Comparative Medicine
Copyright 2007
by the American Association for Laboratory Animal Science

Vol 57, No 2
April 2007
Pages 193–199

Breeding Colony Refinement through Phenotypic and Genotypic Characterization of the SPRD-*Pkdr1*/Rrrc Rat Model of Polycystic Kidney Disease

Beth A Bauer,[1,*] Susan Boedges,[2,†] Cristi R Cook,[3] Elizabeth C Bryda,[1] and Craig L Franklin[1]

The SPRD-Pkdr1 rat model is widely used for the study of human autosomal dominant polycystic kidney disease. This rat model carries the Cy allele of the Pkdr1 gene, which results in polycystic kidney disease. Because the Cy allele is lethal in the homozygous state at weanling age, the breeding colony must be maintained in the heterozygous state. A random breeding scheme in which production of homozygous pups with enlarged kidneys indicates heterozygous breeders is commonly used. This study was performed to determine whether biochemical markers (blood urea nitrogen [BUN] or creatinine), ultrasonography, or genetic analysis could be used to select breeding animals in the SPRD-Pkdr1/Rrrc colony and thus replace the random breeding scheme with a more efficient selective breeding scheme. BUN was predictive of the Cy allele in 8- to 9-wk-old male but not female rats. Ultrasonography identified animals with polycystic kidney disease in both sexes by 9 wk of age. Microsatellite marker polymorphism analysis could not be used to determine carrier status for the Cy allele, but restriction fragment length polymorphism analysis appropriately detected the Cy allele in 100% of the animals examined. In conclusion, multiple methods can be used for detecting the Cy allele, making possible a selective breeding scheme that markedly reduces the necessary number of breeder animals and eliminates the euthanasia of offspring needed with a random test-mating scheme.

Abbreviations: ADPKD, autosomal dominant polycystic kidney disease; bp, basepair; BUN, blood urea nitrogen; SNP, single-nucleotide polymorphism; RFLP, restriction fragment length polymorphism; RRRC, Rat Research and Resource Center

Autosomal dominant polycystic kidney disease (ADPKD) is a prevalent disease that affects 1 in 500 to 1000 people.[1] This inherited disease is characterized by enlargement of focal renal cysts and often progresses to end-stage renal disease by the fifth decade of life.[16] As the cystic renal disease progresses, dialysis and kidney transplantation are the only means of treatment, and currently 10% of all transplants performed in patients with chronic renal failure are the result of polycystic kidney disease.[1] The disease usually is diagnosed by ultrasound evaluation after the onset of clinical symptoms. Mutations in 2 genes, *PKD1* and *PKD2*, are responsible for most cases of ADPKD in humans.[6-8,13,16]

The SPRD-*Pkdr1*/Rrrc rat model (also known as Han:SPRD-*Pkdr1* and Han:SPRD-*Cy* prior to inbreeding) has been characterized and used as a model of human polycystic kidney disease.[5,10,11] The *Cy* mutation arose spontaneously in a colony of Sprague-Dawley rats in Hannover, Germany. Rats carrying the *Cy* mutation in the homozygous state have severe renal enlargement, in which all renal parenchyma is filled with cystic structures. Homozygous animals have a rapidly progressive disease and die by 4 wk of age.[5,10,11] Heterozygous animals, in contrast, have a slowly progressive disease similar to the course of human ADPKD. Cyst formation progresses slowly, and male rats are more severely affected and may show renal failure as early as 6 mo of age. Clinical signs consist of weight loss, polyuria, polydipsia, and finally end stage renal failure and death occur. Female rats have a much slower disease progression, generally remain asymptomatic, and have a normal lifespan despite bilateral cyst formation.

The SPRD-*Pkdr1*/Rrrc rat model[18] is maintained in the heterozygous state because homozygous *Cy/Cy* animals never reach sexual maturity and cannot reproduce. A common method for maintaining breeding colonies is to randomly breed animals and assess the phenotype of the resultant offspring (Figure 1). Because parents are mated randomly, there are 4 possible genetic combinations that can occur when setting up random breeding pairs. The offspring from these breeding pairs are assessed at 2 to 3 wk of age by kidney palpation. If any of the resultant offspring have palpably enlarged kidneys by 3 wk of age, then both parents are confirmed to be heterozygous for the *Cy* mutation. All breeding pairs not producing offspring with enlarged kidneys are shuffled in the hope of eventually mating heterozygous animals to each other. On average, 25% of the breeding pairs will contain *Cy*/+ mated to *Cy*/+, and 75% will contain +/+ mated to either +/+ or *Cy*/+ (Figure 1). In this random breeding scheme, 75% of the initial matings for each generation will result in nonproductive pairings from which all offspring are euthanized because the genetic status of the parents and offspring are unknown.

We undertook the current study to assess possible phenotypic and genotypic markers for identification of *Cy* carrier animals, in order to refine the breeding scheme. If *Cy* carrier status could be determined by biochemical, ultrasound, or genetic analysis, then the random breeding scheme could be replaced by a scheme in which known *Cy* carrier animals were paired, resulting in improved efficiencies of the breeding colony and elimination of the

Received: 18 Aug 2006. Revision requested: 10 Sep 2006. Accepted: 6 Oct 2006.
[1]Research Animal Diagnostic Laboratory, [2]College of Veterinary Medicine, [3]Department of Veterinary Medicine and Surgery, University of Missouri, Columbia, MO.
[†]Present address: Companion Animal Hospital, Hermann, MO.
[*]Corresponding author. Email: bauerbe@missouri.edu

