Association of Mitochondrial Complex I Subunit Gene *NDUFV2* at 18p11 with Bipolar Disorder in Japanese and the National Institute of Mental Health Pedigrees

Shinsuke Washizuka, Kazuya Iwamoto, An-a Kazuno, Chihiro Kakiuchi, Kanako Mori, Mizue Kametani, Kazuo Yamada, Hiroshi Kunugi, Osamu Tajima, Tsuyoshi Akiyama, Shinichiro Nanko, Takeo Yoshikawa, and Tadafumi Kato

Background: *Linkage with 18p11 is one of the replicated findings in molecular genetics of bipolar disorder. Because mitochondrial dysfunction has been suggested in bipolar disorder,* NDUFV2 *at 18p11, encoding a subunit of the complex I, reduced nicotinamide adenine dinucleotide (NADH) ubiquinone oxidoreductase, is a candidate gene for this disorder. We previously reported that a polymorphism in the upstream region of* NDUFV2, *−602G> A, was associated with bipolar disorder in Japanese subjects; however, functional significance of −602G> A was not known.*

Methods: *We screened the further upstream region of* NDUFV2. *We performed a case–control study in Japanese patients with bipolar disorder and control subjects and a transmission disequilibrium test in 104 parent and proband trios of the National Institute of Mental Health (NIMH) Genetics Initiative pedigrees. We also performed the promoter assay to examine functional consequence of the −602G> A polymorphism.*

Results: *The −602G> A polymorphism was found to alter the promoter activity. We found that the other haplotype block surrounding −3542G> A was associated with bipolar disorder. The association of the haplotypes consisting of −602G> A and −3542G> A polymorphisms with bipolar disorder was seen both in Japanese case–control samples and NIMH trios.*

Conclusion: *Together these findings indicate that the polymorphisms in the promoter region of* NDUFV2 *are a genetic risk factor for bipolar disorder by affecting promoter activity.*

Key Words: Bipolar disorder, haplotype, mitochondria, NADH ubiquinone oxidoreductase, promoter assay, transmission disequilibrium test

The etiology of bipolar disorder (BD) is still unknown, but family, twin, and adoption studies strongly suggest the involvement of genetic risk factors (Goodwin and Jamison 1990). Linkage studies have revealed a number of loci to be linked with BD. Of those, several investigators confirmed 18p11 as one susceptibility loci for BD (Berrettini et al 1997; Gershon et al 1996; Nothen et al 1999; Stine et al 1995; Turecki et al 1999). Nominally significant linkage of BD with chromosome 18 was also found in a recent extensive meta-analysis (Segurado et al 2003). Thus, 18p is one of the targets of the genetic association study of BD.

We have proposed a mitochondrial dysfunction hypothesis of BD (Kato and Kato 2000) on the basis of the following evidence: altered brain energy metabolism in patients with BD detected by phosphorus-31 magnetic resonance spectroscopy (Kato et al 1993), increased ratio of the mitochondrial DNA (mtDNA) deletion in the brains of patients with BD (Kato et al 1997), association with mtDNA polymorphisms causing amino acid substitutions in the subunits of complex I (reduced nicotinamide adenine dinucleotide [NADH]: ubiquinone oxidoreductase; Kato et al 2001).

From the Laboratories for Molecular Dynamics of Mental Disorders (SW, KI, AK, CK, KM, MK, TK) and Molecular Psychiatry (KY, TY), Brain Science Institute, RIKEN, Wako, Saitama; Department of Mental Disorder Research (HK), National Institute of Neuroscience; Kyorin University School of Health Sciences (OT); Department of Neuropsychiatry (TA), NTT East Kanto Medical Center; and Department of Psychiatry and Genome Research Center (SN), Teikyo University School of Medicine, Tokyo, Japan.

Address reprint requests to Tadafumi Kato, M.D., Ph.D., Laboratory for Molecular Dynamics of Mental Disorders, Brain Science Institute, RIKEN, Hirosawa 2-1, Wako, Saitama, 351-0198, Japan; E-mail: kato@brain.riken.go.jp.

Received March 9, 2004; revised June 22, 2004; accepted July 5, 2004.

Complex I catalyzes the transfer of electrons from NADH to ubiquinone and the largest and most complicated enzyme in the mitochondrial electron transport chain, consisting of at least 43 subunits. Whereas seven subunits of complex I are coded in the mtDNA, the others are coded in the nuclear genome (Smeitink et al 2001). Of those, *NDUFV2* is located at 18p11 (de Coo et al 1995; Hattori et al 1995) and is a candidate gene for BD. Recently, Nakatani et al (2004) examined the gene expression patterns in the frontal cortex and hippocampus in animal models of depression and reported that *NDUFV2* was one of two genes altered in both regions. Moreover, Karry et al (2004) reported that protein levels of 24kDa subunit of complex I encoded by *NDUFV2* were altered in the autopsied brains of BD patients. These findings suggested a possible role of *NDUFV2* in mood disorders.

