Therapeutic implications of down-regulation of cyclophilin D in bipolar disorder

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Abstract
We previously reported that neuron-specific mutant Polg1 (mitochondrial DNA polymerase) transgenic (Tg) mice exhibited bipolar disorder (BD)-like phenotypes such as periodic activity change and altered circadian rhythm. In this study, we re-evaluated two datasets resulting from DNA microarray analysis to estimate a biological pathway associated with the disorder. The gene lists were derived from the comparison between post-mortem brains of BD patients and control subjects, and from the comparison between the brains of Tg and wild-type mice. Gene ontology analysis showed that 16 categories overlapped in the altered gene expression profiles of BD patients and the mouse model. In the brains of Tg mice, 33 genes showed similar changes in the frontal cortex and hippocampus compared to wild-type mice. Among the 33 genes, SFQ and PPIF were differentially expressed in post-mortem brains of BD patients compared to control subjects. The only gene consistently down-regulated in both patients and the mouse model was PPIF, which encodes cyclophilin D (CypD), a component of the mitochondrial permeability transition pore. A blood–brain barrier-permeable CypD inhibitor significantly improved the abnormal behaviour of Tg mice at 40 mg/kg.d. These findings collectively suggest that CypD is a promising target for a new drug for BD.

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Key words: Bipolar disorder, cyclophilin D, DNA microarray, mtDNA, NIM811.

Introduction
Bipolar disorder (BD) is a serious mental disorder accompanied by extreme mood swings from mania to depression. Recent genome-wide association studies identified candidate genes related to calcium signalling, such as ANK3 and CACNA1C (Ferreira et al. 2008) as being involved in the development of BD. Lithium can prevent relapse, but many patients do not respond to it or cannot tolerate the side-effects. Other drugs currently used to treat BD such as carbamazepine, valproate, or atypical antipsychotics were initially developed for treatment of epilepsy or schizophrenia. So far there has been no instance of successful development of a new mood stabilizer based on the pathophysiological mechanism of the disease (Kato, 2007). This is mainly ascribed to a paucity of animal models.

Several studies have suggested that mitochondria play a role in the pathophysiology of BD (Kato, 2008; Kato & Kato, 2000; Stork & Renshaw, 2005) based on abnormalities found by using magnetic resonance spectroscopy (Dager et al. 2004; Kato et al. 1993; Kato et al. 1994; Stork & Renshaw, 2005). These abnormalities resemble those of mitochondrial diseases. Recently, an elevated lactate level in cerebrospinal fluid was also reported in BD patients (Regenold et al. 2009). Moreover, a hereditary mitochondrial disease, chronic progressive opthalmoplegia (CPEO), sometimes exists as a comorbidity with BD or depression (Kasahara et al. 2006; Suomalainen et al. 1992). Mitochondrial DNA (mtDNA) polymerase (polymerase γ; Polg1) is one of the causative genes for CPEO. We generated transgenic (Tg) mice with forebrain-specific expression of mutant Polg1 (mutPolg1) as a putative animal model for BD (Kasahara et al. 2006). The mutant mice showed distorted diurnal rhythm and periodic fluctuation of activity level in long-term recording of wheel running. Mitochondria isolated from the brains of these mice showed an
enhanced Ca\(^{2+}\) uptake rate (Kubota et al. 2006). These findings suggest that the mutPolg1 Tg mouse could serve as an animal model for BD.

However, most BD patients do not have POLG1 mutations. To establish a new drug target by using the animal model, we believe it is crucial to identify the downstream event of the Polg1 mutation that relates to BD-like phenotypes. The aim of this study was to identify pathways that may be involved in the pathophysiology of BD and potential drug targets for BD.

