Mitochondrial DNA 3243A>G Mutation and Increased Expression of *LARS2* Gene in the Brains of Patients with Bipolar Disorder and Schizophrenia

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Background: *Accumulating evidence suggests mitochondrial dysfunction in bipolar disorder. Analyses of mitochondria-related genes using DNA microarray showed significantly increased* LARS2 *(mitochondrial leucyl-tRNA synthetase) in the postmortem prefrontal cortices of patients with bipolar disorder provided by the Stanley Foundation Brain Collection.* LARS2 *is a nuclear gene encoding the enzyme catalyzing the aminoacylation of mitochondrial* $tRNA^{Leu}$*. A well-studied mitochondrial DNA point mutation, 3243A>G, in the region of* $tRNA^{Leu\ (UUR)}$*, related with MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes), is known to decrease the efficiency of aminoacylation of* $tRNA^{Leu\ (UUR)}$*.*

Methods: *The steady state level of* LARS2 *was examined in the transmitochondrial cybrids carrying 3243A>G. We examined the 3243A>G mutation in these brains using the peptide nucleic acid-clamped polymerase chain reaction restriction fragment length polymorphism method.*

Results: LARS2 *was upregulated in the transmitochrondrial cybrids carrying 3243A>G. The 3243A>G was detected in the postmortem brains of two patients with bipolar disorder and one with schizophrenia. These patients also showed higher levels of the mutation in their livers and significantly higher gene expression of* LARS2 *compared with other subjects.*

Conclusions: *These results suggest that upregulation of* LARS2 *is a hallmark of 324A>G mutation. The accumulation of 3243A>G mutation in the brain may have a pathophysiologic role in bipolar disorder and schizophrenia.*

Key Words: DNA microarray, *leucyl-tRNA synthetase* 2, *mitochondrial (LARS2)*, aminoacylation, mitochondrial DNA 3243A>G, peptide nucleic acid (PNA)-clamped PCR, postmortem brain

We have been studying the relationship between mitochondrial DNA (mtDNA) mutations/polymorphisms and bipolar disorder based on the mitochondrial dysfunction hypothesis (Kato and Kato 2000). Mitochondrial dysfunction was implied by altered energy metabolism detected by phosphorous-31 magnetic resonance spectroscopy (MRS) in the frontal lobes of patients with bipolar disorder (Kato et al 1993, 1994), the possible role of maternal transmission of bipolar disorder (McMahon et al 1995), and comorbidity of chronic progressive external ophthalmoplegia (CPEO), one of the representative mitochondrial diseases, with bipolar disorder (Siciliano et al 2003) and depression (Suomalainen et al 1992). Recent proton magnetic resonance spectroscopy (Dager et al 2004) and gene expression (Konradi et al 2004) studies also supported mitochondrial dysfunction in bipolar disorder.

Human mtDNA is inherited only maternally and encodes 13 protein subunits of the respiratory chain, 22 tRNAs, and 2 rRNAs (Anderson et al 1981). Mutations of mtDNA cause mitochondrial diseases, such as MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes), MERRF (myoclonus epilepsy with ragged-red fibers), and CPEO (Wallace 1999). Mutations and polymorphisms of mtDNA have also been shown to associate with diabetes mellitus or neurodegenerative diseases, such as Alzheimer disease and Parkinson disease (Tanaka et al 2004). Some patients with MELAS caused by the 3243A>G mutation in mtDNA reportedly developed schizophrenia (Prayson and Wang 1998; Ueda et al 2004) and other psychotic disorders (Suzuki et al 1997; Thomeer et al 1998; Yamazaki et al 1991). Patients with mitochondrial diabetes mellitus caused by 3243A>G frequently have depression as comorbidity (Miyaoka et al 1997; Onishi et al 1997). Although it cannot be ruled out that they are coincidental comorbidity of two diseases, it might be possible that 3243A>G is somehow related to mental disorders.

In an attempt to test our mitochondrial dysfunction hypothesis, we initially reanalyzed the DNA microarray data examined in the postmortem prefrontal cortices provided by the Stanley Foundation Brain Collection and consisting of bipolar disorder, major depression, schizophrenia, and control subjects (Iwamoto et al 2004). In the previous report, we could detect only one mitochondria-related gene differentially expressed in bipolar disorder, *YWHAE* (tyrosine 3-monooxygenase epsilon), using stringent criteria such as a change of 1.3-fold or more. Thus, in this study, we selected all mitochondria-related genes, and statistical analysis was performed using less stringent criteria. Among the mitochondria-related genes, *LARS2* (human leucyl-tRNA synthetase 2, mitochondrial NM015340) was increased most significantly in bipolar disorder. The enzyme encoded by this gene catalyzes the aminoacylation of $tRNA^{Leu}$. Mitochondrial 3243A>G mutation causing MELAS was shown to decrease the efficiency of processing and aminoacylation of $tRNA^{Leu}$ (Park et al 2003; Rossmanith and Karwan 1998; Yasukawa et al 2000). We hypothesized that *LARS2* was compensatory upregulated due to the accumulation of 3243A>G. In this study, we showed the accumulation of 3243A>G mutation in the brains of patients with bipolar disorder or schizophrenia with increased expression of *LARS2*.

