厚生労働科学研究費補助金

障害者対策総合研究事業
（精神障害分野）

気分障害の神経病理学に基づく分類を目指した脳病態の解明に関する研究

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平成２２年度　総括研究報告書

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研究代表者　加藤忠史
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平成23（2011）年3月

研究要旨：

うつ病は、大きな社会負担、急増する患者数等から社会問題となっているが、双極スペクトラム、非定型うつ病、血管性うつ病など、多様な病型が存在し、その診療には混乱も生じている。本研究の目的は、遺伝要因、早期養育環境、ストレス、加齢、動脈硬化などのうつ病の危険因子によって引き起こされる脳病態を、死後脳研究、脳画像解析、疫学研究、および動物モデルにおける神経科学的解析などによって明らかにし、脳病態に応じた気分障害の分類を目指すことである。

本研究の特色は、うつ病の危険因子による脳病態を多角的に明らかにすると共に、これを患者死後脳で確認することである。気分障害の疾患概念を病理学に基づいて再構築し、病型に応じた診断法、治療法を開発することができれば、精神医学が病理学に基づく医学へと進化する第一歩となる。現在混沌としている「うつ」が種々の脳疾患と心の悩みに明確に分類され、適切に対応されれば、自殺者数の減少、円滑な人事管理など、多くの波及効果が期待でき、国民の生活の質を向上させると期待される。

平成22年度は、脳卒中患者におけるMRI構造画像を用いた検討により、アパシーが基底核病変と関連する一方、抑うつは特定の脳領域とは関連がないことが示された。また、高齢者ブレインバンクでは、新たに2例の双極性障害患者の剖検を行い、1例では顕著な所見がなく、もう1例は皮質基底核変性症の病理所見を認めた。また、これまでに蓄積された、高齢者ブレインバンクサンプルに関し、神経病理学的所見と気分障害の有無に関する調査を開始した。

また、ミトコンドリアDNA異常を持つモデルマウスにおいて、前頭辺縁系の脳病変部位における神経病理学的変化を、免疫組織化学的手法により検討した。また、うつ病の原因に関わる脳部位を同定するため、強制水泳試験の前に、イミプラミンを投与し、c-Fosの発現が増加した部位を探索した結果、分界条床核の一部を見出した。この部位におけるc-Fosの発現と強制水泳による無動時間との間に、負の相関がみられたことから、この部位の変化が抗うつ薬によるうつ様行動の改善と関連している可能性が考えられた。

気分障害患者の死後脳研究では、モデルマウスにおいて見出された候補脳部位における変化を免疫組織化学的手法により検討可能かどうか、予備的な検討を開始した。

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A 研究目的

社会生活障害を引き起こす疾患として最大の要因であるうつ病の患者数は近年急速に増加し、社会問題となっている。しかし、抗うつ薬によく反応するメランコリー型に加え、抗うつ薬により悪化する双極スペクトラム、抗うつ薬が奏効しにくく心理療法も重要となる非定型うつ病、難治化しやすい血管性うつ病など、治療抵抗性のうつ病が増加し、うつ病診療は混沌とした状況にある。面接に頼った現在の診断法には限界があり、現状の打開には、神経病理学所見に基づいて疾患概念を再構築すると共に亜型分類を確立し、脳病態診断に基づいて治療を最適化する他ない。

うつ病の発症には遺伝、養育、ストレス、加齢など、種々の危険因子が関与する。加藤らはミトコンドリアDNA異常が脳に蓄積する遺伝子改変マウスが双極性障害様の行動異常を示すことを見出した。山下らは基底核に脳血管障害が存在するとうつ病の中でも意欲低下が優勢となることを見出し、血管性うつ病の神経学的基盤を示した。神庭らはインターロイキン1(IL-1)が脳内モノアミン系に影響することや、インターフェロンがIL-1β上昇を介して海馬神経新生を低下させることを見出し、うつ病の神経免疫仮説を確立した。また久山町の65歳以上の全住民を対象としてうつ病の調査を行い、有病率を明らかにした。村山らは1万名近い脳を有する高齢者ブレインバンクを構築し、千例以上の検討で、加齢に伴い徐々にLewy小体が蓄積し、脳機能障害が引き起こされることを示した。

上記の他多くの研究から、遺伝要因によるミトコンドリア機能障害、ストレスによる神経内分泌免疫学的変化、加齢に伴う脳血管障害および異常蛋白蓄積など、各危険因子による脳機能障害がうつ病を引き起こすと考えられる。本研究では、こうした脳病態を臨床研究および動物モデルを用いて明らかにすると共に、これを患者死後脳で検討する。

