufficiency. In addition, case reports suggest that while biliary dysgensis leading to congenital hepatic fibrosis is an invariant finding in ARPKD, it also can occur in ADPKD [3]. And finally, in a minority of children with PKD, the renal and biliary histopathology defy classification according to standard guidelines (L. Guay-Woodford, unpublished data). Therefore, while in human PKD the disease-susceptibility loci for ADPKD and ARPKD are distinct and separate, molecular pathways may be shared.

In this context, our finding that a single PKD gene can be involved in different PKD phenotypes is particularly intriguing. To our knowledge, these data have no prior precedent in PKD although there is ample precedence for this phenomenon with other mouse loci. Perhaps the most compelling example involves mutations in the mouse agouti (a) locus which result in phenotypes ranging from dominant gain-of-function to a series of loss-of-function recessive alleles that can be ranked in order of severity like the model we have suggested for *bpk*/*bpk*, *bpk*/*jcpk*, and *jcpk*/*jcpk* [35]. Therefore, we propose that different mouse PKD models may share aspects of molecular pathogenesis and by extension, genetic investigations in mouse models such as *bpk* have important implications for dissecting the molecular pathogenesis of human PKD.

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