We previously screened mutations and polymorphisms in all exons and the 1-kb upstream region of *NDUFV2* in BD patients and reported that a polymorphism, −602G> A, in the upstream region was significantly associated with BD in Japanese (Washizuka et al 2003). The mRNA expression of *NDUFV2* was also significantly decreased in the lymphoblastoid cells of patients with bipolar I disorder.

In this study, we further screened the 4kb-upstream region of the *NDUFV2* and examined the association with BD in a Japanese case–control samples. Furthermore, we performed a promoter assay to examine the functional significance of the −602G> A polymorphism, which determines the major haplotypes associated with BD. We then examined whether a similar association was found in the National Institute of Mental Health (NIMH) Initiative Genetics Bipolar Pedigrees by the haplotype transmission disequilibrium test (TDT).

Methods and Materials

Japanese Case–Control Samples

The subjects with BD were 189 unrelated patients (117 women and 72 men, 136 with bipolar I disorder (BDI) and 53

0006-3223/04/$30.00
doi:10.1016/j.biopsych.2004.07.004

© 2004 Society of Biological Psychiatry

with bipolar II disorder (BDII; 49.8 ± 13.8 years) who were followed at the hospitals or clinics participating in this study. Their age at onset was 34.9 ± 13.4 years. Consensus diagnosis by at least two senior psychiatrists according to the DSM-IV criteria was made for each patient using a nonstructured interview and by reviewing medical records. The 222 unrelated control subjects (117 women and 105 men, 30.2 ± 8.3 years old) were recruited from hospital staff and students. Control subjects were not assessed for psychiatric symptoms by any structured interview method, but they showed good social functioning and reported themselves to be in good health. All the subjects were Japanese with characteristics as described previously (Washizuka et al 2003). The objective of this study was clearly explained, and written informed consent was obtained from all subjects.

Genomic DNA was extracted from leukocytes using standard methods. There was no evidence for the presence of population substructure in either control subjects or BD using the method of Prichard (2000; Kakiuchi et al 2003). The ethics committees of the Brain Science Institute and participating institutes approved this study.

NIMH Genetics Initiative Pedigrees

For TDT, 105 trio samples (94 trios with BDI probands and 11 trios with BDII probands) were obtained from NIMH Genetics Initiative Bipolar Pedigrees. Each trio was obtained from a larger NIMH family independent of each other. Of those, results of genotyping were inconsistent with the parent–child relationship in one pedigree with BDI. Thus, this pedigree was omitted from the analysis.

Mutation Screening of the *NDUFV2* Gene by Sequencing

Polymorphisms of the upstream region of the *NDUFV2* (GenBank accession number NT_010859) were screened in 20 randomly selected Japanese subjects (9 BDI, 3 BDII, and 8 control subjects). For the scanning of the 5′- upstream region, the following primer sets were used: 5′-TATAGGTCATGAACTCAAAAAGACG and 5′-GCCACACTGTTCACCTTCC. These primers amplified a 3983bp product. Polymerase chain reaction (PCR) was performed in a 25-μL volume containing 20 ng of genomic DNA, .2 μmol/L of each primer, 100 μmol/L of each dNTP, 12.5 μL of 2 × GC buffer I (Takara, Shiga, Japan), and 1.25 units of LA-Taq DNA polymerase (Takara). After an initial denaturation at 95°C for 2 min, 27 cycles consisting of 30 sec at 94°C, 30 sec at 62°C, and 4 min at 72°C were performed. An extension at 72°C for 5 min followed. Sequencing of the PCR products was conducted using the BigDye terminator sequencing kit (Applied Biosystems, Foster City, California) and an ABI 3700 DNA sequencer (Applied Biosystems). For this analysis, 15 sequence primers were used.

Genotyping

Five single nucleotide polymorphisms (SNPs) detected in the screening analysis, −3542G> A, −3245*T*> C, −3041*T*> G, −2694A> G, and −1020G> T, were genotyped in Japanese samples and NIMH bipolar pedigrees. For genotyping of the former four polymorphisms, genomic DNA was amplified by using the upstream primer 5′-AAACTAGCCCTTCCATTCTCCTT and the downstream primer 5′-CCTTCTTGTCTCATTGGCTTACA. These primers amplified a 1547bp product. We performed PCR in a 15-μL volume containing 15 ng of genomic DNA, .1 μmol/L of each primer, 25 μmol/L of each dNTP, 1.5 μL of 10 × Ex-Taq buffer (Takara), and .72 units of Ex-Taq DNA polymerase (Takara). After an initial denaturation at 95°C for 2 min, 35 cycles

