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Motomura K, Kanba S	Lost in translation: Confusion about depression and antidepressant therapy in Japan	Psychiatry Clin Neurosci	67(1)	1-2	2013
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Editorial

Lost in translation: Confusion about depression and antidepressant therapy in Japan

ANTIDEPRESSANT THERAPY IS now the subject of a nation-wide controversy in Japan. Japanese psychiatrists are being accused of dubious diagnoses, excessive prescriptions and insufficient understanding of personal mentalities. What has happened to us?

Japanese psychiatrists had preserved the endogenous–neurotic dichotomy of depression and had regarded intensive pharmacotherapy (with tricyclic antidepressants) as treatment for endogenous depression until around the turn of the century when selective serotonin re-uptake inhibitors were introduced to Japan.[1,2] We then came to know that effects of newer antidepressants were established through randomized controlled trials that recruited patients with *major depression*. Thereafter, results of numerous clinical trials were introduced and the language of the psychiatrist changed drastically, while we hardly grasped the *gestalt* of major depression. Is this a problem inherent in the concept of the disease, or have we failed to import anything attached to it? Apart from the cultural context, here we try to analyze what in the concept of major depression confuses us.

When major depressive disorder appeared in the Research Diagnostic Criteria, it was described as a category encompassing patients of various subtypes, including 'some cases that would be categorized as neurotic depression, and virtually all that would be classified as involutional depression, psychotic depression, and manic depressive illness, depressed type.'[3] Although several subtypes of depressive disorder were provided, the concept of major depression seemed to reject a clear-cut separation of these types. Such criticism of the classification of depression was developed by British psychiatrists early in the last century through meticulous clinical observation,[4] and it is still echoed in several recent epidemiological studies that demonstrate the entangled effects of environmental and genetic factors on the onset of major depression.[5] Empirically, we appreciate the importance of this critical attitude toward simplistic attribution; the more closely a patient is studied, the harder it becomes to justify simple qualitative distinctions. Detailed knowledge about the environmental and constitutional factors of each patient is crucial for treatment. For example, a patient who shows a seemingly reactive onset may actually have considerable genetic risk, whereas another patient with endogenomorphic features may be affected by an unstable relationship with his or her spouse. When we disentangle the threads, we have to draw them one after another. Similarly, for each patient, effective treatment (whether it is pharmacotherapy, cognitive modification or assistance with realistic problem solutions) may vary at each stage.

However, the effectiveness of such personalized methods of treatment is difficult to demonstrate in clinical trials. The evidence level in the treatment of such a heterogeneous disease may reflect methodological difficulties rather than actual importance in clinical practice. Most clinical trials recruit numerous patients with major depression irrespective of such diversity, resulting in generalizable findings with modest significance. When clinicians other than the specialists are informed of these results, they understand only the generalizable relationship between major depression and its treatment response. The critical attitude toward simplification, which established the concept of the disease, is virtually lost, and there remains only a broad category that is susceptible to medicalization. Ironically, what was once a criticism of simplification now serves as the grounds for further simplification.

Recognizing this process may be helpful in analyzing the ongoing conflict over bereavement exclusion criteria. While Kendler argues for the elimination of the criteria relying on the prudence of 'good' psychiatrists,[6] Horwitz and Wakefield address the potential harm of over-diagnosing depressive disorders by general physicians in the primary-care setting[7] who have been informed about simplified concepts

and generalizable research findings. So goes the simplification process of psychiatry during its propagation from specialists to general physicians, from one generation to another[8] and, in our case, from one culture to another.

An additional problem with major depression is its threshold. Based on the current diagnostic criteria, the category of major depressive disorder shows a poor correlation with treatment response, contrary to the expectations of most non-specialist clinicians. With the required number of symptoms, the threshold may be too low to predict responses to antidepressants and too high to judge the requirement for clinical attention.[9]

We suggest that recognition of these two points, the simplification process and the inappropriate threshold, will contribute to the understanding of how various problems related to major depression have emerged and how to minimize confusion related to these problems.

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ARCHIVAL REPORT

A Population-Specific Uncommon Variant in *GRIN3A* Associated with Schizophrenia

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Background: Genome-wide association studies have successfully identified several common variants showing robust association with schizophrenia. However, individually, these variants only produce a weak effect. To identify genetic variants with larger effect sizes, increasing attention is now being paid to uncommon and rare variants.

Methods: From the 1000 Genomes Project data, we selected 47 candidate single nucleotide variants (SNVs), which were: 1) uncommon (*minor allele frequency* <5%); 2) Asian-specific; 3) *missense, nonsense, or splice site variants predicted to be damaging; and* 4) *located* in candidate genes for schizophrenia and bipolar disorder. We examined their association with schizophrenia, using a Japanese case-control cohort (2012 cases and 2781 control subjects). Additional meta-analysis was performed using genotyping data from independent Han-Chinese case-control (333 cases and 369 control subjects) and family samples (9 trios and 284 quads).

Results: We identified disease association of a missense variant in *GRIN3A* (p.R480G, rs149729514, $p = .00042$, odds ratio [OR] = 1.58), encoding a subunit of the *N*-methyl-D-aspartate type glutamate receptor, with study-wide significance (threshold $p = .0012$). This association was supported by meta-analysis (combined $p = 3.3 \times 10^{-5}$, OR = 1.61). Nominally significant association was observed in missense variants from *FAAH, DNMT1, MYO18B,* and *CFB,* with ORs of risk alleles ranging from 1.41 to 2.35.

Conclusions: The identified SNVs, particularly the *GRIN3A* R480G variant, are good candidates for further replication studies and functional evaluation. The results of this study indicate that association analyses focusing on uncommon and rare SNVs are a promising way to discover risk variants with larger effects.

Key Words: Bipolar disorder, *CFB, DNMT1, FAAH, GRIN3A, MYO18B,* NR3A, rare variants

Genome-wide association studies (GWAS), which typically examine millions of common (minor allele frequency [MAF] >5%) single nucleotide polymorphisms (SNPs), have been highly successful in identifying genetic variants reproducibly associated with complex human traits (1). Several large-scale GWAS for schizophrenia have been conducted (2–8) and have provided important findings, for example: 1) the identification of genetic loci such as 6p21-p22.1 (major histocompatibility complex region), 1p21.3 (*MIR137*) and 18q21.2 (*TCF4*), associated with schizophrenia at a genome-wide significance level ($p < 5 \times 10^{-8}$); 2) the proposal of a genetic architecture for schizophrenia, which is most likely extremely polygenic, involving possibly thousands of common SNPs conferring a disease risk; and 3) support for genetic overlap between schizophrenia and bipolar disorder, based on the overall profiles of common SNPs and top hit genes, such as *CACNA1C.*

As is the case for most complex diseases, the risk-conferring common SNPs identified so far only have small effect sizes, with odds ratios (OR) predominately ranging from 1.1 to 1.3. Although a recent study indicated that overall profiles of common SNPs could explain a significant part of the variation in liability to schizophrenia (9), single common SNPs have minimal predictive value and it is uncertain whether they contribute greatly to unraveling the pathogenetic mechanisms that lead to schizophrenia. To discover genetic variants with larger effect sizes, emphasis has shifted toward analyzing uncommon (MAF <5%) or rare (MAF <1% or .1%) single nucleotide variants (SNVs), especially those that directly affect protein coding.

Along with a growing interest in rare variants, recent advances in sequencing technology, particularly the emergence of the next-generation sequencing techniques, have enabled us to obtain large volumes of sequence data in a rapid and cost-effective manner. A number of novel and interesting findings have been reported in pioneering works that utilized next-generation sequencing. In studies of psychiatric and neurodevelopmental diseases, involvement of pathogenic de novo SNVs in intellectual disability, autism spectrum disorder, and schizophrenia has been demonstrated (10–18). In studies of nonpsychiatric complex diseases, for example, sick sinus syndrome (19), inflammatory bowel disease (20), and celiac disease (21), uncommon

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protein-affecting variants with larger effects have been identified by resequencing and fine-mapping analyses of loci detected by GWAS. In a resequencing analysis of candidate genes for impulsivity, an uncommon nonsense variant in *HTR2B* is reported to be associated with psychiatric diseases marked by impulsivity (22). The *HTR2B* nonsense variant reported in that study was exclusive to Finns, highlighting the point that uncommon variants are often population-specific. Therefore, intensive studies using non-Caucasian samples provide an opportunity to discover novel, uncommon risk variants with larger effect sizes that cannot be detected in the Caucasian population, the most extensively investigated population in genetic studies to date.

