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Identification of genes within the *Krd* deletion on mouse Chromosome 19

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The *Krd* deletion, Del(19)TgN8052Mm, was generated by a nontargeted transgene insertion into the distal region of mouse Chr 19 (Keller et al. 1994). The length of the deletion was estimated to be approximately 5 cM from the recombination rate of flanking markers. Three deleted genes were identified in the original report: *Pax2, Scd1,* and the coat color mutation pale ear (*ep*).

The *k*idney and *r*etinal *d*egeneration observed in *Krd*/+ heterozygotes includes early development of renal cysts, a low frequency of unilateral or bilateral agenesis of the kidney, and disorganization of the retinal cell layers (Keller et al. 1994). Retinal development is disrupted owing to the absence of a small population of precursor cells (Otteson et al. 1998). We originally suggested that these abnormalities were caused by haploinsufficiency for the paired box domain transcription factor gene *Pax2,* which is involved in the early stages of organogenesis of the kidney and retina (Dressler 1996). This prediction was confirmed by the identification of human patients with point mutations in PAX2 and abnormalities similar to the *Krd* mouse (Sanyanusin et al. 1995; Schimenti et al. 1997) and by characterization of a point mutation in the mouse (Favor et al. 1996). Mice homozygous for the *Krd* deletion do not survive to birth and appear to be lost at an early, preimplantation stage (Keller et al. 1994).

There is currently renewed interest in mouse chromosomal deletions because of their utility as sensitized background for mutagenesis (Davis and Justice 1998). To evaluate the potential of the *Krd* deletion for use in a sensitized mutation screen, we have analyzed several genes and ESTs mapped to this region of Chromosome (Chr) 19. To determine whether these genes were located within the deletion, we tested the ability of the deletion-bearing chromosome to transmit each locus in a cross between C57BL/6J-*Krd*/+ mice and strain SPRET/Ei or CAST/Ei. The *Krd*/+ offspring of these crosses were identified by PCR with primers derived from the transgene (Keller et al. 1994). Failure to transmit a C3H or C57BL/6J allele along with the *Krd* transgene marker indicates that the locus is deleted from the *Krd* chromosome (Keller et al. 1994). Genomic DNA from the *Krd*/+ offspring was analyzed by use of polymorphic differences between the parental strains (Table 1). Five genes were examined by Southern blotting: *Fgf8, Wnt8b, Tlx1, Pitx3* and *Nkx2-3.* Four of these failed to be transmitted from the *Krd* chromosome (Table 1). The *Cmoat* gene encoding an ion transport protein was demonstrated to be deleted by a single-stranded conformational polymorphism (SSCP) assay. The other genes and ESTs that were tested were observed to be transmitted to the *Krd*/+ F_1 offspring, indicating that they are not deleted from the *Krd* chromosome (Table 1).

The asebia gene (*ab*) was tested for inclusion in the *Krd* deletion by functional complementation. Homozygous mutant *ab/ab* mice demonstrate skin and hair abnormalities that include hair loss, flaky skin, encrusted eyes, and abnormal or absent sebaceous glands. In two litters from a cross between *ab/ab* mice and *Krd*/+ mice, three transgenic *Krd*/+ offspring were obtained. All three of the *Krd*/+ mice demonstrated the characteristic signs of asebia, indicating that the *ab* gene is located within the *Krd* deletion.

The positions of genes known to be deleted from the *Krd* chromosome are shown on the map of Chr 19 in Fig. 1. The ten genes mapped within the deletion include five genes that play a role in regulation of development: *Pax2, Wnt8b, Tlx1* (*Hox11*), *Nkx2-3,* and *Fgf8.* The early lethality of embryos homozygous for the *Krd* deletion may be related to loss of one or more of these genes. In heterozygotes, the kidney and retinal abnormalities can be accounted for by haploinsufficiency of Pax2, since similar abnormalities are seen in human patients heterozygous for PAX2 mutations (Sanyanusin et al. 1995; Schimenti et al. 1997). Furthermore, the other genes in the deletion are not known to exhibit haploinsufficiency.

