ELSEVIER

Neuroscience Letters 251 (1998) 5–8

Neuroscience Letters

A gene essential to brain growth and development maps to the distal arm of rat chromosome 12

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Received 11 May 1998; received in revised form 4 June 1998; accepted 4 June 1998

Abstract

A recently discovered, spontaneous, autosomal recessive mutation in rats, flathead (*fh*), results in greatly reduced brain growth beginning in late fetal development. In this study we have mapped the *fh* mutation by determining the pattern of segregation of polymorphic microsatellite markers with respect to *fh* in 51 affected F_2 offspring from a single interstrain intercross. Two markers on chromosome 12, D12Rat80 and D12Mgh6, cosegregated with the *fh* mutation in all 51 affected animals. The distribution of six additional markers in 40 informative meioses further localizes *fh* approximately 2 cM teleomeric to nos1. There are no known mutations in homologous regions of either mouse or human genomes that result in deficits in late neurodevelopment similar to that observed in *fh*/*fh* animals. The unique phenotype of *fh*/*fh* animals and the location of *fh* suggests the presence of a novel gene essential to normal brain development on the distal end of rat chromosome 12.

Keywords: Mutation; Chromosome 12; Genetic; Microcephaly; Epilepsy; Cell death

Spontaneous mutations have been tremendously valuable in identifying fundamental mechanisms underlying neural development in mammals. Genetic mapping of such mutations in both mice and humans has led to the identification of proteins essential to neuronal migration [7], differentiation, and survival [9,13]. In contrast to mouse, rat has lagged behind as a genetic model organism. Recently, however, there have been significant advances in genetic technology for mapping mutations in rat. In fact, there are now over 4000 microsatellite markers available for the rat genome. Consequently, it is now feasible to use a genetic approach in addition to well established cell culture [4–6], electrophysiological [11,12], and anatomical approaches [2] to study neurodevelopment in rat. Moreover, since novel mutations can often arise in different species, a genetic approach in rat will complement genetic experiments in mice and may lead to the identification of new genes essential to neurodevelopment.

A novel rat mutant, flathead (*fh*), was discovered in an inbred colony of Wistar rats at the University of Connecticut. Through both backcross and intercross experiments within this colony of Wistar rats (WUC1) the mutation was determined to segregate as a single autosomal recessive mutation with complete penetrance. *fh*/*fh* pups can be unambiguously identified at birth by their flattened skull (Fig. 1A). While the bodies of homozygous mutants are the same size as littermates at birth, the brains of *fh*/*fh* animals are dramatically reduced in size relative to unaffected littermates (Fig. 1B). Clinically, rats homozygous for the mutation display resting tremor, severe ataxia, and frequent spontaneous seizures that occur through a proscribed period during early postnatal development [16]. Mutants also have marked cellular dysgenesis in the retina, cerebellum, and dentate gyrus of the hippocampus [14,15].

In order to map this mutation we used a standard homozygosity mapping strategy. For these experiments, we first crossed a *fh*/+ male from the WUC1 colony, with 3 LEWcrl females (WUC1/*fh*/+xLEWcrl/+/+). As expected for an autosomal recessive mutation, none of the offspring from this first cross displayed the flathead phenotype, and through

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0304-3940/98/$19.00
PII S0304-3940(98)00478-9

both intercross and backcross experiments we determined that 1/2 of these F1 animals (8/16, three males and five females) were heterozygous for the *fh* mutation. We then intercrossed these eight heterozygous F1 animals (WUC1/*fh*/+/LEWcrl × WUC1/*fh*/+/LEWcrl). Sixteen litters from these matings contained a total of 219 animals, and consistent with a recessive mutation with nearly complete penetrance, 52 (23.6%) of these F2 offspring showed the flathead phenotype. Genomic DNA was purified from these animals and used in PCR simple sequence length polymorphism (sslp) assays to determine the distribution of polymorphic microsatellites. In an initial screen using polymorphic markers for chromosomes 1 (D1Mgh14), 2 (D2Mit4), 3 (D3Mgh3), 5 (D5Mgh3), 6 (D6Mit10), 7 (D7Mit12), 9 (D9Mgh1) and 12 (D12Mgh3), only D12Mgh3 showed a clear-cut cosegregation with *fh*. Indeed, 31 of the 51 affected F2 animals were homozygous for the allele from the affected parental strain (LOD score 8.6). We, therefore, focused our subsequent analyses on chromosome 12 using seven additional polymorphic sslp markers. Fifty of 51 *fh*/*fh* animals were homozygous for the affected parental alleles for both D12Rat31 and D12Rat25, LOD score 25, and all 51 *fh*/*fh* F2 offspring were homozygous for the affected parental allele for D12Mgh6 and D12Rat80 (LOD, 30.7). While there are several available sslp markers teleomeric to D12Mgh6 and D12Rat80, we have not found one that is polymorphic between the WUC1 and LEWcrl strains. Nevertheless, data from a total of 40 informative meioses (Fig. 2) place the *fh* mutation approximately 1 cM teleomeric to Rat31 and Rat25 in a locus highly linked to D12Mgh6 and D12Rat80.

