Journal of Psychiatric Research 50 (2014) 79–83

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

ITIH3 polymorphism may confer susceptibility to psychiatric disorders by altering the expression levels of *GLT8D1*

Daimei Sasayama [a,b], Hiroaki Hori [a], Noriko Yamamoto [a], Seiji Nakamura [c], Toshiya Teraishi [a], Masahiko Tatsumi [d], Kotaro Hattori [a], Miho Ota [a], Teruhiko Higuchi [e], Hiroshi Kunugi [a,*]

[a] *Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo, 187-8502, Japan*
[b] *Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, Nagano, 390-8621, Japan*
[c] *DNA Chip Research Inc., Yokohama, Kanagawa, 230-0045, Japan*
[d] *Department of Occupational Therapy, School of Nursing and Rehabilitation Sciences, Show University, Tokyo, 142-8555, Japan*
[e] *National Center of Neurology and Psychiatry, Kodaira, Tokyo, 187-8502, Japan*

ARTICLE INFO

Article history:
Received 29 September 2013
Received in revised form
30 November 2013
Accepted 6 December 2013

Keywords:
Schizophrenia
Major depressive disorder
Single nucleotide polymorphism
Gene expression
Microarray
ITIH3
ITIH4
GLT8D1

ABSTRACT

A recent genome-wide analysis indicated that a polymorphism (rs2535629) of *ITIH3* showed the strongest association signal with susceptibility to psychiatric disorders in Caucasian populations. The aim of the study was to replicate the association of rs2535629 with schizophrenia and major depressive disorder (MDD) in Japanese subjects. A total of 611 patients with schizophrenia, 868 with MDD, and 1193 healthy controls were successfully genotyped for rs2535629. A significant difference in allele distribution was found between patients with schizophrenia and controls (odds ratio [OR] = 1.21, 95% confidence interval [CI]: 1.05–1.39, $P = 0.0077$). A similar trend was found for patients with MDD (OR = 1.11, 95% CI: 0.98–1.26, $P = 0.092$). The allele distribution in the combined patient group (schizophrenia and MDD) was significantly different from that of the control group (OR = 1.15, 95% CI: 1.03–1.28, $P = 0.011$). Gene expression microarray analysis of whole blood samples in 39 MDD patients and 40 healthy controls showed that rs2535629 has a strong influence on the expression levels of *ITIH4* and *GLT8D1*. The expression levels of *GLT8D1* were significantly higher in patients with MDD than in controls ($P = 0.021$). To our knowledge, the present study showed for the first time the association of rs2535629 with psychiatric disorders in an Asian population. Our findings suggest that rs2535629 influences the susceptibility to psychiatric disorders by affecting the expression level of *GLT8D1*.

1. Introduction

A recently published genome-wide analysis by Cross-Disorder Group of the Psychiatric Genomics Consortium indicated that a single nucleotide polymorphism (SNP) rs2535629 showed the strongest association signal with susceptibility to 5 psychiatric disorders in the Psychiatric Genomics Consortium: autism spectrum disorder, attention-deficit-hyperactivity disorder, bipolar disorder, major depressive disorder (MDD) and schizophrenia (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). The G allele of rs2535629 was associated with an increased risk of psychiatric disorders (odds ratio [OR]: 1.10, 95% confidence interval [CI]: 1.07–1.12). This SNP is located within an intron of the *ITIH3* gene and is in tight linkage disequilibrium (LD) with SNPs encompassing several genes in the vicinity.

Accumulating evidence indicates an association of *ITIH3–ITIH4* region with schizophrenia (Schizophrenia Psychiatric GWAS Consortium, 2011; Hamshere et al., 2013) and bipolar disorder (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; Scott et al., 2009). A mega-analysis of genome-wide association studies for MDD (Ripke et al., 2013) also obtained the smallest *P* value for rs2535629; however, no association was found in a replication dataset of 6783 patients with MDD and 50,695 controls ($P = 0.70$). Although further studies are necessary to confirm the association between *ITIH3–ITIH4* region and MDD, evidence to date strongly suggests that rs2535629 located in *ITIH3–ITIH4* region is associated with a range of psychiatric disorders.

