Effects of C825T polymorphism of the GNB3 gene on availability of dopamine transporter in healthy volunteers—a SPECT study


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ABSTRACT

Striatal dopaminergic activity is significantly correlated with various cognitive activities, mood regulation, and even metabolic homeostasis, and is modulated by the dopamine transporter (DAT). The availability of DAT could be regulated by presynaptic autoreceptors, which are G-protein coupled receptors; however, whether functional variations in the common downstream signaling molecule, G-protein, could cause individual differences in presynaptic transporter availability remains unclear. To investigate this relationship, the DAT availability in seventy-eight healthy subjects was approximated using single photon emission computed tomography (SPECT) with \[^{99mTc}\] TRODAT-1, a radio-labeled form of tropan derivative for the selective labeling of DAT. The C825T single nucleotide polymorphism (SNP) (rs5443) of the beta subunit of the G-protein second messenger (GN\(\beta_3\)) gene was genotyped, and analysis of variance showed a significant difference in striatal DAT when referenced to the entire occipital lobe among the three genotypes. Post hoc independent t tests were also performed, and showed that the striatal DAT availability of the CC genotype was higher than that of the other two genotypes. These results indicated that genetic variation in the common downstream signaling molecule of the dopamine autoreceptor could affect the functional status of the striatal dopamine system. These results together with the known role of the GN\(\beta_3\) gene might provide further evidence...
to support the common effect of the striatal dopamine system on mood and metabolic regulation.

Key Words: TRODAT-1, dopamine transporter, SPECT, β3 subunit of G-protein.
Introduction

The striatal dopamine system has long been considered to involve circuits that participate in motor coordination (Yang et al., 2003), and more recent studies have shown the system to be involved in the integration of cognitive activities and reward responses through the cortico-thalamic-basal ganglian-cortical loop (Honey et al., 2003). One of the key presynaptic components involved in regulating dopaminergic tone is the dopamine transporter (DAT). Our previous imaging studies using single photon emission computed tomography (SPECT) with $[^{99mTc}]$ TRODAT-1, a radio-labeled form of tropan derivative for the selective labeling of DAT, also indicated that the striatal DAT availability was significantly correlated with the functional significance of various cognitive activities, mood regulation, and even complex social behavior (Hsieh et al., 2010; Yang et al., 2008b; Yeh et al., 2009). A number of studies have indicated a new role of the striatal dopamine system in the regulation of metabolic homeostasis, which may be represented by body mass index (Chen et al., 2008; Wang et al., 2001).

The availabilities of DAT could be regulated by the presynaptic dopamine D$_2$ autoreceptor through direct interaction (Mayfield and Zahniser, 2001). This physical coupling facilitates the recruitment of intracellular DAT to the cell surface and leads to enhanced DAT availability. The presynaptic D$_2$ autoreceptors for dopamine
systems are G-protein-coupled receptors. G-proteins are key components of intracellular signal transduction that stimulate the enzyme phospholipase C to produce intracellular second messengers (Neves et al., 2002). Heterotrimeric G-proteins are formed from three subunits, an $\alpha$ subunit and a $\beta\gamma$-dimer. Variants of any of the subunits appear to be responsible for alteration in downstream signaling. Among the variants, Siffert et al. (1998a; 2000) described a single-nucleotide polymorphism (SNP) (rs5443) of C825T in exon 10 of the gene encoding the $\beta$ subunit of G-protein (GNB3). Expression of the T allele is accompanied by enhanced sensitivity of Gi proteins to receptor activation. In addition, the levels of G-protein activation in the CT genotype were similar to those of the TT genotype, but not to those of the CC genotype (Siffert et al., 1999; Siffert et al., 1998). Studies have demonstrated that DAT and the D$_2$ autoreceptor have a reciprocal presynaptic regulation relationship (Bertolino et al., 2009; Mayfield and Zahniser, 2001). Therefore, we evaluated whether a genetic variation in the dopamine D2 receptor-coupled G-protein beta subunit, the C825T SNP (rs5443), could affect striatal DAT availability in humans. To investigate this relationship, DAT availability was measured using SPECT with [$^{99m}$Tc] TRODAT-1, and the C825T SNP (rs5443) of the beta subunit of its G-protein second messenger (GN$\beta$3) gene chosen from the HapMap database was genotyped.
Materials and Method

Ethics

The Ethical Committee for Human Research at the National Cheng Kung University Hospital approved the study protocol, and informed consent was obtained from the volunteers before any procedure was performed.

Participants

Seventy-eight healthy volunteers recruited from the community through advertisements were enrolled in various studies as healthy controls. The exclusion criteria included any past or present psychiatric or neurological disorder, alcohol or substance abuse (except caffeine and nicotine), serious medical and surgical conditions that could alter cognitive functioning, and a history of head trauma with loss of consciousness. The subjects underwent comprehensive medical and neurological examinations to ensure the absence of disease and were interviewed by a senior psychiatrist using the Mini International Neuropsychiatric Interview to exclude individuals with mental disorders (Sheehan et al., 1998). None of the participants was taking any medication at the time of the study.

