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Behavioral and gene expression analyses of *Wfs1* knockout mice as a possible animal model of mood disorder

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Abstract

Wolfram disease is a rare genetic disorder frequently accompanying depression and psychosis. Non-symptomatic mutation carriers also have higher rates of depression and suicide. Because *WfS1*, the causative gene of Wolfram disease, is located at 4p16, a linkage locus for bipolar disorder, mutations of *WfS1* were suggested to be involved in the pathophysiology of bipolar disorder. In this study, we performed behavioral and gene expression analyses of *Wfs1* knockout mice to assess the validity as an animal model of mood disorder. In addition, the distribution of *Wfs1* protein was examined in mouse brain. *Wfs1* knockout mice did not show abnormalities in circadian rhythm and periodic fluctuation of wheel-running activity. Behavioral analysis showed that *Wfs1* knockout mice had retardation in emotionally triggered behavior, decreased social interaction, and altered behavioral despair depending on experimental conditions. *Wfs1*-like immunoreactivity in mouse brain showed a similar distribution pattern to that in rats, including several nuclei potentially relevant to the symptoms of mood disorders. Gene expression analysis showed down-regulation of *Cdc42ep5* and *Rnd1*, both of which are related to Rho GTPase, which plays a role in dendrite development. These findings may be relevant to the mood disorder observed in patients with Wolfram disease.

Keywords: Wolframin; Wolfram disease; Depression; Bipolar disorder; DNA microarray; Forced swimming test

1. Introduction

Wolfram disease (Online Mendelian Inheritance in Man [OMIM] 222300) is a rare autosomal recessive neurodegenerative disorder characterized by early-onset diabetes mellitus, progressive optic atrophy, diabetes insipidus, and deafness (Domenech et al., 2006); *WfS1*/*wolframin* has been identified as the causative gene (Strom et al., 1998; Inoue et al., 1998). Approximately, 60% of the patients with Wolfram disease have mental symptoms, such as severe depression, psychosis, impulsivity, and aggression (Swift et al., 1990). More importantly, carriers of *WfS1* mutations, who are not affected with Wolfram disease, have a 26-fold higher likelihood of psychiatric hospitalization mainly due to depression (Swift and Swift, 2000). The *WfS1* gene locates at 4p16.1 (Strom et al., 1998; Inoue et al., 1998), a replicated linkage locus of bipolar disorder (Ewald et al., 1998, 2002; Detera-Wadleigh et al., 1999). Some studies showed that bipolar disorder with psychosis (Als et al., 2004; Cheng et al., 2006) or suicidal behavior (Cheng et al., 2006) is linked with this locus. These lines of evidence suggested the possible role of *WfS1* mutations in the pathophysiology of bipolar disorder and related phenotypes.

To date, mutation screening of the *WfS1* gene has been reported in 84 patients with bipolar disorder, 54 with major depression, 119 with schizophrenia, 100 suicide victims, 3 with schizoaffective disorder, and several other patients with other psychiatric diagnoses (Ohtsuki et al., 2000; Martorell et al., 2003; Torres et al., 2001; Crawford et al., 2002; Evans et al., 2000). However, none of these patients had mutations causing Worfram disease.

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Despite the fact that *WfS1* mutations may not be a frequent cause of mental disorders, the mechanism underlying how *WfS1* mutations lead to mental symptoms in patients with Wolfram disease will shed light on the pathophysiology of mood disorders. Mice lacking the *Wfs1* gene might be useful as a genetic animal model of mood disorders.

The symptoms of Wolfram disease resemble those of mitochondrial diseases and, indeed, initial studies suggested mitochondrial dysfunction in Wolfram disease based on mitochondrial DNA (mtDNA) deletions found in patients (Rotig et al., 1993). However, the protein coded by *WfS1* was found to be localized in endoplasmic reticulum (ER) (Takeda et al., 2001; Philbrook et al., 2005). *WfS1* expression was induced by ER stress (Fonseca et al., 2005) or *XBP1* overexpression (Kakiuchi et al., 2006), and disruption of *Wfs1* caused a dysfunctional ER stress response (Fonseca et al., 2005; Riggs et al., 2005; Yamada et al., 2006). Recent studies have provided insight into the function of *WfS1* protein; *WfS1* induces cation channel activity on ER membranes (Osman et al., 2003) and regulates calcium levels in ER (Takei et al., 2006). It also plays a role in stimulus-secretion coupling for insulin exocytosis in pancreatic β cells (Ishihara et al., 2004). Disruption of *Wfs1* increased vulnerability to cell death in the knockout (KO) mice (Ishihara et al., 2004; Philbrook et al., 2005; Riggs et al., 2005; Yamada et al., 2006). In the rat brain, *WfS1* was distributed predominantly in neurons of the so-called limbic system (Takeda et al., 2001). *WfS1* mutations could lead to loss of *WfS1*-expressing neurons in particular brain regions of patients with Wolfram disease, which may underlie progression of mental symptoms.

