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Positional Cloning and Phenotypic Characterization of a New Mutant Mouse with Neuronal Migration Abnormality

Chankyu Park[1] and Susan L Ackerman[2]

[1]*Department of Animal Biotechnology, Cheju National University, Cheju, 690-756, Korea*
[2]*The Jackson Laboratory, Bar Harbor, ME 04609 USA*

Abstract

Positional cloning (map-based cloning) of mutations or genetic variations has been served as an invaluable tool to understand *in-vivo* functions of genes and to identify molecular components underlying phenotypes of interest. Mice homozygous for the cerebellar deficient folia (*cdf*) mutation are ataxic, with cerebellar hypoplasia and abnormal lobulation of the cerebellum. In the *cdf* mutant cerebellum approximately 40% of Purkinje cells are ectopically located within the white matter and the inner granule cell layer (IGL). To identify the *cdf* gene, a high-resolution genetic map for the *cdf*-gene-encompassing region was constructed using 1997 F2 mice generated from C3H/HeSnJ-*cdf*/*cdf* and CAST/Ei intercross. The *cdf* gene showed complete linkage disequilibrium with three tightly linked markers *D6Mit208*, *D6Mit359*, and *D6Mit225*. A contig using YAC, BAC, and P1 clones was constructed for the *cdf* critical region to identify the gene. A deletion in the *cdf* critical region on chromosome 6 that removes approximately 150 kb of DNA was identified. A gene associated with this deletion was identified using cDNA selection. *cdf* mutant mice with the transgenic copy of the identified gene restored the brain abnormalities of the mutant mice. The positional cloning of *cdf* gene provides a good example showing the identification of a gene could lead to finding a new component of important molecular pathways.

Introduction

The goal of structural and functional genomics is to understand functions of genes in the genome and dissect out biological pathways associated with them at the molecular level. Together with the completion or near completion of sequencing of human and mouse genome, there has been a dramatic increase in the number of genes assigned their functions to specific phenotypes in mammals. In this presentation, the procedures and methodologies of positional

cloning will be addressed with the positional cloning of the *cdf* gene as an example.

Phenotypes of *cdf* mutant mice

The cerebellar deficient folia (*cdf*) is a recently identified mutation causing ataxia and cerebellar abnormalities including lobulation defects and Purkinje cell ectopia (Cook et al., 1997). Homozygous *cdf/cdf* mutants are easily classified visually at 2 weeks of age by pronounced slimness throughout the body and a constant side-to-side wobble. *cdf/cdf* mice weigh approximately 50% less than littermate controls at adulthood. Visibly, the cerebellar of *cdf/cdf* mice are noticeably hypoplastic and dysmorphic compared with those of sibling controls. Sagittal sections reveal that the midsagittal area of mutant cerebella is approximately 60% that of control brains. There are also notable differences in foliation pattern. The most interesting phenotype of *cdf* mice is that about 40% of Purkinje cells are ectopically located in granule cell layers and in the white matter of the cerebellum, indicating abnormality in the neuronal migration (Beierbach et al., 2001).

High-resolution genetic mapping of the *cdf* gene

A previous study showed that the *cdf* gene maps between *D6Mit16* and *D6Mit70*, at an estimated genetic distance of 2.64±0.62 cM at the 95% confidence interval (95% CI) (Cook et al., 1997). In order to enable positional cloning of the gene, high-resolution genetic mapping between *D6Mit16* and *D6Mit70* was performed by typing 1997 F2 progeny of (C3H/HeSnJ -*cdf/cdf* x Cast/Ei) F1 intercross representing 3994 meioses. From the mapping cross, 128 recombinants between *D6Mit16* and *D6Mit70* were identified and further genotyped with 18 partially ordered, intervening SSLP markers to finely map the *cdf* gene (Dietrich et al., 1994). The phenotypic data of 129 recombinants between *D6Mit16* and *D6Mit70* were analyzed. For the precise phenotyping, the phenotypes of important recombinants were reconfirmed by immunohistochemistry of the brain sections in addition to their behavioral phenotypes. The *cdf* gene showed complete linkage disequilibrium with three SSLP markers *D6Mit208*, *D6Mit359*, and *D6Mit225* with no recombination (Park et al., 2000). The *cdf* gene is, therefore, mapped to the region between the proximal flanking markers *D6Mit188/D6Mit209* and the distal flanking markers *D6Mit19/D6Mit246* with the recombination frequency of 11/3994 (0.28±0.23 cM, 95% CI).

Construction of a physical map for a *cdf* critical region

Overlapping YAC, BAC, and P1 clones were isolated. Considering the relatively large number of recombinants (n=11) for the nonrecombinant interval and complete cosegregation of the *cdf* gene with the *D6Mit208/D6Mit225/D6Mit359*, the conig building using BAC and P1 clones were concentrated around the *cdf* cosegregating markers for the gene identification analysis (Park et al., 2000). The rest of the nonrecombinant interval was covered by five YAC clones. The 19 new STSs were generated by the end-rescue process to build the contig map between *D6Mit209*

and *D6Mit246*. The entire nonrecombinant region is spanned by a minimum tiling path containing two YACs, one BAC, and one P1 clones. Based on the clone sizes, the maximum size of the nonrecombinant interval is estimated to be about 3 megabase (Mb) (minimum of about 2.5 Mb).

Identification of the *cdf* gene

Several contiguous STSs that were generated from YAC, BAC and P1 clone ends while building a genomic contig from the *cdf* critical region were failed to be amplified in PCR reactions using *cdf/cdf* genomic DNA, suggesting the presence of a deletion in the genome of *cdf* mutant mice. Based on the insert size of associated BAC clones and on pulse field gel analysis of restriction digested *cdf/cdf* DNA, this deletion was approximated at 150 kb. To identify transcripts that might be disrupted by the deletion, cDNA selection was performed using BAC clones and poly-(A)+ RNA from (E)14.5 embryos. Several cDNAs were identified that hybridized back to the BAC clone used for selection. They were corresponded to a gene partially deleted in *cdf* mice.

Transgenic rescue of *cdf* mutant mice

The *cdf*-associated deletion extends well into genomic sequence downstream from sequence encoding the identified gene deleted in *cdf* mice. Therefore, to confirm that mutation of the identified gene and not a linked gene is responsible for the *cdf* mutant phenotype, transgenic mice were generated with a cDNA of the identified gene under the control of the chicken β-actin promoter, previously shown to drive ubiquitous expression. To detect expression of the transgene, an IRES/LacZ sequence was inserted downstream of the *cdf* cDNA and upstream of the SV40 polyadenylation signal. Two founder lines, T53 and T79, were generated that express the P*cdf*-lacZ transgene in both the embryonic and adult brain.

Transgenic mice were mated to *cdf* heterozygotes, and F1 animals were intercrossed to generate *cdf/cdf* mice carrying the P*cdf*-lacZ transgene. Histological analysis of these mice and *cdf/cdf* non-transgenic littermates demonstrated an overall increase in cerebellar size and a normal cerebellar foliation pattern in transgenic animals. To determine if the positioning of Purkinje cells was rescued by the presence of the transgene, immunohistochemistry using anti-calbindin-D28 antibodies was performed. Only occasional Purkinje cells were observed in the white matter of *cdf/cdf* mice carrying the transgene, demonstrating that expression of deleted gene in *cdf* mice almost completely rescues Purkinje cell position. In addition, the packing density and lamination defects observed in the *cdf* mutant hippocampus were rescued by the transgene. The study of *cdf* gene should enable us to expand the knowledge on the molecular mechanisms of neuronal migration during the central nervous system development.

References

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