# Relationship between G-protein β3 Subunit C825T Polymorphism and Citalopram Responses in Korean Patients with Major Depressive Disorder

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## Abstract

This study aimed to determine the relationship between the C825T polymorphism in the G-protein β3-subunit (GNB3) gene and the response to citalopram in a Korean population with major depressive disorder (MDD). Citalopram was administered for 8 weeks to the 84 MDD patients who completed this study. All subjects were examined using the Structured Clinical Interview for DSM-IV, and the severity of depression was assessed using the 21-item Hamilton Depression Rating (HAMD-21) scale. A main effect of an interaction of genotype with time on the decrease in the HAMD-21 score during the 8-week study period was not found. ANOVA revealed no significant effects of the GNB3 C825T polymorphism on the decrease in the HAMD-21 score at each time period. Although the C825T polymorphism of the GNB3 gene may affect the pathogenesis of MDD, our results do not support the hypothesis that this polymorphism is involved in the therapeutic response to citalopram in Korean patients with MDD.

Keywords: GNB3, Polymorphism, Citalopram, MDD

Most antidepressants act on monoaminergic receptors, most of which are linked to G proteins. G proteins control cell reactions by influencing signal transduction in intracellular signaling pathways via intervention in the process of conveying signals from the receptor to the effector protein[1,2]. G proteins comprise α, β, and γ subunits that, once activated, transduce excitation of extracellular receptors into intracellular signals, and thereby play an important role in determining the specificity and other characteristics of reactions in all cells. G protein functions by creating a βγ dimer by separating a α-monomer bound to GTP, with receptor activation resulting in the G protein dividing into a free Gα subunit and a Gβγ subunit, where the latter activates various effector proteins contained therein, including phospholipases, adenylyl cyclases, ion channels, G-protein-coupled receptor kinases, and phosphoinositide 3-kinase[3].

The single-nucleotide *C825T* polymorphism in exon 10 of the G-protein β3-subunit (GNB3) gene was discovered by Siffert[4]. Although the *GNB3 C825T* polymorphism does not affect the amino acid sequence of the β subunit, the T allele is related to defects in nucleotides 498-620 of exon[9]. This polymorphism appears to be associated with enhanced signal transduction and ion-transfer activity. *GNB3* polymorphisms may influence responses to electroconvulsive treatment[5], from which it has been suggested that GNB3 mediates the serotonergic effect at the level of second-messenger cascades[6].

Zill[7] found that the relation between the *GNB3 C825T* polymorphism and responses to treatment with antidepressants was influenced by *TT* isogamy in patients who responded favorably to various antidepressants. Moreover, Serretti[6] and Lee[8] found favorable responses to antidepressant treatment in depressed patients

**Table 1.** Demographic characteristics for MDD group (ITT group analysis, n=84).

| GNB3[a] | Genotype | | | P-value |
|---|---|---|---|---|
| | C/C | T/C | T/T | |
| HAMD at baseline | 25.0±6.7 | 24.8±7.9 | 21.9±4.4 | 0.2148* |
| Onset age | 48.7±13.6 | 46.9±15.9 | 44.3±18.0 | 0.6659* |
| Hospitalization history | 4 (26.7%) | 8 (53.3%) | 3 (20.0%) | 0.7659[†] |
| Suicide attempt | 0 (0.0%) | 4 (80.0%) | 1 (20.0%) | 0.3135[†] |
| Family history | 3 (42.9%) | 3 (42.9%) | 1 (14.2%) | 0.6925[†] |
| Sex (Male) | 4 (16.7%) | 9 (37.5%) | 11 (45.8%) | 0.0293** |
| Education (yr) | | | | |
| Middle school graduate | 11 (32.4%) | 16 (47.0%) | 7 (20.6%) | 0.2267** |
| High school graduate | 5 (26.3%) | 11 (57.9%) | 3 (15.8%) | |
| More than high school | 6 (28.6%) | 6 (28.6%) | 9 (42.8%) | |
| Dose at week 8 | 23.9±9.8 | 28.1±11.8 | 22.4±8.3 | 0.1197* |

