The contribution of autism susceptibility candidate 2 and *N*-methyl-$_D$-aspartate receptor subunit 2B gene polymorphisms to individual differences in alcohol dependence vulnerability and personality traits

（アルコール依存脆弱性や人格特性の個人差に対する autism susceptibility candidate 2 および *N*-methyl-$_D$-aspartate receptor subunit 2B 遺伝子多型の寄与）

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麻布大学大学院　環境保健学研究科

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DE1501　成田　心

The contribution of autism susceptibility candidate 2 and *N*-methyl-$_{D}$-aspartate receptor subunit 2B gene polymorphisms to individual differences in alcohol dependence vulnerability and personality traits

DE1501 Shin Narita

Laboratory of Physiology,

The Graduate School of Environmental Health Sciences,

Azabu University

Abstract

The objective of this research was to elucidate the genetic factors involved in individual differences in alcohol dependence vulnerability and related personality traits by analyzing single nucleotide polymorphisms (SNPs) of two genes, thereby facilitating the development of individually tailored medicines.

Alcohol dependence is a form of drug addiction that involves loss of control as to alcohol consumption, and is hereditary. It is suggested that alcohol dependence is due to an abnormality in the brain reward system relating to anxiety and pleasant emotions. The autism susceptibility candidate 2 (AUTS2) which plays an important role in the development of the cranial nervous system, is broadly involved in various psychiatric disorders including autism. Recent studies reported that the rs6943555 polymorphism in the AUTS2 gene (*AUTS2*) is related to heroin dependence, and this polymorphism also affects individual alcohol consumption as shown by a genome-wide association study meta-analysis. The *N*-methyl-D-aspartate (NMDA) receptor, which is a type of glutamate receptor, is one of the primary targets of ethanol in the brain. It has been reported that the NMDA receptor subunit 2B (GluN2B) of the NMDA receptor increases its expression in response to chronic ethanol exposure. In addition, a case-control study in another population revealed that a polymorphism and several haplotypes of the GluN2B gene (*GRIN2B*) are associated with alcohol dependence. From the above points, a contribution to the brain reward system by both genes is predicted. In this study, to elucidate their effects on individual differences in alcohol dependence vulnerability, the author focused on nine SNPs, including functional polymorphisms in *AUTS2* and *GRIN2B*, and compared the difference in frequencies of these polymorphisms between patients with alcohol dependence and healthy control subjects living in a Japanese provincial prefecture.

The subjects in this study consisted of 64 patients (male: 50, female: 7, not available: 7; mean age ± SD: 57.34 ± 10.18 years) with a diagnosis of alcohol dependence according to the DSM-IV criteria, and 75 unrelated healthy people (male: 23, female: 52; mean age

± SD: 35.36 ± 9.06 years). Blood samples were collected from the subjects, all of whom provided written informed consent for genetic studies. All of the patients and control subjects lived in Yamagata prefecture in Japan. The *AUTS2* (rs6943555 and rs9886351) and *GRIN2B* (rs3764028, rs1019385, rs7301328, rs1806201, rs1805247, rs890 and rs1805502) polymorphisms were genotyped by the polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) method.

In genotype and allele frequency analysis, there were no significant differences in the genotype or allele frequencies of *AUTS2* polymorphisms rs6943555 and rs9886351 between alcohol-dependent and control subjects. On the other hand, haplotype analysis indicated that the frequency of the A-A haplotype consisting of the polymorphisms rs6943555 and rs9886351 was significantly different between the alcohol-dependent and control subjects ($p = 0.019$). The patient group showed a significantly higher frequency of the A-A haplotype as compared with the control group. Besides, there were significant differences in the genotype ($p = 0.019$) and allele ($p = 0.044$) frequencies of *GRIN2B* polymorphisms rs1805247 and rs1805502 (complete linkage disequilibrium in the analyzed population) between the alcohol-dependent and control subjects, and the proportion of C allele carriers of these polymorphisms was significantly higher in the patient group than in the control group ($p = 0.007$). Haplotype analysis indicated a marginally significant association between the C-C-C haplotype in the *GRIN2B* haplotype block (rs1806201-rs1805247-rs1805502) and alcohol dependence ($p = 0.054$). Other *GRIN2B* polymorphisms and haplotypes were not significantly associated with alcohol dependence.

These results suggested that the polymorphisms and haplotypes of the *AUTS2* and *GRIN2B* might be one of the risk factors for alcohol dependence in a Japanese population. Alcohol drinking behavior is influenced by different environmental factors in various countries and races. Therefore, these findings in the first study on a Japanese population are important, and they are expected to help elucidate the pathology of alcohol dependence, and to lead to early prevention and treatment based on features of individuals' genetic backgrounds. In order to obtain more clinically meaningful findings, further

studies with more subjects considering possible biases are needed to confirm the reproducibility of the results of this study.

Personality traits are influenced not only by environmental factors but also by genetic ones. Some genes involved in the characterization of personality traits have aspects affecting various behaviors, and associations with gene polymorphisms that contribute to some disorders such as mood disorders, schizophrenia, and drug addiction have been reported. It has been suggested that the *AUTS2* and *GRIN2B* affect various human mental functions and behaviors, and both genes may be involved in personality traits. To date, a study on the relationship between the *AUTS2* polymorphisms and personality traits has not yet been reported, and there are few studies that have reported the relationship between the *GRIN2B* polymorphisms and personality traits. In this study, the author focused on the *AUTS2* and *GRIN2B* polymorphisms that have been shown to affect human mental function and behavior (including SNPs found to be associated with alcohol dependence in the above-mentioned study), and investigated whether these polymorphisms are related to the individual differences in personality traits.

For analysis of association with *AUTS2* polymorphisms, the participants comprised 190 young people (male: 51, female: 139; mean age ± SD: 20.46 ± 1.15 years), and personality traits were assessed using the Temperament and Character Inventory (TCI). In order to exclude the influence of subjects with depressive symptoms on the results, the Patient Health Questionnaire-9 (PHQ-9) was also performed. For analysis of association with *GRIN2B* polymorphisms, the participants comprised 248 young people (male: 63, female: 185; mean age ± SD: 19.55 ± 1.21 years), and personality traits were assessed using the NEO-Five Factor Inventory (NEO-FFI) and State-Trait Anxiety Inventory (STAI). The *AUTS2* (rs6943555 and rs9886351) and *GRIN2B* (rs7301328, rs1806201, rs1805247 and rs1805502) polymorphisms were genotyped by means of the PCR-RFLP method.

In all subjects, two-way analysis of variance showed that there was a significant main effect of the *AUTS2* polymorphism rs6943555 genotype on reward dependence (p =

0.038) and cooperativeness (p = 0.031) of the TCI dimensions, although the association was lost on Bonferroni correction. In addition, no significant association was found between *AUTS2* polymorphisms and haplotypes and TCI dimensions when subjects with moderate or higher depressive symptoms according to the PHQ-9 were excluded. Similarly, two-way analysis of variance showed no significant effects of the *GRIN2B* polymorphisms on the NEO-FFI and STAI dimensions. In female subjects, the C-T-T haplotype in the *GRIN2B* haplotype block (rs1806201-rs1805247-rs1805502) was significantly associated with extraversion (p = 0.044) of the NEO-FFI dimensions, although the association was lost on Bonferroni correction.

In summary, this study was unable to detect a significant association between the selected *AUTS2* and *GRIN2B* polymorphisms and personality traits. Several studies have revealed that the glutamatergic neurotransmission system plays some role in the characterization of human behavior and personality traits. It is reported that *AUTS2* is expressed in glutamatergic neurons, and this gene may be affecting personality traits by interaction with glutamate-related genes such as *GRIN2B*. Further studies using other personality questionnaires with different characteristics are therefore needed to clarify the relationship between both genes and personality traits, and it would be meaningful also to analyze the effect of interactions between the *AUTS2* and *GRIN2B* polymorphisms.

要旨

本研究では、テーラーメイド医療の一助となることを目的とし、アルコール依存脆弱性や人格特性の個人差にかかわる遺伝的要因の解明のため、遺伝子多型解析による検討を行った。その概要は以下の通りである。

アルコール依存症はアルコール消費に対する制御喪失を伴う薬物依存の一形態であり、その発症脆弱性には遺伝的関与が認められる。これまでの研究から、不安や快情動に関わる脳内報酬システムの異常がアルコール依存形成に繋がることが指摘されている。脳神経系の発達に重要な役割を担うとされる autism susceptibility candidate 2（AUTS2）は自閉症をはじめとする種々の精神疾患に広く関与することが知られている。最近の研究では、AUTS2 遺伝子（*AUTS2*）上に存在する rs6943555 多型が薬物依存症の一種であるヘロイン依存に関与することが報告され、さらにゲノムワイド関連解析のメタ解析において同多型が個人のアルコール消費量にも影響を与えることが明らかとなった。また、グルタミン酸受容体の一種である *N*-methyl-D-aspartate（NMDA）受容体は脳内におけるエタノールの主要な作用部位の一つとされ、そのサブユニットとして知られる NMDA 受容体サブユニット 2B（GluN2B）は慢性エタノール曝露によりその発現が増加することが報告されている。さらに、他国における研究では GluN2B 遺伝子（*GRIN2B*）の多型やハプロタイプがアルコール依存症に関与することが確認された。以上の点から、両遺伝子の脳内報酬システムへの寄与が疑われる。本研究では、*AUTS2* および *GRIN2B* 上に存在する機能性多型を含む 9 つの一塩基多型（Single Nucleotide Polymorphisms: SNPs）に着目し、それらの頻度を日本の一地域におけるアルコール依存症患者群と同一地域の健常者群間で比較解析することで、アルコール依存脆弱性の個人差に対する影響について検討した。

対象は DSM-IV の診断基準においてアルコール依存症と診断されたアルコール依存症患者 64 人（男性 50 人、女性 7 人、不明 7 人、平均年齢 57.34 ± 10.18 歳）およびアルコール依存の経歴のない健常者 75 人（男性 23 人、女性 52 人、平均年齢 35.36 ± 9.06 歳）とし、対象者から同意を得た上で採血を行った。すべ

ての対象者は山形県在住である。また、解析対象となる *AUTS2*（rs6943555、rs9886351）および *GRIN2B*（rs3764028、rs1019385、rs7301328、rs1806201、rs1805247、rs890、rs1805502）多型の遺伝子型は polymerase chain reaction（PCR）- restriction fragment length polymorphism（RFLP）法により決定した。

比較解析の結果、アルコール依存症患者群と健常者群の間で *AUTS2* 多型の rs6943555 および rs9886351 の遺伝子型と対立遺伝子の頻度に有意な差異は認められなかった。一方、ハプロタイプ解析の結果、アルコール依存症患者群と健常者群の間で rs6943555 および rs9886351 からなる A-A ハプロタイプの頻度に有意な差異を認め（$p = 0.019$）、健常者群に比べ患者群において高頻度に検出された。また、本解析集団において完全連鎖不平衡の関係にあった *GRIN2B* 多型の rs1805247 および rs1805502 の遺伝子型（$p = 0.019$）と対立遺伝子（$p = 0.044$）の頻度にアルコール依存症患者群と健常者群の間で有意な差異を認め、C 対立遺伝子保有者の割合が健常者群に比べ患者群において有意に高かった（$p = 0.007$）。ハプロタイプ解析の結果、本解析集団において検出された *GRIN2B* ハプロタイプブロック（rs1806201-rs1805247-rs1805502）の C-C-C ハプロタイプとアルコール依存症との間に関連傾向を認めた（$p = 0.054$）。その他の *GRIN2B* の多型やハプロタイプについては、アルコール依存症との関連は認められなかった。

以上より、*AUTS2* および *GRIN2B* の多型やハプロタイプがアルコール依存症発症の危険因子の一つとして推測され、それらの分子生物学的機能により脳内報酬システムに少なからず影響を与える可能性が示唆された。飲酒行動は様々な国や人種で異なる環境要因の影響を受けるため、日本人を対象とした初めての研究で得た本知見は重要であり、アルコール依存症の病態解明、早期の予防や治療に繋がることが期待される。今後はさらなるエビデンスの構築を目指し、大規模な他集団において結果の再現性を確認することで、より臨床的意義の高い知見が得られるものと示唆される。

人格特性の形成には環境的要因だけでなく遺伝的要因も関与している。人格特性の形成にかかわる遺伝子はヒトの行動に広く影響を与え、その背景には気分障害や統合失調症、薬物依存症などの発症に寄与する遺伝子多型との関連が

報告されている。これまでに *AUTS2* や *GRIN2B* がヒトの様々な精神機能や行動に影響を与えることが示唆されており、人格特性に影響を与える可能性は否定できない。*AUTS2* 多型と人格特性との関連を検討した研究はこれまでに行われておらず、*GRIN2B* 多型との関連についての報告も少ない。本研究では、前述でアルコール依存症との関連を認めた SNPs を含む、ヒトの精神機能や行動への影響が報告されている *AUTS2* および *GRIN2B* 多型に焦点を当て、それらの多型が人格特性の形成に関与する可能性について検討した。

AUTS2 多型を解析対象とした研究では、同意を得た若年者 190 人（男性 51 人、女性 139 人、平均年齢 20.46 ± 1.15 歳）を対象とし、人格特性の評価には自己記入式人格検査の Temperament and Character Inventory（TCI）を用いた。また、抑うつ症状を有する対象者による結果への影響を除外するために抑うつ評価尺度の Patient Health Questionnaire-9（PHQ-9）を実施した。*GRIN2B* 多型を解析対象とした研究では、同意を得た若年者 248 人（男性 63 人、女性 185 人、平均年齢 19.55 ± 1.21 歳）を対象とし、人格特性の評価には自己記入式人格検査の NEO-Five Factor Inventory（NEO-FFI）と State-Trait Anxiety Inventory（STAI）を用いた。*AUTS2*（rs6943555、rs9886351）および *GRIN2B*（rs7301328、rs1806201、rs1805247、rs1805502）多型の遺伝子型は PCR-RFLP 法により決定した。

二元配置分散分析の結果、対象者全員において *AUTS2* 多型 rs6943555 の TCI 気質尺度の報酬依存（$p = 0.038$）と性格尺度の協調（$p = 0.031$）に対する有意な主効果を検出したが、Bonferroni 補正によりその関連性は消失した。さらに、PHQ-9 により中等度以上の抑うつ症状を有する対象者を除外して解析を行ったが、*AUTS2* 多型やハプロタイプと TCI 尺度との有意な関連は認められなかった。同様に、二元配置分散分析の結果、*GRIN2B* 多型の NEO-FFI および STAI 尺度に対する有意な効果は検出されなかった。また、女性において本解析集団で検出された *GRIN2B* ハプロタイプブロック（rs1806201-rs1805247-rs1805502）の C-T-T ハプロタイプと NEO-FFI 尺度の外向性（$p = 0.044$）との間に有意な関連を認めたが、Bonferroni 補正によりその関連性は消失した。

以上より、本研究で解析対象とした *AUTS2* および *GRIN2B* 多型と人格特性との有意な関連を検出することはできなかった。グルタミン酸作動性神経伝達シ

ステムはヒトの行動や人格特性に対し、いくつかの役割を担うことが示唆されている。*AUTS2* はグルタミン酸作動性ニューロンに発現していることが報告されており、同遺伝子が *GRIN2B* などのグルタミン酸関連遺伝子との相互作用により人格特性の形成に影響を与えている可能性も考えられる。今後、人格特性との関連を解明するため、異なる特徴を有する他の人格検査を用いて検討することが必要であり、両遺伝子多型の相互作用についての解析も意義があると考える。

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Association between *AUTS2* haplotypes and alcohol dependence in a Japanese population

Narita S, Nagahori K, Nishizawa D, Yoshihara E, Kawai A, Ikeda K, Iwahashi K. Association between *AUTS2* haplotypes and alcohol dependence in a Japanese population.

