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Research report

Behavioral and neuromorphological characterization of a novel *Tuba1* mutant mouse

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ABSTRACT

As part of the RIKEN large-scale N-ethyl-N-nitrosourea (ENU) mutagenesis project, we screened mice with a dominant mutation that exhibited abnormal behavior using an open-field test and a home-cage activity test. We tested 495 male progeny of C57BL/6J males treated with ENU and untreated C3H/HeJ females using the open-field test and isolated behavioral mutant M101736, which exhibited a significant increase in spontaneous locomotor activity. We identified a missense mutation in the *Tuba1* gene, which encodes the TUBA1 protein, and designated the mutant gene *Tuba1*^{Rgsc1736}. This mutation results in an aspartic acid to glycine substitution in the TUBA1 protein. Detailed analyses revealed that *Tuba1*^{Rgsc1736} heterozygotes exhibited inattention to novel objects and aberrant patterns of home-cage activity. The results of a behavioral pharmacological analysis using methylphenidate and morphological analyses of embryonic and adult brains suggested that *Tuba1*^{Rgsc1736} is a novel animal model for neurodevelopmental disorders.

1. Introduction

N-ethyl-N-nitrosourea (ENU) is an effective chemical mutagen that introduces single base-pair changes into genomic DNA [1,2]. Several large-scale saturation ENU mutagenesis projects have been undertaken to generate large numbers of mutants, enabling the systematic investigation of gene functions *in vivo* [3–5]. The aim of our RIKEN mutagenesis project is to generate mouse models of human diseases, including diabetes, hypertension, cancer, and deformities, and we have been screening dominant mutant mice for visible, clinical biochemical, and hematological abnormalities and have succeeded in establishing unique models of human disease [6,7].

In 2007, the *Jenna* (*Jna*) mutant mouse induced by ENU was reported [8]. This mouse carried a missense mutation in *Tubulin alpha 1* (*Tuba1*) and exhibited hyperactivity in the open-field test and abnormal neuronal migration. The *Jna* mutant mouse displayed a phenotypic similarity to lissencephaly patients, and Keays et al. identified *de novo* mutations in *TUBA3*, the human homolog of *Tuba1* [8]. We have conducted behavioral screenings including the open-field test, the passive-avoidance test, and the home-cage activity test to establish mouse models of psychiatric disorders [7,9]. In this screening, a novel *Tuba1* mutant that exhibited hyperactivity in the open-field test was isolated and named M101736. Here, we report the results of a genetic analysis, a behavioral pharmacological analysis using the psychostimulant methylphenidate (MPH), and neuromorphological analyses of the M101736 mutant. Based on these findings, we discuss the possible application of this mutant as a novel model for a neurodevelopmental disorder.

2. Materials and methods

2.1. Animals and production of ENU mutant mice

All the animal studies were performed in accordance with the guidelines issued by the RIKEN Bioscience Technology Center in their "Outline for Conducting Animal Experiments" (August 1999, revised October 2001). The guidelines were approved by the ethics committee of the RIKEN Tsukuba Institute, and the committee prescribed the minimization of pain and discomfort experienced by the experimental animals. A large-scale mouse ENU mutagenesis was conducted as described previously [7,10] with some modifications. C57BL/6J (B6) and C3H/HeJ (C3H) mice were purchased from a commercial supplier (CLEA Japan, Inc., Tokyo, Japan). The following strategy is outlined in Supplementary data 1. B6 males were treated with ENU (150–250 mg/kg) using an intraperitoneal (i.p.) injection and crossed with C3H females. The progeny generated by this cross were designated as G1 mice (Supplementary data 1A). To obtain control data for genetic mapping and to confirm the phenotypic transmission, C3H females and (C3H × B6)F1 mice were crossed. This cross yielded H × HB mice (Supplementary data 1B). The G1 mice were then backcrossed with C3H mice 6 times for inheritance testing, gene mapping, and the elimination of other ENU-induced mutations (Supplementary data 1C). The N7-male

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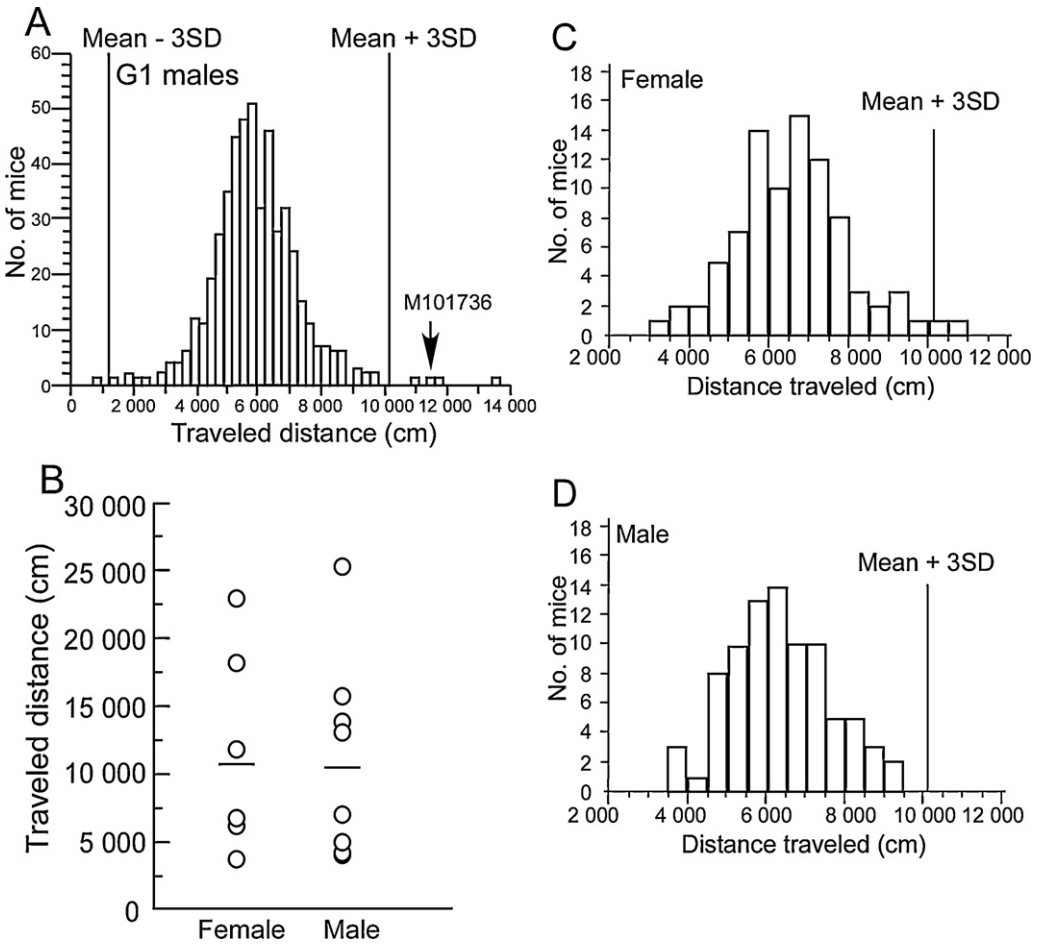

