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The methionine synthase (*Mtr*) gene maps to proximal mouse Chromosome 13

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Species: Mouse
Locus name: methionine synthase or 5-methyltetrahydrofolate-homocysteine methyltransferase
Locus symbol: *Mtr*
Map position: proximal–*D13Mit1*–1.06 cM ± 1.06 SE–*Mtr, D13Bir4, D13Bir6*–1.06 ± 1.06–*D13Abb1e*–2.13 ± 1.49–*D13Bir7*–distal
Method of mapping: *Mtr* was localized by RFLP analysis of 96 animals from an interspecific backcross panel ((C57BL/6JEi × SPRET/Ei)F_1 × SPRET/Ei) provided by The Jackson Laboratory, Bar Harbor, Me. (BSS panel) [1].
Database deposit information: The data are available from the Mouse Genome Database, accession number MGD-JNUM-39061.
Molecular reagents: A 1095-bp mouse cDNA was obtained by reverse transcription/PCR of mouse liver RNA, with degenerate oligonucleotides based on regions of homology within the methionine synthase sequences of lower organisms. The two primers (D1730 and D1733), as described by Leclerc et al. [2], were successful in amplifying both human and mouse cDNAs. The PCR products from both species were subcloned and sequenced; they showed 89% identity. The mouse cDNA was labeled by random priming and hybridized to Southern blots of *Eco*RI-digested mouse genomic DNA.
Allele detection: Allele detection was performed by RFLP analysis of an *Eco*RI polymorphism. The C57BL/6J strain has alleles of approximately 13 kb, while the *Mus spretus* strain has alleles of approximately 9 kb and 4 kb. A constant band of approximately 0.5 kb was seen in both strains.
Previously identified homologs: Human MTR has been mapped to chromosomal band 1q43 by fluorescence in situ hybridization [2–4].
Discussion: Methionine synthase (EC 2.1.1.13, 5-methyltetrahydrofolate-homocysteine methyltransferase) catalyzes homocysteine remethylation to methionine, with 5-methyltetrahydrofolate as the methyl donor and methylcobalamin as a cofactor. Nutritional deficiencies and genetic defects in homocysteine metabolism result in varying degrees of hyperhomocysteinemia. Dramatic elevations in plasma and urinary homocysteine levels are associated with the inborn error of metabolism, homocystinuria. Consequent to the recent isolation of the human cDNA for methionine synthase [2–4], two groups of investigators have identified mutations in methionine synthase in homocystinuric patients [2, 5]. Mild elevations in plasma homocysteine are thought to be a risk factor for both vascular disease and neural tube defects [6–8]. A genetic variant in methylenetetrahydrofolate reductase (MTHFR), the enzyme that synthesizes 5-methyltetrahydrofolate for the methionine synthase reaction, is the most common genetic determinant of hyperhomocysteinemia identified thus far [9]. Mild defects in the methionine synthase reaction are also potential candidates for hyperhomocysteinemia and the associated multifactorial diseases. A common variant has been reported for the human methionine synthase gene, but its physiologic consequences have not yet been determined [2, 4].

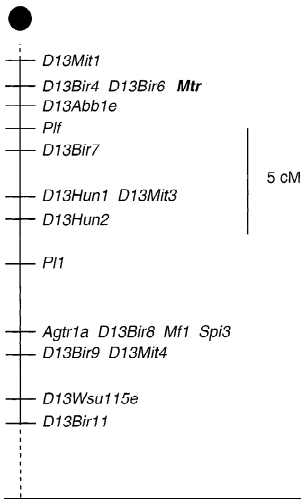

Fig. 1. The localization of *Mtr* to proximal Chr 13. The markers are from the BSS backcross panel. These data and references for mapping the other loci are publicly available from The Jackson Laboratory Mapping Resource at the World Wide Web address: http://www.jax.org/resources/documents/cmdata.

The mapping of the human MTR gene to 1q43 and of the mouse gene to proximal Chromosome (Chr) 13 is consistent with previous findings of human/mouse homologies between these 2 chromosomal regions; the human and mouse nidogen genes have been mapped to 1q43 and proximal Chr 13, respectively [10].
Several genes have already been implicated in neural tube defects in mice [11]. Studies involving the mouse methionine synthase gene will be useful in assessing the role of this important enzyme in the development of birth defects and/or vascular disease.

Acknowledgments: The expert advice of Lucy Rowe (The Jackson Laboratory Backcross DNA Panel Map Resource) and the clerical assistance of Carolyn Mandel are gratefully acknowledged. This work was supported by a grant from the Medical Research Council of Canada to the MRC Group in Medical Genetics.

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Mouse natriuretic peptide receptor 3 gene maps to proximal Chromosome 15

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Species: Mouse
Locus name: Natriuretic peptide receptor 3
Locus symbol: *Npr3*
Map position: 15 centromere–*D15Mit10, D15Bir 3, Npr3*–(2.1 ± 1.5)–*D15Xrf32*–(2.1 ± 1.5)–*D15Bir 5*
Method of mapping: Determined from 94 interspecific backcross progeny of the BSS panel (C57BL/6JEi × SPRET/Ei) × SPRET/Ei of The Jackson Laboratory Backcross DNA Panel Mapping Resource as described [1].
Database deposit information: MGD accession number: MGD-JNUM-38398
Molecular reagents: The mouse *Npr3* gene was cloned from a strain 129 genomic library, with a probe generated by PCR from primers based on the rat *Npr3* sequence [2]. The gene was mapped for restriction enzyme sites and partially sequenced (N. Matsukawa, unpublished). This information was used to design the following probe that corresponds to codons 181–249 of the mouse *Npr3* gene [3]. The probe was an approximately 200-bp PCR product that was generated with primers 5′ GAG ATG ATG CTC GCT CTG TTT CG 3′ and 5′ CTG CCT TGG ATG TAG CGC ACT AT 3′.
Allele detection: The probe recognized restriction fragment length variation between *M. spretus* and C57BL/6J DNA digested with *Eco*RI. The *M. spretus* fragment was approximately 4.0 kb, while the C57BL/6J fragment was approximately 3.7 kb.
Previously identified homologs: The human natriuretic peptide receptor 3 gene (NPR3) was mapped to Chr 5p14-p13 [4]. The human natriuretic peptide receptor 1 gene (NPR1) is on Chromosome (Chr) 1 [4], while the mouse *Npr1* gene is on Chr 3 [5]. We have mapped *Npr1* to mouse Chr 3 in The Jackson Laboratory BSS Backcross panel (S. John, unpublished).
Discussion: The natriuretic peptide family consists of three related peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), that are intimately involved in fluid pressure and volume homeostasis [6]. These peptide hormones are best known for their cardiovascular and renal actions, lowering blood pressure, and promoting natriuresis. Three natriuretic peptide receptors (NPRs) have been identified (NPR1, NPR2, and NPR3) that have different ligand affinities. NPR1 and NPR2 have a guanylate cyclase domain that is activated by natriuretic peptide binding. NPR3 is best known for its clearance function, and may also interact with G-proteins to inhibit adenylate cyclase or to activate the phosphoinositol pathway [6,7].