Objective: Recent genome-wide analysis has indicated that the autism susceptibility candidate 2 (*AUTS2*) gene is involved in the regulation of alcohol consumption. We hypothesised that *AUTS2* might be associated with the development of alcohol dependence. Therefore, in this exploratory study, we compared the genotype and allele frequencies of the polymorphisms rs6943555 and rs9886351 in the *AUTS2* gene between patients with alcohol dependence and healthy control subjects living in a Japanese provincial prefecture. We also examined whether or not the haplotypes consisting of these polymorphisms are related to alcohol dependence.
Methods: The subjects of this study consisted of 64 patients with alcohol dependence and 75 unrelated healthy people. The *AUTS2* genotypes were determined by the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method.
Results: No significant differences in the genotype and allele frequencies of the polymorphisms *AUTS2* rs6943555 and rs9886351 were found between alcohol dependence and control subjects. On the other hand, the frequencies of the *AUTS2* haplotypes were significantly different between them, and the rs6943555 and rs9886351 A-A haplotype was associated with alcohol dependence ($p = 0.0187$).
Conclusion: This suggests that the rs6943555 and rs9886351 A-A haplotype might affect the vulnerability to alcohol dependence pathogenesis. Further studies are needed to confirm the reproducibility of the results of this study with increased numbers of subjects.

Shin Narita[1], Kenta Nagahori[2], Daisuke Nishizawa[3], Eiji Yoshihara[1], Atsuko Kawai[4], Kazutaka Ikeda[3], Kazuhiko Iwahashi[1,3,5]

[1]Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, Sagamihara-shi, Kanagawa, Japan; [2]Department of Anatomy, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan; [3]Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo, Japan; [4]Koutokukai Total Health Clinic, Yamagata, Japan; and [5]Health Administration Center, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara-shi, Kanagawa 252-5201, Japan

Keywords: alcohol dependence; autism susceptibility candidate 2; gene polymorphism; haplotype

Kazuhiko Iwahashi, Laboratory of Physiology (Project of Neurophysiology), the Graduate School of Environmental Health Sciences, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara-shi, Kanagawa 252-5201, Japan.

Tel: +81 42 769 1930;
Fax: +81 42 769 1930;
E-mail: iwahashi@azabu-u.ac.jp

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Significant outcomes

- The frequencies of the *AUTS2* haplotypes were significantly different between alcohol dependence and control subjects.
- The patients with alcohol dependence showed a significantly higher frequency of the A-A haplotype (rs6943555 and rs9886351) as compared with the control group.
- Our study suggested that the rs6943555 and rs9886351 A-A haplotype in the *AUTS2* gene might affect the vulnerability to the alcohol dependence pathogenesis.

Limitations

- The sample size of alcohol-dependent patients and control subjects in our study was small and consequently have low statistical power.
- In addition, it must be considered that the structure of the subject have several bias such as gender and age.
- Larger scale comparison of subjects of the same generation with no gender bias is needed in a further study to confirm our current results.

Introduction

Alcohol dependence is a form of drug addiction that involves physical and psychological dependence on alcohol, and loss of control as to alcohol consumption (1). Several previous reviews have revealed that the heritability of alcohol dependence accounts for 40–60% of the total etiologic variance (2–4). A number of genes are associated with the development of alcohol dependence through interaction with environmental factors (1).

The autism susceptibility candidate 2 (*AUTS2*) gene has been implicated in multiple neurological disorders including autism (5). Recent studies have shown that the *AUTS2* gene is associated with autism (6), schizophrenia (7) and suicide (8). In addition, several lines of evidence suggest that *AUTS2* also plays some role in the development of drug addiction. The results of a previous small-scale study indicated that *AUTS2* expression in lymphoblast cell lines is significantly correlated with nicotine dependence and cannabis dependence (9). The study also suggested that *AUTS2* expression tends to be significantly associated with alcohol dependence (9). In several very recent studies, Chen et al. discovered that the average of relative cDNA level of *AUTS2* in lymphoblastoid cell lines was significantly reduced in patients with heroin dependence compared with healthy controls (10). Additionally, Chen et al. and Dang et al. reported that the polymorphism rs6943555 in the *AUTS2* gene might increase susceptibility to the development of heroin dependence (10,11). Interestingly, Schumann et al. have found that *AUTS2* expression in human brain tissue differs depending on genotype (12). They also found significant differences in mice selected for voluntary alcohol consumption differences in expression of *AUTS2* (12). Furthermore, their large-scale meta-analysis including 12 population-based samples of European ancestry comprising 26 316 individuals with replication genotyping in an additional 21 185 individuals indicated that the polymorphism *AUTS2* rs6943555 is significantly associated with alcohol consumption by individuals, with genome-wide significance (12). They also revealed that down-regulation of an *AUTS2* homolog induced reduced alcohol sensitivity in Drosophila (12). Thus, these observations suggest that the *AUTS2* gene is related to the regulation of alcohol drinking behaviour (10,12), and we hypothesised that polymorphisms in the *AUTS2* gene also affect the vulnerability to alcohol dependence pathogenesis.

The *AUTS2* gene is located on chromosome 7q11.22 and consists of 19 exons, the first six exons being separated by very large introns and the last 13 exons being close (11). Among the several known *AUTS2* gene polymorphisms, we focussed on two single nucleotide polymorphisms (SNPs), which have been studied extensively. One of these common SNPs in the *AUTS2* gene is rs6943555, which comprises a single nucleotide change of T → A. Schumann et al. reported that the minor A allele of rs6943555 significantly increases *AUTS2* gene expression in the prefrontal cortex of the human brain compared with the T allele (12). Meanwhile, Chen et al. found that subjects with the rs6943555 A/A genotype exhibit significantly lower *AUTS2* mRNA level in lymphoblastoid cell lines compared to subjects with the T/T and T/A genotypes (10). Although the *AUTS2* gene expression might not be equal between brain tissue and lymphoblastoid cell lines (10), in any event, these studies suggest that the single nucleotide exchange of T to A in rs6943555 variants may influence transcriptional activity and expression of the *AUTS2* gene. In addition to rs6943555, we selected the polymorphism rs9886351 which was adjacent to rs6943555 (7) and comprises a single nucleotide change of A → G. The selection of this polymorphism was based on previous studies (7,11). We detected SNPs of the *AUTS2* gene in Haploview v4.2 using the HapMap Japanese population and a minor allele frequency (MAF) cut-off ≥ 5%, and selected rs9886351 using the pair-wise tagging only mode and $r^2 \geq 0.8$ (13) as the cut-off for the selection of tagSNPs. Zhang et al. and Dang et al. also selected the polymorphism rs9886351 as tagSNP (7,11). Regarding the association of heroin dependence and the polymorphism rs9886351, an effect of this polymorphism on the development of heroin dependence was not observed, but a statistical association trend has been found (11).

Therefore, in the present study, to elucidate genetic factors for alcohol dependence, we compared the frequencies of the polymorphisms *AUTS2* rs6943555 and rs9886351 between patients with alcohol dependence and healthy control subjects from a Japanese population. In addition, we examined whether or not the haplotypes consisting of these polymorphisms in the *AUTS2* gene are related to alcohol dependence.

Materials and methods

The subjects of this study consisted of 64 patients (male: 50, female: 7, not available: 7; mean age ± SD: 57.34 ± 10.18 years) with a diagnosis of alcohol dependence according to DSM-IV criteria and 75 unrelated healthy people including the alcohol drinker in everyday life (male: 23, female: 52; mean age ± SD: 35.36 ± 9.06 years). Blood samples were collected from the subjects at Koutokukai Sato

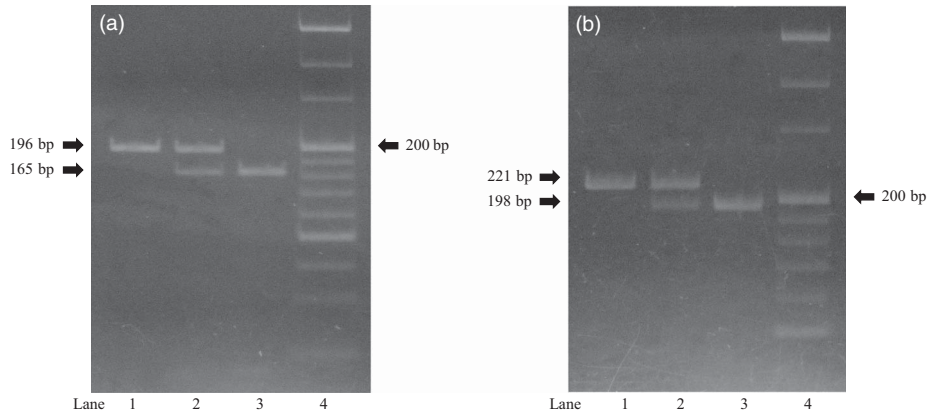

Fig. 1. Representative results for the *AUTS2* gene polymorphisms with the PCR-RFLP method. (a) rs6943555: Lane 1 shows a T/T type. Lane 2 shows a T/A type. Lane 3 shows an A/A type. Lane 4 shows a 20 bp DNA ladder. (b) rs9886351: Lane 1 shows an A/A type. Lane 2 shows an A/G type. Lane 3 shows a G/G type. Lane 4 shows a 20 bp DNA ladder. *AUTS2*, autism susceptibility candidate 2; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

Hospital Group, and all subjects provided written informed consent for genetic studies. All of the patients and control subjects lived in Yamagata prefecture in Japan.

The study was approved by the ethics committees of the Tokyo Metropolitan Institute of Medical Science [13–29] and Azabu University [0648].

Two *AUTS2* gene polymorphisms were genotyped by means of polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) according to the method of Zhang et al. (7). Genomic DNA was amplified with the following primers: rs6943555 (forward: 5'-TGG GTG TTG GAA GAG TTT TGA-3', reverse: 5'-ATA CAG TAT ACA TAA ACA TTG GAA AAG AGG GAA-3') and rs9886351 (forward: 5'-GGT GGA AAA TAA GCC AGT ATG C-3', reverse: 5'-TAG GAA AAT GGA TTA AAC GTA GGA G-3'). The PCR cycling conditions were: 95°C for 10 min, 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s, with final extension at 72°C for 7 min, for rs6943555; and 95°C for 10 min, 35 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with final extension at 72°C for 7 min, for rs9886351. The PCR products (196 bp for rs6943555 and 221 bp for rs9886351) were digested with a restrictive enzyme, *Hinf* I (New England Biolabs, Tokyo, Japan), and the digested products were subjected to electrophoresis on 5% polyacrylamide gels and visualised using the ethidium bromide staining method. Genotypes were determined according to fragment sizes: rs6943555: T/T = 196 bp, T/A = 196 bp + 165 bp + 34 bp, A/A = 165 bp + 34 bp and rs9886351: A/A = 221 bp, A/G = 221 bp + 198 bp + 26 bp, G/G = 198 bp + 26 bp. The major visible bands of these polymorphisms were those of 221, 198, 196, and 165 bp (Fig. 1).

Statistical differences in the allele and genotype frequencies of each polymorphism between the patients with alcohol dependence and healthy control subjects were assessed using the χ^2 test or Yates' correction. In addition, linkage disequilibrium (LD) coefficients (D' and r^2) and haplotype frequencies were calculated with gPLINK 2.050 (http://pngu.mgh.harvard.edu/purcell/plink/) and Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview/index.php) (14,15). Statistical significance was defined as $p < 0.05$.

Results

The distributions of the genotype and allele frequencies of the polymorphisms *AUTS2* rs6943555 and rs9886351 in both patients with alcohol dependence and control subjects are shown in Tables 1 and 2. The genotype distributions of these polymorphisms in both the alcohol dependence and control subjects were in Hardy–Weinberg equilibrium when tested using the χ^2 test (data not shown).

For the polymorphism *AUTS2* rs6943555, there were no significant differences in the genotype or allele frequencies between alcohol dependence and control subjects (total: genotype: $\chi^2(2) = 1.87$, $p = 0.392$, allele: $\chi^2(1) = 1.17$, $p = 0.280$; male: genotype: $\chi^2(2) = 0.154$, $p = 0.926$, allele: $\chi^2(1) = 0.396$, $p = 0.529$; female: genotype: $\chi^2(2) = 3.24$, $p = 0.197$, allele: $\chi^2(1) = 0.365$, $p = 0.546$). For the polymorphism *AUTS2* rs9886351, there were also no significant differences in the genotype or allele frequencies between alcohol dependence and control subjects (total: genotype: $\chi^2(2) = 1.69$, $p = 0.430$, allele: $\chi^2(1) = 1.70$, $p = 0.193$; male: genotype: $\chi^2(2) = 0.0783$, $p = 0.962$, allele: $\chi^2(1) = 0$, $p = 0.996$; female: genotype: $\chi^2(2) = 2.24$, $p = 0.326$, allele: $\chi^2(1) = 3.43$, $p = 0.0641$).

Table 1. Distributions of the genotype and allele frequencies of the polymorphisms *AUTS2* rs6943555 in alcohol dependence and control subjects

Subject	Alcohol dependence	Control	χ^2	df	*p* value
Total					
Genotype					
T/T	22 (34.38)	35 (46.7)	1.87	2	0.392
T/A	36 (56.25)	33 (44.0)			
A/A	6 (9.37)	7 (9.3)			
Allele					
T	80 (62.5)	103 (68.7)	1.17	1	0.280
A	48 (37.5)	47 (31.3)			
Male					
Genotype					
T/T	17 (34.0)	9 (39.13)	0.154	2	0.926
T/A	28 (56.0)	13 (56.52)			
A/A	5 (10.0)	1 (4.35)			
Allele					
T	62 (62.0)	31 (67.4)	0.396	1	0.529
A	38 (38.0)	15 (32.6)			
Female					
Genotype					
T/T	1 (14.3)	26 (50.0)	3.24	2	0.197
T/A	6 (85.7)	20 (38.5)			
A/A	0 (0)	6 (11.5)			
Allele					
T	8 (57.1)	72 (69.2)	0.365	1	0.546
A	6 (42.9)	32 (30.8)			

AUTS2, autism susceptibility candidate 2.
Figures in parentheses are percentages.

