FEBS Letters 587 (2013) 54–59

FEBS Letters

journal homepage: www.FEBSLetters.org

Autistic-like behavioral phenotypes in a mouse model with copy number variation of the *CAPS2/CADPS2* gene

Tetsushi Sadakata [a,b,d,*], Yo Shinoda [c,d], Megumi Oka [a], Yukiko Sekine [b], Teiichi Furuichi [b,c,d,*]

[a] *Advanced Scientific Research Leaders Development Unit, Gunma University, Maebashi, Gunma 371-8511, Japan*
[b] *Laboratory for Molecular Neurogenesis, RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan*
[c] *Department of Applied Biological Science, Tokyo University of Science, Noda, Chiba 278-8510, Japan*
[d] *JST/CREST, Kawaguchi, Saitama 332-0012, Japan*

ARTICLE INFO

Article history:
Received 2 July 2012
Revised 24 September 2012
Accepted 29 October 2012
Available online 15 November 2012

Edited by Jesus Avila

Keywords:
Autism
Copy number variation
Social interaction
Anxiety

ABSTRACT

Ca^{2+}-dependent activator protein for secretion 2 (CAPS2 or CADPS2) facilitates secretion and trafficking of dense-core vesicles. Recent genome-wide association studies of autism have identified several microdeletions due to copy number variation (CNV) in one of the chromosome 7q31.32 alleles on which the locus for *CAPS2* is located in autistic patients. To evaluate the biological significance of reducing *CAPS2* copy number, we analyzed *CAPS2* heterozygous mice. Our present findings suggest that adequate levels of CAPS2 protein are critical for normal brain development and behavior, and that allelic changes due to CNV may contribute to autistic symptoms in combination with deficits in other autism-associated genes.

1. Introduction

Autism is a severe neurodevelopmental disorder marked by profound disturbances in social, communicative, and behavioral functioning [1,2]. Epidemiological studies have shown that the prevalence of autism spectrum disorders is 3–6 per 1000, with a male-to-female ratio of 3:1 [3]. The concordance rate in monozygotic twins is about 90%, whereas that in dizygotic twins is 10% [4], suggesting that autism has a prominent genetic component. Recent genetic and genomic studies have identified a large number of candidate genes for autism [5], many of which encode proteins related to the formation or function of synapses, indicating that synaptic dysfunction may play a critical role in autism [6–12].

The Ca^{2+}-dependent activator protein for secretion (CAPS/CADPS) family consists of two family members, CAPS1 [13] and CAPS2, in mammals [14,15]. Previous studies have indicated that CAPS is involved in the trafficking of dense-core vesicles (DCVs) during their exocytosis [13,16–18]. The human *CAPS2* gene maps to human chromosome 7q31.32 [19], which is located within a susceptibility locus for autism, 7q31–q33, named the autism susceptibility locus 1 (AUTS1) [20]. Mouse CAPS2 protein is immunohistochemically co-localized with brain-derived neurotrophic factor (BDNF) throughout various brain regions [21] and is associated with BDNF-containing secretory vesicles [15]. BDNF is activity-dependently released from neurons [22–25] and plays a key role in many aspects of brain development and function, including the formation of synapses and circuits [26,27]. CAPS2 is involved in the activity-dependent release of BDNF [15] and promotes its secretion kinetics [28]. Moreover, a study of CAPS2 in autism showed that a subgroup of autistic patients expresses aberrant exon 3-skipped CAPS2 that is not properly transported into axons, presumably resulting in a deficit in local synaptic BDNF release [29]. In addition, seven non-synonymous single-nucleotide variations of the *CAPS2* gene were identified in patients with autism [29]. Therefore, we hypothesized that CAPS2 is a candidate autism susceptibility gene [29,30]. Recent genome-wide association studies have shown the presence of several microdeletions within 7q31.32, including the *CAPS2* locus, in single alleles of autistic patients [31,32]. Moreover, it was recently suggested that transcriptional and splicing dysregulation are the mechanisms underlying neuronal dysfunction in patients with autism [33]. In that report, Voineagu and colleagues showed decreased transcription of *CAPS2*

