う脳萎縮の拡大が明らかになっている[3]。統合失調症での脳萎縮の原因はまだ不明であるが，アルツハイマー病のように持続的な慢性炎症を介した神経免疫機構の関与が近年示唆されつつある。統合失調症患者では血液・髄液中の炎症性サイトカイン濃度・酸化ストレスマーカーの異常などが報告されており，統合失調症の病態に炎症の機序の関与が示唆されている[4-6]。

炎症性物質の産生源の一つとしてミクログリアが有力視されており，実際に統合失調症患者の死後脳研究や[^{11}C]PK11195などのリガンドを用いたPET (positron emission tomography) 研究では，ミクログリアの過剰な活性化が示唆されている[7-9]。特にPANSS (positive and negative syndrome scale；統合失調症の症状評価指標) による精神病・陽性症状の度合と末梢性ベンゾジアゼピン受容体の量(ミクログリア活性化の指標)が正相関するという報告は興味深い[9]。他方，ミクログリア活性化抑制作用を有する抗生物質ミノサイクリンが統合失調症患者の精神病症状に効果的であるという臨床薬理学的研究が近年報告されている[10-12]。

これまで筆者らは，齧歯類由来ミクログリア細胞を用いた*in vitro*実験で，interferon (IFN)-γ刺激ミクログリア由来のフリーラジカルや炎症性サイトカインの産生を，D2受容体拮抗作用のある非定型抗精神病薬ばかりでなく，D2受容体部分作動薬ながら抗精神病作用を有する国産精神病薬アリピプラゾールでも抑制することを報告してきた[13-15]。さらに，アリピプラゾールには活性化ミクログリア由来スパーオキシド産生を抑制するという抗酸化作用を見いだし，PC12との共培養実験により，アリピプラゾールがミクログリア活性化阻害を介して神経細胞傷害を抑制することを明らかにした[16]。このほかに筆者らは，オリゴデンドロサイトとの共培養実験でも，アリピプラゾールがミクログリア活性化抑制を介してオリゴデンドロサイト傷害を保護することを報告している[17]。こうした知見は*in vivo*実験により検証されつつあり[18]，筆者らのグループでも齧歯類モデル動物を用いた検証を進めている。

統合失調症におけるミクログリア仮説

胎生期に最も活動性が高いミクログリアは，次第に静止状態として脳内に定常的にとどまることが齧歯類で明らかになっており，胎生期・幼少期におけるミクログリアの過剰な活性化状態の延長・持続は，将来的にミクログリアが再活性化しやすい状態(priming)を作り出すという仮説が提唱されている[19]。統合失調症では遺伝的素因に加えて母胎感染や出生時外傷，あるいは，幼少期の様々な心理社会的ストレスが関与するという説は長年提唱され続けているが，ミクログリアがウイルスなどによる脳内感染ばかりではなく，心理的とも言えるようなストレスによって活性化させることが動物実験で明らかになっており，大変興味深い[20,21]。

胎生期・幼少期からの様々な身体的・心理社会的な環境因子によって誘導される脳内物質を介してミクログリアの悪性の活性化状態が誘発・持続され(プライミング：炎症誘発)，将来的に思春期・青年期に達したときに，健常者においては些細なストレス因子が過剰なミクログリア活性化を誘発し，ミクログリアから産生される炎症性サイトカインやフリーラジカルを介して神経細胞傷害・オリゴデンドロサイト傷害・神経新生抑制など脳内の障害が引き起こされ，結果的に統合失調症に至るのかもしれない。これが現在，筆者らを含む国内外の研究グループが提唱している統合失調症におけるミクログリアを介した病態仮説であり，治療的にはミクログリアの悪い活性化を制御する薬剤が新しい治療薬になりうるという治療仮説である(図1，2)[22-25]。統合失調症以外の精神疾患でもミクログリアの関与が示唆されており，以下に紹介する。

気分障害とミクログリア

大うつ病・双極性障害をはじめとする気分障害では，病像の経時的変化が特徴の一つであり，例えば，躁状態とうつ状態では全く異なる病状を呈し，それぞれで全く異なる脳内動態の存在が想像されるが，その病理基盤は解明されていない。死後脳研究により，大うつ病患者の中でも，自殺し

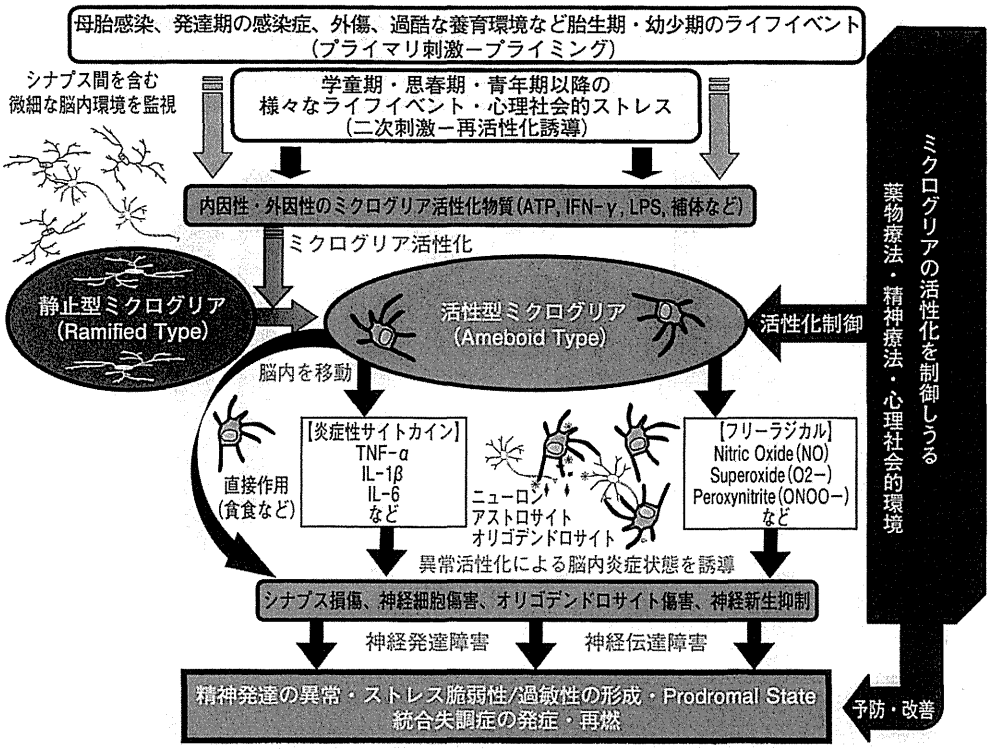

図1　統合失調症におけるミクログリア仮説(脳内ダイナミクスを図式化)

ミクログリアの過剰な活性化は貪食能亢進，あるいは炎症性サイトカイン，ケモカイン，フリーラジカルなどの過剰産生を通じてシナプス損傷，神経細胞傷害，オリゴデンドロサイト傷害，神経新生抑制などを引き起こし，脳内に微細な構造変化をもたらし，結果的に統合失調症の発症・再燃などに関与している可能性がある。さらに，ミクログリア活性化の制御が治療的に働く可能性がある[21]。統合失調症以外の精神疾患においても類似した病理機構が内在している可能性がある。(文献24より一部改変して引用)

た患者に限り前頭前野などの脳部位でミクログリアの過剰活性化が報告されている[7]。自殺は著しい抑うつ症状の最中に起こりやすく，この報告は，ミクログリアの過剰活性化が抑うつの重症度と相関する可能性を示唆している。他方，ミノサイクリンが精神病症状を伴う単極性うつ病患者に有効であったという臨床報告がなされており[26]，ミクログリア活性化抑制が精神病症状と共に抑うつ症状を改善させる可能性が示唆される。筆者らは，齧歯類ミクログリア細胞を用いた *in vitro* 実験において，抗うつ薬によるミクログリア活性化抑制作用[27,28]を報告しており，上述の臨床知見も鑑みると，気分障害の病理機構にミクログリアの過剰活性化が強く想定される。