The functional properties of rs2535629 and the mechanism by which it contributes to susceptibility to psychiatric disorders are yet to be elucidated. In particular, the causative gene remains

* Corresponding author. Tel.: +81 42 341 2712x5132; fax: +81 42 346 1744.
E-mail address: hkunugi@ncnp.go.jp (H. Kunugi).

0022-3956/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jpsychires.2013.12.002

unclear due to a number of genes in LD with the SNP. In addition, previous studies investigating association between *ITIH3–ITIH4* region and psychiatric disorders have been conducted in Caucasian populations. It remains unknown whether there is such an association in Asian populations.

The aims of the present study are twofold. First, we tried to replicate the association of rs2535629 with psychiatric disorders in a Japanese sample. Secondly, we examined the effect of rs2535629 on gene expression to obtain insight into the mechanism by which this SNP increases the susceptibility to psychiatric disorders.

2. Materials and methods

2.1. Subjects

A total of 641 patients with schizophrenia (355 men: mean age ± standard deviation 42.5 ± 13.0 years, mean age at onset 23.3 ± 7.8 years; 286 women: mean age 43.5 ± 14.9 years, mean age at onset 25.3 ± 9.9 years), 908 patients with MDD (408 men: mean age 43.6 ± 13.5 years, mean age at onset 36.9 ± 13.1 years; 500 women: mean age 50.6 ± 16.5 years, mean age at onset 42.4 ± 16.6 years) and 1249 healthy controls (421 men: mean age 45.5 ± 16.1 years; 828 women; 46.3 ± 15.4 years) were genotyped for rs2535629. Samples collected from 39 patients with MDD (18 men and 21 women: mean age 40.2 ± 11.4 years) and 40 healthy controls (20 men and 20 women: mean age 38.7 ± 11.1 years) were available for gene expression microarray analysis. Of those who participated in the microarray analysis, 65 had participated in our previous study (Sasayama et al., 2013b). All subjects were biologically unrelated Japanese individuals, and were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in free local information magazines and by our website announcement. Consensus diagnosis by at least two psychiatrists was made for each patient according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition criteria (American Psychiatric Association, 1994), on the basis of unstructured interviews and information from medical records. The controls were healthy volunteers with no current or past history of psychiatric treatment, and were screened using the Japanese version of the Mini International Neuropsychiatric Interview (M.I.N.I.) (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist to rule out any axis I psychiatric disorders. Participants were excluded if they had prior medical histories of central nervous system disease or severe head injury, or if they met the criteria for substance abuse or dependence, or mental retardation. The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. After description of the study, written informed consent was obtained from every subject.

2.2. Clinical measures

Depressive symptoms of patients with MDD who participated in the microarray study were assessed by an experienced research psychiatrist using the Japanese version of the GRID Hamilton Rating Scale for Depression, 17-item version (HRSD-17) (Hamilton, 1967), which has been demonstrated to show excellent inter-rater reliability (Tabuse et al., 2007).

2.3. Genotyping

Genomic DNA was prepared from the venous blood according to standard procedures. The SNP rs2535629 was genotyped using the TaqMan 5′-exonuclease allelic discrimination assay. Thermal cycling conditions for polymerase chain reaction were 1 cycle at 95 °C for 10 min followed by 50 cycles of 92 °C for 15 s and 60 °C for 1 min. The allele-specific fluorescence was measured with ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster city, CA, USA). Genotype data were read blind to the case–control status. Ambiguous genotype data were not included in the analysis.

2.4. Gene expression microarray

Venous blood was collected between 1100 and 1200 h in PAXgene tubes (Qiagen, Valencia) from each subject and was incubated at room temperature for 24 h for RNA stabilization. RNA was extracted from whole blood according to the manufacturer's guidelines by using the PAXgene Blood RNA System Kit (PreAnalytix GmbH, Hombrechtikon, Switzerland). The RNA was quantified by optical density readings at A260 nm by using the NanoDrop ND-1000 (Thermo Scientific, Rockford). Gene expression analysis was performed using Agilent Human Genome 4 × 44 K arrays (Agilent Technologies, Santa Clara). Raw signal data were analyzed by the GeneSpring GX software (Agilent Technologies). Data were filtered according to the expression level for quality control to eliminate genes that were below the 20th percentile threshold. The expression value of each gene was normalized to the median expression value of all genes in each chip. A total of 30,445 probes were included in the analysis.

