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# Kidney and Retinal Defects (*Krd*), a Transgene-Induced Mutation with a Deletion of Mouse Chromosome 19 That Includes the *Pax2* Locus

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**The semidominant mutation *Krd* (kidney and retinal defects) was identified in transgenic line Tg8052. *Krd*/+ mice have a high incidence of kidney defects including aplastic, hypoplastic, and cystic kidneys. Retinal defects in *Krd*/+ mice include abnormal electroretinograms and a reduction of cell numbers that is most extreme in the inner cell and ganglion layers. Viability of *Krd*/+ mice is strongly influenced by genetic background, and growth retardation is observed in young animals. Homozygosity results in early embryonic lethality. Fluorescence *in situ* hybridization of a transgene-specific probe localized the insertion site to the distal region of mouse Chromosome 19. The sequence of the insertion site revealed transgene insertion into a LINE element with deletion of a single nucleotide from the 3′ terminus of the transgene. A polymorphic microsatellite, *D19Umi1*, was identified in a junction clone and mapped in several large crosses. *D19Umi1* is located 1.7 ± 1.0 cM distal to *Pax2*, which encodes a paired type transcription factor expressed in embryonic kidney and eye. Deletion of *Pax2* from the transgenic chromosome was demonstrated by Southern analysis of genomic DNA from (*Krd*/+ × SPRET/Ei)$F_1$ mice. Additional genetic and molecular data are consistent with an approximately 7-cM deletion that includes the loci stearoyl CoA desaturase (*Scd1*), pale ear (*ep*), *D19Mit17*, *D19Mit24*, *D19Mit27*, *D19Mit11*, and *Pax2*. This deletion, Del(19)TgN8052Mm, will be useful for genetic and functional studies of this region of mouse Chromosome 19.** © 1994 Academic Press, Inc.

## INTRODUCTION

Molecular analysis of spontaneous and induced mutations in inbred mice is an important method for connecting mutant phenotypes with underlying genetic defects (Reith and Bernstein, 1991). Several interesting mutants have been induced by transgene insertion during the generation of transgenic mice (Gridley, 1991; Meisler, 1992; Jones *et al.*, 1993a,b; Ting *et al.*, 1994). The disrupted gene has been isolated for several of these mutants by using the transgene sequence as a molecular probe (Woychik *et al.*, 1990; Weiher *et al.*, 1990; Lee *et al.*, 1992; Hodgkinson *et al.*, 1993; Hughes *et al.*, 1993). Other transgene-induced mutations are associated with chromosome deletions which may be large enough to disrupt expression of more than one gene (Cheng and Costantini, 1993; Karls *et al.*, 1992; Covarrubias *et al.*, 1987; Magram and Bishop, 1991). The larger deletions are often associated with recessive embryonic lethality.

In this paper we describe a new transgene induced mutation, *Krd*, characterized by kidney and retinal defects. Molecular and genetic analysis of the mutant identified a deletion at the transgene insertion site which spans several centimorgans of mouse Chromosome 19. Homozygotes are not viable. The chromosome deletion includes *Pax2*, a locus encoding a transcription factor whose characteristics suggest that it contributes to the developmental abnormalities observed in the mutant.

*Pax2* is a homeobox containing gene of the paired class (Walther *et al.*, 1991) expressed during fetal development in kidney, optic cup, optic stalk, otic vesicle, and neural tube (Dressler *et al.*, 1990; Nornes *et al.*, 1990). In a series of elegant experiments, it has been shown that *Pax2* plays a role in inductive interactions during kidney development (Dressler and Douglass, 1992; Phelps and Dressler, 1993; Dressler *et al.*, 1993). Overexpression of *Pax2* can result in cell transformation and tumorgenesis (Maulbecker and Gruss, 1993). Mutations in other members of the Pax gene family are responsible for the mouse developmental mutants

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**TABLE 1**

**Probes and Restriction Fragment Length Variants for Chromosome 19 Genes**

| Locus | cDNA probe | Enzyme | C57BL/6 | C3H/He | SPRET/Ei | Reference |
|---|---|---|---|---|---|---|
| *Adrb1* | $\beta$1 | *Taq*I | 2.2 | 2.2 | 1.9 | Oakey *et al.*, 1991 |
| *Cyp2c* | pM8-1 | *Taq*I | 10, 2.0 | 10, 2.0 | 9.5, 7, 6 | Meehan *et al.*, 1988 |
| *Cyp17* | Cyp17$\alpha$ | *Taq*I | 4.5, 1.2 | 4.5, 1.1 | 7.8, 1.1 | Youngblood *et al.*, 1991 |
| *Pax2* | c31A + cPX | *Eco*RI | 16, 12 | 16, 12 | 17, 11, 6.4 | Dressler *et al.*, 1990 |
| | | *Sac*I | 4.6, 1.5 | 7.4, 1.5 | 7.4, 2.2 | |
| *Scd-1* | pAL122 | *Eco*RV | 3.8 | 3.8 | 11 | Ntambi *et al.*, 1988 |
| | | *Eco*RI | 9 | 9 | 2.6 | |
| *Tdt* | Tdt-1 | *Taq*I | 3.1 | 3.1 | 3.2 | Landau *et al.*, 1984 |

*Note.* Lengths of hybridizing fragments are indicated in kb.

undulated (Balling *et al.*, 1988), splotch (Epstein *et al.*, 1991), and small-eye (Hill *et al.*, 1991). An unusual characteristic of the Pax genes is the dominant phenotype observed for apparent null alleles, suggestive of haploinsufficiency for Pax gene products (Gruss and Walther, 1992). Pax mutants also vary in severity of phenotypes observed among genetically identical heterozygotes (i.e., Hanson *et al.*, 1994). The *Krd* mutation demonstrates both of these features. It seems likely that haploinsufficiency of *Pax2* contributes to the congenital kidney disease in *Krd*/+ mice, and it may also contribute to the retinal defects.

## MATERIALS AND METHODS

*Animals.* Generation of transgenic line Tg8052 was previously described (Johnson *et al.*, 1993). Transgenic mice were produced by microinjection of a linear 1.9-kb amylase/elastase/CAT construct into fertilized mouse eggs obtained from the mating between (C57BL/6 × C3H/He)$F_1$ individuals. The transgenic founder was crossed to strain YBR/Ki to generate N1 animals, and subsequent generations (N2–N6) were produced by crossing transgenic offspring to strain C57BL/6J. Strains SPRET/Ei, C57BL/6J, RSV/Le-Re/Re, Sd/+, and C57BL/6J-*Ph*/+ were obtained from The Jackson Laboratory (Bar Harbor, ME).

*Identification of transgenic mice.* Genomic DNA isolated from tails was analyzed by the polymerase chain reaction (PCR) using primers complementary to the CAT reporter gene as previously described (Keller *et al.*, 1990).

*Southern blot analysis.* Ten-microgram aliquots of genomic DNA were digested with restriction endonucleases, electrophoresed on agarose gels, and transferred to nylon filters (Zetaprobe GT, Bio-Rad Laboratories). DNA fragments used as hybridization probes were purified from agarose gels and radiolabeled by the random oligo primer method (Feinberg and Vogelstein, 1983). Filters were hybridized and washed according to the manufacturer's instructions. The transgene probe was an 0.9-kb *Msp*I fragment corresponding to the 3′ end of the chloramphenicol acetyltransferase gene. Other probes are described in Table 1.