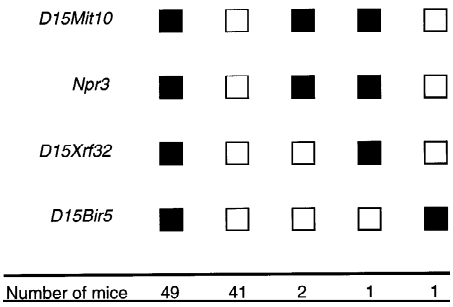

Fig. 1. Localization of *Npr3* to proximal Chr 15. The marker *D15Mit10* has no recombinants with *Npr3*. Other markers having no recombinants with *Npr3* but not shown include *D15Mit13, D15Bir2,* and *D15Bir3.* The filled boxes indicate the presence of both C57BL/6J and SPRET/Ei alleles, while the open boxes indicate the presence of only SPRET/Ei alleles. The number of offspring carrying each type of chromosome is indicated at the bottom. Complete raw data for the Jackson BSS backcross are available on The World Wide Web at the URL address http://www.jax.org/resources/documents/cmdata.

In this study, we used a probe specific to the mouse *Npr3* gene to follow allelic segregation in an interspecific backcross panel. Comparison of the allele distribution pattern to those of other loci mapped in this cross located *Npr3* to proximal Chr 15. Map Manager [8] was used to determine linkage, to calculate marker distances, and to draw haplotypes. The most likely marker order was determined by inspection of the data and minimization of double recombinants (Fig. 1). No recombinants were found between *Npr3* and the most proximal markers (for example, *D15Mit10, D15Mit13,* and *D15Bir3*) previously mapped on this backcross panel. Two recombinants were found between *Npr3* and *D15Xrf32.* These data place *Npr3* 2.1 centiMorgans proximal to *D15Xrf32.*

The human NPR3 gene has been mapped to Chr 5p14-p13 [4]. The present study indicates that the mouse *Npr3* gene maps to proximal Chr 15. Mouse proximal Chr 15 contains other genes that map to human Chr 5p, such as the genes for the interleukin-7 receptor, the leukemia inhibitory factor receptor, and growth hormone [9]. This study, therefore, extends the homology between mouse Chr 15 and human Chr 5.

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Localization of ubiquitin gene family members to mouse Chromosomes 5, 11, and 18

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Species: Mouse
Locus names: Ubiquitin B, Ubiquitin C, and Ubiquitin C-related sequence 1
Locus symbols: *Ubb, Ubc,* and *Ubc-rs1*
Map positions: *Ubb* is localized on mouse Chr 11, *Ubc* on Chr 5, and *Ubc-rs1* on Chr 18 (see Fig. 1)
Method of mapping: Linkage analysis with a panel of 67 DNA samples from a [(C57BL/6J × *Mus spretus*)F_1 × C57BL/6J] backcross [1]. This backcross has been typed for more than 350 markers, and linkage was detected with Map Manager v.2.6.5 [2].
Database deposit information: MGD accession number MGD-JNUM-36975.
Molecular reagents: An expressed sequence tag was isolated in a screen for mRNAs expressed at altered levels in the liver of neonatal BALB/cByJ-*fld* mice (M. Klingenspor and K. Reue, unpublished). From comparisons with GenBank sequences, the probe was identified as a portion of the cDNA encoding ubiquitin C, having 90% nucleotide identity with the human polyubiquitin C nucleotide sequence (GenBank accession #D63791, nucleotides 5313–5437).
Allele detection: RFLVs between C57BL/6J and *Mus spretus* were identified with *Hin*dIII. Variant alleles of 3.2 kb (*Ubb*), 10.5 kb (*Ubc*), and 2.7 kb (*Ubc-rs1*) were scored in *Mus spretus.*
Previously identified homologs: UBB, the human homolog of *Ubb,* maps to Chr 17p12-p11.1 [3]. UBC, the human homolog of *Ubc,* maps to Chr 12q24.3 [4].
Discussion: Ubiquitin is present in all eucaryotic cells and has a critical function in targeting proteins for intracellular proteolytic degradation (reviewed in [5]). Whereas proteins entering the cell from outside are degraded in lysosomes, the selective turnover of intracellular proteins occurs largely through a nonlysosomal proteolytic pathway in which proteins are tagged by covalent linkage of ubiquitin molecules to lysine amino groups of proteins destined for degradation. The ubiquitin-tagged proteins are subsequently targeted to a protease complex and rapidly degraded. Recent evidence indicates that modification of proteins by ubiquitin may also play a role in protein activation through proteolytic processing to remove a precursor domain, or through selective degradation of inhibitors to liberate an active protein from a complex (reviewed in [6]).

The 76 amino acid ubiquitin molecule is synthesized as a precursor containing tandemly repeated ubiquitin subunits. Human and mouse cells express multiple ubiquitin gene transcripts that have been classified into three families based on size and sequence [7,8]. The UbA family of gene transcripts encode a single ubiquitin subunit fused with ribosomal proteins; the UbB and UbC families of transcripts encode a variable number of tandem repeats of the ubiquitin coding unit. We have isolated an expressed sequence tag from mouse liver having 90% identity to a portion of the human ubiquitin C cDNA, and which hybridizes to multiple mouse liver mRNA transcripts of 6.4, 5.0, 1.7, and 1.2 kb (data not shown). The 6.4-kb transcript is identical in size to one of the transcripts previously reported for mouse UbC, whereas the 1.7-kb and 1.2-kb transcript have previously been detected for mouse UbB [8]. The 5.0-Kb transcript does not correspond to previously reported

