Increased Levels of a Mitochondrial DNA Deletion in the Brain of Patients with Bipolar Disorder

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Mutations in mitochondrial DNA (mtDNA) have been implicated in the pathophysiology of affective disorders. To examine possible pathophysiological significance of mtDNA deletions in bipolar disorder, the concentration of the 4977-base-pair deletion in mtDNA in the autopsied brains of 7 patients with bipolar disorder, 9 suicide victims, and 9 controls was examined using a quantitative polymerase chain reaction method. The ratio of deleted to wild-type mtDNA in cerebral cortex was significantly higher in patients with bipolar disorder [0.23 ± 0.18 (mean ± SD)%] compared with that in age-matched controls (0.06 ± 0.07%, p < 0.05). This result supports a hypothesis that mtDNA deletions may play a role in the pathophysiology of bipolar disorder. © *1997 Society of Biological Psychiatry*

Key Words: Affective disorder, depression, suicide, molecular genetics, energy metabolism, mutation

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Introduction

Mitochondrial DNA (mtDNA) deletions have an etiological role in several disorders, including chronic progressive external ophthalmoplegia (CPEO) and Pearson's disease (Holt et al 1988; Rotig et al 1989). Two pedigrees of familial CPEO in which depressive symptoms were one characteristic of the syndrome were reported (Ciafaloni et al 1991; Suomalainen et al 1992). These reports prompted us to examine the deletion in mtDNA in the brains of patients with bipolar disorder; however, we could not detect any mtDNA deletion using Southern blot analysis in the autopsied brains of 7 patients with bipolar disorder or 9 subjects who committed suicide (Stine et al 1993).

In spite of this initial negative finding, we have accumulated evidence that supports the pathophysiological significance of a mtDNA abnormality in bipolar disorder. We examined brain high-energy phosphate metabolism by phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS), and found that phosphocreatine was decreased in the frontal lobes in patients with bipolar disorder (Kato et al 1992, 1994, 1995). This finding is similar to that observed in mitochondrial myopathies. We also found that mitochondrial inheritance may be involved in the transmission of bipolar disorder (McMahon et al 1995), although the interpretation of such a clinical genetic finding is not straightforward (Kato et al 1996). Moreover, we examined the 4977-base-pair deletion (so-called "common deletion") in leukocyte mtDNA in patients with bipolar disorder and found that 2 of 35 patients had the deletion, but it was found in none of 29 normal controls (Kato and

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Takahashi 1996). We examined the proportion of the common deletion using a quantitative polymerase chain reaction (PCR) method in these 2 patients and an additional 34 patients with affective disorder, and found 1 of 2 bipolar patients previously reported and another patient with unipolar depression who had markedly increased levels of the deletion (Kato et al 1997).

After our initial negative report on mtDNA deletion in the brains of patients with bipolar disorder, several studies of the levels of the mtDNA deletion in brain have been reported using quantitative PCR. These studies revealed that the common deletion in mtDNA increases with age (Corral-Debrinski et al 1992; Soong et al 1992), and it was markedly increased in the brains of patients with Huntington's disease (Horton et al 1995). This deletion may also be increased in the brains of patients with Alzheimer's disease (Corral-Debrinski et al 1994), although this is still controversial (Cavelier et al 1995).

The development of experimental techniques for the quantitation of mtDNA deletions allowed us to quantitate the common deletion in the brains of patients with bipolar disorder that we had previously examined by Southern blot analysis, because Southern blot analysis has a much lower sensitivity ($1/10^2$) for the detection of the deletion compared with quantitative PCR methods ($1/10^8$).

In this study, we examined the relative quantity of the 4977-base-pair deletion in mtDNA in the autopsied brains of patients with bipolar disorder and suicide victims using the quantitative PCR method. Our results support a significant increase in the proportion of deleted mtDNA in bipolar patients, but not in suicide victims or age-matched controls.

Methods and Materials

Materials

The brain tissue examined was the same as that in our previous report (Stine et al 1993). Brain tissue was collected from cases in the District of Columbia Medical Examiners Office and from former patients at St. Elizabeth's Hospital. Samples were taken from the cerebral cortex of 7 individuals diagnosed by medical records as having bipolar disorder, 9 individuals who committed suicide (5 had antidepressants in their blood at autopsy), and 8 normal controls. There was no significant difference of age and sex among these three groups.