Table 2. Distributions of the genotype and allele frequencies of the polymorphisms *AUTS2* rs9886351 in alcohol dependence and control subjects

Subject	Alcohol dependence	Control	χ^2	df	*p* value
Total					
Genotype					
A/A	26 (40.6)	26 (34.7)	1.69	2	0.430
A/G	32 (50.0)	35 (46.6)			
G/G	6 (9.40)	14 (18.7)			
Allele					
A	84 (65.6)	87 (58.0)	1.70	1	0.193
G	44 (34.4)	63 (42.0)			
Male					
Genotype					
A/A	19 (38.0)	9 (39.13)	0.0783	2	0.962
A/G	25 (50.0)	11 (47.83)			
G/G	6 (12.0)	3 (13.04)			
Allele					
A	63 (63.0)	29 (63.0)	0	1	0.996
G	37 (37.0)	17 (37.0)			
Female					
Genotype					
A/A	5 (71.4)	17 (32.70)	2.24	2	0.326
A/G	2 (28.6)	24 (46.15)			
G/G	0 (0)	11 (21.15)			
Allele					
A	12 (85.7)	58 (55.8)	3.43	1	0.0641
G	2 (14.3)	46 (44.2)			

AUTS2, autism susceptibility candidate 2.
Figures in parentheses are percentages.

The frequencies of haplotypes consisting of the polymorphisms *AUTS2* rs6943555 and rs9886351 in both the patients with alcohol dependence and control subjects are shown in Table 3. The distributions of the A-A haplotype combinations were statistically significantly different between alcohol dependence and control subjects ($\chi^2(1) = 5.53$, $p = 0.0187$). The patients with alcohol dependence showed a significantly higher frequency of the A-A haplotype (rs6943555 and rs9886351) as compared with the control group (26.73% of patients, 15.03% of controls). The frequencies of other haplotypes did not show significant differences between alcohol dependence and control subjects.

In addition, the pairwise *D*' and r^2 values for the polymorphisms *AUTS2* rs6943555 and rs9886351 in this study were 0.033 and 0.001, respectively. These polymorphisms showed weak LD as to each other.

Table 3. Haplotype frequencies for the polymorphisms *AUTS2* rs6943555 and rs9886351 in alcohol dependence and control subjects

	Polymorphism		Frequency (%)			
Haplotype	rs6943555	rs9886351	Alcohol dependence	Control	χ^2	*p* value
1	A	G	11.86	16.31	1.04	0.308
2	T	G	22.35	25.69	0.395	0.530
3	A	A	26.73	15.03	5.53	0.0187*
4	T	A	39.06	42.97	0.410	0.522

AUTS2, autism susceptibility candidate 2.
The distributions of the A-A haplotype showed a statistically significant difference between alcohol dependence and control subjects.
*$p < 0.05$.

Discussion

In this study, we investigated the association of *AUTS2* gene polymorphisms and alcohol dependence in a Japanese population. The genotype and allele frequencies of the *AUTS2* gene polymorphisms observed in our healthy subjects were consistent with the genotype (rs6943555: $\chi^2(2) = 0.0494$, $p = 0.976$; rs9886351: $\chi^2(2) = 0.352$, $p = 0.839$) and allele (rs6943555: $\chi^2(1) = 0.248$, $p = 0.618$; rs9886351: $\chi^2(1) = 0.0928$, $p = 0.761$) frequencies observed in the HapMap (http://hapmap.ncbi.nlm.nih.gov/index.html.ja) Japanese population, respectively.

Our results showed that there were no significant differences in the genotype and allele frequencies of the polymorphisms *AUTS2* rs6943555 and rs9886351 between the patients with alcohol dependence and healthy control subjects. Recent studies have shown that the *AUTS2* gene polymorphisms, especially

rs6943555, are broadly associated with schizophrenia (7), suicide (8), and heroin dependence (10,11). All of these case-control reports stated that A/A homozygotes and/or the minor A allele of this polymorphism is a risk factor in such cases. Regarding the association of heroin dependence, Chen et al. measured the cDNA level of *AUTS2* in lymphoblastoid cell lines with heroin-dependent patients and control subjects, and they discovered that it was significantly reduced in the patient group compared with the control group (10). In addition, they found that the rs6943555 A/A genotype lowered the *AUTS2* mRNA level in lymphoblastoid cell lines compared with the T/T and T/A genotypes (10). For these findings, reduced *AUTS2* gene expression is likely to be a risk factor for the development of heroin dependence (10,11). On the other hand, Schumann et al. revealed that down-regulation of an *AUTS2* homolog induced reduced alcohol sensitivity in Drosophila (12). The low level of response (reduced sensitivity) to alcohol contribute to an increased risk for alcohol use disorders (10–12,16). Therefore, there is a possibility that reduced *AUTS2* gene expression induces an increasing risk of developing alcohol drinking disorders in humans (10,11). In this study, although no relationship was found between the polymorphism *AUTS2* rs6943555 alone and alcohol dependence, the haplotype A-A including the minor A allele of the rs6943555 is one of the factors that reduce the *AUTS2* gene expression in lymphoblastoid cell lines associated with the disease. Thus, the polymorphism rs6943555 might have affected more than a little to alcohol dependence, though not to the extent of heroin dependence.

Meanwhile, Schumann et al. reported that the rs6943555 A allele increases *AUTS2* gene expression in the prefrontal cortex of the human brain compared with the T allele (12). Furthermore, in genome-wide association study in Europeans, they also have revealed that the minor ancestral A allele of the rs6943555 was associated with 5.5% lower alcohol consumption (12). For these observations, we expected that the A allele of this polymorphism would be associated with protective effects against alcohol dependence, although no association was found between them in a Japanese population. In molecular biological research on genetic factors influencing alcohol drinking disorders, including alcohol dependence, when consistent results can not be observed between the various countries and populations, the differences in genetic factors as well as in environmental factors such as alcohol availability, people's thinking about alcohol, and social system constructed by law that affects alcohol drinking behavior, will need to be considered (1). Incidentally, there was a tendency towards a slightly lower rs6943555 minor A allele frequency (24%) of Europian subjects in Schumann's study (12) compared with those of Japanese subjects in our study (case: 37.5%, control: 31.3%). Thus, the genotype and allele frequencies of the polymorphism *AUTS2* rs6943555 might differ between races (10). The A/A genotype (9.3%) and A allele frequencies (31.3%) in our healthy subjects showed not much difference from that in Han Chinese subjects (A/A genotype: 8.31–13.0%; A allele: 29.3–35.8%) (7,10,11). On the other hand, in Polish Caucasian subjects the A/A genotype accounts for only 4.7% and the A allele 21% (8). The genotype and allele frequencies of the polymorphism *AUTS2* rs6943555 in Polish Caucasian subjects show a significant difference from that in the Japanese determined in this study (genotype: $\chi^2(2) = 7.69$, $p = 0.0213$; allele: $\chi^2(1) = 9.34$, $p = 0.00224$) (8). Therefore, if different results from those in our study on additional exploration of the polymorphism *AUTS2* rs6943555 and alcohol dependence are obtained, such differences in genotype and allele frequencies among races might be one of the reasons. However, because the number of healthy subjects in our study was smaller than that in Chojnicka's study (8) of Polish Caucasians, it will be necessary to take this point into consideration.

For the polymorphism rs9886351, several studies have concerned the association with schizophrenia and heroin dependence, but they failed to reveal significant associations with these diseases (7,11). Furthermore, no association was found between the polymorphism rs9886351 and alcohol dependence in this study. Although it is currently unknown whether the rs9886351 affects the function of *AUTS2*, it is unlikely that this polymorphism alone affects the development of alcohol dependence.

On the other hand, the frequencies of the *AUTS2* haplotypes were significantly different between the patients with alcohol dependence and healthy control subjects, and the rs6943555 and rs9886351 A-A haplotype was associated with alcohol dependence. In this study, we observed that two SNPs in all subjects did not exhibit strong LD. In an association study of *AUTS2* gene polymorphisms and schizophrenia, although Zhang et al. also revealed that the LD values between the polymorphisms rs6943555 and rs9886351 was low ($D' = 0.06$ and $r^2 = 0$), they suggested that calculation of the haplotype frequencies of these SNPs is not required (7). Additionally, Dang et al. investigated the correlation of heroin dependence and 21 SNPs in the *AUTS2* gene, and they compared the haplotype frequencies in heroin dependence and healthy control subjects focusing on five haplotype blocks (strong LD) (11). According to that report, the haplotypes

consisting of the polymorphisms rs6943555 and rs10251416 were significantly associated with heroin dependence (11). Conversely, in the presence of multiple susceptibility alleles, analyses based on haplotypes have advantages over analysis based on one SNP, particularly when the degree of LD between the SNPs is weak (17,18). Morris and Kaplan have suggested that the power advantage of haplotype analysis can be minimal or lost when there is strong correlation among SNPs (17). Furthermore, they also concluded that statistical methods based on haplotypes when the LD between SNPs is weak may be promising for identifying and locating disease genes in the presence of multiple susceptibility alleles with general features of complex disease genes (17). Haplotype analysis is thought to be meaningful not only when the LD between SNPs is strong but also when it is weak, and important factors that were not found on analysis to have strong correlation among SNPs might be detected with weak correlation among SNPs. Although the present study was exploratory research, the new finding that the A-A haplotype consisting of the polymorphisms rs6943555 and rs9886351 is significantly associated with alcohol dependence, may be one of the important findings in molecular biological studies for elucidation of genetic factors for alcohol dependence.

When performing analyses to examine the association between alcohol dependence and the *AUTS2* gene polymorphisms, we did not perform correction for multiple comparisons, such as Bonferroni correction. Several studies have indicated that Bonferroni adjustment would be too conservative for genetic association studies (19), and the likelihood of type II errors is increased by such adjustment, meaning that truly important differences may be deemed nonsignificant (20). Furthermore, when the sample size of a study is also small, there is a low possibility of committing a type II error (1). For the reasons stated above, for this exploratory study, we present results with no correction.

It must be considered that for the subjects in this study, there were several limitations and bias. First, the sample sizes of alcohol-dependent patients and control subjects in our study were small and consequently have low statistical power. Second, the alcohol-dependent subjects ($n = 64$) were mostly males ($n = 50$) and, contrary to this, in the healthy control subjects, the number of females ($n = 52$) was more than twice than that of males ($n = 23$). Third, there was a significant difference in the average ages ($p < 0.001$) of alcohol-dependent patients (57.34 ± 10.18) and healthy control subjects (35.36 ± 9.06). However, all of the subjects in this study were from a particular provincial population (i.e. Yamagata prefecture) in Japan. Because alcohol drinking behavior differs among populations, genetic factors for alcohol dependence can be hidden when various populations are mixed in a study (1). Furthermore, there is also a possibility that different results as to the frequency of a minor allele are obtained for different regions of the same country (1). Larger scale comparison of subjects of the same generation with no gender bias is needed in a further study to confirm our current results. Furthermore, because we could not obtain detailed clinical data on the alcohol-dependent patients, it will also be worth examining the association between the clinical characteristics of alcohol dependence such as tendency to violence and the age of onset.

In conclusion, our results suggested that the frequencies of the *AUTS2* haplotypes were significantly different between patients with alcohol dependence and healthy control subjects, and the A-A haplotype consisting of the polymorphisms rs6943555 and rs9886351 might be a risk factor for alcohol dependence in a Japanese population. However, the results of an exploratory study should be interpreted cautiously. If genes that are involved in the development of alcohol dependence are revealed in future studies, as a tailor-made medical procedure, early prevention and treatment based on features of the genetic background of individuals determined through genetic polymorphisms may be possible.

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Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the

relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Nihon Arukoru Yakubutsu Igakkai Zasshi
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Association between *N*-methyl-D-aspartate Receptor Subunit 2B Gene Polymorphisms and Alcohol Dependence in a Japanese Population

Shin NARITA[1], Eiji YOSHIHARA[1], Daisuke NISHIZAWA[2], Atsuko KAWAI[3], Kazutaka IKEDA[2] and Kazuhiko IWAHASHI[1,2,4]*

1) *Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara-shi, Kanagawa 252-5201, Japan*
2) *Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan*
3) *Koutokukai Total Health Clinic, 948-1 Kunugiduka, Nanyo, Yamagata 999-2221, Japan*
4) *Health Administration Center, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara-shi, Kanagawa 252-5201, Japan*

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Summary

The *N*-methyl-D-aspartate (NMDA) receptor, especially the NMDA receptor subunit 2B (GluN2B), plays an important role in regulation of alcohol action. Several studies have revealed the relationship between GluN2B gene (*GRIN2B*) polymorphism and alcohol dependence, but many of them mainly focused on the polymorphism rs1806201. Thus it is necessary to perform multifaceted analysis regarding its relevance using other meaningful polymorphisms in various populations. Therefore, we examined whether seven *GRIN2B* polymorphisms (including rs1806201) are related to alcohol dependence in a Japanese population. We compared the genotype and allele frequencies of seven *GRIN2B* polymorphisms between patients with alcohol dependence and healthy control subjects living in a Japanese provincial prefecture. The *GRIN2B* genotypes were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. There were significant differences in the genotype (P = 0.019) and allele (P = 0.044) frequencies of the rs1805247 and rs1805502 polymorphisms (complete linkage disequilibrium in the analyzed population) between alcohol-dependent and control subjects. On the other hand, other *GRIN2B* polymorphisms were not significantly asso-

* Corresponding author: Kazuhiko Iwahashi, Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara-shi, Kanagawa 252-5201, Japan
mail: iwahashi@azabu-u.ac.jp

ciated with alcohol dependence. In addition, haplotype analysis indicated a marginally significant association between haplotype C-C-C (rs1806201-rs1805247-rs1805502) in the *GRIN2B* haplotype block and alcohol dependence. These results suggest that the *GRIN2B* polymorphisms and haplotype might affect the vulnerability to alcohol dependence, but the results of this exploratory study should be interpreted cautiously. Further studies with more subjects considering possible bias are needed to confirm the reproducibility of the results in this study.

Key words: NMDA receptor subunit 2B, polymorphism, haplotype, alcohol dependence

Introduction

It is generally known that there are individual differences in vulnerability to and severity of substance dependence such as alcohol dependence, and in responsiveness to substances causing such diseases[1]. Both environmental and genetic factors have complicated effects on the pathogenesis of alcohol dependence[1], and a previous review indicated that the heritability of alcohol dependence accounts for approximately close to 60%[2]. So far, various genetic polymorphisms that contribute to individual differences in vulnerability to alcohol dependence have been identified[1].

The *N*-methyl-D-aspartate (NMDA) receptor is a kind of glutamate-gated ion channel[3], and is one of the primary targets of ethanol in the brain[4]. Recent review studies have shown that the NMDA receptor plays some roles in regulation of alcohol action and the development of alcohol dependence[5,6,7]. In particular, the NMDA receptor subunit 2B (GluN2B) of the NMDA receptor is affected by chronic ethanol exposure, and its expression increases[8,9]. Devaud and Morrow discovered that GluN2B levels are significantly increased in the cerebral cortex of ethanol-dependent male and female rats[10]. Significant hyperexcitability that would be mediated by a hyperglutamatergic state, e.g., up-regulation of GluN2B-containing NMDA receptor activity, leads to alcohol dependence and withdrawal[11]. A recent animal study revealed that an effect of ethanol sensitivity in the bed nucleus of the stria terminalis is dependent on GluN2B-containing NMDA receptors, and that this subunit is an important factor in regulation of alcohol action[12]. This finding supports the result that treatment with the selective GluN2B-containing NMDA receptor antagonist ifenprodil significantly suppressed the expression of ethanol withdrawal signs in mice[13]. Thus, the GluN2B, which is highly sensitive to ethanol, has been identified as a potential pharmacological target for the treatment of alcohol dependence[14]. From these observations, it is suggested that the GluN2B may affect the pathogenesis of alcohol dependence.