In addition to analysis of de novo variants, fine mapping of loci previously identified in GWAS, and resequencing of targeted candidate regions, we can now utilize dense catalogs of human genetic variations, including uncommon SNVs created by next-generation sequencing projects, such as the 1000 Genomes Project (23) and the Exome Sequencing Project (24), albeit that the latter was confined to European American and African American samples. Uncommon SNVs in these projects were found in seemingly healthy subjects, contrary to extremely rare variants that are robustly associated with psychiatric disorders, such as autism spectrum disorder and schizophrenia, and have been identified exclusively in case samples. However, these uncommon SNVs could represent another class of variants that go some way to provide an explanation for the as yet undiscovered genes responsible for disease heritability. Association analysis of these variants is another promising strategy for identifying genetic risks for common diseases.

In this study, we selected candidate SNVs from the 1000 Genomes Project data, focusing on uncommon, population-specific, and protein-damaging variants. A total of 47 candidate SNVs satisfied the criteria of being: 1) uncommon; 2) missense, nonsense, or splice site variants predicted to be damaging; 3) Asian-specific; and 4) located in candidate genes for schizophrenia and bipolar disorder. These SNVs were subjected to association analysis using a Japanese case-control cohort, consisting of 2012 patients with schizophrenia and 2781 healthy control subjects. We also performed meta-analysis using genotyping data from an independent Han-Chinese case-control set (results from 333 cases and 369 control subjects, previously reported by another group) and family samples (9 trios and 284 quads, genotyped by our laboratory for this study), to analyze the most significantly associated SNV in a Japanese case-control study.

Methods and Materials

Subjects

We used a Japanese case-control sample, consisting of 2012 unrelated patients with schizophrenia (1111 men, 901 women; mean age 48.1 ± 14.4 years) and 2781 control subjects (1197 men, 1584 women; mean age 43.7 ± 14.4 years). We also used 865 samples from unrelated Japanese patients with bipolar disorder (425 men, 440 women; mean age 50.2 ± 14.4 years; 584 with bipolar I disorder, 276 with bipolar II disorder, and 5 with schizoaffective disorder bipolar type) for additional analysis. Samples from bipolar patients and some of the control participants were recruited through the Collaborative Study of Mood Disorders consortium (25). All participants were of Japanese origin and were recruited from the Hondo area of Japan. Populations in the Hondo area are known to fall into a single genetic cluster (26). In our previous analysis using a subset of the same participants, it was shown that $Pr(K = 1)$ (probability that the number of populations present in the sample = 1 [27]) was larger than .99 (28,29) and λ (genomic control factor [30]) was 1.074 (31). These data indicated a negligible population stratification effect in our Japanese samples. All patients had a consensual diagnosis of schizophrenia, according to DSM-IV criteria, from at least two experienced psychiatrists. All healthy control subjects were psychiatrically screened in unstructured interviews. All control subjects and patients gave informed, written consent to participate in the study after being provided with and receiving an explanation of study protocols and objectives.

We also genotyped Han-Chinese samples from mainland China and Taiwan, consisting of 293 schizophrenia pedigrees (1163 subjects: 9 trios and 284 quads, offspring were all affected) (32). This sample set was collected by the National Institute of Mental Health initiative (http://nimhgenetics.org/). Diagnoses of these samples were made using the Diagnostic Interview for Genetic Studies (33) and the Family Interview for Genetic Studies (34), based on DSM-IV-Text Revision or DSM-IV criteria.

In the meta-analysis, genotyping data collated in a report by Shen *et al.* (35) was included. In that study, case-control samples of Han-Chinese in Taiwan (333 cases and 369 control subjects) were used. All patients in this cohort were diagnosed by senior psychiatrists using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders. Exclusion criteria included psychosis due to general medical conditions, substance-related psychosis, and mood disorder with psychotic features. Mental status of control participants was evaluated by senior psychiatrists in diagnostic interviews.

This study was approved by the ethics committees of all participating institutes.

Selection of Single Nucleotide Variants

We selected candidate SNVs from the 1000 Genome Project data, released on December 16, 2010 (23). In this version, 22,891,767 SNVs with PASS flags for quality controls were included. Among them, 11,573,027 SNVs were detectable in Asians, of which 1,911,710 were Asian-specific. From these, we selected variants using the following criteria: 1) missense or splice site variants; 2) variants located within the 1568 genes registered in SZGene (36) or the Bipolar Disorder Gene Database (37) (accessed in January 2011); 3) variant calls with a Phred score >10, indicating a call accuracy of >90%; 4) variants with MAF <5% in Asians; and 5) variants with MAF >0 and <5% in the Japanese population. A total of 308 variants remained after the selections. These included one variant located at an essential splice site. There were no nonsense variants. To narrow down the list further, we evaluated functionality of the remaining missense variants, using conservation scores from 46 vertebrate species (phyloP score, collected from the University of California Santa Cruz (UCSC) genome browser [38] [University of California, Santa Cruz, California]) and two programs predicting the impact of missense variants (PolyPhen2 [39] and SIFT [40]). Finally, 47 SNVs, comprising one essential splice site variant and 46 putatively functional missense variants (a phyloP score >2, predicted to be probably or possibly damaging by PolyPhen2 and predicted to be damaging by SIFT) were selected. The process of SNV selection is summarized in Table 1.

Genotyping

Genotyping of Japanese case-control samples was performed using the iPlex Assay on the Sequenom MassARRAY platform (SEQUENOM San Diego, California), according to the manufacturer's

Table 1. Number of SNVs Passing Each Filtering Criterion

Filtering Criteria	Number of SNVs Remaining
With PASS Flag	22,891,767
In Asian	11,573,027
Only in Asian	1,911,710
In Coding Regions (UCSC Genes hg19)	21,679
Nonsense, Missense, or Splice Site	14,305
In SZGene or Bipolar Disorder Gene Database (January 2011, 1568 Genes)	1195
Phred Score >10	392
MAF in Asian <.05	308
MAF in Japanese 0 < and < .05	
phyloP >2 and Damaging in PolyPhen2 and SIFT Prediction or in Essential Splice Sites (or Nonsense)	47 (one splice site and 46 missense variants)

MAF, minor allele frequency; SNV, single nucleotide variant; UCSC, University of California Santa Cruz.

instructions. When primers for the iPlex assay could not be designed or genotyping scatter plots were not well clustered, we first tested the candidate base positions for variant alleles using Sanger sequencing. For this test, we used 384 samples consisting of 128 samples each of patients with schizophrenia, patients with bipolar disorder, and controls subjects. These were a part of the samples described in the Subjects section. Single nucleotide variants validated by this analysis were then genotyped using the TaqMan SNP genotyping assay (Applied Biosystems, Grand Island, New York). To evaluate the genotyping accuracy of the multiplex iPlex Assays, we included two previously analyzed SNPs (rs2279381 and rs3763627 [41] and unpublished data [Kazuo Yamada, MD, PhD, *et al.*]) that had been genotyped using the TaqMan assay. Genotyping of Chinese schizophrenia pedigree samples collected by the National Institute of Mental Health initiative was performed using the TaqMan assay.

Quality Control and Statistical Analyses

Quality control of genotyping data was performed using the following exclusion criteria: 1) samples with a call rate of <80%; 2) SNVs with a call rate of <98%; 3) SNVs showing significant deviation from Hardy-Weinberg equilibrium (HWE) in control samples ($p < .001$); and 4) SNVs showing a significant difference of success rate (that is, the proportion of individuals with successful genotype data) between cases and control subjects ($p < .001$). Allelic association was analyzed using Fisher's exact test. For analysis of the *CACNA1F* S671C variant (rs143938580) located on chromosome X, one allele per male sample and two alleles per female sample were counted. Analyses of deviation from HWE, success rate for genotyping, allelic association, and the transmission disequilibrium test (TDT) were performed using PLINK software (version 1.0.7) (42). McNemar's exact test for TDT was performed using the exact2×2 package for R (43) (http://www.r-project.org/). Meta-analysis of case-control and TDT data sets and evaluation of sample heterogeneity were performed using the case-control and TDT meta-analysis package, catmap for R (44). The *Q* statistic (45) calculated by catmap was used for the assessment of heterogeneity among data sets. Post hoc calculations of statistical power were performed using the Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) (46) with the multiplicative model.

Results

Allelic Association Analyses

The results of Japanese case-control allelic association analyses are shown in Table 2, while detailed information about selected SNVs and the filtering criteria are shown in Table 1. The *GRIN3A* R480G variant (rs149729514) was most significantly associated with schizophrenia ($p = .00042$, OR = 1.58; two-tailed Fisher's exact test). From the number of SNVs whose association could be tested in this study (43 SNVs), we defined a study-wide significance threshold as .0012 (= .05/43). Association of the *GRIN3A* R480G variant ($p = .00042$) was more significant than this study-wide significance threshold. Nominal association was observed for *FAAH* A356V (rs77101686, $p = .010$, OR = .71), *DNMT1* G876R (rs62621087, $p = .031$, OR = 1.53), *MYO18B* E559K (rs117243697, $p = .032$, OR = 2.35), and *CFB* R576H variants (rs117314762, $p = .046$, OR = .7).