In this study, we performed behavioral analysis of *Wfs1* KO mice to characterize their behavioral abnormality. We previously developed neuron-specific mutant polymerase γ-transgenic mice (mPolg Tg mice) based on a mitochondrial dysfunction hypothesis of bipolar disorder (Kato and Kato, 2000) and demonstrated that these mice had bipolar disorder-like phenotypes, such as altered circadian rhythm and periodic fluctuation of wheel-running activity (Kasahara et al., 2006). Whether or not the *Wfs1* KO mice show such wheel-running activity was examined. A behavioral test battery was also conducted to search for other behavioral phenotypes. Distribution of *Wfs1* in the brain was examined to search for the neural basis of behavioral alteration. In addition, gene expression analysis was performed to search for the molecular basis of behavioral phenotypes of *Wfs1* KO mice.

2. Experimental procedures

2.1. Generation of Wfs1 KO mice

The methods for the generation of *Wfs1* KO mice have been described elsewhere (Ishihara et al., 2004). In brief, a neomycin-resistance gene was inserted into exon 2 of the *Wfs1* gene in the targeting vector. The targeting vector was injected into 129Sv embryonic stem (ES) cells, and the ES cells with homologous recombination were obtained. By crossing the chimeric mice with C57BL/6J (B6) mice, *Wfs1* heterozygous KO mice were obtained. Genotyping was performed as previously described (Ishihara et al., 2004). The heterozygous KO mice were crossed with the B6 mice for at least eight generations before the analysis. The mice were maintained in a 12-h light:12-h dark cycle, except for several specific experiments as indicated. Wild-type (WT) littermates were used for the control whenever possible. All animal experiments were approved by the local animal experiment committees of RIKEN and Behavioral and Medical Sciences Research Consortium (BMSRC) (Akashi, Japan). Animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize the number of animals used and their suffering.

2.2. Wheel-running activity

For this analysis, 11 homozygous KO mice ($Wfs1^{-/-}$) and 9 WT littermates ($Wfs1^{+/+}$) were used. All were males aged 34 weeks at the initiation of the analysis. The groups did not differ significantly in body weight.

The methods for the analysis of wheel-running activity were described in detail elsewhere (Kasahara et al., 2006). In brief, mice were individually housed in cages (width, 24 cm; depth, 11 cm; height, 14 cm) equipped with a steel wheel (width, 5 cm; diameter, 14 cm) (O'Hara & Co., Tokyo, Japan). Wheel-running activity was monitored by measuring the rotation of the wheel (3 counts/1 rotation). Food and water were available ad libitum. The data of initial 7–10 days were omitted from the analysis. Delayed and anticipatory activity indices, referring to the wheel-running activity during the initial 3 h of a light phase and that during the last 3 h of a light phase, were calculated. The periodicity of wheel-running activity was assessed by Lomb-Scargle periodogram (Kasahara et al., 2006).

The Mann–Whitney *U*-test was used for statistical analyses. Significance levels were set at 0.05 (two-tailed; d.f., degree of freedom). The average and standard error of mean (S.E.M.) were presented for each experimental parameter in one group.

2.3. Behavioral analysis: phase I. Screening by a test battery

This analysis was performed at BMSRC (Akashi, Japan). For this analysis, 14 homozygous KO mice ($Wfs1^{-/-}$), 14 heterozygous KO mice ($Wfs1^{-/+}$), and 13 WT littermates ($Wfs1^{+/+}$) were analyzed. All were males aged 12 weeks at the initiation of the behavioral analysis. The analyses were performed in the order of open-field test, startle response and prepulse inhibition test, elevated plus maze, Morris water maze, passive avoidance learning, active avoidance learning, and forced swimming test. After the behavioral test battery, the non-fasting blood glucose level was examined to rule out the possibility that elevated blood glucose levels might affect the results of behavioral analysis. There was no significant difference among the genotypes, consistent with a previous report that there was no apparent increase in blood glucose levels in *Wfs1* KO mice on the B6 background (Ishihara et al., 2004).

2.3.1. Open-field test

A transparent cubic box without a ceiling (30 cm × 30 cm × 30 cm) was placed in a ventilated sound-attenuating chamber. A 40-W white lamp provided room lighting, which was approximately 110 lx on the floor of the chamber. In addition, a fan attached on the upper part of the wall at one end of the chamber presented a masking noise of 45 dB. Two infrared beams were set on each wall 2 cm above the floor with an interval of 10 cm. The total number of successive interceptions of two adjoining beams on each bank was scored as locomotion behavior. The other 12 infrared ray beams were attached 4.5 cm above the floor in 2.5-cm intervals, and the total number of vertical beam interceptions was scored as rearing behavior. Each mouse was allowed to explore freely in the open-field area for 20 min.

For statistical analysis, repeated measures analysis of variance (ANOVA) with the intrasubject factor of time (1–20 min) and the intersubject factor of genotype (−/−, +/−, and +/+) was applied.

2.3.2. Startle response and prepulse inhibition (PPI)

Each mouse was enclosed in a transparent acrylic box (7 cm × 7 cm × 10 cm). Startle response was detected as vibration of the box, using an accelerometer (GH-313A, Keyence, Osaka, Japan). The acoustic startle pulse of broadband burst (115 dB, 50 ms) and tone prepulse (85 dB, 30 ms) were presented via a speaker located in front of the box. Light prepulse (30 ms) was