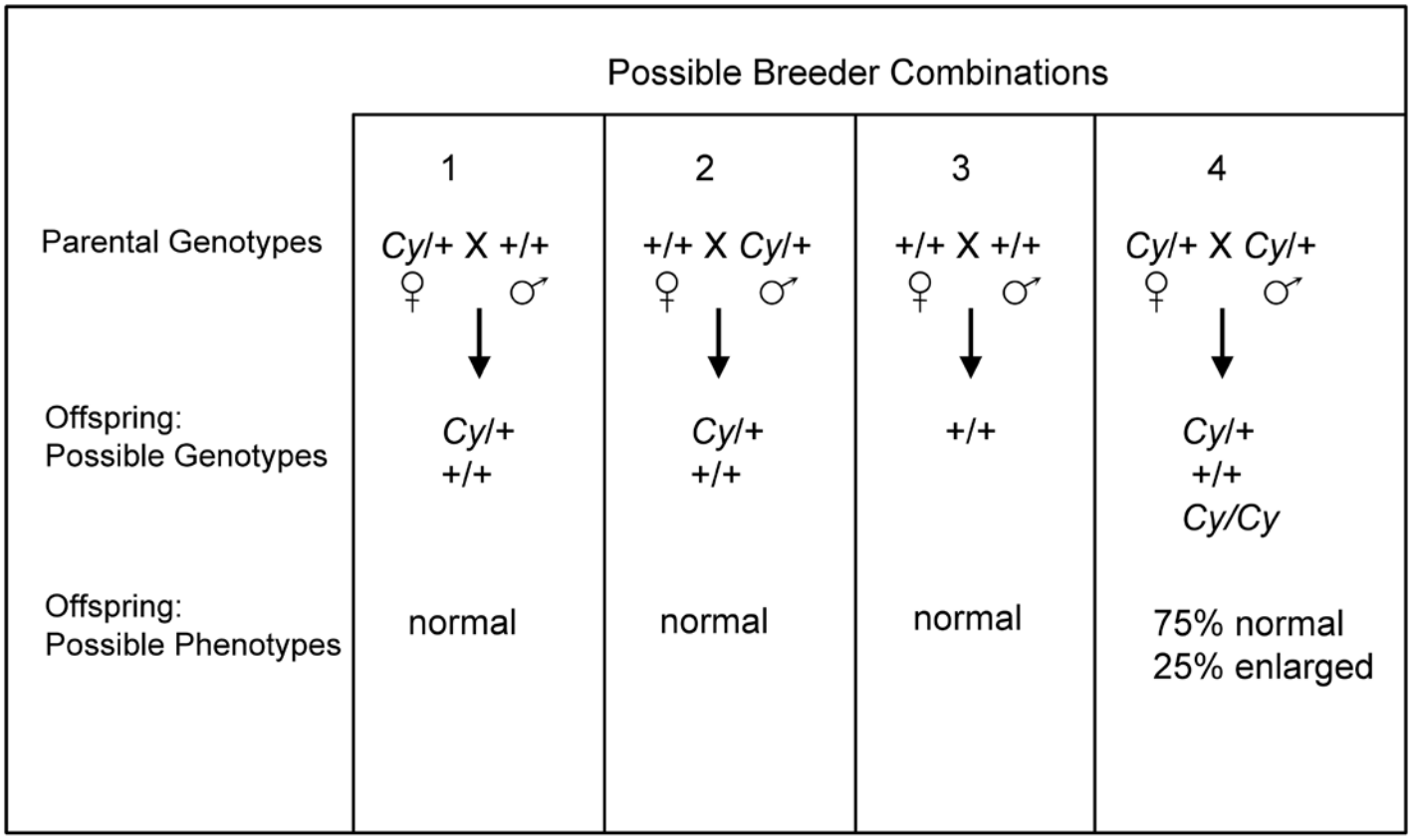

Figure 1. Schematic of breeding schemes and offspring genotypes. The 4 possible random combinations of breeders are depicted with the genotypes of the offspring produced by each combination. The possible kidney phenotypes found in 2- to 3-wk-old offspring on abdominal palpation are listed on the bottom line.

production of test offspring destined for euthanasia. We examined blood urea nitrogen (BUN) and creatinine levels to determine whether biochemical changes could be used to determine *Cy* carrier status in young breeding age animals. In addition, we performed ultrasound evaluation of kidneys to characterize the renal changes seen in weanling and young adult rats. We evaluated microsatellite markers for use in assessing the *Cy* genotype, and we developed a polymerase chain reaction (PCR) followed by restriction digestion based on genetic information previously reported.[23]

Materials and Methods

Animals. Two proven heterozygous SPRD-*Pkdr1*/Rrrc breeder pairs, deposited at the Rat Resource and Research Center (RRRC), were bred to produce the offspring used in this study (depositors: Drs J Grantham and B Cowley, University of Kansas Medical Center, Kansas City, KS). This colony, originally obtained from Hannover, Germany, was maintained by brother-sister mating for 33 generations at the University of Kansas Medical Center prior to transfer to the University of Missouri RRRC. Pedigree information for the animals transferred to the RRRC can be found at http://www.nrrrc.missouri.edu/StrainInfo.asp?appn=46. All animal studies were performed in accordance with the University of Missouri's Animal Care and Use Committee guidelines and the ILAR *Guide for the Care and Use of Laboratory Animals*.[15] On arrival, animals were housed in microisolation cages in breeding pairs, fed a standard pelleted rodent chow, and housed in an environmentally controlled room with a 12:12-h light:dark cycle. All resulting offspring were palpated at 2 to 3 wk of age, and pups with palpably enlarged kidneys (*Cy*/*Cy*) were euthanized by inhalation of carbon dioxide (CO_2) followed by cervical dislocation.