From 47 selected SNVs, we observed no variant alleles for three base positions in our population (*ADRA1A* R376H: rs140512348, *FAT1* T3326A: no rs number, and *HDAC9* G32R: rs79608746). One SNV (*DDX39B* R259C: rs145191873) failed quality control, due to a low call rate. Genotypic distribution of the *CD36* P90S variant (rs75326924) in control subjects deviated from HWE, with nominal significance ($p = .010$). Significant difference in the success rate of genotyping was not observed for any SNVs. The consistency between multiplex iPLEX genotyping in this study and TaqMan genotyping in our previous experiments was >99.9% for rs2279381 and 100% for rs3763627, proving the accuracy of genotyping by the iPLEX Assay.

Replication Analysis and Meta-Analysis of the *GRIN3A* R480G Variant

To test the validity of association between *GRIN3A* R480G variant and schizophrenia, we performed replication and meta-analyses, using two sets of data from independent samples.

In 2009, Shen *et al.* (35) resequenced *GRIN3A* using samples from the Han-Chinese population in Taiwan, consisting of 333 patients with schizophrenia and 369 control subjects. They detected the R480G variant as an uncommon SNV, along with a nonsignificant overrepresentation of the G allele (Table 3, $p = .145$, OR = 1.52, one-tailed Fisher's exact test). Although it has been demonstrated that the genetic clusters generated by Japanese and Han-Chinese populations are different from each other (26), MAFs of the *GRIN3A* R480G variant in our Japanese samples and their Han-Chinese samples from Taiwan were similar (2.1% in Japanese control subjects and 1.9% in Han-Chinese control subjects).

We also analyzed Han-Chinese pedigree samples, consisting of 9 trios and 284 quads with affected offspring. We successfully genotyped 565 out of 577 offspring and their parents and observed a nominally significant overtransmission of the G allele (Table 3, $p = .036$, OR = 1.92, one-tailed McNemar's exact test).

Combining the three data sets, meta-analysis further supported association between the R480G variant and schizophrenia (Table 3 and Fig. 1, combined $p = 3.3 \times 10^{-5}$, OR = 1.61). In this meta-analysis, we included all probands from trio and quad samples. This result did not change significantly when we randomly selected one proband from each pedigree (combined $p = 6.5 \times 10^{-5}$, OR = 1.60). As there was no significant heterogeneity among three data sets (*Q* statistic $p = .85$), the fixed-effect model was applied for meta-analysis.

Association Analysis of the *GRIN3A* R480G Variant with Bipolar Disorder

There is accumulating evidence to support the existence of overlap in the genetic susceptibility to schizophrenia and bipolar

Table 2. Results of the Allelic Association Analysis

Chr	Position	dbSNP ID	First Appeared[a]	Reference	Variant	Gene Symbol	SNV Property	PhyloP	PolyPhen2	SIFT	MAF		*p* Value	OR
											Cases	Control Subjects		
1	19,071,356	rs57227966	Build 129	G	A	*PAX7*	R484H	5.68	Probably damaging	Damaging[b]	.95	1.28	.14	.74
1	46,874,246	rs77101686	Build 131	C	T	*FAAH*	A356V	4.18	Probably damaging	Damaging	2.19	3.06	.010[c]	.71
2	216,248,878	rs147655202	Build 134	C	T	*FN1*	V214M	2.57	Probably damaging	Damaging	1.61	1.51	.74	1.07
3	58,135,711	rs141559684	Build 134	G	A	*FLNB*	A2107T	6.42	Probably damaging	Damaging	.90	.91	1.00	.99
3	58,140,563	rs138327769	Build 134	C	G	*FLNB*	S2258C	4.46	Probably damaging	Damaging	.12	.18	.61	.69
3	170,732,328	rs1800572	Build 89	C	T	*SLC2A2*	V101I	5.86	Probably damaging	Damaging	3.37	2.68	.050	1.27
4	187,531,047	Not registered	/	T	C	*FAT1*	T3326A	4.95	Probably damaging	Damaging		Variant allele was not observed		
5	131,325,201	rs3763118	Build 107	C	T	*ACSL6*	V126M	3.27	Probably damaging	Damaging	.55	.89	.070	.62
5	168,201,351	rs2288792	Build 100	C	T	*SLIT3*	R395Q	6.1	Probably damaging	Damaging	1.00	1.12	.61	.89
6	31,503,104	rs145191873	Build 134	G	A	*DDX39B*	R259C	2.21	Probably damaging	Damaging[b]		QC failed		
6	31,914,306	rs117314762	Build 132	G	A	*CFB*	R576H	2.5	Probably damaging	Damaging	1.27	1.80	.046[c]	.70
6	87,725,674	rs3828741	Build 107	G	A	*HTR1E*	A208T	5.47	Probably damaging	Damaging	1.11	1.11	1.00	1.00
6	123,101,544	rs2279381	Build 100	C	T	*FABP7*	T61M	3.39	Probably damaging	Damaging	2.62	2.76	.70	.95
6	132,910,485	rs80078646	Build 131	T	A	*TAAR5*	D114V	3.36	Probably damaging	Damaging	3.30	4.08	.055	.80
6	135,644,371	rs148000791	Build 134	T	C	*AHI1*	E536G	4.86	Possibly damaging	Damaging	4.29	4.24	.92	1.01
6	152,614,868	rs80265744	Build 131	C	T	*SYNE1*	R480H	2.08	Probably damaging	Damaging[b]	.30	.29	1.00	1.04
6	155,458,738	rs116960376	Build 132	C	T	*TIAM2*	T541M	4.26	Probably damaging	Damaging	4.07	4.68	.16	.86
7	18,535,896	rs79608746	Build 131	G	A	*HDAC9*	G32R	5.76	Possibly damaging	Damaging		Variant allele was not observed		
7	21,892,164	rs150631721	Build 134	C	T	*DNAH11*	A3666V	4.22	Probably damaging	Damaging	.60	.76	.38	.79
7	48,412,084	rs143050255	Build 134	A	T	*ABCA13*	Q3708L	2.63	Probably damaging	Damaging	1.19	1.51	.21	.78
7	80,286,003	rs75326924	Build 131	C	T	*CD36*	P90S	5.03	Probably damaging	Damaging	4.14	4.79	.15	.86
7	99,366,093	rs12721627	Build 121	G	C	*CYP3A4*	T185S	3.28	Possibly damaging	Damaging	1.64	2.09	.13	.78
8	16,853,208	rs3793405	Build 107	C	G	*FGF20*	G116R	6.27	Probably damaging	Damaging	3.33	3.28	.91	1.02
8	22,421,982	rs77246845	Build 107	C	T	*SORBS3*	P255L	2.92	Probably damaging	Damaging	.78	.51	.11	1.54
8	22,426,701	rs3758036	Build 131	C	T	*SORBS3*	P449L	5.49	Probably damaging	Damaging	2.57	2.48	.79	1.04
8	23,301,426	rs150919990	Build 134	G	A	*ENTPD4*	P202S	3.84	Probably damaging	Damaging	.97	1.26	.20	.77
8	26,627,940	rs140512348	Build 134	C	T	*ADRA1A*	R376H	2.74	Probably damaging	Damaging[b]		Variant allele was not observed		
8	133,634,908	rs76147813	Build 131	G	T	*LRRC6*	P288H	2.05	Possibly damaging	Damaging	5.16	4.85	.50	1.07
9	104,433,256	rs149729514	Build 134	G	C	*GRIN3A*	R480G	4.16	Probably damaging	Damaging	3.28	2.11	.00042[c]	1.58
10	55,663,053	rs149478475	Build 134	C	T	*PCDH15*	G1156R	4.72	Probably damaging	Damaging	.43	.47	.88	.90
10	61,840,324	rs74777754	Build 131	C	T	*ANK3*	R593H	4.36	Probably damaging	Damaging[b]	2.45	1.96	.12	1.26
10	121,286,832	rs117042762	Build 132	C	T	*RGS10*	V44M	2.11	Probably damaging	Damaging	1.05	1.34	.22	.78
12	56,495,023	rs2271188	Build 100	G	A	*ERBB3*	R1127H	5.13	Probably damaging	Damaging[b]	1.52	1.81	.30	.84
12	70,932,745	rs138916804	Build 134	G	A	*PTPRB*	P1725L	4.51	Possibly damaging	Damaging	1.20	1.11	.70	1.08
12	123,022,996	rs75373025	Build 131	A	G	*KNTC1*	T121A	3.69	Probably damaging	Damaging[b]	.65	.54	.50	1.20
12	123,028,739	rs61751320	Build 129	A	G	*KNTC1*	T198A	3.91	Probably damaging	Damaging[b]	1.71	2.05	.25	.83
13	99,356,584	rs2274827	Build 100	G	A	*SLC15A1*	R459C	3.23	Possibly damaging	Damaging	2.66	2.72	.90	.98
13	103,343,179	rs140948695	Build 134	G	T	*METTL21C*	A89E	3.9	Probably damaging	Damaging	.28	.24	.69	1.17
17	67,079,352	rs149614799	Build 134	C	T	*ABCA6*	Splice site	5.28	–	–	.87	.82	.82	1.07
18	21,353,474	rs76572574	Build 131	G	C	*LAMA3*	G399A	5.15	Probably damaging	Damaging[b]	2.50	2.30	.54	1.09
18	70,417,304	rs118113391	Build 132	C	T	*NETO1*	V512I	5.8	Possibly damaging	Damaging[b]	2.24	2.20	.94	1.02
19	10,259,654	rs62621087	Build 129	C	T	*DNMT1*	G876R	3.7	Probably damaging	Damaging	1.44	.95	.031[c]	1.53
21	37,417,981	rs145926295	Build 134	C	T	*SETD4*	V209M	3.56	Probably damaging	Damaging	.61	.51	.57	1.19
22	26,166,934	rs117243697	Build 132	G	A	*MYO18B*	E559K	4.15	Probably damaging	Damaging	.42	.18	.032[c]	2.35
22	26,706,697	rs117917851	Build 132	G	A	*SEZ6L*	E526K	3.29	Possibly damaging	Damaging	2.13	2.05	.83	1.04
22	42,294,751	rs17848351	Build 123	G	C	*SREBF2*	V902L	3.94	Possibly damaging	Damaging	1.49	1.69	.46	.88
X	49,079,494	rs143938580	Build 134	G	C	*CACNA1F*	S671C	4.86	Probably damaging	Damaging	1.38	1.54	.62	.89