Abbreviations: CAPS2, Ca^{2+}-dependent activator protein for secretion 2; CNV, copy number variation; DCVs, dense-core vesicles; AUTS1, autism susceptibility locus 1; BDNF, brain-derived neurotrophic factor; USV, ultrasonic vocalization

* Corresponding authors. Address: Advanced Scientific Research Leaders Development Unit, Gunma University, Maebashi, Gunma 371-8511, Japan (T. Sadakata). Fax: +81 4 7124 2150.

E-mail addresses: sadakata-1024@umin.ac.jp (T. Sadakata), tfuruichi@rs.tus.ac.jp (T. Furuichi).

0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2012.10.047

in autistic brains [33]. However, the biological effects of reduced transcription of the *CAPS2* gene are unknown.

In this report, we examined the effects of the loss of the *CAPS2* gene from one allele on behavioral phenotypes by utilizing *CAPS2* heterozygous mice expected to be equivalent to patients with a copy number reduction and decreased transcription. We found that *CAPS2* heterozygotes showed impairments in adaptation to a novel environment, ultrasonic vocalization (USV) and circadian behavior, suggesting that reduction of *CAPS2* expression partly contributes to the onset of autistic-like symptoms.

2. Materials and methods

2.1. Behavioral tests

Breeding and all tests were basically performed as described in a previous study [29]. Briefly, mice were housed with a 12:12 h light–dark cycle, with the dark cycle occurring from 20:00 to 8:00. All mice used in the experiments below were littermates from mated heterozygotes unless otherwise noted. The experimenter was blind to the genotype in all behavioral tests.

2.1.1. Open field test and novel object recognition test

The test was performed as previously described [34,35] with minor modifications. Two-month-old male mice were used. Locomotor activity was measured with an open field apparatus (60 × 60 cm) at 50 lux (at the surface level of the area). Each mouse was placed in the center of the open field, and its horizontal movements were monitored for 15 min with a video camera. The images were processed using NIH IMAGE O.F. software (O'Hara & Co., Tokyo, Japan). Total activity was used in the statistical analysis.

2.1.2. Vocalization

Ultrasonic vocalization (USV) was monitored before behavioral testing between P5 and P7. Wild-type females were mated with wild-type or homozygote males. The pups in the home cage were separated from their dam immediately before recording and were positioned in the sound-attenuating chamber below a Condenser Ultrasound Microphone (cat. No. 40011, Avisoft Bioacoustics) to detect USVs at 50–100 kHz. The number of calls was recorded and quantified using Avisoft-RECORDER software (Avisoft Bioacoustics). Call frequency was measured as the number of USVs an animal made during the 10-min test.

2.1.3. Recording of circadian rhythms

The circadian rhythms were recorded as previously described [36]. Two-month-old male mice were used. Wheel-running was measured using a wheel-meter (WW-3302, O'Hara & Co.) with 20 sets of a room (14.3 cm wide × 14.8 cm high × 29.3 cm deep) and a wheel-cage (50 cm lap × 5 cm wide). Mice were able to move freely and given free access to solid food and water. Every 1/3 revolution of the wheel-cage was recorded as 1 count. The circadian period was calculated using the chi-square periodogram.

Other methods are provided in the Supplementary data.

3. Results

Fig. 1A shows the region of two different microdeletions reported in autistic patients [31,32]. One of the deletions (del-1, 748 kb) almost corresponds to the entire region of the *CAPS2* gene. Thus, we thought that *CAPS2* heterozygous mutant mice would be almost equivalent to patients with the del-1 microdeletion with respect to any dosage effect (= copy number) of the *CAPS2* gene. We first examined the expression levels of CAPS2 protein in the brains of heterozygous mice by western blotting. Heterozygous mice showed a reduced intensity of CAPS2-immunopositive bands in protein extracts from the cerebellum, hippocampus and neocortex (Fig. 1B and C), indicating that heterozygous mice have reduced CAPS2 protein levels in the brain.

