Clinical and Pathologic Findings in Two New Allelic Murine Models of Polycystic Kidney Disease

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Abstract. Patients with inherited cystic kidney diseases have progressive cystic dilation of nephrons with concomitant loss of functional renal parenchyma and renal failure. Animal models of inherited cystic kidney disease are useful for study of the pathogenesis and molecular basis of cystic renal diseases. This article describes the clinical and pathologic features in two spontaneously occurring murine models of inherited polycystic kidney disease due to independent allelic mutations on mouse chromosome 8. The mutations, designated kat and kat2J, affect a chromosomal segment homologous to a region of human chromosome 4q35; the altered gene has not yet been identified. An allelism test showed that the mutations are at the same locus. The phenotype, inherited as an autosomal recessive, is more severe in kat2J/kat2J mice. Their kidneys are morphologically normal at birth, but by 3 mo of age, cysts affect all levels of the nephron. Adult males have testicular hypoplasia and they are sterile. A few of the oldest kat2J/kat2J mice have focal portal bile duct proliferation and dilation. kat2J/kat2J mice develop anemia and uremia and die before 1 yr of age. In kat/kat mice, the renal cystic disease progresses more slowly but is morphologically similar to that of kat2J/kat2J mice. The progressive cystic transformation of the kidneys in these allelic murine models resembles that seen in humans with autosomal dominant polycystic kidney disease.

The polycystic kidney diseases (PKD) are a heterogeneous group of disorders inherited either as autosomal recessive (ARPKD) or autosomal dominant (ADPKD) traits. In PKD, renal function deteriorates due to diffuse cystic dilation of all or a segment of the nephron and loss of normal parenchyma. In ADPKD, renal cysts are usually not apparent at birth; they begin to develop in childhood and are numerous and large in adults. Extrarenal manifestations of ADPKD include cerebral arterial aneurysms, cardiac valve abnormalities, intestinal diverticula, and biliary, splenic, and pancreatic cysts (1). ARPKD affects infants and children with massive kidney enlargement due to dilated collecting ducts and, in some patients, portal biliary tract dilation and proliferation. In addition to clinical variability, there is genetic heterogeneity in PKD (2). Mutations in at least three distinct loci are known to cause ADPKD (3).

Multiple spontaneously occurring animal models of PKD have been described (3–8). Each may define a novel gene important for maintenance of normal renal structure and epithelial differentiation. Cellular processes associated with signal transduction, transcriptional regulation, and cell-cycle control are involved in cyst formation in PKD (3), and models can be used to investigate the complex cellular, genetic, and molecular bases of cystic kidney disease. Studies of animal models have provided insight into the pathogenesis of human PKD. Seminal information on extracellular matrix composition, epithelial cell proliferation, and differentiation and trans-tubular fluid transport has been obtained using animal models of PKD (3,4). Animals with PKD can also be used to assess effects of pharmacologic and molecular therapies on cyst formation and parenchymal destruction (9). Such studies may provide important information for the treatment of human PKD.

Here we describe the clinical and pathologic findings in two murine models of PKD caused by independent allelic mutations. The mutations, kat and kat2J, arose spontaneously in RBF/Dn and C57BL/6J mice, respectively, and map to chromosome (Chr) 8 in a segment homologous to human Chr 4q35 (10,11). The kat mutation was backcrossed first to C3HeB/FeJ three times, followed by five backcrosses to C57BL/6J, resulting in the Stock kat+/strain. Matings of heterozygote Stock kat+/ with C57BL/6J kat2J/+ mice produced compound heterozygotes with the mutant phenotype, confirming that the two mutations are allelic (10). The phenotype in both kat/kat (KAT) and kat2J/kat2J (KAT2J) mice is inherited as an autosomal recessive trait and includes progressive cystic kidney disease, anemia, uremia, testicular hypoplasia and male infertility, and choroid plexus cysts. The progressive renal cystic disease resembles that seen in ADPKD, and these mice can be used as models of human PKD. The delayed onset and progression of disease in these mice provides an opportunity for assessing temporal effects of therapeutic modalities.

Materials and Methods

The C57BL/6J-kat2J and Stock kat mice were maintained as pedigreed strains by the progeny test method at the Jackson Laboratory.
The highest standards of humane animal care were used. The Jackson Laboratory is fully accredited by the American Association for Accreditation of Laboratory Animal Care. Approximately half of KAT2J and KAT mice die before weaning for unknown reasons (10). To define survival after weaning in the two strains, 34 KAT2J and 52 KAT mice were followed from weaning or shortly after to their death. The survival distribution of KAT and KAT2J mice were compared using the Kaplan–Meier method.