In this study, we searched for genes whose expression was commonly altered in the brains of mutPolg1 Tg mice and in the post-mortem brains of BD patients, without limiting the analysis to mitochondria-related genes. Although we searched for such genes using a comprehensive, unbiased approach, we finally determined that Ppif, encoding cyclophilin D (CypD) or mitochondrial peptidyl-prolyl cis-trans isomerase, is the only gene altered in both mutPolg1 Tg mice and BD patients. Thus, we further investigated whether a CypD inhibitor improves the BD-like phenotypes in Tg mice. We found that the CypD inhibitor ameliorated the behaviour of Tg mice, which suggests that CypD inhibition may be a possible new treatment strategy for BD.

Materials and methods

Animals

Mutant Polg1 lacking proofreading activity due to a D198A mutation was attached with the promoter of calmodulin kinase IIz. The method for generating mutPolg1 Tg mice was as previously described (Kasahara et al. 2006). Male mutant mice were used for mating to avoid possible transmission of mtDNA mutations from the maternal side. All experimental procedures involving animals were approved by the RIKEN Brain Science Institute (BSI) Animal Committee. Male and female mutPolg1 Tg mice used in the present study were aged 18–50 wk at the beginning of the experiments. For DNA microarray analysis, five pairs of littermates were used.

DNA microarray analysis in mutPolg1 Tg mice

All of the procedures were as previously described (Kubota et al. 2006). Briefly, the bilateral frontal cortices and hippocampi were dissected from five pairs of male mutPolg1 Tg mice and their wild-type littermates. RNA samples were extracted with TRIzol reagent (Invitrogen, USA). Five micrograms of total RNA from each sample was reverse-transcribed into cDNA, and biotinylated cRNA was synthesized from the cDNA by in-vitro transcription.

DNA microarray experiments were performed with the MG_430 2.0 array (Affymetrix, USA). The hybridization signal on the chips was scanned by a GeneArray scanner and processed by GeneSuite software (Affymetrix). The raw data were initially analysed by MAS5 (Affymetrix) and then imported into GeneSpring 7.3.1 software (Silicon Genetics, USA). The fluorescence intensity of each probe on the chips was divided by its median value and normalized by GeneSpring. For statistical analysis, a two-tailed paired t test was performed between the mutPolg1 Tg mice and their littermates; p < 0.05 was considered statistically significant.

Quantitative real-time polymerase chain reaction (qRT–PCR)

qRT–PCR analysis was performed with commercially available probe-primer sets (TaqMan technology; Applied Biosystems, USA) as previously described (Kakiuchi et al. 2003). The relative expression levels of each mRNA were normalized to the corresponding expression levels of \(\beta\)-actin mRNA level. Each reaction was performed in quadruplicate. Results were presented as mean ± S.E.M., and p < 0.05 was considered statistically significant.

DNA microarray analysis in post-mortem brain samples

Samples of post-mortem prefrontal cortex (Brodmann’s area 46) were donated by the Stanley Medical Research Institute (SMRI) from the institute’s Array Collection. Detailed information of the original set of subjects may be found on the SMRI website (http://www.stanleyresearch.org/dnn/BrainResearchLaboratorybrBrainCollection/ArrayCollection/tabid/89/Default.aspx). The gene expression profile of these samples obtained with an Affymetrix HGU133A array was as previously reported (Iwamoto et al. 2005) and is available through the SMRI website. Among the expression data of 33 BD patients and 34 control subjects that we profiled, we chose only high-pH samples (pH ≥ 6.5) for data analysis in this study to avoid any effect of agonal factors on gene expression (Iwamoto et al. 2005; Li et al. 2004; Tomita et al. 2004). These samples accounted for 18 BD patients and 25 control subjects. Detailed information on these high-pH samples has been described previously (Iwamoto et al. 2005).
The mouse MG430 2.0 probe IDs were converted to human HGU133A probe IDs by the NetAffyx analysis centre website (http://www.affymetrix.com/analysis/index.affx). To examine the expression change in centre website (http://www.affymetrix.com/analysis/index.affx). To examine the expression change in patients, we used the t test (p < 0.05). In order to consider the possible effect of the confounding factors on gene expression, we used Pearson’s correlation (p < 0.05) for continuous variables including age, age at onset, duration of illness, and post-mortem interval. For categorical variables including sex, medication, and suicide status, we used the Mann–Whitney U test (p < 0.05). For each of these variables, statistical analysis was performed by using all available high-pH samples regardless of the individuals’ diagnoses.