気分障害の病態仮説として従来のセロトニン神経伝達の異常仮説に加えて，最近では，セロトニン系にも関与しているトリプトファンを出発点とするキヌレニン経路が注目されており，動物モデルによる研究や臨床サンプルを用いた研究が進められている[29]。キヌレニンからの代謝物の一つであるキノリン酸はNMDA受容体アゴニストとして作用し強い神経毒性を有することが知られているが，ミクログリアはキノリン酸を合成する際に不可欠なKMO(kynurenine 3-monooxygenase)と呼ばれる酵素を脳内で生成できる唯一の細胞であり，気分障害のキヌレニン仮説においてもミクログリアの関与は無視できず，今後の研究での深化が望まれる[29]。

自閉症とミクログリア

X染色体上に存在する *MECP2*(methyl-CpG-binding protein-2)遺伝子の変異によって引き起こされるレット症候群では自閉症様症状を呈することが多く，自閉症研究では *MECP2* 欠損モデル

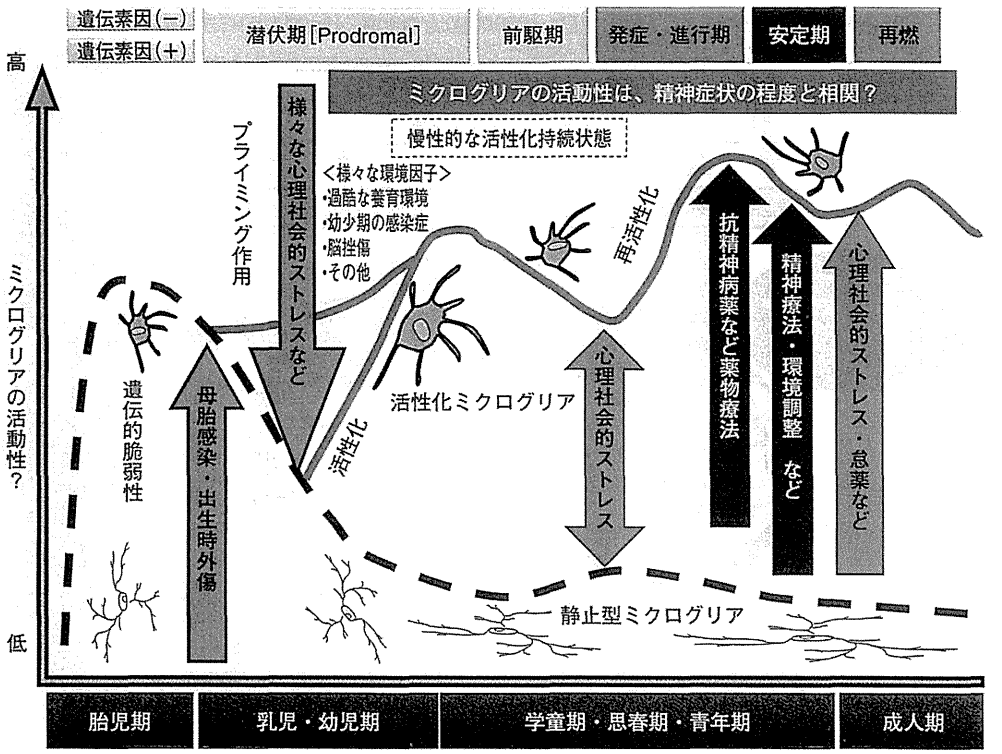

図 2　精神疾患におけるミクログリア仮説(経時的変化を模式化)

通常，ミクログリアは胎生期において最も活性化しており，その後，徐々に静止型として脳内にとどまると考えられている(青線)。遺伝的素因，母胎感染や出生時外傷，幼少期の感染症や外傷，さらには，過酷な養育環境による各種ストレスなどによって，ミクログリアの活性化持続状態(プライミング)が形成されると筆者らは想定している(赤線)。すると，脳内では活性化状態が潜在的に持続し，思春期・青年期における様々な心理社会的ストレスなどの二次刺激によって，過大なミクログリア活性化が誘導され，精神疾患の発症・症状形成の直接的な引き金になるのではないかと筆者らは考えている[25]。プライミングや二次刺激の時期やその脳内部位によって，統合失調症，あるいは自閉症など特定の精神疾患が誘導される可能性があり，今後の研究で解明されるべき課題である。(文献25より一部改変して引用)

マウスが活用されている。本モデルマウスにおけるミクログリア貪食能の異常が報告されており，脳内のミクログリアを野生型に入れ替えることで行動異常が改善したという報告[30]は興味深い。死後脳研究では小児自閉症患者におけるミクログリアの活性化が報告されている[31]。高機能自閉症を持つ成人患者と健常者を対象とした生体PET研究においても，高機能自閉症患者群において脳全体的にミクログリアの過剰な活性化を認めるという報告がなされている[32]。自閉症の病理機構は解明されていないが，上述の研究成果は，遺伝的素因に加えて胎生期やその後の脳内環境因子が重要であり，ミクログリアの過剰活性化がその病理基盤に重要な役割を果たしている可能性が強く示唆される。

強迫性障害とミクログリア

強迫性障害とミクログリアとの関連について臨床研究による直接的なエビデンスはないが，チックを主症状とし強迫症状を高頻度で合併することが知られるトゥレット症候群患者の死後脳研究において，基底核におけるMCP-1・IL-2の遺伝子発現上昇が報告されている[33]。こうしたサイトカインはミクログリアの活性化と共に発現が上昇するため，トゥレット症候群におけるミクログリアの関与が間接的に示唆される。近年，モデル動物実験においてセンセーショナルな報告がなれている[34]。Capecchiらが開発したHoxB8ノックアウトマウスでは強迫的な脱毛行動が出現することが知られており，野生型マウスの骨髄を移植するこ

とで異常行動が軽減したという報告である[34]。HoxB8は脳内においてはミクログリアに特異的に発現しており，HoxB8欠損マウスでは正常ミクログリアが減少しており，骨髄移植による正常に機能するミクログリアの増加に伴い異常行動が改善している[34]。小児期の溶連菌感染の後に強迫症状などの精神症状を呈する症候群が提唱されており，PANDAS (pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections)と呼ばれている[35]。PANDASでは強迫症状と炎症免疫との関与が示唆されており，その病理機構にミクログリアが関与しているのかもしれない。

せん妄とミクログリア

意識混濁を背景に幻覚・妄想など多彩な精神・行動症状を引き起こすせん妄(delirium)は，癌や様々な全身性の炎症性疾患に併発する。病態生理仮説として，抗コリン薬によりせん妄が誘発されるという経験的事実からコリン作動性神経の低活動性がせん妄を誘発するという仮説[36]が提唱されており，コリンエステラーゼ阻害薬リバスチグミンの有効性が検証されたが，せん妄期間が短縮することはなく，その仮説に限界が指摘されている[37]。せん妄患者の死後脳研究でミクログリアが過剰に活性化しているとの報告があり[38]，近年ではミクログリア過剰活性化を介したせん妄の炎症免疫仮説[39]が提唱されている。

筆者らは最近，終末期癌患者のせん妄に対してミノサイクリンが有効であった症例を報告している[40]。せん妄モデル動物においても，ミクログリア活性化制御を介したCOX-1阻害剤の治療的効果が報告されており[41]，せん妄の病態治療機序にミクログリアの関与が強く示唆される。

おわりに

本稿では，統合失調症はじめ精神疾患におけるミクログリア研究の現状を概説した。精神疾患をターゲットとしたミクログリア研究は始まったばかりであり，今後さらなる研究が望まれる。ミクログリアは，多くの神経伝達に関与する受容体を発現しており[42]，シナプス間を監視する機能も明らかになっている[2]。シナプス間を含む神経回路網は神経だけが司っていると従来信じられていたが，今後は，神経回路網の理解においてもミクログリアの関与を無視できない。アストロサイト・オリゴデンドロサイトなど他のグリア細胞も脳内には存在しており，神経-グリア相関という複雑系での脳内ネットワークを介した精神疾患への理解が今後不可欠であろう。筆者らは，健常者を対象としたミノサイクリン内服によるニューロエコノミクス実験において，ミクログリアの活動が性格由来の社会的行動[43]や，異性との間での社会的意思決定機構[44]に影響を与える可能性を見いだしている。精神疾患ばかりではなく，われわれの日常的な精神活動や社会活動においてもミクログリアの関与は無視できず，細胞レベルから健常者，精神疾患患者を対象としたトランスレーショナル研究によるミクログリア仮説の解明が望まれる[45]。