For pathologic study, 32 KAT2J and 32 gender-matched normal littermate controls from 5 to 290 d of age were killed by CO₂ asphyxia. The body, kidneys, heart, spleen, gonads, and liver were weighed. The left kidney cyst fluid was aspirated by multiple punctures with a 30-gauge needle and tuberculin syringe, after which the kidney was lyophilized for 24 h and dry weight was determined. Hematocrit (Hct) of blood collected from the retro-orbital sinus immediately before asphyxia was measured. Blood urea nitrogen was quantified and peripheral blood smears were examined from five gender- and age-matched pairs of KAT2J and control mice at 1, 5, 6, 7, and 8 mo of age.

For light microscopy, the right kidney, testis, ovary, and liver were fixed in 10% formalin or Telyesnieczyk/Fekete fixative, processed routinely, and stained with hematoxylin and eosin. Selected sections were stained for iron with Prussian blue stain and Jones methenamine silver stain to demonstrate basement membrane (12). Samples of renal cortex from a 5-mo-old KAT2J and a control mouse were fixed in glutaraldehyde, post-fixed in osmium, processed routinely, and examined ultrastructurally.

Kidneys and liver from 19 KAT and 12 control mice from 19 to 630 d of age and testis from four KAT males and four littermate

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**Figure 1.** An adult KAT2J mouse (right) is smaller than the normal littermate (left) and has a blunted nose and abdominal distention due to enlarged kidneys.

**Figure 2.** There were no statistical differences in survivorship between male and female mice in the KAT \((P = 0.053)\) or KAT2J \((P = 0.1333)\) groups. Based on the log-rank test, survival was poorer for KAT2J mice \((P = 0.0027)\). All KAT2J mice die by 1 yr of age, and as a group, KAT mice survive longer than KAT2J mice do.

**Figure 3.** Many KAT2J mice are anemic even before they are weaned, and hematocrit (Hct) falls progressively as they age. Hct of KAT mice fell late in life, after about 200 d of age. The 95% predictive interval for normal control mice is between the solid lines.

**Table 1.** Blood urea nitrogen (mg/dl) in KAT2J and control mice

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<tr>
<th>Age (months)</th>
<th>Control</th>
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The survival distribution of KAT and KAT2J mice were compared using the Kaplan–Meier method. For pathologic study, 32 KAT2J and 32 gender-matched normal littermate controls from 5 to 290 d of age were killed by CO₂ asphyxia. The body, kidneys, heart, spleen, gonads, and liver were weighed. The left kidney cyst fluid was aspirated by multiple punctures with a 30-gauge needle and tuberculin syringe, after which the kidney was lyophilized for 24 h and dry weight was determined. Hematocrit (Hct) of blood collected from the retro-orbital sinus immediately before asphyxia was measured. Blood urea nitrogen was quantified and peripheral blood smears were examined from five gender- and age-matched pairs of KAT2J and control mice at 1, 5, 6, 7, and 8 mo of age.

For light microscopy, the right kidney, testis, ovary, and liver were fixed in 10% formalin or Telyesnieczyk/Fekete fixative, processed routinely, and stained with hematoxylin and eosin. Selected sections were stained for iron with Prussian blue stain and Jones methenamine silver stain to demonstrate basement membrane (12). Samples of renal cortex from a 5-mo-old KAT2J and a control mouse were fixed in glutaraldehyde, post-fixed in osmium, processed routinely, and examined ultrastructurally.

Kidneys and liver from 19 KAT and 12 control mice from 19 to 630 d of age and testis from four KAT males and four littermate...
controls were examined histologically as described above. Hct of 55 male and 54 female KAT mice from 41 to 642 d old were measured.