Gene ontology (GO) analysis of DNA microarray data in humans and mice

GO terms were investigated among the differentially expressed genes by using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) annotation tool, version 2.0 (Dennis et al. 2003; Huang da et al. 2009). The Affymetrix probe IDs that differentially expressed in the frontal cortex of the BD patients compared to the control subjects or those differentially expressed in frontal cortex of Tg mice compared to wild-type mice were converted to the list of DAVID gene IDs. Only GO categories with enrichment scores ≥ 1.4 were considered for further analysis. Fisher’s exact test was adopted to measure the gene enrichment in annotation terms in DAVID. A Bonferroni correction for multiple testing was applied by multiplying the p value by the number of GO terms tested. The analysis determined overrepresentation of GO terms by computing the probability (p < 0.05). A statistical analysis was individually applied to the number of categories of biological processes, cellular components, and molecular functions.

Recording of wheel-running activity

The methods for analysing wheel-running activity are described in detail elsewhere (Kasahara et al. 2006). In brief, male and female mutPolg1 Tg 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., Japan). Wheel-running activity was monitored by measuring the rotation of the wheel (3 counts/1 rotation). The animals were maintained under a 12-h light/dark cycle (lights on 08:00 hours JST) with food and water available ad libitum. Data from the initial 7–10 d were not included in the analysis. The basal wheel-running activity was calculated for 14 d before vehicle or drug treatment. The delayed activity index (DAI), referring to the wheel-running activity during the initial 3 h of a light phase, was calculated as previously described (Kasahara et al. 2006). Six pairs of Tg and wild-type littermates were placed in either the vehicle- or drug-treatment groups. For statistical comparison of the vehicle- and drug-treatment groups, the averages of the activity levels during day 7 and day 16 were used as the values after the treatment. In order to assess the effect of drug treatment by excluding the inter-individual difference of basal activity, we standardized DAI s for each day by the averages of the DAI s before the treatment for each mouse.

Treatment with CypD inhibitor

NIM811 (N-methyl-4-isoleucine-cyclosporin) was kindly provided by Novartis Pharma (Switzerland). NIM811 (5–50 mg/kg) or vehicle was injected intraperitoneally once a day under light ether anaesthesia. A stock solution of 50 mg/ml NIM811 dissolved in vehicle containing 76% cremophore EL (Nakarai Chemicals, Japan) and 24% ethanol was prepared in advance, and then diluted to the final concentration of 5 mg/ml with saline immediately prior to administration. The injection was given at 13:00 ± 2 hours for 16 d. Two hours after the final injection, the animals were perfused with phosphate buffer including 0.1% EDTA (pH 7.4). Then the brain was removed, weighed, and stored at −80 °C until preparation.

Brain tissue was homogenized in distilled water in a Teflon-glass homogenizer (1000 rpm, 5 strokes); sonicated for 30 s by a probe-type sonicator (VCX-130-PB; Sonics & Materials, USA) on ice; and then centrifuged at 15000 rpm for 10 min. The concentration of NIM811 in the supernatant was measured (SRL Inc., Japan) by radioimmunoassay (Diasorin Inc., USA) with the use of a gamma scintillation counter (ARC-950; Aloka, Japan). The NIM811 content was normalized by wet tissue weight. A standard curve was constructed by using brain homogenates from untreated wild-type mice. Mixtures of serial dilutions of NIM811 in the range of 0–2 μg/ml with the brain homogenates were analysed in duplicate to determine known amounts of the drug.