本計画の特徴は、うつ病の異種性の高さを前提とし、各危険因子と脳病態の関連を明らかにし、脳病態を臨床診断（亜型分類）と対応づけることにより、気分障害の神経病理学的な分類の基盤を固めることである。本研究によりうつ病の神経病理学的所見に基づく分類が可能となれば、診療やメンタルヘルス管理の最適化が可能となり、自殺者減少、生活の質の向上につながる。

B. 研究方法

動物実験では、ミトコンドリアDNA変異が脳内に蓄積するモデルマウスを用いて、ミトコンドリアDNA蓄積を指標として、気分障害類似の行動異常と関連する脳の異常を同定すると共に、こうした変化を可視化する技術を開発する。

また、うつ病の原因脳部位を探索するため、c-fos免疫染色を用いて、抗うつ薬の評価系として用いられている強制水泳試験時に、抗うつ薬投与によりc-fos反応が変化する脳部位を探索する。

脳卒中患者における、定量的MRIを用いて、病変部位と症状の関連を検討する。

また、高齢者ブレインバンクの登録症例で、気分障害の調査を行うと共に、新たな剖検例について、神経病理学的検索を行う。

C.研究結果

ミトコンドリアDNA(mtDNA)合成酵素の変異を脳特異的に発現させた双極性障害モデルマウスにおいて、mtDNA変異が蓄積している前頭辺縁系の諸部位において、mtDNA欠失が蓄積した細胞を可視化するため、mtDNA由来蛋白質であるチトクローム酸化酵素(Cox)、および核DNA由来蛋白質である、クエン酸脱水素酵素の抗体、および核を染色するDAPIを用いた三重染色を行った。その結果、mtDNA変異が蓄積している部位において、Cox陰性細胞が散見され、モデルマウスでは有意に多く見られた。

気分障害患者の死後脳で同様の検討を行うべく、スタンレー脳バンクより供与された凍結視床切片を用いて、まずはニッスル染色、ヘマトキシリンエオジン染色による検討を行った。今後、このサンプルを用いて、マウスと同様の三重染色を行う方法を検討する予定である。

うつ病の原因となる脳部位を同定するため、マウスに抗うつ薬を投与後、強制水泳試験を行い、脳を摘出し、灌流固定を行って、c-Fos免疫組織化学による検討を行った。

その結果、分界条床核の内側域後内側部（STMPM）において、イミプラミン前投与後の強制水泳試験で、c-Fosの発現が増加した。この部位におけるc-Fosの発現と強制水泳による無動時間との間に、負の相関がみられたことから、この部位の変化が抗うつ薬によるうつ様行動の改善と関連している可能性が考えられた。

脳卒中患者における、定量的MRIを用いて、

病変部位と症状の関連を検討した研究では、アパシーが基底核病変と関連していることを見出した。一方、抑うつと関連する特定の脳領域とは関連がないことが示された。

これまでに蓄積された、高齢者ブレインバンクサンプルに関し、神経病理学的所見とうつ病の有無に関する調査を開始した。

高齢者ブレインバンクサンプルのうち、双極性障害の症例1は、67歳男性で、臨床診断は双極性障害、死因は Non- Hodkin large B cell lymphoma であった。33歳時、躁うつ病を発症し、合計9回の入院歴がある。多弁、多動、気分高揚、過干渉などがみられた。66歳時、非ホジキンリンパ腫に罹患し、化学療法を受けていたが、死亡の二ヶ月前より嚥下障害、経口摂取困難となった。躁病による不隠に対し、抗精神薬を投与されたが、その後、死亡した。死後の病理学的検索では、脳には顕著な所見は見られなかった。

症例2は、79歳女性で、臨床診断は、パーキンソン病、双極性感情障害であった。50歳頃に，玄関に死んだ人が横たわっている、誰かに追いかけられているといった訴えで、精神科病院に措置入院。53歳頃より、うつ状態と躁状態を繰り返すようになった。また、73歳頃より、パーキンソン症候群の診断で投薬を受けた。73歳時、時間を構わず電話をかける等の躁症状のため、精神科に入院。その後、誤嚥性肺炎にて入院したが、発熱が改善せず、パーキンソン症状も悪化したため、入院。十二指腸潰瘍の併発、貧血、褥創、低栄養状態のため、5ヶ月の経過で死亡した。病理学的検索では、皮質基底核変性症と診断された。　この結果より、双極性障害の中には、既知の神経病理学的変化に伴う場合があると考えられた。