2.5. Statistical analysis

Deviations of genotype distributions from the Hardy–Weinberg equilibrium (HWE) were assessed with the χ^2 test for goodness of fit. Genotype and allele distributions were compared between patients and controls by using the χ^2 test for independence. Differences between groups were compared using *t*-test or Mann–Whitney *U* test for continuous variables and χ^2 test for categorical variables. Spearman's rank correlation coefficient (ρ) was used to assess the correlation between two variables. All statistical tests were two-tailed, and $P < 0.05$ indicated statistical significance unless otherwise specified. The correlation between the genotype of rs2535629 (coded as the number of G alleles, i.e., 0 = A/A, 1 = G/A, and 2 = G/G) and gene expression level was considered significant if the Spearman's rank correlation coefficient (ρ) corresponded to a Bonferroni-corrected *P* value of <0.05. Analyses were performed using IBM SPSS Statistics Version 21 (IBM SPSS, Tokyo, Japan).

Power calculations were performed using Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) (Purcell et al., 2003). Assuming disease allele frequencies of 0.651 in controls and allelic odds ratio of 1.1 as reported previously (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013) and assuming a prevalence of 0.1, our sample had 65% statistical power for the general 2 df test (GG versus GA versus AA) and 44% for the allelic test (G versus A) to detect the difference between the combined patient group and the controls under an alpha level of 0.05. Statistical power to detect the difference in schizophrenia assuming a prevalence of 0.01 was 36% and 25% for general 2 df and allelic tests, respectively. Statistical power to detect the difference in MDD assuming a prevalence of 0.1 was 51% and 35% for general 2 df and allelic tests, respectively.

3. Results

A total of 611 patients with schizophrenia, 868 with MDD, and 1193 healthy controls were successfully genotyped for rs2535629. The overall call rate of genotyping was 95.5%. Genotype and allele distributions of rs2535629 are shown in Table 1. The genotype

Table 1
Genotype and allele distributions of rs2535629.

	N	Genotype count (frequency)			Allele count (frequency)		HWE		Statistics versus controls						
									Genotype			Allele			Allelic OR (95% CI)
		G/G	G/A	A/A	G	A	χ^2	P value	χ^2	df	P value	χ^2	df	P value	
Schizophrenia	611	211 (0.35)	291 (0.48)	109 (0.18)	713 (0.58)	509 (0.42)	0.25	0.62	7.32	2	0.026	7.09	1	0.0077	1.21 (1.05–1.39)
MDD	868	276 (0.32)	426 (0.49)	166 (0.19)	978 (0.56)	758 (0.44)	0.0050	0.94	2.86	2	0.24	2.85	1	0.092	1.11 (0.98–1.26)
Schizophrenia + MDD	1479	487 (0.33)	717 (0.48)	275 (0.19)	1691 (0.57)	1267 (0.43)	0.15	0.70	6.55	2	0.038	6.47	1	0.011	1.15 (1.03–1.28)
Controls	1193	343 (0.29)	595 (0.50)	255 (0.21)	1281 (0.54)	1105 (0.46)	0.010	0.92							

HWE: Hardy–Weinberg equilibrium; OR: odds ratio; CI: confidence interval; df: degrees of freedom; MDD: major depressive disorder.

distributions did not significantly deviate from the HWE in the control group or in the patient groups. Significant differences in genotype and allele distributions were found between patients with schizophrenia and controls. The G allele was significantly more common in patients than in controls. A nonsignificant trend in the same direction was found in patients with MDD. The genotype and allele distributions in the combined patient group were significantly different from that of the control group.

Gene expression microarray analysis of whole blood samples was performed in 39 MDD patients and 40 healthy controls. The clinical characteristics of those examined for the gene expression levels are shown in Table 2. The rs2535629 SNP was successfully genotyped in 38 MDD patients and 39 controls. None of the 30,445 gene probes examined in the microarray analysis had expression levels significantly associated with the genotype of rs2535629 after Bonferroni correction for the number of probes. So we focused on nearby genes to examine the *cis* expression quantitative trait locus (eQTL) effects. Since majority of eQTL SNPs are located within 100 kilobases (kb) of the gene (Dixon et al., 2007), we examined the genes within 100 kb upstream or downstream of rs2535629 to investigate the *cis* effect. A total of 8 genes (i.e., *GLT8D1*, *SPCS1*, *NEK4*, *ITIH1*, *ITIH3*, *ITIH4*, *MUSTN1*, *TMEM110*), according to HapMap data (release 28, Phase III), were located within 100 kb upstream or downstream of rs2535629. Of those, *ITIH4* and *GLT8D1* expression levels were significantly correlated with the number of G alleles of rs2535629. The expression level of *ITIH4* was negatively correlated with the number of G alleles ($\rho = -0.38$, $P = 0.0006$), indicating that the G allele was associated with decreased expressions. Conversely, the expression level of *GLT8D1* was positively correlated with the number of G alleles ($\rho = 0.33$, $P = 0.003$), indicating that the G allele was associated with increased expressions. No significant correlation was observed for the other 6 genes examined.