Student’s t test and two-way repeated-measures analysis of variance (rm-ANOVA) with a between-group factor of drug (NIM811 or vehicle) and a within-group factor of time (before and after drug treatment) were used. When a significant interaction was found by rm-ANOVA, a paired t test was applied for post-hoc analysis.
were commonly down-regulated (Table 2). Notably, two regions, 33 transcripts were altered in the same in the frontal cortex and the hippocampus. In these and wild-type mice, 60 showed a common alteration cortex (1471 transcripts) between the hippocampus (922 transcripts) and the frontal BD. Among the transcripts differentially expressed in order to identify a similarity to gene expression in limiting the analysis to mitochondria-related genes in re-evaluated the gene expression changes without type mice (Kubota et al. 2006). In the present study, we

**Results**

**Comparison of gene expression profiles between post-mortem brains of BD patients and mutPolg1 Tg mice**

To search for similarity of a pathophysiological process in the brain between BD patients and BD model mice, we compared the gene expression profiles between patients and the mouse model, according to two strategies, GO analysis of differentially expressed genes, and gene level analysis.

We previously conducted a DNA microarray analysis in the post-mortem prefrontal cortex of BD patients and control subjects. The expression levels of 764/11 920 transcripts were significantly different in the frontal cortex. GO analysis was applied to this dataset. We also previously performed gene expression analysis of the frontal cortex and hippocampus in mutPolg1 Tg and wild-type mice. For comparison, GO analysis was applied to the genes differentially expressed in the frontal cortex in the Tg mice (1471 out of 22 643 transcripts).

We found that 30 categories in the human dataset and 30 categories in the mouse dataset were significantly overrepresented. Among them, 16 categories of the GO terms overlapped in the human and mouse datasets (Table 1). The overrepresented categories included functional modules related to RNA processing and organelles, as well as other general biological processes (Table 1).

**Differentially expressed genes in the brains of mutPolg1 Tg compared to wild-type mice**

Previously we had reported a preliminary analysis of the difference in the expression levels of mitochondria-related genes in mutPolg1 Tg mice compared to wild-type mice (Kubota et al. 2006). In the present study, we re-evaluated the gene expression changes without limiting the analysis to mitochondria-related genes in order to identify a similarity to gene expression in BD. Among the transcripts differentially expressed in the hippocampus (922 transcripts) and the frontal cortex (1471 transcripts) between mutPolg1 Tg mice and wild-type mice, 60 showed a common alteration in the frontal cortex and the hippocampus. In these two regions, 33 transcripts were altered in the same direction; 15 were commonly up-regulated and 18 were commonly down-regulated (Table 2). Notably, the glucocorticoid receptor (GR) gene [nuclear receptor subfamily 3, group C, member 1 (Nr3c1)] was down-regulated in the two regions. The change was slightly larger in the frontal cortex but statistically more significant in the hippocampus ($p<0.001$) as confirmed by qRT–PCR ($p=0.02$).

**Shared gene expression changes in mutPolg1 Tg mice and post-mortem brains of BD patients**

We then searched for the genes with altered expression in both Tg mice and BD patients. The 33 mouse probes showing consistent alteration in the cortex and the hippocampus corresponded to 39 human probes, and three of the latter showed statistically significant changes ($p<0.05$) in post-mortem brains of BD patients compared to control subjects. These probes corresponded to two genes; one probe for SFPQ and two probes for PPIF showed expression changes in the same direction as that of Tg mice (Tables 2 and 3). SFPQ is included in several GO categories related to RNA processing in humans and mice, and PPIF is included in several GO categories related to organelles, in humans.

Expression levels of the two PPIF probes in the post-mortem brains were not significantly affected by confounding factors such as age, age at onset, duration of illness, post-mortem interval, and sex (data not shown). Although the number of samples ($n=4$) was too small to apply statistical analysis, these probes also showed a tendency for decreased expression in the medication-free BD patients. Taken together, these results suggest that decreased expression of PPIF was a change shared by the patients and the animal model.

In the present study, we further focused on the pharmacological analysis of PPIF in vivo using Tg mice.