The GluN2B gene (*GRIN2B*) is located on chromosome 12p12 and consists of 13 exons, the coding sequence being encompassed by exons 2 to 13[15,16]. Thus far, several case-control studies of the relationship between the *GRIN2B* polymorphism and alcohol dependence have been reported[17,18,19,20,21,22], and many of those studies mainly focused on the polymorphism rs1806201 (2664C/T), which is located on exon 13 encoding the carboxyl-terminal intracellular domain of the GluN2B[16,23], in a particular race. However, since *GRIN2B* has a large gene size and shows

considerable genetic variability, it is necessary to examine the relevance of *GRIN2B* polymorphisms and alcohol dependence using a wide variety of population models and other polymorphisms, thereby enabling more detailed analysis[21]. Therefore, in addition to the polymorphism rs1806201, we focused on six meaningful single nucleotide polymorphisms (SNPs): rs3764028 (-421C/A, promoter region), rs1019385 (-200T/G, 5'-upstream region), rs7301328 (366C/G, exon 2), rs1805247 (4197T/C, exon 13), rs890 (5072T/G, 3'-UTR), and rs1805502 (5988T/C, 3'-UTR), of the *GRIN2B*. Previous case-control studies have revealed that these polymorphisms are associated with various neuropsychiatric disorders, e.g., Alzheimer's disease[24], schizophrenia[16,25,26], obsessive-compulsive disorder[27], major depression (treatment resistant)[28], and bipolar disorder[29].

In the present study, to elucidate genetic risk factors for alcohol dependence, we compared the genotype and allele frequencies of multiple *GRIN2B* polymorphisms between patients with alcohol dependence and healthy control subjects for the first time in a Japanese population. Furthermore, we also examined whether the *GRIN2B* haplotypes are related to alcohol dependence.

Materials and Methods

The subjects in this study consisted of 64 patients (male: 50, female: 7, not available: 7; mean age ± SD: 57.34 ± 10.18 years) with a diagnosis of alcohol dependence according to DSM-IV criteria and 75 unrelated healthy people (male: 23, female: 52; mean age ± SD: 35.36 ± 9.06 years). Blood samples were collected from the subjects at Koutokukai Sato Hospital Group, and all subjects provided written informed consent for genetic studies. All of the patients and control subjects lived in Yamagata prefecture in Japan. The study was approved by the ethics committees of the Tokyo Metropolitan Institute of Medical Science (13-29) and Azabu University (0168).

Seven *GRIN2B* polymorphisms were genotyped by means of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method[16,24,30,31]. The primer sequences and restriction enzymes used for genotyping are shown in Table 1. The PCR cycling conditions were: 95℃ for 10 min, 35 cycles of 95℃ for 30 s, 56℃ (rs1806201), 60℃ (rs3764028, rs7301328, rs1805247, rs890 and rs1805502) or 63℃ (rs1019385) for 30 s, and 72℃ for 30 s, with final extension at 72℃ for 7 min. The PCR products were digested with a restrictive enzyme, and the digested products were subjected to electrophoresis on a polyacrylamide gel and then visualized using the ethidium bromide staining method.

Hardy-Weinberg disequilibrium was assessed using the χ^2 test. Because there was only a small number of subjects of each gender, the subjects of both genders were combined for statistical analyses in this study. Statistical differences in the genotype and allele frequencies of the *GRIN2B* polymorphism between the patients with alcohol dependence and healthy control subjects were assessed using the χ^2 test or Yates' correction. In addition, linkage disequilibrium (LD) coefficients (D' and r^2) and haplotype frequencies were calculated with gPLINK 2.050 (http://zzz.bwh.harvard.edu/plink/index.shtml) and Haploview 4.2 (http://www.broad.

Table 1 Primer sequences and restriction enzymes for PCR-RFLP method.

SNP	Primer sequence		PCR product size (bp)	Restriction enzyme
rs3764028	Forward: CGCTCTCCGTCGGTGCTGTT	Reverse: CTGGGGAAGTGGGGTGGTAACG[a]	115	*Tai*I
rs1019385	Forward: CTGGGAGCAGAAGCAGTATC	Reverse: ACACACACAGACACACAGGCAC[a]	98	*BshN*I
rs7301328	Forward: TCAGCACAGACTCTCACCTC[a]	Reverse: CCTCAGCACAAACCCTCAGG	112	*Taq*I
rs1806201	Forward: AGACTATTCGCTTCATGC	Reverse: GTGTGTTGTTCATGGCTG[a]	210	*Pst*I
rs1805247	Forward: CGGACATCACCACCACAACA	Reverse: TGAAAGCCCTGGGGTTTTTG	320	*Nco*I
rs890	Forward: AGTGAAGCTGGGAGAACCA	Reverse: CTCTGCCACCAATGACCTTT	301	*Psu*I
rs1805502	Forward: CCCCCAAAACTGATTACAAC	Reverse: TGTTAAGTGAAGGGAGCATC	353	*Aci*I

[a]Mismatch primer.

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism,

SNP: single nucleotide polymorphism.

mit.edu/mpg/haploview/index.php)[32,33]. Statistical significance was defined as $P < 0.05$.

Results

The distributions of the genotype and allele frequencies of the *GRIN2B* polymorphisms in both patients with alcohol dependence and healthy control subjects are shown in Table 2. The genotype distributions of six polymorphisms in the control subjects were in Hardy-Weinberg equilibrium when tested using the χ^2 test (rs3764028: χ^2 (1) = 0.133, P = 0.715; rs1019385: χ^2 (1) = 0.404, P = 0.525; rs7301328: χ^2 (1) = 0.383, P = 0.536; rs1806201: χ^2 (1) = 0.164, P = 0.686; rs1805247: χ^2 (1) = 0.042, P = 0.838; rs1805502: χ^2 (1) = 0.042, P = 0.838). However, the observed genotype frequencies of the rs890 polymorphism in the control subjects were not in Hardy-Weinberg equilibrium (χ^2 (1) = 4.297, P = 0.038). Therefore, this polymorphism was excluded from the subsequent discussion.

For the rs3764028, rs1019385, rs7301328 and rs1806201 polymorphisms, there were no significant differences in the genotype or allele frequency between alcohol-dependent and control subjects (rs3764028: genotype: χ^2 (2) = 0.058, P = 0.971, allele: χ^2 (1) = 0.027, P = 0.871; rs1019385: genotype: χ^2 (2) = 0.570, P = 0.752, allele: χ^2 (1) = 0.098, P = 0.755; rs7301328: genotype: χ^2 (2) = 1.265, P = 0.531, allele: χ^2 (1) = 0.891, P = 0.345; rs1806201: genotype: χ^2 (2) = 1.489, P = 0.475, allele: χ^2 (1) = 1.403, P = 0.236). For the rs1805247 and rs1805502 polymorphisms, there were significant differences in the genotype and allele frequency between alcohol-dependent and control subjects (rs1805247: genotype: χ^2 (2) = 7.876, P = 0.019, allele: χ^2 (1) = 4.041, P = 0.044; rs1805502: genotype: χ^2 (2) = 7.876, P = 0.019, allele: χ^2 (1) = 4.041, P = 0.044). On analysis with the subjects divided into two groups regarding these polymorphisms, i.e., T/T

Table 2 Distributions of the genotype and allele frequencies of the *GRIN2B* polymorphisms in alcohol-dependent and control subjects.

SNP	*n*	Genotype (%)			*P*-value	Allele (%)		*P*-value
rs3764028		C/C	C/A	A/A		C	A	
Alcohol dependence	64	21 (32.81)	31 (48.44)	12 (18.75)	0.971	73 (57.0)	55 (43.0)	0.871
Control	75	26 (34.7)	35 (46.7)	14 (18.6)		87 (58.0)	63 (42.0)	
rs1019385		T/T	T/G	G/G		T	G	
Alcohol dependence	64	19 (29.69)	33 (51.56)	12 (18.75)	0.752	71 (55.5)	57 (44.5)	0.755
Control	75	26 (34.7)	34 (45.3)	15 (20.0)		86 (57.3)	64 (42.7)	
rs7301328		C/C	C/G	G/G		C	G	
Alcohol dependence	64	15 (23.44)	31 (48.44)	18 (28.12)	0.531	61 (47.7)	67 (52.3)	0.345
Control	75	20 (26.7)	40 (53.3)	15 (20.0)		80 (53.3)	70 (46.7)	
rs1806201		C/C	C/T	T/T		C	T	
Alcohol dependence	64	17 (26.6)	34 (53.1)	13 (20.3)	0.475	68 (53.1)	60 (46.9)	0.236
Control	75	15 (20.0)	39 (52.0)	21 (28.0)		69 (46.0)	81 (54.0)	
rs1805247		T/T	T/C	C/C		T	C	
Alcohol dependence	64	29 (45.3)	35 (54.7)	0 (0)	0.019*	93 (72.7)	35 (27.3)	0.044*
Control	75	51 (68.0)	22 (29.3)	2 (2.7)		124 (82.7)	26 (17.3)	
rs890		T/T	T/G	G/G		T	G	
Alcohol dependence	64	41 (64.06)	22 (34.38)	1 (1.56)	0.240	104 (81.25)	24 (18.75)	0.423
Control	75	48 (64.0)	20 (26.7)	7 (9.3)		116 (77.3)	34 (22.7)	
rs1805502		T/T	T/C	C/C		T	C	
Alcohol dependence	64	29 (45.3)	35 (54.7)	0 (0)	0.019*	93 (72.7)	35 (27.3)	0.044*
Control	75	51 (68.0)	22 (29.3)	2 (2.7)		124 (82.7)	26 (17.3)	

*P <0.05, *GRIN2B*: N-methyl-D-aspartate receptor subunit 2B gene,
SNP: single nucleotide polymorphism.

versus T/C + C/C for rs1805247 and rs1805502, there was also a significant difference in the frequency between alcohol-dependent and control subjects (χ^2 (1) = 7.276, P = 0.007).

The LD pattern for six *GRIN2B* polymorphisms in the analyzed population is shown in Fig 1. Haploview indicated that the rs1805247 and rs1805502 polymorphisms exhibited complete LD (D' = 1.000, r^2 = 1.000), and haplotype block 1 composed of the rs1806201, rs1805247, and rs1805502 polymorphisms was detected. In addition, the rs3764028 and rs1019385 polymorphisms showed strong LD (D' = 1.000, r^2 = 0.957) and haplotype block structure (block 2). Therefore, we performed haplotype analysis for each block consisting of two- or three-markers. The haplotype frequency of two blocks in both patients with alcohol dependence and healthy control subjects is shown in Table 3. The three observed haplotype frequencies in block 1 (rs1806201-rs1805247-rs1805502) indicated no statistically significant difference between alcohol-dependent and control subjects, but the alcohol-dependent group showed a marginally significant higher frequency of haplotype C-C-C as compared with the control group (χ^2 (1) = 3.703, P = 0.054; 27.05% of patients, 17.22% of controls). On the other hand, none of the haplo-

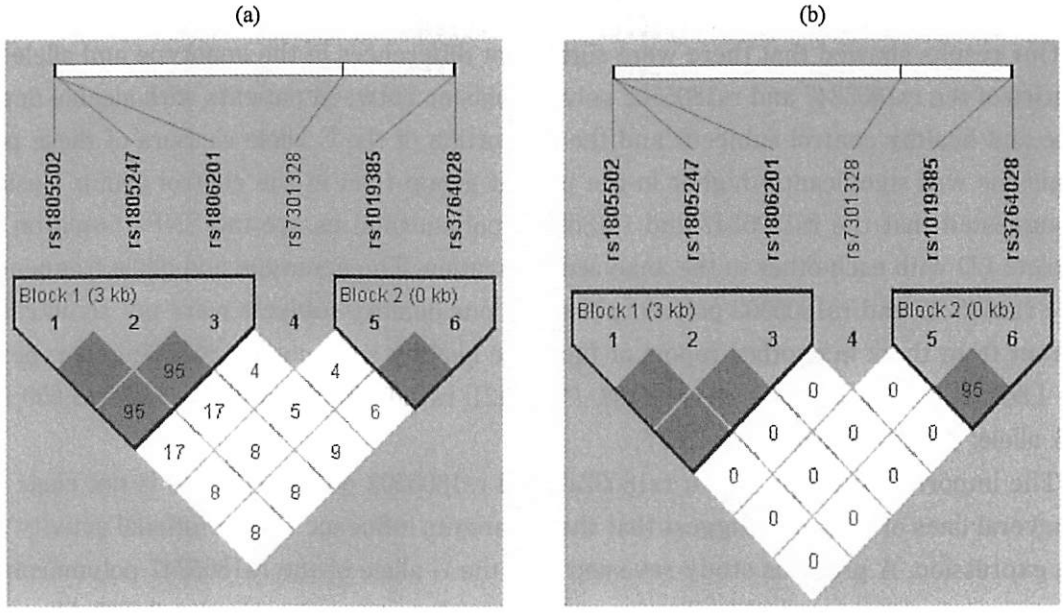

Fig 1 Linkage disequilibrium (LD) map of six SNPs at the *GRIN2B* locus in the analyzed population.
Pairwise LD between SNPs measured using the D' (a) and r^2 (b) values.
The figures in the squares are rounded percentages of the calculated D' and r^2 values (e.g., a white square with a "9" indicates $D' = 0.091$).
SNPs: single nucleotide polymorphisms, *GRIN2B*: *N*-methyl-D-aspartate receptor subunit 2B gene.

Table 3 The *GRIN2B* haplotype of each block frequencies in alcohol-dependent and control subjects.

Block	Haplotype	Frequency			χ^2	*P*-value
		Alcohol dependence	/	Control		
1	C - C - C	0.271	/	0.172	3.703	0.054 †
	C - T - T	0.266	/	0.288	0.166	0.684
	T - T - T	0.464	/	0.539	1.476	0.224
2	A - G	0.412	/	0.420	0.016	0.900
	C - G	0.018	/	0.007	0.682	0.409
	C - T	0.570	/	0.573	0.003	0.959

Block 1: rs1806201-rs1805247-rs1805502, Block 2: rs3764028-rs1019385.
† A significant trend. *GRIN2B*: *N*-methyl-D-aspartate receptor subunit 2B gene.

types detected in block 2 (rs3764028-rs1019385) showed a meaningful association with alcohol dependence.

Discussion

In this study, we investigated the association of *GRIN2B* polymorphisms and alcohol depen-

dence in a Japanese population.

