Association of C825T Polymorphism of G Protein β3 Subunit With the Autonomic Nervous System in Young Healthy Japanese Individuals

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Background: The T allele of the C825T polymorphism of the G protein β3 subunit gene (*GNB3*) is reported to be associated with increased intracellular signal transduction and the prevalence of essential hypertension. Because the two major receptors in the autonomic nervous system (ANS), the adrenergic and muscarinic acetylcholine receptors, are G protein–coupled receptors, it was expected that the *GNB3* C825T polymorphism was associated with ANS function. In the present study, we have investigated the association of this polymorphism with ANS in young, healthy Japanese male individuals.

Methods: A total of 94 young, healthy subjects underwent the genotyping for the *GNB3* C825T polymorphism and electrocardiogram R-R interval power spectral analysis in supine rest and standing positions.

Results: There were no significant differences among genotypes in any of the characteristics investigated (body mass index, blood pressure, plasma glucose, insulin, lipids, and family history of hypertension, diabetes, or obesity). However, in power spectral analysis of heart rate variability, the very-low–frequency component when standing was higher in TT carriers than in CC carriers, and TT and CT carriers had a significantly higher sympathetic nervous system (SNS) index and lower parasympathetic nervous system (PNS) index when standing than CC carriers. In addition, we found that TT carriers showed no chronological variations in either SNS or PNS index after postural change.

Conclusions: These observations suggested that *GNB3* C825T polymorphism is associated with ANS in youth. These findings raise the possibility that individuals who are T allele carriers are at increased risk for developing hypertension in relation to ANS function. Am J Hypertens 2005;18:523–529 © 2005 American Journal of Hypertension, Ltd.

Key Words: *GNB3*, polymorphism, heart rate variability, power spectral analysis, sympathetic nervous system, parasympathetic nervous system.

Heterotrimeric G proteins are essential components of heptahelical transmembrane receptor–mediated intracellular signaling cascades that are relevant to various physiologic functions, such as cardiovascular homeostasis and peripheral vascular resistance.[1] Recently, a single nucleotide polymorphism (C825T) in exon 10 of the gene encoding the G protein β3 subunit (*GNB3*) has been identified.[2] The C825T substitution is associated with alternative splicing of exon 9, which eliminates 41 amino acids and results in the expression of a novel splice variant (Gβ3-s), related to enhancement of G protein activation.[2] Previous studies have reported that the 825T allele was associated with essential hypertension,[2,3] obesity,[4] left ventricular hypertrophy,[5] and lower plasma renin activity.[3] However, such an association has not been demonstrated in some studies,[6,7] probably due to differences such as ethnic background, sample size, and age bracket. Considering the inconsis-

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tency of these results, the physiologic roles of C825T polymorphism of *GNB3* are unclear.

The autonomic nervous system (ANS) plays essential roles in regulation of heart rate, blood pressure (BP), body temperature, circulation, digestion, and other physiologic functions. Various disorders such as hypertension, obesity, and diabetes are closely related to several polymorphisms in ANS receptors.[8] Adrenergic and muscarinic acetylcholine receptors are major receptors of nurotransmitters in sympathetic nervous system (SNS) and parasympathetic nervous system (PNS), respectively, and couple to G protein. Therefore, it is thought that functional variations in adrenergic receptors or G protein are associated with variation in ANS function. The C825T polymorphism of *GNB3* was related to enhanced activation of G protein–mediated signaling in vitro,[2] and noradrenaline-induced vasoconstriction.[9] Therefore, we hypothesized that the altered functions of G protein in vivo, according to the *GNB3* polymorphism, could be detected by investigating ANS function in subjects with and without the 825T allele.

Power spectral analysis is a useful, noninvasive, and sensitive method that can be used as an index of autonomic cardiac modulation[10–15] and that has been applied to a functional indicator of ANS in many pathophysiologic states including status of arterial hypertension.[16] In general, power spectral analysis of heart rate variability has shown two major distinct regions of periodicity in electrocardiogram (ECG) R-R intervals: namely, a high-frequency component (HI, >0.15 Hz) and a low-frequency component (LO, <0.15 Hz), which provide markers of vagal and sympathetic nervous activities and sympatho-vagal modulation in relation to their dynamic balance.[10–15] Some previous works have indicated that the high-frequency component is mediated solely by PNS and that the low-frequency component is influenced by both SNS and PNS,[11,13] although the specific physiologic significance of both frequency components are still controversial. In addition, the ratio of the very-low– and low-frequency to the high-frequency component and the ratio of high to total power (TOTAL) have been considered as indices of sympathetic and parasympathetic activities (SNS index and PNS index, respectively).[11,14–16] The very low frequency components are not well described; however, we and others have reported that very low frequencies (VLO, <0.03 Hz) are associated with thermoregulation and the renin-angiotensin system.[12,17–19]

In this study, we have determined the prevalence of the C825T polymorphism in young healthy Japanese male subjects. We have also investigated the association of this polymorphism with the power spectral components of heart rate variability as indices of autonomic function, assessed in supine rest conditions and in response to standing. In addition, we analyzed the differences among genotypes in the longitudinal sympatho-vagal responsiveness reflected in SNS and PNS indices to a sympathetic activation state induced by postural change.

Methods

Study Population

A total of 94 young healthy Japanese male individuals were recruited at random from Kyoto University for participation in each examination after written informed consent was obtained. Ages of subjects ranged from 18 to 25 years (mean ± SEM, 20.8 ± 0.2). All subjects were normotensive (causal supine BP <140/90 mm Hg) and nonobese (body mass index [BMI] <30 kg/m^2). As determined by interview, the subjects were not taking any medication and had no history of any organic disease such as cardiovascular disease, metabolic disorder, renal disease, or neuropathy. Body mass index, systolic and diastolic BP, heart rate (supine rest/standing), blood glucose, insulin, total cholesterol, and triglycerides were measured as baseline characteristics, and family history was investigated through interviews including whether subjects had relatives within the third degree who had hypertension, diabetes, or obesity (BMI >30). All subjects underwent electrocardiograpy and power spectral analysis of heart rate variability.

The study protocol was reviewed by the appropriate institutional review committee of Kyoto University School of Medicine, and the guidelines of the Declaration of Helsinki were followed.

Genotyping of *GNB3* C825T Polymorphism

Genomic DNA was extracted from whole blood (DNA Extractor WB Kit, Wako, Osaka, Japan). The *GNB3* C825T polymorphism was genotyped by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) as previously described.[2,6]

Electrocardiographic R-R Interval Power Spectral Analysis

All subjects were studied in the morning (9 to 11 AM) after an overnight fast, in a quite room with an ambient temperature of 25°C. The subjects rested supine for at least 20 min before ECG recording.

The CM5 lead ECG was continuously recorded during supine rest and postural change to standing. After 10 min of supine rest, the subjects stood up by the bedside and remained at standing rest for another 10 min. During the test, the respiratory rate was controlled at 0.25 Hz by an electric metronome to avoid interference by the low-frequency component in the parasympathetic component.[17,20–22]

The R-R interval power spectral analysis procedures have been described previously.[17,20–22] Briefly, the ECG R-R interval data obtained from the CM5 lead was digitized at 1000 Hz and the derived R-R interval time series were then aligned in 2-Hz sequence for power spectral analysis. The DC component and linear trend were completely eliminated by digital filtering for band-pass be-