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Location of the 9257 and ataxia mutations on mouse Chromosome 18

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Abstract. The location of three mutations on proximal Chromosome (Chr) 18 was determined by analysis of the offspring of several backcrosses. The results demonstrate that ataxia and the insertional mutation TgN9257Mm are separated by less than 1 cM and are located approximately 3 cM from the centromere, while the balding locus is 7 cM more distal. Previous data demonstrated that the twirler locus also maps within 1 cM of ataxia. The corrected locations will contribute to identification of appropriate candidate genes for these mutations. Two polymorphic microsatellite markers for proximal Chr 18 are described, *D18Umi1* and *D18Umi2*. The *Lama3* locus encoding the α3 subunit of nicein was mapped distal to ataxia and did not recombine with *Tg9257*.

Introduction

Four interesting mutations have been mapped to the most proximal 10 cM of Chr 18: ataxia (*ax;* Lyon 1955), balding (*bal;* Davisson et al. 1994), twirler (*Tw;* Lyon 1958), and the transgene induced mutation TgN9257Mm (*Tg9257*) (Ting et al. 1994). The ataxia mutation causes a generalized retardation of central nervous system development with progressive paralysis and death in *ax/ax* animals (Lyon 1955). Balding causes hair loss and immunological anomalies in affected animals (Davisson et al. 1994). Twirler and *Tg9257* produce bidirectional circling behavior due to abnormal development of the vestibular apparatus of the inner ear. The inner ear defects observed in twirler and *Tg9257* include hypoplasia of the semicircular canals, with the lateral canal most severely affected, and flattening or invagination of the crista ampullaris (Lyon 1958; Ting et al. 1994). The related phenotypes of twirler and *Tg9257* suggest that these mutations may be alleles of the same locus, or mutations in two closely linked genes affecting craniofacial development.

To map the transgene insertion in line *Tg9257* more precisely, we have developed two microsatellite markers from DNA flanking the insertion site. The position of *Tg9257* relative to ataxia (*ax*) and balding (*bal*) has been determined. The results will be important for future consideration of candidate genes for these mutations. In addition, the hypothesis of allelism of twirler and *Tg9257* has been supported by the demonstration that the transgene insertion site is located within 1 cM of ataxia.

Materials and methods

Genetic crosses. The 9257 transgene backcross [(C57BL/6J-Tg/+ × SPRET/Ei)F_1 × C57BL/6J] was previously described (Ting et al. 1994). The balding backcross [(C57BL/6J-*bal/bal* × CAST/Ei)F_1 × C57BL/6J-*bal/bal*] was described by Davisson and associates (1994). The ataxia backcross [(C57BL/6J-ax^J/+ × DBA/2J)F_1 × C57BL/6J-ax^J/+ was generated at Kyoto University and the Massachusetts Institute of Technology by G. Radice. The beige-satin backcross [(SB/Le-*bg sa/bg sa* × *M. spretus*)F_1 × SB/Le-*bg sa/bg sa*] was previously described (Justice et al. 1990).

Microsatellite markers. Cosmid clones were isolated from libraries of Tg9257 DNA by colony hybridization with a transgene probe (Ting et al. 1994). P1 clones (Pierce et al. 1992) from the region of the Tg9257 insertion site were isolated with PCR primers derived from the cosmid clones (Genome Systems, St. Louis). Primers were synthesized by the Oligonucleotide Facility of the University of Michigan or purchased from Research Genetics (Huntsville, Ala.). PCR amplification was carried out as previously described (Ting et al. 1993). One primer from each pair was end-labeled with T4 polynucleotide kinase (Boehringer-Mannheim) and [γ-^{32}P]-ATP (Amersham; Sambrook et al. 1989). Reaction mixtures were incubated at 94°C for 3 min, and subjected to 30 cycles of 55 s each at 94°C, 60°C, and 72°C with a model PTC-150 thermal cycler (MJ Research). Products were detected by autoradiography after electrophoresis on 6% polyacrylamide/urea gels. In some cases, microsatellite markers were detected with nonradioactive assays, as previously described (Liu et al. 1994).

Other genetic markers. The *Cdh2* (cadherin 2) locus (formerly *Ncad*) was detected by digestion of genomic DNA with *Acc*I and hybridization with a 3.5-kb *Hin*dIII fragment from the mouse N-cadherin cDNA (Miyatani et al., 1989). DBA/2J contains strain-specific *Cdh2* fragments of 8.1 kb and 3.4 kb which were typed on the *ax* backcross. *Evi3* (ecotrophic viral insertion 3), *Egr1* (early growth response 1), *Grl1* (glucocorticoid receptor 1), and the Tg9257 transgene were analyzed as previously described (Justice et al. 1994; Ting et al. 1994). *Lama3* was assayed by digestion of genomic DNA with *Taq*I or *Msp*I and hybridization with MN97, a 2.0 kb mouse cDNA encoding the α3 subunit of laminin-5 (Aberdam et al. 1994b). An RFLP at the Tg9257 insertion site was assayed by hybridization with the AT1 probe (Ting et al. 1994), which detected a 6.8-kb *Taq*I fragment in strain SB/Le and a 5.4-kb fragment in *M. spretus* DNA.