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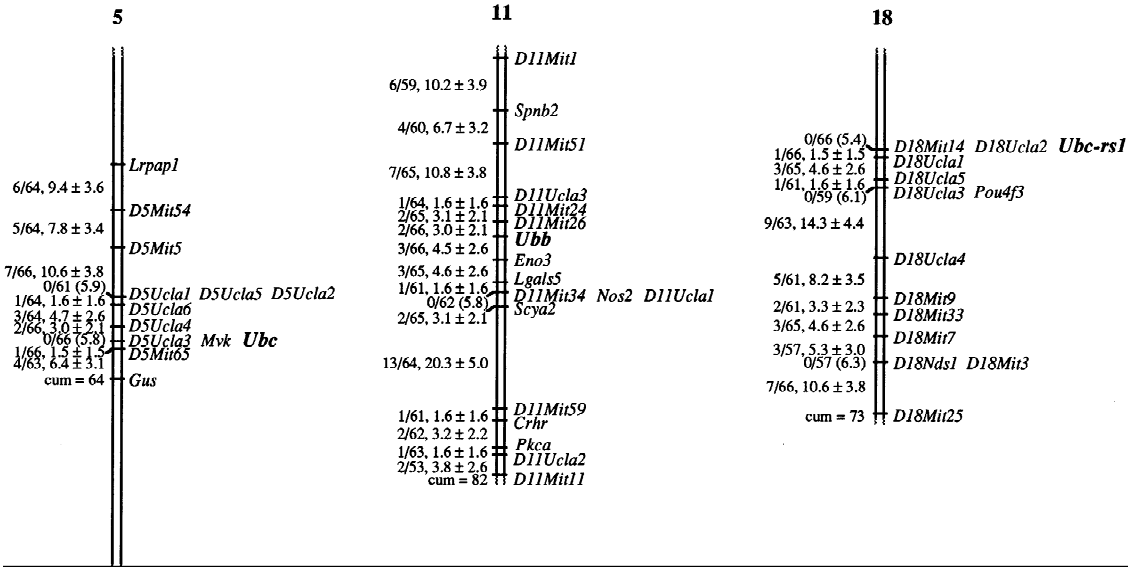

Fig. 1. Mapping of ubiquitin gene family members to Chrs 5, 11, and 18 in an interspecific mouse backcross. Chromosomes are drawn to scale with the ratios of the number of recombinants to the total number of informative mice, as well as the recombination frequencies ± standard errors (in cM), indicated to the left. Numbers in parentheses represent the upper 95% confidence interval for pairs of loci that cosegregate. For each chromosome, the cumulative (cum) distance (in cM) of the most distal locus from the centromere is indicated to the left of the corresponding locus. Loci are linked with lod scores >5.0. *Ucla* markers are reported in Warden and associates [1] or are unpublished data. The Chr 11 marker *Eno3* (enolase 3) is an unpublished locus, and specific information can be obtained from the authors. References for other linked loci can be obtained from the Mouse Genome Database [10].

mouse ubiquitin mRNA species and may represent an additional member of the gene family (see below).

Using the mouse ubiquitin sequence tag, we detected three loci in the mouse genome and followed the segregation of RFLVs for these loci in an interspecific mouse backcross panel. On the basis of mRNA transcript sizes and available mouse–human chromosome homology data, two of the loci most probably correspond to *Ubc* [4] (Chr 5) and *Ubb* [9] (Chr 11). The third locus (Chr 18), which does not correspond to any previously mapped mouse or human ubiquitin genes, has been designated *Ubc-rs1*.

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Sur2 and *Kcnj8* genes are tightly linked on the distal region of mouse Chromosome 6

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Species: Mouse
Locus name: ATP-sensitive K^+ channel subunits, SUR2 and Kir6.1
Locus symbols: *Sur2* and *Kcnj8*
Map position: Chromosome (Chr) 6: Centromere—*Gucy2c*–2.5 ± 1.4–[*Sur2, Kcnj8, Lmo3*]–1.6 ± 1.2–*Kras2* (Fig. 1).
Method of mapping: Interspecific backcross analysis with progeny derived from matings of [(C57BL/6J × *Mus spretus*) F_1 × C57BL/6J] mice [1].
Molecular reagents: A 1.7-kb *Sac*I fragment of mouse SUR2B (*Sur2*) [2] and a 1.7-kb *Eco*RI/*Xho*I fragment containing the entire coding region of mouse Kir6.1 (*Kcnj8*) [3] were used as probes to determine the chromosomal locations. Probes for the genes linked to *Sur2* and *Kcnj8* have been reported previously [4,5].
Allele detection: The *Sur2* probe detected fragments of 19.0, 3.2, 2.2, and 1.5 kb in *Bam*HI-digested C57/6J DNA and fragments of 9.3, 6.8, 5.1, 3.2, 2.2, and 1.5 kb in *Bam*HI-digested *M. spretus* DNA. The three *M. spretus*-specific *Bam*HI fragments, which cosegregated, were followed in backcross mice.

The *Kcnj8* probe detected major *Pvu*II fragments of 3.8 and 3.0 kb in C57BL/6J DNA and 5.0 and 3.8 kb in *M. spretus* DNA. The presence or absence of the 5.0-kb *Pvu*II *M. spretus*-specific fragment was followed in backcross mice.
Previously identified homologs: The human homologs of *Sur2* and *Kcnj8* have been mapped by FISH to Chr 12p12.1 [6] and 12p11.23 [7], respectively.
Discussion: The ATP-sensitive K^+ (K_{ATP}) channels are inhibited by intracellular ATP and activated by intracellular nucleoside diphosphates, which are the targets of the antidiabetic sulfonylureas and several vasorelaxant drugs, called K^+ channel openers. These channels have been identified in a variety of tissues and participate in diverse functions, such as insulin secretion from pancreatic β-cells, cardioprotection in ischemic preconditioning, smooth muscle relaxation, regulation of skeletal muscle contraction, and neurotransmitter release [reviewed in 8].

K_{ATP} channels are heteromeric proteins composed of at least two subunits, sulfonylurea receptor (SUR) and two-transmembrane type inwardly rectifying K^+ (Kir) channel. SUR2 is believed to have three variants by alternative splicing [2,6,9]. One of them, SUR2B, was shown to form K_{ATP} channel of smooth muscle with Kir6.1 [3].

Human SUR2 and Kir6.1 genes (*Sur2* and *Kcnj8*) were mapped to 12p12.1 and 12p11.23, respectively [6,7]. In this study, we determined that the mouse chromosomal locations of *Sur2* and *Kcnj8* were tightly linked in the distal region of mouse Chr 6, corresponding with human Chr 12p [10]. No obvious candidate mouse mutations in the vicinity of *Sur2* and *Kir6.1* have been found.

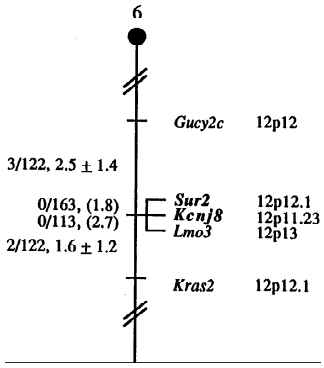

Fig. 1. *Sur2* and *Kcnj8* map to the distal region of mouse Chr 6. The genes were mapped by interspecific backcross analysis. The linkage map shows the location of *Sur2* and *Kcnj8* in relation to linked genes. The number of N2 animals is presented over the total number of N2 animals typed to the left of the chromosome map between each pair of loci. The recombination frequencies expressed as genetic distance in cM (± one standard error) are also shown. The upper 95% confidence limit of the recombination distance is given in parentheses when no recombinants were found between loci. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution pattern. The positions of the human homologous loci are shown to the right of the chromosome. References for the map positions of the human loci can be obtained from GDB (Genome Data Base), a computerized database of human linkage information maintained by the William H. Welch Medical Library of The Johns Hopkins University (Baltimore, Md.).