Crosses between *Krd*/+ heterozygotes and chemically mutagenized males could be used to generate allelic series for these developmental regulatory proteins. Multiple alleles can provide important functional information about the pleiotrophic functions of mammalian genes, as exemplified in recent studies of mutations in mouse *Myo5a* (Huang et al. 1998a, 1998b). The human orthologs of the genes in the *Krd* deletion are located on Chr 10q23-10q24. Genetic disorders mapped to this region include a locus for the Hermansky Pudlak syndrome, which corresponds to the mouse pale ear locus (Feng et al. 1997; Gardner et al. 1997), as well as several human disease genes that have not yet been cloned: partial epilepsy (EPT), progressive external ophthalmoplegia type 1 (PEO1), corneal dystrophy (CDB2), and urofacial syndrome (UFS). The *Krd* mouse may be useful in the future for development of models of these inherited disorders.

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Table 1. Polymorphisms used to detect gene deletion in (C57Bl/6J-*Krd*/+ × SPRET/Ei)F_1 mice **(A)** Genomic DNA was analyzed as previously described (Keller et al. 1994). RE, restriction endonuclease; E, *Eco*RI; T, *Taq*I; S, *Sph*I. **(B)** Primers were selected to amplify 150–250 bp of genomic DNA, and single-stranded polymorphisms (SSCPs) were detected as described by Semina et al. (1997). References: (1) Unpublished sequence from The WashU-HHMI Mouse EST Project, available from GenBank; (2) EST from mouse physical mapping project available at http://www-genome.wi.mit.edu/cgi-bin/mouse/indexL; (3) Semina et al. 1998; (4) Lammert et al. 1998.

A. Restriction fragment length polymorphisms (RFLPs).

Locus	RE	C3H/HeJ, C57BL/6J	SPRET/Ei	(*Krd* × SPRET/Ei)F1	Result
Fg/8	E	15	9.0, 6.0	9.0, 6.0	Deleted
Wnt8b	T	4.6, 1.4, 0.9	4.6, 2.6	4.6, 2.6	Deleted
Tlx1 (Hox11)	T	15	17	17	Deleted
Nkx2-3	T	2.2	1.0, 0.8	1.0, 0.8	Deleted
Pitx3	S	9	12	9, 12	Not deleted

B. Single-stranded conformational polymorphisms (SSCPs)

Locus	Ref.	Primers	Product (bp)	Result
CMOAT	(2)	F: 5′-cctagacagcggcaagattgt-3′ R: 5′-ttacagggtggttgagaccag-3′	250	Deleted
AA05080	(1)	F: 5′-aatagaagagagaagggtgg-3′ R: 5′-ggacacgaaaatgaatgg-3′	122	Not deleted
AA060281	(1)	F: 5′-gatgacttgaagtctttcatcc-3′ R: 5′-tcttgaatcgctctccc-3′	158	Not deleted
AA259484	(1)	F: 5′-ttcgctttcccttgccttc-3′ R: 5′-aatgtttatttcacacgcgctc-3′	132	Not deleted
PITX3	(3)	F: 5′-tgtggtcaagaaccggc-3′ R: 5′-ttgaccgagttgaaggcgaa-3′	212	Not deleted
D18387	(4)	F: 5′-cttacaccaccagcaacacccct-3′ R: 5′-gagggtggaggctgtacaaa-3′	161	Not deleted

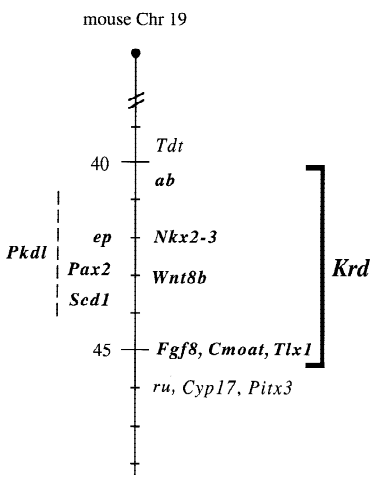

Fig. 1. Genetic map of mouse Chr 19, including genes located within the *Krd* deletion. Genes mapped to the deletion in this report are shown to the right of the chromosome; those to the left were previously mapped (Keller et al. 1994; Nomura et al. 1998). Approximate distances from the centromere are indicated in cM (Poirier and Guénet 1997). *Wnt8b* was also mapped to this region by use of the previously described interspecific backcross (Copeland and Jenkins 1991; data not shown). *Cmoat* was previously assigned to Chr 19 but was not previously localized. Human orthologs of some of these genes have been mapped to human Chr 10q23-24.

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