SNP Detection

Genomic DNA was extracted from each blood sample using a QIAamp DNA blood kit according to the manufacturer’s instructions. The quality of the extracted
genomic DNA was checked by agarose gel electrophoresis analysis, and the extracted
DNA was then stored at –80°C until use.

The SNP C825T (rs5443) of the GNβ3 gene was analyzed using a
commercially-available TaqMan® SNP Genotyping Assay (ABI Inc., Taiwan)
according to the manufacturer’s instructions. Amplification and dissociation
were carried out using a 7900HT Fast Real-Time PCR System (ABI, USA), and
the ABI7900 calculated the negative derivative of the change in fluorescence
automatically. The SNP genotype of each tested sample was determined by
computer software and was confirmed manually. In cases of disagreement, the
analysis was repeated.

DAT availability in the striatum

For brain imaging, each subject was intravenously administered 740 MBq (20mCi)
[^99mTc] TRODAT-1 (a radio-labeled form of tropan derivative for the selective
labeling of DAT) in a quiet environment about ten minutes after insertion of an
intravenous line. The SPECT data were obtained using an energy window of 15%
centered on 140 keV for [^99mTc]. Imaging of [^99mTc] TRODAT-1 was initiated
approximately 240 minutes after injection, and SPECT images were acquired over a
circular 360° rotation in 120 steps, 50 seconds per step, in a 128×128×16 matrix. The
images were then reconstructed using Butterworth and Ramp filters (cut-off frequency $= 0.3$ Nyquist; power factor = 7) with attenuations by Chang’s method, and the reconstructed transverse images were realigned parallel to the canthomeatal line. The slice thickness of each transverse image was 2.89 mm. In addition, all subjects underwent magnetic resonance imaging (Signa CV-I, 1.5 Tesla, GE Medical Systems, Milwaukee, WI, USA). Using the commercial software PMOD (PMOD Technologies, Zurich, Switzerland), each subject’s SPECT image was co-registered with the corresponding T2-weighted MRI image automatically and was then finely-adjusted manually by an experienced nuclear medicine physician.

The MRI image was loaded as a reference, so the slice thickness of the co-registered images was the thickness of the T2-weighted MRI images (3.3 mm). For the co-registration, rigid transformations were defined by 6 parameters, the rotation angles and translation distances in the three spatial directions. The interpolation method was trilinear. On the co-registered images, the two contiguous transverse slices that contained the most intense striatal radioactivity were further examined in order to ascertain whether the SPECT and MRI images were co-registered accurately and whether the striatum was best seen on the two slices of the MRI images. If that was not the case, further adjustment of co-registration was performed manually until a satisfactory outcome was achieved.

Regions of interest (ROIs), including the striatum and occipital cortex, were then
drawn on the two contiguous MRI transverse slices, and these ROIs were projected onto the co-registered SPECT images. The ratio of the radioactivity [the (St-Oc)/Oc ratio] was then derived by dividing the difference between the average activity in the striatum (St) and the average activity in the occipital cortex (Oc) by the average activity in the occipital cortex (Oc) (Hwang et al., 2004).

Statistical analysis

The proportions of genotypes and allele frequencies between genders were evaluated by chi-squared statistics. Continuous variables were expressed as mean±SD and compared across genotypes by analysis of variance, or covariance when suitable, and the LSD post hoc test was also performed, adjusting for confounders and calculating the 95% confidence intervals when appropriate. The CT and TT genotypes were analyzed together in a dominant genetic model for post hoc comparison, as the T allele has been found to exert a dominant effect (Siffert et al., 1999; Siffert et al., 1998).
Results

The distributions of age, gender and body mass index (BMI) did not differ significantly between the genotypes (Table 1). The availabilities of DAT are shown in Table 1a as the mean±SD deviation: a significant difference in DAT availability was observed between the two genders in the whole study group ($t_{DAT}=2.05$, $p=0.04$) but not in the smoking-free group ($t_{DAT}=1.65$, $p=0.10$). Analysis of variance showed a marginally significant difference in striatal DAT when referenced to the entire occipital lobe among the three genotypes (Table 1a). Independent t tests were performed, which showed that the striatal DAT availability of the CC genotype was higher than that of the other two genotypes (all $p<0.03$, Table 2a). As our previous study indicated that BMI and gender may affect the DAT availability (Chen et al., 2008), we then used BMI and gender as covariates for further analyses, and the results indicated that the striatal DAT availability of the CC genotype was still higher than that of the CT/TT genotypes (Table 2a).