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Strain-specific deletions in exon 10 of rat K-kininogen and T1-kininogen genes allow mapping of both genes to rat Chromosome 11

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Species: *Rattus norvegicus*
Locus names: K-kininogen, T1-kininogen
Locus symbols: *Kngk, Kngt1, D11Elh1*[1]
Chromosomal location: Chromosome 11
Database deposit information: RATMAP
Molecular reagents: Forward primer 5′-GAGAGGGATCCAGGAAATGAACAA-3′ (KKin+) and reverse primer 5′-CCTCTGTCCTCCCCTGTATCTGTG-3′ (KKin−) corresponding to bases 249–272 and 610–633 of RATKINKG, rat high molecular weight (HMW), and low molecular weight (LMW) K-kininogen genes, 3′ end; Genbank Accession number: M14369 [1] were synthesized. The primers simultaneously amplify part of the histidine-rich coding region of K-kininogen exon 10 and a highly homologous region of rat T1-kininogen; Genbank Accession number M14370 [1]. Forward primer 5′-GGGTCCCATAGTGACAAACGA-3′ (Kex4+) and reverse primer 5′-TCCGAGAGCAAAGAGGTG-3′ (Kex4−) were synthesized to amplify a 145-bp fragment surrounding base 504 of RATKINKH, HMW K-kininogen mRNA complete cds; Genbank Accession number: L29428.
Allele detection: PCR amplification of the exon 10 fragments was carried out with genomic DNA from rats of the New Zealand genetically hypertensive (GH), brown Norway (BN), spontaneously hypertensive (SHR), spontaneously hypertensive stroke prone (SHRSP), Wistar-Kyoto (WKY), Donryu (DRY), albino surgery (AS), AS2, inbred Dahl salt-sensitive (SS/Jr), inbred Dahl salt-resistant (SR/Jr), Buffalo, and Lewis strains. KKin+/KKin− products were analyzed by electrophoresis in 0.5 × TBE on 3% Metaphor gels [2]. Fragments of the predicted approximate sizes of 385 bp and 463 bp for K- and T1-kininogen respectively were amplified from all the strains except DRY, where smaller than expected products were observed for both K- and T1-kininogen. Direct sequencing of the purified PCR product of DRY K-kininogen demonstrated a 6-bp deletion of bases 507–512, numbering as for M14369. The following point mutations were also observed in DRY; base 287 G→C, base 318 A→G, and base 598 A→G. Direct sequencing of the purified $KKin^{+}/KKin^{-}$ T1-kininogen product from DRY revealed a 42-bp deletion of bases 501–542 of T1-kininogen, M14370, and further changes of CA→TG at bases 352–353 and GGG→TGA at bases 573–575. The later change introduces a *Bcl*I cutting site, which was experimentally confirmed.
Sequence analysis of K-kininogen Kex4+/Kex4− fragments from GH, BN, SHR, SHRSP, WKY, DRY and SS/Jr found complete concordance with the Kitazato BN K-kininogen gene [3], demonstrating that none of these strains carries the G→A point mutation which renders the Katholiek BN strain kininogen deficient [4].
A simple sequence repeat in a T-kininogen gene sequence, Genbank Accession number: M29087 [5], previously used to map T-kininogen to rat Chromosome (Chr) 11 [6], did not exhibit any polymorphisms in the strains tested: GH, BN, SHR, WKY, SS/Jr, SR/Jr, and DRY.
Mapping: The K- and T1-kininogen genes mapped to a position between *D11Mgh1* (vCATOMT) and *D11Mgh3* (SATELLA) on Chr 11 with 46 F_2 outlier progeny [7] from an intercross between SHR and DRY [8]. By the Kosambi function there was 3.5 cM between *D11Mgh1* and the kininogen genes and 8.0 cM between the kininogen genes and *D11Mgh3*. The K- and T1-kininogen genes were genetically inseparable in 155 F_2 rats, and no cosegregation could be detected with any phenotype (mean arterial blood pressure, left and right ventricular weight, kidney weight, and body weight) that had been measured for this cross. The genotyping data are available in RATMAP.
Discussion: Kininogens are pluripotent macromolecules that generate kinins such as bradykinin and serve as cysteine protease inhibitors [9]. The high molecular weight form of kininogen is required for the initiation of the intrinsic blood clotting pathway and adhesion to anionic surfaces [10]. Bradykinin is a potent vasodilator, and recently differences in the kinin metabolism in rats of the SHR and DRY strains have been reported [11], thus making the kininogen genes candidates for hypertension.

One gene encodes K-kininogen, and three or more genes that have been found only in the rat encode the T-kininogens [1,5,12]. Differential splicing of exons 10 and 11 of the K-kininogen transcripts gives rise to the low-molecular-weight and high-molecular-weight forms of these proteins [13,14]. Sequence changes at or near the splice sites in the T-kininogen genes create impediments to the alternative splicing of the HMW form of T-kininogen that result in production of only the LMW form of the T-kininogens [13,14].

With access to the F_2 generation of an SHR × DRY cross, it was possible to use the DRY deletion polymorphisms in the K- and T1-kininogen genes to map the chromosomal location of K-kininogen for the first time in the rat and to confirm the mapping of T-kininogen to rat Chr 11 [6]. The mapping of these genes in a cross between a hypertensive and a normotensive strain also made it possible to look for cosegregation of kininogen alleles with blood pressure and other cardiovascular parameters [8]. The linkage of the two genes suggests that they have developed from a

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[1]The nucleotide sequence data reported in this paper have been assigned the GenBank accession numbers AF003623 for the 379 bp fragment of the DRY *Kngk* gene AF003624 for the 421 bp fragment of the DRY *Kngl1* gene. Locus names and mapping information have been submitted to the Rat Genetic Nomenclature Committee and RATMAP respectively.

gene duplication as hypothesized by Nakanishi [1]. Our mapping of K-kininogen in the rat is consistent with the mapping of kininogen to human Chr 3q27 [15] in a conserved synteny group homologous to a group on rat Chr 11 [16] and previous results mapping a T-kininogen gene in the rat by linkage analysis [7]. The failure to detect any cosegregation with any phenotype available is consistent with the observation that no QTL influencing blood pressure has been mapped to rat Chr 11 in a total of 5 crosses involving three different hypertensive strains that have been subjected to genome scans [17]. These results suggest that altered kininogen genes are unlikely to be causative for hypertension in these rat models.

The 42-bp deletion in the DRY T1-kininogen gene is in the silent exon 10 region of T-kininogen and therefore will probably not impact on the production of T-kininogen in this strain. The CA→TG mutation in the T1-fragment of the DRY strain corresponds to a similar change between T1-kininogen and T2-kininogen in the Kitazato BN strain [1].