**Treatment of mutPolg1 Tg mice with CypD inhibitor**

CypD encoded by Ppif is a component of the mitochondrial permeability transition pore (PTP) and regulates the PTP opening. Cyclosporin A (CsA) is a well-known CypD inhibitor, but it does not penetrate the blood-brain barrier (BBB) because of its high affinity to the P-glycoprotein transporter (Sakata et al. 1994). CsA also inhibits calcineurin. In contrast, NIM811 is a BBB-permeable CsA analog that potently inhibits CypD but has negligible effect on calcineurin. We confirmed that NIM811 potently inhibited PTP in mitochondria isolated from brains of wild-type mice (data not shown). Treatment with NIM811 is protective against mitochondrial dysfunction in vitro (Hansson et al. 2004; Waldmeier et al. 2002).
<table>
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<th>Category</th>
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<th>Human</th>
<th>Mouse</th>
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<td>31, 4.3, 3.27</td>
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<td></td>
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<td>GOTERM_BP_ALL mRNA metabolic process</td>
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<td>GOTERM_CC_ALL Organelle part</td>
<td>211, 29.0, 1.63</td>
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<td></td>
<td>GOTERM_CC_ALL Intracellular organelle part</td>
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<td>&lt;0.00001</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
<td></td>
<td>GOTERM_CC_ALL Endomembrane system</td>
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<td>GOTERM_CC_ALL Envelope</td>
<td>48, 6.6, 2.32</td>
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<td></td>
<td>GOTERM_CC_ALL Nuclear part</td>
<td>81, 11.1, 2.07</td>
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<td>GOTERM_BP_ALL Macromolecule localization</td>
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<td>GOTERM_BP_ALL Protein transport</td>
<td>51, 7.0, 2.01</td>
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</table>

Among 764 probe sets (t test p < 0.05), 727 probe sets were used for GO analysis in human study.
Among 1471 probe sets (paired t test p < 0.05), 1,437 probe sets were used for GO analysis in mouse study.

* Count: number of genes in the gene list mapping to a specific term.

b EASE score (a modified Fisher’s exact p value) with Bonferroni correction was used to determine statistical significant GO terms (p < 0.05) in DAVID annotation tool.
Furthermore, its neuroprotective effect has been proven in vivo (Korde et al. 2007; Ravikumar et al. 2007). Mood stabilizers effective for BD are known to have neuroprotective effects (Chen et al. 1999). Thus, we hypothesized that the down-regulation of PPiF is an

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Gene title</th>
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<th>Frontal cortex</th>
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<tr>
<td></td>
<td></td>
<td>Fold change</td>
<td>p value (a)</td>
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<td>Splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)</td>
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<td>Top1mt</td>
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<td>Zinc finger CCCH type containing 13</td>
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<td>Pspc1</td>
<td>Paraspeckle protein 1</td>
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<td>Solute carrier family 35, member E1</td>
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<td>Rbm25</td>
<td>RNA binding motif protein 25</td>
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<td>Ptpn2</td>
<td>Protein tyrosine phosphatase, receptor type, N polypeptide 2</td>
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<td>Polymerase (DNA directed), gamma</td>
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<td>Contactin 3</td>
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<td>Glutamate dehydrogenase 1</td>
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</table>

\(a\) Paired t test (<0.05).
adaptive change associated with mitochondrial dysfunction, and that CypD inhibitor may be effective against the behaviour of mutPolg1 Tg mice.

First, we determined the tissue concentration of the drug in mice treated with 20–50 mg/kg NIM811 for 2 wk (Fig. 1a). The concentration of NIM811 increased dose dependently ($r = 0.913, p < 0.001$) in the brain after the injection, implying that NIM811 can penetrate into the brain at higher doses. Similar to the levels in the brain, there was a dose-dependent increase of the drug concentration in the liver (data not shown).