The *fh* mutation maps to a region of rat chromosome 12 that is homologous to human chromosome 12 and mouse chromosome 5 in a locus between nos1 and tcf1 (Mouse Genome Database, The Jackson Laboratory) (Fig. 3). None of the several genes that have been mapped to this region in mouse have been shown to be specifically expressed in brain. In addition, mutations in genes in this interval in human and mouse have phenotypes that do not involve abnormalities in the central nervous system (CNS) [1,8,10]. In fact, the *fh*/*fh* phenotype does not appear to have an analogous one in the mouse. Histological analysis of *fh*/*fh* mutant rats through development indicate that the mutation is CNS specific and does not appear until late fetal development, approximately E18 [3,14,15]. At this time, there is a dramatic decrease in growth throughout the CNS and this is associated with increased apoptotic cell death in mutants [14,15]. While the approximately 10-fold increase in cell death in the mutant animals is greatest in proliferative zones, cell death in the mutant is increased in cell populations in all stages of development and throughout the CNS. It is unknown at this point whether the increased cell death is also associated with inappropriate migration, or differentiation. However, dysplasias in retina and cerebellum indicate that migration is disrupted by this mutation. The uniqueness of the *fh*/*fh* phenotype and the lack of brain specific candidate genes in this region of rat chromosome 12 suggests that the *fh*

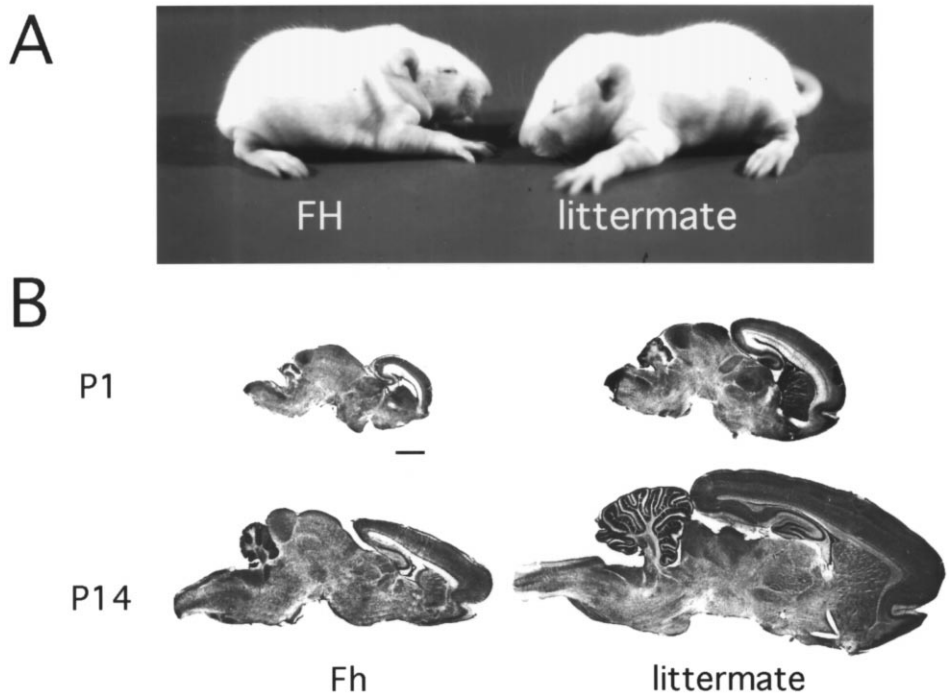

Fig. 1. The *fh*/*fh* phenotype. (A) A *fh*/*fh* animal, left, and an unaffected littermate, right, on postnatal day 10. (B) Sagittal sections through the brain of *fh*/*fh* animals and unaffected littermates at P1 and P14. The entire CNS is reduced in size and the cerebral cortices and cerebellum are greatly reduced in size relative to the midbrain. Sections were prepared on a vibratome and stained with cresyl violet. Scale bar, 1 mm.