D.考察

動物モデル研究では、気分障害の原因脳部位として、前頭辺縁系の脳構造の関与が疑われた。一方、脳卒中患者における MRI の研究では、うつ病を引き起こす特定の脳部位は同定されなかったが、これは,MRI の分解能の限界によるものである可能性が考えられる。

また、老年期の双極性障害患者における検討から、さまざまな神経病理学的過程が関与する可能性が示唆され、双極性障害を引き起こす病理学的変化の性質はさまざまである可能性が考えられた。従って、病変の性質もさることながら、責任部位を検討することが特に重要であると考えられた。

E.結論

これまでのうつ病研究では、抗うつ薬の作用機序などに注目される場合が多く、脳のどの部位にどのような病理学的異常が生じるのかは、わかっていなかった。

本研究の結果、気分障害に前頭辺縁系の諸部位の関与が疑われること、神経変性など、さまざまな要因による病理学的変化がうつ病の背景となっている可能性が示唆された。

今後、これらの要因の関与について、より詳細に検討していく予定である。

F.研究発表

1.　論文発表

（平成22年度）

Kubota M, Kasahara T, Iwamoto K, Komori A, Ishiwata M, Miyauchi T, Kato T. Therapeutic implications of down-regulation of cyclophilin D in bipolar disorder. Int J Neuropsychopharmacol. 2010 Nov;13(10):1355-68.

Fuke S, Kubota-Sakashita M, Kasahara T, Shigeyoshi Y, Kato T. Regional variation in mitochondrial DNA copy number in mouse brain. Biochim Biophys Acta. 2011 Mar;1807(3):270-4. Epub 2010 Dec 8.

Iwamoto K, Ueda J, Bundo M, Kojima T, Kato T. Survey of the effect of genetic variations on gene expression in human prefrontal cortex and its application to genetics of psychiatric disorders. Neurosci Res. 2011 Mar 4.

Sugawara H, Iwamoto K, Bundo M, Ueda J, Ishigooka J, Kato T. Comprehensive DNA methylation analysis of human peripheral blood leukocytesand lymphoblastoid cell lines. Epigenetics. 2011 Apr 1;6(4):508-15.

Horikawa H, Kato TA, Mizoguchi Y, Monji A, Seki Y, Ohkuri T, Gotoh L, Yonaha M, Ueda T, Hashioka S, Kanba S. Inhibitory effects of SSRIs on IFN-γ induced microglial activation through the regulation of intracellular calcium. Prog Neuropsychopharmacol Biol Psychiatry. 2010 Oct 1;34(7):1306-16

Hama S, Yamashita H, Yamawaki S, Kurisu K. Post-stroke depression and apathy: Interactions between functional recovery, lesion location, and emotional response. Psychogeriatrics. 2011 Mar;11(1):68-76.

Yamashita H, Fujikawa T, Takami H, Yanai I, Okamoto Y, Morinobu S, Yamawaki S. Long-term prognosis of patients with major depression and silent cerebral infarction. Neuropsychobiology. 2010 Aug;62(3):177-81.

Ishibashi K, Kanemaru K, Saito Y, Murayama S, Oda K, Ishiwata K, Mizusawa H, Ishii K. Cerebrospinal fluid metabolite and nigrostriatal dopaminergic function in Parkinson's disease. Acta Neurol Scand. 2010 Jul;122(1):46-51. Epub 2009 Nov 28.