The remaining offspring (*Cy*/+ or +/+) consisted of 5 female and 7 male rats. These animals were anesthetized at 4, 6, 9, and 12 wk of age with inhaled isoflurane (Halocarbon Laboratories, River Edge, NJ) delivered by mask, and blood was collected from the lateral saphenous vein for BUN and creatinine determination, after which abdominal ultrasonography was performed. Tissue (distal tail tip or liver) was collected at euthanasia from Sprague-Dawley rats (Harlan, Indianapolis, IN, and Charles River, Wilmington, MA) used on other research studies. Tail biopsies were collected from SPRD-*Pkdr1*/Rrrc pups at 14 to 18 d; adult animals were anesthetized with isoflurane, and ear punch biopsies were collected. All tissue samples were frozen and stored at –20 °C (tail biopsies) or –80 °C (liver) for subsequent genotyping analysis.

Serum chemistry analysis. Whole blood was collected in capillary collection tubes that contained lithium heparin (Becton Dickinson, Franklin Lakes, NJ). Samples then were submitted to the clinical pathology laboratory of the Veterinary Medical Diagnostic Laboratory at the University of Missouri for BUN and creatinine analysis. Plasma BUN and creatinine were determined using an automated clinical chemistry system (Johnson and Johnson Vitros 250 Dry Chemistry System Analyzer, Ortho-Clinical Diagnostics, Raritan, NJ).

Ultrasonography. Animals were placed in dorsal recumbency, and the ventral abdomen was shaved. Acoustic ultrasound gel was applied to the ventral abdomen to improve contact. Ultra-

Figure 2. Schematic of the 248-bp PCR product for RFLP analysis. The arrows indicate the forward and reverse primer. Bases in bold indicate the *Nci*I restriction endonuclease recognition site present in the wild-type (+) allele. The box indicates the position of the mutated base (C to T transition) in the *Cy* allele resulting in the loss of the *Nci*I restriction site.

sonography was performed using a 10-MHz linear transducer (Logic 500, GE Medical, Milwaukee, WI) and the preset 'standard small parts' protocol. Images were recorded, and kidneys were assessed for size, shape, echogenicity, and presence of cysts.

Genotyping assays. For microsatellite analysis, DNA was extracted from tissue samples (DNeasy Tissue Kit, Qiagen, Valencia, CA) according to manufacturer's instructions and by using a single elution volume of 200 µl. Sequences of the primers for microsatellite markers *D5Rat9*, *D5Mit9*, and *D5Rat11* can be found at http://www.rgd.mcw.edu/objectSearch/sslpQuery.jsp and have been published previously.[3,12,20] Primers were synthesized (Integrated DNA Technology, Coralville, IA) and used in PCR reactions to determine whether these closely linked markers could be used to assess the genotype of the SPRD-*Pkdr1*/Rrrc rats for the *Cy* allele. The PCR reaction mixture (total volume, 25 µl) contained 5 µl of the eluted DNA (approximately 200 ng), 2.5 µl of 10× FastStart *Taq* Buffer with 20 mM $MgCl_2$ (Roche, Indianapolis, IN), 0.2 mM of each dNTP, 0.5 µM of each primer, and 0.5 U of FastStart *Taq* (Roche). PCR amplifications were performed using the following cycling conditions: 95 °C for 9 min; 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 30 s; and 72 °C for 7 min. PCR products were analyzed by denaturing high-performance liquid chromatography (Transgenomic Wave System, Omaha, NE).

For genotyping analysis, in which restriction fragment length polymorphism (RFLP) analysis of PCR products was performed, DNA was extracted by use of either the kit described earlier or the Sigma REDExtract-N-Amp (Sigma-Aldrich, St Louis, MO) according to the manufacturer's instructions. Primers (Integrated DNA Technology) for this reaction were designed that flanked the suspected point mutation in the *Pkdr1* gene (Figure 2). The reaction mixture (total volume, 50 µl) contained 5 µl of the eluted DNA from the DNeasy kit (approximately 200 ng), 5 µl of 10× FastStart *Taq* Buffer with 20 mM $MgCl_2$ (Roche), 0.2 mM of each dNTP, 1.0 µM of each primer, and 1 U of FastStart *Taq* (Roche). Alternatively, when DNA extracted with the Sigma REDExtract-N-Amp kit was used, the reaction mixture (total volume, 40 µl) volume contained 4 µl of DNA (approximately 160 ng), 20 µl of 2× REDExtract-N-Amp PCR reaction mixture, and 1.0 µM of each primer. PCR amplifications were performed at the following cycling conditions: 95 °C for 4 min; 35 cycles of 94 °C for 30 s, 59 °C for 30 s, and 72 °C for 1 min; and 72 °C for 7 min. After PCR amplification, a 20 µl restriction digestion reaction was performed that included 10 µl of the PCR products (including the 248-base-pair [bp] target), 10 U *Nci*I enzyme (New England Biolabs, Ipswich, MA), and 2 µl of 10× Buffer 4 (New England Biolabs). After incubation overnight at 37 °C, restriction digests underwent 3% agarose gel electrophoresis, ethidium bromide staining, and visualization with ultraviolet light.