Our results showed that there were significant differences in the genotype and allele frequencies of the rs1805247 and rs1805502 polymorphisms between patients with alcohol dependence and healthy control subjects, and the proportion of the C allele carriers of these polymorphisms was significantly higher in the patient group than in the control group. Besides, we suggested that the rs1805247 and rs1805502 polymorphisms are tag SNPs based on the complete LD with each other in the analyzed population. The genotype and allele frequencies of the rs1805247 and rs1805502 polymorphisms in our healthy subjects were not significantly different from those in another report of Japanese healthy subjects (rs1805247: genotype: χ^2 (2) = 1.868, P = 0.393, allele: χ^2 (1) = 2.408, P = 0.121; rs1805502: genotype: χ^2 (2) = 1.650, P = 0.438, allele: χ^2 (1) = 2.296, P = 0.130)[16].

The important function of the rs1805247 and rs1805502 polymorphisms is not clear yet, but several lines of evidence suggest that these variants influence transcriptional activity and gene expression. A previous study revealed that the G allele of the rs1805247 polymorphism produces greater intracortical facilitation and greater long-term potentiation (LTP) -like cortical plasticity after intermittent theta-burst stimulation, and that individuals carrying this allele are likely to have enhanced glutamate NMDA receptor function[34]. It was shown that chronic ethanol exposure enhances LTP in the bed nucleus of the stria terminalis via paradoxical extrasynaptic NMDA receptor involvement[12]. That is, the alcohol-dependent state is associated with increased sensitivity (i.e., up-regulation) of NMDA receptors including GluN2B[11,35], and a mutant allele of the rs1805247 polymorphism may be one of the factors for promoting the formation of this system. Meanwhile, the 3'-untranslated regions (3'-UTRs) in which the rs1805502 polymorphism is located play important roles in translation, localization and stability of messenger RNA (mRNA)[36]. A microRNA binding site that induces translational repression or transcript degradation usually lies in 3'-UTRs[37]. Mammalian brain tissue, particularly *GRIN2B*, exhibits extensive lengthening of 3'-UTRs, and it is suggested that these extensions contain many microRNA binding sites and components involved in post-transcriptional regulation[38]. A postmortem brain study recently also revealed that the C allele of the rs1805502 polymorphism is associated with significantly reduced NMDA receptor subunit 1 mRNA and protein expression in schizophrenia[39]. Although its functions in alcohol dependence have not been verified, the rs1805502 polymorphism strongly correlated with rs1805247, which may affect vulnerability to the disease.

On the other hand, Kim et al. reported that the frequency of *GRIN2B* polymorphism rs1806201, but not those of rs1805247 and rs1805502, showed significant differences between alcohol-dependent patients and healthy subjects in a Korean population[21]. Several studies other than that of Kim et al. have concerned the relationship between the rs1806201 polymorphism and alcohol dependence, but failed to find any significant association[17,20], and showed a possible association with subgroups of alcohol-dependent patients[18,22]. The different findings in these previous reports may be due to methodological differences such as sample size, selected sample populations, and statistical procedure. In addition, it was observed that the frequency of the *GRIN2B* rs1806201 polymorphism differed among races. For instance, there was a lower

T allele frequency of the rs1806201 polymorphism in healthy Caucasian subjects (25.5%) compared with that in the Japanese healthy subjects examined in this study (54.0%)[20]. The frequencies of three polymorphisms in healthy Korean controls in Kim's study (rs1805247: T = 78%, C = 22%; rs1805502: T = 79%, C = 21%; rs1806201: C = 50%, T = 50%) are not greatly different from those in our Japanese healthy subjects (rs1805247 and rs1805502: T = 83%, C = 17%; rs1806201: C = 46%, T = 54%), while both studies gave conflicting results as to the association between the three *GRIN2B* polymorphisms and alcohol dependence.[21] As background to this phenomenon, even in the same East Asian region, different environmental factors in various countries and populations that include alcohol availability and social systems such as law and tradition may also affect alcohol drinking behavior[40]. Indeed, in Caucasians in the European region, Tadic et al. reported that the rs1806201 polymorphism is not associated with alcohol dependence, including clinical characteristics[20], whereas Wernicke et al. showed that the T allele of this polymorphism is significantly reduced in alcohol-dependent patients with Cloninger type 2 and early onset compared with control subjects[18]. Thus, multilateral information aggregation under various conditions seems necessary in molecular biological research for elucidation of alcohol dependence-related genetic polymorphisms. Kim et al. nevertheless revealed that not only the rs1806201 polymorphism but also the rs1805247-rs1805502 haplotypes are associated with alcohol dependence[21]. Importantly, they also showed that the C-C-C (rs1806201-rs1805247-rs1805502) haplotype, but not the T-T-T and C-T-T ones, is one of the risk factors for this disease[21]. The new finding in this study is that the C alleles of rs1805247 and rs1805502 polymorphisms are part of the haplotype that may have a more-or-less important role in genetic susceptibility to alcohol dependence.

As a matter of caution, the genotype distributions of the rs1805247 and rs1805502 polymorphisms deviated significantly from Hardy-Weinberg equilibrium in the alcohol-dependent patient group. The genotyping call rate for assessments of these polymorphisms was 100% in both case and control groups. This deviation is probably due to the small sample size, since the present study was exploratory research. However, in our previous study with the same sample, we confirmed that the genotype distributions of other gene polymorphisms in the alcohol-dependent subjects were in Hardy-Weinberg equilibrium[41]. Exclusion of markers indicating deviation from Hardy-Weinberg equilibrium has the potential to discard valuable information for identification of disease susceptibility polymorphisms in case-control studies, and significant Hardy-Weinberg disequilibrium in the cases but not in the controls suggests the actual association of genetic polymorphisms and disease in appropriate situations[42,43]. In any case, it is necessary to confirm with other alcoholic populations whether our findings are supported by additional studies.

The rs3764028 and rs1019385 polymorphisms have been demonstrated to affect transcriptional activity of the *GRIN2B*[24,25]. Previous case-control studies revealed that these polymorphisms are significantly related to several neuropsychiatric disorders such as Alzheimer's disease[24], schizophrenia[25], and obsessive-compulsive disorder[27]. However, our study suggested that the rs3764028 and rs1019385 polymorphisms are unlikely to affect the pathogenesis pathway for alcohol dependence. Foley et al. have reported the relationship between the

rs7301328 polymorphism and alcohol dependence in Caucasians, but they failed to reveal significant associations with the disease[19].

In the process of analysing the relationship between the *GRIN2B* polymorphisms and alcohol dependence, we did not perform correction for multiple comparisons, such as the Bonferroni correction. Previous studies have suggested that Bonferroni correction would be overly conservative, especially when there is high correlation (LD) between SNPs, in genetic case-control association studies[44], and such correction is likely to inflate the risk for type II error[45]. Moreover, a small sample size also increases the likelihood of committing a type II error[46]. For the reasons mentioned above, this exploratory study presented results with no correction, and a *P*-value slightly exceeding 0.05 was evaluated as a borderline significant trend.

It must be considered that for the subjects in this study there were several limitations and biases. First, the sample sizes of patients with alcohol dependence and healthy control subjects were small and consequently have low statistical power. Second, there were differences in the numbers of each gender and age between alcohol-dependent patients and healthy subject groups. Therefore, further large-scale studies involving subjects of the same generation with no gender bias are needed to confirm our current results. Furthermore, it will also be worth examining the effect of *GRIN2B* polymorphisms on the clinical characteristics of alcohol dependence. On the other hand, all of the subjects in this study were from a particular population (i.e., Yamagata prefecture) in Japan. Because alcohol drinking behavior differs among populations, genetic factors for alcohol dependence may not be detectable when various populations are mixed in a study[40]. In addition, there is also a possibility that different results will be obtained in different regions of the same country[40].

In conclusion, our results suggested that the variant C allele of the rs1805247 and rs1805502 polymorphisms and the haplotype C-C-C based on rs1806201-rs1805247-rs1805502 in the *GRIN2B* might be risk factors for alcohol dependence in a Japanese population. However, the results of this exploratory study should be interpreted cautiously. If genes that affect the vulnerability to alcohol dependence are revealed in future studies, early prevention and treatment based on features of the genetic background of individuals determined through genetic polymorphisms may be possible as a "tailor-made" medical procedure.

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Statement of interest

None.

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No Association between the Polymorphism rs6943555 in the *AUTS2* Gene and Personality Traits in Japanese University Students

Shin Narita[1], Kazutaka Ikeda[2], Daisuke Nishizawa[2], Eiji Yoshihara[1], Maki Numajiri[1], Yuuya Onozawa[1], Nobuyo Ohtani[3], and Kazuhiko Iwahashi[1,2,4] ✉

[1]Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, Kanagawa, Japan
[2]Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
[3]Laboratory of Effective Animals for Human Health, Azabu University, Kanagawa, Japan
[4]Health Administration Center, Azabu University, Kanagawa, Japan

Objective The autism susceptibility candidate 2 (*AUTS2*) gene has been implicated in multiple neurological disorders. Several recent studies have revealed that the polymorphism rs6943555 in the *AUTS2* gene is broadly associated with human mental function and behavior. Therefore, in the present study we investigated whether the polymorphism rs6943555 is associated with human personality traits in Japanese university students. In addition, our previous study reported that the *AUTS2* rs6943555-rs9886351 haplotype is associated with alcohol dependence. As a preliminary analysis, we also examined whether the *AUTS2* haplotypes are related to personality traits.

Methods After written informed consent had been obtained from the participants, two *AUTS2* polymorphisms were analyzed, and personality was assessed using the Temperament and Character Inventory (TCI) in 190 university students. In addition, in order to exclude the influence of the results for students with mental health problems, we gave the Patient Health Questionnaire-9 (PHQ-9) to all subjects.

Results In all the subjects, there was a main effect of the polymorphism rs6943555 genotype on reward dependence (p=0.038) and cooperativeness (p=0.031), although the significance was lost on Bonferroni correction. Similarly, on analysis that excluded the subjects with PHQ-9 scores≥10, no significant association with any TCI dimension score among the rs6943555 genotypes was seen. There was no effect of the rs6943555-rs9886351 haplotypes on the TCI dimension scores.

Conclusion This study suggests that the polymorphism *AUTS2* rs6943555 is not associated with personality traits. Further large-scale studies with more subjects using other self-report questionnaires are needed. **Psychiatry Investig 2017;14(5):681-686**

Key Words Autism susceptibility candidate 2, Gene polymorphism, Personality traits, Temperament and Character Inventory, Patient Health Questionnaire-9.

INTRODUCTION

Twin studies on heritability of personality traits demonstrated that personality traits measured by means of self-report questionnaires show moderate heritability.[1] Genetic factors as well as environmental ones also contribute to the determination of human personality traits.[2] According to Bouchard's report, approximately two-thirds of personality traits are estimated to be due to genetic influence.[3] Some genes related to the characterization of personality traits are known to affect a wide range of human behavior including abnormal types, and polymorphisms of these same genes are associated with some disorders such as attention deficit hyperactivity disorder, alcohol and heroin dependence, autism, and schizophrenia.[1] Therefore, these genes are thought to regulate aspects of impulsiveness and attention-process that are common to a normal personality as well as disturbances reflected in such diverse disorders.[1]

The autism susceptibility candidate 2 (*AUTS2*) gene has been implicated in multiple neurological disorders including au-

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✉ **Correspondence:** Kazuhiko Iwahashi, MD, PhD
Laboratory of Physiology (Project of Neurophysiology), the Graduate School of Environmental Health Sciences, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara-shi, Kanagawa 252-5201, Japan
Tel: +81-42-769-1930, **Fax:** +81-42-769-1930, **E-mail:** iwahashi@azabu-u.ac.jp

tism,[4,5] and others such as attention deficit hyperactivity disorder,[6] schizophrenia,[7] bipolar disorder.[8] A very recent study revealed that *AUTS2* is present not only in nuclei but also in the cytoplasm and neurites, especially at the growth cones, and that it is involved in the regulation of cortical neuronal migration and neuritogenesis in the developing brain.[9] Therefore, these functional abnormalities caused by *AUTS2* gene mutations in the brain development process, as a common base, might induce the pathogeneses of a variety of psychiatric disorders.[9] Interestingly, several recent studies have shown that the polymorphism rs6943555 in the *AUTS2* gene is associated with schizophrenia,[10] heroin dependence,[11,12] suicide under the influenceof ethanol,[13] and alcohol consumption.[14] These findings suggest that *AUTS2*, especially the polymorphism rs6943555, might be involved in human mental function and behavior, and it is predicted to be one of the important factors affecting human personality traits.

The *AUTS2* gene is located on chromosome 7q11.22 and consists of 19 exons, the first 6 exons being separated by very large introns and the last 13 exons being close.[12] One of these common single nucleotide polymorphisms (SNPs) in the *AUTS2* gene is rs6943555, which comprises a single nucleotide change of T to A in intron 4.[12] Schumann et al.[14] reported that the A allele of rs6943555 significantly increases *AUTS2* gene expression in the prefrontal cortex of the human brain compared with the T allele. Meanwhile, Chen et al.[11] found that subjects with the rs6943555 A/A genotype exhibit a significantly lower *AUTS2* mRNA level in a lymphoblastoid cell lines (LCL) compared to subjects with the T/T and T/A genotypes.[11] Although the expression of the *AUTS2* gene might not be equal between brain tissue and LCL, the cause of this inconsistency is needed for further research.[11] However, in any event, these studies suggest that the rs6943555 variants may influence transcriptional activity and expression of the *AUTS2* gene.

Cloninger proposed that the three heritable dimensions of personality comprise novelty seeking, harm avoidance, and reward dependence.[15] In subsequent research, the Temperament and Character Inventory (TCI), which is one of the self-report questionnaires, and has four temperament dimensions (novelty seeking, harm avoidance, reward dependence, and persistence) and three character dimensions (self-directedness, cooperativeness, and self-transcendence), was developed to assess the personality traits of individual.[16] These dimensions are assumed to be as follows: the temperament traits are moderately heritable and stable throughout life, and the character traits are weakly heritable and moderately influenced by social learning.[16]

To our knowledge, a study on the relationship between *AUTS2* gene polymorphisms and personality traits has not yet been reported. In the present study, we investigated whether the polymorphism rs6943555 in the *AUTS2* gene is associated with human personality traits, as assessed by the TCI in Japanese university students. In addition, our recent study reported that the *AUTS2* haplotype consisting of the polymorphisms rs6943555 and rs9886351 might affect the pathogenesis of alcohol dependence.[17] Therefore, as a preliminary analysis, we also examined whether the *AUTS2* haplotypes are related to personality traits.