加藤忠史（2010）生物リズムと双極性障害　脳21　13: 383-389

加藤忠史（2010）総論：うつ病の多様性と生物学—特集にあたって—　Medical Bio 7: 21-25

加藤忠史（2010）双極性障害における神経生物学的研究　ビオフィリア　6: 25-28

加藤忠史（2011）ミトコンドリアDNAと気分障害　別冊・医学のあゆみ「ここまでわかったミトコンドリア研究の新展開」97-101

高田篤、加藤忠史（2011）視床室傍核　分子精神医学　11: 62-67

G.知的財産権の出願・登録状況

1. 特許取得
   なし

2. 実用新案登録
   なし

3. その他

研究成果の刊行一覧表（平成 22 年度）

加藤　忠史

氏名	タイトル	雑誌／書籍名	巻	頁	年
Kubota M, Kasahara T, Iwamoto K, Komori A, Ishiwata M, Miyauchi T, Kato T,	Therapeutic implications of down-regulation of cyclophilin D in bipolar disorder.	Int J Neuropsycho pharmacol.	13 (10)	1355 -68	2010
Fuke S, Kubota-Sakashita M, Kasahara T, Shigeyoshi Y, Kato T,	Regional variation in mitochondrial DNA copy number in mouse brain.	Biochim Biophys Acta.	1807 (3)	270-4	2011
Iwamoto K, Ueda J, Bundo M, Kojima T, Kato T,	Survey of the effect of genetic variations on gene expression in human prefrontal cortex and its application to genetics of psychiatric disorders.	Neurosci Res.			2011
Sugawara H, Iwamoto K, Bundo M, Ueda J, Ishigooka J, Kato T,	Comprehensive DNA methylation analysis of human peripheral blood leukocytes and lymphoblastoid cell lines.	Epigenetics.	6(4)	508-15.	2011
Horikawa H, Kato TA, Mizoguchi Y, Monji A, Seki Y, Ohkuri T, Gotoh L, Yonaha M, Ueda T, Hashioka S, Kanba S,	Inhibitory effects of SSRIs on IFN-γ induced microglial activation through the regulation of intracellular calcium.	Prog Neuropsycho pharmacol Biol Psychiatry	34(7)	1306 -16	2010

Hama S, Yamashita H, Yamawaki S, Kurisu K,	Post-stroke depression and apathy: Interactions between functional recovery, lesion location, and emotional response.	Psychogeriatrics.	11(1)	68-76.	2011
Yamashita H, Fujikawa T, Takami H, Yanai I, Okamoto Y, Morinobu S, Yamawaki S ,	Long-term prognosis of patients with major depression and silent cerebral infarction.	Neuropsychobiology.	62(3)	177-81.	2010
Ishibashi K, Kanemaru K, Saito Y, Murayama S, Oda K, Ishiwata K, Mizusawa H, Ishii K,	Cerebrospinal fluid metabolite and nigrostriatal dopaminergic function in Parkinson's disease.	Acta Neurol Scand	122 (1)	46-51	2010
加藤忠史	生物リズムと双極性障害	脳 21	13	383 -389	2010
加藤忠史	総論：うつ病の多様性と生物学—特集にあたって—	Medical Bio	7	21-25	2010
加藤忠史	双極性障害における神経生物学的研究	ビオフィリア	6	25-28	2010
加藤忠史	ミトコンドリア DNA と気分障害	別冊・医学のあゆみ「ここまでわかったミトコンドリア研究の新展開」		97-101	2011
高田篤、加藤忠史	視床室傍核	分子精神医学	11	62-67	2011

資　料

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ARTICLE

CINP

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, *SFPQ* 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.

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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 IIα. 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 β-actin mRNA level. Each reaction was performed in quadruplicate. Results were presented as mean$\pm$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$\geq$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 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 $\geqslant 1.4$ 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 DAIs for each day by the averages of the DAIs 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 $13{:}00 \pm 2$ 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. Statistical calculation was performed with KyPlot version 4.0 (KyensLab Inc., Japan) and SPSS software version 16.0 (SPSS Inc., USA).

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 1. Gene ontology (GO) analysis of DNA microarray datasets in human and mouse

			Human				Mouse			
Category		Term	Count[a]	%	Fold enrichment	*p* value[b]	Count[a]	%	Fold enrichment	*p* value[b]
RNA processing										
GOTERM_MF_ALL	GO:0003723	RNA binding	65	8.9	2.49	<0.00001	80	5.6	2.07	<0.00001
GOTERM_BP_ALL	GO:0006396	RNA processing	44	6.1	2.64	0.00006	57	4.0	2.34	0.00002
GOTERM_BP_ALL	GO:0006397	mRNA processing	31	4.3	3.27	0.00012	38	2.6	2.56	0.00117
GOTERM_BP_ALL	GO:0008380	RNA splicing	30	4.1	3.62	0.00002	32	2.2	2.74	0.00268
GOTERM_BP_ALL	GO:0016071	mRNA metabolic process	36	5.0	3.19	0.00001	42	2.9	2.49	0.00057
Organelle										
GOTERM_CC_ALL	GO:0031090	Organelle membrane	95	13.1	1.86	<0.00001	81	5.6	1.91	0.00002
GOTERM_CC_ALL	GO:0031967	Organelle envelope	48	6.6	2.33	0.00010	55	3.8	1.95	0.00247
GOTERM_CC_ALL	GO:0044422	Organelle part	211	29.0	1.63	<0.00001	250	17.4	1.50	<0.00001
GOTERM_CC_ALL	GO:0044446	Intracellular organelle part	209	28.8	1.62	<0.00001	248	17.3	1.49	<0.00001
Others										
GOTERM_CC_ALL	GO:0005737	Cytoplasm	329	45.3	1.43	<0.00001	512	35.6	1.42	<0.00001
GOTERM_CC_ALL	GO:0044444	Cytoplasmic part	204	28.1	1.49	<0.00001	309	21.5	1.43	<0.00001
GOTERM_CC_ALL	GO:0012505	Endomembrane system	63	8.7	1.78	0.00940	55	3.8	2.14	0.00013
GOTERM_CC_ALL	GO:0031975	Envelope	48	6.6	2.32	0.00011	56	3.9	1.97	0.00140
GOTERM_CC_ALL	GO:0044428	Nuclear part	81	11.1	2.07	<0.00001	117	8.1	1.64	0.00007
GOTERM_BP_ALL	GO:0033036	Macromolecule localization	58	8.0	1.89	0.02083	86	6.0	1.64	0.03115
GOTERM_BP_ALL	GO:0015031	Protein transport	51	7.0	2.01	0.01894	75	5.2	1.70	0.03654