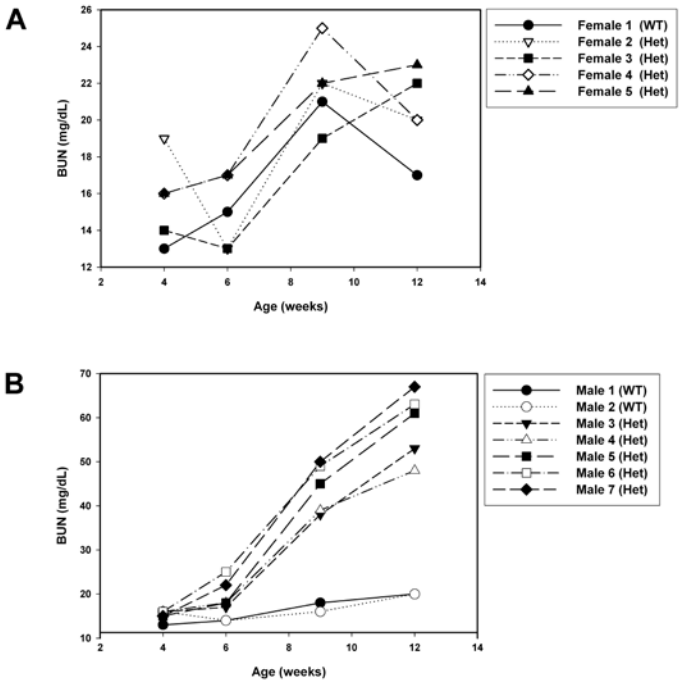

Figure 3. Plasma BUN in rats of various ages. (A) Female rats. (B) Male rats.

Statistical analysis. The Student *t* test was used to compare BUN values between wild-type (+/+) and heterozygous (*Cy*/+) rats (Sigma Stat software for Windows, version 3.10, Rockware, Golden, CO). A *P* value of 0.05 was used as the threshold for statistical significance.

Results

Serum chemistry analysis. In a pilot study, BUN and creatinine were evaluated in 12 (5 female and 7 male) SPRD-*Pkdr1*/Rrrc rats, starting at 4 wk of age, to determine whether either creatinine or BUN could be used as a biochemical marker to determine carrier status of the *Cy* mutation. In both sexes of rats, creatinine was not predictive of *Cy* genotype between the ages of 4 to 12 wk (data not shown). In contrast, 9-wk-old *Cy*/+ male rats but not *Cy*/+ female rats showed an increase in BUN (Figure 3).

On the basis of these preliminary findings, we assessed BUN in male rats at 8 to 9 wk of age to determine whether *Cy*/+ males consistently had an elevated BUN at this age. An additional 65 8- to 9-wk-old male SPRD-*Pkdr1*/Rrrc rats underwent BUN evaluation, and kidney morphology was assessed at the time of euthanasia to determine whether animals carried the *Cy* mutation (Figure 4). On necropsy examination, 43 of the 65 rats had cystic kidneys, indicating they carried the *Cy* mutation, and 22 had normal kidneys and thus did not carry the *Cy* mutation. The average BUN of 8- to 9-wk-old male rats with the *Cy* mutation was 51 mg/dl (1 standard deviation, 10.8), whereas that of 8- to 9-wk-old male rats with normal kidneys was 18 mg/dl (1 standard deviation, 2.3). This difference was statistically significant ($P < 0.001$), demonstrating that BUN at 8 to 9 wk can be used as a biochemical marker to determine *Cy* carrier status in male rats.

Ultrasonography. We examined 3 (2 male and 1 female) homozygous wild-type (+/+) SPRD-*Pkdr1*/Rrrc rats at 4, 6, 9, and 12 wk of age to verify the normal size, shape, echogenicity, and architecture of their kidneys. We also similarly evaluated 9 (5 male

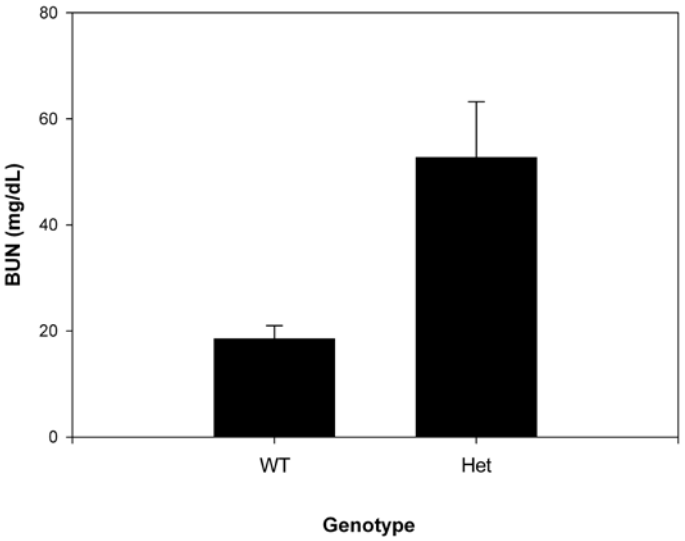

Figure 4. Mean BUN levels from 65 male rats, 8 to 9 wk old. WT, wild-type (+/+) rats not carrying *Cy* allele; Het, heterozygous (*Cy*/+) rats carrying 1 copy of the *Cy* allele. Bar, 1 standard deviation.

and 4 female) heterozygous (*Cy*/+) SPRD-*Pkdr1*/Rrrc rats (Table 1). All *Cy*/+ rats had normal appearing kidneys on renal ultrasound evaluation at 4 wk of age. At 6 wk of age, 80% (4 of 5) of the male *Cy*/+ rats had abnormal, enlarged kidneys on ultrasound examination, with hyperechoic renal cortices in comparison to the darker cortices of normal kidneys (Figure 5 A, B). In addition, numerous small, circular cysts, apparent as black circular structures with a surrounding thin white rim, were present (Figure 5 C). On ultrasonography, cysts appear as black structures because the fluid within the cyst absorbs the ultrasonic waves, which thus are not reflected back to the probe. The surrounding thin white layer is the epithelial lining of the cyst, which reflects ultrasonic waves back to the probe and thus is visualized. Only 2 of the 6-wk-old female rats had abnormal, hyperechoic kidneys.