To investigate the effect of decreased CAPS2 protein on BDNF secretion, we examined constitutive and regulated BDNF release from primary cell cultures of wild-type and heterozygous mice. We exogenously overexpressed BDNF in cerebellar cultures at 5 DIV using a recombinant adenoviral vector [15]. In this system, granule cells, which are the predominant cell type expressing CAPS2 in mouse cerebellum [15,21], are preferentially infected with the adenovirus vector [37]. At 48 h after infection with the adenovirus vector, cultures expressing either BDNF-GFP (Ad-BDNF-GFP) or GFP alone (Ad-GFP) at 7 DIV were treated for 15 min with media containing 5 mM KCl as a control, or 50 mM KCl to induce membrane depolarization. BDNF secretion from heterozygous cultures was decreased compared with that from wild-type cultures by both 5 mM KCl and 50 mM KCl media (Fig. 1D). These results suggest that a decrease in CAPS2 protein affects both constitutive and regulated BDNF release from cerebellar dissociated cultures.

We then examined the behavioral phenotypes of *CAPS2* heterozygous mice. We used male mice for the analyses of behavior except for the analysis of maternal behavior. *CAPS2* heterozygous mice placed in an open field in the light cycle showed normal locomotor activity (Fig. 2A) and speed (Fig. 2B) in comparison with their wild-type littermates. There was no significant difference between wild-type and *CAPS2* heterozygous mice in terms of the time spent in a central area (Fig. 2C), which is generally accepted as a measure of fear in rodents [38]. However, as shown in Fig. 2D, *CAPS2* heterozygous mice became less active than wild-type mice when placed in an open field containing a novel object (the black-and-white vertical object shown in the inset of Fig. 2D) at its center (3958.1 ± 181.7 cm in wild-type vs. 3071.7 ± 217.4 cm in *CAPS2* heterozygotes, mean ± SEM, $P = 0.0053$, by Student's *t*-test). Moreover, *CAPS2* heterozygous mice showed decreased speed in comparison with their wild-type littermates (4.40 ± 0.20 cm/s in wild-type vs. 3.42 ± 0.24 cm/s in *CAPS2* heterozygotes, mean ± SEM, $P = 0.0052$, by Student's *t*-test) (Fig. 2E). However, there was no significant difference in the time spent in a central area between wild-type and *CAPS2* heterozygous mice (Fig. 2F).

We next administered elevated plus maze tests. There was no difference in moving distance, suggesting that locomotor activity was not affected by the decrease in the level of CAPS2 protein (Fig. 3A). There was also no difference in time spent in the open and closed arms of the apparatus (Fig. 3B, C). In addition, the number of open arm entries was not decreased (data not shown).

A light/dark box experiment was also carried out (Fig. 3D–F). There were no differences in anxiety-like behavior, as measured in the light/dark box experiment for distance (Fig. 3D), in time spent in the light portion of the box (Fig. 3E), or in the latency to transition between the light and dark portions (Fig. 3F).

Next, social interaction tests were performed. Wild-type and *CAPS2* heterozygous mice were placed in the center of an open field. Another C57BL/6J mouse in a small cage was placed in the corner of the open field. *CAPS2* heterozygous mice placed in an open field showed normal locomotor activity (Fig. 4A) and speed (Fig. 4B). Moreover, there was no difference between wild-type and *CAPS2* heterozygous mice in the time spent interacting with the small cage containing the unfamiliar mouse (Fig. 4C). In a control experiment, we found that there was also no difference between wild-type and *CAPS2* heterozygous mice in the time spent interacting with an empty small cage (i.e. not containing an unfamiliar mouse) (data not shown).