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.

[a] 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.

Table 2. Differentially expressed genes in the brains of *mutPolg1* Tg mice compared to wild-type mice

		Hippocampus		Frontal cortex			
Symbol	Gene title	Fold change	*p* value[a]	Fold change	*p* value[a]	Public ID	Probe set ID
Sfpq	Splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)	1.26	0.003	1.27	0.039	BG061796	1439058_at
Erdr1 protein	Clone IMAGE: 3983821	2.12	0.005	1.98	0.022	BC021831	1427820_at
Top1mt	DNA topoisomerase 1, mitochondrial	1.13	0.005	1.19	0.002	AF362952	1460370_at
Zc3h13	Zinc finger CCCH type containing 13	1.18	0.006	1.18	0.031	AW536655	1434894_at
Pspc1	Paraspeckle protein 1	1.37	0.013	1.27	0.009	BB590675	1423192_at
Hist2h2aa1	Histone cluster 2, H2aa1	1.33	0.032	1.48	0.024	BC010564	1418367_x_at
ImmP2l	Mitochondrial inner membrane protease subunit 2 (IMP2- like protein).	1.38	0.039	1.27	0.029	BB291417	1458099_at
EG633640	Predicted gene, EG633640	1.28	0.039	1.12	0.040	BG068672	1426607_at
Slc35e1	Solute carrier family 35, member E1	1.20	0.041	1.21	0.038	BB041864	1434103_at
Ube3c	Ubiquitin protein ligase E3C	1.22	0.041	1.29	0.019	BE690666	1444562_at
Rbm25	RNA binding motif protein 25	1.18	0.042	1.31	0.024	AI159652	1437862_at
Ptprn2	Protein tyrosine phosphatase, receptor type, N polypeptide 2	1.16	0.045	1.45	0.009	U57345	1425724_at
Polg	Polymerase (DNA directed), gamma	1.42	0.045	1.23	0.007	BG064799	1423272_at
4930447A16Rik	RIKEN cDNA 4930447A16 gene	1.28	0.046	1.35	0.027	BB012182	1431671_at
Tradd	TNFRSF1A-associated via death domain	1.20	0.047	1.39	0.050	AA201054	1452622_a_at
Nr3c1	Nuclear receptor subfamily 3, group C, member 1	−1.12	<0.001	−1.33	0.035	NM_008173	1421866_at
Ero1l	ERO (endoplasmic reticulum oxidoreductin) 1-like (S. cerevisiae)	−1.08	0.001	−1.21	0.043	BM234652	1419029_at
Med26	Mediator complex subunit 26	−1.46	0.005	−1.62	0.007	AK017726	1452282_at
Npdc1	Neural proliferation, differentiation and control gene 1	−1.12	0.008	−1.12	0.028	NM_009849	1418259_a_at
2900052N01Rik	RIKEN cDNA 2900052N01 gene	−1.36	0.009	−1.32	0.026	AU067665	1436231_at
Kif5c	Kinesin family member 5C	−1.32	0.009	−1.50	0.047	AI844677	1450804_at
Flnb	Filamin, beta	−1.15	0.016	−1.29	0.002	AW538200	1426750_at
Rapgef6	rap guanine nucleotide exchange factor (GEF) 6	−1.10	0.017	−1.11	0.038	BQ177183	1427412_s_at
Usp31	Ubiquitin specific peptidase 31	−1.09	0.017	−1.19	0.038	BM227490	1442099_at
Prmt3	Protein arginine N-methyltransferase 3	−1.21	0.020	−1.24	0.005	AK008118	1431768_a_at
Aif1l	Allograft inflammatory factor 1-like	−1.22	0.035	−1.07	0.016	BC024599	1424263_at
Cntn3	Contactin 3	−1.09	0.036	−1.13	0.024	NM_008779	1420739_at
Ppif	Peptidylprolyl isomerase F (cyclophilin F)	−1.12	0.038	−1.18	0.008	NM_134084	1416940_at
Copg	Coatomer protein complex, subunit gamma	−1.16	0.044	−1.13	0.009	BC024686	1415670_at
Enoph1	Enolase-phosphatase 1	−1.10	0.044	−1.28	0.002	BC021429	1423705_at
Smad3	MAD homolog 3 (Drosophila)	−1.25	0.045	−1.28	0.019	BI150236	1450472_s_at
BC003266	cDNA sequence BC003266	−1.04	0.049	−1.08	0.039	NM_030252	1449189_at
Glud1	Glutamate dehydrogenase 1	−1.10	0.049	−1.19	0.040	BI329832	1416209_at

