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Astrotactin (ASTN), a Gene for Glial-Guided Neuronal Migration, Maps to Human Chromosome 1q25.2

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Astrotactin is a recently cloned neuronal adhesion molecule required for glial-guided neuronal movement in cortical regions of developing brain, and it is therefore an excellent candidate gene for neuronal migration defects in humans. A number of human brain malformations result from disturbed neuronal movement during CNS histogenesis (reviewed in 3). Abnormalities may range from failure to form normal convolutions (lissencephaly, pachygyria, or polymicrogyria) or folia with disturbed cortical lamination, to more subtle mal-

formations in which neuronal subpopulations move to inappropriate locations (neuronal heterotopias). The clinical significance of neuronal migration defects is increasingly apparent with improvements in high-resolution MR imaging. Recent studies suggest that heterotopias account for 15–25% of adult epilepsy (9). In one autopsy survey of 591 patients with intractable seizures, 47% were found to manifest neuronal migration defects (10). However, little is known about the molecular genetic basis for these developmental disorders.

To identify genes that direct the migration of neurons along glial fibers during brain histogenesis, Hatten and colleagues developed an *in vitro* model system with cells purified from murine cerebellar cortex (4, 6). Using blocking antibodies to identify neuron–glial ligands required for neuronal migration, they have cloned astrotactin,² a CNS gene that supports neuronal movement along glial fibers (19). The structure and function of astrotactin are consistent with those anticipated from the large body of work by Rakic and others showing that a system of radially oriented glial fibers provides a scaffold for the directed migration of young neurons in developing human brain (reviewed in 17, 11). Its demonstrated function and the restriction of astrotactin expression to cortical areas where immature neurons undergo radial glial-directed migration suggest that it will be important in the development of these regions. FISH analysis was therefore used to determine the chromosomal location of astrotactin in humans and mice as a first step toward identifying a brain malformation associated with mutation(s) of this gene.

Metaphases were obtained from human peripheral blood lymphocytes and normal mouse fibroblasts by routine culturing, harvesting, slide-making, and G-banding techniques (1). Metaphase images were captured using a high-performance CCD camera and GeneVision software (Applied Imaging). The slides were destained, fixed, dehydrated, and denatured. FISH was performed with a biotinylated 4-kb mouse astrotactin cDNA using routine *in situ* suppression techniques (18). Detection utilized fluorescein-labeled avidin and anti-avidin antibodies (Oncor). The slides were counterstained with propidium iodide (Oncor). The previously captured metaphases were relocated, and fluorescent microscopic images were captured using ProbeVision (Applied Imaging).

FISH with the 4-kb mouse astrotactin cDNA probe demonstrated a fluorescent signal in the middle of the long arm of both chromosome 1 homologues in 39 of 40 human metaphases. No other specific hybridization signals were

² The HGMW-approved symbol for the gene described in this paper is ASTN.

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FIG. 1. Physical mapping of a 4-kb mouse astrotactin cDNA to mouse and human chromosomes. (A) Prometaphase spread of human chromosomes. (B) FISH analysis of the spread shown in A. (C) Metaphase spread of mouse chromosomes. (D) FISH analysis of the spread shown in C. Arrows point to the sites of hybridization of the astrotactin cDNA probe (fluorescein label). Chromosomes are counterstained with propidium iodide. (E) Idiograms of human and mouse chromosomes 1. The shaded region is known to be syntenic and includes both the human (1q25) and the mouse (1H2) astrotactin loci.

seen. Sequential G-banding to FISH analysis was used to localize accurately the map position of the astrotactin gene. Fifteen G-banded prometaphase cells (550–850 band level) were identified in which at least one of the chromosome 1 homologues was involved in a chromosomal overlap involving bands 1q24–q31. By comparing the location of the fluorescent signal to the region of overlap, the astrotactin gene was precisely mapped to band 1q25.2 (Figs. 1A and 1B). Similar experiments in mouse metaphase spreads localized the signal to 1H2 (Figs. 1C and 1D). These locations have been shown to be syntenic in comparative mapping studies (16) and serve to validate our localizations as diagrammed in Fig. 1E.

Interest in genes orchestrating neuronal migration has recently been rekindled by the identification of the gene defects in the *reeler* and *weaver* mouse mutants (reviewed in 12). While the reelin protein is an extracellular matrix molecule expressed in Cajal–Retzius and early postmigrational neurons of developing brain (2), the *weaver* gene product is a neuronal inward rectifying potassium channel (8). In contrast to these molecules, astrotactin is the first gene product shown to function directly in neuronal migration along the glial fiber system (19).

The deduced amino acid structure of astrotactin contains a signal peptide, a putative transmembrane domain, and three EGF-like and two fibronectin type-III extracellular domains, consistent with its proposed role in adhesion and cell–cell signaling at the migration junction (19). Functional studies *in vitro* have shown that exposure of young cerebellar granule neurons to blocking anti-astrotactin antibodies halts their migration along glial processes (4, 19). The cellular distribution of astrotactin mRNA expression indicates that it is involved in neuronal migration in cerebral and cerebellar cortex, hippocampus, and olfactory cortex, but not in subcortical regions, thalamus, or brain stem (19).