Table 1. Pairwise Linkage Disequilibrium Between Polymorphisms in the *NDUFV2*

	Frequency of minor allele	−3542G>A	−3245T>C	−3041T>G	−2694A>G	−1020G>T	−796C>G	−795T>G	−602G>A	−233T>C	86C>T
−3542G>A	.32		r^2 = .97	r^2 = .17	r^2 = .97	r^2 = .10	r^2 = .33	r^2 = .33	r^2 = .91	r^2 = .20	r^2 = .59
−3245T>C	.32	D' = 1.00		r^2 = .16	r^2 = 1.00	r^2 = .10	r^2 = .29	r^2 = .29	r^2 = .63	r^2 = .16	r^2 = .59
−3041T>G	.28	D' = 1.00	D' = .93		r^2 = .17	r^2 = .06	r^2 = .23	r^2 = .23	r^2 = .11	r^2 = .08	r^2 = .21
−2694A>G	.32	D' = .98	D' = 1.00	D' = 1.00		r^2 = .11	r^2 = .34	r^2 = .34	r^2 = .97	r^2 = .21	r^2 = .60
−1020G>T	.16	D' = 1.00	D' = 1.00	D' = 1.00	D' = 1.00		r^2 = .29	r^2 = .29	r^2 = .09	r^2 = .04	r^2 < .01
−796C>G	.41	D' = .95	D' = .96	D' = .94	D' = .97	D' = 1.00		r^2 = 1.00	r^2 = .21	r^2 = .43	r^2 = .16
−795T>G	.41	D' = .95	D' = .96	D' = .94	D' = .97	D' = 1.00	D' = 1.00		r^2 = .21	r^2 = .43	r^2 = .16
−602G>A	.32	D' = .96	D' = .81	D' = .77	D' = .98	D' = .92	D' = .78	D' = .78		r^2 = .13	r^2 = .56
−233T>C	.28	D' = .96	D' = .84	D' = .65	D' = 1.00	D' = .29	D' = .86	D' = .86	D' = .83		r^2 = .27
86C>T	.39	D' = .86	D' = .87	D' = 1.00	D' = .87	D' < .01	D' = .59	D' = .59	D' = .83	D' = 1.00	

Table 2. Genotypic and Allele Distributions of the Additional *NDUFV2* Gene Polymorphisms and −602G>A in Japanese Controls and Bipolar Patients

Polymorphisms		Subject Counts (%)				
		Controls	BP Total	BPI	BPII	*p* Value[b]
−3542G>A						
Genotype	G/G	15 (.07)	25 (.13)	13 (.09)	12 (.23)	
	G/A	102 (.46)	80 (.42)	63 (.46)	17 (.32)	.02
	A/A	104 (.47)	85 (.45)	61 (.45)	24 (.45)	
P Value			.09	.62	.003	
Allele	G	132 (.30)	130 (.34)	89 (.32)	41 (.39)	.21
	A	310 (.70)	250 (.66)	185 (.68)	65 (.61)	
P Value			.20	.50	.08	
−3245T>C						
Genotype	T/T	15 (.07)	24 (.13)	12 (.09)	12 (.23)	
	T/C	101 (.47)	80 (.42)	64 (.47)	16 (.30)	.01
	C/C	97 (.46)	84 (.45)	59 (.44)	25 (.47)	
P Value			.14	.80	.002	
Allele	T	131 (.31)	128 (.34)	88 (.33)	40 (.38)	.38
	C	295 (.69)	248 (.66)	182 (.67)	66 (.62)	
P Value			.32	.61	.20	
−3041T>G						
Genotype	T/T	106 (.49)	98 (.52)	73 (.54)	25 (.47)	
	T/G	88 (.40)	86 (.46)	60 (.44)	26 (.49)	.009
	G/G	23 (.11)	4 (.02)	2 (.02)	2 (.04)	
P Value			.001	.002	.23	
Allele	T	300 (.69)	282 (.75)	206 (.76)	76 (.72)	.12
	G	134 (.31)	94 (.25)	64 (.24)	30 (.28)	
P Value			.07	.04	.63	
−2694A>G						
Genotype	A/A	16 (.07)	24 (.13)	12 (.09)	12 (.23)	
	A/G	99 (.46)	79 (.42)	62 (.46)	17 (.32)	.03
	G/G	100 (.47)	84 (.45)	60 (.45)	24 (.45)	
P Value			.19	.86	.006	
Allele	A	131 (.31)	128 (.34)	88 (.33)	40 (.38)	.38
	G	295 (.69)	248 (.66)	182 (.67)	66 (.62)	
P Value			.32	.61	.20	
−1020G>T						
Genotype	G/G	160 (.72)	131 (.69)	93 (.66)	38 (.72)	
	G/T	58 (.26)	52 (.28)	39 (.29)	13 (.25)	.77
	T/T	4 (.02)	6 (.03)	4 (.03)	2 (.03)	
P Value			.64	.62	.60	
Allele	G	378 (.85)	314 (.83)	225 (.83)	89 (.84)	.66
	T	66 (.15)	64 (.17)	47 (.17)	17 (.16)	
P Value			.44	.39	.76	
−602G>A[a]						
Genotype	G/G	17 (.08)	27 (.14)	15 (.11)	12 (.23)	
	G/A	106 (.48)	77 (.41)	60 (.44)	17 (.32)	.02
	A/A	99 (.44)	85 (.45)	61 (.45)	24 (.45)	
P Value			.07	.51	.003	
Allele	G	140 (.32)	131 (.35)	90 (.33)	41 (.39)	.37
	A	304 (.68)	247 (.65)	182 (.67)	65 (.61)	
P Value			.34	.66	.15	