With regard to the effect of CsA, the lower dose is reportedly therapeutic as an immunosuppressant for patients after liver transplantation (Rasmussen et al. 1996). NIM811 at a lower dose was only reported to be effective in a case accompanied by a breach of the BBB.
(Sullivan et al. 2000). In the present study, we examined the effect of NIM811 on the behaviour of the Tg mice at two doses, 5 and 40 mg/kg. However, the lower dose (5 mg/kg), did not achieve a detectable concentration in the brain, possibly due to the intact BBB.

NIM811 treatment at a lower dose (5 mg/kg) did not significantly affect the DAI, the measure of excessive wheel-running activity at the beginning of the light phase, compared to the vehicle group. Rm-ANOVA showed no significant interaction between drug (NIM811 and vehicle) and time (before and after the treatment) (d.f. = 1, F = 0.811, p = 0.383) (Fig. 2e). On the other hand, after the 40 mg/kg NIM811 treatment, there was a significant interaction between drug and time (d.f. = 1, F = 18.15, p = 0.002) (Fig. 3e). The DAI was decreased in the NIM811 group (Post/Pre = 0.658 ± 0.102) whereas it was increased in the vehicle group (Post/Pre = 1.720 ± 0.289). There was a significant difference in the Post/Pre ratio between the two groups (p < 0.05) (Fig. 3f).

Discussion

GO analysis of the genes differentially expressed in BD patients and model mice showed some overlap of the enriched GO categories. This might suggest the similarity of gene expression patterns between the patients and the animal model.

Notably, we found that PPIF encoding CypD, mitochondrial peptidyl-prolyl cis-trans isomerase, was consistently down-regulated both in mPolg1 Tg mice (Table 2) and in BD patients (Table 3). Furthermore, pharmacological inhibition of CypD by NIM811 alleviated the behavioural phenotype of the mutPolg1 Tg mice (Fig. 3). Because low-dose NIM811 did not have an effect against the behavioural phenotype of the mutPolg1 Tg mice, this effect was regarded as reflecting a direct effect on the brain (Fig. 2).

CypD is a component of mitochondrial PTP and is localized in a mitochondrial matrix. A Ca²⁺ overload induces CypD binding to the adenine nucleotide translocator resulting in the opening of the PTP, which has a key role in apoptotic or necrotic cell death. CsA, a potent CypD inhibitor, inhibits PTP opening. We confirmed that CsA enhanced mitochondrial Ca²⁺ uptake in isolated mitochondria (Kubota et al. 2006). NIM811 also inhibits PTP opening (Hansson et al. 2004; Waldmeier et al. 2002). In addition, NIM811 reduces the infarct volume and the release of cytochrome c from mitochondria after ischaemia (Korde et al. 2007; Ravikumar et al. 2007) and is effective for experimental traumatic brain injury (Mbye et al. 2008). Furthermore, brains of CypD knockout mice are resistant to ischaemia/reperfusion injury (Baines et al. 2005; Basso et al. 2005; Nakagawa et al. 2005; Schinzel et al. 2005). Cell death in animal models of neuromuscular diseases was also attenuated by crossbreeding with CypD knockout mice (Fortet et al. 2007; Millay et al. 2008; Palma et al. 2009). In a mouse model of Alzheimer’s disease, lack of CypD restores synaptic and cognitive function (Du et al. 2008). These findings suggest that CypD inhibition is a key therapeutic approach against central nervous system diseases.

Although simple logic suggests that down-regulation of CypD, being a downstream event of Polg1 mutation, should contribute to the abnormal phenotypes observed in Tg mice, then, an activator, instead of an inhibitor of CypD, should be used to increase CypD level to improve the phenotypes. However, most of the drugs that are effective for maintenance treatment of BD, such as lithium (Nonaka et al. 1998), valproate (Jeong et al. 2003), clonazapine and quetiapine (Qing et al. 2003), reportedly have neuroprotective effects. Thus, it would be plausible to assume that a CypD inhibitor is effective for BD, and its down-regulation observed in the BD model mice is compensatory in nature.