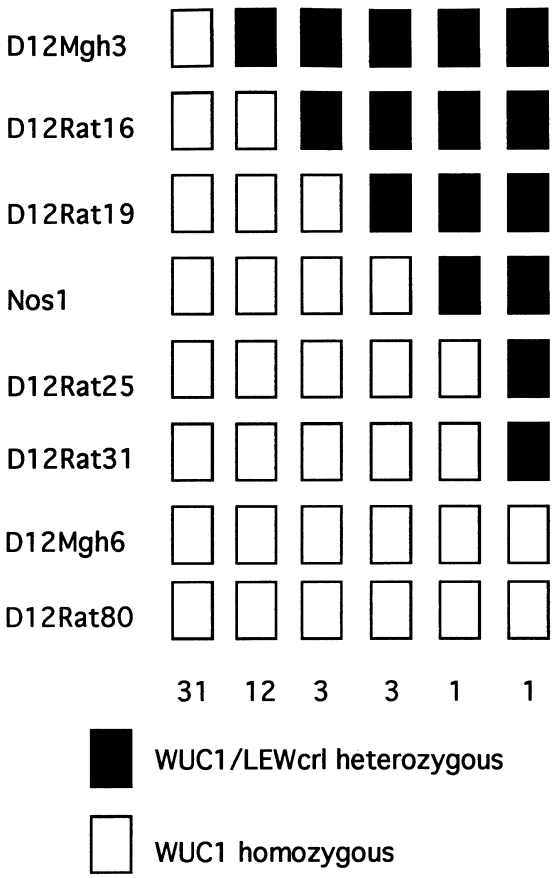

Fig. 2. Distribution of eight sslp markers on chromosome 12 in all 51 *fh*/*fh* F2 animals. Thirty-one animals were homozygous for all markers. Twenty offspring were heterozygous for some markers, and all 51 of 51 animals were homozygous for the WUC1 allele at D12Mgh6 and D12Rat80. MapPairs[tm] (Research Genetics) PCR primers were used to amplify simple sequence length polymorphisms (sslps). A total of 32 MapPairs were used in this study, of which we experimentally determined that 16 were polymorphic between the WUC1 and LEWcrl strains. Target DNA was purified from spleens and amplified by PCR, 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s for 35 cycles, and analyzed by polyacrylamide gel electrophoresis.

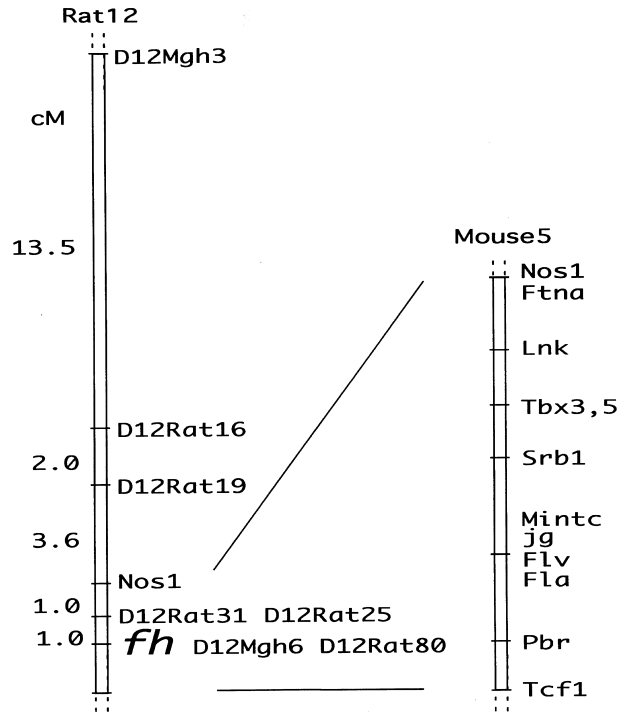

Fig. 3. Map of rat chromosome 12 as determined in this study. The *fh* mutation is approximately 2 cM distal from nos1 and linked to Mgh6 and Rat80. On the right is the homologous region of the mouse genome, chromosome 5, and the genes that have been mapped to this interval. All data obtained from the distribution of sslp markers was analyzed with the aid of MapManager. For linkage and map distances we used intercross statistics with 99% confidence interval. Markers were ordered on the map of chromosome 12 to minimize the number of crossovers. The order of markers was found to be identical to the order of markers in release 4 of the rat genome project reported by the Whitehead Institute/MIT Center for Genome research (http://www.genome.wimit.edu/rat/public/).

mutation involves a novel gene essential to coordinated growth throughout the CNS.

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