BP, bipolar disorder
[a]Data of Washizuka et al (2003).
[b]Differences in genotype distributions or allele frequencies among patients with BPI, BPII, and controls.

consisting of 30 sec at 94°C, 30 sec at 61°C, and 2 min at 72°C were performed. An extension at 72°C for 3 min followed.

For genotyping of the −1020G> T polymorphism, the primer sets were used as follows: 5′-ACCAAGGCATTTGGTATCTATTCT and 5′-ATGTTTGTTTGGTTATCTCTGGAAA. We performed PCR in a 25-μL volume containing 25 ng of genomic DNA, .1 μmol/L of each primer, 25 μmol/L of each dNTP, 2.5 μL of 10 × Ex-Taq buffer, and 1.2 units of Ex-Taq DNA polymerase. After an initial denaturation at 95°C for 2 min, 35 cycles consisting of 20 sec at 94°C, 30 sec at 60°C, and 30 sec at 72°C were performed. An extension at 72°C for 3 min followed.

In addition, our previously reported polymorphisms of the *NDUFV2* gene (−796C> G, −795*T*> G, −602G> A, −233*T*> C, and 86C> T) were also genotyped in NIMH samples. The primer set and the PCR condition that were used in genotyping of these SNPs are shown in our previous article (Washizuka et al 2003).

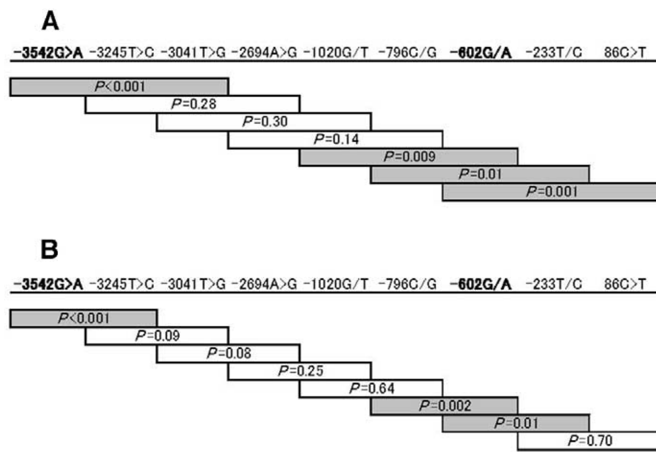

Figure 1. Haplotype associations in Japanese case control samples: **(a)** three-marker haplotype analysis; **(b)** two-marker haplotype analysis. *P* value indicates the global *P* analyzed using COCAPHASE program. Gray squares indicate statistically significant global *p* value.

Sequencing of the PCR products was conducted using the BigDye terminator sequencing kit (Applied Biosystems) and an ABI 3700 DNA sequencer (Applied Biosystems).

Statistical Procedures

Deviations from Hardy–Weinberg equilibrium (HWE) were evaluated by use of the Arlequin program (http://anthropologie.Unige.Ch/arlequin/methods.html). Genotype distributions and allele frequencies between patients and control subjects were computed using Fisher's Exact Probability Test, which was applied using SPSS software (SPSS, Tokyo, Japan). For other analyses, UNPHASED programs (COCAPHASE and TDTPHASE; http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/) were used. The normalized LD coefficient D' and the squared correlation coefficient r^2 were calculated using COCAPHASE program. For TDT of NIMH trio samples, the McNemar Test was used. For the computation of haplotype frequencies, evaluation of haplotypic distributions, and TDT analysis of the multimarker haplotypes, the COCAPHASE and TDTPHASE programs were used. To evaluate the data appropriately, we reanalyzed the significant result using the permutation test implemented in COCAPHASE and TDTPHASE. Sequences were searched for potential transcription factor binding sites using the Match program (http://www.gene-regulation.de/).

