Mouse Chromosomal Localization of the Cortexin (*Ctxn*) Gene

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Cortexin is a novel 82-amino-acid protein of unknown function that is highly enriched in precursor neurons of the cortical plate of fetal rodent brain and in pyramidal neurons of adult rodent cerebral cortex and hippocampus (1–3; GenBank Accession No. L15011). A putative single membrane-spanning domain in the middle of the cortexin amino acid sequence suggests that it is an integral membrane protein that may mediate extracellular or intracellular signaling of cortical neurons during forebrain development. In the present study, we determined the mouse chromosomal localization of the cortexin gene to test whether it resides near any of the known mouse neurological loci (4).

The cortexin gene, referred to as *Ctxn*, was mapped by linkage analysis of restriction fragment length variants (RFLVs) in interspecific backcrosses of C57BL/6J × *Mus spretus* F1 mice with C57BL/6J mice. The cross has previously been typed for several hundred RFLVs and simple sequence length polymorphisms (5). To identify informative RFLVs, Southern blots containing restriction-digested genomic DNA from the parental strains were hybridized to a ^{32}P-random labeled cDNA insert (1.01 kb) of the mouse cortexin mRNA (2) and washed at 50°C in 1.0× SSC, 0.1% SDS. An informative RFLV was obtained following digestion with *Eco*RI, yielding a 20-kb hybridizing band in C57BL/6J, a 3.7-kb band in *M. spretus*, and both bands in F1 hybrids (data not shown).

TABLE 1

Segregation Pattern of *Ctxn* with Chromosome 8 Markers

Genotype[a]					
D8Mit3	*Ctxn*	*D8Mit24*	*D8Mit9*	*D8Mit11*	Number
B	B	B	B	B	16
S	S	S	S	S	14
B	B	B	B	S	2
B	B	B	S	S	5
B	B	S	S	S	2
B	S	S	S	S	2
S	B	B	B	B	2
S	S	B	B	B	5
S	S	S	B	B	2
S	S	S	S	B	1
				Total	51

[a] Alleles inherited from the hybrid parent [(C57BL/6J × *M. spretus*) F1] are designated as "B" or "S" to indicate their origin in either C57BL/6J or *M. spretus*, respectively.

Comparison of the segregation pattern of *Ctxn* with other markers indicated linkage to several chromosome 8 markers, the nearest flanking markers being *D8Mit3* and *D8Mit24* (Table 1) (6, 7). *Ctxn* exhibited no significant linkage with markers typed on other chromosomes. The results indicate the following gene order: centromere–*D8Mit3*–7.8 ± 3.8 cM–*Ctxn*–13.7 ± 4.8 cM–*D8Mit24*–13.7 ± 4.8 cM–*D8Mit9*–5.9 ± 3.2 cM–*D8Mit11*.

One recessive neurological mutant in the mouse, nervous (*nr*), maps in the vicinity of the *Ctxn* gene. The nervous mutation has been mapped to chromosome 8, 24.6 ± 1.5 cM proximal to oligosyndactylism (*Os*) (8). The *Os* gene maps about 4.3 cM proximal to the *Mt-2* gene (9), which is closely linked to *D8Mit11* and about 40 cM distal to *D8Mit3* (6). Thus, these results suggest that the *nr* gene is about 30 cM proximal to *D8Mit11* and 10 cM distal to *D8Mit3*, placing it very near the *Ctxn* gene. Although additional linkage studies in the same crosses will be required to map *Ctxn* relative to *nr* more precisely, we propose that the cortexin gene is a candidate for the nervous mutation.

The nervous mouse exhibits a neurological phenotype characterized by progressive cerebellar and retinal degeneration, exhibiting mitochondrial swelling predominantly in cerebellar Purkinje cells (8, 10, 11). Inconsistent with the sites of nervous pathology, cortexin mRNA is detected primarily in forebrain regions, most notably cerebral cortex and hippocampus, but there are no detectable levels of cortexin mRNA in cerebellum (1–3). Western immunoblotting, on the other hand, does detect cortexin protein at low levels in cerebellar membrane-enriched fractions and, as expected, at much higher levels in membrane-enriched fractions from cerebral cortex (unpublished observations). Future investigations will address whether cortexin expression is altered in nervous mice.

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