On structural and functional grounds, astrotactin is a strong candidate gene for neuronal migration disorders of cortical CNS regions. We have therefore begun to search for malformations involving this locus. The map location on human chromosome 1 indicates that astrotactin is not the gene involved in human lissencephaly associated with mutations on chromosome 17p13 (13) or on Xq22 (14), nor is it the gene involved in bilateral periventricular nodular heterotopia, which maps to Xq28 (5).

A search has also begun for mouse mutations that may involve the astrotactin gene. One such mutant is the *dreher* mouse (*dr*), which bears an autosomal recessively inherited mutation previously mapped in test crosses to mouse chromosome 1 (7). The *dr* phenotype includes neuronal heterotopias in the cerebellum, hippocampus, and cerebral cortex, consistent with disrupted neuronal migration in these areas (15). Structural and linkage analyses will determine whether the astrotactin gene is mutated in this strain.

Among the genes recently identified that affect neuronal migration in brain cortical structures, astrotactin is the first to be clearly required for glial-guided mechanisms. Malfunction of astrotactin would likely disrupt histogenesis in both cerebral and cerebellar cortices. Therefore, the gene is being examined in patient families expressing cerebral malformations, particularly when accompanied by cerebellar hypoplasia. Identification of brain malformations due to astrotactin mutation will be an important step in understanding the functional interrelationships of genes

that establish appropriate neuronal position in developing brain.

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Molecular Cloning of Murine Homologue Dentatorubral-Pallidoluysian Atrophy (DRPLA) cDNA: Strong Conservation of a Polymorphic CAG Repeat in the Murine Gene

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Dentatorubral-pallidoluysian atrophy (DRPLA) (MIM No. 125370) is an autosomal dominant neurodegenerative disease caused by expansion of an unstable CAG repeat encoding a polyglutamine tract in a gene mapped to chromosome 12p (1, 3, 7). Although the nucleotide sequences of full-length cDNA for the human and rat DRPLA genes have been determined, sequence analysis did not reveal any homologous domains suggesting the function of the products of these genes (4–6). Although expression of the mutant protein harboring an expanded polyglutamine tract is assumed to lead to selective neuronal death due to the aberrant nature of this protein, it remains unclear how neurons undergo degeneration. To elucidate the function of the DRPLA gene product and to develop animal models for DRPLA, we have isolated full-length mouse DRPLA cDNA clones and determined the complete coding sequence of the mouse DRPLA gene.

Sequence data from this article have been deposited with the GenBank/EMBL Data Libraries under Accession No. D87744.

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By screening a mouse brain cDNA library (ICR outbred strain) (Stratagene) and a mouse teratocarcinoma cDNA library (PCC4 cell line) (Stratagene) constructed in Lambda ZAPII vector using two human cDNA fragments as probes, we isolated 32 overlapping clones encompassing the full-length DRPLA cDNA. The nucleotide and the deduced amino acid sequence data have been deposited with the GenBank, EMBL, and DDBJ Data Libraries under Accession No. D87744. The consensus murine cDNA spans 4103 bp and includes an open reading frame encoding 1175 amino acids with a predicted molecular mass of 125 kDa. Alignment of the coding region of the nucleotide sequence revealed 86% identity between human and mouse DRPLA cDNA and 92% identity between mouse and rat DRPLA cDNA. Both of the cDNA clones isolated from the two cDNA libraries contained three CAG repeat units, indicating that the CAG repeat of the mouse DRPLA gene is much shorter than that of the human DRPLA gene, which consists of 8 to 35 repeat units. Analysis of the deduced amino acid sequences revealed 92% identity between human and mouse DRPLA protein and 95% identity between mouse and rat protein, respectively. The polyglutamine tract in the mouse DRPLA protein consisted of six glutamine residues interrupted by a single proline residue. The fact that the polyglutamine tracts have been conserved among mouse, rat, and human suggests that they may play some essential role in the function of the DRPLA protein.

Chromosomal localization of the mouse DRPLA gene was determined by segregation analysis using 130 interspecific backcross mice between strains C57BL/6 and MSM. MSM is an inbred strain derived from a Japanese wild mouse *Mus musculus molossinus*. Using a polymorphism located within the 3'-untranslated region, the genotypes of the 130 interspecific backcross mice were analyzed. A clear lineage of the mouse DRPLA gene was established using the three markers GLUT3, D6Jpk2, and D6Jpk3 on chromosome 6 (Fig. 1A).

To examine whether the CAG repeat in the mouse DRPLA gene shows intraspecific variability in length, the CAG repeats of seven species of wild mice belonging to different phylogenetic groups were analyzed. The names of samples examined are shown in Fig. 1B and denoted under the taxonomical name of each phylogenetic group. The dendrogram of genus *Mus* was constructed based on the polymorphism found in nuclear and mitochondrial DNA (2, 8). To determine the number of CAG repeat units of the DRPLA gene, DNA fragments containing the CAG repeat were amplified in the presence of [α -³²P]dCTP by polymerase chain reaction (PCR). The PCR products were electrophoresed in 5% denaturing polyacrylamide gel along with PCR product DNAs containing known numbers of CAG repeat units as size standards. As shown in Fig. 1B, the number of CAG repeat units among seven different phylogenetic groups of wild mice exhibited polymorphisms ranging from three to eight repeat units, but were constant within the same groups. Interestingly, nucleotide sequence analysis of the PCR products demonstrated that the length variation occurs exclusively upstream of the interruption by CCA. The phylogenetic distribution pattern of the polymorphism of the CAG repeat of the DRPLA gene showed good