By 9 wk of age, all animals had enlargement of the kidneys with other abnormal ultrasound characteristics. These findings tended to be more severe in males, and the presence of hyperechoic renal cortices with cyst formation and loss of the corticomedullary junction were the most common findings in male rats at this age (Figure 5 C). Female rats lacked visible cysts at this age, and enlarged, hyperechoic renal cortices were the most common abnormality (Figure 5 B). However, by 12 wk of age, cysts were ultrasonographically evident in the female rats as well.

Genotyping. Microsatellite analysis with markers *D5Rat9*, *D5Mit9*, and *D5Rat11* did not correlate with *Cy* carrier status. Product size did not vary with *Cy* genotype, therefore polymorphism at these marker locations was not evident in this colony. However, RFLP analysis correlated 100% with *Cy* carrier status. Figure 6 demonstrates the expected products that are found for the 3 different genotypes (+/+, *Cy*/+, and *Cy*/*Cy*). Homozygous wild-type (+/+) animals produce 2 products (75 and 173 bp) due to digestion of the 248-bp PCR product by restriction enzyme *Nci*I. Homozygous mutant (*Cy*/*Cy*) animals produce a single 248-bp PCR product that has lost the *Nci*I restriction recognition site due to mutation of a single C nucleotide to T in exon 16 of the *Pkdr1* gene (Figure 2). Heterozygous animals (*Cy*/+) produce 3 products (75, 173, and 248 bp) on RFLP analysis. Because the *Cy* mutation originated in a colony of Sprague Dawley rats, we then examined 106 Sprague Dawley rats from 2 different vendor sources for the presence of the C-to-T mutation in the *Pkdr1* gene predicted to cause the *Cy* mutation. This mutation was not found in any Sprague Dawley rats obtained from either Harlan or Charles River Laboratories, demonstrating that this substitution is not a single-nucleotide polymorphism (SNP) in the Sprague-Dawley rat population. We also tested 82 inbred SPRD-*Pkdr1*/Rrrc rats for the presence of this mutation. According to RFLP results, this mutation was found in 50 of the 82 SPRD-*Pkdr1*/Rrrc rats examined, and all 50 rats that contained the mutation also had polycystic kidneys. The 32 SPRD-*Pkdr1*/Rrrc rats that lacked this C-to-T mutation had normal kidneys. These results demonstrated that the RFLP assay can be used with 100% correlation to determine which rats within the SPRD-*Pkdr1*/Rrrc colony carry the *Cy* allele.

Discussion

The random breeding scheme used previously to maintain the SPRD-*Pkdr1*/Rrrc rat colony was inefficient, costly, and produced a large number of unusable offspring. To refine the breeding scheme in this colony, we assessed biochemical markers, ultrasound findings, and genetic analysis. We selected multiple methods to allow flexibility in the approach to determining *Cy* carrier status depending on the resources of the facility maintaining the colony. In addition, having multiple testing methods means that an alternative assay can be performed if difficulty is encountered with primary testing results. Previous studies examining creatinine and BUN levels in SPRD-*Pkdr1*/Rrrc rats yielded conflicting findings. Schafer and colleagues found that creatinine and BUN levels were not significantly different between heterozygous and wild-type animals until 8 mo of age, when animals would be retired from breeding.[19] However, Torres and colleagues found that BUN was elevated in heterozygous rats of both sexes as early as 8 wk of age.[21] A third pattern also has been described, in which BUN is elevated in heterozygous male but not female rats as early as 8 wk of age.[5]

The dramatic differences in published BUN levels in SPRD-*Pkdr1*/Rrrc rats may have been the result of genetic variability. The *Cy* mutation arose in an outbred colony of rats, and the model was not inbred when it was distributed to other investigator laboratories, resulting in a number of sublines for this model that may have different disease progression characteristics. These dramatic differences in the literature led us to characterize the BUN and creatinine levels found in the fully inbred strain deposited at the RRRC. BUN was elevated in male rats and could be used as a marker for the *Cy* allele in male rats as young as 8 to 9 wk of age. This consistent BUN elevation facilitated effective selection of appropriate breeders. We assessed BUN in all male rats at 8 wk of age determine which carried the *Cy* allele. Male carriers then were used in breeding pairs; doing so resulted in 50% of the offspring being heterozygous (*Cy*/+) carriers.

Ultrasound examination also revealed differences in disease progression between male and female rats. Male rats had visible evidence of cyst formation at an earlier age than did female rats. By 9 wk of age all male rats examined had cysts that were visible on ultrasound examination. Female rats had a much slower progression, and the earliest changes seen were enlarged kidneys with hyperechoic cortices. It should be noted that increased echogenicity is a consistent finding in several mouse models of autosomal recessive polycystic kidney disease.[17,22] In humans, increased echogenicity of the kidney is a nonspecific finding that is associated with parenchymal disease but has also been noted as a

Table 1. Ultrasound findings

Age (wk)	Sex	Ultrasound findings (no. abnormal/no. examined)	Ultrasound findings in abnormal animals
4	Male	Normal (0/5)	—
	Female	Normal (0/4)	—
6	Male	Abnormal (4/5)	hyperechoic, dilated renal pelvis, cysts
	Female	Abnormal (2/4)	hyperechoic, dilated renal pelvis
9	Male	Abnormal (5/5)	cysts, dilated renal pelvis
	Female	Abnormal (4/4)	hyperechoic, dilated renal pelvis
12	Male	Abnormal (5/5)	cysts, dilated renal pelvis
	Female	Abnormal (4/4)	hyperechoic, cysts

common finding in utero for autosomal recessive polycystic kidney disease.[9] Ultrasound evaluation was successful in detecting *Cy*-carrier male rats by 8 wk of age (similar to BUN evaluation), but we found BUN assessment to be more convenient and easier to perform than ultrasound evaluation. Although BUN was easy to use to assess *Cy* carrier status in male rats, it was not a useful indicator in female rats. Therefore, we explored genetic testing to look for the presence of the *Cy* allele.