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COMMON VARIANTS IN *BCL9* GENE AND SCHIZOPHRENIA IN A JAPANESE POPULATION: ASSOCIATION STUDY, META-ANALYSIS AND COGNITIVE FUNCTION ANALYSIS

UOBIČAJENE VARIJANTE *BCL9* GENA I ŠIZOFRENIJA U JAPANSKOJ POPULACIJI: STUDIJA POVEZANOSTI, METAANALIZA I ANALIZA KOGNITIVNIH FUNKCIJA

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Summary

Background: Schizophrenia is a relatively common disorder, with a lifetime prevalence of about 1%. Family history is the most important risk factor for schizophrenia, consistent with a genetic contribution to its etiology. Recent human genetic studies reported that some common variants located within *BCL9* are associated with schizophrenia in the Chinese population, but not associated with bipolar disorder in the Caucasian population.
Methods: Single nucleotide variant (SNP) prioritization sample was comprised of 575 patients with schizophrenia and 564 healthy controls with no personal or family history of psychiatric illness. For SNP association analysis, we used an independent Japanese sample set (replication sample) comprising 1464 cases and 1171 controls. For the analysis of cognitive performance, we investigated 115 cases and 87 controls using Continuous Performance Test (CPT-IP) and the Wisconsin Card Sorting Test Keio version (WCST). Meta-

Kratak sadržaj

Uvod: Šizofrenija je relativno čest poremećaj, sa rasprostranjenošću od oko 1% u ukupnoj populaciji. Porodična istorija bolesti predstavlja najvažniji faktor rizika za nastanak šizofrenije, što je u skladu sa genetičkom osnovom njene etiologije. Nedavne genetičke studije pokazuju da su neke uobičajene varijante u okviru gena *BCL9* u vezi sa šizofrenijom u kineskoj populaciji, ali ne i sa bipolarnim poremećajem u populaciji belaca.
Metode: Uzorci za analizu tačkastih polimorfizama (SNP) potiču od 575 pacijenata sa šizofrenijom i 564 zdravih kontrolnih subjekata bez lične ili porodične istorije psihijatrijskih oboljenja. Za SNP analizu korišćen je nezavisni japanski set uzorak (replikacioni uzorak) koji sadrži 1464 slučaja bolesti i 1171 kontrolu. Za analizu kognitivnih funkcija, ispitivali smo 115 slučajeva bolesti i 87 kontrolnih slučajeva, korišćenjem kontinualnog testa funkcija (CPT-IP) i *Wisconsin Card Sorting* testa, Keio verzije (WCST). Metaanaliza je

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analysis was performed using a combined Japanese total sample (N=3735) and a Chinese sample from a previous study.
Results: In the replication sample set, we did not detect any association in 2 SNPs (rs672607 and rs10494252) and schizophrenia. Meta-analysis of rs672607 showed significant association (p-value 0.012, odds ratio 0.855). There was a significant ($p<0.01$) difference between the A/A and G carrier group of rs672607 in CPT mean d' ($p=0.0092$).
Conclusions: We were able to detect evidence for an association between rs672607 in BCL9 and schizophrenia in the meta-analysis of Japanese and Chinese populations. Additionally, this common variant may affect cognitive performance, as measured by the CPT-IP in schizophrenia patients.

Keywords: *BCL9*, Chinese, cognitive impairment, genome-wide association study, Japanese, meta-analysis, schizophrenia

urađena korišćenjem kombinovanog japanskog ukupnog uzorka (N=3735) i kineskog uzorka iz prethodne studije.
Rezultati: U replikacionom uzorku nije otkrivena nikakva veza između 2 SNP-a (rs672607 i rs10494252) i šizofrenije. Metaanaliza rs672607 je pokazala njegovu značajnu povezanost sa šizofrenijom (p-vrednost 0,012, 0,855 *odds ratio*). Utvrđena je značajna ($p<0,01$) razlika između A/A i G grupe nosilaca rs672607 u CPT srednjoj vrednosti d' ($p=0,0092$).
Zaključak: Dokazana je veza između rs672607 u genu BCL9 i šizofrenije u metaanalizi japanske i kineske populacije. Pored toga, ova zajednička varijanta može da utiče na kognitivne funkcije, što je utvrđeno testom CPT-IP kod šizofrenih bolesnika.

Ključne reči: *BCL9*, Kinezi, kognitivne funkcije, GWAS, Japanci, metaanaliza, šizofrenija

Introduction

Schizophrenia is a chronic, more or less enervating illness characterized by impairments in cognition, affect and behavior, all of which have a pronounced bizarre aspect (1). Delusions, which are generally bizarre, and hallucinations, generally auditory in type, typically occur during the clinical course of schizophrenia (2).

Schizophrenia is a relatively common disorder, with a lifetime prevalence of about 1% (3). Although the overall sex ratio is almost unbiased, males tend to have an earlier onset than females, a finding accounted for by the later age of onset in those females who lack a family history of the disease (4). Family history is the most important risk factor for schizophrenia, consistent with a genetic contribution to its etiology (5) and the heritability of schizophrenia is estimated to be 64% (6). Although genes relevant for schizophrenia or variants that may modulate risk for the disease have been identified using both linkage- and candidate-based or whole genome association studies, the genetic basis of schizophrenia is still unclear (7–10).

Recent human genetic studies reported that some B-cell CLL/lymphoma 9 gene (*BCL9*) variants are associated with schizophrenia in the Chinese population (11), but not associated with bipolar disorder in the Caucasian population (12). In addition, another study showed evidence for genetic association between common variants within *BCL9* and negative symptoms in schizophrenia patients (13). *BCL9* maps to chromosome 1q21.1 (NCBI37: 145,479,806-145,564,639), a region that was shown to be associated with schizophrenia (14). In addition, about 75% of all children with a 1q21.1 microdeletion have delayed development, particularly affecting the development of motor skills such as sitting, standing, and walking, while the intellectual disability and learning problems associated with this genetic change are usually mild (15). Furthermore, schizophrenia is significantly more common in combination with the 1q21.1 deletion syndrome, while autism is significantly more common with the 1q21.1 duplication syndrome (16).

From a biological point of view, the *BCL9* is required for efficient T-cell factor-mediated transcription in the Wnt signaling pathway (17). The Wnt signaling pathway influences neuroplasticity, cell survival, and adult neurogenesis (11), and several studies have suggested that mental disorders may involve impairments in these functions (18). As *BCL9* is indeed an attractive candidate gene for schizophrenia that has not been investigated in the Japanese population, we examined the relationship of common SNPs in *BCL9* and the risk for schizophrenia in a large Japanese case-control sample and conducted a meta-analysis between the Chinese (11) and Japanese sample set used in the current study. We also explored potential relationships between SNPs in *BCL9* and the aspects of human cognitive function.

Materials and Methods

Participants

This study was approved by the Ethics Committees of the Nagoya University Graduate School of Medicine and associated institutes and hospitals. Written informed consent was obtained from all participants. In addition, the patients' capacity to consent was confirmed by a family member when needed. Subjects with legal measure of reduced capacity were excluded. Patients were included in the study if they (1) met the DSM-IV criteria for schizophrenia, (2) were physically healthy and (3) had no mood disorders, substance abuse, neurodevelopmental disorders, epilepsy or known mental retardation. A general characterization and psychiatric assessment of subjects is available elsewhere (19). Controls were select-

ed from the general population. Control subjects had no history of mental disorders, based on questionnaire responses from the subjects themselves during the sample inclusion step, and based on an unstructured diagnostic interview done by an experienced psychiatrist during the blood collection step.

The JGWAS sample was comprised of 575 patients with schizophrenia (43.5±14.8 years (mean±s.d.), male 50%) and 564 healthy controls with no personal or family history of psychiatric illness (44.0±14.4 years (mean±s.d.), male 49.8%). All subjects were unrelated, living in the central area of the Honshu island of Japan and self-identified as members of the Japanese population.

For SNP association analysis, we used an independent Japanese sample set (replication sample) comprising 1464 cases (aged 45.9±14.2 years, male 54.5%) and 1171 controls (aged 48.06±14.48 years, male 47.3%). For the analysis of cognitive performance, we investigated 115 cases (aged 45.3±14.2 years, male 64.3%) and 87 controls (aged 26.3±7.7 years, male 63.2%).