Chr, chromosome; dbSNP, Single Nucleotide Polymorphism database; MAF, minor allele frequency; OR, odds ratio; QC, quality control; SNV, single nucleotide variant.

[a]The release version of dbSNP in which each variant appeared first. dbSNP later than the Build129 contains data from the 1000 Genomes project.

[b]Prediction with low confidence.

[c]$p < .05$.

Table 3. Results of the Meta-Analysis

	Japanese Case-Control				Han-Chinese (Taiwan) Case-Control				Han-Chinese (Mainland China and Taiwan) Pedigree				Meta-Analysis	
Allele	Cases	Control Subjects	*p* Value	OR [95% CI]	Cases	Control Subjects	*p* Value[a]	OR [95% CI]	Transmitted[b]	Untransmitted[b]	*p* Value[c]	OR [95% CI]	*p*	OR [95% CI]
G	132	117	.00042	1.58 [1.23–2.03]	19	14	.145	1.52 [.76–3.05]	25	13	.036	1.92 [.95–4.09]	3.3×10^{-5}	1.61 [1.28–2.01]
C	3888	5435			647	724			13	25				
MAF	.033	.021			.029	.019			NA	NA				

CI, confidence interval; MAF, minor allele frequency; NA, not applicable; OR, odds ratio.
[a]Calculated by one-tailed Fisher's exact test.
[b]Transmission from heterozygous parent.
[c]Calculated by one-tailed McNemar's exact test.

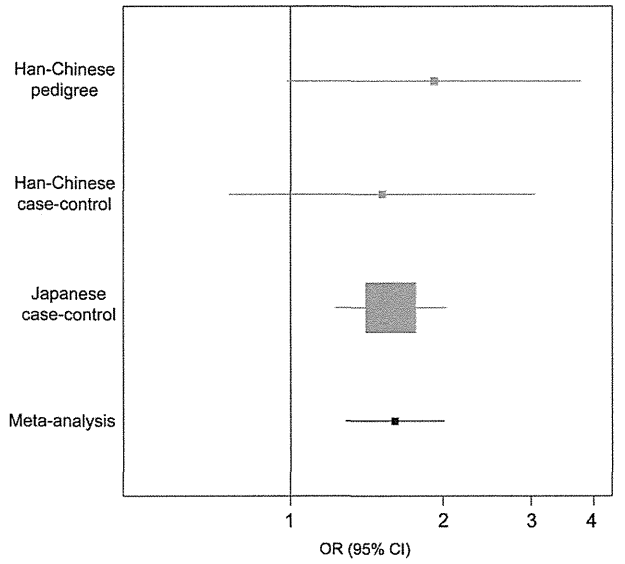

Figure 1. Forest plots of odds ratios (OR) and their 95% confidence intervals (CI) for individual studies and meta-analysis.

disorder (4,47,48). To test whether *GRIN3A* R480G shows positive association with bipolar disorder, we genotyped samples from Japanese bipolar disorder patients (865 samples). While the G allele, which was overrepresented in schizophrenia, was more frequent in bipolar case samples, we did not observe significant association ($p = .138$, MAF in cases = 2.72%, OR = 1.3; two-tailed Fisher's exact test). Statistical power obtained from the sample size of bipolar cases and control subjects was 33% for nominal significance ($\alpha = .05$). Association analysis using combined Japanese schizophrenia and bipolar disorder cases and control subjects (2877 cases and 2781 control subjects) showed study-wide significant association ($p = .00077$, Bonferroni-corrected $p = .033$. OR = 1.50), but the association was slightly less significant than that observed with schizophrenia.

Discussion

In this study, we performed association analyses of uncommon, putatively functional, and Asian-specific SNVs with schizophrenia. We observed five significantly associated SNVs with ORs ranging from 1.41 to 2.35. Among them, the *GRIN3A* R480G variant showed the strongest association with study-wide significance ($p = .00042$, Bonferroni-corrected $p = .018$). Positive association of the *GRIN3A* R480G variant was observed in independent Han-Chinese pedigree samples ($p = .036$). Meta-analysis using these data sets and published data from Han-Chinese case-control samples collected in Taiwan further supported this association (combined $p = 3.3 \times 10^{-5}$).

On the other hand, we observed no significant association of the *GRIN3A* R480G variant with bipolar disorder. While calculation of statistical power indicated that the sample size of our bipolar cases was not sufficient to appropriately assess association, it is possible that this variant may be more relevant to schizophrenia than bipolar disorder.

The *GRIN3A* R480G variant was not detected in Caucasian and African American populations (23,24) and thus far seems Asian-specific. However, if aberrant *GRIN3A* function, driven by genomic

variations in this gene, does contribute to the pathogenesis of schizophrenia, other risk variants within this gene could be identified in non-Asian populations (and if so, those variants might be non-Asian specific in some cases).

The association signals in *GRIN3A* have not been detected with genome-wide significance in large-scale GWAS conducted so far. This is not surprising, considering the sample sizes used for the reported GWAS and the MAF and OR of the *GRIN3A* R480G variant observed in this study. For example, if there is a common SNP with 10%, 25%, or 50% MAF in complete linkage disequilibrium (LD) with the R480G variant, the OR of this common SNP would be 1.13, 1.05, and 1.03, respectively, and the number of cases required for 80% power should be 29,012, 83,979, and 248,882, respectively.

Therefore, the sample sizes used in the reported Chinese (7,8) and Japanese (49,50) GWAS have not been sufficient to identify common marker SNPs in LD with the *GRIN3A* R480G variant. Even the largest Caucasian GWAS to date (6) would not have enough power to detect marker SNPs, if other risk-contributing SNVs existed in *GRIN3A* did not generate ORs and/or MAFs much larger than those of the *GRIN3A* R480G variant. This point clearly indicates the advantage of direct genotyping of disease risk-contributing SNVs, especially in studies aiming to identify risk variants with moderate effect sizes, using medium-sized samples.

GRIN3A encodes the NR3A subunit of the *N*-methyl-D-aspartate type glutamate receptor (NMDAR). Involvement of NMDAR hypofunction in the pathophysiology of schizophrenia was first proposed, on the observation that NMDAR antagonists, such as phencyclidine, ketamine, and MK801, induced psychiatric abnormalities that mimic both positive and negative symptoms of schizophrenia (51). The NMDAR hypofunction theory is supported by various lines of evidence from pharmacologic, genetic, postmortem histopathologic, and brain imaging studies (52).

N-methyl-D-aspartate type glutamate receptor is a heteromeric tetramer protein, composed of two obligate NR1 subunits and two subunits from the NR2 or NR3 families. NR3A acts in a dominant-negative manner to suppress NMDAR activity (53,54). NR3A-containing NMDARs display reduced calcium ions (Ca^{2+}) permeability and low sensitivity to magnesium ion (Mg^{2+}) blockade (55,56).