Initially we examined closely linked microsatellite markers, and we were surprised to find that the microsatellite marker previously published for determining the *Cy* allele was not polymorphic in the RRRC colony. The *Pkdr1* gene was previously mapped to chromosome 5 near marker *D5Mit10*, and marker *D5Rat9* was demonstrated to be polymorphic in the SPRD-*Pkdr1* rat model.[2,14] Nagao and colleagues found that *Cy/Cy* rats had a 136-bp allele at the *D5Rat9* locus, +/+ rats had a 126-bp allele, and *Cy*/+ animals had alleles of both sizes. However, the RRRC colony, obtained from the same original colony (University of Kansas Medical Center) as the Nagao animals but several years later, failed to demonstrate polymorphism at the *D5Rat9* locus. The *D5Rat9* locus is approximately 0.8 cM from the *Cy* allele, which in theory would result in <1% recombination, but rare crossover events can still occur within this area of approximately 1,681,000 bp. Whenever a closely linked marker, such as an SNP or microsatellite, is used for detection of a mutation, it is critically important to confirm that the marker is still linked with the gene of interest. This linkage usually is confirmed by assessing both the marker and the phenotype of the breeding animals. We found that the microsatellite marker in our colony was no longer polymorphic and therefore no longer useful to distinguish the wild-type allele from the mutant *Cy* allele. We suspect that this closely linked polymorphic marker was lost during inbreeding between the time that animals were sent to Dr Nagao and when deposited in the RRRC.

During our studies, preliminary data presented by David Woo indicated a single-nucleotide difference within the *Cy* allele.[23] We developed a RFLP assay to detect this single-nucleotide change. Because this spontaneous mutation occurred in a Sprague-Dawley rat colony, we examined Sprague-Dawley rats from 2 different vendors to confirm that this single-basepair change was associated with polycystic kidney disease rather than with a SNP in Sprague-Dawley rats. We found that the mutation was absent in 106 unrelated normal Sprague-Dawley rats, making it unlikely to be a SNP present within Sprague-Dawley stocks. In addition, all of the 50 SPRD-*Pkdr1*/Rrrc rats that had polycystic kidneys at the time of necropsy also had the C-to-T transition, and the 32 SPRD-*Pkdr1*/Rrrc rats that had normal kidney morphology on necropsy all lacked this mutation.

During the course of the current study, Brown and colleagues published their work on the positional cloning of the *Pkdr1* gene that confirmed the C-to-T transition, whose protein product, SamCystin, is expressed in renal proximal tubules.[4] This mutation results in a missense mutation within the sterile α motif (SAM) domain of the SamCystin protein. Although definitive proof that the mutation in the SamCystin protein is the cause of polycystic kidney disease in the SPRD-*Pkdr1* rats has not been presented yet, the findings of Brown and colleagues support our data regarding Sprague-Dawley and SPRD-*Pkdr1* rats. The findings of these 2 studies demonstrate that it is unlikely that the C-to-T mutation is a closely linked SNP marker and make this mutation the most likely candidate for the cause of polycystic kidney disease in this rat model.

In conclusion, *Cy* carrier status can be assessed in SPRD-*Pkdr1*/Rrrc rats by several different mechanisms. Plasma BUN, ultrasonography, and genetic testing all can be used to assess male *Cy* carrier status, whereas ultrasonography and genetic testing can be used to assess the *Cy* carrier status of female rats. Detection of the *Cy* allele by plasma BUN, ultrasonography, or genetic testing allowed us to immediately reduce the number of breeding pairs by 75% because we no longer had to set up random test-mating breeding cages (Figure 1). We found genetic testing to be a reliable and direct way to genotype both male and female offspring as young as 14 d of age so that specific breeding strategies could be implemented to maintain the *Cy* allele. For example, mating heterozygous (*Cy*/+) female rats with wild-type (+/+) male rats offers a way to produce litters in which 50% of the offspring are heterozygous and 50% are wild-type and therefore can be used as age-matched controls. This breeding scheme has the advantage of not producing homozygous offspring that will become severely ill and die at 3 to 4 wk of age, and it eliminates using heterozygous breeder males that develop chronic renal failure at 6 mo of age while allowing production of carrier offspring for research studies. This breeding scheme has been adopted for colony maintenance at the RRRC. However, it is important to remember that this breeding scheme cannot be implemented for all SPRD-*Pkdr1* breeding colonies and that this scheme may need to be altered based on the demands of the research experiment. For instance investigators that need homozygous offspring for analysis would need to use a *Cy*/+ × *Cy*/+ breeding scheme. Investigators evaluating paternal or maternal imprinting may need to examine offspring from 2 different breeding schemes in which *Cy*/+ female rats are mated with +/+ male rats and Cy/+ male