Our result that NIM811, a CypD inhibitor, is effective for the BD-like phenotype in the animal model is consistent with the previous findings that pharmacological inhibition or genetic ablation of CypD has neuroprotective effects.

In heart-specific mutPolg1 Tg mice, heart mitochondria are reportedly resistant to PTP opening, and CsA, a PTP inhibitor, prevented heart failure (Mott et al. 2006). This suggests that the resistance to PTP opening in Tg mice might not be a cause of heart failure but is instead a compensatory phenomenon caused by accumulation of mtDNA deletions.

Thus, the down-regulation of CypD in the brain in BD patients or in neuron-specific mutPolg1 Tg mice might be an adaptive response to mitochondrial dysfunction, rather than the cause of the disorder, and thus blockade of CypD might have counteracted the BD-like behaviour of the mutPolg1 Tg mice. Indeed, the behavioural phenotypes of CypD knockout mice, such as enhanced anxiety (Lusis et al. 2008) and cognitive impairment (Mouri et al. 2009), are different from those of mutPolg1 Tg mice.

Two mood stabilizers, lithium and valproate, up-regulate B-cell lymphoma protein-2 (Bcl-2), an anti-apoptotic factor (Chen et al. 1999; Corson et al. 2004; Hiroi et al. 2005). Similar to CypD down-regulation, up-regulation of BCL-2 also inhibits PTP opening.
Fig. 2. Effect of 5 mg/kg NIM811 on wheel-running activity in *mutPolg1* Tg mice. Individual activity record of (a) vehicle-treated or (c) NIM811-treated *mutPolg1* Tg mice. The light and dark periods (12:12 hours) are indicated by white and black bars. Each bar represents the total count of wheel running in a 10-min interval. An arrowhead indicates the first day of injection (day 15). Standardized delayed activity index (DAI) of (b) vehicle-treated or (d) NIM811-treated *mutPolg1* Tg mice. DAI during the treatment (Post) was standardized by the mean value before the treatment (Pre). A broken line shows the basal activity level. Day 0 indicates the first day of injection. A horizontal bar represents the period of the drug injection. (e) Change of DAI of individual animals. The index before the treatment was averaged over 14 d (Pre: days -14 to -1) and after the treatment for 10 d (Post: days 7–16). (f) Effect of the vehicle or NIM811 treatment on DAI. The effect of drug treatment was estimated by the ratio of the index values before (Pre) and after (Post) the treatment. Values indicate mean ± S.E.M. (vehicle treatment group, *n* = 7; NIM811 treatment group, *n* = 9). A broken line shows the averaged level of DAI before the treatment.
Fig. 3. Effect of 40 mg/kg NIM811 on wheel-running activity in mutPolg1 Tg mice. Individual activity record of (a) vehicle-treated or (c) NIM811-treated mutPolg1 Tg mice. An arrowhead indicates the first day of injection (day 15). Standardized delayed activity index (DAI) of (b) vehicle-treated or (d) NIM811-treated mutPolg1 Tg mice. DAI during (Post) the treatment was standardized by the mean value before the treatment (Pre). A broken line shows the basal activity level. Day 0 indicates the first day of injection. A horizontal bar represents the period of the drug injection. (e) Change of DAI of individual animals. The indices before the treatment was averaged for 14 d (Pre: days 1 to 14) and after the treatment for 10 d (Post: days 7–16). (f) Effect of the vehicle or NIM811 treatment on DAI. The effect of drug treatment was estimated by the ratio of the index values before (Pre) and after (Post) treatment. *p < 0.05 (Aspin–Welch’s modified t test). Values indicate mean ± S.E.M. (n = 6 for each group). A broken line shows the averaged level of DAI before the treatment.
Mitochondria isolated from cells overexpressing BCL-2 were more resistant to PTP opening (Shimizu et al. 1998). Further, mitochondria from Tg mice with overexpression of BCL-2 showed a higher membrane potential after treatment with Ca²⁺ (Shimizu et al. 1998). In both studies, overexpression of BCL-2 prevented apoptotic processes. The mRNA expression and protein levels of Bcl-2 were increased in myocytes of heart-specific mutPolg1 Tg mice (Mott et al. 2001; Zhang et al. 2005). The increased Bcl-2 was speculated to be an adaptive protective response (Mott et al. 2004). These findings suggest that inhibition of PTP opening, in general, might be a therapeutic strategy against BD.