Results

Clinical and Laboratory Findings in KAT2J and KAT Mice

The KAT2J mice were runts at birth and had persistent growth failure and facial dysmorphism (Figure 1). Although KAT2J mice gained weight as they aged, they were consistently smaller than control mice and had abdominal distention due to enlarged kidneys. Of the KAT2J mice that survived weaning, approximately one-third died suddenly without apparent cause before 100 d of age. All KAT2J mice were dead by 1 yr of age. KAT mice had similar growth failure and dysmorphism, but 22% of KAT mice survived past 1 yr of age (Figure 2). Survival was poorer for KAT2J mice as a group (median survival, 211 d) than for the KAT mice (median survival, 286 d) ($P = 0.0027$). There was no statistical difference in the survivorship between male and female mice in either group (KAT males versus females $P = 0.053$, KAT2J males versus females $P = 0.1333$).

Anemia affected even very young KAT2J mice, and progressed throughout life. Hct was slightly less than normal in many of the young KAT mice and fell progressively after 200 d of age (Figure 3). The peripheral red cells were normochromic and normocytic in the three youngest KAT2J mice examined. The 7- and 8-mo-old KAT2J mice had marked poikilocytosis and also had increased blood urea nitrogen (Table 1).

Pathology of KAT2J and KAT Mice

Both wet and dry kidney weight as a proportion of total body weight increased with age in KAT2J mice, and the wet kidney weight/body weight ratio increased at a very rapid rate (Figure 4, A and B). The older mice had large pale kidneys with almost complete replacement of the parenchyma by clear fluid-filled cysts of up to 1 mm in diameter (Figure 5). Cystic transformation was bilateral and symmetrical in all but one adult KAT2J mouse, a 60-d-old male with severe cystic change at one pole of one kidney; the balance of that and the other kidney had only mild cystic change, similar to other like-aged KAT2J mice.

Microscopically, the youngest KAT2J mouse examined (5 d old) had no cysts, but by 1 mo of age, KAT2J mice had small clusters of glomerular cysts, dilated proximal tubules lined by eosinophilic epithelium with a brush border, and cysts lined with cuboidal or flat epithelium. In the oldest KAT2J mice, much of the renal cortex was replaced by variably sized cysts at all levels of the nephron, and medullary tubules were also dilated (Figure 6, A through C). Males and females were similarly affected until approximately 6 mo of age, when cystic lesions advanced more rapidly in females. Mitotic figures were
inconspicuous in cyst epithelium, and neither adenomas nor intracystic epithelial polyps were seen, although there were occasional infoldings of epithelial-lined ridges in tubules. Focally thickened, duplicated cyst basement membrane surrounded cysts. Interstitial edema, clusters of lymphocytes, plasma cells, and hemosiderin-laden macrophages were common in older mice. KAT mice had renal cystic lesions similar to, although less extensive, than those of similarly aged KAT2J mice (Figure 6D). The extent of cystic change in 13-mo-old KAT mice was similar to that of 5- to 6-mo-old KAT2J mice. There was no difference in the extent of cystic change in male and female KAT mice. Ultrastructurally, a single layer of simplified epithelium lined most cysts in the KAT2J mouse examined with focal zones of irregular, duplicated tubular basement membrane (Figure 7).

Testes in the KAT2J males weighed less than normal controls (data not shown). Histologically, spermatogenesis, although present, was less than in normal mice. In the oldest KAT2J and KAT male mice, tubules were lined by Sertoli cells only or had decreased spermatogenesis (Figure 8). The weights

Figure 6. (A) A 33-d-old KAT2J mouse kidney had clusters of cystically dilated Bowman’s capsules and tubules limited to the cortex. (B) Progressive cystic change occurred as mice aged. Cysts were primarily cortical but also affected the medulla in a 7-mo-old KAT2J mouse. (C) Both glomerular and tubular cysts were present, and the interstitium from an 8-mo-old KAT2J mouse was loose and edematous with occasional lymphocytes. (D) A 7-mo-old KAT mouse had focal cortical cysts but the morphologic alterations are much less than that of a similarly aged KAT2J mouse. Hematoxylin and eosin stain. Magnification: ×24 in A, B, and D; ×60 in C.

Figure 7. In the kidney of the KAT2J mouse examined ultrastructurally, a single layer of simplified epithelium lined large cysts and there were focal zones where the tubular basement membrane was thickened, irregular, and duplicated. Uranyl acetate-lead citrate stain. Magnification: ×2600; ×12,000 inset.
of liver, heart, spleen, and ovaries from KAT2J mice were not different from controls and ovaries were histologically normal (data not shown). In four of the 12 KAT2J mice over 170 d old, occasional portal spaces had prominent irregular bile ducts (Figure 9) without associated portal fibrosis, cirrhosis, or canalicular or ductular cholestasis.

