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Conserved linkage of neurotrophin-3 and von Willebrand factor on mouse Chromosome 6

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Neurotrophin-3 and other neurotrophic factors contribute to the development and maintenance of the vertebrate nervous system by promoting localized neuronal survival. The human gene encoding neurotrophin-3, a highly conserved protein with complete amino acid identity in human, mouse, and rat, has been mapped to Chromosome (Chr) 12p13 (Maisonpierre et al. 1991). The mouse homolog, *Ntf-3,* was assigned to Chr 6 by use of a somatic cell panel (Ozcelik et al. 1991). We undertook the regional localization of *Ntf-3* in order to test it as a candidate for the *mnd2* mutation which we recently mapped to Chr 6; homozygosity for *mnd2* results in motor neuron disease due to degeneration of spinal motor neurons (Jones et al. 1993).

von Willebrand factor is a glycoprotein involved in blood coagulation that is both a carrier for factor VIII (antihemophilic factor) and a mediator of platelet–vessel wall interaction (Ginsburg and Bowie 1992). The human gene *VWF* (previous symbol *F8VWF*) is the most distally mapped gene on Chr 12p13.3 (NIH/CEPH Collaborative Mapping Group 1992). The locations of *NTF3* and *VWF* suggested that they might be members of the conserved linkage group on mouse Chr 6 that includes 11 other genes from human 12p12-13 (Nadeau et al. 1992; Elliott and Moore 1992).

Ntf-3 was mapped by Southern blot analysis with a *Bgl*II RFLP between strains C57BL/6J (3.6 kb) and SPRET/Ei (9.7 kb; Fig. 1). The *Ntf-3* probe was a 550-bp genomic fragment that was amplified from genomic DNA from strain C57BL/6J with primers based on the published nucleotide sequence (Hohn et al. 1990): primer 1, 5′ (+250) AGG AGT TTG CCG GAA GAC TCT CTC AAT TCC (+279) 3′; and primer 2, 5′ (+799) GCA ACC GTT TTT GAC CGG CCT GGC TTC (+772) 3′. Amplification was carried out for 30 cycles with 1 min at 94°C and 1 min at 72°C.

Two PCR-based genetic variants were used to map the *Vwf* locus. First, a length polymorphism between C57BL/6J (115 bp) and CAST/Ei (119 bp) was detected by direct amplification of 0.2 μg of genomic DNA with primer 3 (5′ TCC GGT GCC TTA CAG TCT GCT G 3′) and primer 4 (5′ TGT ACT CAG TAG TTC TTC CTA GGA G 3′). Primer 3 was end-labeled with γ^{32}P-ATP (>5000 Ci/mmol, Amersham) by use of polynucleotide kinase (New England Biolabs) as previously described (Roth et al. 1990). Amplification was carried out for 30 cycles with 1 min at 94°C, 30 s at 60°C, and 30 s at 72°C. PCR products were diluted threefold with formamide loading buffer and electrophoresed on 8% denaturing polyacrylamide gels. Second, a PCR RFLP between C57BL/6J and SPRET/Ei was detected by amplification of 0.2 μg of genomic DNA with primer 5 from exon 28 (5′ CCT TCA ATG GAT CCC AGT CCA AGG AGG AGG 3′) and primer 6 from intron 28 (5′-GTT CTA GAC TCA AGC TTC TGG ATC TGT GTG 3′) in a 50-μl reaction under previously described conditions (Ginsburg et al. 1989). Amplification was carried out for 35 cycles of 1 min at 94°C, 30 s at 55°C, and 2 min at 72°C. The PCR products were then digested with *Rsa*I and analyzed on a 4% agarose gel containing 3% FMC NuSieve and 1% BRL agarose (Fig. 1; Nichols et al., manuscript in preparation).