Methods and Materials

We first reanalyzed the previously published DNA microarray data in the postmortem brains focusing on mitochondria-related genes. Because observed upregulation of *LARS2* was speculated

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Table 1. Characteristics of the Subjects

	Control (n = 14)	Bipolar Disorder (n = 15)	Schizophrenia (n = 13)	Major Depression (n = 15)
Profile of Subjects				
Age, years (M, SD)	49.0, 10.4	42.3, 11.7	44.4, 12.9	46.5, 9.3
Age at onset, years (M, SD)		21.6, 8.6	23.3, 8.4	33.9, 13.3
Postmortem interval, hours (M, SD)	23.8, 10.3	32.5, 16.1	35.4, 14.6	27.5, 10.7
Side of the Brain (n)	R6, L8	R8, L7	R5, L8	R6, L9
Gender (n)	F5, M9	F6, M9	F6, M7	F6, M9
Suicide (n)	0	9	3	7
Medication (n)				
Lithium	0	4	2	2
Valproate	0	6	0	0
Carbamazepin	0	3	1	0
Antidepressants	0	8	4	9
Typical antipsychotics	0	4	8	0
Atypical antipsychotics	0	3	6	0
Any Drug	0	12	10	10
Results of DNA Microarray (Mean, SD)				
LARS2 (MAS5)	.61, .12	.80, .18[a]	.75, .27[c]	.67, .21
LARS2 (Affy)	81.5, 11.2	97.2, 23.4[b]	90.0, 21.5	77.4, 12.4

F, female; L, left; M, male; R, right.
[a] $p < .01$.
[b] $p < .05$.
[c] $p < .10$, compared with control subjects.

to be caused by the mtDNA 3243A>G mutation, we tested whether the 3243A>G mutation actually upregulates *LARS2* using reverse transcription polymerase chain reaction (RT-PCR) in transmitochondrial cybrids. The mtDNA 3243A>G mutation in the postmortem brains was examined using two methods, conventional PCR restriction fragment length polymorphism (RFLP) and a more sensitive method, peptide nucleic acid (PNA)-clamped PCR. Finally, the DNA micoarray data was reanalyzed with regard to the presence or absence of mtDNA 3243A>G mutation.

Postmortem Samples of Brain and Liver

Postmortem prefrontal cortex (Brodmann area 10) and liver were donated by the Stanley Foundation Brain Collection. The primary sample set included 15 each of control, major depression, bipolar disorder, and schizophrenia subjects, whose details are described elsewhere (Torrey et al 2000). DNA microarray was performed using a part of the sample set, 15 control subjects, 11 patients with bipolar disorder, 11 with major depression, and 13 with schizophrenia as described previously (Iwamoto et al 2004). The samples, which were used to detect the 3243A>G mutation, consisted of 14 control, 15 bipolar disorder, 15 major depression, and 13 schizophrenia subjects, from the sample set of the brain collection. A summary of the subjects' demographic information is shown in Table 1.

Expression of Mitochondria-Related Probe Sets in Bipolar Disorder

The probe sets for mitochondria-related genes were selected by NetAffx (http://www.affymetrix.com/analysis/index.affx) with the key words, "mitochondria" or "mitochondrion." A total of 493 probe sets were selected in the HU95A chip. Student's t test with no correction of multiple testing was applied to the expression level of each gene between bipolar disorder (n = 11) and control subjects (n = 15).

Cell Culture and Generation of Cybrids

We used Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum, penicillin/streptomycin, pyruvate (Gibco-BRL), and uridine (Sigma) as the growth medium. The transmitochondrial cybrids carrying the 3243G were generated by fusing the platelet of a patient with MELAS and 143B.TK$^-\rho^0$206 cells lacking mtDNA as established by King and Attardi (1989). Peripheral whole blood of a patient with MELAS carrying 3243G was obtained after informed consent and used to generate the cybrids. The ethics committees of RIKEN approved this study. We collected platelets from the blood sample by centrifugation at 1500 g for 20 min. This fraction of platelets was fused with the ρ^0 cells and cybrids were obtained as previously described (Chomyn et al 1994). These cybrid cells were cultured in growth medium and cloned by limiting dilution. Each colony was picked up and grown until the cell count reached 1×10^6. After harvesting individual cybrid cell lines, the integration of mtDNA was confirmed by Southern blot and PCR as described previously (Munakata et al 2004). These cybrids carried approximately 50%–100% of 3243G.

Preparation of cDNA and Total DNA

cDNA was synthesized as previously described (Iwamoto et al 2004). In short, total RNA was extracted from frozen postmortem tissues and 1×10^6 cells of each cybrid clone using Trizol (Invitrogen, Carlsbad, California), contaminated DNA in the total RNA was excluded using DNaseI (Takara Bio, Otsu, Japan), and cDNA was synthesized from the RNA using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Total genomic DNA was extracted from the remaining Trizol solution after RNA extraction.

Detection and Quantification of Mitochondrial 3243A>G Mutation

PCR-RFLP was designed to detect 3243A>G mutation. In summary, the mitochondrial DNA fragment encompassing