*Cloning and sequencing of the transgene insertion site.* Genomic DNA from spleen of a *Krd*/+ transgenic mouse was digested with *Xba*I. Fragments between 4 and 7 kb in length were isolated from agarose gels and ligated into the *Xba*I site of plasmid pSP72 (Promega Biotech). Clones containing transgene sequences were identified by colony hybridization with a transgene specific probe and analyzed as described by Keller (1994). The junction clone, pKIS, contains the predicted 5.8-kb *Xba*I fragment that, when digested by *Bgl*II, yields a 3.8-kb product containing transgene sequences. The 3.8- and 2.0-kb *Xba*I/*Bgl*II fragments were subcloned and partially sequenced using vector primers and primer (5′-ACC TCC CCC TGA ACC TG-3′) from the 3′ end of the transgene. A 500-bp nonrepetitive mouse DNA fragment was amplified from pKIS for use as a probe on Southern blots using the primers 5′-CTA CCC TGA AAT GTG TGA GAG TTC TGA ACC-3′ and 5′-CAT GTG TGA GGT CAA AGG ACA GCT TGT CAG G-3′. Plasmid DNA was sequenced by the DNA Sequencing Core Facility of the University of Michigan with an Applied Biosystems 370A sequencer.

*Fluorescence in situ hybridization.* Metaphase chromosomes were prepared from spleen cells from a heterozygous transgenic animal after culture for 2 days in the presence of concanavalin A, as previously described (Boyle *et al.*, 1990). The 0.9-kb transgene probe was labeled by nick translation with biotin–11-dUTP. The L1 banding probe KS13A was labeled with digoxigenin–11-dUTP. Fluorescence *in situ* hybridization was carried out exactly as described (Boyle *et al.*, 1992; Ting *et al.*, 1994). Image acquisition and processing was done on a Macintosh IIci computer (Boyle *et al.*, 1992).

*Microsatellite markers. D19Umi1* was amplified using the primers 5′-AAA GGC TTT TAA TGT ATG TGT GTC A-3′ and 5′-CAG GGC AGG TGA GTT G-3′. *D19Nds1* was amplified as described (Hearne *et al.*, 1991). *D19Mit11* was amplified with the primers 5′-TAT CCT CAA AGT CAA GGT GGG CAG CTG AGG-3′ and 5′-TTA TGT TGG AAG ACT TTC CAG ATG TTG GGC-3′ (Dietrich *et al.*, 1992) which gave a stronger signal than the commercially available primer set. Additional primers were purchased from Research Genetics (Huntsville, AL). PCR reactions contained 0.1 $\mu$Ci/$\mu$l [$^{32}$P]dCTP (Amersham). Amplified products were resolved on 6% polyacrylamide sequencing gels or on 6% nondenaturing polyacrylamide gels.

*Histology.* For retinal histology, animals were anesthetized with a lethal dose of sodium pentobarbital and perfused through the heart with phosphate-buffered saline followed by 0.1 *M* phosphate buffer, pH 7.4, containing 2.5% glutaraldehyde and 2.0% paraformaldehyde. Eyes were fixed overnight at 4°C, dehydrated, and infiltrated and embedded in glycomethyacrylate (Polysciences). Five-micrometer sections were cut with a rotary microtome, mounted on glass slides, and stained with 2.5% toluidine blue.

Kidneys from 1- and 4-day-old mice were fixed in 10% neutral buffered formalin, bisected along the greatest dimension, and embedded in paraffin. Photomicrographs were prepared from representative hematoxylin and eosin stained sections.

*Electroretinograms.* Mice were dark-adapted for at least 12 h and then anesthetized by intraperitoneal injection of Avertin, 0.1 ml per 7 g. Surgery and illumination with 40-ms flashes from a white xenon arc lamp ($I$ = 3.1 log cd/m$^2$) were carried out as previously described (Green *et al.*, 1991). Electroretinograms (ERGs) were recorded with a cotton-wick electrode placed on the cornea. Electrical potentials were recorded with an AC coupled amplifier, bandwidth 0.2 to 250 Hz.

## RESULTS

*Kidney abnormalities.* Kidney abnormalities in transgenic line Tg8052 were discovered in the course