The brain tissue was minced, and treated with sodium dodecyl sulfate and proteinase K. The DNA was extracted with phenol and chloroform, and precipitated with ethanol.

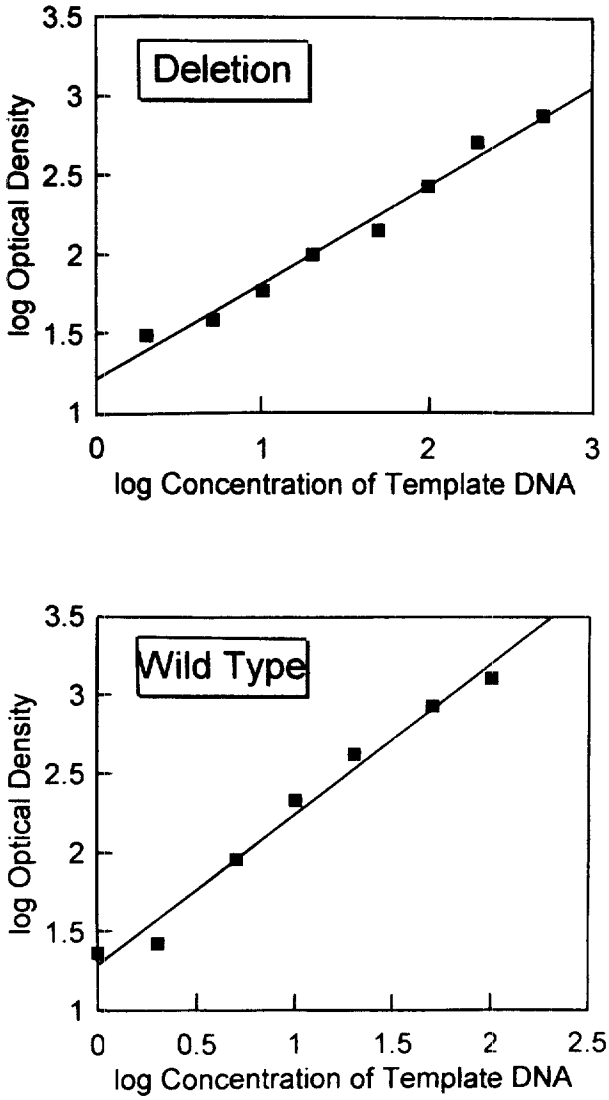

Figure 1. Log-log plots of densitometric data and concentration of standard solutions.

Methods

The mtDNA deletion was quantitated by the method of Soong et al (1992). Standard solutions of known concentrations were made from PCR products amplified from leukocyte DNA of a normal subject and muscle DNA of a patient with CPEO. These standard solutions were simultaneously amplified in each PCR experiment, and a standard curve was made from each gel.

Wild-type mtDNA was quantitated using the primer set, L13176 (corresponding to the nucleotide numbers 13176–13199) and H13501 (13501–13477). The DNA solution of 0.8 μL was amplified in an 8-μL reaction containing 2 mmol/L of Tris–HCl (pH 8.4), 50 mmol/L of KCl, 1.5 mmol/L of MgCl, 1 μmmol/L each of oligonucleotide primer, 0.31 mmol/L each of deoxycytidine triphosphate,

Table 1. Quantitation of the 4977 bp Mitochondrial DNA Deletion in the Brains in Patients with Bipolar Disorder and Suicide Victims

Diagnosis	Age (mean ± SD)	Percentage of the mtDNA deletion (mean ± SD)
Bipolar disorder (n = 7, F 4, M 3)	44.5 ± 11.9	0.233 ± 0.180[a]
Suicide victims (n = 9, F 5, M 4)	39.1 ± 11.7	0.136 ± 0.167
Controls (n = 9, F 5, M 4)	40.4 ± 12.3	0.069 ± 0.074

F, female; M, male. No significant difference in age was found among the three groups. No significant difference of the ratio of mtDNA deletion was found between suicide victims and controls (t = 1.04, p = .3).

[a] $p < .05$ vs. controls (t = 2.3, by Student's t test).

deoxythymidine triphosphate, and 2-deoxyguanosine-5′-triphosphate, 0.078 mmol/L of deoxyadenosine triphosphate (dATP), 2.5 μCi of ^{35}S-dATP, and 0.5 U of Taq polymerase. The parameters of the PCR were as follows; 94°C for 20 sec and 60°C for 20 sec for 16 cycles.

