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

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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 subjects.

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). Haploype 1 (T -A) was associated with a higher lithium response (haplotype-specific P = 0.03447), whereas haplotype 2 (C -A) was associated with a lower lithium response (haplotype-specific P = 0.03443).

The pairwise D’ and r² 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

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 antidepressants and produces antidepressivelike activity in preclinical behavioral models, is able to inhibit GSK-3β. The GSK-3β substrate cyclic adenosine monophosphate regulatory element–binding protein transcription factor has been shown to modulate antidepressant activity. A recent study revealed a genetic interaction between 2 functional SNPs in the GSK-3β gene and the microtubule-associated protein τ (GSK3B haplotype, suggesting a possible combative role of τ and GSK-3β in Parkinson disease and/or Alzheimer disease pathology.

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. 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.

The Hardy-Weinberg disequilibrium was assessed by the χ² test. For statistical analysis of GSK3B haplotypes, gPLINK (http://pngu.mgh.harvard.edu/~purcell/plink/) and Haplovip (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haplovip/haplovip.html) were used.

RESULTS

As shown in Table 1, the distributions of the GSK3B haplotypes (−50T/C and −1727A/T) showed a trend with significant
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 (haplotype-specific P<0.01; 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).

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 al5,6 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% (T) and 13% (T), respectively, and we 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 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.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 | Responder | Nonresponder | \( \chi^2 \) | P | Empirical P |
|-----------|--------|---------|----------|-----------|-------------|-------|-----|-----------|
| 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=0.0704; 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).*

**DISCUSSION**

The reported for white populations. Benedetti et al5,6 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 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.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 2. Allelic and Genotypic Distribution According to Lithium Therapeutic Response**

| Variant | Responder | Nonresponder | \( \chi^2 \) | df | P  | Empirical 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 al5,6 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 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.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.

**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 al10 (1999).

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|-----------|--------|---------|----------|-----------|-------------|-------|-----|-----------|
| 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=0.0704; 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.16 In addition, Benedetti et al17 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 GSK-3 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 disease19,20 pathology but also in bipolar illnesses9 has already been documented. On the other hand, Yoona and Kim20 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.

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