Recent studies demonstrated that CypD immunoreactivity in the hippocampus was primarily localized in neurons rather than in astrocytes (Mouri et al. 2009; Naga et al. 2007). CypD is abundant in synaptic mitochondria compared to non-synaptic mitochondria (Naga et al. 2007). Mitochondria contribute to Ca²⁺ buffering in the synaptic terminal (Billups & Forsythe, 2002; Kim et al. 2005). Release of glutamate and acetylecholine was decreased in CypD knockout mice (Mouri et al. 2009). Taken together, these findings suggest that CypD down-regulation may also affect local synaptic dysfunction.

Interestingly, up-regulation of SFPQ, as shown in Table 2, was also reported in the other set of post-mortem frontal cortices of BD patients (Nakatani et al. 2006). Based on the gene expression analysis of DBP (D-box binding protein) knockout mice and the convergent functional genomics approach, Le-Niculescu and colleagues suggested that SFPQ is a novel candidate gene for BD (Le-Niculescu et al. 2008). A protein, polyprimidine tract-binding protein-associated splicing factor (PSF) encoded by SFPQ has been identified as regulating gene expression of a mitochondrial phosphate carrier (Iacobazzi et al. 2005), which is also involved in PTP opening (Leung et al. 2008). Expression of SFPQ was enriched through brain development and highly detected in differentiated neurons rather than in non-neuronal cells in zebrafish (Lowery et al. 2007). Immunoreactivity of PSF was also apparent in the brains and was much stronger at the stage of neuronal differentiation (Chanas-Sacre et al. 1999). This suggests that SFPQ could play a role in neuron-specific splicing or transcriptional regulation even in the adult brain. Additionally, the increased cell death by low level of SFPQ expression at the embryonic stage in zebrafish indicated that PSF protein normally suppresses apoptosis (Lowery et al. 2007). In our data, SFPQ was up-regulated in the brains of Tg mice and patients, which exerts an anti-apoptotic effect. This result is in line with the biological function of CypD down-regulation. Thus, it is plausible to assume that mutant Polg1 causes accumulation of mtDNA deletions, which exerts compensatory changes of PP1F and SFPQ. The functional significance of the up-regulation of SFPQ should be clarified. Recently, it has been reported that an aberrant splicing mechanism is relevant to the pathophysiology of affective disorders (Glatt et al. 2009; Watanuki et al. 2008).

In the present results, 3/39 (7.6%) human probes corresponding to the altered mice probes were also differentially expressed in the patients. Because 682/11920 (5.7%) probes were differentially expressed in patients, the number of altered genes is not significantly larger than that expected by chance (p=0.48 by Fisher’s exact probability test). Thus, it cannot be excluded that the overlap between the mouse gene profiling and the human one is due to chance. However, the role of down-regulation of Ppif was supported by the pharmacological analysis of Ppif in vivo using Tg mice.

In the present study, we also found that GR mRNA was down-regulated in the mutPolg1 Tg mice (Table 2). Considering that down-regulation of GR has been reported in several animal models of depression (Boyle et al. 2005; Herman et al. 1995; Kitraki et al. 1999), we think this finding further supports the validity of our model as an animal model of mood disorders.

In summary, we found that down-regulation of CypD with up-regulation of SFPQ was the common molecular signature in the mouse model and post-mortem brains of BD patients. A CypD inhibitor, NIM811, improved the behavioural phenotype of the mice. This suggests that CypD is a promising drug target for BD. This is the first application of the neuron-specific mutPolg1 Tg mice in the study of drug development.

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Statement of Interest
None.

References


