Haplotype Analysis of *GSK-3β* Gene Polymorphisms in Bipolar Disorder Lithium Responders and Nonresponders

Kazuhiko Iwahashi, MD, PhD,†‡§ Daisuke Nishizawa, PhD,‡ Shin Narita,* Maki Numajiri,* Ohoshi Murayama, PhD,‖ Eiji Yoshihara, PhD,* Yuuya Onozawa, PhD,* Kenta Nagahori,* Fumihiko Fukamauchi, MD, PhD,¶ Kazutaka Ikeda, PhD,‡ and Jun Ishigooka, MD, PhD§*

Abstract: The *GSK-3β* gene, *GSK3B,* codes for an enzyme that is a target for the action of mood stabilizers, lithium and possibly valproic acid.

In this study, the relationship between haplotypes consisting of single nucleotide polymorphisms (SNPs) of *GSK3B* −50T/C and −1727A/T and the effect of lithium was studied among Japanese bipolar disorder lithium nonresponders and responders.

The distributions of the *GSK3B* haplotypes (−50T/C and −1727A/T) showed a trend for significant difference between the lithium nonresponders and responders (global P=0.07074). Haplotype 1 (T-A) was associated with a higher lithium response (haplotype-specific P=0.03477), whereas haplotype 2 (C-A) was associated with a lower lithium response (haplotype-specific P=0.03443).

The pairwise D' and r^2 values between the 2 SNPs in this study were 1.0 and 0.097, respectively. The 2 SNPs showed weak linkage disequilibrium with each other.

Key Words: *GSK-3β*, bipolar disorder, lithium response

(*Clin Neuropharm* 2014;37: 108–110)

Recent findings suggest that glycogen synthase kinase–3β (*GSK-3β*) may play a role in the pathophysiology and treatment of mood disorders. Mood stabilizers, lithium and valproic acid, have been used for the treatment of bipolar disorder, and their ability to inhibit *GSK-3β* has been implicated as the mechanism of action in bipolar disorder.[1] Various genetic studies have shown the association of genetic polymorphisms for *GSK-3β* with mood disorders.[2]

The *GSK-3β* gene, *GSK3B*, was mapped to 3q13.3,[3] and a linkage of regions on chromosomes 3q to not only schizophrenia but also bipolar disorder was suggested.[4] In addition, *GSK3B* has been known as one of the candidate genes for both schizophrenia and bipolar disorder.

Russ et al[5] detected 5 single nucleotide polymorphisms (SNPs) in the *GSK3B*. They identified 2 common SNPs at positions −50 C/T and −1727 A/T localized in the promoter region, with minor allele frequencies in white controls of 35% and 13%, respectively. It was reported that −50T/C of *GSK3B* influenced the long-term response to lithium salts in bipolar illness and that carriers of the mutant (C) allele (*GSK3B* C/C genotype) improved on lithium therapy.[6,7]

Because *GSK3B* −50T/C and −1727A/T were detected in the *GSK3B* promoter region,[8] the 2 SNPs were selected for the present study to determine their association with bipolar disorder (Fig. 1).

Brain-derived neurotrophic factor, which is modulated by antidepressants and produces antidepressivelike activity in preclinical behavioral models, is able to inhibit *GSK-3β*.[6] The *GSK-3β* substrate cyclic adenosine monophosphate regulatory element–binding protein transcription factor has been shown to modulate antidepressant activity.[5] A recent study revealed a genetic interaction between 2 functional SNPs in the *GSK-3β* gene and the microtube-associated protein τ H1/H1 haplotype, suggesting a possible combinative role of τ and *GSK-3β* in Parkinson disease and/or Alzheimer disease pathology.[9,10]

In this study, we hypothesized that genetic variants of the *GSK-3β* gene could partially underlie the response susceptibility to lithium treatment in bipolar disorder. In this study, we examined the possible association of the 2 previously studied *GSK3B* polymorphisms, −50T/C (rs334558) and −1727A/T (rs3755557), with bipolar disorder in Japanese lithium-treated patients, using an update of a previous study on the *GSK3B* haplotype undertaken in our laboratory.

METHODS

The relationship between haplotypes consisting of SNPs of *GSK3B* −50T/C and −1727A/T and the effect of lithium was studied for lithium responders and nonresponders among Japanese patients affected by bipolar disorder (*Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*).