Fig. 1 shows the expression levels of *GLT8D1* and *ITIH4* of patients with MDD and the controls. The expression levels of *GLT8D1* were significantly higher in patients with MDD than in controls ($P = 0.021$; Mann–Whitney U test). Furthermore, the expression levels of *GLT8D1* were significantly positively correlated with the HRSD-17 score ($\rho = 0.32$, $P = 0.047$), as shown in Fig. 2. The expression levels of *ITIH4* were not significantly correlated with the HRSD-17 score ($\rho = 0.16$, $P = 0.67$).

No significant difference between men and women was observed for the expression levels of *GLT8D1* (MDD: $U = 235$, $P = 0.20$; controls: $U = 181$, $P = 0.62$) or *ITIH4* (MDD: $U = 251$, $P = 0.083$; controls: $U = 178$, $P = 0.57$). No significant correlation with body mass index was observed for the expression levels of *GLT8D1* (MDD: $\rho = -0.16$, $P = 0.43$; controls: $\rho = 0.18$, $P = 0.38$) or *ITIH4* (MDD: $\rho = -0.13$, $P = 0.53$; controls: $\rho = 0.28$, $P = 0.16$). No significant difference between smokers and non-smokers was observed for the expression levels of *GLT8D1* (MDD: $U = 213$, $P = 0.42$; controls: $U = 182$, $P = 1.0$) or *ITIH4* (MDD: $U = 211$, $P = 0.45$; controls: $U = 240$, $P = 0.10$). No significant difference in the expression levels was observed between patients with MDD prescribed antidepressants and those not prescribed antidepressants (*GLT8D1*: $U = 54$, $P = 0.083$; *ITIH4*: $U = 71$, $P = 0.29$). The expression levels of *GLT8D1* and *ITIH4* did not significantly correlate with each other ($\rho = 0.091$, $P = 0.43$).

Table 2
Clinical characteristics of those who participated in the microarray study.

	Healthy controls	Patients with MDD	Statistics
	(N = 40)	(N = 39)	
Sex (men/women)	20/20	18/21	$\chi^2 = 0.12$, df = 1, $P = 0.73$
Average age (years)	38.7 ± 11.1	40.2 ± 11.4	$t = 0.58$, df = 77, $P = 0.56$
Smoking status (smokers/nonsmokers)	14/26	16/23	$\chi^2 = 0.30$, df = 1, $P = 0.58$
BMI[a]	22.4 ± 3.9	24.1 ± 6.7	$t = 1.12$, df = 50, $P = 0.27$
Age at onset (years)		32.7 ± 11.5	
Treatment duration (years)		5.1 ± 6.0	
HRSD-17 score		16.0 ± 6.8	
Current medication status			
On antidepressants		84.6%	
On antipsychotics		28.2%	
On lithium		10.3%	

MDD: major depressive disorder; HRSD-17: Hamilton rating scale for depression (17-item version); BMI: body mass index.
The values are shown as mean ± standard deviation.
[a] BMI data were available for only 27 of the controls and 25 of the patients.

4. Discussion

The present study showed that the G allele of rs2535629 was associated with an increased risk for schizophrenia in a Japanese population. Although not significant, a similar trend was found in patients with MDD. Our findings are consistent with the findings in Caucasian populations which demonstrated that the G allele of rs2535629 was associated with an increased risk of psychiatric disorders with an OR of 1.1 (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013). To our knowledge, the present study showed for the first time the association of rs2535629 with psychiatric disorders in an Asian population. Furthermore, our results showed that rs2535629 influenced the expression levels of *ITIH4* and *GLT8D1* in whole blood. The expression levels of *GLT8D1* were significantly higher in patients with MDD than in controls and were positively correlated with the severity of depression assessed by HRSD-17. The finding suggests that rs2535629 may influence the susceptibility to psychiatric disorders by affecting the expression levels of *GLT8D1* gene.