METHODS

Subjects

The participants comprised 190 volunteers (male: 51; female: 139). In order to rule out confounding factors such as age and general intelligence level differences, all candidates for this research consisted of students in Azabu University, Japan. The mean age was 20.46±1.15 (mean±SD) years (male: 20.75±1.45 years; female: 20.36±1.00 years). The study was approved by the ethics committee of Azabu University, Japan (0648). After obtaining written informed consent, blood samples were obtained from all the subjects. In addition, we performed the Japanese versions of the TCI and Patient Health Questionnaire-9 (PHQ-9, 2013 NCNP version) for all subjects. The shortened version of the TCI is used to assess personality traits by means of a questionnaire comprising 125 items with four possible answers.[18] The 4-point answer scale for each item comprises 1 (strongly disagree) to 4 (strongly agree). In addition, although the subjects in our study were ostensibly healthy students, it has been reported that depressive symptoms are accounted for the high frequency among the mental disorders found in university students.[19] Therefore, in order to exclude the influence on the results of students with mental health problems, the PHQ-9 was also performed. The PHQ-9 is a self-report questionnaire based on the diagnosis of Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) depressive disorders, and consists of nine questions related to the depression module of the Patient Health Questionnaire (PHQ) that is used assess eight diagnoses (major depressive disorder, panic disorder, other anxiety disorder, bulimia nervosa, other depressive disorder, probable alcohol abuse/dependence, probable somatoform disorder, binge eating disorder).[20,21] Each of the nine questions of the PHQ-9 is scored from 0 (not at all) to 3 (nearly every day), therefore the PHQ-9 total score ranges from 0 to 27.[22] The validity and reliability of the Japanese version of the TCI and PHQ-9 has already been confirmed in the Japanese population.[18,23]

DNA analysis

We performed extraction and purification of genomic DNA by the phenol/chloroform method. The polymorphisms *AUTS2* rs6943555 and rs9886351 were genotyped by means of poly-

merase chain reaction-restriction fragment length polymorphism (PCR-RFLP) according to the method of Zhang et al.[10] In regard to the polymorphism rs9886351, the 188 subjects of genotyping has been completed. Genomic DNA was amplified with the following primers: rs6943555 (forward: 5'-TGG GTG TTG GAA GAG TTT TGA-3', reverse: 5'-ATA CAG TAT ACA TAA ACA TTG GAA AAG AGG GAA-3') and rs9886351 (forward: 5'-GGT GGA AAA TAA GCC AGT ATG C-3', reverse: 5'-TAG GAA AAT GGA TTA AAC GTA GGA G-3'). The PCR product (196 bp for rs6943555 and 221 bp for rs9886351) was digested with a restrictive enzyme, *Hinf* I (New England Biolabs, Tokyo, Japan), and the digested products were subjected to electrophoresis on 5% polyacrylamide gels and visualized using the ethidium bromide staining method. Genotypes were determined according to fragment sizes: T/T=196 bp, T/A=196 bp+165 bp+34 bp, A/A=165 bp+34 bp, and rs9886351: A/A= 221 bp, A/G=221 bp+198 bp+26 bp, G/G= 198 bp+26 bp.

Statistical analyses

The Hardy-Weinberg disequilibrium was assessed using a chi-square test. First, in all the subjects, we compared the TCI dimension scores among the rs6943555 genotypes by performing statistical analysis using two-way analysis of variance with genotypes and gender as independent variable, and with the TCI dimension scores as dependent variable. Second, because a PHQ-9 score of 10 or higher is the threshold for major depression,[21,24,25] we excluded the subjects with this criterion from the analysis. Furthermore, as a preliminary analysis, we analyzedthe effect of the haplotypes consisting of the polymorphisms rs6943555 and rs9886351 on the TCI dimension scores in the subjects with PHQ-9 scores <10. Statistical analyses were performed using SPSS 12.0J for Windows. In addition, linkage disequilibrium (LD) coefficients (*D*' and r^2) and haplotype effects were calculated with gPLINK 2.050 (http://zzz.bwh.harvard.edu/plink/index.shtml) and Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview/index.php).[26,27] In order to consider multiple issues, p-values were adjusted by means of Bonferroni correction. Statistical significance was defined as $p<0.05$.

RESULTS

The genotype frequencies of the polymorphisms *AUTS2* rs6943555 and rs9886351 were as follows: rs6943555 (T/T type: 80, T/A type: 88, A/A type: 22) and rs9886351 (A/A type: 57, A/G type: 95, G/G type: 36). The rs6943555 and rs9886351 genotypes distribution was in Hardy-Weinberg equilibrium (rs6943555: χ^2 (1)=0.0879, p=0.767; rs9886351: χ^2 (1)=0.103, p=0.748).

The TCI scores in all the subjects as to the rs6943555 genotype are shown in Table 1.

There was no main effect of the polymorphism rs6943555

Table 1. TCI dimension scores in all the subjects grouped as to the *AUTS2* rs6943555 genotype

Genotype	N	NS	HA	RD	P	SD	C	ST
All subjects	190							
T/T	80	51.08±7.07	56.87±9.20	41.18±6.02	12.66±2.37	59.13±10.53	70.81±11.07	31.22±7.28
T/A	88	49.49±7.55	58.67±8.84	42.99±5.77	13.12±2.66	58.63±10.05	74.42±10.21	30.50±8.10
A/A	22	48.86±6.45	58.82±9.52	43.36±3.82	13.18±2.44	57.45±8.50	74.77±7.62	31.82±6.56
F		1.986	1.919	3.321	0.241	0.309	3.538	0.570
p		0.140	0.150	0.038*	0.786	0.734	0.031*	0.566

TCI dimension scores are showed as mean±SD. *p<0.05, this significance was lost on Bonferroni correction (p>0.05). TCI: Temperament and Character Inventory, *AUTS2*: autism susceptibility candidate 2, NS: novelty seeking, HA: harm avoidance, RD: reward dependence, P: persistence, SD: self-directedness, C: cooperativeness, ST: self-transcendence

Table 2. TCI dimension scores in the subjects with PHQ-9 scores <10 grouped as to the *AUTS2* rs6943555 genotype

Genotype	N	NS	HA	RD	P	SD	C	ST
PHQ-9 scores <10	156							
T/T	67	50.81±6.79	55.79±9.05	41.75±5.87	12.78±2.44	60.28±10.56	71.91±10.73	31.57±7.68
T/A	72	49.61±7.67	56.74±8.07	43.29±5.85	13.11±2.50	60.37±9.01	74.31±9.87	30.79±8.37
A/A	17	48.65±6.10	57.65±9.83	42.71±4.04	12.59±2.21	58.18±8.38	73.12±7.35	31.00±6.13
F		1.103	1.066	1.577	0.177	0.367	1.898	0.218
p		0.335	0.347	0.210	0.838	0.693	0.153	0.805

TCI dimension scores are showed as mean±SD. TCI: Temperament and Character Inventory, PHQ-9: Patient Health Questionnaire-9, *AUTS2*: autism susceptibility candidate 2, NS: novelty seeking, HA: harm avoidance, RD: reward dependence, P: persistence, SD: self-directedness, C: cooperativeness, ST: self-transcendence

genotype on the novelty seeking (NS), harm avoidance (HA), persistence (P), self-directedness (SD), and self-transcendence (ST) scores. On the other hand, there were significant association with reward dependence (RD) and cooperativeness (C) among the rs6943555 genotypes, although the significance was lost on Bonferroni correction (p>0.05). Interaction between the rs6943555 genotype and gender was not found for any TCI dimension score (NS: F=0.785, p=0.457; HA: F=1.495, p=0.227; RD: F=0.774, p=0.463; P: F=0.758, p=0.470; SD: F=0.159, p=0.853; C: F=1.001, p=0.369; ST: F=0.316, p=0.729).

The TCI scores in the subjects with PHQ-9 scores <10 as to the rs6943555 genotype are shown in Table 2.

In an analysis that excluded the subjects with PHQ-9 scores ≥10, no main effect of the polymorphism rs6943555 genotype on any TCI dimension score was seen. Similarly, interaction between the rs6943555 genotype and gender also was not found for any TCI dimension score (NS: F=0.369, p=0.692; HA: F=1.627, p=0.200; RD: F=0.532, p=0.588; P: F=1.006, p=0.368; SD: F=0.001, p=0.999; C: F=1.457, p=0.236; ST: F=1.173, p=0.312).

The effect of the *AUTS2* haplotypes on the TCI scores in the subjects with PHQ-9 scores <10 are shown in Table 3.

There was no effect of the haplotypes consisting of the polymorphisms rs6943555 and rs9886351 on the TCI dimension scores.

In addition, the pairwise *D*' and r^2 values for the polymorphisms *AUTS2* rs6943555 and rs9886351 in our subjects were 0.250 and 0.042, respectively.

Table 3. The effect of the *AUTS2* haplotypes on the TCI dimension scores in the subjects with PHQ-9 scores <10

TCI dimension	Haplotype		Beta	p
	rs6943555	rs9886351		
NS	A	G	-0.2217	0.838
	T	G	-0.2681	0.788
	A	A	-1.873	0.149
	T	A	1.214	0.168
HA	A	G	0.2546	0.849
	T	G	-0.1212	0.921
	A	A	1.92	0.231
	T	A	-0.9528	0.380
RD	A	G	1.267	0.140
	T	G	-0.8522	0.282
	A	A	0.02181	0.983
	T	A	-0.183	0.794
P	A	G	0.1254	0.735
	T	G	-0.3463	0.309
	A	A	-0.1841	0.679
	T	A	0.2716	0.367
SD	A	G	-1.879	0.197
	T	G	-0.08072	0.952
	A	A	0.6402	0.715
	T	A	1.012	0.393
C	A	G	0.8489	0.576
	T	G	-0.6491	0.643
	A	A	1.228	0.501
	T	A	-0.617	0.618
ST	A	G	0.7267	0.547
	T	G	-0.1704	0.878
	A	A	-2.105	0.146
	T	A	0.6165	0.530

AUTS2: autism susceptibility candidate 2, TCI: Temperament and Character Inventory, PHQ-9: Patient Health Questionnaire-9, NS: novelty seeking, HA: harm avoidance, RD: reward dependence, P: persistence, SD: self-directedness, C: cooperativeness, ST: self-transcendence, Beta: regression coefficient

DISCUSSION

In this study, we investigated the association of the polymorphism rs6943555 in the *AUTS2* gene and personality traits, as assessed by the TCI in Japanese university students.

Our results showed that there was no significant association between the polymorphism rs6943555 in the *AUTS2* gene and personality traits measured by the TCI in all the subjects. Furthermore, even when subjects with more or less mental health problems (PHQ-9 scores ≥10) were excluded, no significant association was seen between them including the rs6943555-rs9886351 haplotypes. Incidentally, there was also no significant association between the polymorphism rs9886351 and personality traits (data not shown).

The *AUTS2* gene has been implicated in multiple neurological disorders.[28] Several recent studies have shown that the polymorphism rs6943555 in the *AUTS2* gene is broadly associated with schizophrenia,[10] heroin dependence,[11,12] suicide under the influence of ethanol,[13] and alcohol consumption.[14] Therefore, we expected that the single nucleotide exchange of T → A affect the individual differences of human personality traits, although no association was found between them in our study. Regarding other polymorphisms in the *AUTS2* gene, a very recent genome-wide association study revealed that the intronic polymorphisms rs7785360 and rs12698828 are significantly associated with antidepressant responses to selective serotonin reuptake inhibitors (SSRIs) and mirtazapine (NaSSA: noradrenergic and specific serotonergic antidepressant).[29] This finding indicates the possibility that the *AUTS2* gene might be closely related to the activity of serotonergic and noradrenergic systems. Originally, Cloninger proposed that the three heritable dimensions of personality, i.e., novelty seeking, harm

avoidance, and reward dependence, are linked to dopaminergic, serotonergic and noradrenergic neurons, respectively.[15,30] Therefore, the polymorphisms rs7785360 and rs12698828 might be more likely associated with personality traits, while it is not clear whether these polymorphisms affect *AUTS2* gene expression. However, a previous study has suggested that important elements for regulation of gene expressionis contained within introns.[31] In fact, since the polymorphism rs6943555 has been reported to affect gene expression,[11,14] such intronic polymorphisms in the *AUTS2* gene might be important to elucidate the relationship with human personality traits. As further evidence of this, a recent study revealed that the intronic haplotypes in the *AUTS2* gene including rs6943555 are related to heroin dependence.[12] In addition, our previous study also reported that the haplotype consisting of the intronic polymorphisms rs6943555 and rs9886351 might affect the pathogenesis of alcohol dependence.[17] Thus, although an effect of the rs6943555-rs9886351 haplotypes on the development of personality traits was not observed in this study, it would be interesting for further analysis to be focused on *AUTS2* haplotypes in future research.

The genotype and allele frequencies of the polymorphism *AUTS2* rs6943555 might differ among races.[11,17] Genotype and allele frequencies of this polymorphism in this study were not significantly different compared to that of our previous study (male: 23, female: 52, mean age: 35.36±9.06) (genotype: χ^2 (2)=0.271, p=0.873; allele: χ^2 (1)=0.557, p=0.455).[17] In addition, the A/A genotype (11.6%) and A allele frequencies (34.7%) in our subjects showed very little difference from those in Han Chinese subjects [Zhang et al.: male: 192, female: 243, mean age: 37.6±10.8; Chen et al.: male: 390 (genotyping in 373 males), mean age: 42.8±14.4; Dang et al.: male: 355, female: 61, mean age: 37.13±5.23] (A/A genotype: 8.31−13.0%; A allele: 29.3−35.8%).[10-12] On the other hand, in Polish Caucasian subjects (male: 1819, female: 2042, mean age: 45.72±14.91) the A/A genotype accounts for only 4.7% and the A allele 21%.[13] The genotype and allele frequencies of the polymorphism *AUTS2* rs6943555 in Polish Caucasian subjects show significant differences from those in the Japanese population examined in this study [genotype: χ^2 (2)=39.4, p<0.01; allele: χ^2 (1)=40.1, p<0.01].[13] However, because the number of subjects in this study was far fewer than that in Chojnicka's study of Polish Caucasians,[13] such a difference in methodology will should be considered. Likewise, the age ranges of the subjects in Chojnicka's[13] and our studies (20.46±1.15) were also greatly different, although a significant difference in genotype [χ^2 (2)=48.4, p<0.01] and allele [χ^2 (1)=46.9, p<0.01] frequencies of the polymorphism rs6943555 between Chojnicka's and Chen's[11] studies that have roughly the same age range was also observed. Therefore, if the different results to our study are confirmed in further studies, such differences in genotype and allele frequencies among races might be one of the reasons.

It must be considered that there are several limitations in this study. First, the sample size of all the subjects (n=190) in our study was small and consequently the statistical power was low. We have calculated whether the number of samples is appropriate in this study by using the G*Power version 3.1.9.2 (http://www.gpower.hhu.de/). The effect size in the two-way analysis of variance [3 (genotype)×2 (gender)] was set to 0.25 (medium effect),[32] and statistical power was 0.8.[32] Consequently, when analyzing the main effect of the genotype on TCI dimension (α=0.05), the required number of samples was calculated to be 158 subjects (approximately 27 subjects per group). However, the required number of samples per group could not be obtained (e.g., the number of males with the A/A genotype of the polymorphism rs6943555 was 10 subjects). Second, to study the relationship between the *AUTS2* gene polymorphisms and personality traits, we used only the shortened version of TCI as a self-report questionnaire. In addition, to clarify the effects of these polymorphisms on personality traits, it is necessary to use various self-report personality questionnaires with different characteristics.[33] However, the high validity and reliability of the Japanese version of the TCI-125 items with a 4-point answer scale has been confirmed in Japanese university students (mean age: 20.37).[18] Third, in order to exclude the influence of the results for students with mental health problems, we performed the PHQ-9. Because the PHQ-9 is a self-report questionnaire that evaluates only depressive disorders,[34] there is a possibility that it could not exclude subjects with other mental health problems such as anxiety disorders. Meanwhile, it has been reported that the PHQ-9 exhibits higher sensitivity and specificity in comparison with other self-report questionnaires for depression screening.[35] Therefore, by excluding subjects with depressive symptoms that are observed at high frequency in university students,[19] the possibility that more reliable results can be obtained is suggested.