The subjects had received lithium treatment for at least 24 months. The lithium treatment efficacy was evaluated by calculating the difference between the symptoms before and during lithium treatment, using a structured clinical rating scale, namely, the Young Mania Rating Scale.[11] Responder analysis revealed that 64% of the patients showed a reduction of 50% or more from baseline to endpoint in the Young Mania Rating Scale score (responder).

Genomic DNA samples were obtained from 42 patients (responders, 27 [11 men and 16 women]; nonresponders, 15 [4 men and 11 women]; mean [SD] age, 35.8 [8.8] years) after written informed consent had been obtained. The *GSK3B* −50T/C and −1727A/T genotyping was performed by the polymerase chain reaction method.[5,6]

The Hardy-Weinberg disequilibrium was assessed by the χ^2 test.

For statistical analysis of *GSK3B* haplotypes, gPLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) and Haploview (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview) were used.[12,13]

RESULTS

As shown in Table 1, the distributions of the *GSK3B* haplotypes (−50T/C and −1727A/T) showed a trend with significant

*Laboratory of Physiology, Psychophysiology/Psychiatry Project, the Graduate School of Environmental Health, and †Health Administration Center, Azabu University, Sagamihara; ‡Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science; §Department of Neuropsychiatry, Tokyo Women's Medical University, Tokyo; ‖Laboratory of Molecular Biology, School of Life and Environmental Science, Azabu University, Sagamihara; and ¶Enomoto Clinic, Tokyo, Japan.

Address correspondence and reprint requests to Kazuhiko Iwahashi, MD, PhD, Department of Psychophysiology, Azabu University, Sagamihara 252-5201, Japan; E-mail: iwahashi@azabu-u.ac.jp; and Daisuke Nishizawa, PhD, Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo 156-8506, Japan; E-mail: nishizawa-ds@igakuken.or.jp

Supported by a research project grant awarded by the Azabu University.

Conflicts of Interest and Source of Funding: The authors have no conflicts of interest to declare.

DOI: 10.1097/WNF.0000000000000039

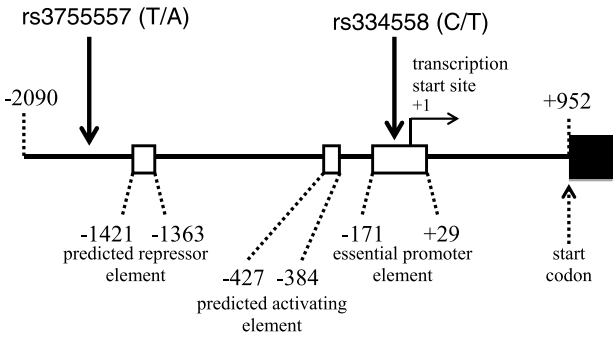

FIGURE 1. SNPs in the promoter region of the *GSK-3β* gene. Regulatory *cis*- elements for transcription are indicated according to the report of Lau et al[8] (1999).

difference between the lithium nonresponders and responders (global P=0.07074; empirical global P=0.1305). Haplotype 1 (T-A) was associated with a higher lithium response (haplotype-specific P=0.03477; empirical P=0.0673), whereas haplotype 2 (C-A) was associated with a lower lithium response (haplotype-specific P=0.03443; empirical P=0.079).

Pairwise D' and r^2 values between the 2 SNPs in this study were 1.0 and 0.097, respectively. The 2 SNPs showed weak linkage disequilibrium with each other.

Hardy-Weinberg equilibrium was tested by means of the goodness-of-fit χ^2 test. The 2 SNPs, *GSK3B* −50T/C and −1727A/T, were polymorphic, and their minor allele frequencies were 31% and 18%. There was no evidence of deviation from Hardy-Weinberg equilibrium for both SNPs.

There was no significant difference in genotypic or allelic frequencies of single SNPs (rs334558 and rs3755557) between the responder and nonresponder groups (Table 2). Genotypic and allelic frequencies of −50T/C polymorphism observed in this study were consistent with the genotypic (χ^2=0.8577, P=0.6512, df=2) and allelic frequency (χ^2=0.8432, P=0.3585, df=1) observed in the HapMap Japanese population; however, those of −1727A/T were not (χ^2=46.0482, $P < 0.01$, df=2; χ^2=68.6213, $P < 0.01$, df=1).

DISCUSSION

The results of the present study reinforce the association between *GSK-3β* and bipolar illness because *GSK3B* haplotype 1 (T-A) was associated with a higher lithium response and haplotype 2 (C-A) was associated with a lower lithium response.