Results

Polymorphic microsatellite markers for the transgene insertion.A (CA)n repeat (D18Umi1) and a (GA)n repeat (*D18Umi2*) were identified by partial sequencing of a P1 clone that spans the 9257 transgene insertion site (Table 1). *D18Umi1* and *D18Umi2* are separated by approximately 20 kb of genomic DNA and can be considered as markers for the *9257* insertion site. Interstrain variation in the dinucleotide repeat length was used to follow the segregation of the *9257* locus in genetic crosses.

Close linkage of ataxia and Tg9257. The ataxia mutation arose on the kreisler strain (Lyon 1955) and has been maintained as a con-

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Table 1. Polymorphic microsatellite markers from the Tg9257 insertion site. The primers for amplification of *D18Umi1* are, from 5′ to 3′: F, (ATG GTG AAT GTG CAT TCC ATT TAG GAA TCT C) and R, (GCA ATC CAG CCA CTC AGT CTT AGC AGT GC). The primers for amplification of *D18Umi2* are: F, (AGA GTG CAA CTC TGA ATA GTG TCT CAG TGG), and R, (TTC TTT TCT GGC TTG ATT TGT AGT GTG CAC).

Locus	Repeat	Strain	Allele (bp)
D18Umi1	$(CA)_n$	CAST/Ei	260
		CASA/Rk	260
		C57BL/6J	275
D18Umi2	$(GA)_n$	SPRET/Ei	190
		C57BL/6J	196
		C3H/HeJ	198
		kreisler	200
		DBA/2J	204
		CAST/Ei	208

genic strain by backcrossing to C57BL/6J for more than 40 years. We detected two alleles of *D18Umi2* in the ataxia congenic line, the 196-bp allele from C57BL/6J (Table 1) and a 200-bp allele derived either from the original kreisler strain or by mutation. Retention of a kreisler allele would suggest that *D18Umi2* was located within a few cM of ataxia. For direct determination of the distance between these loci, 262 animals from an ataxia backcross were typed for *D18Umi2* (Fig. 1A). The observation of a single recombinant indicates that the distance between *ax* and *D18Umi2* is 0.4 ± 0.4 cM.

A subset of 28 backcross animals were also typed for *Cdh2.* The haplotype of one recombinant animal (Fig. 1A) demonstrated the gene order: *ax–D18Umi2–Cdh2* (Figure 1A). The orientation of the gene cluster with respect to the centromere was determined by analysis of the marker *Evi3,* which was reported to be 0.5 cM proximal to *Cdh2* (Justice et al. 1994). RFLP analysis of *Tg9257* on a beige-satin backcross that was previously typed for *Evi3* (Fig. 1B) demonstrated that *Tg9257* is proximal to *Evi3.* Since *Tg9257* and *D18Umi2* are physically linked, the combined data from Fig. 1A and 1B indicate that the gene order is: (centromere)–*ax–Tg9257, D18Umi2–Evi3–Cdh2*. The data indicate that *ax* and *Tg9257* are among the most proximal loci on Chr 18.

Analysis of additional microsatellite markers. To confirm that Tg9257 is located close to the centromere, we mapped it relative to three additional microsatellite markers from proximal Chr 18 (Dietrich et al. 1994; Fig. 1C). The observed gene order and distances are: (centromere)–*Tg9257, D18Mit19*–(2.9 ± 2.0)–*D18Mit20, D18Mit30*–(5.9 ± 2.9)–*Egr1, Grl1.* Since *D18Mit19* was previously mapped 3 cM distal to the centromere (Dietrich et al. 1994; Johnson and Davisson 1994), the location of *Tg9257* close to the centromere was confirmed.

Balding locus. The mutation balding was mapped to proximal Chr 18 (Davisson et al. 1994) and placed within 1 cM of *Tw* on the consensus map (Johnson and Davisson 1994). To measure the distance between *Tg9257* and balding, a balding backcross was typed for *D18Umi1, D18Mit19, D18Mit20,* and *D18Mit30* (Fig. 1D). The observed gene order and distances were: (centromere)–*Rnr18*–(1.1 ± 1.0)–*D18Mit19*–(2.1 ± 1.5)–*D18Umi1*–(2.1 ± 1.5)–*D18Mit30*–(3.2 ± 1.8)–*D18Mit20*–(2.1 ± 1.5)–*bal*–(11.6 ± 3.3)–*Grl1.* These results confirm the location of the transgene marker *D18Umi1* approximately 3 cM from the centromere and indicate that balding is located approximately 7 cM distal to the *Tw/ax/Tg9257* cluster.