In conclusion, our study suggests that the polymorphism *AUTS2* rs6943555 is not associated with personality traits, as assessed by the TCI, in Japanese university students. Additionally, there was no significant association between the rs6943555-rs9886351 haplotypes and personality traits. Further large-scale studies with more subjects using not only the TCI but also other self-report questionnaires are needed, and it would be valuable also to clarify the relationship between the other important *AUTS2* polymorphisms (including haplotypes) and human personality traits.

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Association between *N*-methyl-D-aspartate Receptor Subunit 2B Gene Polymorphisms and Personality Traits in a Young Japanese Population

S Narita, Y Onozawa, E Yoshihara, D Nishizawa, M Numajiri, K Ikeda, K Iwahashi

Abstract

Objective: The *N*-methyl-D-aspartate receptor subunit 2B (GluN2B) is involved in regulation of anxiety and depression and nervous activity in the brain. Single nucleotide polymorphisms of the GluN2B gene (*GRIN2B*) are associated with human mental function and behaviour. We investigated whether four *GRIN2B* polymorphisms (rs7301328, rs1806201, rs1805247, and rs1805502) affect characterisation of personality traits.

Methods: In 248 young people, *GRIN2B* polymorphisms were analysed, and personality traits were assessed using the Neuroticism Extraversion Openness-Five Factor Inventory (NEO-FFI) and State-Trait Anxiety Inventory (STAI).

Results: There was no main effect of the *GRIN2B* polymorphisms on the NEO-FFI and STAI dimension scores. Interaction between polymorphism and sex was found in rs1805247 ($p = 0.034$) and rs1805502 ($p = 0.040$) in terms of the conscientiousness score of the NEO-FFI. However, post hoc simple main effect analysis showed no significant effect. The preliminary haplotype analysis indicated that haplotype CTT (rs1806201-rs1805247-rs1805502) in the haplotype block was associated with the extraversion score of the NEO-FFI in female participants ($p = 0.044$), but the significance was lost on correction for multiple testing.

Conclusion: There was no significant association between selected *GRIN2B* polymorphisms and personality traits, but this may be due to low statistical power. Further studies involving a larger study population are needed to clarify this.

Key words: *Haplotypes; NR2B NMDA receptor; Personality; Polymorphism, genetic*

Dr Shin Narita, MS, Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, Kanagawa, Japan.
Dr Yuuya Onozawa, PhD, Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, Kanagawa, Japan.
Dr Eiji Yoshihara, PhD, Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, Kanagawa, Japan.
Dr Daisuke Nishizawa, PhD, Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.
Dr Maki Numajiri, MS, Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, Kanagawa, Japan.
Dr Kazutaka Ikeda, PhD, Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.
Dr Kazuhiko Iwahashi, MD, PhD, Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, Kanagawa, Japan & Addictive Substance Project, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan & Health Administration Center, Azabu University, Kanagawa, Japan.

Address for correspondence: *Dr Yuuya Onozawa, Laboratory of Physiology (Project of Neurophysiology), Course of Environmental Health Science, Graduate School of Environmental Health, Azabu University, 1-17-71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan.*
Tel/Fax: +81-42-769-1930; Email: onzwy@kitasato-u.ac.jp

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Introduction

Personality traits are influenced by environmental and genetic factors. Up to 60% of the variance in personality traits might be inherited.[1] Genetic factors have a significant effect on personality traits as measured by the Cloninger model and five-factor model.[2] Some genes involved in the characterisation of personality traits may affect various human behaviours, including mood disorders and schizophrenia.[2] Genetic polymorphisms of neurotransmission-related genes are hypothesised to contribute to the formation of personality traits.[3]

N-methyl-D-aspartate (NMDA) receptors are glutamate-gated ion channels that are widely expressed in the central nervous system. They play central roles in synapse development and plasticity, and learning and memory.[4,5] The glutamate-binding site has been localised to the NMDA receptor subunit 2, which is the main molecular determinant of NMDA receptor functional diversity in the brain.[6,7] The activity of NMDA receptor subunit 2B (GluN2B) contributes to vulnerability to neural excitotoxicity and psychiatric disorders, as well as working

memory function in the prefrontal cortex.[8] GluN2B is involved in mental function and behaviour; rats that are more anxious have altered patterns of GluN2B expression in the prefrontal cortex, amygdala, and hippocampus, all of which control emotional behaviour.[9] In the prefrontal cortex of patients with major depressive disorder, expression of GluN2B is significantly reduced compared with controls.[10] GluN2B antagonists are efficacious in the treatment of depressive states.[11,12]

The GluN2B gene (*GRIN2B*) is located on chromosome 12p12 and consists of 13 exons, the coding sequence being encompassed by exons 2 to 13.[13,14] Molecular genetic studies have identified GRIN2B as a candidate gene for bipolar disorder.[15,16] In a sample of bipolar patients, the C allele of the rs1805502 polymorphism in *GRIN2B* was transmitted more frequently.[17] Subsequent case-control analysis of *GRIN2B* polymorphisms and bipolar disorder revealed that the rs1805247 polymorphism and the haplotype composed of rs1805247 and rs1805502 were significantly associated with bipolar disorder in a Chinese Han population.[18] Patients with and without treatment-resistant depression differ significantly in the allele and genotype frequencies of the rs1805502 polymorphism.[19] The rs1806201 polymorphism has been shown to affect Cloninger type 2 alcohol-dependent patients.[20] In a study of *GRIN2B* mutations on decision-making using the Iowa Gambling Task, healthy German women with the C/C genotype of the rs1806201 polymorphism made less use of a win-stay strategy and demonstrated more exploratory behaviour during task execution.[21] The rs7301328 polymorphism has been linked to schizophrenia in Japanese patients[14] and disruptive behaviour in Taiwanese volunteers.[22]

GRIN2B polymorphisms may affect the characterisation of personality traits. This study aimed to investigate whether *GRIN2B* polymorphisms are associated with personality traits in a young Japanese population. We focused on 4 single nucleotide polymorphisms (SNPs): rs7301328 (366C/G, exon 2), rs1806201 (2664C/T, exon 13), rs1805247 (4197T/C, exon 13), and rs1805502 (5988T/C, exon 13).

Methods

This study was approved by the ethics committee of Azabu University, Japan. Written informed consent was obtained from each participant. A total of 248 participants (63 males and 185 females) with a mean ± standard deviation age of 19.55 ± 1.21 years were recruited. Their blood samples were obtained. The personality traits of the participants were evaluated using the Japanese version of the Neuroticism Extraversion Openness-Five Factor Inventory (NEO-FFI) and the State-Trait Anxiety Inventory (STAI). The NEO-FFI is a 60-item self-report questionnaire based on the five-factor model of personality. It assesses the relationship between gene polymorphisms and 5 major dimensions of personality traits (neuroticism, extraversion, openness, agreeableness, and conscientiousness) and provides a comprehensive evaluation of personality traits.[23] The STAI is a 40-item self-report questionnaire used to measure 2 major scales of anxiety (state anxiety and trait anxiety).[24] State anxiety is the strength of anxiety at the moment, and trait anxiety is the tendency to anxiety as a personality trait. Both the NEO-FFI and the STAI are concise and thus facilitate accurate evaluation.[23,25] Their validity and reliability have been confirmed in a Japanese population.[24,26]

Extraction and purification of genomic DNA was performed using the phenol/chloroform method. *GRIN2B* polymorphisms rs7301328, rs1806201, rs1805247, and rs1805502 were genotyped by means of polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP).[14,27] The PCR cycling conditions were 95°C for 10 min, 35 cycles of 95°C for 30 s, 56°C (rs1806201) or 60°C (rs7301328, rs1805247, and rs1805502) for 30 s, and 72°C for 30 s, and final extension at 72°C for 7 min. The PCR product (112bp for rs7301328, 210bp for rs1806201, 320bp for rs1805247, and 353bp for rs1805502) was digested with a restriction enzyme, *Taq*I, *Pst*I, *Nco*I, or *Aci*I, and the digested products were subjected to electrophoresis, and visualised using the ethidium bromide staining method (Figure 1).

The Hardy-Weinberg disequilibrium was assessed using the χ^2 test. We compared the NEO-FFI and STAI dimension scores among the *GRIN2B* genotypes using two-way analysis of variance, with genotypes and sex as independent variables and NEO-FFI or STAI dimension scores as dependent variables. As a preliminary analysis, we analysed the effects of the haplotypes consisting of the *GRIN2B* polymorphisms on the NEO-FFI and STAI dimension scores. Linkage disequilibrium (LD) coefficients (D' and r^2) and haplotype effects were calculated with gPLINK 2.050 (http://zzz.bwh.harvard.edu/plink/index.shtml) and Haploview 4.2 (http://www.broad.mit.edu/mpg/haploview/index.php).[28,29] A p value of <0.05 was considered statistically significant.

Results

In the 248 participants, the genotype frequencies of the analysed *GRIN2B* polymorphisms were as follows: rs7301328 (C/C type: 28.2%, C/G type: 51.2%, G/G type: 20.6%), rs1806201 (C/C type: 31.5%, C/T type: 45.2%, T/T type: 23.3%), rs1805247 (T/T type: 55.7%, T/C type: 38.3%, C/C type: 6.0%), and rs1805502 (T/T type: 56.1%, T/C type: 37.9%, C/C type: 6.0%). The genotype distribution of the four *GRIN2B* polymorphisms was in Hardy-Weinberg equilibrium (rs7301328: χ^2 (1) = 0.227, p = 0.634; rs1806201: χ^2 (1) = 2.047, p = 0.152; rs1805247: χ^2 (1) = 0.064, p = 0.800; rs1805502: χ^2 (1) = 0.029, p = 0.866). There was no significant difference in the frequency of the *GRIN2B* genotype between sexes (rs7301328: χ^2 (2) = 3.245, p = 0.197; rs1806201: χ^2 (2) = 1.969, p = 0.374; rs1805247: χ^2 (2) = 5.590, p = 0.061; rs1805502: χ^2 (2) = 5.689, p = 0.058).

The NEO-FFI and STAI dimension scores in the

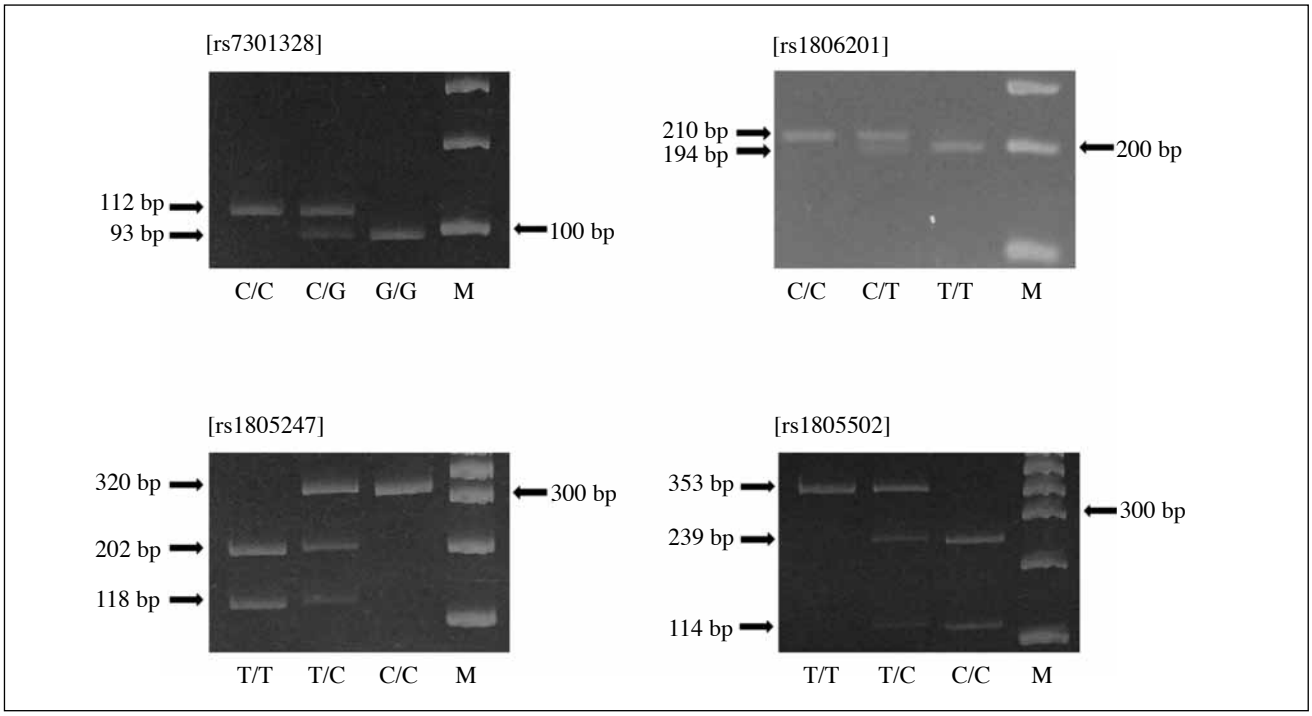

Figure 1. Representative results for the GRIN2B polymorphisms with the PCR-RFLP method.
Abbreviations: *GRIN2B = N-methyl-D-aspartate receptor subunit 2B gene; M = 100 bp DNA ladder; PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.*

participants grouped as *GRIN2B* genotype are shown in Table 1. In the two-way analysis of variance, there was no main effect of the *GRIN2B* polymorphisms on the NEO-FFI and STAI dimension scores. Interaction between the rs7301328 or rs1806201 polymorphisms and sex was not found for any NEO-FFI and STAI dimension scores. In contrast, interaction between the rs1805247 or rs1805502 polymorphisms and sex was found for the conscientiousness score of the NEO-FFI (rs1805247: F = 3.426, p = 0.034; rs1805502: F = 3.259, p = 0.040). However, there was no simple main effect of the rs1805247 or rs1805502 polymorphisms in either sex on the conscientiousness score (rs1805247: male: F = 2.268, p = 0.106, female: F = 1.429, p = 0.242; rs1805502: male: F = 2.263, p = 0.106, female: F = 1.200, p = 0.303). For other dimension scores, no interaction was observed between the rs1805247 or rs1805502 polymorphisms and sex.

The LD pattern for the four *GRIN2B* polymorphisms is shown in Figure 2. Haploview indicated that the rs1805247 and rs1805502 polymorphisms showed nearly complete LD ($D' = 1.000, r^2 = 0.989$), and haplotype block 1 composed of the rs1806201, rs1805247, and rs1805502 polymorphisms was detected. Therefore, we performed haplotype analysis for block 1 consisting of 3 markers.