For the quantitation of deleted mtDNA, 0.8 μl of the DNA solution was amplified using the primer pair L8224 (8224–8247) and H13501 in the same reaction buffer as that used in the quantitation of wild-type mtDNA, except for Taq polymerase of 0.7 U. The PCR was done at 92°C for 20 sec and 60°C for 20 sec for 29 cycles. In all PCR, heat-denaturation was performed at 94°C for 3 min before the first cycle, and final extension was done at 72°C for 3 min.

Taq polymerase was purchased from Gibco BRL and ^{35}S-dATP was purchased from Amersham Co. Ltd. The oligonucleotide primers were made at the DNA Core Facility of the University of Iowa College of Medicine. A Thermal Cycler 480 (Perkin Elmer) was used for the PCR.

PCR products were electrophoresed in 6% polyacrylamide gels and visualized by autoradiography. The signal intensities of these PCR products were examined by densitometry. The linear regression was calculated between the logarithm of concentration of standard DNA solutions and logarithm of optical density of the PCR products (Figure 1). Concentrations of wild-type or deleted mtDNA were calculated from these standard curves.

The concentrations of wild-type mtDNA and deleted mtDNA were examined in duplicate in all samples. The ratio of deletion/wild-type mtDNA (D/W ratio) was calculated from these values. For statistical analysis, two-tailed Student's t test and Mann–Whitney U test were used.

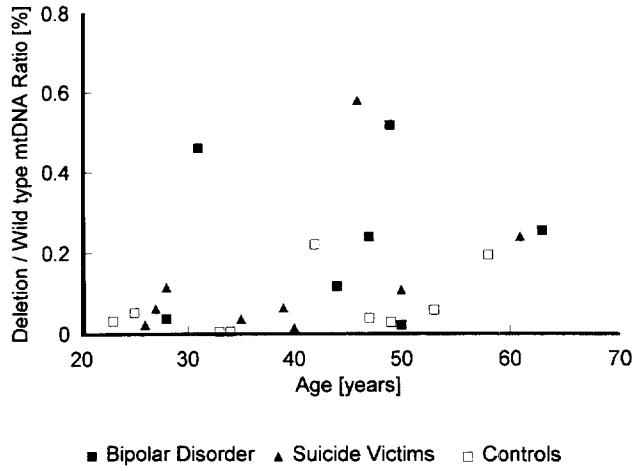

Figure 2. Levels of the mtDNA deletion in the brain in relation to age in patients with bipolar disorder, suicide victims, and normal controls.

Results

The results are summarized in Table 1. The levels of the mtDNA deletion in the brain in relation to age are shown in Figure 2.

The D/W ratio in bipolar disorder cases was significantly higher than that in age-matched controls (t = 2.31, df = 14, $p < .05$ by Student's t test). This difference was close to significance with Mann–Whitney U test (U = 14, p = .06). Although the D/W ratio in suicide victims did not differ significantly from controls (t = 1.04, df = 16, p = .31 by t test, U = 27, p = .23 by Mann–Whitney U test), 1 subject who died of suicide had the highest proportion of deleted mtDNA seen in this study (Figure 2), which resulted in a somewhat higher average proportion of deleted mtDNA in suicide victims. When the two groups, bipolar disorder and suicide victims, were combined, they tended to have higher levels of the deletion (0.178 ± 0.179%, n = 16) compared with controls (t = 1.67, df = 23, p = .10 by t test, U = 41, p = .07 by Mann–Whitney U test).

Discussion

We found significantly increased levels of the common deletion of mtDNA in the brains of patients with bipolar disorder compared with age-matched controls. The mean level of the mtDNA deletion in bipolar disorder was three times higher than that in normal controls. This is the first direct evidence that a mtDNA abnormality may play an important role in the pathophysiology of bipolar disorder.

Why should the quantity of mtDNA deletion be increased in the brains of patients with bipolar disorder? The 4799-base-pair deletion in mtDNA increases with age in normal brain (Corral-Debrinski et al 1992; Soong et al 1992). This may be due to mtDNA damage from oxygen free radical generation. Increased mtDNA deletion in the brains of Huntington's disease (Horton et al 1995) and Alzheimer's disease (Corral-Debrinski et al 1994) may

also be due to a similar mechanism; however, bipolar disorder has a much lower age at onset than these neurodegenerative disorders.