Promoter Assay

A 1106-bp fragment (−1111 to −6) of the upstream from the initiation codon of the *NDUFV2* gene was amplified by PCR and cloned into the *Mlu*I/*Bgl*II site of pGL3-Basic vector (Promega, Madison, Wisconsin). Two kinds of reporter plasmids, having either −602G or −602A were prepared. A 586-bp fragment (−591 to −6) lacking the −602G> A site was also amplified and cloned into the same vector. Hela-S3 and HEK293 cells cultured in a 96-well plate were transfected using Superfect (Qiagen, Valencia, California) with .5 mg of the reporter plasmid, .05 mg of a reference plasmid (pRL-TK), and the pGL3-Basic vector carrying no insert. After 36 hours incubation, luciferase activities were measured with the aid of Dual-Glo luciferase assay system (Promega). Four independent experiments were performed for each condition, and the mean and SEM values were presented.

Results

We previously reported that −602G> A, among four polymorphisms (−796C> G, −795T> G, −602G> A, −233T> C) in

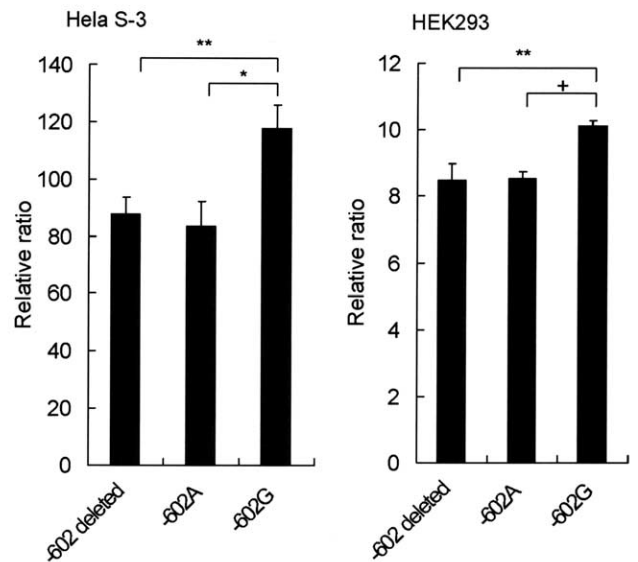

Figure 2. Promoter assay. Promoter activity of three kinds of reporter plasmids, having −602G or −602A and lacking the −602G> A site, were examined by the luciferase assay. Two kinds of cells, Hela-S3 and HEK293, were used. Four independent experiments were performed for each condition, and the mean and SEM values were presented. The transcription activity of the −602G construct was significantly higher than that of the −602A in both Hela-S3 and HEK293. The short construct lacking in the −602G> A site presented nearly equal activity of the −602A construct and had significantly smaller activity compared with −602G constructs. ** $p < .01$. * $p < .05$. + $p = .05$.

the upstream region of *NDUFV2,* showed the significant association with BD (Washizuka et al 2003). In this study, four novel (−3542G> A, −3245T> C, −3041T> G, −2694A> G) and one known (−1020G> T) polymorphisms were detected by sequencing the upstream region extending up to 3963 bp of the transcription initiation site in the *NDUFV2*. We then genotyped these polymorphisms in Japanese patients with BD ($n = 189$) and control subjects ($n = 222$). The genotype frequencies of these five polymorphisms were in HWE in control subjects, although −3041T> C polymorphism was not in BDI patients, and −3245T> G was not in BDII patients. There was no significant difference of genotype frequencies of each SNP between male and female subjects. Detected polymorphisms in this study and in our previously reported polymorphisms (−796C> G, −795T> G, −602G> A, −233T> C, and 86C> T) were in strong linkage disequilibrium with each other (Table 1).

The genotype and allele distributions of the polymorphisms in Japanese population are shown in Table 2. The data for −602G> A polymorphism were cited from our previous paper (Washizuka et al 2003). Statistically significant differences in genotype distribution were observed between patients with BD and control subjects for −3041T> G ($p = .001$). The −3542G> A, −3245T> G, and −2694A> G polymorphisms showed significant genotypic association with BDII ($p = .003$, $p = .002$, and $p = .006$, respectively). These SNPs tended to be associated with BD or BDII even after Bonferroni correction. There was also a nominally significant difference in allelic distribution of −3041T> G polymorphism between patients with BDI and control subjects ($p = .04$).

