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Lysyl oxidase (*Lox*) maps between *Grl-1* and *Adrb-2* on mouse Chromosome 18

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Lysyl oxidase is an extracellular copper enzyme that catalyzes the oxidation of peptidyl lysine to α-amino-adipic-δ-semialdehyde, which condenses with neighboring aldehydes or ε-amino groups to form covalent interchain or intrachain crosslinks between elastin and collagen fibers (Kagan and Trackman 1991). These crosslinkages convert soluble elastin and collagen monomers into insoluble fibers in the extracellular matrix. Lysyl oxidase may regulate the development and repair of the matrix in lung, aorta, and other connective tissues (Kagan and Trackman 1991). In addition a role for lysyl oxidase in tumor suppression is suggested by the finding that the sequence of lysyl oxidase matches that of a regulator of the *ras* oncogene, ras recision gene *rrg* (Kenyon et al. 1991).

Low levels of lysyl oxidase have been reported in patients with several X-linked, recessively inherited human connective tissue disorders including Ehlers-Danlos type V and IX (cutis laxa) and Menkes syndromes. However, lysyl oxidase is encoded by a single-copy autosomal gene, *LOX,* that has been assigned to human Chromosome (Chr) 5 by somatic cell hybrid analysis (Mariani 1992) and localized to human 5q.23.3-31.2 by in situ hybridization (Hamalainen et al. 1991; Svinarich et al. 1992). This localization eliminated *Lox* as a candidate gene for the X-linked disorders with lysyl oxidase deficiency. Synteny homology between mice and humans suggested that *Lox* would map to mouse Chr 11 or 18 (Nadeau et al. 1992).

We mapped *Lox* to mouse Chr 18 using a *M.m. castaneus* intersubspecific backcross (DF/B-*df*/*df* × CASA/Rk)F_1 × (DF/B-*df*/*df*) that was previously characterized for Chr 11 (Buckwalter et al. 1991). We report the typing of this cross for *Lox* and three markers previously localized on Chr 18: early growth response 1, *Egr-1;* glucocorticoid receptor 1, *Grl-1;* and adrenergic receptor β2, *Adrb-2* (Johnson and Davisson 1992; Oakey et al. 1991). The polymorphisms used to type each locus are described in Fig. 1. The unambiguous gene order was based on haplotype analysis of 36 animals (Fig. 2). The intergene distances were deter-