In postmortem brains of patients with schizophrenia, increased *GRIN3A* expression in layer V of the dorsolateral prefrontal cortex (57), along with decreased spine density in the same region (58,59), were reported. Removal of NR3A increases spine density (56) and promotes synapse maturation and memory consolidation (60,61). Male NR3A knockout mice show increased prepulse inhibition, a measure of sensorimotor gating, which is impaired in schizophrenia and other neuropsychiatric disorders (62). These findings collectively suggest the involvement of aberrant, possibly enhanced, *GRIN3A* function in the pathophysiology of schizophrenia, while also implying that the R480G variant of *GRIN3A* promotes a gain of function.

Other significantly associated SNVs are located in *FAAH*, *DNMT1*, *MYO18B*, and *CFB*. *FAAH* encodes the integral membrane enzyme fatty acid amide hydrolase. Another missense variant in this gene (P129T) was reported to be associated with drug addiction (63–65). *DNMT1* encodes DNA (cytosine-5-)-methyltransferase 1, which plays an important role in maintaining methylation patterns in the mammalian genome (66). *MYO18B* encodes an unconventional myosin, myosin-XVIIIb. This gene harbors rs5761163, which is one of the most significantly associated SNPs in GWAS, conducted by the International Schizophrenia Consortium (4). It is noteworthy that the *MYO18B* E559K variant and rs5761163 are in complete LD in the 1000 Genomes Project data. *CFB* encodes complement factor B, a component of the complement system. This gene resides in the major histocompatibility complex class III region on chromosome 6p21.3, a locus associated with schizophrenia, with genome-wide significance (3–6,8). Nevertheless, association of these variants was only nominal and the significant probability of false positivity should be recognized.

To interpret the results of this study more appropriately, two main limitations need to be considered. First, the number of SNVs analyzed in this study was limited. As a selection criterion for SNVs in this study was location in candidate disease genes, it was not surprising to find association of missense variants in promising genes, such as *GRIN3A*. Given the poor replication status of candidate gene association studies for schizophrenia and other psychiatric diseases, more comprehensive and hypothesis-free analyses, particularly genome-wide association studies of uncommon and putatively functional variants are warranted, especially if we are to uncover new genes. Second, the statistical power obtained from our samples was insufficient for testing association of rare and uncommon variants with a stringent threshold of significance. While we applied study-wide significance ($p < .0012$) defined by the number of investigated SNVs in this study, genome-wide significance ($p < 5 \times 10^{-8}$) is needed to demonstrate concrete association. Post hoc statistical power for analysis of the *GRIN3A* R480G variant, calculated from the overall allele counts used in meta-analysis, was 99.3% for nominal significance ($\alpha = .05$) and 88.5% for study-wide significance ($\alpha = .0012$) but only 15.6% for genome-wide significance ($\alpha = 5 \times 10^{-8}$). Moreover, if we consider our Japanese cohort as a sample set for discovery, the statistical power that can be obtained from both sets of Han-Chinese samples for replication are 62.7, 17.0, and .1% for α levels of .05, .0012, and 5×10^{-8}, respectively. To corroborate the association observed in this study, analyses using further independent samples should be conducted.

As that has already been demonstrated in genetic studies of other complex diseases, analysis of uncommon protein-damaging SNVs is a valid method of isolating risk variants with larger effects. The identification of study-wide significant association and the observed ORs of associated SNVs in this study would also support this strategy. More comprehensive genome-wide analysis and replication studies are necessary to overcome the limitations of this study, and they will undoubtedly provide further solid and informative biological insight into the pathophysiology of schizophrenia. This caveat aside, our finding that this putatively functional variant of NR3A, a subunit of the NMDAR, showed significant association with schizophrenia paves the way for functional characterization of this mutation in animal models and sets the stage for the discovery of other uncommon disease-associated variants.

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Minocycline Modulates Human Social Decision-Making: Possible Impact of Microglia on Personality-Oriented Social Behaviors

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Abstract

Background: Microglia, one of the glial cells, play important roles in various brain pathologies including psychiatric disorders. In addition, microglia have recently been proved to monitor synaptic reactions via direct-touching even in normal brain. Human microglia may modulate various social/mental functions, while microglial social/mental roles remain unresolved especially in healthy humans. There is no known drug with the specific effect of modulating microglia. Therefore, using minocycline, a tetracycline antibiotic and the most famous microglial inhibitor, is one of the best alternative approaches to clarify microglial functions on human social/mental activities.

Methodology/Principal Findings: We conducted a double-blind randomized trial of trust game, a monetary decision-making experiment, with ninety-nine human adult males who decided how much to trust an anonymous partner after a four-day administration of minocycline. Our previous pilot trial indicated a positive effect of minocycline, while the underlying mechanisms were not clarified. Therefore, in this trial with larger samples, we additionally measured the effects of anxiety and personality. The monetary score in trust game was significantly lower in the minocycline group. Interestingly, participants' ways of decision-making were significantly shifted; cooperativeness, one component of personality, proved to be the main modulating factor of decision-making in the placebo group, on the other hand, the minocycline group was mainly modulated by state anxiety and trustworthiness.

Conclusions/Significance: Our results suggest that minocycline led to more situation-oriented decision-making, possibly by suppressing the effects of personality traits, and furthermore that personality and social behaviors might be modulated by microglia. Early-life events may activate human microglia, establish a certain neuro-synaptic connection, and this formation may determine each human's personality and personality- oriented social behaviors in later life. To explore these mechanisms, further translational research is needed.

Trial Registration: UMIN clinical trial center UMIN000004803

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Introduction

Microglia are one of the glial cells with immunological/inflammatory functions, and contribute to various brain pathologies; not only in neurodegenerative diseases [1,2,3] but also in psychiatric disorders such as schizophrenia and autism [4,5,6]. Minocycline, a tetracycline antibiotic, is known as the most famous microglial inhibitor [7], which has recently been applied to brain diseases such as stroke and neurodegenerative diseases [8,9]. In addition, minocycline has been suggested to be an effective drug for psychiatric disorders [10,11]. These reports suggest that inhibiting microglial activation may modulate human social and mental activities, and rodent studies have indicated this possibility [12,13].

Rodent microglia have recently been shown to monitor synaptic reactions via direct-touching not only in pathological brain but also in normal brain [14,15,16,17], and have proved to play important roles in normal brain development such as synaptic pruning [18]. Neurons and neuronal networks including synapses have been dominantly believed to play crucial roles in human social/mental activities. The above-mentioned evidence indicates

that human microglia may modulate various social/mental functions, while microglial social/mental roles continue to remain unresolved especially in healthy humans.

There is no known drug with the specific effect of modulating microglia. Therefore, using minocycline, a tetracycline antibiotic and the most famous microglial inhibitor, is one of the best alternative approaches to clarify microglial functions on human social/mental activities. One human study suggests that minocycline attenuates the subjective reward effects of dextroamphetamine [19], while, to our knowledge, the effects of minocycline on human social/mental activities are not well understood.

Crockett et al have revealed that serotonin modulates behavioral reactions to unfairness, via a monetary decision-making game with healthy volunteers who were administered tryptophan-depleted amino acid which induces lower serotonin levels [20]. In order to measure human social/mental activities, these monetary decision-making experiments have been actively applied because such experiments enable the analysis of the interaction between social/mental activities and actual social behaviors [21,22]. Pharmacology-based neuro-economic research is showing that human social behaviors are modulated by neurotransmitters such as serotonin and oxytocin [20,23,24,25]. In addition, a significant link has recently been reported between the dopamine D4 receptor gene and fairness preference in ultimatum game [26]. However, the pharmacological interaction of social decision-making beyond neurotransmitters remains to be clarified [27].

As a first step in this direction, we recently conducted a pilot experiment with trust game, one of the decision-making experiments, with minocycline [28]. The forty-nine participants, healthy adult humans, made a monetary decision about whether or not to trust an anonymous partner after a four-day oral administration of minocycline or placebo. The minocycline group showed a strong and positive correlation between their scores in trust game and their pre-evaluation scores in others' trustworthiness, but the placebo group did not. These pilot data have suggested that inhibitory effects of microglial activation may sharpen a sense of trust in social behavior, and this effect would enhance situation-oriented behaviors according to immediate social situations. In trust game, a player's optimal decision depends on his/her prediction about the other player's decision. Thus, social environment, including the other's behavior, determines what behavior the player should take. In our actual life, however, our decisions are determined not only by social environment but also by our fundamental mental factors such as temperament and character (i.e. personality), which are independent from situational factors and may strongly impact on decision-making. These factors may act as a "noise" in trust game and during similar human decision-making situations [29]. Our pilot data demonstrated that only the minocycline group showed situation- oriented decision-making, which suggests that microglia may be inducing the "noise" consistently and inhibiting microglial activation could reduce this "noise" effect. However, the underlying mechanisms of "noise" were not clarified in our previous trial [28], thus the next appropriate step is the measurement of the effects of not only trustworthiness but also anxiety and personality.