Discussion

The morphologic similarity of the renal cystic disease in the KAT2J and KAT mice to that of ADPKD makes these mice useful models. In both ADPKD and the models we describe, kidneys are morphologically normal very early in life but enlarge gradually and usually bilaterally with cysts developing in multiple segments of the nephron, leading to renal failure.

The mechanism of cyst formation in the KAT2J and KAT mice requires further study. The rapid increase in wet kidney weight with age in KAT2J mice indicates that renal enlargement is largely due to cyst fluid accumulation. However, the dry weight increase suggests that cellular hyperplasia and/or increased extracellular matrix may also contribute. We did not observe increased mitotic activity or epithelial cell hyperplasia as described in other PKD models (7,13), but future characterization of epithelial cell proliferative activity will be important in these models. A focally altered matrix, a localized environmental factor (14), or abnormal clones of cells that give rise to cysts could explain our observation of clusters of cysts in the younger KAT2J mice and the one mouse with focal severe renal cystic disease. The gender-dependent expression with more severe renal lesions in older female compared with male KAT2J mice suggests that hormonal influences may also affect cyst formation. In pcy mice, females have more severe uremia and die sooner than male mice (15). Since male and female KAT mice have similar renal lesions as they age, the increased cystic change in older KAT2J females may be mutation-dependent.

Progressive anemia in KAT and KAT2J mice may be due to decreased erythroid stem cell response, increased erythrocyte fragility, or erythropoietin deficiency as proposed in the pcy model (16). In addition to the pcy murine model (16), anemia has been described in a rat model of PKD (17) and is common in patients with PKD (18). Poikilocytosis in older KAT2J mice reflects their uremia. Although the cause of testicular hyperplasia and loss of germinal epithelium in KAT2J and KAT males is uncertain, it does explain why male KAT2J and KAT mice are sterile (10). Previous reports of murine PKD models have not included descriptions of testicular abnormalities, and this lesion may be unique to the models we describe. The skeletal abnormalities in KAT2J and KAT mice are similar to those described in an autosomal recessive rat model of PKD (19), and the skeletal alterations may indicate that the abnormal gene in these mice also disturbs normal bone growth. Both KAT and KAT2J mice are runts at birth and their growth failure persists into adulthood.

The mutations in the mice we describe are distinct from those in previously reported PKD models including the cpk (congenital polycystic kidney) gene on Chr 12 (20), the pcy mutation on Chr 9 (15), or the jck (juvenile cystic kidney) mutation on Chr 11 (21). The murine homologue for the Chr 16p13.3 human PKD-1 locus has been mapped to mouse Chr 17 (22). No spontaneously occurring animal PKD mutations have been mapped to this region, although a knockout mouse with a truncated PKD1 gene has been described (23).

There are two possible explanations for the more severe renal disease in the KAT2J mice compared with the KAT mice. In KAT2J mice, there may be a null mutation, whereas the KAT mutation may result in a reduced amount of an active protein or an altered protein with reduced function. Alternatively, during the transfer of the KAT mutation from its strain of origin, RBF/Dn, to the C57BL/6J background, transfer of a modifier locus closely linked to the KAT locus may have occurred, and the linked modifier gene could delay the progression of the phenotype in the KAT mice. Identification of the genetic and molecular defects in the KAT and KAT2J mice will be important in explaining their phenotypic differences.

Figure 8. The testis from an 8-mo-old KAT2J mouse was hypoplastic with some seminiferous tubules lined by Sertoli cells only and others with a marked decrease in the number of spermatogonia. Hematoxylin and eosin stain. Magnification: ×166.

Figure 9. Liver from a 7-mo-old female KAT2J mouse shows a portal space with an increased number of irregular, dilated bile ducts. Hematoxylin and eosin stain. Magnification: ×150.
Regardless of the genetics, the KAT and KAT2J mice provide excellent models to assess progressive development of PKD and the associated anemia.

Our description of the clinical and pathologic findings in KAT2J and KAT mice will support further characterization of the gene altered by the kat2J and kat mutations, longitudinal studies of mechanisms of cyst formation, matrix composition, and renal ultrastructural alterations in these mice. Furthermore, definition of the phenotype will serve as a benchmark for evaluating the effect of pharmacologic and molecular therapeutic strategies directed at reducing the formation and progression of cystic lesions in these models.

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References


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