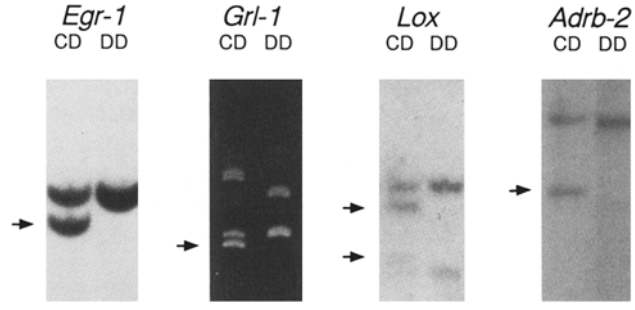

Fig. 1. Polymorphisms used to type backcross progeny on Chr 18. *Egr-1, Lox,* and *Adrb-2* were typed with RFLPs detected by Southern blot analysis, and *Grl-1* was typed with a simple sequence repeat polymorphism detected by PCR. Southern blots were performed as previously described (Sambrook et al. 1989). All gels were transferred to Zeta-Probe nylon membranes (Bio-Rad). Probes were labeled by the random hexanucleotide method (Feinberg and Vogelstein 1982). Hybridizations with a mouse cDNA clone for *Egr-1,* pRSV3.1, provided by V.P. Sukhatme, Chicago (Sukhatme et al. 1988), were carried out at 65°C. Genomic DNA digested with *Sspl* revealed a 6.1-kb DF/B allele of *Egr-1* and a 5.4-kb CASA/Rk allele. A rat lysyl oxidase cDNA probe, 13 L-O, provided by P.C. Trackman, Boston (Trackman et al. 1990), was hybridized at 65°C to Southern blots of genomic DNA digested with *Bam*HI. The DF/B allele of *Lox* produced fragments of 5.4, 4.0, and 1.9 kb, and the fragments from the CASA/Rk allele were 4.9, 4.2, and 1.9 kb. A human ADRB2 cDNA, pTF, was obtained from R.J. Lefkowitz, North Carolina (Kobilka et al. 1987) and hybridized at 57°C to Southern blots of genomic DNA digested with *Eco*RI. A DF/B fragment of 6.6 kb and a CASA/Rk fragment of 5.2 kb were detected. *Grl-1* was mapped by PCR with 500 ng of mouse genomic DNA used as template in a 25-μl reaction volume with 10 μmol of each primer and 2 μmol each of dATP, dCTP, dGTP, and dTTP. Primers for *Grl-1 (D18Mit17)* were extended with 1-2 U of *Taq* polymerase (Dietrich et al. 1992). The amplification products were 192 bp for DF/B DNA and 180 bp for CASA/Rk DNA. The products were separated on a 7.5% native polyacrylamide gel. Arrowheads designate the *M. castaneus*-specific alleles present in genomic DNA from (DF/B-*df*/*df* × CASA/Rk)F_1 mice (DC) but absent in DF/B-*df*/*df*(DD) mice.

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Egr-1		*Grl-1*		*Lox*		*Adrb-2*	number of mice
M		M		M		M	15
T		T		T		T	15
M	X	T		T		T	1
T		M		M		M	0
M		M	X	T		T	0
T		T		M		M	3
M		M		M	X	T	0
T		T		T		M	2

Fig. 2. The haplotype distribution of 36 backcross progeny was used to determine gene order. Genes are shown in the proximal-to-distal order. "M" designates homozygosity for the DF/B allele; "T" indicates heterozygosity. All alternative gene orders result in multiple double recombinants over short intervals.

mined by analysis of these and additional animals: *Egr-1*–2.7 ± 1.9–*Grl-1*–9.2 ± 3.6–*Lox*–4.8 ± 3.3–*Adrb-2* (Fig. 3). The distances between the Chr 18 markers observed in our cross are comparable to those reported by others in crosses with *M. spretus: Egr-1–Grl-1,* 4.3 cM and *Grl-1–Adrb-2,* 12.1 cM (Johnson and Davisson 1992) to 17.5 cM (Oakey et al. 1991).

We predict that mutations in *Lox* would result in connective tissue abnormalities and/or enhanced tumor susceptibility. Plucked is a recessive mouse mutation that maps to the general region of mouse Chr 18, where we localized *Lox* (Davisson et al. 1991). Plucked mice have unusual skin features (Lyon and Searle 1989), as do people with lysyl oxidase deficiency syndromes. However, since the human syndromes result from lesions in X-linked genes, targeted disruption of *Lox* by homologous recombination in embryonic stem cells may be necessary to determine the phenotype of *Lox* mutations.

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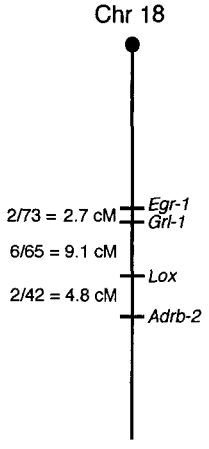

Fig. 3. A map of Chr 18 is presented with the loci reported here. The genetic distance was calculated from the number of recombinants observed per total number of individuals examined (left).

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Note added in proof. Analysis of an interspecific backcross with *M. spretus* placed *Lox* on Chr 18 with the following gene order: centromere–*Camk-4–Lox–Ii–Mbp* (Mock et al. 1992). This is consistent with our data. Mock, B.A., Contente, S., Kenyon, K., Friedman, R.M., and Kozak, C.A. (1992). The gene for lysyl oxidase maps to mouse chromosome 18. Genomics 14, 822–823.