The 6-bp deletion of bases 507–512 in the K-kininogen fragment of DRY deletes a histidine–glycine from the important histidine-rich region of HMW K-kininogen, a region involved in the binding of HMW K-kininogen to endothelial cells, neutrophils, and platelets. Furthermore, the G→C base change detected in DRY K-kininogen corresponds exactly to a sequence change in T-kininogen, which has been shown to lower the ratio of HMW: LMW transcript in a set of chimeric kininogen genes transfected into heterologous COS cells [13,14]. These results suggest that the DRY strain will make an extremely interesting model for the study of expression of the kininogen genes.

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Assignment of the gene encoding the serotonin $5HT_{1B}$ receptor to rat Chromosome 8q31 by fluorescence in situ hybridization

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Species: Rat
Locus name: Serotonin (or 5-hydroxytryptamine (5HT)) receptor, type 1B
Locus symbol: *Htr1b*
Map position: Chromosome (Chr) 8, band q31
Method of mapping: Fluorescence in situ hybridization (FISH), essentially as described by Pinkel et al. [1] with minor modifications [2].
Molecular reagents: A 4.2-kb genomic DNA fragment from rat $5HT_{1B}$ receptor, cloned in pBluescript SK+ vector, was used as a probe and labeled by biotin-14-dATP with the BioNick labeling kit (GibcoBRL). The position of the probes was visualized with a Zeiss Axiophot microscope, with an antifade mounting medium containing propidium iodide (as counterstain) and DAPI (for chromosome identification).
Previously identified homologs: The human homolog is the gene encoding the $5HT_{1D\beta}$ receptor, mapping at 6q14.3-16.3 [3]. The mouse homolog maps at 9E [4].
Discussion: Serotonin (5HT) is a neuromodulator that is involved in various functions such as sleep, appetite, pain perception, and vascular contraction, and in several pathologies such as depression, anxiety, and alcoholism. The multiple actions of serotonin are mediated by the specific interaction of this amine with numerous receptors, classified on the basis of their pharmacological profile, their physiological effects, and more recently their molecular structure [5].

The $5HT_{1B}$ receptor, the rodent homolog of the human $5HT_{1D\beta}$ receptor, has been involved in food intake, sexual activity, locomotion, and more recently in aggressiveness and alcohol consumption by the use of mice lacking $5HT_{1B}$ receptor [6,7]. The receptor has a seven-transmembrane-domain structure and belongs to the large family of receptors that interact with G protein and are negatively coupled to adenylate cyclase [5]. The gene encoding the $5HT_{1B/1D\beta}$ receptor is an intronless gene, like other members of the $5HT_1$ family, which has been cloned in the rat [8], in the mouse [9], and in human [10,11].

Double spots (two labeled sister chromatids) were found on Chr 8 only, at 65–75% of the chromosome length, that is, in the upper part of band q31.

Rat Chr 8 is highly homologous to mouse Chr 9 and partially to human Chr 6 [12]. The mouse Chr 9 region homologous to human Chr 6 is in band E [4], and our result thus defines a rat corresponding region.

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Prolactin receptor maps to pig Chromosome 16

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Species: Pig
Locus name: Prolactin receptor
Locus symbol: *PRLR*
Map position: The *PRLR* locus was closely linked to three markers that are mapped to pig Chromosome (Chr) 16 of the published PiGMaP linkage map (LOD score in parentheses): *S0006* (10.29), *GHR1* (6.35), *S0077* (3.23). A multiple point analysis was done to produce a best Chr 16 map (Fig. 1) involving all linked markers. The order and recombination fraction of these loci are *PRLR*–9.9–*S0006*–14.2–*GHR1*–0–*S0077* (log likelihood = −34.55). *PRLR* was also physically localized to pig Chr 16 with a probability of 0.94, with regional assignment to 16q1.4 or 16q2.2-2.3.
Method of mapping: PCR-RFLP analysis of the PiGMaP reference family [1] and somatic cell hybrid panel [2].
Database deposit information: GenBank accession number U96306
Molecular reagents: Human [3] and rabbit [4] cDNA sequences were used to design degenerate primers overlapping the 3′ coding and untranslated region. The primers amplified a fragment of approximately 500 base pairs in pig genomic DNA samples and a human control. The forward primer 5′-TCA CAA GGT CAA C/TAA AGA TG-3′ and the reverse primer 5′-TGG/A AGA AAG/A AGG CAA G/ATG GT-3′ were used in the following PCR conditions: 93°C for 3 min, 6 cycles of 93°C 30 s, 47°C 2 min, 72°C 3 min, 36 cycles of 93°C 30 s, 53°C 2 min, 72°C 5 min, and a final 72°C 5 min. The *Taq* polymerase was added last while samples were held at 80°C. Fragments from two animals were purified and sequenced in forward and reverse directions. The pig sequence from the coding region was conceptually translated to amino acids and compared with known sequences. A database search reported the rabbit and human *PRLR* sequences as the two best matches, with 74% and 67% identity respectively. From the pig DNA sequence, primers (forward primer 5′-CCC AAA ACA GCA GGA GAA CG-3′ and the reverse primer 5′-GGC AAG TGG TTG AAA ATG GA-3′) were designed to amplify a 457-bp fragment in the following PCR conditions: 93°C for 3 min, and 35 cycles of 93°C 30 s, 60°C 1 min, 72°C 1 min, and a final 72°C 3 min. The *Taq* Polymerase was added last while samples were held at 80°C. The pig-specific primers were used in the above conditions to amplify DNA from a pig × rodent somatic cell hybrid panel [2]. Hybrids 2, 4, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, and 19 showed positive signals for *PRLR.*

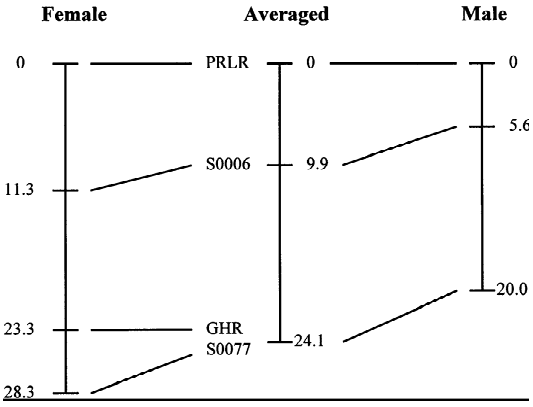

Fig. 1. Position of *PRLR* in pig Chr 16. Multiple-point linkage was done with CriMap to produce a sex-averaged best map, with a LOD score of 3 or greater being considered as significant.