Haplotype analysis consisting of all 10 polymorphisms revealed a statistically significant association in Japanese samples (global $p < .0001$). To explore which part of the *NDUFV2* gene

Table 3. Estimated Haplotype Frequencies of *NDUFV2* in Japanese Analyzed by Using the COCAPHASE Program

Haplotype[a]	Case	Frequency	Control	Frequency	χ^2	*P* Value	Common
A-A	244	.64	270	.61	.34	.55	+
G-A	3	.008	31	.07	20.35	<.0001	+
A-G	4	.01	37	.08	23.81	<.0001	+
G-G	127	.33	99	.22	10.79	.001	+
Global *P*						<.0001 (<.0001)[b]	

[a]Haplotypes of −3542G>A and −602G>A.
[b]The global *P* value in parentheses shows the global significance by permutation test.

contributes most to this overall association, we employed the sliding window approach in which each set of two or three consecutive polymorphisms were tested for association with BD (two- or three-marker haplotype analysis). This analysis showed evidence of association with BD in two limited regions around −3542G> A and −602G> A (most significant haplotype $p <$ .0001, global $p <$ .0001, and most significant haplotype $p =$ .008, global $p =$ .001, for the three-marker analysis, and global $p <$ .0001 and global $p =$ .002, respectively, for the two-marker analysis; Figure 1).

Because these two SNPs were located at the putative promoter region, we supposed that these polymorphisms might alter the transcription activity. At the beginning, we prepared a 3983bp fragment containing those two polymorphisms and tried to ligate this fragment into the pGL3-basic vector; this was not successful, however. Then we examined whether the −602G> A polymorphism had functional significance. Based on our previous analysis indicating that the two major haplotypes, C-T-A-T and C-T-G-T (consisting of −796C> G, −795*T*> G, −602G> A, and −233*T*> C polymorphisms of *NDUFV2*) were associated with BD, constructs of these two haplotypes were analyzed. Promoter activity was examined in two cell lines, Hela-S3 and HEK293. The transcription activity of the −602G construct was significantly higher than that of the –602A both in Hela-S3 and HEK293 ($p =$ .03 for Hela-S3, and $p =$ .05 for HEK293). The short construct lacking in the –602G> A site presented nearly equal activity of the −602A construct but had significantly smaller activity compared with the −602G construct ($p =$ .0009 and $p =$ .005, respectively; Figure 2).

Because we could not experimentally examine the functional significance of the −3542G> A, we examined whether this site affects the putative binding sites of transcription factors using the Match program. The −3542G> A was predicted to be within the putative binding site for HSF (heat shock transcription factor). HSF1 is known to affect the expression of several other nuclear encoded mitochondrial complex I subunit genes (e.g., *NDUFB8, NDUFA10, NDUFAB1,* and *NDUFS1*). Recently, the binding sequence of HSF1 was well characterized (TT*C*[*T*/C][G/*A*]*GAANNT*-*T*C[T/*C*]; the bases similar to this site of *NDUFV2* promoter was italicized; Trinklein et al 2004). When the −3542 site is G, the core sequence of putative binding site for HSF1, GAA, is lost, and probability of binding was predicted to be decreased.

Thus, the frequency of haplotypes consisting of −3542G> A and −602G> A polymorphisms was also estimated. Distributions of haplotype frequencies differed significantly between patients with BD and control subjects (global $p <$ .0001) (Table 3). Among the haplotypes, the G-G haplotype was significantly more frequently seen in BD ($p =$ .001), whereas G-A and A-G haplotypes were significantly less common in patients with BD compared with control subjects ($p <$ .0001). The results were basically similar when younger control subjects were excluded to match ages of the subjects.

We then performed a TDT in NIMH Genetics Initiative Bipolar Pedigrees. The distributions of genotypes of all 10 detected polymorphisms of the probands, fathers, and mothers were in HWE. We could not detect any allele that was significantly overtransmitted from patients to affected offspring in the NIMH trio samples of BD (Table 4).

We then examined the transmission of haplotypes consisting of −3542G> A and −602G> A polymorphisms from patients to affected offspring by using TDTPHASE program. We found significant association of the *NDUFV2* haplotypes with BD (global $p <$ .0001). Two haplotypes (G-A and A-G) tended to be undertransmitted in parents–proband trios of NIMH samples (nominal $p =$ .04 and $p =$ .01, respectively; Table 5).

Discussion

We identified four novel polymorphisms (−3542G> A, −3245*T*> G, −3041*T*> C, and −2694A> G) associated with BD in this study. Haplotype analysis revealed that two haplotype blocks surrounding the −3542G> A and −602G> A polymor-

Table 4. Transmission Disequilibrium Test in National Institute of Mental Health Initiative Bipolar Pedigrees

Polymorphism	Allele	Tr	Not Tr	Ratio	χ^2	*P*	Number of Trios
−3542G>A	G	20	27	.74	1.04	.30	85
−3245T>C	T	20	30	.67	2.00	.15	91
−3041T>G	T	39	44	.89	.30	.58	90
−2694A>G	A	19	26	.73	1.08	.29	81
−1020G>T	G	10	16	.63	1.38	.23	95
−796C>G	C	42	48	.88	.40	.52	94
−602G>A	G	31	24	1.29	.89	.34	98
−233T>C	T	43	50	.86	.52	.46	99
86C>T	C	34	25	1.36	1.37	.24	99

Tr, transmitted.