[a]Data represents mean±std or n (%) appropriately

*Genotype comparisons are made by ANOVA*, Chi-square test**, or Fisher's exact test[†]

with the *GNB3 TT* genotype or *T* allele. In the reports about *GNB3 C825T* polymorphism, however, effects of the SNP were evaluated in depressive patients including bipolar patients, and various treatment methods were employed which are including various pharmacological medications such as noradrenergic and serotonergic specific agents (NaSSa)[7,8], serotonin and noradrenaline reuptake inhibitors (SNRI)[8], tricyclic antidepressants (TCA)[7,8] and selective serotonin reuptake inhibitors (SSRI)[6-8] as well as non-pharmacological treatment such as electroconvulsive theraphy and transcranial magnetic stimulation[7]. In addition, Zill[7] and Serretti[6] followed up the patients for 4 and 6 weeks of treatments, respectively.

Thus, we tested association between *GNB3 C825T* polymorphism and response to long-term treatment with single antidepressant, especially citalopram which is a kind of SSRI that targets the serotonin transporter and has been shown to exhibit antidepressant activity that is superior to that of TCAs, in homogenous population with major depressive disorder (MDD).

## Clinical Characteristics of Study Subjects and Hardy-Weinberg Equilibrium for the *GNB3 C825T* Polymorphism

Of the 134 patients chosen to participate in this study, 60 withdrew because of a failure to draw blood, lack of efficacy, personal conflict or other personal decision, loss to treatment, or adverse events during treatment. Table 1 summarizes patient data for gender, age at onset, and frequency of hospitalization history, suicide attempts, and family history of MDD or other psychotic diseases. None of these parameters differed significantly among the three *GNB3 C825T* genotypes (C/C, C/T, and T/T) except gender distribution (P=0.0293). Chi-square tests applied to the three genotype

**Table 2.** Distribution of genotype and allele frequencies of GNB3 polymorphism among responsive and non-responsive patients.

| ITT[a] group | Genotype frequencies[†] | | | Allele frequencies[‡] | |
|---|---|---|---|---|---|
| | C/C | T/C | T/T | C | T |
| Responsive (n=58) | 17 (29.3%) | 22 (37.9%) | 19 (32.8%) | 56 (48.3%) | 60 (51.7%) |
| Non-responsive (n=26) | 7 (26.9%) | 16 (61.6%) | 3 (11.5%) | 30 (57.7%) | 22 (42.3%) |

[a]Intent-to-treat group. LOCF (last-observation-carried-forward) analysis is performed for missing data in HAMD scores

[†]Comparison of genotype frequencies between responsive and non-responsive group: $\chi^2$=5.3340, d. f.=2, P=0.0695

[‡]Comparison of allele frequencies between responsive and non-responsive group: $\chi^2$=1.2742, d. f.=1, P=0.2590, Odd ratio (OR)=1.461, 95% Confidence interval (CI) for OR=0.755-2.826

frequencies revealed that the subjects were in Hardy-Weinberg equilibrium ($\chi^2$=0.754, P=0.686).

## Association Analysis for the *GNB3 C825T* Polymorphism with Response to Citalopram Medication

At first, we compared the distributions of genotypes and alleles between responders (Rp) and nonresponders (Non-Rp). As shown in Table 2, we could observe a trend of association, however, which was not statistically significant between genotype frequencies and response to citalopram medication during 8 weeks ($\chi^2$=5.334, d. f.=2, P=0.070). The allele frequencies are also comparable in the two groups ($\chi^2$=1.274, d. f.=1, P=0.259, OR=1.461, 95% CI for OR=0.755-2.826).

In addition, we also tested association between the frequencies of genotypes and alleles and remission

status at 8 weeks after the initiation of citalopram treatment (Table 3). In similar with the result of response status (Table 2), we could observe a trend of association, however, which was not statistically significant between genotype frequencies and remission status by citalopram medication ($\chi^2$=4.890, d. f.=2, $P$=0.087). The allele frequencies are also comparable in the two groups ($\chi^2$=0.771, d. f.=1, $P$=0.380, OR=1.322, 95% CI for OR=0.708-2.468).