Therefore, to clarify the microglial "noise" effect during human social decision-making, we newly explored whether anxiety and personality as a "noise" influences outcomes of trust game on humans with minocycline or placebo. To improve the small sample size and the weaker statistical power of our previous trial, we newly conducted the trial with larger samples (about one hundred participants).

Methods and Materials

The protocol for this trial and supporting CONSORT checklist are available as supporting information; see Checklist S1, Protocol S1 and Protocol S2. This double-blind randomized study was approved by the Kyushu University Ethical Committee under the administration of the UMIN clinical trial center (**UMIN000004803**). All the participants of the present experiment, which was conducted in December 2010, were unique to this study and differ from the previous participants who enrolled in an earlier experiment in March 2010 under the administration of the UMIN clinical trial center (UMIN000003281: published in Watabe et al. Psychopharmacology 2012). Flow diagram of this study is listed on **Figure 1**. All participants gave written informed consent to participate after a complete description of the study. Participants were administered minocycline or placebo for four days, after which they played a trust game with an anonymous partner.

Subjects

Participants were recruited by advertisements on campus. Inclusion criterion was as follows; healthy adult males from 20 to 30 years old who can obtain informed consent. Exclusion criteria were the following five items; 1) those who have had side effects to antibiotics including minocycline, 2) those who have severe heart, liver or kidney disease, 3) those who have a tendency to develop allergies, and 4) those who have been diagnosed with psychiatric disorders. Their mental and physical health was confirmed by interview with a psychiatrist (TAK). All the participants were qualified for this study (**Table S1**).

Drug Administration

Participants received a sheet describing their detailed dosing schedule. They were then asked to write the exact time of every dosing, and to submit every capsule package as evidence of dosing. Participants started to take a capsule in the evening of the first day and twice daily (morning and evening) for four days afterward. On the day of the game experiment (the fifth day), they were instructed to take the last capsule three-hours prior to the appointment time for the experiment so that all participants played the trust game under the similar drug effect. Each capsule contained 100 mg minocycline (in the treatment group) or 100 mg lactose (in the placebo group). This minocycline dose (200 mg/day) is within the range of the usual daily dose used for treatment of infections [30], and this dose has also been applied in recent clinical trials [10,19]. Participants were randomly assigned to the treatment group or to the placebo group in advance, with a double-blind procedure.

Procedure

Prior to drug administration, participants completed a set of questionnaires (details in **Scales**). After four days of drug administration, participants were interviewed by physicians regarding side effects, other medications, and adherence to the drug administration protocol. They then played a trust game [21] and responded to the same set of questionnaires they had completed before administration.

Trust Game

Figure 2 shows the structure of trust game. In this two-player game, each player was initially given 1300 JPY (nine hundred JPY had been used in our previous trial [28], but to let participants recognized clearer incentive and make their decisions more seriously, we used 1300 JPY (about 15 USD) in this new trial so

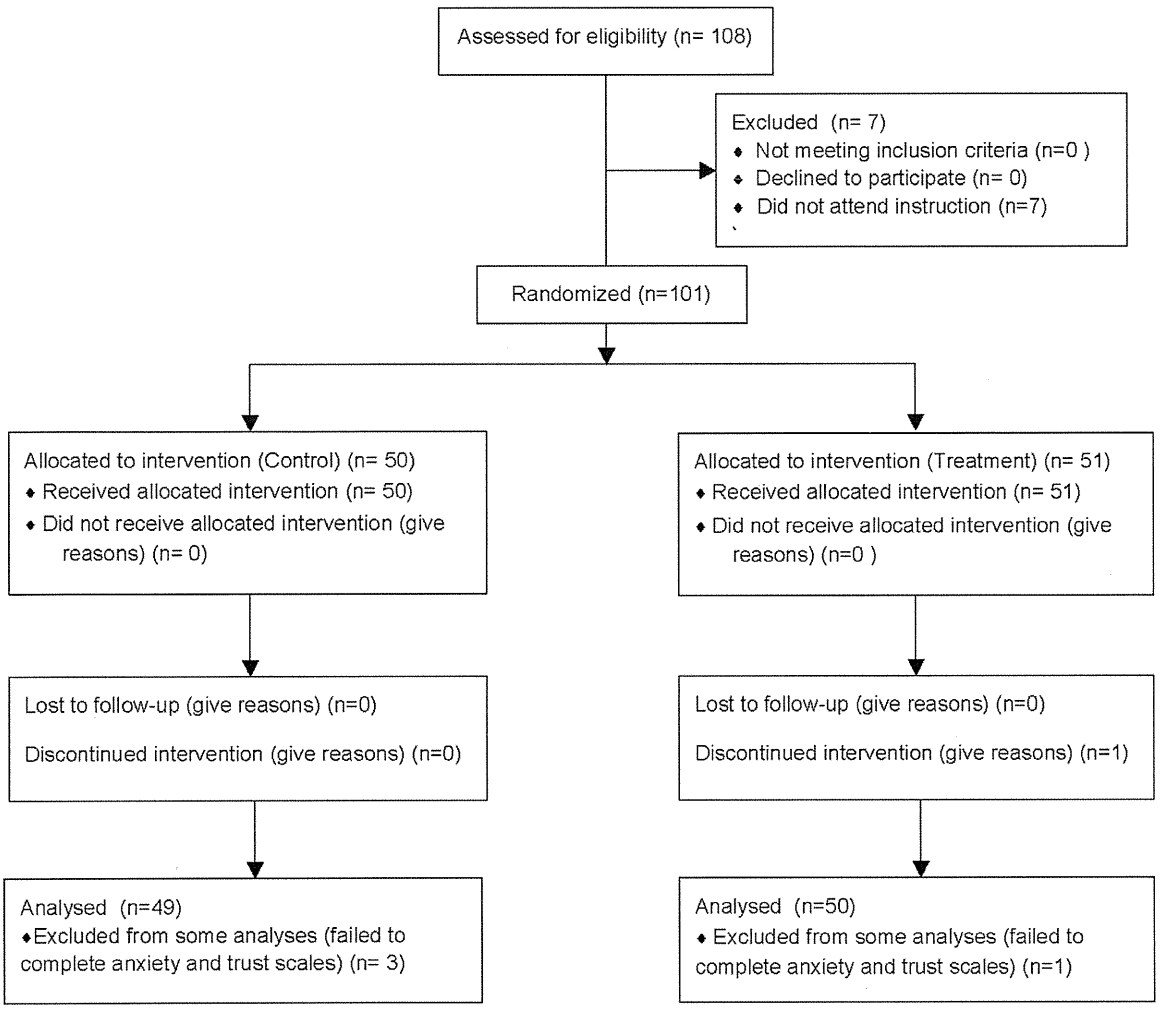

Figure 1. Flow Diagram of This Study.
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that we can obtain more reliable behavioral data). The first player then decided how much of the 1300 JPY to give to the second player. The second player then went to another room, where the amount of money given to him by the first player was tripled. The second player then decided whether to split his money equally with the first player or to take all of his money. In this experiment, all the participants were actually assigned to be the first player. The first player's decision as to how much money to give to the second player is thought to be the first player's level of trust in his partner. The amount of money given was expected to be a behavioral measure of the first player's trustfulness.

In this experiment, participants had no information about the partner except that he was male. The participants thus were likely to have made their decisions based primarily on how much they trusted others in general. All the participants' partner was actually a research confederate and always the same person, a 22-year-old Japanese male. In order to control the participant's impression of the partner, the partner acted and talked exactly in the same way throughout all the experimental sessions.

Scales

Our previous trial showed the positive correlation between participants' scores in trust game and their pre-evaluation scores in others' trustworthiness, while we did not examined other psychological factors and thus the underlying mechanisms were not clarified [28]. Therefore, we examined the effects of anxiety and personality, in addition to the trust scores, in this trial. The following self-rated questionnaires were completed by the participants at pre- and post-treatment.

Temperament and Character Inventory (TCI)

TCI is based on the seven-factor model of temperament and character in personality [31]. According to TCI model, personality is classified into temperament, which consists of Novelty Seeking (NS), Harm Avoidance (HA), Reward Dependence (RD), Persistence (PS), and character, which consists of Self-Directedness (SD), Cooperativeness (C), Self-Transcendence (ST) with a four-point Likert type scale. We used a Japanese version with 125 questions (TCI-125) [32], which was kindly provided for use in this study from the HUMAN CAPITAL CONSULTING Corporation, Tokyo, Japan.