SNP prioritization step

From the previous genetic study of *BCL9* in a Chinese population, we selected a SNP with the lowest p-value (rs672607 A>G, $p=1.23*10^{-11}$). From the JGWAS data set, there were 3 SNPs (rs17160256, rs17160264 and rs10494252) with $p<0.05$ in BCL9 and +10% region. We selected only one SNP (rs10494252 A>G, p=0.0369) from the 3 SNPs because of high r^2 (>0.95) in the Japanese population (SNPinfo Web Server, http://snpinfo.niehs.nih.gov/snpinfo/index.html).

Genotyping and data analysis

DNA was extracted from peripheral blood according to a standard protocol (20, 21). Genotyping was performed using a fluorescence-based allelic discrimination assay (Taqman, Applied Biosystems, Foster City, CA). To exclude low-quality DNA samples or genotyping probes, data sets were filtered on the basis of SNP genotype call rate (more than 90%) or deviation from the HWE in the control sample. Subjects whose percentage of missing genotypes was >10% or who had evidence of possible DNA contamination were excluded from subsequent analyses. All allele-wise association analyses (JGWAS or replication sample set) were carried out by calculating the p-values for each candidate SNP. Significance was determined at the 0.05 level using Fisher's exact test. All p-values were two-sided. In this joint analysis, p-values were generated by Cochran-Mantel-Haenszel stratified analysis, and the Breslow-Day test was performed for evaluation of heterogeneous associations as implemented in PLINK v1.07 (22). Statistical significance was set at a nominal level ($p<0.05$) in an association study. Comprehensive Meta-Analysis Version 2 Professional version (Biostat, Inc., http://www.meta-analysis.com/index.html) was used to conduct a meta-analysis of the Japanese and Chinese sample sets in rs672607.

Neurocognitive assessment

We used the Continuous Performance Test–Identical Pairs Version Release 4.0 (CPT-IP) (New CPT.exe, Copyright 1982–2004 by Barbara A. Cornblatt, All Rights Reserved). The size of the PC monitor used for the test was 10.4 inches as each letter was at least 2.2×1.5 cm (23, 24). Stimuli were flashed on the screen at a constant rate of 1 per second, with a stimulus »on« time of 50 ms. Stimuli were four-digit numbers and were presented 150 times. In each 150-trial condition, 30 of the trials (20%) were target trials and required a response. Target trials were those on which the second of a pair of two identical stimuli appeared (23). The outcome measure was a mean d'.

The Wisconsin Card Sorting Test (WCST) (25) mainly assesses executive function including cognitive flexibility in response to feedback. We used a modified and computerized version of the test: Wisconsin Card Sorting Test (Keio Version) (KWCST) (26–28). The outcome measures were numbers of categories achieved (CA), total errors (TE), and perseverative errors of Milner (PEM) and Nelson types (PEN) in the first trial. We selected outcomes in the WCST, following a prior study, which used KWCST as a measure of cognitive function (29): (1) CA, which is the number of categories for which six consecutive correct responses are achieved (eight is the maximum number of categories which can be achieved), and which is the sum measure of the level of conceptual shifts in the KWCST; (2) PEN, which is the number of incorrect responses in the same category as the immediately preceding incorrect response (maximum of 47 perseverative errors); (3) PEM, which is the number of incorrect responses in the same category as the immediately preceding correct response after the category changes; and (4) TE, which is the total number of incorrect responses.

Chlorpromazine (CPZ) equivalent doses were calculated based on the report by Inagaki et al. (30, 31). The Positive and Negative Symptom Scale (PANSS) was used to evaluate patients (32). From the sample used in the current study, we made a subset of randomly selected participants older than 18 years of age for an analysis of cognitive performance. Cognitive data analysis was done for the participants who completed both WCST and CPT-IP. We checked the effect of two SNPs (rs672607 and rs10494252) on cognitive performance measured by the CPT-IP and the WCST (115 schizophrenic patients, 87

healthy controls). IBM SPSS statistical software, version 20, was used for all analyses. We compared sex, age, education, CPZ equivalent doses, age at onset, duration of illness, positive scale, negative scale and General Psychopathology Scale between schizophrenia cases and control subjects using a Fisher's exact test, two-tailed t-test and Welch's t-test. Next, we compared d' in the CPT and CA, PEM, PEN, TE in the WCST between the case and control groups using a two-tailed t-test and Welch's test (*Table III*).

Patients' records were used to obtain relevant clinical information (e.g. age, education, CPZ equivalent doses, age at onset and duration of illness). Medication status of patients was investigated on the day when cognitive tests were conducted. Patients' medication status and positive and negative symptom scale (PANSS) (32) scores were obtained at the time of cognitive assessment.

Significance level in clinical information was set at p=0.0055 after Bonferroni correction (p=0.05/9). Significance level in five cognitive outcomes was set at p=0.01 after Bonferroni correction (p=0.05/5).

Results

In JGWAS and replication sample set, we did not detect any association in 2 SNPs (rs672607 and rs10494252) and schizophrenia (*Table I*). Joint analysis by PLINK also did not show significantly low p-value in both SNPs (*Table II*). Meta-analysis of the Japanese total sample and Chinese sample in rs672607 showed a significant association (p-value 0.012, odds ratio 0.855).

We investigated the genetic effects of rs672607 and rs10494252 on the CPT-IP and WCST. There was no significant (p<0.0055) difference in clinical information. There was a significant (p<0.01) difference between the A/A and G carrier group of rs672607 in CPT mean d' (p=0.0092) (*Table III*).

Discussion

In this study, we investigated the association between two SNPs within *BCL9* and schizophrenia in the Japanese population. We detected a significant (p=0.012) association between *BCL9* and schizophrenia in the meta-analysis of Japanese and Chinese sample set, however, as the Chinese GWAS dataset was included in the meta-analysis, evidence for the association might be overestimated. The minor allele of rs672607 may be a common variant associated with schizophrenia in the Asian population. Thus, further studies in different populations are needed.

In addition, one of the main obstacles in the identification of genetic variants for schizophrenia is its heterogeneous diagnostic entity, which is clinically relevant, though less appropriate for etiological and genetic research. Therefore, it was of interest to focus on alternative indicators of liability, or endophenotypes. We chose the CPT-IP that is designed to assess highly heritable traits (working memory and visual sustained attention) that are shown to be impaired in schizophrenic patients (33). The WCST was selected in order to evaluate executive function. We tested the association between candidate SNPs from our meta-analysis and cognitive performance measured by the

Table I Association study in JGWAS and the replication sample set.

		Case N	Control N	Total N	Case[b]	Control[b]	p-value[c]	Or[d]	L95[e]	U95[e]	HWEp[f]
rs672607 A>G (Ch1, 145519964a)	JGWAS	548	552	1100	0.375	0.393	0.37	0.92	0.78	1.10	0.47
	Replication	1464	1171	2635	0.374	0.392	0.22	0.93	0.83	1.05	0.87
rs10494252 C>A (Ch1, 145483560a)	JGWAS	548	552	1100	0.190	0.226	0.04	0.80	0.65	0.99	0.06
	Replication	1464	1171	2635	0.221	0.221	1.00	1.00	0.87	1.15	0.53

a. based on NCBI 36
b. minor allele frequency
c. p-value of Fisher's exact test
d. Odds ratio
e. Lower (L95) and upper (U95) 95% confidence intervals
f. Hardy-Weinberg Equilibrium test p-value in control

Table II Joint analysis of JGWAS and the replication sample set.