## Association Analysis for the *GNB3 C825T* Polymorphism with the % Decline of HAMD-21 Score in MDD Patients by Citalopram Medication

To clarify the observed trends of the association between *C825T SNP* and response to citalopram or remission status, we tested association of the SNP

with decline of HAMD-21 score after citalopram treatment. As expected, repeated measures ANOVA showed significant decline of mean HAMD-21 score in MDD patients ($P < 0.001$, Table 4). The mean of HAMD-21 score at baseline was $24.1 \pm 6.9$, which was decreased to $9.5 \pm 7.1$ after 8 weeks by citalopram treatment. The decline of HAMD-21 were statistically significant in all period of medication compared with baseline score. However, there were no associations between genotypes and the decline in total HAMD-21 across time period as well as at each of time period (Table 4).

## Discussion

Numerous studies have provided no consistent explanation for the effects of antidepressants on neurotransmitters[11-16].

Recent investigations of antidepressant mechanisms have focused on intracellular signaling pathways controlled by the long-term administration of antidepressants[17]. Some studies have shown that long-term treatment with an antidepressant can activate G proteins, which is related to an antidepressant effect. This is supported by three reports that the GNB3 gene is related to the functional mechanism of antidepressants[6,8,18].

However, although polymorphism of the GNB3 gene is known to be related to functional differences of G proteins, we found no relationship between GNB3 gene polymorphism and the response to treatment with citalopram. In addition, we did not detect any differences in antidepressant effects among the three genotypes of the GNB3 gene or with the progression of time. Moreover, the genotype, allele, and allele-carrier frequencies did not differ between Rp and Non-Rp or between Rm and Non-Rm.

**Table 3.** Distribution of genotype and allele frequencies of GNB3 polymorphism among patients with remission and without remission.

| ITT[a] group | Genotype frequencies[†] | | | Allele frequencies[†] | |
|---|---|---|---|---|---|
| | C/C | T/C | T/T | C | T |
| Remission* (n=32) | 10 (31.3%) | 10 (31.3%) | 12 (37.4%) | 30 (46.9%) | 34 (53.1%) |
| No remission (n=52) | 14 (26.9%) | 28 (53.9%) | 10 (19.2%) | 56 (53.9%) | 48 (46.1%) |

[a]Intent-to-treat group. LOCF (last-observation-carried-forward) analysis is performed for missing data in HAMD scores
*Remission group is defined by patients whose HAMD score at week 8 is less than 7
[†] Comparison of genotypes between patients with remission and without remission $\chi^2$=4.8901, d. f.=2, $P$=0.0867
[†] Comparison of alleles between patients with remission and without remission $\chi^2$=0.7706, d. f.=1, $P$=0.3800, OR=1.322, 95% CI for OR=0.708-2.468

**Table 4.** Comparison of changes of HAMD scores across study period among genotypes (ITT group analysis, n=91).

| Genotype | HAMD score | | | | | | |
|---|---|---|---|---|---|---|---|
| | Baseline | Week 1 | Week 2 | Week 4 | Week 8 | $P$-value* | $P$-value** |
| C/C | $25.0 \pm 6.7$ | $19.9 \pm 8.1$ | $15.5 \pm 7.8$ | $10.7 \pm 6.6$ | $9.4 \pm 6.8$ | $< 0.001$ | 0.7644 |
| T/C | $24.8 \pm 7.9$ | $18.8 \pm 7.7$ | $15.3 \pm 7.2$ | $12.3 \pm 6.8$ | $11.1 \pm 7.9$ | | |
| T/T | $21.9 \pm 4.4$ | $17.0 \pm 4.7$ | $12.4 \pm 4.9$ | $8.8 \pm 5.7$ | $7.1 \pm 5.6$ | | |
| Overall [†,§] | $24.1 \pm 6.9$ | $18.6 \pm 7.2$ | $14.6 \pm 6.9$ | $10.9 \pm 6.5$ | $9.5 \pm 7.1$ | | |
| $P$-value[†] | 0.2148 | 0.3812 | 0.2166 | 0.1427 | 0.1163 | | |

*Repeated measures ANOVA p-value for testing overall mean HAMD score changes across time period
**Repeated measures ANOVA p-value for testing mean HAMD score changes by genotypes across time period
[†] ANOVA test at each of time period
[†] Testing overall mean HAMD score changes comparing to that of baseline: week 1 vs. baseline ($P < 0.0001$); week 2 vs. baseline ($P < 0.0001$); week 4 vs. baseline ($P < 0.0001$); week 8 vs. baseline ($P < 0.0001$)
[§] Testing cubic trend of overall mean HAMD score changes: $P$=0.0115