[a] Paired *t* test (<0.05).

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 3. Shared gene expression changes in the brains of *mutPolg1* Tg mice and post-mortem brains of the bipolar disorder patients

		pH ⩾ 6.5 samples (bipolar $n=18$, control $n=25$)		All samples (bipolar $n=33$, control $n=34$)				
Symbol	Gene title	Fold change	p value[a]	Fold change	p value[a]	Public ID	Probe set ID	Locus
PPIF	Peptidylprolyl isomerase F (cyclophilin F)	−1.20	0.016	−1.19	0.005	NM_005729	201490_s_at	10q22-q23
PPIF	Peptidylprolyl isomerase F (cyclophilin F)	−1.17	0.042	−1.11	0.095	BC005020	201489_at	10q22-q23
SFPQ	Splicing factor proline/glutamine-rich (polypyrimidine tract binding protein associated)	1.33	0.002	1.10	0.215	AV705803	221768_at	1p34.2

[a] t test (<0.05).

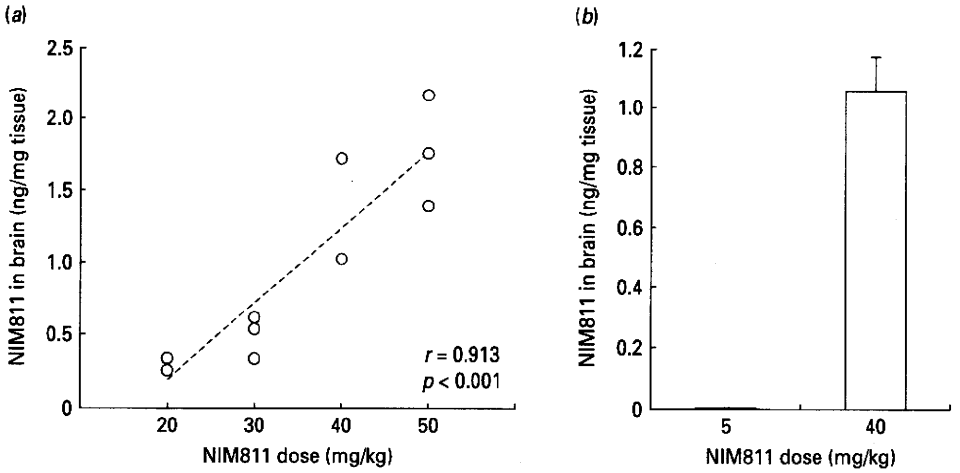

Fig. 1. Detection of NIM811 in the brain after chronic treatment. (*a*) Each dose of NIM811 (20, 30, 40, 50 mg/kg) was injected intraperitoneally into wild-type mice for 12 d. The animals were housed in home cages. Values indicate mean ± S.E.M. (20, 30, 50 mg/kg, $n=3$; 40 mg/kg, $n=2$). The NIM811 concentration in the brain was well correlated with the dose ($r=0.913$, $p<0.001$, by Pearson's correlation coefficient). (*b*) The *mutPolg1* Tg mice were injected with 5 or 40 mg/kg of NIM811 for 2 wk. After their wheel-running activity was recorded, the drug concentration was measured as described in the Materials and methods section. For detection of NIM811 in the brain after 5 mg/kg treatment, three brains were homogenized together and analysed in duplicate, because the drug content was undetectable in our analysis when individual tissue was used (data not shown). The NIM811 concentration was corrected for tissue weight. Values indicate mean ± S.E.M. (5 mg/kg, $n=2$; 40 mg/kg, $n=6$).