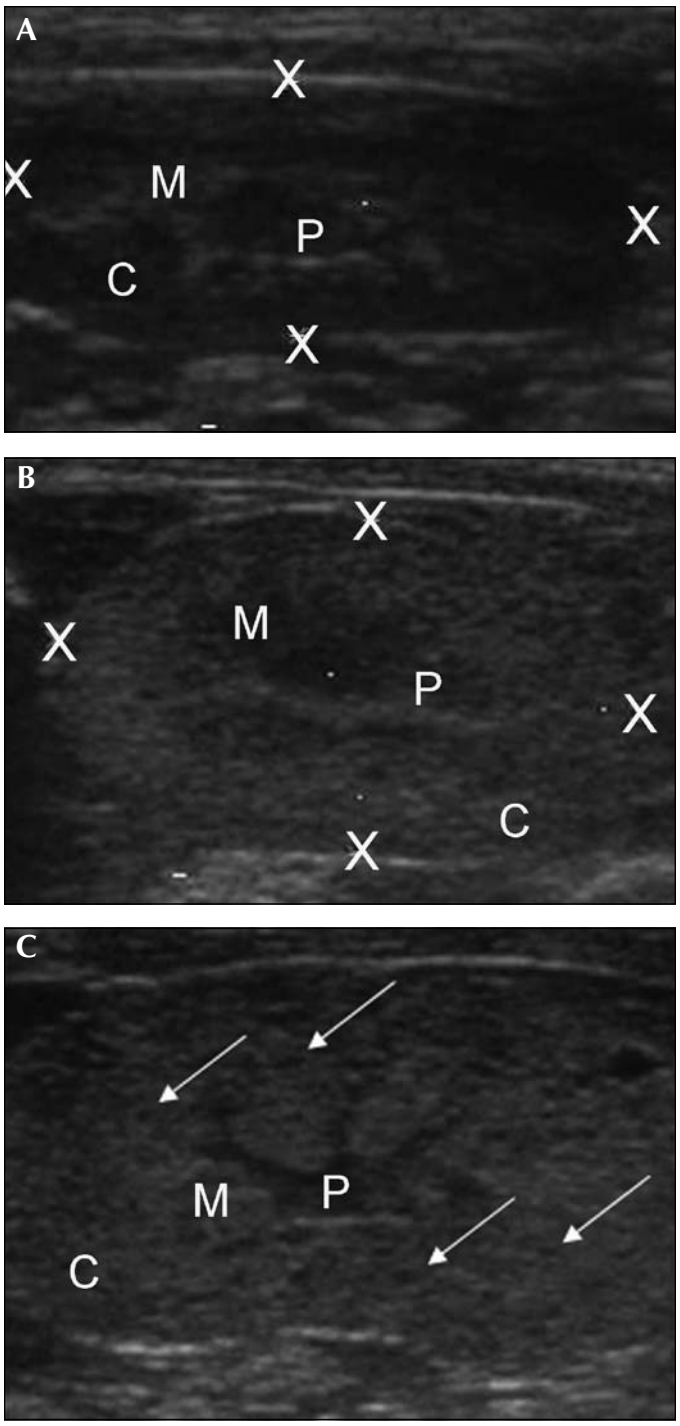

Figure 5. Renal ultrasound images. (A) Normal kidney. (B) Kidney with a hyperechoic (brighter white) cortex. The kidney outline is marked with the letter X in panels A and B. (C) Hyperechoic kidney with multiple small cysts (arrows) and loss of the corticomedullary junction. C, renal cortex; M, renal medulla; P, renal pelvis.

rats are mated with +/+ female rats. Which breeding scheme is the most efficient to use in a mutant rodent model to assist in reducing animal numbers and reduction of the disease morbidity and mortality associated with the mutation should always be evaluated in conjunction with the experimental design and the research hypothesis that is being studied.

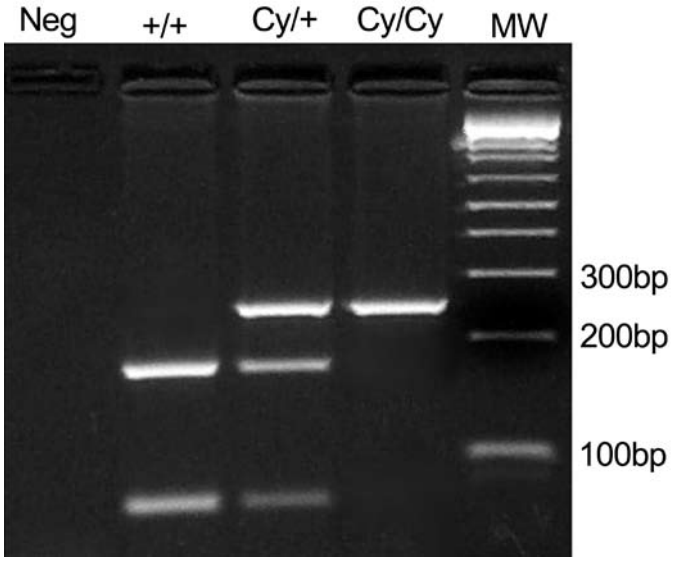

Figure 6. Agarose gel electrophoresis results of RFLP genotyping analysis. The first lane contains the negative (no template) DNA control from the PCR reaction, the second lane shows restriction digest products of a wild-type (+/+) rat that results in 2 fragments (75 and 173 bp). The third lane shows restriction digest products from a heterozygous (*Cy*/+) rat in which 3 products are present (75, 173, and 248 bp); the fourth lane shows restriction digest product from a homozygous mutant (*Cy/Cy*), which yields a single uncut product (248 bp). The lane containing the molecular weight marker is indicated by MW.

Acknowledgments

This study was funded by Rat Resource and Research Center grant P40 RR16939 from the National Institutes of Health. Funds for Susan Boedges were provided by the Veterinary Research Scholars Program funded in part by Merck-Merial, the College of Veterinary Medicine, and the Research Animal Diagnostic Laboratory. The authors gratefully acknowledge the assistance provided by the technical staff in the clinical pathology laboratory of the Veterinary Medicine Diagnostic Laboratory, radiology unit in the Veterinarian Medicine Teaching Hospital, and the necropsy and genotyping laboratories of the Research Animal Diagnostic Laboratory.