Because a recent SPECT with TRODAT-1 study found significant decreases in striatal DAT binding in current smokers as compared with non-smokers (Newberg et al., 2007; Yang et al., 2008a), we therefore reanalyzed the data of the 64 subjects who were non-smokers, and the results showed that the effects of the C825T
polymorphism of the GNB3 gene on the availability of DAT remained the same in these subjects ($F=3.32, p=0.04$) (Table 1b, 2b).
Discussion

This is the first SPECT study to show that the C825T (rs5443) SNP of the GNβ3 gene is significantly associated with striatal DAT availability. The results suggested that genetic variation in the common downstream signaling molecule of dopamine autoreceptors may affect the functional status of the striatal dopamine system, as represented by DAT availability. Further study would be useful in order to investigate the underlying mechanism and interactions between dopamine transporters, receptors, and the downstream G-protein in the striatum.

The SNP C825T of the GNβ3 gene has been reported to be associated with elevated metabolic indexes in different groups of patients suffering from hypertension, diabetes or cardiovascular disease (Brand et al., 2003; Casiglia et al., 2008; Danoviz et al., 2006; Hayakawa et al., 2007; Kopf et al., 2008; Meirhaeghe et al., 2005).

Moreover, the variant has also been found to be associated with medication-induced metabolic disturbances (Hauner et al., 2003; Peters et al., 2008; Souza et al., 2008; Wang et al., 2005). The preliminary results from our laboratory also showed the variant to be associated with valproate-induced metabolic disturbance in patients with bipolar disorder (Chang et al., 2009; Chang et al., 2010a; Chang et al., 2010b). Interestingly, previous brain imaging studies have indicated a role of the striatal
dopamine system in energy homeostasis (Chen et al., 2008; Wang et al., 2001). We also found previously that BMI was the only significant predictor of striatal DAT availability (Chen et al., 2008). Taken together, the results of the current study indicated possible links between the GNB3 gene, the striatal dopaminergic system and energy homeostasis, the underlying mechanisms of which merit further investigation. In addition to energy homeostasis, the SNP C825T of the GNβ3 gene has also been found to be correlated with major depressive disorder, which indicated the involvement of G-proteins in mood regulation (Lee et al., 2005; Willeit et al., 2003). This polymorphism has also been reported to be associated with short-term antidepressant treatment outcome (Lin and Chen, 2008). Subjects with the GNB3 T/T variant exhibited a better response to treatment than those with the T/C and C/C variants (Serretti et al., 2003). Moreover, we recently reported that interactions between GNB3, serotonin receptor and SERT variants were significantly associated with treatment response in major depressive disorder (Lin et al., 2009). Mesolimbic dopaminergic circuitry is also known to be involved in mediating mood under normal and abnormal conditions (Clausius et al., 2009; Nestler and Carlezon, 2006; Ruhe et al., 2007; Yang et al., 2008b). The serotonin system is critical for mood regulation and also interacts with the dopaminergic system in the striatum (Beal and Martin, 1985; Sershen et al., 2000). The results of the current study indicated a link between the
GNB3 gene and the striatal dopaminergic system, and further investigation is required in order to ascertain whether or not the link plays a particular role in mood regulation.

Previous studies have suggested that common mechanistic mediators may be involved in the etiology and progression of metabolic disturbances and stress-related mood disorders (Michalsen et al., 2009; Reagan, 2007). Accumulating evidence demonstrates that both the variant of the GNB3 gene and striatal DAT availability are associated with mood and metabolic regulation, and the results reported herein indicate a link between GNB3 gene variation and striatal DAT availability, possibly providing further evidence in support of the common role of the striatal dopamine system in mood and metabolic regulation (Chen et al., 2008; Yang et al., 2008b).

The results of the present study need to be interpreted with caution due to the following limitations. First, the number of subjects enrolled in this study was relatively small. Second, we need to perform further genetic manipulation experiments in an animal model in order to assess the causal relationship between the C825T polymorphism of the GNB3 gene and the striatal dopamine transporter availability. Third, the partial volume effect (PVE) was not corrected for in this study. It is known that the PVE affects the measurement of the count concentration, generally leading to an underestimation of the true values. This underestimation depends on the size of the object. In this study, the SPECT and MRI images were
co-registered automatically and further cautiously fine adjusted manually. We drew ROIs on the MRI images carefully. Furthermore, we analyzed the striatal ROI sizes of the CC, CT and TT genotype groups and found no significant differences between the three groups (all subjects, $p>0.42$; smoking free subjects, $p>0.70$); therefore, the PVE is considered to be minimized. Fourthly, voxel-wise analysis (such as SPM) was not performed. This approach may corroborate our current ROI-based analysis and may provide a better spatial demonstration of the DAT availability difference between the groups. However, voxel-size analysis might introduce errors owing to spatial normalization and multiple comparisons (Bonne et al., 2003; Loring et al., 2002).

Finally, the reference area in this study differed to that of the study by Mozley and colleagues (Mozley et al., 2000), and therefore the localization of the background region may not be accurate.

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References


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