Ntf-3 and *Vwf* were mapped with respect to the Chr 6 reference loci *mi* and *Raf-1,* as well as several anonymous DNA markers. Microophthalmia (*mi*) was typed by hybridization with the 2.5-kb genomic clone p24RI2.5, which directly flanks the insertion site of a transgenic insertional mutation at this locus (Krakowsky et al. 1993). This probe hybridizes with *Hin*dIII fragments of 4.5 kb in C57BL/6J and 3.9 kb in SPRET/Ei (Fig. 1). A *Taq*I polymorphism (Jones et al. 1993) at the anchor locus *Raf-1* (Kozak et al. 1984) was

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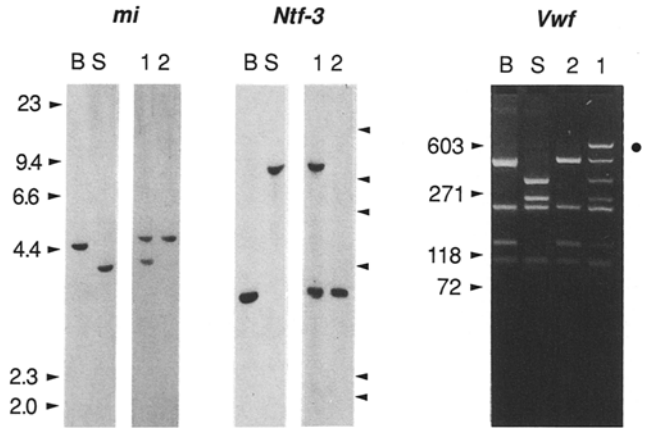

Fig. 1. Genetic variation at three loci on Chr 6. For Southern blot analysis of *Ntf-3* and *mi*, genomic DNA was digested with *Bgl*II and *Hin*dIII, respectively, and hybridized as described below. The positions of bacteriophage λ *Hin*dIII fragments are indicated (kb). For analysis of *Vwf*, genomic DNA was amplified by PCR, followed by digestion with *Rsa*I as described in the text. A heteroduplex band is visible in heterozygous samples **(solid circle).** The positions of ΦX174 *Hae*III fragments are indicated (bp). **B,** C57BL/6J; **S,** SPRET/Ei; **1,** heterozygous backcross individual; **2,** homozygous backcross individual. *Methods.* Genomic DNA was extracted by a salting out procedure (Miller et al. 1988), digested overnight with the appropriate restriction enzymes, and electrophoresed on 0.7% agarose gels with 10 μg DNA per lane. DNA fragments were transferred to Zetaprobe GT (Bio-Rad Laboratories) nylon filters by capillary blotting (Southern 1975) and hybridized for 18 h at 65° in 20 ml of hybridization solution (0.5 M dibasic sodium phosphate, 1 mM EDTA, 7% SDS, pH 7.2) with a final wash of 0.1 × SSC, 0.1% SDS at 65°C before the filters were exposed to X-ray film for 2–4 days. Probes were labeled with α^{32}P-dCTP (>3000 Ci/mmol, Amersham) by random oligoprimed labeling to 10^9 cpm/μg (Feinberg and Vogelstein 1983).

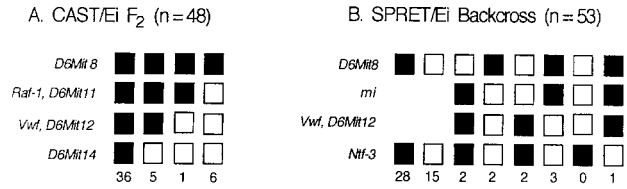

Fig. 2. Linkage data for loci on Chr 6. **(A)** Haplotypes from (C57BL/6J-*mnd2* × CAST/Ei) F_2 animals. **(B)** Tierred mapping of backcross progeny from the cross (C57BL/6J-tg9257 × SPRET/Ei)F_1 × C57BL/6J. Each column represents one haplotype, and the number of mice with each haplotype is given at the bottom of each column. All of the F_2 progeny typed were homozygous for the Chr 6 locus *mnd2* (Jones et al. 1993); haplotypes were inferred by assuming the absence of double crossovers between markers in this 20 cM region. **Solid symbols,** C57BL/6J homozygotes; **open symbols,** heterozygotes with C57BL/6J and CAST/Ei or SPRET/Ei alleles.

detected by hybridization with the probe p627 (ATCC No. 41050). Primers for the microsatellite markers *D6Mit8, D6Mit11, D6Mit12,* and *D6Mit14* were obtained from Research Genetics (Huntsville, Ala.). PCR analysis was carried out as described (Dietrich et al. 1992) except that 100 ng of template DNA was used, and products were labeled by incorporation of α^{32}P-dCTP (>3000 Ci/mmol, Amersham) (1 μCi per 10-μl reaction) with a 40-fold reduction of unlabeled dCTP in the reactions.