State-Trait Anxiety Inventory (STAI)

This anxiety scale with 20 questions consists of two factors; state anxiety, which refers to relatively unstable emotional threat to

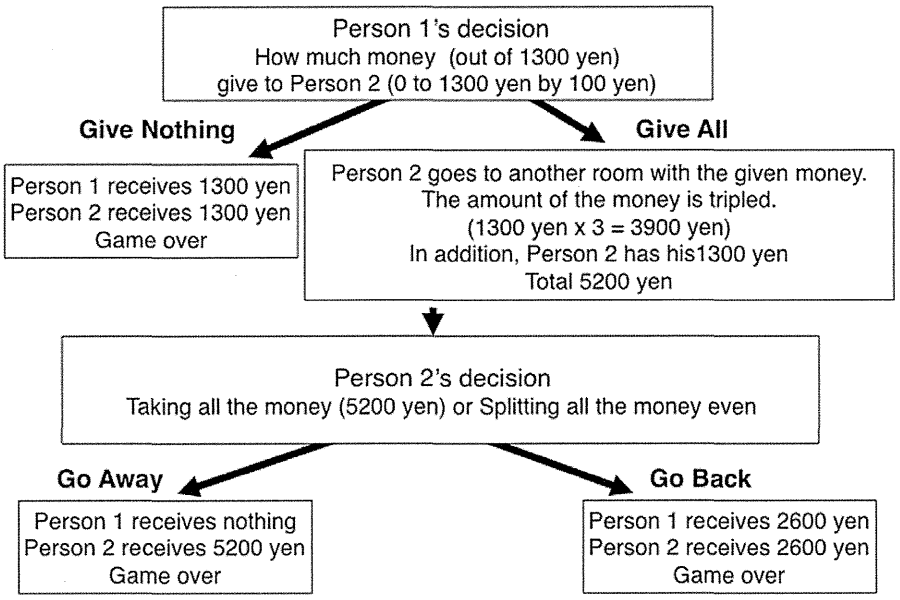

Figure 2. Trust Game Structure.
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current situations, and trait anxiety, which refers to relatively stable emotional threat consistently felt in daily life [33].

General Trust Scale (GTS)

GTS consists of six questions with a seven-point Likert type scale developed by Yamagishi and Yamagishi [34]. This scale measures respondents' estimation of others' trustworthiness. The reliability and validity of GTS have been confirmed across many countries [35]. According to past research on GTS, the major confounders of general trust are culture, sex and education level [34]. To eliminate the effects of these confounders, we recruited a homogenous sample as possible. As a result, all the participants were Japanese males and who had collage/university level educations so that we could test the effect of general trust without these confounders.

Data Analysis

Ninety-nine Japanese males, out of 101 entries, completed our experiments (mean age 21.52 years, SD 1.65 years), and analysis was conducted on this data. Among the participants, four (three in the minocycline condition, one in the control condition) failed to complete the questionnaires of STAI and GTS, so the analyses including these two scales were performed with the data of the 95 participants. All of the data analyses were performed with SPSS ver.19.

Results

In our previous trial, the statistical power was 0.766, and the statistical power in the present trial is 0.847. Therefore, the present trial exceeds the suggested efficient power of 0.8. The following analyses are shown with this more appropriate power.

Behavior in Trust Game

We compared the mean amount of participants' monetary offers in trust game by a t-test (**Table 1**). The monetary score in trust game was significantly lower in the minocycline group compared to the placebo group ($t(97) = 2.08$, $p<.05$). This result is consistent with our pilot study [28].

Effects of Minocycline on Personality, Anxiety and Trust

The effects of minocycline on personality, anxiety and trust were evaluated with the seven subscales of TCI, the two subscales of STAI, and GTS. We performed ANOVA with a repeated measure; the scores of the subscales as the dependent variable, and drug condition (Minocycline vs. Control), repeated measure of the subscales' scores (*Before* vs. *After* treatment) and their interaction as independent variables (**Table 1**).

There was no significant interaction term on each of the subscale of TCI. The main effect of time (*Before* vs. *After* treatment) was significant for *Persistence*. The score of *Persistence* is higher *After* (mean score = 13.09, SD = .237) than *Before* (mean score = 13.46, SD = .236). No effect was found on the rest of the items. These results indicate that participants' personality itself was not significantly affected by minocycline.

On STAI, interaction effect and main effect were significant on *Before-After* for state anxiety. Compared to the control group, the state anxiety score increased steeply in the minocycline group. According to simple main effect test, the score after the treatment was significantly higher in the minocycline group than in the control group ($p<.001$). Thus, this result may explain the cause of the lower trusting behavior for minocycline group in trust game. We found no significant effect on trait anxiety.

On GTS, there were no main or interaction effects.

Effects of Minocycline on Decision-Making Style

Next, to examine the effects of minocycline on decision-making style, we performed a multiple linear regression analysis of the amount of money offered (monetary score) in trust game as the dependent variable, and subscales of TCI, STAI and GTS as independent variables by conditions (**Table 2**). We revealed that state anxiety ($\beta = -.795$, $t = -4.42$, $p = .001$) and trust ($\beta = .321$, $t = 2.35$, $p = .023$) have significant effects in the minocycline group ($R^2 = .288$, $F(3,46) = 9.30$, $p = .001$) while only cooperation scale of

Table 1. Behavior in Trust Game, and Effects of Minocycline on Personality, Anxiety and Trust.

Category	Subcategory	Before Treatment		After Treatment		Before-After	Control-Minocycline	Interaciton
		Control	Minocycline	Control	Minocycline			
Monetary Score in Trust Game (%)	–	N/A	N/A	61.38 (32.43)	48.77 (27.70)	N/A	$t(97) = 2.08$, $p<.05$	N/A
TCI (from 1 to 20)	**Self-Transcendence**	10.69 (2.05)	10.82 (2.19)	10.52 (2.47)	10.57 (2.63)	*ns.*	*ns.*	*ns.*
	Cooperative-ness	14.98 (1.79)	14.98 (1.89)	14.80 (1.91)	14.79 (1.98)	*ns.*	*ns.*	*ns.*
	Self-Directedness	12.87 (0.20)	12.71 (0.22)	12.62 (1.98)	12.22 (2.09)	*ns.*	*ns.*	*ns.*
	Persistence	12.86 (2.14)	13.54 (2.53)	13.31 (2.34)	13.76 (2.25)	*ns.*	*ns.*	*ns.*
	Reward Dependence	14.07 (1.88)	14.28 (2.38)	14.10 (1.75)	14.10 (2.21)	*ns.*	*ns.*	*ns.*
	Harm Avoidance	13.51 (2.47)	13.30 (2.37)	13.55 (2.50)	13.26 (2.56)	*ns.*	*ns.*	*ns.*
	Novelty Seekng	12.97 (1.69)	12.99 (1.76)	12.94 (1.77)	13.02 (1.62)	*ns.*	*ns.*	*ns.*
STAI (from 1 to 4)	**State Anxiety**	2.04 (0.45)	2.00 (0.53)	2.11 (0.51)	2.28 (0.57)	$F(1, 93) = 18.60$ $p<.01$	*ns.*	$F(1, 93) = 6.57$ $p<.05$
	Trait Anxiety	2.33 (0.52)	2.21 (0.56)	2.27 (0.51)	2.27 (0.58)	*ns.*	ns.	ns.
General Trust Score (from 1 to 7)	-	4.31 (1.06)	4.51 (1.12)	4.41 (1.07)	4.53 (1.04)	*ns.*	*ns.*	*ns.*

We performed t-test on the behavior (monetary score) in trust game, and the average scores are shown in the Table. The effects of minocycline on personality, anxiety and trust were evaluated with the seven subscales of TCI, the two subscales of STAI, and GTS. We performed ANOVA with a repeated measure; the scores of the subscales as the dependent variable, and drug condition (Minocycline vs. Control), repeated measure of the subscales' scores (*Before* vs. *After* treatment) and their interaction as independent variables. As four participants (three for control, one for minocycline group) failed to complete the questions of STAI and GTS, 95 sets of data were analyzed. Significant and/or marginal effects are shown in the Table. Results were expressed as means (S.D.).

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TCI ($\beta = .486$, $t = 2.58$, $p = .013$) was significant in the control group ($R^2 = .092$, $F(3,42) = 2.51$, $p = .078$). Our novel finding in the present study is that the effect of state anxiety was stronger than that of trustworthiness. In sum, for the minocycline group, the less state anxiety and the more trustful, the more trusting behavior; while for the control group, the more cooperativeness, the more trusting behavior.