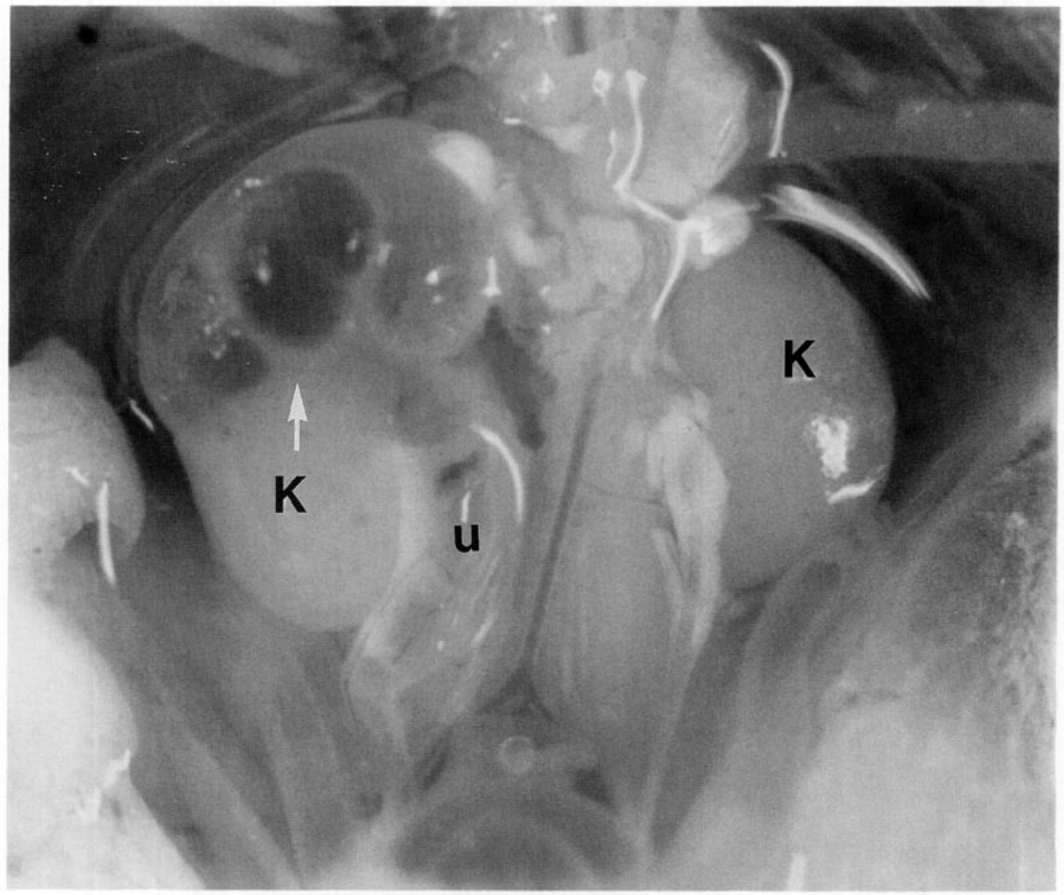
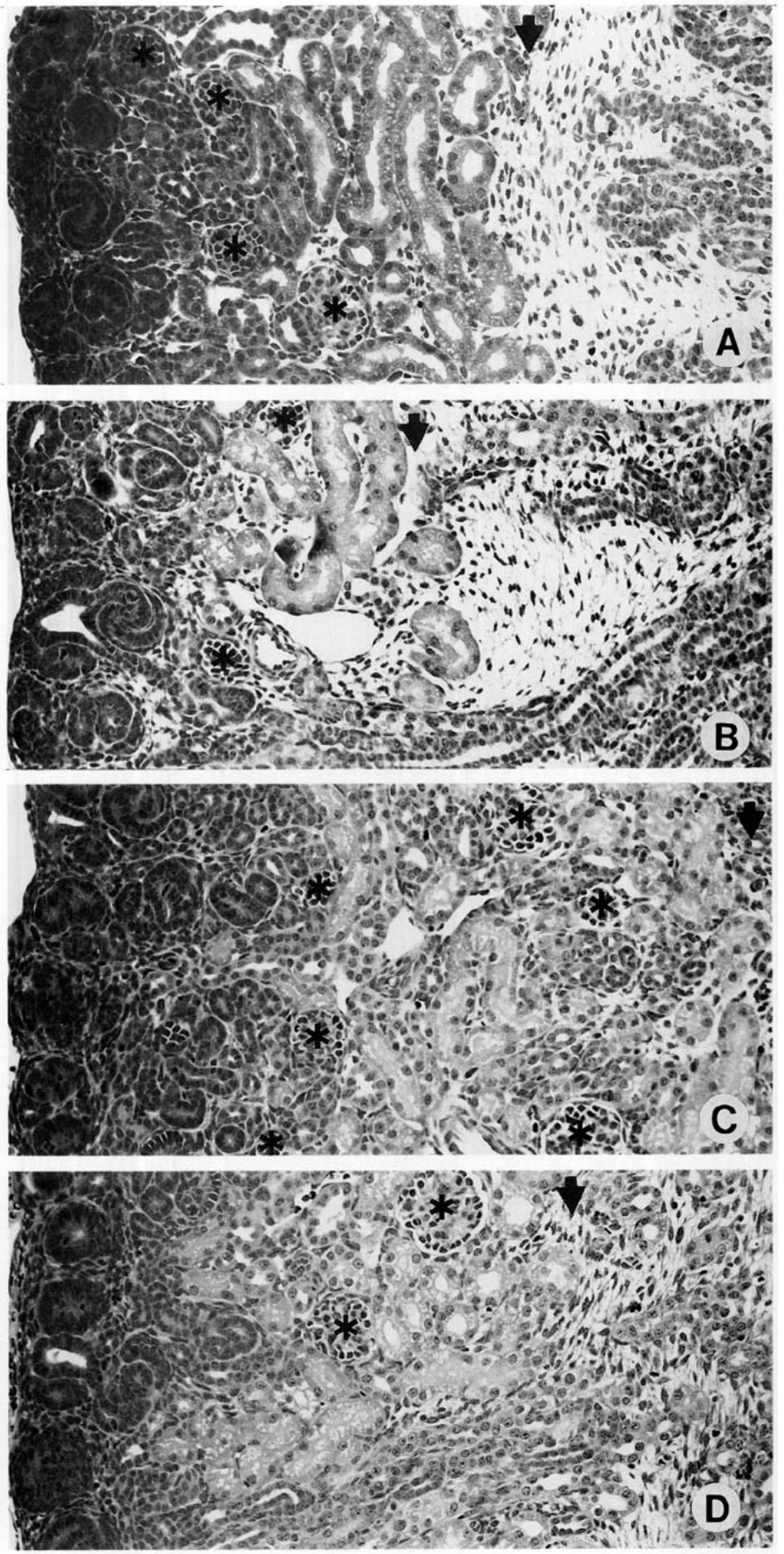



**FIG. 1.** Cystic kidney in transgenic mice of line Tg8052. Multiple cysts are apparent on the right kidney (**k**) of a 5-day-old hemizygous transgenic mouse (white arrow). The swollen ureter is also visible (**u**).

of screening transgenic lines for new insertional mutations (Meisler, 1992). First- and second-generation (N1 and N2) hemizygous transgenic individuals were mated and their offspring were examined at 6 weeks of age. Three cases of renal agenesis were observed among the first 23 progeny, two animals lacking the right kidney and one stillborn animal lacking both kidneys. Unilateral kidney cysts and dilated ureters were common in hemizygous transgenic mice from these and other crosses (Fig. 1). Visible kidney defects (cysts and agenesis) were present in approximately 25% of transgenic hemizygotes, but were never observed in nontransgenic littermates. The relative kidney weight was significantly reduced in those transgenic mice that did not have obvious kidney abnormalities, 4.8 ± 0.7 mg/g body weight ($n$ = 48) in transgenic mice at 3 to 8 weeks of age compared with 6.2 ± 0.8 ($n$ = 28) in littermates ($P < 0.001$, unpaired $t$ test).

Light microscopic examination revealed striking differences in the histologic appearance of *Krd*/+ and +/+ kidneys (Fig. 2). On Day 1 after birth, the *Krd*/+ kidneys demonstrated considerable immaturity compared with +/+ littermates. The nephrogenic zone appeared attenuated and somewhat disorganized. The thickness of the more mature cortical tissue was decreased due in part to decreased numbers of glomeruli and profiles of proximal convoluted tubules. Immature mesenchymal tissue was more prominent at the corticomedullary junction (arrows, Figs. 2A, 2B). Although the cortex was thicker in all animals at 4 days of age, the *Krd*/+ cortex was thinner than that in +/+ littermates, with fewer glomeruli and less well developed proximal convoluted tubules (Figs. 2C, 2D). Immature interstitial tissue was still persistent at 4 days in the *Krd*/+ animals. At later times, the thickness of the renal cortex noted in surviving *Krd*/+ animals approached that observed in normal littermates but the number of mature nephrons appeared to be smaller. Occasionally, cystic changes were noted in the medullary tissue of *Krd*/+ animals independent of the histologic differences described above. The observed reduction in the cortex appears to be sufficient to account for the reduced kidney weight in transgenic animals.

*Growth and viability.* The viability of hemizygous transgenic mice is dependent upon genetic background. When transgenic first generation (N1) individuals were crossed with strain C57BL/6J, the expected 50% transgenic offspring was observed at weaning. In subsequent generations the percentage of transgenic offspring fell to below 10% (Fig. 3). When N2 mice were crossed to strain C3H/HeJ, 46% offspring were transgenic (26/56), compared with 35% in the cross to


A
B
C
D

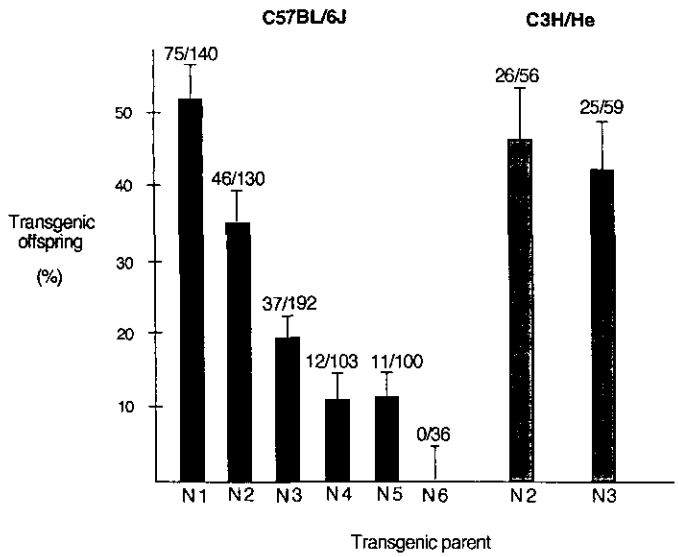



**FIG. 3.** Effect of genetic background on viability of *Krd*/+ mice. Genomic DNA was collected from offspring of the crosses C57BL/6J × *Krd*/+ and C3H/HeJ × *Krd*/+ at 2 to 3 weeks of age. Transgenic mice were identified by PCR using primers complementary to the transgene. Thin bars indicate the standard errors of the mean values plotted.