Fig. 1. Histograms of the open-field activity of G1 mice and control mice (H × HB), and phenotypic distribution of N2 progeny of the M101736 founder mouse. (A) Histograms of the open-field activity of G1 mice. The ordinate and abscissa indicate the number of mice and the distance traveled, respectively. The vertical line in the histograms indicates the population greater than 3 standard deviations above the mean. Spontaneous activity during the open-field test was normally distributed. Mice whose activity level was more than 3 standard deviations above the mean (the mean was 5887.2 cm for G1 males) were isolated as mutant candidates. The vertical arrow indicates the activity level of the M101736 mouse. (B) Distribution of the locomotor activity of the N2 progeny of the M101736. The ordinate indicates distance traveled. The horizontal line indicates the locomotor activity criterion that divides wild-type mice and mutant mice (3 standard deviations above the mean in the H × HB population). (C and D) Histograms of the open-field activity of control mice. The vertical line in the histograms indicates the population greater than 3 standard deviations above the mean. Spontaneous activity during the open-field test was normally distributed. N2 progeny of M101736 whose activity level was more than 3 standard deviations above the mean (the mean was 6595.7 cm for females, 6350.1 for males) obtained from H × HB (female, $n = 87$; male, $n = 84$) were designated as hyperactive mice.

mice were used in the behavioral characterization to eliminate the influence of the female estrous cycle.

2.2. Phenotype screening and gene mapping

We used locomotor activity in the open field as an index of abnormal behavior. A total of 495 G1 male mice were screened for abnormal open-field activity at 9 weeks of age. Mice that showed high or low levels of activity (greater than the mean ± 3 S.D. of the G1 population) were classified as behavioral phenodeviants and crossed with wild-type C3H mice to test for phenotypic transmission and genetic mapping. A total of 236 N2-7 mice (Supplementary data 1C) between 10 and 12 weeks of age were collected and tested for spontaneous locomotor activity in the open field. A genome-wide scan was conducted as described previously, with some modifications [7]. Single nucleotide polymorphism (SNP) markers spaced at 10 cM were chosen from the Mouse SNP database (http://www.broad.mit.edu/snp/mouse/) and used for genome-wide scanning. We used microsatellite or SNP markers for a detailed linkage analysis. Candidate causative genes located near the mapped locus were identified by searching a positional cloning assistant database (Positional MEDLINE, otherwise known as PosMed; http://omicspace.riken.jp/PosMed/") [11]. The genomic structures of the candidates were determined using the mouse Ensembl database (http://www.ensembl.org/Mus_musculus/index.html), and the coding regions were directly sequenced.

2.3. Mutation analysis of the Tuba1 gene

The A–G point mutation in exon 2 of *Tuba1* was identified using the allele-specific primer-polymerase chain reaction (ASP-PCR) and direct sequencing. ASP-PCR was performed using the following primers: forward primer for the wild-type allele, ACCATTGGGGGAGGAGATGA; forward primer for the mutant-type allele, ACCATTGGGGGAGGAGATGG; reverse primer for the wild-type and mutant alleles, AAGCTCACTCACCCTTCTGG.

2.4. Open-field test

Naive mice were placed in the corner of an open-field apparatus (400 mm wide × 400 mm long × 300 mm high; O'Hara & Co., Ltd., Tokyo, Japan) made of white polyvinyl chloride. The distance traveled by each animal in the open field was recorded for 20 min with a video imaging system (Image OF9; O'Hara & Co., Ltd.).

2.5. Home-cage activity test

Each mouse was placed alone in a testing cage (227 mm × 329 mm × 133 mm) under a 12-h light–dark cycle (lights on at 8:00) and had free access to both food and water. After one day of acclimation, the spontaneous activity in the cage was measured for 5 days (starting at 08:00) with an infrared sensor (AB system 4.0; Neuroscience Co., Ltd., Tokyo, Japan).

2.6. Object-exploration test

We used a previously described procedure [7,12] with some modifications. Each mouse was placed in an open-field apparatus without a novel object for 20 min of acclimation before testing. A transparent acrylic tube (bottom diameter, 66 mm; top diameter, 44 mm; height, 154 mm) containing marbles was placed in the center of the open field. The total time spent exploring the object and the frequency of exploration during a 10-min period were determined using a video imaging system