Lama3. The *Lama3* locus encoding the α3 subunit of laminin-5 was recently mapped cytogenetically to proximal Chr 18 (Aberdam et al. 1994a). *Lama3* is known to be expressed in epithelial structures and in the floorplate of the developing neural tube, and was suggested as a candidate gene for *bal, Tw,* and *ax* (Aberdam et al. 1994b). No recombinations were observed between *Lama3* and *D18Umi1* (0/95) or the Tg9257 transgene (0/68), while a single recombinant placed *Lama3* distal to ataxia (Fig. 1). *Lama3* is thus closely linked to *Tg9257* and may be eliminated as a candidate gene for *ax* and *bal.*

Discussion

Genetic mapping data from four backcrosses are consistent with placement of the *Tg9257* insertion and the ataxia locus at a position 2–3 cM distal to the centromere of Chr 18. This is more proximal than the assigned positions on recent consensus maps (Fig. 2A; Johnson and Davisson 1994). Since the twirler locus was previously mapped to a position less than 1 cM from the ataxia locus (Lyon 1958, 1975; Lane et al. 1981), its position on the consensus map should also be changed to reflect the more proximal location of ataxia. The previously reported distance of 3.9 + 3.7 cM (n = 70) between twirler and the centromeric marker *Dp*(18Hc) (Lane et al. 1981) is consistent with the new location. The higher estimate of 9.6 ± 4.1 cM (n = 52) between twirler and another centromeric marker, *Rb6Rma* (Lane et al. 1981) does not differ significantly. A revised map consistent with all of the available data is presented in Figure 2B. The revised mapping infor-

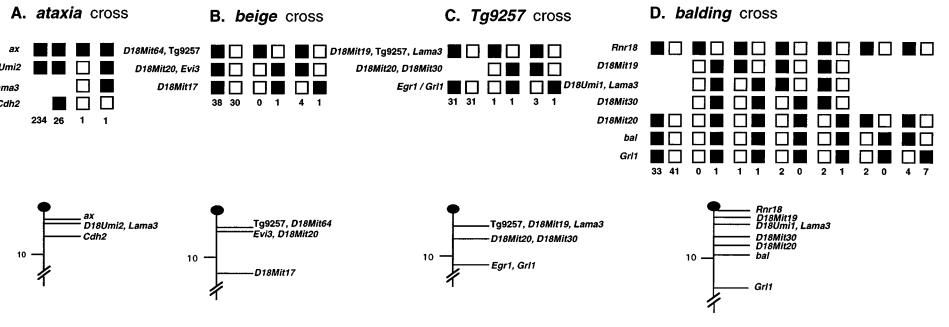

Fig. 1. Gene order on proximal Chr 18. The crosses are described in Materials and methods. Each column represents an observed haplotype; the number of mice with each haplotype is indicated at the bottom of each column. **(A)** Only affected *ax/ax* backcross progeny were typed. Solid box, kreisler or C57BL/6J allele; open box, DBA/2J allele. **(B)** Beige-satin backcross progeny. Solid box, SB allele; open box, *M. spretus* allele. **(C)** Tg9257 backcross progeny. *D18Mit20* and *D18Mit30* were typed on the recombinant animals that were previously identified (Ting et al. 1994). Solid box, C57BL/6J allele; open box, *M. spretus* allele. **(D)** Balding backcross progeny. *D18Umi1, D18Mit19,* and *D18Mit30* were typed on previously identified recombinant animals (Davisson et al. 1994). Solid box, C57BL/6J allele; open box, *M.m. castaneus* allele.

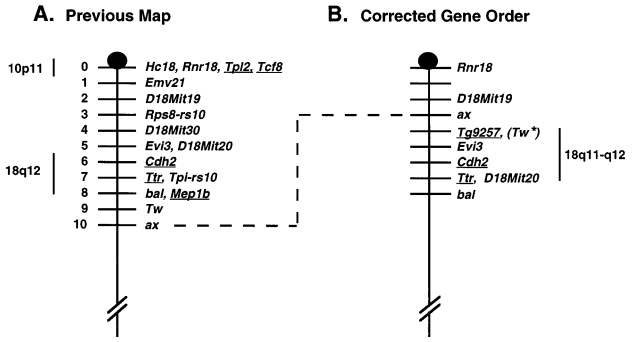

Fig. 2. Maps of proximal Chr 18. **(A)** Adapted from the Chromosome Committee consensus map (Johnson and Davisson, 1994). **(B)** Corrected position of *ax*. The human homolog of 9257 was recently mapped to 18q11 (Griffith et al. 1996). *Tw is located within 1 cM of ax, but their order is not known.

mation will contribute to the identification of appropriate candidate genes for the 9257, ataxia, twirler, and balding mutations.