C57BL/6J (46/130). In the following generation, the difference in the fraction of transgenic offspring was greater, with 42% from the cross to C3H/HeJ (25/59) and only 19% from the cross to C57BL/6J (37/192) (Fig. 3).

To investigate the timing of reduced viability in the C57BL/6J line, offspring of the N3 × C57BL/6J cross were examined prenatally. In contrast with the 19% frequency at the time of weaning (37/192), the frequency of transgenic offspring at 12.5 and 14.5 days of gestation did not differ significantly from 50% (23/53; $P = 0.34$). This observation indicates that late fetal or early postnatal lethality is responsible for the imbalance seen at weaning. Unilateral kidney aplasia was seen in several of the transgenic mice who died before Postnatal Day 10.

The growth of transgenic animals is retarded for the first 2 months after birth, and transgenic mice can be recognized by their smaller size during this period, which is approximately 80% of normal. Normal weight is eventually reached.

*Structure and function of the eye.* To assess the visual function of transgenic mice, electroretinograms (ERGs) were measured (Green *et al.*, 1991). The typical a and b waves observed in normal animals are illustrated in Fig. 4C. The ERGs of eight transgenic mice varied from three in the low normal range to three that were extremely abnormal (i.e., Fig. 4D). In the affected animal shown in Fig. 4, the b-wave is nearly absent and the a-wave is greatly attenuated. The reduced b-wave indicates altered function in the bipolar cells and the reduced a-wave demonstrates that photoreceptor function is altered as well.

Retinas of some transgenic mice were noticeably thinner than normal on gross examination. Histological sections revealed a striking depletion of cells (Figs. 4A and 4B). In the affected animals all nuclear layers contained fewer somata than normal, although this was most noticeable for the ganglion cells and inner nuclear layers. The degree of cell loss varied at different positions in the retina, and from animal to animal. No defects were seen in the retinal pigmented epithelium, a monolayer of epithelial cells that are derived from the same germ layer as the retina. In the affected animal shown in Fig. 4, the functional deficit in the ERG is accompanied by extreme hypocellularity.

In addition to the variable degree of cellular depletion, all of the retinas from transgenic mice contained malformations of the cellular layers (Fig. 5), which were not seen in sections from control retinas. These took the form of a local narrowing of all retinal layers, a local "excess" of somata in a single nuclear layer, photoreceptor nuclei lying ectopically within the photoreceptor layer, and "rosettes" of photoreceptors enclosed between retinal layers that appeared to be cross sections of spheres. The laminar malformations were restricted to the central retina, while the cellular depletion was apparent across the centroperipheral extent of the retina.

Transgenic retinas were obtained from N3 and N4 mice produced by repeated backcrossing of the (C57BL/6J × C3H/He)$F_2$ founder. To evaluate the potential effect of residual heterozygosity for the unlinked recessive retinal degeneration mutation, *rd,* carried by C3H/He, we examined retinas from (C57BL/6J × C3H/HeJ)$F_1$ mice. The abnormalities characteristic of transgenic mice were not observed in these *rd*/+ controls.

*Chromosomal mapping of the transgene insertion site.* The initial chromosome assignment of the transgene insert was obtained by fluorescence *in situ* hybridization of metaphase chromosomes with biotin-

**FIG. 2.** Abnormal morphology of kidney cortex. Representative photomicrographs of renal tissue from +/+ and *Krd*/+ mice are arranged with the nephrogenic zone on the left and the medulla on the right. The corticomedullary junction is depicted with an arrow. Glomeruli are identified with asterisks. (**A**) One-day-old +/+ kidney. Note the gradient of tissue maturation from immature nephrogenic zone on the left to the more mature nephrons near the corticomedullary junction (arrow). (**B**) One-day-old *Krd*/+ kidney. The thickness of mature cortical tissue is diminished. The number of glomeruli and profiles of proximal tubular segments are decreased. (**C**) Four-day-old +/+ kidney cortex. Continued maturation of nephrons is evident with mature cortical tissue extending to the right margin of the photomicrograph (arrow). Numerous glomeruli are dispersed throughout the field. (**D**) Four-day-old *Krd*/+ kidney cortex. Compared with the littermate control in **C**, the amount of mature cortical tissue is decreased with relatively few glomeruli and reduced number of proximal convoluted tubule profiles. Magnification, 235×.

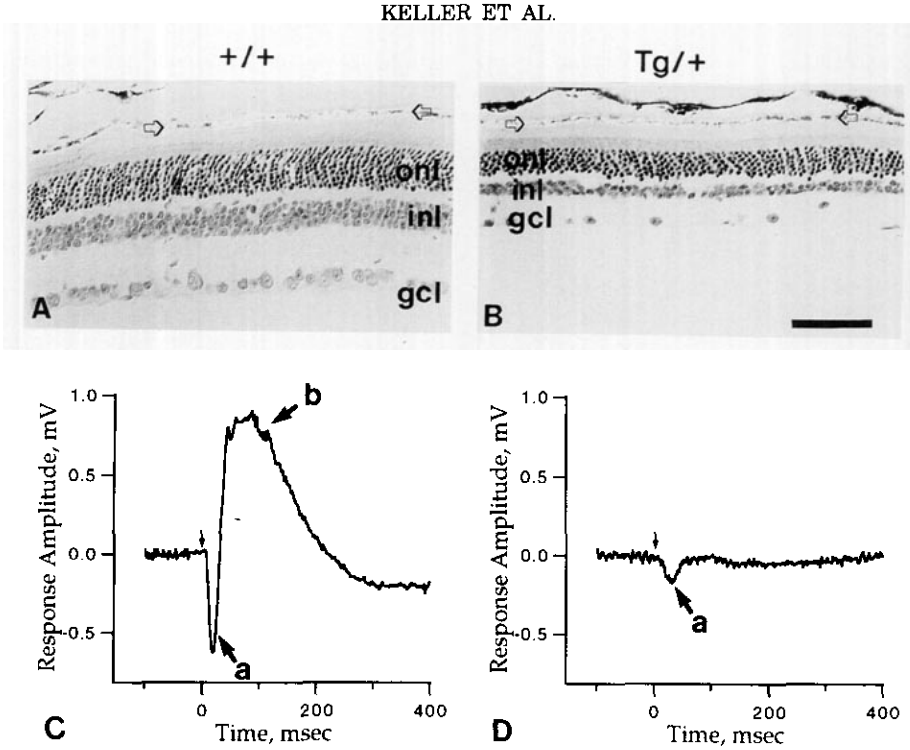



**FIG. 4.** Abnormal retinal structure and function. (**Top**) Photomicrographs of peripheral retina stained with toluidine blue. (**A**) Non-transgenic control; (**B**) transgenic. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Open arrows, retinal pigmented epithelium layer; scale bar, 50 $\mu$m. (**Bottom**) Full-field electroretinograms in response to a bright flash ($I$ = 3.1 log cd/m$^2$). The responses in **C** and **D** are from the mice whose retinas are shown immediately above. a, a-wave; b, b-wave.

ylated transgene probe as described under Materials and Methods. The signal was localized to the distal third of Chromosome 19, which was identified by DAPI and L1 banding. Approximately 50 metaphase spreads were examined, and 10 were imaged. Signal was observed on a single Chromosome 19 in each metaphase (data not shown). In no case was signal observed on other chromosomes.