Table 5. Transmission of Haplotypes in National Institute of Mental Health Initiative Bipolar Pedigrees Analyzed by using TDTPHASE

Haplotype[a]	Tr	Frequency	Not Tr	Frequency	χ^2	*P* Value	Common
A-A	146	.84	137	.80	1.61	.20	+
G-A	<.01	<.01	3	.02	4.18	.04	+
A-G	<.01	<.01	4	.02	5.59	.01	+
G-G	26	.15	28	.16	.08	.76	+
Global *P*						.01 (.11)[b]	

[a]Haplotypes of −3542G>A and −602G>A.
[b]The global *P* value in parentheses shows the global significance by permutation test.

phisms were associated with BD. The haplotype of these two SNPs were significantly associated with BD in Japanese subjects. In NIMH trios, no individual SNP was associated, and the overtransmission of the risk haplotype in Japanese, G-G, was not observed. Although the observed trend of undertransmission of two haplotypes, G-A and A-G, might be due to the small number of trios, it is noteworthy that the trends of undertransmission of these two haplotypes seen in the NIMH bipolar trio samples were in the same direction to the significant decrease of these haplotypes in Japanese BD subjects.

Although the mechanism by which −602A> G changed the promoter activity is unknown, it would be of interest to note that the −602G polymorphism loses the putative binding site of a transcription factor, p300 (CCACTC). The finding that the −602G haplotype is more common in BD, although yielding a significantly higher promoter activity, is apparently inconsistent with our mitochondrial dysfunction hypothesis in BD; however, the direction of change of promoter activity in the luciferase assay cannot be directly compared with that in vivo. First, promoter activity can be affected by neighboring sequences because several transcription factors form a complex. When only a part of the promoter sequence is subcloned into the luciferase vector, as in this study, the promoter activity does not directly represent the activity in vivo. Second, regulation of gene expression is complex. Although we reported that mRNA expression of *NDUFV2* was decreased in the lymphoblastoid cells, Karry et al (2004) reported that the protein encoded by *NDUFV2* was up-regulated in autopsied BD brains. Thus, it cannot be concluded what kind of mitochondrial dysfunction is caused by polymorphisms of *NDUFV2* promoter. Even though the direction of the change may not represent the promoter activity in vivo, the results of promoter assay indicate that this region has some functional activity only when the −602 position is G.

We could not determine whether −3542G> A affects promoter activity, and this would be a worthwhile topic for future study. Because it has been reported that lithium enhances HSF1 activity (Carmichael et al 2002), it would be particularly interesting to examine the effects of HSF1.

It is most important to test whether the association of *NDUFV2* with BD is replicated using independent BD case–control or trio samples. In addition, 18p11 is a common linkage locus for BD and schizophrenia (Berrettini 2000; Lewis et al 2003), and mRNA and protein expression of *NDUFV2* is also altered in schizophrenia (Karry et al 2004). It would thus be interesting to examine the association between *NDUFV2* and schizophrenia.

In conclusion, the haplotypes consisting of −3542G> A and −602G> A polymorphisms in the upstream region of *NDUFV2* were associated with BD commonly in two ethnicities. Together with altered promoter activity, these findings indicate the role of *NDUFV2* as a genetic risk factor of bipolar disorder.

Data and biomaterials of the National Institute of Mental Health (NIMH) pedigrees were collected in four projects that participated in the NIMH Bipolar Disorder Genetics Initiative. From 1991 to 1998, the principal investigators and co-investigators were as follows: Indiana University, Indianapolis, Indiana, U01 MH46282, John Nurnberger, M.D., Ph.D., Marvin Miller, M.D., and Elizabeth Bowman, M.D.; Washington University, St. Louis, MO, U01 MH46280, Theodore Reich, M.D., Allison Goate, Ph.D., and John Rice, Ph.D.; Johns Hopkins University, Baltimore, Maryland U01 MH46274, J. Raymond DePaulo, Jr., M.D., Sylvia Simpson, M.D., MPH, and Colin Stine, Ph.D.; NIMH Intramural Research Program, Clinical Neurogenetics Branch, Bethesda, Maryland, Elliot Gershon, M.D., Diane Kazuba, B.A., and Elizabeth Maxwell, M.S.W.

Berrettini WH (2000): Susceptibility loci for bipolar disorder: Overlap with inherited vulnerability to schizophrenia. *Biol Psychiatry* 47:245–251.