	Case N	Control N	Total N	p-value[a]	OR[b]	L95[e]	U95[c]	BDp[d]
rs672607 A>G	2012	1723	3735	0.13	0.93	0.84	1.02	0.95
rs10494252 C>A	2012	1723	3735	0.26	0.94	0.83	1.05	0.08

a. p-value of Cochran–Mantel–Haenszel stratified analysis by PLINK v1.07
b. Odds ratio
c. Lower (L95) and upper (U95) 95% confidence intervals
d. p-value of Breslow-Day test

Table III Cognitive performance of two SNPs in BCL9.

	rs672607 A>G						rs10494252 C>A					
	Cases (n=110)			Controls (n=76)			Cases (n=110)			Controls (n=76)		
	A/A[a] (n=31)	G carrier (n=79)	p-value[b]	A/Aa (n=21)	G carrier (n=55)	p-value[b]	C/Ca (n=61)	A carrier (n=49)	p-value[b]	C/Ca (n=48)	A carrier (n=28)	p-value[b]
Sex (Males/Females)	21/10	49/30	0.66	13/8	36/19	0.79	37/24	33/16	0.55	26/22	23/5	0.024
Age (years)	48.2	44.8	0.25	26.6	27.2	0.76	44.4	47.5	0.25	26.2	28.4	0.24
	13.6	14.2		7.6	8.1		13.5	14.6		6.9	9.3	
Education (years)	12.1	12.1	0.94	15.6	15.3	0.66	12.2	11.9	0.53	15.4	15.4	0.96
	2.5	2.2		2.8	2.5		2.4	2.2		2.5	2.7	
CPZeq (mg/day)[c]	630.5	627.5	0.97				640.6	612.9	0.69			
	378.4	355.1					340.9	386.1				
Age at onset (years)	26.6	26.7	0.97				25.9	27.5	0.43			
	10.7	10.3					9.0	11.9				
Duration of illness (years)	21.5	18.0	0.24				18.3	19.8	0.58			
	13.9	14.0					13.4	14.8				
PANSS[d] Positive (7–49)	15.5	16.0	0.63				16.4	15.1	0.12			
	4.8	4.3					4.7	4.0				
PANSS[d] Negative (7–49)	20.0	18.5	0.19				19.8	17.8	0.05			
	5.7	5.2					5.6	4.9				
PANSS[d] General (16–112)	36.2	35.8	0.82				36.8	34.9	0.25			
	10.2	7.7					8.9	7.9				
CPT-IP[e] mean d'	0.9	1.4	0.009	2.9	2.7	0.33	1.2	1.3	0.74	2.7	2.8	0.60
	0.9	0.8		0.7	0.7		0.8	0.9		0.7	0.8	
WCST CA[f]	2.6	3.5	0.08	5.7	5.7	0.82	3.1	3.5	0.36	5.7	5.7	0.93
	2.1	2.1		0.5	0.4		2.1	2.2		0.5	0.5	
WCST PEN[g]	7.6	7.3	0.86	0.7	0.6	0.69	7.4	7.4	0.99	0.7	0.5	0.38
	5.8	7.1		1.1	0.9		6.7	6.9		1.1	0.9	
WCST PEM[h]	5.8	4.8	0.54	0.4	0.3	0.98	5.7	4.3	0.31	0.4	0.3	0.77
	5.9	7.6		0.5	0.6		8.6	4.9		0.6	0.5	
WCST TE[i]	23.1	21.3	0.44	11.0	10.8	0.74	22.2	21.2	0.60	10.7	11.0	0.63
	10.1	10.2		1.2	2.1		9.9	10.5		1.9	1.9	

a. Results shown as mean and standard deviation (absolute number for row »sex«)
b. P-value of Student's t-test (p-value of Fisher exact test for row »Sex«/p-value of Welch's t test for row WCST PEN)
c. Chlorpromazine equivalent dose
d. Positive and negative syndrome scale
e. Continuous performance test–identical pairs version
f. Wisconsin card sorting test categories achieved
g. Wisconsin card sorting test perseverative errors – Nelson's type
h. Wisconsin card sorting test perseverative errors – Milner's type
i. Wisconsin card sorting test total errors

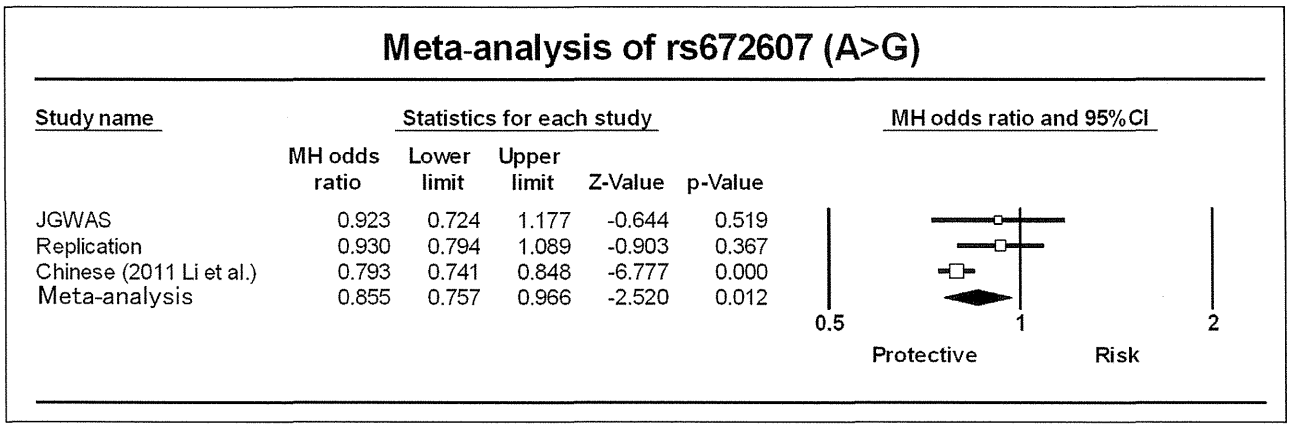

Figure 1 Meta-analysis of the Japanese and Chinese sample set in rs672607. MH: Cochran–Mantel–Haenszel test; lower limit: 95% confidence intervals; upper limit: 95% confidence intervals.

CPT and WCST. In the CPT-IP, the group with the minor allele of rs672607 (protective allele, odds ratio= 0.855 in our meta-analysis of Japanese and Chinese sample sets) showed significantly impaired working memory in schizophrenia patients.

Several caveats should be noted. Firstly, we did not include a systematic genome-wide mutation scan in either the 5 flanking region or exon regions to search for novel functional variants that may exist within the BCL9 locus, but had not been registered in the databases of common variants. Secondly, our phenotypic diagnosis is not based on structured interviews, and the control samples are significantly younger than the case samples. Thirdly, the sample sizes of cognitive tests were relatively small and the results of cognitive tests may be biased.

As a conclusion, we were able to detect evidence for an association between rs672607 in BCL9 and schizophrenia in the meta-analysis of Japanese and Chinese populations. Additionally, this common variant may affect cognitive performance, as measured by the CPT-IP in schizophrenia patients. Further studies using the sample collected in a non-Asian population are needed.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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SHORT COMMUNICATION

Effects of repeated dosing with mirtazapine, trazodone, or placebo on driving performance and cognitive function in healthy volunteers

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Objective This study aimed to evaluate the effects of repeated treatments with the sedative antidepressants mirtazapine and trazodone on driving performance and cognitive function.
Methods Nineteen healthy men received continuous nocturnal doses of 15-mg mirtazapine , 25-mg trazodone, or placebo for 8 days in a double-blinded, three-way crossover trial. Subjects were asked to perform three driving tasks (road tracking, car following, and harsh braking) using a driving simulator and cognitive tasks (the Wisconsin Card Sorting Test, Continuous Performance Test, and N-back Test) at baseline and on Days 2 and 9. Stanford Sleepiness Scale scores were also assessed.
Results Mirtazapine significantly increased the standard deviation of lateral position in the road-tracking task as compared with trazodone on Day 2. Mirtazapine significantly increased Stanford Sleepiness Scale scores as compared with trazodone and placebo. For the remaining tasks, no significant effects of treatment were observed.
Conclusions Acute treatment of mirtazapine impaired road-tracking performance and increased sleepiness, but sedative effects disappeared under repeated administrations. Trazodone did not affect driving performance or cognitive function under acute or repeated administrations. Both initial sedative effects and pharmacological profiles should be taken into consideration when using sedative antidepressants. Copyright © 2013 John Wiley & Sons, Ltd.

KEY WORDS—sedative antidepressant; mirtazapine; trazodone; driving performance; cognitive function

INTRODUCTION

Many antidepressants are available for psychiatric treatment, but pharmacological profiles of these drugs differ widely. The choice of antidepressant is determined by safety, tolerability, efficacy, payment, and simplicity, which are summarized by the mnemonic STEPS (Preskorn, 1996). Although sedation is one of the unpleasant side effects (Bourin and Briley, 2004), sedative antidepressants can represent a useful treatment option for some patients with agitation or insomnia (Mann, 2005; Linden and Westram, 2010).