To further localize the transgene on distal Chromosome 19, we constructed the interspecific backcross [(*Krd*/+ × SPRET/Ei)$F_1$ × C57BL/6J]. Backcross offspring were typed for the transgene by PCR, and for the Chromosome 19 genes *Tdt*, *Cyp17*, and *Adrb1* using the restriction fragment length polymorphisms described in Table 1. Haplotypes from the backcross demonstrating linkage are presented in Fig. 6. The observed gene order is centromere–*Tdt*–5.9 ± 3.3–transgene, *Cyp17*–5.9 ± 3.3–*Adrb1*.

Two apparent double recombination events involving the transgene were observed, one in which the transgene was deleted from a chromosomal region inherited from the transgenic parent and one in which the transgene was apparently transferred to a chromosomal region inherited from SPRET/Ei (Fig. 6). The observed frequency of double recombinants, 2/51 or 4%, is much higher than expected for the interval between

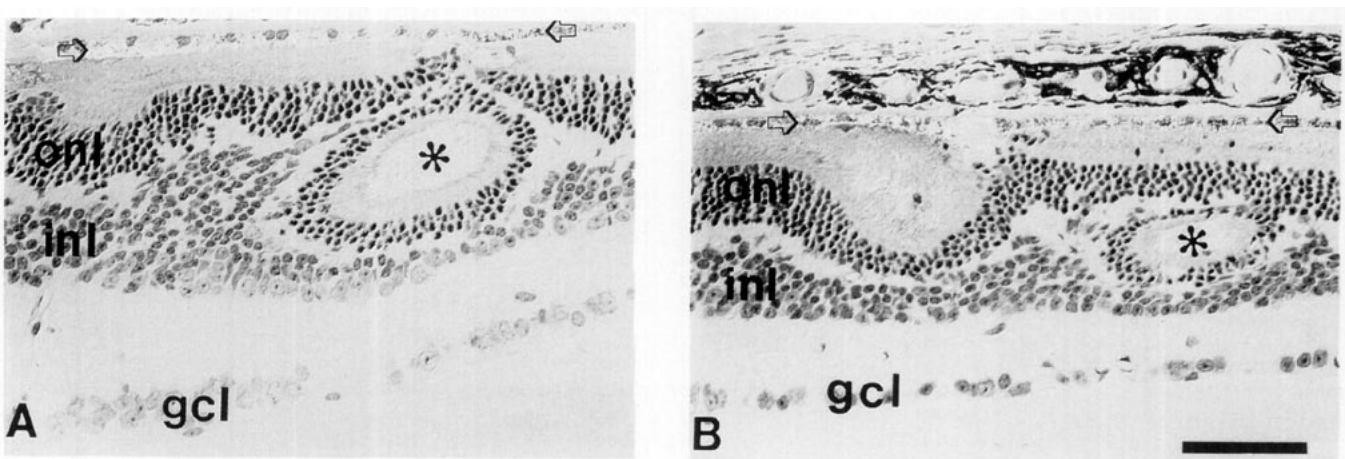



**FIG. 5.** Lamellar malformations in the central retina of transgenic mice. The normal layered structure of the retina shown in Fig. 4 is interrupted by abnormal cellular arrays. The asterisks identify rosettes of photoreceptors embedded within the retina. Sections from two transgenic mice. Symbols as in Fig. 4; scale bar, 50 $\mu$m.

*Tdt* and *Cyp17,* which is estimated at less than 10 cM (see below). The rate is especially high if the negative interference observed in this species is taken into account. Since the double recombinants may not reflect normal crossovers between homologous chromosomal regions, but are likely to be related to the deletion described below, they were not included in the calculation of the genetic distance between *Tdt* and the transgene insertion site above.

*Structure of the transgene insert.* Genomic DNA from transgenic mice was analyzed by Southern blotting using a transgene-specific probe and the enzymes *Bgl*II, which does not cleave the 1.9-kb transgene, and *Xba*I, which cleaves the transgene at a single site upstream of the hybridization probe. A single hybridizing *Bgl*II fragment of 28 kb was observed in transgenic DNA, defining the upper limit for the size of the transgene array (not shown). Digestion with *Xba*I produced 5 hybridizing fragments (Fig. 7 Lane 1). The 1.9-kb *Xba*I fragment corresponds in length to the predicted product of tandem transgene copies in head to tail array. The 5.8-kb fragment was identified as a junction fragment (see below). Fragments corresponding to the tail-to-tail orientation were not detected. The remaining three fragments may be derived from partial or rearranged transgene copies, from nontransgenic DNA located within the transgene array, or from the other junction with mouse DNA. From the number and intensity of hybridizing fragments, the insert appears to contain between 4 and 8 copies of the transgene.

Double digestion of genomic DNA with *Xba*I and *Bgl*II reduced the length of the 5.8-kb *Xba*I fragment to 3.8 kb (Fig. 7, Lane 2), indicating that it is a junction fragment. The 5.8-kb fragment was cloned and partially sequenced as described under Materials and Methods. A nonrepetitive probe from this clone hybridizes with a 9-kb *Xba*I fragment in nontransgenic DNA and an additional 5.8-kb *Xba*I fragment in transgenic hemizygotes (Fig. 7B), confirming that the clone is derived from the insertion site.

The junction between transgene and mouse DNA was sequenced using a primer complementary to the 3′ end of the transgene (Fig. 8A). A single base pair is deleted from the 3′ terminus of the transgene. The adjacent mouse sequence is closely related to a mouse LINE element (Genbank Accession No. M13002). The sequence also revealed rearrangement of the 3′ terminal transgene copy, which contains the transgene sequence +28 to +8 followed directly by +830 to +963 and then by +266 to +563. At least two rearrangement events within the transgene are indicated.

*A polymorphic microsatellite marker for the transgene insertion site.* A $(CA)_n$ microsatellite was identified in the junction clone approximately 2 kb from the transgene (Fig. 8B). The primers selected to amplify this microsatellite are underlined in the figure. A 154-bp amplification product was obtained from strains C3H/HeJ and SPRET/Ei, and a 171-bp product from strains C57BL/6J and CAST/Ei. This polymorphic microsatellite, designated *D19Umi1,* has been used as a genetic marker for the transgene insertion site. Amplification of the junction clone produces the 154-bp product predicted by the sequence, indicating that the transgene inserted into a region of Chromosome 19 derived from strain C3H/He in the microinjected (C57BL/6 × C3H/He)$F_2$ egg.