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. 1*a*). 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. 2*e*). 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. 3*e*). 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. 3*f*).

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 ameliorated 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 Ca^{2+} 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 Ca^{2+} 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 (Forte *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), olanzapine 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 (Luvisetto *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.

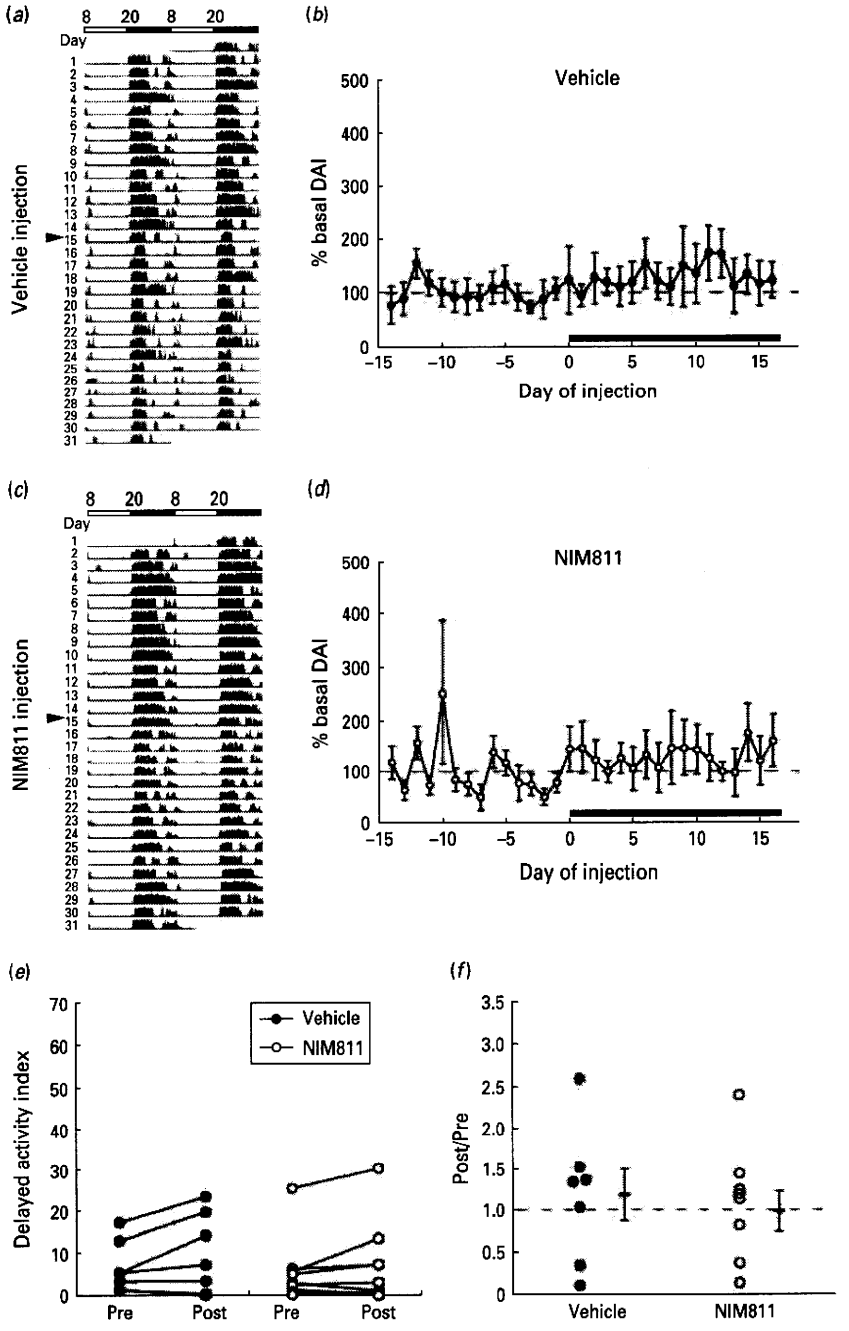

Fig. 2. Effect of 5 mg/kg NIM811 on wheel-running activity in *mutPolg1* Tg mice. Individual activity record of (*a*) vehicle-treated or (*c*) NIM811-treated *mutPolg1* Tg mice. The light and dark periods (12:12 hours) are indicated by white and black bars. Each bar represents the total count of wheel 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.