The effects of the *GRIN2B* haplotypes on participants' NEO-FFI and STAI dimension scores are shown in Table 2. A haplotype CTT (rs1806201-rs1805247-rs1805502) was significantly associated with the extraversion score of the NEO-FFI in female participants ($R^2 = 0.022$, p = 0.044). However, after correction for multiple testing ($p < 0.05 / 3$ [haplotype number] = 0.0167), the significance was lost. There was no significant effect of the haplotype consisting of the three *GRIN2B* polymorphisms on the NEO-FFI and STAI dimension scores in either the male participants or total participants.

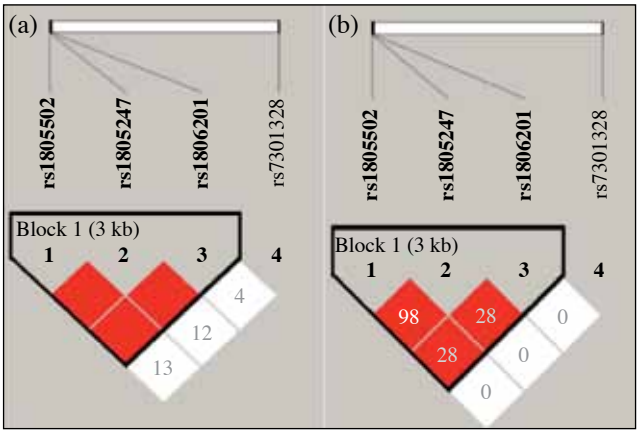

Figure 2. Linkage disequilibrium map of 4 polymorphisms at the GRIN2B locus. Pairwise linkage disequilibrium between SNPs was measured using the (a) D' and (b) r^2 values.
Abbreviations: *GRIN2B = N-methyl-D-aspartate receptor subunit 2B gene; SNP = single nucleotide polymorphism.*

Table 1. NEO-FFI and STAI dimension scores in participants grouped as GRIN2B genotypes.

SNP	Mean ± SD NEO-FFI score					Mean ± SD STAI score
	Neuroticism	Extraversion	Openness	Agreeable-ness	Conscien-tiousness	Trait anxiety
rs7301328						
Genotype (male)						
C/C	27.29 ± 6.52	23.57 ± 8.60	26.43 ± 7.60	27.43 ± 7.42	25.29 ± 8.32	53.73 ± 9.43
C/G	30.27 ± 7.90	23.43 ± 6.51	25.78 ± 5.14	28.38 ± 5.46	25.73 ± 6.42	50.74 ± 10.03
G/G	28.11 ± 9.10	27.78 ± 5.67	24.78 ± 9.09	30.67 ± 6.42	27.33 ± 7.07	51.00 ± 9.09
Genotype (female)						
C/C	31.82 ± 7.27	26.07 ± 7.28	27.87 ± 6.69	29.44 ± 5.55	25.82 ± 6.34	51.89 ± 9.51
C/G	30.60 ± 7.43	24.90 ± 6.45	28.79 ± 5.08	29.75 ± 5.87	25.70 ± 6.35	50.95 ± 10.87
G/G	31.45 ± 9.60	24.14 ± 6.60	28.57 ± 5.58	27.88 ± 5.84	24.76 ± 6.50	50.67 ± 10.72
Main effect of the genotype	0.799	0.437	0.883	0.788	0.949	0.530
Interaction between the genotype and sex	0.286	0.130	0.661	0.176	0.571	0.849
rs1806201						
Genotype (male)						
C/C	29.90 ± 7.18	24.43 ± 8.56	25.67 ± 5.48	28.86 ± 6.06	26.62 ± 8.45	52.36 ± 9.13
C/T	28.61 ± 8.39	23.79 ± 6.34	26.21 ± 7.42	28.00 ± 6.22	25.57 ± 6.26	50.07 ± 10.22
T/T	29.64 ± 7.84	24.36 ± 5.73	24.91 ± 5.28	29.09 ± 6.20	25.18 ± 5.47	53.55 ± 9.75
Genotype (female)						
C/C	31.04 ± 6.83	23.96 ± 6.83	28.02 ± 5.52	29.25 ± 5.57	26.05 ± 6.00	51.73 ± 9.94
C/T	30.94 ± 8.58	25.10 ± 6.71	28.85 ± 6.18	29.21 ± 5.93	25.73 ± 6.32	51.13 ± 10.93
T/T	31.68 ± 7.97	26.34 ± 6.60	28.32 ± 5.06	29.23 ± 5.90	24.53 ± 6.85	50.57 ± 10.19
Main effect of the genotype	0.806	0.713	0.668	0.853	0.556	0.635
Interaction between the genotype and sex	0.902	0.636	0.912	0.873	0.927	0.609
rs1805247						
Genotype (male)						
T/T	28.90 ± 7.97	24.52 ± 6.59	26.17 ± 5.92	28.72 ± 6.93	26.83 ± 6.64	52.27 ± 9.41
T/C	29.96 ± 7.91	23.42 ± 7.04	24.63 ± 7.20	28.08 ± 5.65	23.83 ± 7.39	50.52 ± 10.24
C/C	28.29 ± 7.41	24.86 ± 9.21	28.14 ± 4.67	29.00 ± 3.92	28.86 ± 4.60	51.71 ± 10.16
Genotype (female)						
T/T	31.05 ± 8.10	25.05 ± 6.51	28.46 ± 5.32	28.87 ± 6.26	24.95 ± 6.46	50.91 ± 10.49
T/C	31.47 ± 7.51	25.09 ± 7.13	28.26 ± 6.34	29.46 ± 4.73	26.53 ± 5.91	51.73 ± 10.31
C/C	29.71 ± 9.62	25.43 ± 7.23	30.71 ± 4.61	32.43 ± 7.53	24.14 ± 8.61	49.57 ± 11.43
Main effect of the genotype	0.706	0.845	0.202	0.500	0.688	0.928
Interaction between the genotype and sex	0.963	0.864	0.765	0.566	0.034	0.687
rs1805502						
Genotype (male)						
T/T	28.90 ± 7.97	24.52 ± 6.59	26.17 ± 5.92	28.72 ± 6.93	26.83 ± 6.64	52.27 ± 9.41
T/C	29.96 ± 7.91	23.42 ± 7.04	24.63 ± 7.20	28.08 ± 5.65	23.83 ± 7.39	50.52 ± 10.24
C/C	28.29 ± 7.41	24.86 ± 9.21	28.14 ± 4.67	29.00 ± 3.92	28.86 ± 4.60	51.71 ± 10.16
Genotype (female)						
T/T	31.06 ± 8.06	25.20 ± 6.68	28.56 ± 5.40	28.92 ± 6.25	25.02 ± 6.47	50.77 ± 10.53
T/C	31.46 ± 7.57	24.84 ± 6.88	28.10 ± 6.25	29.39 ± 4.73	26.45 ± 5.91	51.96 ± 10.21
C/C	29.71 ± 9.62	25.43 ± 7.23	30.71 ± 4.61	32.43 ± 7.53	24.14 ± 8.61	49.57 ± 11.43
Main effect of the genotype	0.709	0.757	0.175	0.498	0.655	0.951
Interaction between the genotype and sex	0.962	0.938	0.837	0.588	0.040	0.623

Abbreviations: *GRIN2B = N-methyl-D-aspartate receptor subunit 2B gene; NEO-FFI = Neuroticism Extraversion Openness-Five Factor Inventory; SNP = single nucleotide polymorphism; STAI = State-Trait Anxiety Inventory.*

Table 2. Effect of GRIN2B haplotypes on NEO-FFI and STAI dimension scores.

Dimension	Haplotype			Beta (regression coefficient)	p Value
	rs1806201	rs1805247	rs1805502		
NEO-FFI					
Neuroticism					
Total	T	T	T	0.244	0.723
Total	C	C	C	-0.127	0.880
Total	C	T	T	-0.190	0.794
Male	T	T	T	-0.313	0.827
Male	C	C	C	0.125	0.933
Male	C	T	T	0.194	0.891
Female	T	T	T	0.308	0.696
Female	C	C	C	0.015	0.988
Female	C	T	T	-0.381	0.655
Extraversion					
Total	T	T	T	0.921	0.119
Total	C	C	C	-0.288	0.690
Total	C	T	T	-0.966	0.124
Male	T	T	T	-0.126	0.922
Male	C	C	C	-0.230	0.864
Male	C	T	T	0.332	0.794
Female	T	T	T	1.186	0.076
Female	C	C	C	-0.190	0.829
Female	C	T	T	-1.457	0.044
Openness					
Total	T	T	T	0.193	0.711
Total	C	C	C	-0.120	0.850
Total	C	T	T	-0.221	0.688
Male	T	T	T	-0.236	0.840
Male	C	C	C	0.187	0.878
Male	C	T	T	0.063	0.957
Female	T	T	T	0.175	0.758
Female	C	C	C	0.104	0.888
Female	C	T	T	-0.398	0.517
Agreeableness					
Total	T	T	T	0.014	0.978
Total	C	C	C	0.529	0.395
Total	C	T	T	-0.458	0.397
Male	T	T	T	-0.033	0.977
Male	C	C	C	-0.107	0.927
Male	C	T	T	0.129	0.907
Female	T	T	T	-0.012	0.984
Female	C	C	C	0.941	0.210
Female	C	T	T	-0.689	0.269
Conscientiousness					
Total	T	T	T	-0.762	0.176
Total	C	C	C	0.456	0.508
Total	C	T	T	0.460	0.443
Male	T	T	T	-0.769	0.544
Male	C	C	C	-0.248	0.851
Male	C	T	T	0.978	0.435
Female	T	T	T	-0.746	0.238
Female	C	C	C	0.750	0.364
Female	C	T	T	0.285	0.678
STAI					
Trait anxiety					
Total	T	T	T	-0.429	0.629
Total	C	C	C	0.149	0.890
Total	C	T	T	0.500	0.596
Male	T	T	T	0.113	0.949
Male	C	C	C	-0.755	0.682
Male	C	T	T	0.574	0.744
Female	T	T	T	-0.577	0.578
Female	C	C	C	0.537	0.691
Female	C	T	T	0.481	0.668

Abbreviations: *GRIN2B = N-methyl-D-aspartate receptor subunit 2B gene; NEO-FFI = Neuroticism Extraversion Openness-Five Factor Inventory; STAI = State-Trait Anxiety Inventory.*

Discussion

The genotype and allele frequencies of the analysed polymorphisms in the participants were not significantly different from those reported in healthy Japanese participants (rs7301328: genotype: p = 0.145, allele: p = 0.075; rs1806201: genotype: p = 0.475, allele: p = 0.396; rs1805247: genotype: p = 0.637, allele: p = 0.416; rs1805502: genotype: p = 0.700, allele: p = 0.427).[14]

To the best of our knowledge, analysis using NEO-FFI and STAI dimension scores for assessing personality traits has not been reported. In a young Chinese Han population, the rs1806201 polymorphism was positively associated with shrewdness measured by the 16 Personality Factor Questionnaire, but the significance was lost after correction for multi-testing.[3] Although the rs1806201 polymorphism is a silent (synonymous) mutation,[30] it contributes to the selective regulation of the response inhibition process at the behavioural and neurophysiological levels.[31] The response inhibition process is stronger in the combined CT/TT genotype group than in the CC genotype group; this suggests that the variant T allele is associated with increased glutamatergic transmission.[31] The glutamatergic neurotransmission system is involved in the characterisation of human behaviour and personality traits. Glutamate concentration in the anterior cingulate cortex is associated with sensation seeking, harm avoidance, and anxiety.[32-34] Glutamate concentration in the dorsolateral prefrontal cortex is linked to extraversion among human personality traits.[35] Our study failed to find any association between functional rs1806201 polymorphism and personality traits in terms of NEO-FFI and STAI dimension scores; this may have been caused by the methodological approach. According to the Temperament and Character Inventory model developed by Cloninger et al,[36] the three heritable personality dimensions (novelty seeking, harm avoidance, and reward dependence) are thought to reflect the activity of dopaminergic, serotonergic, and norepinephrinergic neurons, respectively.[37] Meanwhile, glutamate in the central nervous system is closely related to monoamine neurons as a co-transmitter, and the presence of a glutamate neurotransmitter pool in serotonin, dopamine, and norepinephrine neurons has been confirmed.[38] Under this system, the function of NMDA receptors including the GluN2B has been shown to be influenced by monoamine neurotransmitters.[39-41] Thus, analysis of personality traits based on the Cloninger theory may be able to detect a significant association with *GRIN2B* polymorphisms. In fact, glutamatergic-related genes such as the excitatory amino acid transporter 2 (*EAAT2*) and ionotropic glutamate receptor kainate 3 (*GRIK3*) polymorphisms are associated with several personality dimensions/subdimensions such as reward dependence and harm avoidance, as measured by Temperament and Character Inventory.[42,43]

In our study, LD analysis identified a nearly complete LD between the rs1805247 and rs1805502 polymorphisms, and a haplotype block based on the rs1806201, rs1805247, and rs1805502 polymorphisms. The G allele of the rs1805247 polymorphism induces greater synaptic facilitation and greater long-term potentiation-like synaptic plasticity after intermittent theta-burst stimulation, and it has been suggested that individuals carrying this allele have enhanced glutamate NMDA receptor function.[44] The exact effect of the rs1805502 polymorphism on the expression or function of *GRIN2B* is unknown,[45] but the polymorphism is located in the same LD block as rs1806201 and rs1805247, which might affect glutamatergic pathways and gene expression. Furthermore, as an extension of this, a haplotype composed of some SNPs could be a meaningful mediator for elucidating genes involved in the characterisation of personality traits.[46,47] The effects of a haplotype CTT (rs1806201-rs1805247-rs1805502) on extraversion in female participants was lost after correction for multiple testing. Thus, it is necessary to increase the number of male participants to improve the statistical power of the analysis of the effect by sex.

This study has several limitations. The number of participants needed was calculated using G*Power version 3.1.9.2 (http://www.gpower.hhu.de/).[48] The effect size in the two-way analysis of variance was set to 0.25 (medium effect), and the power was 0.8 ($\alpha = 0.05$).[49] Consequently, when analysing the interaction between genotypes and sex for the NEO-FFI or STAI dimension scores, the required total number of participants was 158 (158 / (3 [genotypes] × 2 [sexes]) = approximately 27 participants per cell). For instance, the number of male participants with the C/C genotype of the rs1805247 and rs1805502 polymorphisms was 8. A significant association could not be detected probably because of low statistical power. Although all participants were supposedly healthy young people, their mental health was not confirmed by a psychiatrist. Therefore, we are unable to completely eliminate a confounding factor for the presence or absence of a mental disorder in participants.

Conclusion

There was no significant association between selected *GRIN2B* polymorphisms and personality traits in a young Japanese population, based on NEO-FFI and STAI dimension scores. Further large-scale studies involving more participants and using a full version of the Revised NEO Personality Inventory and Temperament and Character Inventory are needed to clarify the relationship between *GRIN2B* polymorphisms and personality traits.

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Declaration

The authors have no conflicts of interest to disclose.

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出典

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