*Krd is a recessive lethal mutation.* Transgenic offspring of the cross C57BL/6J × *Krd*/+ are hemizygous for the transgene and heterozygous for the C3H/He and C57BL/6 alleles of *D19Umi1.* Mice of this genotype were crossed, and embryos were collected between 9.5 and 12.5 days of gestation. DNA was prepared from yolk sac and amplified with the primers for *D19Umi1.* Of 38 embryos examined, none were homozygous for the C3H/He allele (Table 2). This observation differs significantly from the expected 25% ($P < 0.001$). There were no homozygotes among the 13 embryos of 10.5 days gestation or younger (significantly different than 25%, $P = 0.01$), and we did not observe a significant number of resorbed embryos, suggesting that *Krd* is lethal prior to implantation. The observed frequency of transgenic embryos (22/38) was not significantly different from the 67% expected if homozygotes do not survive ($P = 0.25$).

*Deletion of Pax2 in line Tg8052.* The location of *Pax2* on Chromosome 19 and its known role in kidney development suggested that it might contribute to the phenotype of the *Krd* mutation. Southern blot analysis of genomic DNA from *Krd*/+ mice with a *Pax2* cDNA probe did not reveal novel bands after digestion with several restriction endonucleases. To test for deletion of *Pax2,* genomic DNA from (Tg8052 × SPRET/Ei)$F_1$ animals was probed with a *Pax2* cDNA probe. DNA from strains C57BL/6 and C3H/He contain two hybridizing *Eco*RI fragments of 12 and 16 kb that are not present in strain SPRET/Ei (Fig. 9A). Transgenic $F_1$ mice inherited only the SPRET/Ei fragments, demonstrating deletion of *Pax2* in line Tg8052 (Fig. 9A). Similar observations were made using the enzyme *Sac*I.

*Deletion of additional Chromosome 19 loci.* To estimate the size of the deletion, we examined the status of additional markers. When the (*Krd*/+ × SPRET/Ei)$F_1$

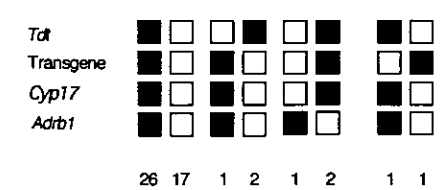



**FIG. 6.** Linkage of the transgene with loci on Chromosome 19. Offspring from the backcross [(*Krd*/+ × SPRET/Ei)$F_1$ × C57BL/6J] were typed for the transgene by PCR and for *Tdt, Cyp17,* and *Adrb1* by Southern blotting as described under Materials and Methods. Each column contains the haplotype of chromosomes which were observed in backcross progeny; the number of mice with each haplotype is given at the bottom. Open boxes, individuals carrying one SPRET/Ei allele; solid boxes, individuals lacking a SPRET/Ei allele.

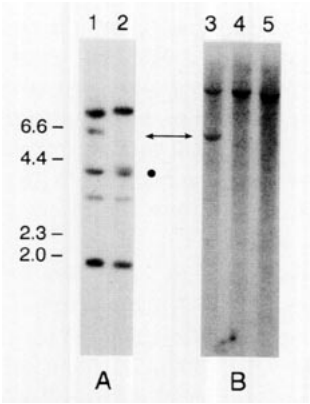



**FIG. 7.** Southern blot analysis of the insertion site. DNA was digested with *Xba*I alone or with *Xba*I and *Bgl*II (Lane **2**). Filters were hybridized with probes derived from transgene (**A**) or flanking sequence (**B**). Lanes **1–3,** *Krd*/+; lane **4,** C57BL/6J; lane **5,** C3H/HeJ. Arrow, 5.8-kb *Xba*I fragment; filled circle, 3.8-kb *Xba*I/*Bgl*II fragment.

genomic DNA was amplified by PCR, the SPRET/Ei allele but not the C3H or C57BL/6 alleles were produced with primers for *D19Mit11, D19Mit17, D19Mit24,* and *D19Mit27,* indicating that these microsatellite loci are deleted from the transgenic chromosome (data not shown). In control experiments, both alleles of these loci were amplified from 1:1 mixtures of SPRET/Ei DNA with C57BL/6 or C3H DNA. The locus *D19Nds1* was also found to be deleted by this method. *D19Nds1* is derived from the gene for stearoyl–CoA desaturase (*Scd1*) (Ntambi *et al.,* 1988). Deletion of *Scd1* was confirmed by Southern blot analysis of the $F_1$ DNA using an *Scd1* cDNA probe. The 3.8-kb hybridizing *Eco*RV fragment present in C57BL/6J and C3H/He was not detected in the (*Krd*/+ × SPRET/Ei)$F_1$ genomic DNA (Fig. 9B).

The *Krd*/+-derived allele of the following nondeleted loci could be detected in $F_1$ genomic DNA, *Tdt, Cyp2c, Adrb1, Cyp17, D19Umi1,* and *D19Mit 1, 3, 4, 5, 9, 10,* and *19.* We were able to distinguish between the C57BL/6 and C3H/He alleles of *Cyp17* and *D19Mit1, 9,* and *10.* In each case, the $F_1$ animals inherited the C3H allele from the transgene parent, indicating that the region around the transgene insertion is derived from the C3H parent. This Chromosome 19 deletion is designated Del(19)TgN8052Mm (Keller, 1994), in accord with the recommendations of the International Committee on Mouse Nomenclature (Lois Maltais, The Jackson Laboratory, personal communication).

*Complementation analysis of pale ear (ep) and ruby-eye (ru).* The pale ear locus, *ep,* characterized by pigment dilution and platelet storage pool disease, was recently mapped to the Chromosome 19 interval between the loci *D19Mit11* and *D19Mit24* (O'Brien *et al.,* 1994), both of which are deleted from the transgenic chromosome. To test directly for deletion of *ep, Krd*/+ mice were crossed to C57BL/6J-*ep*/*ep* homozygotes. Three of the 12 offspring had the diluted pigmentation characteristic of pale ears, and 9 were wildtype. Affected animals inherited the C3H/He allele of *D19Umi1* carried by the transgenic chromosome, while unaffected animals were homozygous for the C57BL/6J allele. The inability of the transgenic chromosome to complement the *ep* mutation is consistent with deletion of *ep* from the transgenic chromosome. The *Krd*/*ep* animals were runted, with body weights ranging from 50 to 70% of those of littermates of the same sex. The smaller size of the *Krd*/*ep* mice, compared with *Krd*/+, *ep*/+, or *ep*/*ep,* suggests that the *ep* allele retains some function and is not a null allele.