However, Benedetti et al[6,7] showed that carriers of the mutant (C) allele of −50T/C (rs334558) improved on lithium salt therapy in 88 bipolar type I patients, supporting the hypothesis that GSK is a target for the therapeutic action of lithium.

TABLE 2. Allelic and Genotypic Distribution According to Lithium Therapeutic Response

Variant	Responder	Nonresponder	χ^2	*df*	*P*
GSK-3β −50T/C (rs334558)					
Genotypes					
CC	12	10	2.5	2	0.28
CT	9	5			
TT	6	0			
Alleles					
C	33	25	3.48	1	0.06
T	21	5			
GSK-3β −1727A/T (rs3755557)					
Genotypes					
AA	19	11	0.31	2	0.86
AT	6	3			
TT	2	1			
Alleles					
A	44	25	0.77	1	0.93
T	10	5			

There is a significant racial difference in the *GSK3B* polymorphisms between Japanese and white populations. A significantly lower frequency of the T allele of −50T/C (rs334558) and a significantly higher frequency of the C allele of −50T/C (rs334558) were found in the Japanese patients than those reported for white populations. Benedetti et al[6,7] showed that the genotype frequencies were T/T 38%, T/C 45%, and C/C 15% and that the allele frequencies were T 60.5% and C 39.5% for Italian bipolar type I patients. In this study, the observed genotype frequencies T/T 14.3%, T/C 33.3%, and C/C 52.4% and the allele frequencies T 31.0% and C 69.0% for the Japanese bipolar disorder patients were not significantly different from those for Japanese healthy subjects (19%,63%,18%; 49%:51%).[14] There was a significant difference in the genotype frequency of -50T/C between the Italian and Japanese patients. As for −1727A/T, the allele frequencies (A 87% and T 13%) for white healthy subjects shown by Russ et al were not significantly different from those for the Japanese healthy subjects (A 81% and T 19%). They identified 2 common SNPs at positions −50T/C and −1727A/T localized in the promoter region of the gene, with minor allele frequencies in white controls of 35% (C) and 13% (T), respectively, and we identified in the Japanese controls 49% (T) and 19% (T), respectively.[5,14] In this study, genotypic and allelic frequencies of −1727A/T polymorphism observed were inconsistent with the genotypic and allelic frequencies observed in the HapMap Japanese population. However, the frequencies observed in our data are consistent with previous studies conducted in an Asian population,

TABLE 1. Haplotype Frequencies in Lithium Responders and Nonresponders

Haplotype	−50T/C	−1727A/T	Frequency		χ^2	*P*	Empirical *P*
			Responder	Nonresponder			
1	T	A	0.3889	0.1667	4.456	0.03477	0.0673
2	C	A	0.4259	0.6667	4.473	0.03443	0.079
3	C	T	0.1852	0.1667	0.04509	0.8318	0.851

The haplotype distributions showed a trend with significant difference between the lithium nonresponders and responders (global P=0.07074; empirical global P=0.1305). Haplotype 1 (T-A) was associated with a higher lithium response (haplotype-specific P=0.03477; empirical P=0.0673), whereas haplotype 2 (C-A) was associated with a lower lithium response (haplotype-specific P=0.03443; empirical P=0.079).

and the frequencies of −1727 A/A genotype in our Japanese control subjects (64%) were similar to those in Korean subjects (70%–73%).[14–16]

In previous genetic and functional studies on *GSK-3β*, it was revealed that the major physiological mechanism that regulates the activity of GSK3 is the phosphorylation of the N-terminal serine of GSK3.[17] It was shown that the T allele of −50T/C (rs334558) *GSK3B* polymorphism gives greater transcriptional activity, which can be associated with the hyperphosphorylation of τ, resulting in neurodegeneration.[9] In addition, Benedetti et al[6,7] reported that, in humans, the promoter variant (rs334558*C) was associated with reduced activity and better antidepressant response. Furthermore, lithium has been used for the treatment of bipolar disorder, and its ability to inhibit *GSK-3β* has been implicated as the mechanism of action in bipolar disorder.[1]

Therefore, the *GSK3B* transcriptional activity regulation by lithium may also be associated with the susceptibility to lithium treatment in bipolar disorder. Our finding that *GSK3B* haplotype 1 (T-A) was associated with a higher lithium response may suggest that patients with the T allele of −50T/C (rs334558), which gives greater transcriptional activity, are more affected by lithium, which inhibits *GSK-3β* activity.