References

1. **Arnaout MA.** 2001. Molecular genetics and pathogenesis of autosomal dominant polycystic kidney disease. Annu Rev Med **52:**93–123.
2. **Bihoreau MT, Ceccherini I, Browne J, Kranzlin B, Romeo G, Lathrop GM, James MR, Gretz N.** 1997. Location of the first genetic locus, PKDr1, controlling autosomal dominant polycystic kidney disease in Han:SPRD cy/+ rat. Hum Mol Genet **6:**609–613.
3. **Bihoreau MT, Gauguier D, Kato N, Hyne G, Lindpaintner K, Rapp JP, James MR, and Lathrop GM.** 1997. A linkage map of the rat genome derived from three F2 crosses. Genome Res **7:**434–440.
4. **Brown JH, Bihoreau MT, Hoffmann S, Kranzlin B, Tychinskaya I, Obermuller N, Podlich D, Boehn SN, Kaisaki PJ, Megel N, Danoy P, Copley RR, Broxholme J, Witzgall R, Lathrop M, Gretz N, Gauguier D.** 2005. Missense mutation in sterile alpha motif of novel protein SamCystin is associated with polycystic kidney disease in (cy/+) rat. J Am Soc Nephrol **16:**3517–3526.
5. **Cowley BD Jr, Gudapaty S, Kraybill AL, Barash BD, Harding MA, Calvet JP, Gattone VH, 2nd.** 1993. Autosomal-dominant polycystic kidney disease in the rat. Kidney Int **43:**522–534.
6. **European Polycystic Kidney Disease Consortium.** 1994. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium. Cell **77:**881-894.

7. **Harris PC.** 2002. Molecular basis of polycystic kidney disease: PKD1, PKD2, and PKHD1. Curr Opin Nephrol Hypertens **11:**309–314.
8. **Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San Millan JL, Gamble V, Harris PC.** 1995. The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. Nat Genet **10:**151–160.
9. **Jain M, LeQuesne GW, Bourne AJ, Henning P.** 1997. High-resolution ultrasonography in the differential diagnosis of cystic diseases of the kidney in infancy and childhood: preliminary experience. J Ultrasound Med **16:**235–240.
10. **Kaspareit-Rittinghausen J, Deerberg F, Wcislo A.** 1991. Hereditary polycystic kidney disease. Adult polycystic kidney disease associated with renal hypertension, renal osteodystrophy, and uremic enteritis in SPRD rats. Am J Pathol **139:**693–696.
11. **Kaspareit-Rittinghausen J, Rapp K, Deerberg F, Wcislo A, Messow C.** 1989. Hereditary polycystic kidney disease associated with osteorenal syndrome in rats. Vet Pathol **26:**195–201.
12. **Kwitek AE, Gullings-Handley J, Yu J, Carlos DC, Orlebeke K, Nie J, Eckert J, Lemke A, Andrae JW, Bromberg S, Pasko D, Chen D, Scheetz TE, Casavant TL, Soares MB, Sheffield VC, Tonellato PJ, Jacob HJ.** 2004. High-density rat radiation hybrid maps containing over 24,000 SSLPs, genes, and ESTs provide a direct link to the rat genome sequence. Genome Res **14:**750–757.
13. **Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, Kimberling WJ, Breuning MH, Deltas CC, Peters DJ, Somlo S.** 1996. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. Science **272:**1339–1342.
14. **Nagao S, Ushijima T, Kasahara M, Yamaguchi T, Kusaka M, Matsuda J, Nagao M, Takahashi H.** 1999. Closely linked polymorphic markers for determining the autosomal dominant allele (Cy) in rat polycystic kidney disease. Biochem Genet **37:**227–235.
15. **National Research Council**. 1996. Guide for the care and use of laboratory animals. Washington (DC): National Academy Press.
16. **Ong AC, Harris PC.** 2005. Molecular pathogenesis of ADPKD: the polycystin complex gets complex. Kidney Int **67:**1234–1247.
17. **Pollard R, Yunis R, Kultz D, Martin P, Griffey S, Ferrara K.** 2006. Ultrasound detection and characterization of polycystic kidney disease in a mouse model. Comp Med **56:**215–221.
18. **Rat Research and Resource Center** [Internet]. Rat strains available listing and rat strain information [cited July 2001]. Available at http://www.nrrrc.missouri.edu.
19. **Schafer K, Gretz N, Bader M, Oberbaumer I, Eckardt KU, Kriz W, Bachmann S.** 1994. Characterization of the Han:SPRD rat model for hereditary polycystic kidney disease. Kidney Int **46:**134–152.
20. **Steen RG, Kwitek-Black AE, Glenn C, Gullings-Handley J, Van Etten W, Atkinson OS, Appel D, Twigger S, Muir M, Mull T, Granados M, Kissebah M, Russo K, Crane R, Popp M, Peden M, Matise T, Brown DM, Lu J, Kingsmore S, Tonellato PJ, Rozen S, Slonim D, Young P, Jacob HJ.** 1999. A high-density integrated genetic linkage and radiation hybrid map of the laboratory rat. Genome Res **9:**AP1–AP8.
21. **Torres VE, Bengal RJ, Nickander KK, Grande JP, Low PA.** 1998. Renal concentration of alpha-tocopherol: dependence on gender and lack of effect on polycystic kidney disease in Han:SPRD rats. Am J Kidney Dis **31:**687–693.
22. **Winters WD, McDonald RA, Krauter LD.** 1997. The detection of murine autosomal recessive polycystic kidney disease using real-time ultrasound. Pediatr Nephrol **11:**337–338.
23. **Woo D.** 2004. Genetics of polycystic kidney disease in cy rat. Poster abstract no 69. Presented at the Genetic Systems in the Rat XVth International Workshop, Copenhagen, Denmark.