The pigmentation mutant ruby-eye (*ru*) is also closely linked to the transgene insertion marker *D19Umi1,* with no recombinants observed among 500 offspring (O'Brien *et al.,* 1994). Crosses between *Krd*/+ and *ru*/*ru* mice produced 15 unaffected offspring, including both homozygotes and heterozygotes for *D19Umi1.* These data indicate that *ru* is not deleted and is the closest known locus distal to *D19Umi1.*

*Genetic location of deleted loci.* To determine its location relative to the deleted loci, *D19Umi1* was typed on recombinant animals from the European Collabora-

**A**

```
                                                        A
TTGTTGTTGTTAACTTGTTTATTGCAGCTTATAATGGTTACNAATA

AAGCAATAGCATCACAAATTTCACAAATAAAGCATTTTTTTCACTG

CATTCTAGTTGTGGTTTGTCCAAACTCATCAATGTATCTTATCATG

                                  T
TCTGGATCCCCGGGTACCGAGCCAGGGCCAAAAAGCNGGAGTGGGT
                      ************** *********

GTGTAGGGGAGCNGGGCNGAGGGAGGGTNTAGGGAACTNTCGGGAT
* * ******** *** *^^** ** * * *** *** * ** **

AGCATTTGNAATGTATATAAAGAAAATATCTAACNAATAAA
****** * ******  * *   ***** ****  ** ***
```

**B**

```
CATAACAAAACTGATTCTATTAGGTAGCCACTTAAGATGGGCTGTTCCCT

CACAATCAGCTGTGGATCTCACCCACTGTTTCCCTGGTAACTCTAAAACT

GGCTATGTCACAAATGCCTCTCCTTAAGAGTCAAAATACCAGGGCAGGTG

AGTTGGCTCGTGGCATAAACGTGCCTGGGCTCACATGGTGGAAGGAGACA

GAGAAGTGACACCTGACAAGCTGTCCTTTGACCTCACACATGACAGGTGT

GTGTGTGTGTGTGTGTTGACACACATACAATAAAAGCCTTTTTAAGGGGC
```

**FIG. 8.** Genomic DNA sequence flanking the transgene insert. (**A**) The junction between the transgene and mouse genomic DNA, contained in clone pKIS, was sequenced using a primer corresponding to the 3′ end of the transgene. The transgene derived sequence is underlined. Two altered nucleotides are indicated above. Nucleotides identical to residues 7258 to 7365 of a mouse LINE element (M13002) are marked with asterisks. (**B**) The simple sequence repeat $(GT)_{10}$ was identified in downstream sequence from pKIS. The underlined sequences were used as primers to amplify this polymorphic microsatellite, *D19Umi1.*

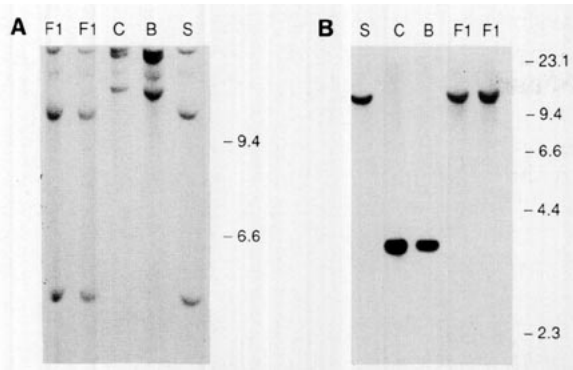



**FIG. 9.** Deletion of *Pax2* (**A**) and *Scd1* (**B**) from the transgenic chromosome. Genomic DNA was digested with *Eco*RI (**A**) or *Eco*RV (**B**) and filters were probed with the indicated cDNA (details in Table 1). S, SPRET/Ei; C, C3H/HeJ; B, C57BL/6J; F1, (*Krd*/+ × SPRET/Ei)$F_1$.

tive Interspecific Backcrosses [(C57BL/6 × SPR)$F_1$ × SPR and (C57BL/6 × SPR)$F_1$ × C57BL/6] (J. L. Guénet, personal communication) and the Roswell Park backcross [(B6C3Fe-*a*/*a*-*bm, ep, ru*/*bm, ep, ru* × PWK-*A*/*A*)$F_1$ × B6C3Fe-*a*/*a*-*bm, ep, ru*/*bm, ep, ru*] (O'Brien *et al.*, 1994). The results demonstrate that the deleted loci are clustered in a region proximal to *D19Umi1*, and none of the markers distal to *D19Umi1* are deleted (Fig. 10). The data are consistent with a simple deletion whose distal breakpoint is contained in the transgene junction clone containing *D19Umi1*. The minimal size of the deletion is the distance between *D19Umi1* and *D19Mit11*, the most centromeric deleted locus; this distance is 4.9 cM on the European Collaborative cross and 6.4 cM on the backcross BSB generated in our laboratory (Fig. 10). The maximal size of the deletion is the distance between *D19Umi1* and the closest centromeric nondeleted marker, *D19Mit20*, which is 7.0 ± 2.0 cM on the BSB cross. The length of the deletion is thus approximately 7 cM. This estimate is consistent with the reported distances between the Mit microsatellite markers measured on a (C57BL/6J-*ob*/*ob* × CAST)$F_2$ intercross (Dietrich *et al.*, 1992).

The same gene order was observed in all crosses, but the genetic distances observed on the Roswell Park cross are smaller than those on the other crosses. The reduced recombination frequency in this cross may be related to the different wild derived parent, strain PWK, or to the effect of the mutant alleles of Chromosome 19 loci *bm, ep,* and *ru* that are segregating in this cross.

## DISCUSSION

The transgene insertion in line Tg8052 was accompanied by a large deletion of approximately 7 cM, equivalent to 10 to 15% of the length of Chromosome 19 (Guénet and Poirier, 1993). Assuming that the mouse genome contains 100,000 genes and a total length of 1600 cM, the deletion may include as many as 400 genes. Recent analysis of radiation induced deletions of similar magnitude found growth retardation to be a common feature, and indicated that as much as 8% of the total haploid genome is susceptible to viable deletion (Cattanach *et al.*, 1993). This is the first large deletion described on Chromosome 19, and may be useful for future studies of gene dosage and for genetic and physical mapping.

In spite of the potentially large number of deleted genes, there is reason to think that some of the features of *Krd* may be due to deletion of *Pax2*. It has been demonstrated in *Drosophila,* and more recently in vertebrates (Niehrs *et al.*, 1994), that the concentration of transcription factors is critical to correct gene expression. Haploid doses of other members of the Pax gene family are known to produce developmental abnormalities in the mouse (Gruss and Walther, 1992). *Pax2* is expressed during organogenesis of the kidney. The expression of *Pax2* in the metanephrogenic mesenchyme is dependent on interaction with the ureteric bud, and the evidence suggests that *Pax2* is required for the conversion of mesenchyme to epithelial cells (Dressler and Douglas, 1992; Phelps and Dressler, 1993). These epithelial cells are the progenitors of the nephron, including the glomerulus, proximal, and distal tubules. Structural defects and impaired renal function have been observed in transgenic mice with deregulated overexpression of *Pax2* (Dressler *et al.*, 1993). The predicted phenotype for a mouse with reduced gene dosage for *Pax2* might therefore include developmental abnormalities such as decreased nephron number leading to hypoplastic kidneys or, in the most severe case, aplasia. The variability in the extent of kidney defects among *Krd*/+ animals, a phenomenon also observed for other *Pax* gene mutations, is of considerable interest and appear to result from stochastic developmental processes which are not well understood.