Allele detection: A polymorphism was detected in the PCR fragment when digested with the restriction endonuclease *Alu*I. Resolution of the bands was obtained with a 6% NuSieve (FMC) agarose gel at 120 volts for 4 h at room temperature. Gels were stained with ethidium bromide. The fragment sizes observed were approximately 124, 110, 79, 77, and 67 base pairs with the polymorphic site being located in the 110-bp fragment. When the polymorphic *Alu*I site was present, a 90 bp fragment was produced.
Previously identified homologs: PRLR has been mapped to human Chr 5p13-5p14 [5], Chr 15 in the mouse [6], and Chr 2 in the rat [6].
Discussion: Prolactin (PRL) is an anterior pituitary peptide hormone involved in many different endocrine activities and is essential for reproductive success. This action is mediated by its receptor (PRLR) [6]. Samples from seven breeds of pig were genotyped with the *Alu*I polymorphism. The allele frequencies for the A allele are as follows: Chester White (n = 10): .25; Duroc (n = 10): .79; Hampshire (n = 11): .05; Landrace (n = 9): .72; Yorkshire (n = 12): .37; Chinese Meishan (n = 9): .56; and European Large White (n = 11): .32. It appears that some breed differences exist for gene frequencies at *PRLR.* The linkage and physical mapping results placed *PRLR* on pig Chr 16. The two discordant positive hybrids (8 and 18) were also found to be positive for two microsatellite markers, *SW81* and *SW419,* both physically and linkage mapped to chromosome 16 [8]. This is further evidence that a portion of this chromosome is present in at least a fraction of hybrid 8 and 18 cells and is detectable by PCR. The mapping of

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PRLR adds a Type I locus to the PiGMaP genetic linkage map and increases the synteny of this region with human 5p. The allele frequency differences among breeds suggest that one allele may have been selected for in some populations and against in others and deserves further investigation into the significance of these differences and the traits they may affect.

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The bovine glutamine synthase gene (*GLUL*) maps to 10q33 and a pseudogene (*GLULP*) to 16q21

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Species: Cattle (*Bos taurus*)

Locus name: Glutamate-ammonia ligase (glutamine synthase) and pseudogene
Locus symbols: *GLUL, GLULP* (alias *GLNS, GLNSP*)
Map positions: 10q33 (*GLUL*) and 16q21 (*GLULP*)
Method of mapping: Fluorescence in situ hybridization (FISH)
Database deposit information: EMBL accession numbers Y10347 (GLUL) and Y10348 (GLULP)
Molecular reagents: A forward (5′-GTCCATATTACTGTGGTGTGGGAG-3′) and a reverse primer (5′-TCTCCTCCCGCATGGCCTTGGTGC-3′) were synthesized based on the human glutamate-ammonia ligase sequence after comparison of the rat and human sequences. The primers were designed to amplify a 317-bp fragment corresponding to bases 595–912 of the human cDNA (GenBank accession number Y00387) and bases 607–924 of the rat cDNA (GenBank accession number X07921). The PCR reactions were carried out in a Hybaid thermocycler in a volume of 25 μl containing 100 ng of template DNA, 10 pmol of each primer, each dNTP at 50 μM, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 2.5 units Taq polymerase (Boehringer). Cycling conditions were 5 min at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 65°C, and 30 s at 72°C. PCR from bovine genomic DNA yielded a 500-bp fragment in addition to a fragment of similar size as the expected 317-bp fragment amplified from human genomic DNA. A bovine genomic library constructed in SuperCos1 (Stratagene) and consisting of more than 300,000 independent clones was screened with the smaller PCR-fragment. Of four cosmid clones isolated, two contained the smaller (ca. 317 bp) and two the larger fragment (ca. 500 bp) as shown by PCR with the above-mentioned primers. A subclone of one of the 500-bp fragment- and a subclone of one of the 317-bp fragment-containing cosmids was sequenced and the resulting sequence information deposited (EMBL accession numbers Y10347 and Y10348). Comparison of the two bovine sequences revealed the deletion of a 198-bp stretch in the smaller fragment and 97% identity in the flanking regions. Comparison with the human sequence suggests the smaller fragment is derived from a processed pseudogene, since the 198-bp deletion corresponds in size approximately to the intron between exon 5 and exon 6 of the rat glutamate-ammonia ligase sequence (GenBank accession M29594 and M28542). Rat exons 5 and 6 are 85% and 89% similar, respectively, to the bovine exons flanking the 198-bp intron. One cosmid containing the 317-bp fragment and one cosmid containing the 500-bp fragment were used for fluorescence in situ hybridization mapping as described previously [1]. Specific signals were detected on bovine Chromosome (Chr) (BTA) 10q33 (500-bp fragment-containing cosmid, locus *GLUL*) and 16q21 (317-bp fragment-containing cosmid, locus *GLULP*) (Fig. 1).
Previously identified homologs: GLUL and GLULP have been assigned to human Chr (HSA)1q25 and 9p13, respectively, and three related loci (GLULL1, GLULL2, GLULL3) on human Chr HSA 5q33, 11p15, and 11q24 [2]. In the mouse, a pseudogene (*Glns-ps1*) is on Chr 11 [3].
Discussion: Glutamine synthase plays a key role in the elimination of free ammonia and converts the neurotransmitter and excitotoxic amino acid glutamate to glutamine, which is not neurotoxic. Mutations in GLUL may therefore be the basis of neurodegenerative disorders. The location of the functional gene on BTA10q33 conflicts with findings from comparative mapping, since the functional GLUL has been assigned to HSA1q25, a region that has been shown to correspond to BTA16 [4]. Interestingly, the bovine pseudogene has been assigned to BTA16. A possible explanation, if we have correctly assigned the functional *GLUL* gene to BTA 10 and a pseudogene to BTA 16, is that the locus at HSA1q25 may contain a processed pseudogene rather than the functional gene.

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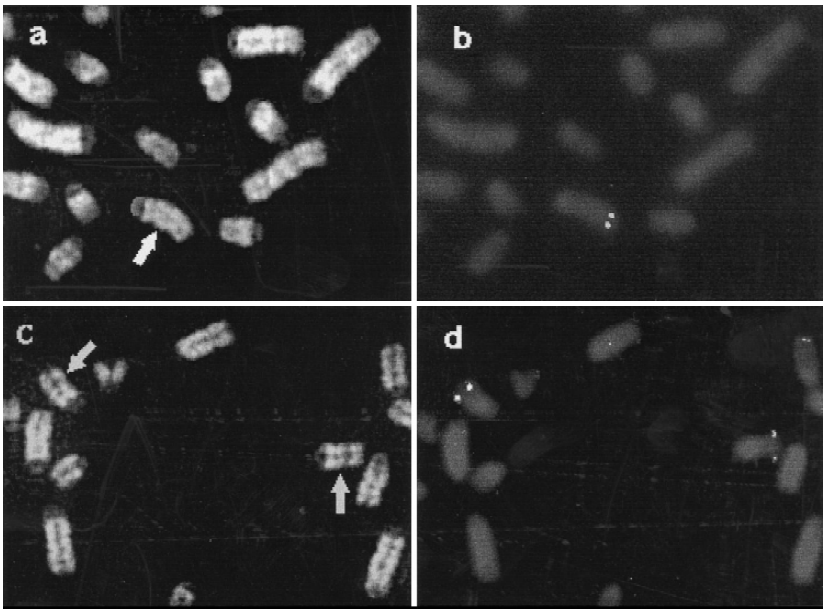

Fig. 1. Partial bovine metaphase spreads before (a, c; QFQ-banded) and after (b, d; DAPI-counterstaining) hybridization with a *GLUL* (b) and a *GLULP* (d) containing cosmid. The arrows indicate Chrs 10 (a) and 16 (c).