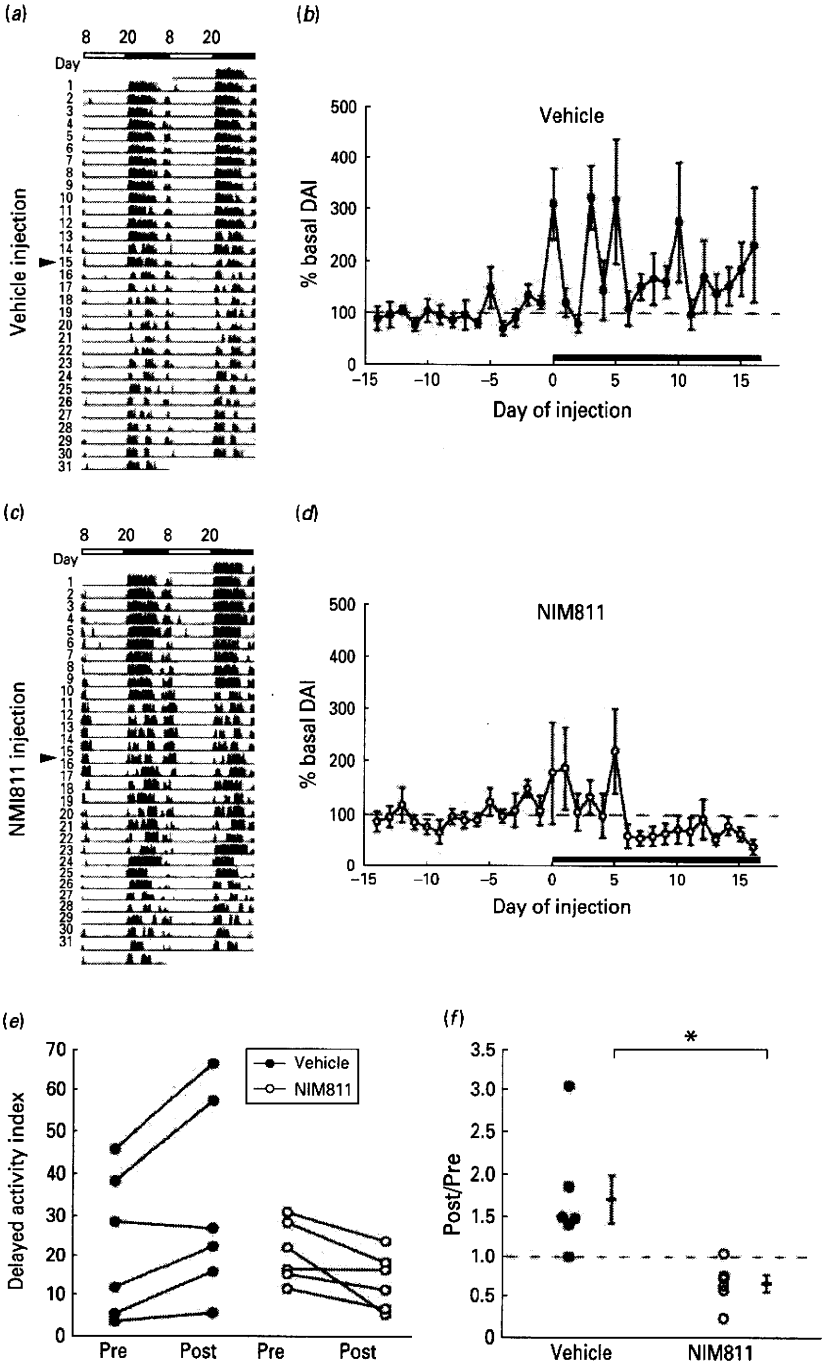

Fig. 3. Effect of 40 mg/kg NIM811 on wheel-running activity in *mutPolg1* Tg mice. Individual activity record of (*a*) vehicle-treated or (*c*) NIM811-treated *mutPolg1* Tg mice. An arrowhead indicates the first day of injection (day 15). Standardized delayed activity index (DAI) of (*b*) vehicle-treated 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 −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) 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 Ca^{2+} (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 Ca^{2+} buffering in the synaptic terminal (Billups & Forsythe, 2002; Kim *et al.* 2005). Release of glutamate and acetylcholine was decreased in CypD knockout mice (Mouri *et al.* 2009). Taken together, these finding 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 *PPIF* 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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