Among the sedative antidepressants, tricyclic antidepressants (TCAs) show anticholinergic properties as well as sedative properties. Because of these properties, TCAs have been repeatedly shown to impair cognitive and psychomotor performance (Serretti *et al.*, 2010), including car driving (Ramaekers, 2003; Iwamoto *et al.*, 2008). Thus, non-sedating antidepressants may represent a better option (Bourin and Briley, 2004; Versiani *et al.*, 2005). However, the sedative antidepressants trazodone and mirtazapine are among the most commonly used drugs for chronic insomnia in the USA because of safety and lower dependence potential. Therefore, these two drugs need to be examined with respect to psychomotor performance in daily life, including car-driving skills.

Previous studies have suggested that mirtazapine could impair road-tracking performance (Wingen *et al.*, 2005). However, the effects of mirtazapine on driving skills associated with traffic accidents have not been fully investigated. Moreover, the effects of trazodone on

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driving skills have rarely been studied (Roth *et al.*, 2011). The aim of the study was to evaluate the effects of repeated treatment with mirtazapine or trazodone on driving performance using methods designed to test the risk of rear-end collisions, the most common type of traffic accidents.

MATERIAL AND METHODS

Nineteen healthy male volunteers (26–49 years old, mean ± standard deviation, 38.8 ± 6.8 years) were included through health interviews and the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition. All participants had a driving license for ≥5 years and regularly drove a car (minimum, 5000 km/year). The study was approved by the ethics committee of the Nagoya University Graduate School of Medicine and Nagoya University Hospital, and written informed consent was obtained from each individual before participation.

This study used a double-blind, placebo-controlled, three-way crossover design. Each subject received 8 days of continuous bedtime dosing with either 15-mg mirtazapine, 25-mg trazodone, or matched placebo in identical capsules across three different treatment series. The doses selected were clinical recommended starting doses (Sadock *et al.*, 2005). Dosing started at bedtime on Day 1, preceding the first test day (Day 2). A washout period of ≥7 days was provided between treatment series.

Baseline assessments were conducted only once before the treatment session. After baseline assessments without treatment, subsequent assessments were performed on Days 2 and 9 at 09:30 AM for each treatment series. We used a driving simulator (DS) (Toyota Central R&D Labs, Nagakute, Japan) to examine three driving skills that appeared to be associated with traffic accidents, including frequent rear-end collisions. The details of DS configuration and tasks have been described previously (Iwamoto *et al.*, 2008). The road-tracking test measures the standard deviation of lateral position (SDLP) on a gently winding road at a constant speed of 100 km/h. The car-following test measures the coefficient of variation of the distance between a preceding car and subject's own (Uchiyama *et al.*, 2003). Subjects were required to maintain a constant distance between cars. The harsh-braking test measures mean brake reaction time in seven braking trials to avoid crashing into the humanoid models that randomly ran into the road. Each test was recorded every 20 ms and lasted for 5 min. The three cognitive tests, described in detail previously (Iwamoto *et al.*, 2008), were examined using a computer. The modified version of the Wisconsin Card Sorting Test (Heaton, 1981) was used to measure executive function. This performance was measured by category achievement, perseverative errors of Nelson, and difficulty of maintaining set. The Continuous Performance Test, Identical Pairs version (Cornblatt *et al.*, 1988), was used to measure sustained attention. A series of four-digit stimuli were used, and performance was measured by the signal detection index *d*-prime, a measure of discriminability computed from "hits" and "false alarms." The *N*-back test (Callicott *et al.*, 2000, 2003) was used to assess working memory. A two-back condition was used, and performance was measured as the percentage of correct responses. All subjects were trained in both driving and cognitive tests 1–2 weeks before first testing until the plateau level. The Stanford Sleepiness Scale (SSS) (Hoddes *et al.*, 1973) is used to examine the level of alertness.

A two-way repeated-measures analysis of variance with time and drug as factors was used to analyze percentage changes in outcome variables over 8 days. If a significant interaction between factors was observed, these variables at each evaluation point were examined with one-way repeated-measures analysis of variance, followed by the Bonferroni *post hoc* test. All tests were two tailed, and the alpha value was set at 0.05.

RESULTS

In the road-tracking test, one subject administered mirtazapine failed to complete the test on Day 2. Because of technical malfunctions, car-following test, road-tracking test, and Continuous Performance Test data were incomplete for one subject, and harsh-braking test data were incomplete for two subjects. Only complete data sets were included in the analyses.

A summary of the results is shown in Table 1. There is a significant Drug × Time interaction in the road-tracking test ($F = 3.520$, $df = 2, 46$, $p = 0.023$). The SDLP of the mirtazapine condition was significantly greater than that observed in the trazodone condition on Day 2 ($p = 0.001$). The results of SDLP are presented in Figure 1. There is no significant Drug × Time interaction in other driving tests and cognitive tests.

There is a significant Drug × Time interaction in sleepiness ($F = 10.630$, $df = 1, 34$, $p < 0.001$). SSS scores under mirtazapine conditions were significantly greater than those observed under trazodone and placebo conditions on Day 2 ($p < 0.001$ each). Results of the SSS are presented in Figure 2.

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Table 1. Summary of the results of driving tests, cognitive tests, and subjective measurements in healthy subjects enrolled in a crossover trial of 15-mg mirtazapine, 25-mg trazodone, and placebo ($N = 19$)

Measure	Test time	Mean (*SD*)		
		Placebo	Mirtazapine (15 mg)	Trazodone (25 mg)
Driving test				
SDLP (cm)[a]	Baseline	42.4 (11.02)		
	Day 2	42.2 (12.32)	48.5 (11.61)	41.1 (11.65)
	Day 9	42.2 (11.26)	43.1 (10.69)	39.9 (9.59)
DCV[b]	Baseline	37.4(24.50)		
	Day 2	55.6 (87.45)	64.8 (75.46)	37.7 (30.79)
	Day 9	31.1 (19.45)	32.2 (26.33)	44.5 (42.44)
BRT (ms)[a]	Baseline	536.5 (46.57)		
	Day 2	528.4 (70.81)	539.6 (44.20)	526.5 (43.31)
	Day 9	524.1 (49.04)	543.6 (52.24)	529.8 (41.22)
Cognitive test				
CPT (d')[b]	Baseline	2.9 (0.75)		
	Day 2	3.3 (0.71)	3.0 (0.80)	3.3 (0.75)
	Day 9	3.2 (0.81)	3.2 (0.85)	3.4 (0.61)
WCST (CA)[c]	Baseline	5.6 (0.67)		
	Day 2	5.7 (0.71)	5.7 (0.64)	5.8 (0.52)
	Day 9	5.7 (0.57)	5.7 (0.57)	5.8 (0.67)
WCST (PEN)[c]	Baseline	0.7 (1.07)		
	Day 2	0.7 (1.07)	1.0 (2.27)	0.4 (0.67)
	Day 9	1.1 (1.48)	0.7 (1.16)	0.5 (0.94)
WCST (DMS)[c]	Baseline	0.3 (0.73)		
	Day 2	0.2 (0.52)	0.2 (0.49)	0.3 (0.73)
	Day 9	0.1 (0.31)	0.3 (0.57)	0.3 (0.44)
Two-back (accuracy, %)[c]	Baseline	93.6 (15.35)		
	Day 2	94.4 (10.76)	90.6 (12.05)	87.2 (23.12)
	Day 9	97.0 (6.27)	92.1 (12.67)	94.0 (11.65)
Subjective measurement (SSS)[c]	Baseline	2.3 (0.46)		
	Day 2	2.4 (0.74)	3.8 (1.15)	2.3 (0.46)
	Day 9	2.4 (0.49)	2.7 (0.65)	2.4 (0.58)

Baseline data were assessed once before treatment.
SDLP, standard deviation of lateral position; DCV, distance coefficient of variation; BRT, brake reaction time; CPT, Continuous Performance Test; WCST, The Wisconsin Card Sorting Test; CA, category achievement; PEN, perseverative errors of Nelson; DMS, difficulty of maintaining set; SSS, Stanford Sleepiness Scale.
[a] $n = 17$
[b] $n = 18$
[c] $n = 19$

DISCUSSION

The present results demonstrated that mirtazapine significantly impaired road-tracking performance and increased subjective sleepiness in acute dosing. No other performances were significantly affected by any treatment condition during the 8 days. The effects of mirtazapine on diving performances in this study are roughly consistent with data shown in previous studies (Ramaekers *et al.*, 1998; Ridout *et al.*, 2003; Wingen *et al.*, 2005).