Since *Pax2* expression has been detected during fetal development in otic vesicle and optic cup (Nornes *et al.*, 1990), we tested the hearing and vision of *Krd*/+ mice. Measurements of auditory brain response were normal (unpublished observations), but recording of retinal response to light revealed a severe deficiency in some

**TABLE 2**

**Deficiency of Transgenic Homozygotes during Prenatal Development**

| | Genotype of embryos | | |
|---|---|---|---|
| Age (dpc) | B/B | C/B | C/C |
| 9.5 | 2 | 2 | 0 |
| 10.5 | 2 | 7 | 0 |
| 11.5 | 1 | 4 | 0 |
| 12.5 | 11 | 9 | 0 |

*Note.* Offspring were obtained from the mating of two hemizygous transgenic mice. The parents were heterozygous (B/C) at the transgene flanking locus *D19Umi1*. Embryos were collected at the indicated day postcoitus (dpc) and genotypes were determined by PCR.

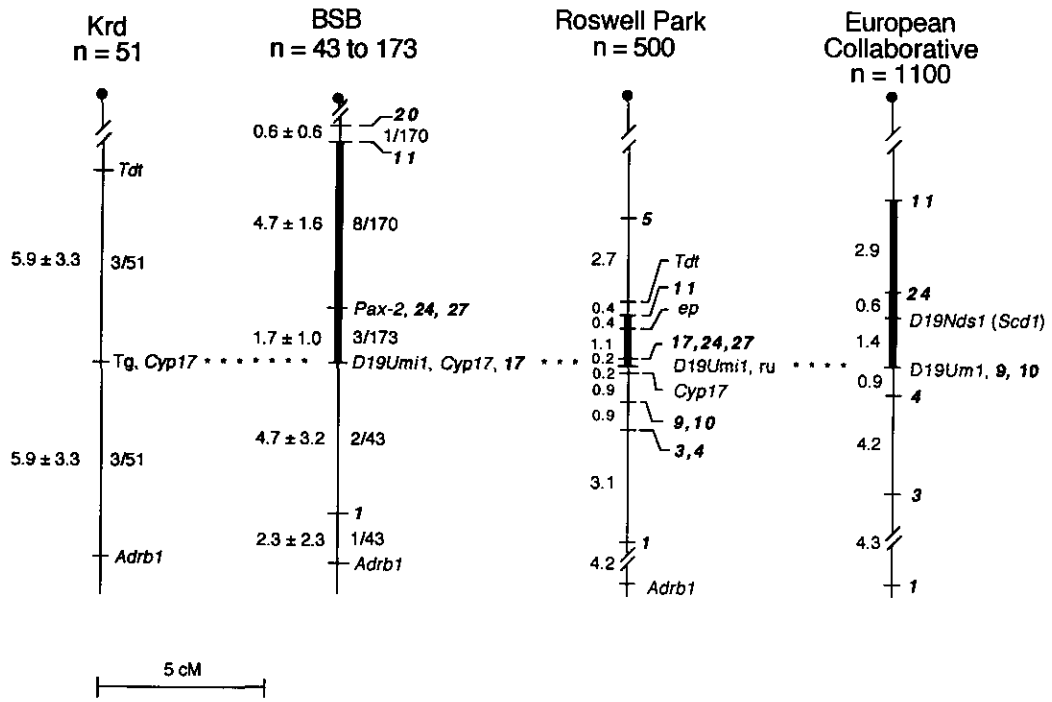



**FIG. 10.** Genetic map of the deleted region of mouse Chromosome 19. Haplotypes from the *Krd*/+ cross are presented in Fig. 8. The BSB cross was generated by crossing transgenic lines C57BL/6J-9257 (Ting *et al.*, 1994) and C57BL/6J-283 (Jones *et al.*, 1993a) to strain SPRET/Ei and backcrossing the $F_1$ to C57BL/6J. *D19Umi1* was typed on the European Collaborative Interspecific Backcross in the laboratory of Jean-Louis Guénet (personal communication) as described in this paper. The data for the Roswell Park cross is from O'Brien *et al.* (1994). Distances between adjacent loci (cM ± SE) are indicated at the left of each map, and recombination frequences are given at the right. The maps are aligned at the transgene (Tg) and the insertion site marker, *D19Umi1* (asterisks). Formal names of the Mit microsatellite loci are abbreviated; i.e., *D19Mit1* is shown as **1.** The chromosome region that is deleted from the transgenic chromosome appears in bold.

heterozygous mice. The hypocellularity and abnormal cell aggregates observed in these mice suggests that *Pax2* may play a role in cell differentiation in the retina. Analysis of earlier stages of development in *Krd*/+ mice will be required to distinguish between failure of retinal development and later retinal degeneration. The viability and normal behavior of *Krd*/+ mice demonstrates that haploid dosage of *Pax2* is consistent with fairly normal development of brain and ear, although minor abnormalities have not been ruled out.

Crosses of *Krd*/+ with other mutants may be used to identify additional genes involved in developmental processes regulated by *Pax2*. To test the hypothesis that the platelet-derived growth factor receptor alpha encoded by the patch locus (*Ph*) might be one such locus, we crossed *Krd*/+ mice with C57BL/6J-*Ph*/+. Kidney size in *Krd*/+ offspring was unaffected by genotype at *Ph*, and no increase in frequency of agenesis was observed in the double heterozygotes. On the other hand, preliminary experiments with the Danforth's short tail locus (*Sd*) were suggestive of an increased rate of renal agenesis in doubly heterozygous *Krd*/+, *Sd*/+ mice.

The large effect of genetic background on viability of *Krd*/+ mice is intriguing. The viability is close to 100% on strain C3H/He, but less than 10% on strain C57BL/6J. The gradual reduction in viability observed during several generations of crossing suggests that homozygosity for C57BL/6J alleles at two or more modifier loci outside of the deleted region is deleterious to survival. Modifier loci might be involved in kidney development directly, such as *Pax8*, or might be related to other deleted loci. The effect of genetic background on the frequency of kidney defects has not been extensively compared, but no change in frequency of agenesis was evident during two generations of crossing to strains YBR/Ki and C3H/HeJ.

The transgene insert in line Tg8052 terminates at the 3′ end of the 1.9-kb injected fragment with loss of a single nucleotide. Other examples of loss of a small number of nucleotides from the end of inserted transgenes have been reported, suggesting that transgene arrays are derived from linear rather than circular intermediates (Hamada *et al.*, 1992). We did not observe homology between the transgene sequence and the flanking mouse DNA, which is derived from a member of the LINE element family of repeated sequences. At least two other examples of transgene insertion into related elements have been observed, in a LINE element in line Tg9257 (Ting *et al.*, 1994, and unpublished observations) and near an element with 60% homology to a LINE (Mark *et al.*, 1992). Since LINE elements may account for as much as 10% of the mouse genome, preferential insertion is probably not required to account for these cases.

Mutations in the human Pax genes are responsible for the Waardenburg syndrome type I and for aniridia (Tassabehji *et al.*, 1992; Ton *et al.*, 1991). The pheno-

type of *Krd/+* mice suggests that *Pax2* may play a role in human congenital kidney and eye disease. Hereditary renal adysplasias, autosomal dominant disorders of variable penetrance and expression, are characterized by unilateral or bilateral renal agenesis or dysplasia. Some of these occur as syndromes in which other sites of *Pax2* expression, including otic vesicles, are affected. The human homologs of genes in the deletion Del(19)TgN8052Mm are part of a conserved linkage group on human chromosome 10q. Although one recent study excluded this region as causative for congenital nephrotic syndrome of the Finnish type (Kestilä *et al.*, 1994), this region should be considered in future linkage studies of other inherited kidney and eye disorders.

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