Discussion

As a first step to explore how microglia modulates human social/mental activities, we showed the novel effect of minocycline, the most famous inhibitor of microglial activation, on human monetary decision-making in trust game. Our previous trial, with smaller sample size and weaker statistical power, indicated the positive effect of minocycline on trust game, while the significant results were limited. In the present trial, we newly revealed that the monetary score in trust game was significantly lower in the minocycline group. Another novel finding was that minocycline treatment itself did not change personality, while, surprisingly participants' ways of decision-making were significantly shifted; cooperativeness, one component of personality, was the main modulating factor of decision-making in the placebo group, on the other hand, the minocycline group was mainly modulated by state anxiety and trustworthiness, both of which are known to be mainly dependent on real-time environments such as present social situation. In addition, the effect of state anxiety was stronger than that of trustworthiness. These results suggest that minocycline led to more situation-oriented decision-making, supporting our "noise reduction" hypothesis [28]; participants' personality may act as a "noise" during human social decision-making and minocycline may mimic personality- oriented behaviors.

Impact of Microglia on Personality-Oriented Social Behaviors

The novel effects of minocycline may explain the unknown role of microglia in social/mental activities. Until now, no study has reported microglial activities in healthy human subjects, while microglia have proved to play important roles in normal brain by communicating with neurons via releasing mediators and synaptic direct contact in rodent studies [14,15,16]. Therefore, human microglia may perform actively even in healthy brains, and inhibiting microglial activation with minocycline may create a shift from personality-oriented behaviors to situation-oriented behaviors by modulating neuro-synaptic-microglial networks. Rodent microglia play essential roles in synaptic pruning [18], which has pointed to the cryptic roles of microglia in human brain development. A recent study suggests that rodent microglial activation by infections during early developmental periods last, and these pre-activated microglia will be re-activated rapidly compared to normal state microglia [36]. Another study has suggested that microglia have a crucial role in the process of early-life memory in rats [37]. Early-life events can significantly modulate normal learning-dependent cytokine activity within the hippocampus, via a specific, enduring impact on brain microglial function, and preventing microglial activation by minocycline during learning prevents memory impairment in neonatally infected rats. Microglia are known to be activated not only by infection but also by physical and psychological stress in rodent studies [12,38,39,40]. In addition, Wei et al. reported that early life stress inhibits expression of a novel innate immune pathway in the developing hippocampus in pups [41]. Based on these recent findings, we suggest the possible existence of the following mechanism on personality and social behaviors; early-life environmental experiences such as psychological stress and traumatic events may activate human microglia, establish a certain neuro-synaptic-microglial connection, which may be memorized unconsciously as a primer for an extended period, and this formation in the human brain may determine each human's personality and personality- oriented social behaviors in later life (**Figure 3**). In addition, we can interpret the present results as follows; the control group's personality- oriented behaviors could be formulated by microglial priming effects, and the minocycline group's situation- oriented behaviors may be induced by suppressing microglial contribution to social behaviors. Further studies are needed to clarify contributions of microglia to human development including personality formation, and social/mental activities in later life.

Clinical Implication

Minocycline has been suggested to be an effective drug for psychiatric disorders [10,11]. Disturbed decision-making is a common symptom of various psychiatric disorders [42,43], and this disturbance is treated by psychotropic drugs such as antipsychotics and antidepressants, which have proved to inhibit microglial activation from *in vitro* studies [44,45,46,47,48]. In addition, a recent study suggests that effort-based decision-making in rat is modulated by estradiol [49], a sex hormone, which also has inhibitory effects on microglial activation [50,51]. These data support our minocycline results, and indicate that psychiatric

Table 2. Multiple Regression Analysis on Behavior in Trust Game.

Independent Variable	Control Group	Minocycline Group
	Beta	Beta
Cooperativeness (TCI)	.486*	
Reward Dependence(TCI)	−.281	
Self-Directedness (TCI)	−.284	
State Anxiety (STAI)		−.583**
General Trust		.321*
	$N = 46$, $R^2 = .092$, $F(3, 42) = 2.51$, $p < .10$	$N = 49$, $R^2 = .288$, $F(2, 46) = 9.30$, $p < .001$

Note: *p<.05,
**p<.01.
We performed a multiple linear regression analysis of the amount of money offered in trust game as the dependent variable, and subscales of TCI, STAI and GTS as independent variables by conditions. Remarkable effects are shown in the Table.
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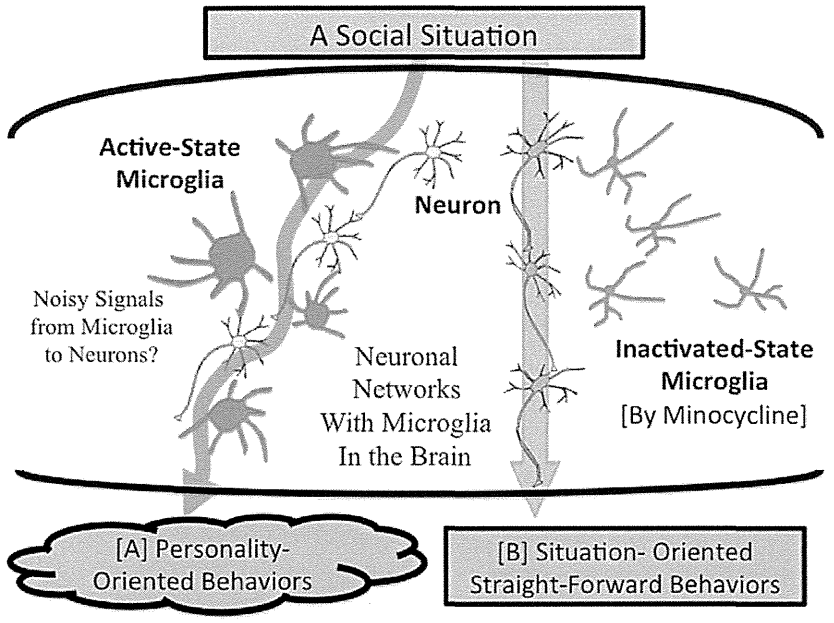

Figure 3. Possible Impact of Microglia on Personality and Social Behaviors. Early-life environmental experiences such as psychological stress and traumatic events may activate human microglia, establish a certain neuro-synaptic-microglial connection, which is memorized unconsciously as a primer for an extended period, and this formation in the human brain determines each human's personality and personality-oriented social behaviors in later life. In sum, neuronal networks with active microglia may induce noisy-decision-making, which is equivalent to personality- oriented behaviors (A). On the other hand, decision-making with neuronal dominant networks may induce straightforward behaviors, which are less affected by personality (B). In the present study, the control group's personality- oriented behaviors could be formulated by microglial priming effects (A), and the minocycline group's situation- oriented behaviors may be induced by suppressing microglial contribution to social behaviors (B).
doi:10.1371/journal.pone.0040461.g003

treatments may modulate microglial contribution to disturbed decision-making in social behaviors. To develop our results and these perspectives, animal based decision-making experiments with minocycline (or other microglial inhibitors) and histological analysis of microglia are called for in the near future. In addition, clinical trials of social decision-making experiments focusing on microglia should be attempted.

Limitation

First, this study did not examine the dose-dependent effects of minocycline. Second, this study was conducted only with adult males, while there may be a difference when players are female. Third, we did not measure microglia activity in the brain via imaging methods, while minocycline may inhibit some brain regional activities which are thought to be linked to trust and social decision-making [52,53]. Therefore, brain imaging studies are needed to clarify these regional activation mechanisms. Finally, other possible minocycline effects should be taken into account. Apart from inhibiting microglial activation, minocycline also has been reported to interact with brain glutamate and dopamine neurotransmission [54,55] and to have direct effects on neuronal cell line, PC12 [56]. Some reports suggest positive links between microglia, glutamate and dopamine interaction [57,58]. Further research should be performed to clarify this interaction mechanism. No specific inhibitor of microglia exists to date, therefore we selected minocycline as the most appropriate and safest drug to be used in humans at present. When a safe, specific inhibitor of microglial activation is developed, microglial human function will be clarified more effectively.

Conclusion

Based on the results of the present human social decision-making experiment, we have proposed a novel microglial contribution to personality and social behaviors. Our present study may shed new light on microglial roles in the social and mental life of healthy humans and also of people with psychiatric disorders. To explore these perspectives, further *in vitro/in vivo* studies and translational research are needed.

Ethics Statement

This double-blind randomized study was approved by the Kyushu University Ethical Committee under the administration of the UMIN clinical trial center (**UMIN000004803**). All participants gave written informed consent to participate after a complete description of the study.

Supporting Information

Table S1 Participants List.
(XLS)

Checklist S1 CONSORT Checklist.
(DOC)

Protocol S1 Trial Protocol.
(DOC)

Protocol S2 Japanese Version of Trial Protocol.
(DOC)