Mirtazapine also did not impair car-following performance in this study. To the best of our knowledge, this represents the first study to demonstrate the effects of mirtazapine on car-following performance. Whereas the road-tracking test requires subjects to handle a wheel rather than manipulate the pedals, the car-following test requires subjects to constantly switch between accelerator and brake pedal rather than handle a wheel. This means that the road-tracking test is a comparatively monotonous visuomotor task, whereas the car-following test is a more complex executive function task. Wezenberg *et al.* (2007) suggested that mirtazapine was likely to impair simpler cognitive tasks requiring less cognitive effort. Mirtazapine may tend to affect monotonous driving tasks such as the road-tracking test. Meanwhile, amitriptyline, a TCA, impaired both road-tracking and car-following performances in our DS (Iwamoto *et al.*, 2008). Its anticholinergic activity may harm car-following performance, as more cognitive effort is required (Sakulsripong *et al.*, 1991, Curran *et al.*, 1998). The difference of the effects of these sedative antidepressants on driving performance may be explained by the pharmacological properties. On the other hand, for braking performance, mirtazapine and amitriptyline did not impair brake reaction time (Iwamoto *et al.*, 2008), but diazepam, a benzodiazepine,

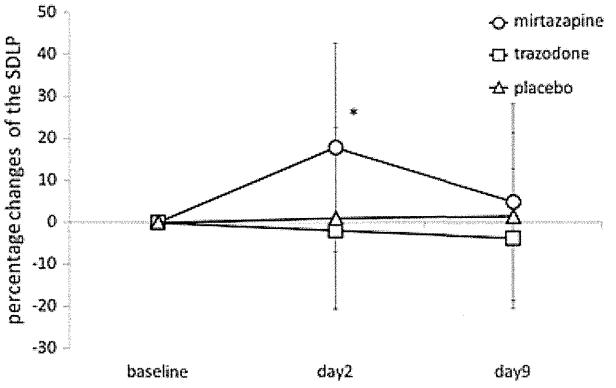

Figure 1. Mean (standard deviation) standard deviation of lateral position (SDLP) after 2 and 9 days of crossover treatment with 15-mg mirtazapine, 25-mg trazodone, and placebo ($N = 17$). Differences in SDLP were examined by a two-way repeated-measures analysis of variance. Differences in SDLP at each evaluation point were examined with a one-way repeated-measures analysis of variance followed by a Bonferroni test (*post hoc* test). A significant Drug × Time interaction was noted among the three conditions ($F = 3.520$, $df = 2,46$, $p = 0.023$). **Post hoc* testing demonstrated that SDLP of the 15-mg mirtazapine condition was significantly greater than that of the 25-mg trazodone condition on Day 2 ($p = 0.001$).

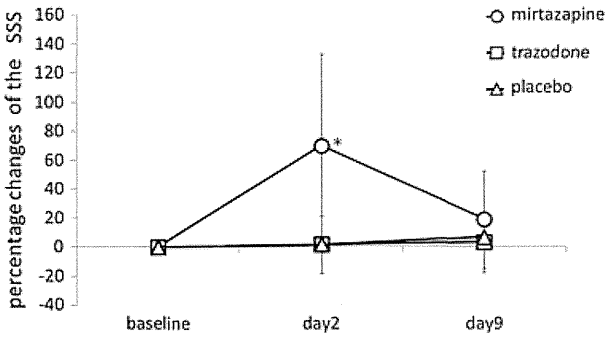

Figure 2. Mean (standard deviation) Stanford Sleepiness Scale (SSS) after 2 and 9 days of crossover treatment with 15-mg mirtazapine, 25-mg trazodone, and placebo ($N = 19$). Differences in SSS were examined by a two-way repeated-measures analysis of variance. Differences in SSS at each evaluation point were examined by a one-way repeated-measures analysis of variance followed by a Bonferroni test (*post hoc* test). Significant Drug Time interactions were seen among the three conditions ($F = 10.630$, $df = 1$, 34, $p < 0.001$). **Post hoc* testing demonstrated that SSS of the 15-mg mirtazapine condition was significantly greater than that observed in the placebo and 25-mg trazodone conditions on Day 2 ($p < 0.001$ each).

did result in impairments (Takahashi *et al.*, 2010). The harsh-braking task is likely to be affected by a peripheral muscle relaxant effect rather than a cognitive detrimental effect. Because subjective assessments and psychometric tests did not fully predict drug effects on driving performance (Verster and Roth, 2012a, 2012b), further researches are needed to elucidate the impact of psychotropics on car-driving performance.

The present study showed that 25-mg trazodone did not impair both driving and cognitive performances, although the previous study showed that 50-mg trazodone did not impair driving performance but affected memory and learning (Roth *et al.*, 2011) and that more than 100 mg of trazodone affected memory and attention (Curran *et al.*, 1998; Sakulsripong *et al.*, 1991). Although these differences may be attributable to the dosage of trazodone and cognitive tasks, pharmacological profiles of trazodone may be also in part responsible for these results. The sedative effect of trazodone is associated with its high affinity to the histamine H1 receptor; however, trazodone has features of a weak anticholinergic activity and short half-life (Bryant and Ereshefsky, 1982). Therefore, a low dose of trazodone may produce no detrimental effects on psychomotor performance as antihistamines have dose-dependent effects on psychomotor performance including driving performance (Theunissen *et al.*, 2004).

In the present study, the sedative effects of mirtazapine were no longer apparent on Day 9. According to pharmacological profiles, mirtazapine is a strong histamine H1 receptor antagonist without anticholinergic activity, and its activity contributed to detrimental effects. In assessing sedative properties with medications, an important issue is the degree to which tolerance to the sedative effect develops. Tolerance to sedative effects of mirtazapine may develop rapidly, as with histamine H1 antihistamines (Richardson *et al.*, 2002). Development of tolerance may apply equally to repeated doses of trazodone. Meanwhile, TCAs often exert an anticholinergic activity that can cause different detrimental effects on cognitive performance. In the case of amitriptyline, tolerance to sedative effects, based on subjective and behavioral measures, develops within 1–2 weeks (Deptula and Pomara, 1990; Veldhuijzen *et al.*, 2006), although several studies have indicated intolerance of amitriptyline based on several cognitive measures (Sakulsripong *et al.*, 1991; van Laar *et al.*, 2002). Anticholinergic properties should thus be considered in cases of tolerance to sedative antidepressants.

The effects of antidepressant on driving performance are different in healthy subjects and psychiatric patients and are also influenced by age and gender of the subjects. In addition, both the psychopharmacological treatment and the pathology itself may impair driving ability. Recent epidemiological studies showed that exposure to antidepressants including selective serotonin reuptake inhibitors was associated with an increased risk of motor vehicle accidents, unlike with past studies (Meuleners *et al.*, 2011; Chang *et al.*, 2013). As for the experimental studies, newer antidepressants, unlike TCAs, have no detrimental effects on driving performance (Ramaekers, 2003), and mirtazapine could also improve driving ability in depressed patients (Brunnauer *et al.*, 2008; Shen *et al.*, 2009). These discrepancies may

be explained in part by age, dosage, dosing period, active depressive symptom, comorbid psychotropic drugs, and methodological variances (Sansone and Sansone, 2009). Especially, benzodiazepines often prescribed in clinical settings may increase the risk of motor vehicle accidents (Dassanayake *et al.*, 2011). Meanwhile, many depressed patients before hospital discharge showed impairments in psychomotor functions related to driving abilities, and those were influenced by different classes of antidepressants (Brunnauer *et al.*, 2006). The effects of antidepressants on driving ability in depressed patients under treatment have not yet been fully defined because of many confounding factors such as psychopharmacological treatment and the depression itself. Thus, it is important to examine the effects of antidepressants on driving performance in healthy subjects to find the inherent influences of antidepressants for driving impairments. However, future studies need to elucidate the impact of similar antidepressants in depressed patients in a similar experimental line and make a comparison with depressed patients.