Vwf was first mapped on a previously described (C57BL/6J-*mnd2* × CAST/Ei)F_2 mapping panel comprised of individuals homozygous for the Chr 6 mutation *mnd2* (Jones et al. 1993). Haplotypes from this panel are presented in Fig. 2A. The data localize *Vwf* to the interval between *Raf-1* and *D6Mit14,* with the indicated gene order: (centromere)–*D6Mit8*–(12.5 ± 4.8)–*Raf-1, D6Mit11*–(2.1 ± 2.1)–*Vwf, D6Mit12*–(10.4 ± 4.4)–*D6Mit14*. Additional loci including *Ntf-3* were analyzed on a backcross between C57BL/6J-tg9257 and SPRET/Ei (Bain et al. 1993). Again, there were no recombinants between *Vwf* and *D6Mit12* (Fig. 2B). The results demonstrate the gene order: (centromere)–*D6Mit8*–(7.5 ± 3.6)–*mi*–(9.4 ± 4.0)–*Vwf, D6Mit12*–(1.9 ± 1.9)–*Ntf-3*.

The results of the two crosses are combined in the map in Fig. 3, demonstrating gene order and relative positions of these loci. The positions of *mi* and *Raf-1* are consistent with the Chromosome 6 Committee map (Elliott and Moore 1992). Since *mnd2* is located proximal to *Raf-1* (Jones et al. 1993), the data eliminate *Ntf-3* as a candidate gene for this disorder. *Ntf-3* may be considered a candidate for the mutation *opisthotonus,* which maps to this region and results in loss of balance and early death (Green 1989; Elliott and Moore 1992).

Our data add two new loci to the conserved linkage group on human Chr 12p12-13 and mouse Chr 6 (Nadeau et al. 1992; Elliott and Moore 1992). It is interesting that the von Willebrand factor gene is located close to the telomere on human Chr 12 but at an internal position on mouse Chr 6. Mapping this gene in

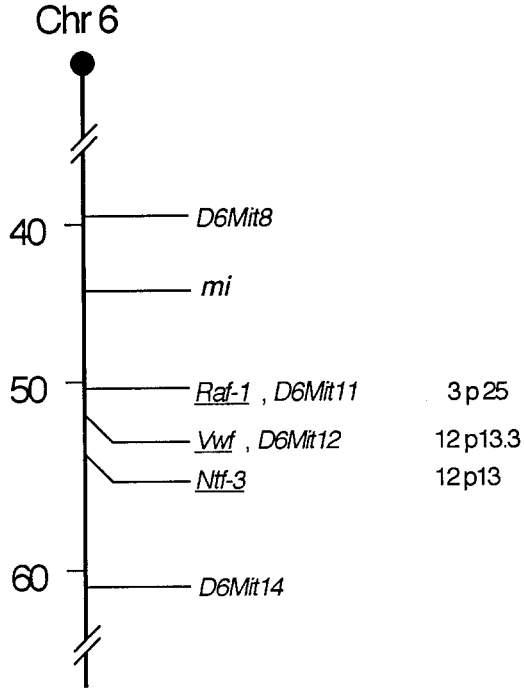

Fig. 3. Order of Chr 6 loci based on data in Fig. 2. The approximate map positions are indicated in cM from the centromere (Elliott and Moore 1992). The positions of human genes homologous to the underlined mouse loci are indicated at the right (Bonner et al. 1984; NIH/CEPH Collaborative Mapping Group 1992; Maisonpierre et al. 1991).

additional mammalian species would be of interest with regard to evolution of the 12p telomere. The localization of *Vwf* reported here will facilitate ongoing investigations of clotting disorders in the mouse, including characterization of a mouse model for von Willebrand disease (Sweeney et al. 1990). There are no known clotting mutations in this region of Chr 6.

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