Initially, GSK-3 was identified as a phosphorylating and inactivating glycogen synthase that is critical to the regulation of glucose storage.[18] It was recently discovered that GSK3 is a serine/threonine-specific protein and that it plays an important role in regulating neuronal plasticity, gene expression, and cell survival.[19]

The importance of *GSK-3β* and τ protein seen not only in Parkinson disease and/or Alzheimer disease[9,10] pathology but also in bipolar illness[6] has already been documented. On the other hand, Yoona and Kima[20] suggested that 2 promoter polymorphisms of the *GSK-3β* gene may not be related to the pathogenesis of major depression disorder and the risk for suicidal behavior in Korean depressive patients.

The sample size in this study including Japanese bipolar disorder lithium responders and nonresponders was not large enough for clinical situation, even if this study is a pilot study for personalized medicine (tailor-made therapy) for bipolar disorder. Therefore, larger-scale comparison is needed to confirm the actual relationship between susceptibility to lithium and *GSK-3β* haplotypes among bipolar disorder patients.

REFERENCES

1. Gould TD, Manji HK. Glycogen synthase kinase-3: a putative molecular target for lithium mimetic drugs. *Neuropsychopharmacology* 2005;30:1223–1237.
2. Saus E, Soria V, Escaramís G, et al. A haplotype of glycogen synthase kinase 3β is associated with early onset of unipolar major depression. *Genes Brain Behav* 2010;9:799–807.
3. Shaw PC, Davies AF, Lau KF, et al. Isolation and chromosomal mapping of human glycogen synthase kinase-3 alpha and -3 beta encoding genes. *Genome* 1998;41:720–727.
4. Bailer U, Leisch F, Meszaros K, et al. Genome scan for susceptibility loci for schizophrenia and bipolar disorder. *Biol Psychiatry* 2002;52:40–52.
5. Russ C, Lovestone S, Powell JF. Identification of sequence variants and analysis of the role of the glycogen synthase kinase 3 beta gene and promoter in late onset Alzheimer's disease. *Mol Psychiatry* 2001;6:320–324.
6. Benedetti F, Serretti A, Pontiggia A, et al. Long-term response to lithium salts in bipolar illness is influenced by the glycogen synthase kinase 3-beta -50T/C SNP. *Neurosci Lett* 2005;376:51–55.
7. Benedetti F, Dallaspezia S, Lorenzi C, et al. Gene-gene interaction of glycogen synthase kinase 3-β and serotonin transporter on human antidepressant response to sleep deprivation. *J Affect Disord* 2012;136:514–519.
8. Lau KF, Miller CC, Anderton BH et al. Molecular cloning and characterization of the human glycogen synthase kinase-3beta promoter. *Genomics* 1999;60:121–128.
9. Kwok JB, Hallupp M, Loy CT, et al. *GSK3B* polymorphisms alter transcription and splicing in Parkinson's disease. *Ann Neurol* 2005;58:829–839.
10. Kalinderi K, Fidani L, Katsarou Z, et al. GSK3β polymorphisms, MAPT H1 haplotype and Parkinson's disease in a Greek cohort. *Neurobiol Aging* 2011;32:546–545.
11. Young RC, Biggs JT, Ziegler VE, et al. Rating scale for mania: reliability, validity, and sensitivity. *Br J Psychiatry* 1978;133:429–435.
12. Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21:263–265.
13. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–575.
14. Numajiri M, Nishizawa D, Ikeda K, et al. The relationship between glycogen synthase kinase − 3beta -1727A/T × -50T/C genetic polymorphisms and nicotine dependence. *Nihon Arukoru Yakubutsu Igakkai Zasshi* 2013;48:293–299.
15. Lee KY, Ahn YM, Joo EJ, et al. No association of two common SNPs at position -1727 A/T, -50 C/T of GSK-3 beta polymorphisms with schizophrenia and bipolar disorder of Korean population. *Neurosci Lett* 2006;395:175–178.
16. Yoon HK, Kim YK. Association between glycogen synthase kinase-3beta gene polymorphisms and major depression and suicidal behavior in a Korean population. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:331–334.
17. Frame F, Cohen P, Biondi RM. A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation. *Mol Cell* 2001;7:1321–1327.
18. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem* 1980;107:519–527.
19. Jope RS, Johnson GV. The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci* 2004;29:95–102.
20. Yoona HK, Kima YK. Association between glycogen synthase kinase-3β gene polymorphisms and major depression and suicidal behavior in a Korean population. *Prog Neuropsychopharmacol Biol Psychiatry* 2010;34:331–334.