Brief Communication

Localization of the Mouse Lissencephaly-1 Gene to Mouse Chromosome 11B3, in Close Proximity to D11Mit65

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Abstract—Lissencephaly is a human brain malformation manifested by a smooth cerebral surface and severe mental retardation. Some of the patients have been shown to have deletions in chromosome 17p13.3, and recently, LIS-1 has been proposed to be the disease-associated gene. We have now mapped the mouse homolog of LIS-1 to mouse chromosome 11B3 by using fluorescence in situ hybridization to metaphase chromosomes. The analysis of yeast artificial chromosome clones placed Lis-1 in close proximity to the microsatellite marker D11Mit65.

INTRODUCTION

Lissencephaly represents gyral malformations of the human brain including the presence of broad and flat gyri (macrogyria) or the absence of gyri (agyria) (1). The underlying cause of this disorder is thought to be an impaired neuronal migration during gestation which results in an immature cerebral cortex, leading to profound mental retardation and early death of the patients (2). The mechanism of the arrest of neuronal migration, however, is not yet known.

Although lissencephaly occurs most frequently as an isolated birth defect (isolated lissencephaly sequence, ILS), it can also be part of multiple congenital anomalies, referred to as Miller–Dieker syndrome (MDS). In MDS patients lissencephaly is associated with a characteristic abnormal facial appearance and often other abnormalities (3). MDS was proposed to be a contiguous gene syndrome in which the codeletion of several genes results in the complex phenotype observed (4).

It has been recently demonstrated that 40% of patients with ILS and over 90% of those with MDS have deletions in chromosome 17p13.3 (5). For both ILS and MDS a candidate disease-associated gene, LIS-1, has been serendipitously identified by a PCR-based approach designed to clone genes containing β–transducin-like repeats (6). LIS-1 belongs to the family of WD-repeat containing proteins (7), and it was found to be 99% identical to the 45 kDa subunit of the intracellular form of the bovine platelet-activating factor (PAF) acetylhydrolase (8), suggesting a regulatory role in PAF metabolism. It will be interesting to

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elucidate how an altered regulation of PAF acetylhydrolase activity can lead to disturbances of neuronal development.

In this communication we report the chromosomal localization of the mouse homolog of LIS-1 by fluorescence in situ hybridization (FISH). In addition, we physically map Lis-1 to a chromosome 11 specific microsatellite marker using yeast artificial chromosome (YAC) clones. These YACs will be useful in the analysis of the genomic region to identify nearby genes, the codeletion of which in humans might contribute to the MDS. The functional analysis of such genes in mouse should facilitate the genetic dissection of MDS.

MATERIALS AND METHODS

Fluorescent In Situ Hybridization (FISH) to Metaphase Chromosomes. FISH was carried out using a 15 kb λ-phage insert as a probe. The λ-phage clone was obtained by the screening of a mouse genomic library with a human LIS-1 cDNA (6) as a probe. This clone represents part of the mouse Lis-1 gene (manuscript in preparation) as confirmed by sequencing and comparison to the mouse Lis-1 cDNA (9). The probe for the FISH was labeled with biotin-16-dUTP by nick translation, and the hybridization performed as described (10). The hybridization signal was detected after one cycle of signal amplification procedure using biotinylated anti-avidin antibody and FITC-avidin as described (11).

Isolation of YAC Clones. A mouse genomic YAC library (Research Genetics) was screened by PCR. Primer sequences were obtained from an electronic database (12), and the PCR conditions were as described (13). PCR products were analyzed on ethidium bromide-stained polyacrylamide gels.

Southern Hybridization of YAC Clones. Agarose-embedded yeast chromosomal DNA was prepared according to standard methods. Chromosomes were separated by the Chef Mapper PFGE system (BioRad), and after UV irradiation, the gels were blotted to Nytran+ membranes (Schleicher and Schuell) under alkaline conditions. Hybridization was performed in Express Hybridization solution (Clontech) according to the manufacturer's instructions. Mouse Cot-1 DNA (Gibco-BRL) and Lis-1 cDNA (9) were labeled using the random hexamer primed procedure (14). Final washes were performed in 1 × SSC at 60°C, and autoradiography was for 6–12 hours at −80°C using Kodak XAR-5 film.

RESULTS AND DISCUSSION

As a result of our efforts to identify the mouse homolog of the human LIS-1 gene we obtained a λ-phage clone from a mouse genomic library (manuscript in preparation). Sequence comparison with the Lis-1 cDNA sequence (8) indicated that it was part of the mouse Lis-1 gene. To localize the gene, we performed FISH experiments using the λ-insert as a probe. Figure 1 demonstrates that the Lis-1 gene is localized to the B3 subband (15) of mouse chromosome 11, a position that is in accordance with the extensive synteny conservation between this mouse chromosome and human chromosome 17 (16).

To confirm the result of the FISH and to map the Lis-1 gene more precisely we collected a panel of YAC clones from a mouse genomic YAC library (Research Genetics). For the PCR screening we used primer pairs specific for five microsatellite markers (D11Mit116, D11Mit65, D11Mit7, D11Mit117, D11Mit35) mapping to the 11B3 chromosomal subband (12, 16). Figure 2 shows 17 different YAC clones obtained, visualized by hybridization with mouse Cot-1 DNA. To determine if any of these YACs contained the Lis-1 gene we hybridized the