The present study has several limitations. First, participation was restricted to healthy adult volunteers, and the sample size is relatively small. Neither elderly nor patient populations were included in the study. The elderly are more vulnerable to the side effects of pharmacological treatments. In addition, depression and insomnia can affect driving performance (Brunnauer *et al.*, 2008; Shen *et al.*, 2009) and cognitive function. Both properties of antidepressant and disorder should be considered in clinical settings. Second, the validity and sensitivity of the DS need to be considered; however, our past results using same DS are roughly consistent with preceding results (Iwamoto *et al.*, 2008; Takahashi *et al.*, 2010). Although cognitive tasks used in this study were employed in many psychiatric researches and our past studies, the sensitivity of these tasks regarding the assessment for drug effects should be considered, too. Third, dosage selection may be lower than that of past studies, because we used the initial starting dose for clinical practice. Considering an affinity for histamine H1 receptor in particular, the dose of trazodone may be low in comparison with that of mirtazapine.

Finally, acute treatments of mirtazapine did not impair car-following or harsh-braking performances but did impair road-tracking performance, although this impairment disappeared under repeated administrations. The lower dose of trazodone did not affect driving or cognitive performances under acute or repeated administrations. Both initial sedative effects and pharmacological profiles should be taken into consideration in prescribing sedative antidepressants.

CONFLICT OF INTEREST

The authors have no conflicts of interest directly relevant to the content of this study.

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ORIGINAL INVESTIGATION

Effects of sedative antidepressants on prefrontal cortex activity during verbal fluency task in healthy subjects: a near-infrared spectroscopy study

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Abstract
Rationale Japanese researchers have recently conducted studies using near-infrared spectroscopy (NIRS) to help diagnose psychiatric disorders based on changes in brain activity. However, the influence of psychotropic drugs on NIRS measurements has not been clarified.
Objective To assess the effects of sedative antidepressants on prefrontal cortex activity in healthy subjects using NIRS in a double-blinded, placebo-controlled, crossover trial.
Methods Nineteen healthy males received nocturnal doses of mirtazapine 15 mg, trazodone 25 mg, or placebo for eight consecutive days in rotation, with a washout period of more than 1 week between each rotation. Subjects performed a verbal fluency task during NIRS on a total of seven occasions during the study period: more than a week prior to receiving the first dose of the first medication; and on days 2 and 9 of each rotation. The number of words correctly generated during the task (behavioral performance) was also recorded. Stanford Sleepiness Scale (SSS) scores were determined each day.
Results Mirtazapine 15 mg significantly increased oxyhemoglobin (oxy-Hb) concentration change in NIRS on day 9, compared to trazodone 25 mg and placebo. Mirtazapine 15 mg significantly increased SSS on day 2, compared to the other conditions. No significant differences in behavioral performance were observed.
Conclusions Administration of mirtazapine for eight consecutive days affected oxy-Hb changes on NIRS. This result indicates that researchers should consider how certain types of antidepressant could influence brain function when the brain activity of patients with psychiatric disorders is assessed.

Keywords Antidepressant · Mirtazapine · Trazodone · Near-infrared spectroscopy (NIRS) · Brain activity · Sleepiness · Neuroimaging

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Introduction

Near-infrared spectroscopy (NIRS) is a noninvasive functional brain imaging technique that utilizes the absorbance of light in the near-infrared spectrum by hemoglobin (Hb) to determine blood volumes in an anatomical region of interest. NIRS has been gaining attention recently because of its relatively high temporal resolution and the compactness of the measurement devices. These features make NIRS

suitable for testing, as experimental procedures can be performed under conditions that are close to natural. Many studies have confirmed the suitability of NIRS for various types of investigations. Frontal lobe activity as measured by NIRS has been suggested to be decreased in many psychiatric disorders (i.e., major depressive disorder, bipolar disorder, schizophrenia, panic disorder, eating disorder, attention deficit hyperactivity disorder, Alzheimer's dementia, and alcoholism).(Kameyama et al. 2006; Schecklmann et al. 2007; Suda et al. 2010; Suto et al. 2004).

Age, sex, and sleepiness have been indicated as factors that may influence Hb concentration changes in NIRS, in addition to the influences attributable to disease (Kameyama et al. 2004; Suda et al. 2008). We have previously reported that insufficient sleep could lower the peak oxyhemoglobin (oxy-Hb) concentration in the lateral frontal lobes (Miyata et al. 2010). In addition, the effects of psychotropic drugs on NIRS measurements have yet to be clarified.

Many studies have investigated the influence of drugs on brain function using functional brain imaging. Such studies have been performed using positron emission tomography (PET), single photon emission computed tomography, and functional magnetic resonance imaging (fMRI). Conversely, only a few studies have used NIRS to directly verify these influences. Tsujii et al. (2009, 2007) examined the effects of antihistamines on brain activity. Comparing the first-generation histamine H1-receptor antagonist ketotifen to the second-generation epinastine, they revealed that ketotifen significantly impaired cortical activation in the lateral prefrontal cortex.

Given these findings, the present study used NIRS to examine whether taking an antidepressant for consecutive days can affect brain activity in healthy subjects. In light of revelations from previous studies that insufficient sleep could lower cortical activation and that the sedative effects of antihistamines impair neural response, we verified the effects of an antidepressant itself on brain activity in healthy volunteers using multichannel NIRS with the sedative antidepressants mirtazapine and trazodone, both of which exert strong sedative/hypnotic effects.

Subjects and methods

Subjects

Participants in this study comprised 19 healthy, male Japanese volunteers who were right-handed (mean age, 38.8 years; SD, 6.8 years; range, 26–49 years). The study protocol was approved by the ethics review committees at Nagoya University Graduate School of Medicine and Nagoya University Hospital. Written informed consent was obtained from all participants prior to enrolment in the study. All subjects were interviewed to confirm the absence of any psychiatric disorders using the Structured Clinical Interview for DSM-IV by one of the experimenters. All subjects were found to be in good health without any significant clinical history of physical or mental illness and were not receiving any concomitant medications likely to affect brain function.

Drug administration and study design

A double-blind, placebo-controlled, crossover design was used. Before going to bed, subjects took orally either mirtazapine 15 mg, trazodone 25 mg, or placebo for eight consecutive days. After finishing eight consecutive days of the first drug, the subject then went more than 1 week without medication as a washout period before proceeding to the next drug. The second and third drugs were administered in the same manner as the first drug. The order of drugs that subjects took was allocated based on a pre-determined randomization schedule. In total, subjects were required to come to the study room seven times. The first visit was more than a week prior to receiving the first medication (day-pre). Subsequently, each subject came on day 2 (three times during the study) and day 9 (three times during the study) during administration. The study schedule is shown in Fig. 1. Doses were determined according to the initial dosages recommended in a manual for psychiatric drug treatment, because we considered that one of the aims of this study was to evaluate sleepiness as a side effect (Sadock et al. 2005). Each examination started at 0900 hours. Subjects completed a verbal fluency task, during which prefrontal cortical activity was measured using a NIRS recorder. In addition, subjective sleepiness at the time of the examination was evaluated using the Stanford Sleepiness Scale (SSS).

Activation task

Hb concentration changes were measured during a letter-version verbal fluency task that has been administered in many previous studies. The subject sat on a comfortable chair in a

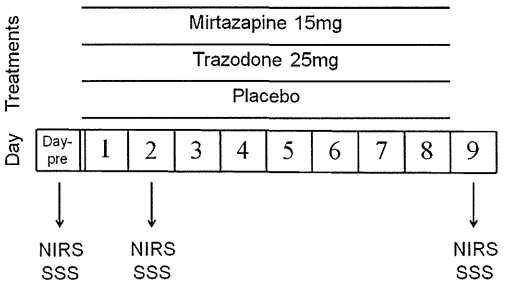

Fig. 1 Study design. Subjects were to be measured more than a week prior to receiving the first medication (day-pre), on days 2 (three times during the study) and 9 (three times during the study). *NIRS* NIRS measurement, *SSS* Stanford Sleepiness Scale