This study was subject to several limitations. First, the T allele is reportedly correlated with the severity of depression and improvement by treatment with antidepressants[8], but also with a poor response to nortriptyline[18]. The absence of such correlations in the present study may have been due to the administered doses being too low, and hence future studies should consider the drug concentration in the blood. Second, the use of a small sample in this study may lower the statistical powers of analyses. Thus, possible effects of GNB3 C825T on the function of citalopram should be evaluated in future research using a larger sample or functional validation. Third, although the C825T polymorphism of the GNB3 gene affected the response to treatment with citalopram, it may still play only a partial role in a complex mechanism involving multiple genes-this limitation applies to all single-gene approaches. Fourth, there are reports[7,19,20] that the response to antidepressant treatment is better in Caucasian MDD patients than in Japanese[21] and Korean[8] MDD patients, indicating that the frequency of the T allele may differ between Asian and Caucasian populations. It is therefore possible that ethnicity differences were responsible for the results of this study differing from those of previous studies.

In conclusion, although the C825T polymorphism of the GNB3 gene may affect the pathogenesis of MDD, our study found that GNB3 gene variants did not affect the antidepressant function of citalopram. This observation needs to be confirmed in future studies that are not subject to the above-mentioned limitations.

## Materials & Methods

### Subjects

Trained psychiatrists examined all the potential subjects using the Structured Clinical Interview for the fourth revision of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)[9] and the K-DIGS (Korean version of the Diagnostic Interview for Genetic Studies)[10]. The severity of depression was assessed using the 21-item Hamilton Depression Rating (HAMD-21) scale. Only subjects with a minimum score of 18 on the HAMD-21 scale entered the study, and subjects with primary or comorbid diagnoses of schizophrenia, schizoaffective disorder, rapid cycling bipolar disorder, dementia, or alcohol or substance dependence based on DSM-IV criteria within the previous 6 months were excluded. We also excluded subjects showing a personal or family history of substance abuse/dependence and major psychiatric disorders (schizophrenia, schizoaffective disorder, rapid

cycling bipolar disorder, or dementia) and those with serious or unstable medical illness. Patients who were receiving medications were subjected to a 2-week washout period. Demographic data, medical history, and laboratory data were documented. All subjects were at least 18 years of age, and comprised a mixture of in- and outpatients. During the treatment period of the study, all subjects took citalopram daily (range: 10-60 mg/day). Psychotropic drugs such as benzodiazepines and mood stabilizers were not allowed. Venous blood was drawn from each subject after obtaining written informed consent using a protocol approved by the Ethics Committee of the Korea University Medical Center.

The clinical symptoms of the subjects were evaluated based on the HAMD-21 scores at baseline and after 1, 2, 4, and 8 weeks of treatment. Responders (Rp) were defined as a reduction in the HAMD-21 scores from baseline of at least 50%, and remitters (Rm) were defined as a HAMD-21 total score of 7 points or less after either 4 or 8 weeks of treatment.

### DNA Analysis

DNA was extracted from peripheral blood and a polymerase chain reaction (PCR) was performed with the sense primer 5′-TGA CCC ACT TGC CAC CCG TGC-3′ and the antisense primer 5′-GCAGCA GCC AGG GCT GGC-3′. The amplification mixture contained 3 μL of DNA, 5 μL of 10× PCR buffer, 4 μL of 2.5 mM dNTP, 1 μL of each primer, 35.75 μL of distilled water, and 0.25 μL of *Taq* polymerase. Samples were amplified using a thermocycler with an initial 5 min at 94°C, followed by 35 cycles of 40 sec at 94°C, 40 sec at 62°C, and 40 sec at 72°C. After a final 5 min at 72°C, the reaction was terminated at 4°C. The amplified DNA was digested with the restriction endonuclease *Bse DI*, which cuts at the -825T site, and the product was electrophoresed on 3% agarose gels and stained with ethidium bromide. The DNA-amplified fragment was 268 bp long. The presence or absence of the restriction *Bse DI* site determines whether a single 268-bp fragment (allele T) or two fragments of 116 and 152 bp (allele C) are produced.