CPEO, the disease caused by mtDNA deletions, is usually sporadic, but sometimes maternally inherited (Holt et al 1988). In some pedigrees, CPEO is inherited in an autosomal-dominant manner (Suomaleinen et al 1992). In this case, several populations of mtDNA deletions (multiple deletions) can be found in muscles. This disease, autosomal-dominant CPEO, is thought to be caused by a mutation in a nuclear gene that interacts with mtDNA. This autosomal locus was recently assigned to 10q23.3–24.3 (Suomalainen et al 1995).

We have postulated that maternal inheritance may be involved in the transmission of bipolar disorder (McMahon et al 1995). Therefore, this aberrant mtDNA in the brains of patients with bipolar disorder might be transmitted from a mother.

The pedigree in which CPEO and affective disorder were transmitted together displayed autosomal-dominant inheritance (Suomalainen et al 1992). Furthermore, the deletion we found in leukocyte mtDNA occurred in a proband with bipolar disorder and his father (Kato et al 1997). Therefore, it may also be possible that patients with bipolar disorder have some abnormality in nuclear genes predisposing to deletions in mtDNA.

Does the deletion in a small percentage we observed in mtDNA play an etiological role in bipolar disorder? Figure 2 indicates that 3 patients having higher levels of the deletion caused statistically significant results. These 3 patients had a D/W ratio around 0.5%. If this deletion was more abundant in specific regions of the brain, it could play a pathophysiological role. Although we could not examine other brain regions such as basal ganglia in this study, other brain regions should be examined in the future.

Is the observed phenomenon a contributing cause to or an effect of the disorder? Even if we found an association between the common deletion in mtDNA and bipolar disorder, this association could be interpreted not only as a cause, but also as an epiphenomenon (e.g., secondary to lithium treatment or drug use in these bipolar patients) or a consequence of the illness.

It is possible that mitochondrial dysfunction in the brain causes affective disorder, because there are many case reports that patients with mitochondrial myopathy or carriers of mutations in mtDNA had affective disorder (Wallace 1970; Stewart and Naylor 1990; Shanske et al 1993; Sweeney et al 1993; Onishi et al 1997). The authors have reported a decrease in phosphocreatine in the left frontal lobe in patients with bipolar disorder, correlating with higher scores on the Hamilton Depression Rating Scale (Kato et al 1995). This also suggests that mitochondrial dysfunction plays a role in progression of affective symptoms. Some symptoms of affective disorder, such as psychomotor retardation and fatigability, might be explained by a dysfunction in oxidative phosphorylation. One may think that it is hard to reconcile mania, a state of overactivity, with impairment of brain energy metabolism; however, it has been reported that brain injury also causes mania (Robinson et al 1988). We previously reported that intracellular pH in the frontal lobes detected by ^{31}P-MRS was decreased in bipolar disorder (Kato et al 1993), and hypothesized that alteration of monoamine activity in bipolar disorder may be a secondary phenomenon caused by decreased intracellular pH, because intracellular pH is regulated by Na^+/H^+ antiport activity, and this transport system is regulated by monoaminergic systems and protein kinase C (Alpern et al 1993). Thus, subclinical impairment of brain energy metabolism might have caused compensatory activation of monoaminergic systems, via increased presynaptic activity, altered receptor function, or alterations of second-messenger systems such as protein kinase C (Friedman et al 1993), although this is very speculative.

Is this finding specific to bipolar disorder or common in other psychiatric disorders? In this study, suicide victims did not have higher levels of the mtDNA deletion. Considering the estimate that 70% of completed suicides had major depression, we can assume that about 6 of our 9 suicide cases had major depression. An increase of the mtDNA deletion was not found in the brains of schizophrenic patients (Cavelier et al 1995). Therefore, this finding may not be observed in other psychiatric disorders.

The weakness of this study is that the exact region examined in the cerebral cortex was not known, and brain tissues of other regions were not available. Because regional differences in the proportion of deleted mtDNA have been reported, study of other brain regions in bipolar cases is indicated.

All the problems discussed above need to be addressed before the 4977-base-pair deletion in mtDNA can be suggested to play an important role in the pathophysiology of bipolar disorder.

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