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Fine scale mapping places DLG1, the gene encoding hDlg, telomeric to the OPA1 candidate region

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Species: Human
Locus name: Discs large tumor suppressor gene
Locus symbol: DLG1
Map position: DLG1 is located on human Chromosome (Chr) 3q28-q29. The order of markers is as follows CEN–D3S2344–D3S1265–D3S1311–D3S1272–D3S3707–**DLG1**–D3S3326–D3S3550 (Fig. 1).
Method of mapping: The GeneBridge 4 radiation hybrid mapping panel (Research Genetics, Inc., Huntsville, AL) was screened by PCR with two different primer pairs specific for DLG1. The CEPH Mega Yac library was screened with microsatellites situated at the OPA1 locus.
Database deposit information: The cDNA sequences for hDlg (DLG1) have been deposited in the GenBank data base under accession numbers U13896 and U13897.
Molecular reagents used for mapping: A 5-kb upstream genomic fragment (5′-probe) and an 11-kb downstream fragment (3′-probe) of the DLG1 locus.
Previously identified homologs: Drosophila discs large tumor suppressor, *dlg* [1].
Discussion: Dominant optic atrophy (DOA), type Kjer, is an inherited disease of the optic nerve and is the most common of all hereditary optic atrophies [2]. It shows autosomal dominant inheritance with nearly complete penetrance, but the disease shows variable severity, with some patients showing no symptoms and others being legally blind [3].

The gene for DOA, type Kjer (designated OPA1), is located at 3q28-q29 in the region between D3S3669 and D3S3562 [4]. The hDlg locus, DLG1, has also been mapped to 3q29 [1] and appeared to be an attractive candidate gene for OPA1. DLG1 encodes a membrane-associated guanylate kinase homolog (MAGUK), belonging to a group of highly related proteins important in controlling epithelial and neuronal cell junctions. Several MAGUKs, including rat PSD95, rat Chapsyn110, and Drosophila *Dlg*, have critical functions in the structural organization and function of neuronal synapses [5,6] suggesting that the same could be true of hDlg. Furthermore, the PDZ2 domain of rat PSD95 and human Chapsyn110 interact with the cytoplasmic tail of N-methyl-D-aspartate (NMDA) receptors [7,8]. Since the primary structure of the PDZ2 domain of hDlg is virtually identical to that of rat PSD95 and human Chapsyn 110, hDlg may also bind to NMDA receptors. The expression of NMDA receptors in retinal cells [9] is consistent with the idea that abnormalities of hDlg-NMDA receptor interactions could be responsible for DOA, type Kjer.

We mapped DLG1 by screening the GeneBridge 4 radiation hybrid mapping panel (Research Genetics, Inc.), using two different primer pairs specific for DLG1. Data from two independent PCR screenings for each primer pair were scored against the STS marker database from the Whitehead Institute for Biomedical Research/MIT Center for Genome Research, by use of the statistical program RHMAP. The data confirmed the location of DLG1 at chromosomal region 3q29, and more precisely placed the locus between markers D3S2344 and D3S3550 (Fig. 1). Further analysis using the databases of the Genome Database (gdb), the Cooperative Human Linkage Center (CHLC) and the Genetic Location

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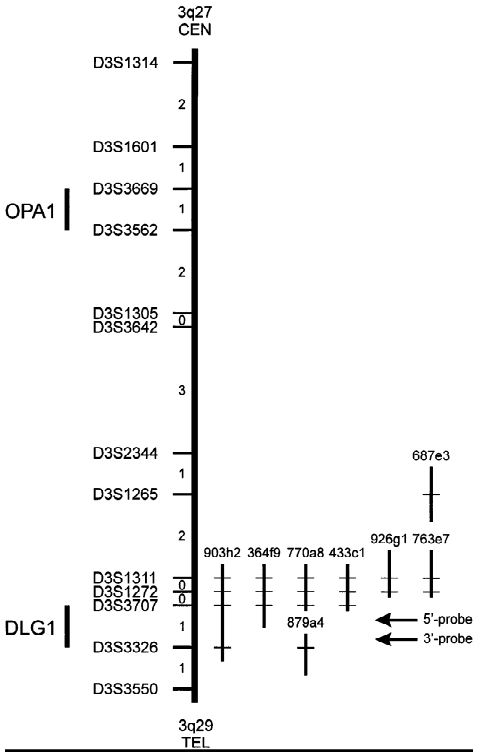

Fig. 1. The genetic organization and YAC contig of the DLG1 locus. Microsatellite markers for this approximate 14 cM region are indicated with genetic distance between markers given in cM. The physical locations of YACs mapped in this study are indicated to the right of the marker map by thick vertical bars, with positive PCR amplification for markers on YACs shown by thin horizontal bars. The regions containing the OPA1 candidate gene and DLG1 are indicated to the left of the marker map.

Database (LDB) identified additional microsatellite markers that mapped within this region. The order of these markers is as follows CEN–D3S2344–D3S1265–D3S1311–D3S1272–D3S3707–**DLG1**–D3S3326–D3S3550 (Fig. 1).

To confirm the order of these markers in relation to DLG1 and determine their location with respect to the OPA1 locus, we screened for CEPH Mega YACs which contained DLG1. Purified DNA from each YAC clone (5.0 μg) was spotted on membranes that were then hybridized with radiolabeled probes derived from a 5-kb upstream genomic fragment (5′-probe) and an 11.0 kb downstream genomic fragment (3′-probe) of the DLG1 locus. Both probes hybridized to the 903-h-2 YAC, and PCR screening of this YAC resulted in positive amplification using D3S1311, D3S1272, D3S3707, and D3S3326 (Fig. 1). Additionally, the 5′-probe detected a second YAC clone (364-f-9), which was positive for D3S1311, D3S1272, and D3S3707, and the 3′-probe identified a third YAC clone (879-a-4), which was positive for D3S3326 (Fig. 1). Five additional YACs (770-a-8, 433-c-1, 926-g-1, 763-e-7, and 687-e-3) were negative with respect to DLG1. The analysis confirms the marker order, places DLG1 between D3S3707 and D3S3326, and indicates DLG1 is transcribed in a proximal-to-distal direction.