### Statistical Analysis

The categorical data were analyzed using the chi-square test or Fisher's exact test as necessary. Genotype differences for continuous variables were evaluated using the *t*-test or one-way ANOVA, followed by the LSD multiple-range test for comparisons among groups. Odd ratios with 95% confidence intervals (CIs) were calculated to estimate the effects of high-risk genotypes and alleles. The effect of genotype, time, or a time-genotype interaction was determined using

a two-way repeated-measures ANOVA with time as a covariate. The cutoff probability value was set at 0.05. All statistical analyses were performed using SPSS (version 10.0 for Microsoft Windows).

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## References

1. Neer, E. J. Heterotrimeric G proteins: organizers of transmembrane signals. *Cell* **80**:249-257 (1995).
2. Hamm, H. E. The many faces of G protein signaling. *J Biol Chem* **273**:669-672 (1998).
3. Ford, C. E. *et al*. Molecular basis for interactions of Gprotein beta gamma subunits with effectors. *Science* **280**:1271-1274 (1998).
4. Siffert, W. *et al*. Enhanced G protein activation in immortalized lymphoblasts from patients with essential hypertension. *J Clin Invest* **96**:759-766 (1995).
5. Anttila, S. *et al*. Interaction between 5-HT1A and BDNF genotypes increases the risk of treatment-resistant depression. *J Neural Transm* **114**:1065-1068 (2007).
6. Serretti, A. *et al*. SSRIs antidepressant activity is influenced by G beta 3 variants. *Eur Neuropsychopharmacol* **13**:117-122 (2003).
7. Zill, P. *et al*. Evidence for an association between a G-protein beta3-gene variant with depression and response to antidepressant treatment. *Neuroreport* **11**: 1893-1897 (2000).
8. Lee, H. J. *et al*. Association between a G-protein beta 3 subunit gene polymorphism and the symptomatology and treatment responses of major depressive disorders. *Pharmacogenomics J* **4**:29-33 (2004).
9. First, M. B., Spitzer, R. L., Gibbon, M. & Willams, J. B. W. Structured Clinical Interview for DSM-IV Axis I Disorders SCID I: Clinician Version, Administration Booklet. Washington, DC: *American Psychiatric Press* (1997).
10. Han, O. S. & Hong, J. P. Structured clinical interview for DSM-IV axis I disorder-Korean version. *Hana Medical* (2000).
11. Vetulani, J. & Sulser, F. Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. *Nature* **257**:495-496 (1975).
12. Sulser, F., Vetulani, J. & Mobley, P. L. Mode of action of antidepressant drugs. *Biochem Pharmacol* **27**:257-261 (1978).
13. Bergstrom, D. A. & Kellar, K. J. Adrenergic and serotonergic receptor binding in rat brain after chronic desmethylimipramine treatment. *J Pharmacol Exp Ther* **209**:256-261 (1979).
14. Blackshear, M. A. & Sanders-Bush, E. Serotonin receptor sensitivity after acute and chronic treatment with mianserin. *J Pharmacol Exp Ther* **221**:303-308 (1982).
15. Kendall, D. A. & Nahorski, S. R. 5-Hydroxytryptamine-stimulated inositol phospholipid hydrolysis in rat cerebral cortex slices: pharmacological characterization and effects of antidepressants. *J Pharmacol Exp Ther* **233**:473-479 (1985).
16. Blier, P. & de Montigny, C. Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* **15**:220-226 (1994).
17. Manji, H. K., Drevets, W. C. & Charney, D. S. The cellular neurobiology of depression. *Nature Medicine* **7**:541-547 (2001).
18. Joyce, P. R. *et al*. Age-dependent antidepressant pharmacogenomics: polymorphisms of the serotonin transporter and G protein [beta]3 subunit as predictors of response to fluoxetine and nortriptyline. *Int J Neuropsychopharmacol* **6**:339-346 (2003).
19. Bondy, B. *et al*. Combined action of the ACE D- and the G-protein beta3 T-allele in major depression: a possible link to cardiovascular disease? *Mol Psychiatry* **7**:1120-1126 (2002).
20. Siffert, W. G-protein beta3 subunit C825T allele and hypertension. *Curr Hypertens Rep* **5**:47-53 (2003).
21. Kunugi, H. *et al*. Association study of C825T polymorphism of the G-protein beta3 subunit gene with schizophrenia and mood disorders. *J Neural Transm* **109**:213-218 (2002).