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References

Aberdam, D., Galliano, M.F., Mattei, M.-G., Pisani-Spadafora, A., Ortonne, J.P., Meneguzzi, G. (1994a). Assignment of mouse nicein genes to Chromosomes 1 and 18. Mamm. Genome 5, 229–233.

Aberdam, D., Aguzzi, A., Baudoin, C., Galliano, M.-F., Ortonne, J.-P., Meneguzzi, G. (1994b). Developmental expression of nicein adhesion protein (laminin-5) subunits suggests multiple morphogenic roles. Cell Adhesion and Comm. 2, 115–129.

Davisson, M.T., Cook, S.A., Johnson, K.R., Eicher, E.M. (1994). Balding: a new mutation on mouse chromosome 18 causing hair loss and immunological defects. J. Hered. 85, 134–136.

Dietrich, W.F., Miller, J.C., Steen, R.G., Merchant, M., Damron, D., Nahf, R., Gross, A., Joyce,D.C., Wessel, M., Dredge, R.D., Marquis, A., Stein, L.D., Goodman, N., Page, D.C., Lander, E.S. (1994). A genetic map of the mouse with 4,006 simple sequence length polymorphisms. Nature Genet. 7, 220–245.

Griffith, A.J., Burgess, D.L., Kohrman, D.C., Yu, J., Blaschak, J., Blanton, S.H., Boehnke, M., Hecht, J.T., Overhauser, J. and Meisler, M.H. (1996). Localization of the homology of a mouse craniofacial mutant to human chromosome 18q11 and evaluation of linkage to human CLP and CPO. Genomics, in press.

Johnson, K.R., Davisson, M.T. (1994). Mouse Chromosome 18. Mamm. Genome 5 (Suppl.), S259–S265.

Justice, M.J., Silan, C.M., Ceci, J.D., Buchberg, A.M., Copeland, N.G., Jenkins, N.A. (1990). A molecular genetic linkage map of mouse chromosome 13 anchored by the beige (*bg*) and satin (*sa*) loci. Genomics 6, 341–351.

Justice, M.J., Morse, H.C., III, Jenkins, N.A., Copeland, N.G. (1994). Identification of *Evi-3*, a novel common site of retroviral integration in mouse AKXD B-cell lymphomas. J. Virol. 68, 1293–1300.

Lane, P.W., Searle, A.G., Beechey, C.V., Eicher, E.M. (1981). Chromosome 18 of the house mouse. J. Hered. 72, 409–412.

Liu, C.-Y., Zhu, G., Coverse, R., Kao, C.W.-C., Nakamura, H., Tseng, S.C.-G., Mui, M.-M., Seyer, J., Justice, M.J., Stech, M.E., Hansen, G.W., Kao, W. (1994). Characterization and chromosomal location of the cornea-specific murine keratin gene: Krt1.12. J. Biol. Chem. 269, 24627–24636.

Lyon, M.F. (1955). Ataxia—a new recessive mutant of the house mouse. J. Hered. 46, 77–80.

Lyon, M.F. (1958). Twirler: a mutant affecting the inner ear of the house mouse. J. Embryol. Exp. Morphol. 6, 105–116.

Lyon, M.F. (1975). Recombination between Tw and ax. Mouse News Lett. 52, 36.

Miyatani, S., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Takeichi, M. (1992). Genomic structure and chromosomal mapping of the mouse N-cadherin gene. Proc. Natl. Acad. Sci. USA 89, 8443–8447.

Miyatani, S., Shimamura, K., Hatta, M., Nagafuchi, A., Nose, A., Matsunaga, M., Hatta, K., Takeichi, M. (1989). Neural cadherin: role in selective cell-cell adhesion. Science 245, 631–635.

Pierce, J.C., Sauer, B., Sternberg, S. (1992). A positive selection vector for cloning high molecular weight DNA by the bacteriophage P1 system: improved cloning efficiency. Proc. Natl. Acad. Sci. USA 89, 2056–2060.

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, 2nd ed. (Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory Press).

Ting, C.-N., Burgess, D.L., Chamberlain, J.S., Keith, T.P., Falls, K., Meisler, M.H. (1993). Phosphoenolpyruvate carboxykinase (GTP): characterization of the human PCK1 gene and localization distal to MODY on chromosome 20. Genomics 16, 698–706.

Ting, C.-N., Kohrman, D., Burgess, D.L., Boyle, A., Altschuler, R.A., Gholizadeh, G., Samuelson, L.C., Jang, W., Meisler, M.H. (1994). Insertional mutation on mouse chromosome 18 with vestibular and craniofacial abnormalities. Genetics 136, 247–254.