Berrettini WH, Ferraro TN, Goldin LR, Detera-Wadleigh SD, Choi H, Muniec D, et al (1997): A linkage study of bipolar illness. *Arch Gen Psychiatry* 54:27–35.

Carmichael J, Sugars KL, Bao YP, Rubinsztein DC (2002): Glycogen synthase kinase-3beta inhibitors prevent cellular polyglutamine toxicity caused by the Huntington's disease mutation. *J Biol Chem* 277:33791–33798.

de Coo R, Buddiger P, Smeets H, Geurts van Kessel A, Morgan-Hughes J, et al (1995): Molecular cloning and characterization of the active human mitochondrial NADH: Ubiquinone oxidoreductase 24-kDa gene (*NDUFV2*) and its pseudogene. *Genomics* 26:461–466.

Gershon ES, Bander JA, Detera-Wadleigh SD, Ferraro TN, Berrettini WH (1996): Maternal inheritance and chromosome 18 allele sharing in unilineal bipolar illness pedigrees. *Am J Med Genet* 67:202–207.

Goodwin FK, Jamison KR (1990): *Manic-Depressive Spectrum*. New York: Oxford University Press.

Hattori N, Suzuki H, Wang Y, Minoshima S, Shimizu N, Yoshino H, et al (1995): Structural organization and chromosomal localization of the human nuclear gene (*NDUFV2*) for the 24-kDa iron-sulfur subunit of complex I in mitochondrial respiratory chain. *Biochem Biophys Res Commun* 216:771–777.

Kakiuchi C, Iwamoto K, Ishiwata M, Bundo M, Kasahara T, Kusumi I, et al (2003): Impaired feedback regulation of XBP1 as a genetic risk factor for bipolar disorder. *Nat Genet* 35:171–175.

Karry R, Klein E, Ben Shachar D, (2004): Mitochondrial complex I subunits expression is altered in schizophrenia: A postmortem study. *Biol Psychiatry* 55:676–684.

Kato T, Kato N (2000): Mitochondrial dysfunction in bipolar disorder. *Bipolar Disord* 2:180–190.

Kato T, Kunugi H, Nanko S, Kato N (2001): Mitochondrial DNA polymorphisms in bipolar disorder. *J Affect Disord* 62:151–164.

Kato T, Stine OC, McMahon FJ, Crowe RR (1997): Increased levels of a mitochondrial DNA deletion in the brain of patients with bipolar disorder. *Biol Psychiatry* 42:871–875.

Kato T, Takahashi S, Shioiri T, Inubushi T (1993): Alterations in brain phosphorous metabolism in bipolar disorder detected by in vivo 31P and 7Li magnetic resonance spectroscopy. *J Affect Disord* 27:53–59.

Lewis CM, Levinson DF, Wise LH, DeLisi LE, Straub RE, Hovatta I, et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: Schizophrenia. *Am J Hum Genet* 73:34–48.

Nakatani N, Aburatani H, Nishimura K, Semba J, Yoshikawa T (2004): Comprehensive expression analysis of a rat depression model. *Pharmacogenomics J* 4:114–126.

Nothen MM, Cichon S, Rohleder H, Hemmer S, Franzek E, Fritze J, et al (1999): Evaluation of linkage of bipolar affective disorder to chromosome 18 in a sample of 57 German families. *Mol Psychiatry* 4:76–84.

Pritchard JK, Stephens M, Donnelly P (2000): Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.

Segurado R, Detera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger JI, et al (2003): Genome scan meta-analysis of schizophrenia and bipolar disorder, part III: Bipolar disorder. *Am J Hum Genet* 73:49–62.

Smeitink J, Sengers R, Trijbels F, van den Heuvel L (2001): Human NADH: ubiquinone oxidoreductase. *J Bioenerg Biomembr* 33:259–266.

Stine OC, Xu J, Koskela R, McMahon FJ, Gschwend M, Friddle C, et al (1995): Evidence for linkage of bipolar disorder to chromosome 18 with a parent-of-origin effect. *Am J Hum Genet* 57:1384–1394.

Trinklein ND, Murray JI, Hartman SJ, Botstein D, Myers RM (2004): The role of heat shock transcription factor 1 in the genome-wide regulation of the mammalian heat shock response. *Mol Biol Cel* 15:1254–1261.

Turecki G, Grof P, Cavazzoni P, Duffy A, Grof E, Martin R, et al (1999): Lithium responsive bipolar disorder, unilineality and chromosome 18: A linkage study. *Am J Med Genet* 88:411–415.

Washizuka S, Kakiuchi C, Mori K, Kunugi H, Tajima O, Akiyama T, et al (2003): Association of mitochondrial complex I subunit gene *NDUFV2* at 18p11 with bipolar disorder. *Am J Med Genet* 120B:72–78.