The fine scale mapping of DLG1 between D3S3707 and D3S3326 places it telomeric to the OPA1 candidate region, whose most telomeric flanking marker is D3S3562 [4]. Therefore, DLG1 is not a candidate gene for DOA, type Kjer. A more refined map of the region containing the DLG1 locus will be essential in defining the involvement of hDlg in other human diseases. Markers D3S1311, D3S1272, D3S3707, D3S3326, and D3S3550 span a region of approximately 2 cM and should be useful in PCR-based genotyping assays with respect to DLG1. In addition, the development of a YAC contig for this region will be useful in determining the complete genomic structure of DLG1.

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Murine *D17H6S45* (Rd) gene: polymorphism and overlap with complement factor B

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Species: Mouse
Locus name: Anonymous segment *D17H6S45*
Locus symbol: *D17H6S45*
Map position: Chromosome (Chr) 17, 18.84cM (from MGD at Jackson Laboratory).
Database deposit information: The sequences reported in this paper have been submitted to GenBank and have been assigned the accession numbers: U82693, U83841, and U83842.
Method of analysis and molecular reagents: DNA from 38 strains of inbred mice were used in this study, 35 of which were obtained from The Jackson Laboratory mouse DNA resource, and the remainder were extracted from our own breeding colonies. The repeat region of exon 7 was amplified by PCR from genomic DNA with a primer from the 5′ end of exon 7 (mRd/7+: 5′-GAGCTATGAAGAGCATGGTAGCGC-3′), corresponding to nucleotide positions 643 to 666 of the cDNA [1], and the 5′ end of intron 7 (mRd/7i−: 5′-TAAACCCTGGCTAAGATCACTCAC-3′), derived from our own unpublished sequence data. The primer mRd/7+ was 5′ labeled with the fluorescent dye 6-FAM. PCR products were sized on a Perkin Elmer ABI 377 DNA sequencer with respect to the size standard GS-350 (ABI, Inc., Foster City, Calif.).
Allele detection: VNTR analysis. PCR product sizes are 274bp, 268bp, and 250bp.
Previously identified homologs: Human homolog is D6S45, mapped to 6p21.3.
Discussion: The *D17H6S45* gene (formerly known as Rd) encodes

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The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession numbers: U82693, U83841, U83842.

a protein that contains a very unusual region of precise repeats of the dipeptide RD (arginine-aspartic acid). Comparison to the human D6S45 cDNA highlighted a high level of evolutionary conservation of the amino acid sequence between the two species [2,3], which indicates a strong selective pressure to maintain this unusual repeat structure. Currently there is no known function of the RD protein. Sequence similarity has been detected between the RD protein and U1 snRNP and *tra-2*, both of which are proteins involved in splicing [2]. The localization of a family of RD proteins to the spliceosomal complexes [4] is also suggestive of a role for RD in mRNA splicing. The strong evolutionary conservation and proposed biological role suggest that the gene may be essential and that minimal structural alterations may not be compatible with life.

The published sequence of the murine *D17H6S45* cDNA predicts 26 dipeptide repeats, and no polymorphisms have been reported so far. In humans the homolog D6S45 gene has 24 dipeptide repeats [2], although two less common alleles containing 22 and 23 RD repeats have been described [5]. These variants have been found only in the heterozygous state, and homozygosity of these variants was estimated to be present in 1 in 4000 individuals, so no predictions could be made about the phenotypic consequences of homozygosity for these polymorphisms.

Our aim was to examine the *D17H6S45* gene repeat region in inbred mice for similar polymorphisms, which would exist in the homozygous state.

Screening of the 38 different inbred strains revealed two new alleles smaller than the original allele. PCR products representative of the three sizes of allele were sequenced to characterize the nature of the variation. One allele was shown to have a 24-bp deletion at nucleotide positions 827–850 of the cDNA, which removes four dipeptide repeats, codons 230 to 237 inclusive, maintaining the reading frame and the RD repeat. The variation in the second new allele consisted of a 6-bp deletion at positions 772–777 of the cDNA, which removes one dipeptide repeat, codons 212 and 213, with no disruption of the reading frame or RD repeat. The 24-bp deletion was found in all mice of the H-2^b haplotype tested and also in the two mice with H-2^{i7} and H-2^{ia1} haplotypes. Haplotypes H-2^{i7} and H-2^{ia1} are both derived from recombination involving the H-2^b haplotype. The 6-bp deletion was characteristic of both mice derived from an RIII background containing the H-2^r haplotype (Table 1).

In addition to the polymorphism described above, PCR amplification from the last exon (exon 11) of the *D17H6S45* gene to the last exon (exon 18) of the gene for complement factor B, *H2-Bf*, showed that the 3′-ends of the two genes are very close together, and sequence analysis of the *D17H6S45* gene revealed that the last four nucleotides of the 3′-untranslated region of the *D17H6S45* gene are complementary to the last four nucleotides of the 3′-untranslated region of *H2-Bf*.

In summary, we have described the existence of two novel murine *D17H6S45* alleles, one containing 22 RD repeats and the other 25, compared with the 26 repeats originally reported. The presence of these new alleles in the homozygous state indicates that a certain degree of variation in the number of repeats is compatible with the biological function of the protein. We have also shown for the first time that the *D17H6S45* gene and the *H2-Bf* gene overlap at their 3′ ends.

Table 1. A summary of the inbred strains of mice analyzed, their H-2 haplotypes, and the predicted number of dipeptide repeats encoded by the *D17H6S45* gene.

Strain	H-2 Haplotype	Number of RD repeats
B10.A/SgSn	a	26
A.BY/SnJ	b	22
B6.SJL/2Cy	b	22
C3H.SW/Sn	b	22
D1.LP/Sn	b	22
129Sv	b	22
129O1a	b	22
BXSB	b	22
B6.C-H2^{bm1}/By	bm1	22
B6.C-H2^{d}/aBy	d	26
B10.D2/nSn	d	26
D1.C/Sn	d	26
B10.D2-H2^{dm1}/Eg	dm1	26
A.CA/Sn	f	26
B10.M/Sn	f	26
C3H.NB/Sn	f	26
C3H.HTG/Sn	g	26
B10.D2(R103)/Eg	g3	26
B6.C-H2^{g6}	g6	26
B10.A(2R)/SgSn	h2	26
B10.A(5R)/SgSn	i5	26
B10.D2(R107)/Eg	i7	22
B10.D2(R106)/Eg	ia1	22
C3H.JK/Sn	j	26
B10.BR/SgSnJ	k	26
AKR.M/nSn	m	26
B10.AKM/SnJ	m	26
C3H-H2^{o2}/SfSnJ	o2	26
B10.DA(80NS)/Sn	p	26
B10.Y/Sn	pa	26
D1.DA/Sn	qp	26
B10.RIII(71NS)/Sn	r	25
LP.RIII/Sn	r	25
A.SW/Sn	s	26
B6.SJL/1Cy	s	26
B10.PL(73NS)/Sn	u	26
B10.SM(70NS)/Sn	v	26

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