The *GLT8D1* gene encodes the protein glycosyltransferase 8 domain-containing protein 1 (GLT8D1), a member of the glycosyltransferase family 8. Glycosyltransferases are known to be involved in the synthesis of glycoconjugates by transferring mono- or oligosaccharide residue to an acceptor molecule for the elongation

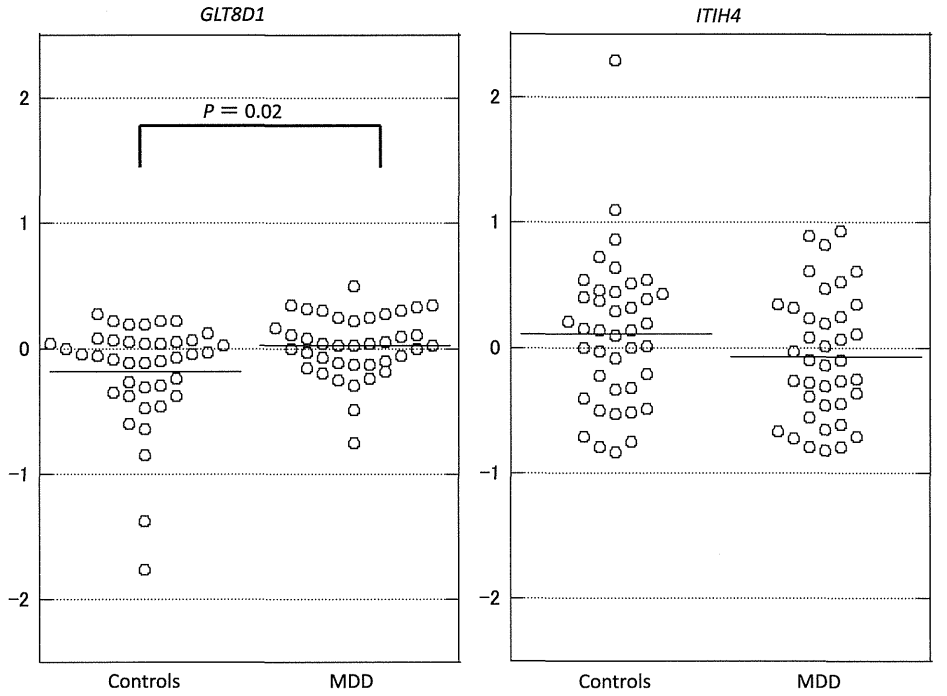

Fig. 1. Gene expression levels of *GLT8D1* and *ITIH4* in whole blood sample The gene expression levels of *GLT8D1* and *ITIH4* of patients with MDD and controls are shown. The expression levels of *GLT8D1* were significantly higher in patients with MDD than in controls ($P = 0.021$; Mann–Whitney *U* test). The expression levels of *ITIH4* were not significantly different between patients with MDD and healthy controls. Horizontal bars indicate the mean value.

or initiation of the carbohydrate chain. According to the human protein atlas (Uhlen et al., 2010), GLT8D1 is expressed in most normal tissues including brain, with especially high levels in hepatocytes and renal tubules. However, the specific function of GLT8D1 has not been determined. Further studies are needed to elucidate the role of GLT8D1 in the etiology and pathogenesis of psychiatric disorders.

The mechanisms of how rs2535629 located at intron of *ITIH3* is associated with the expression levels of *ITIH4* and *GLT8D1* are as yet to be elucidated. There are numerous SNPs in strong LD ($r^2 > 0.8$) with rs2535629. These SNPs spread across regions approximately 24 kb upstream to 15 kb downstream of rs2535629, according to HapMap 3 (release 2) JPT + CHB data (examined using SNAP; http://www.broadinstitute.org/mpg/snap/ (Johnson et al., 2008)). It is possible that some of these nearby SNPs have functional effects regulating gene expression levels. Therefore, further examination of the functional roles of these SNPs is necessary to clarify the mechanism of gene expression regulation.

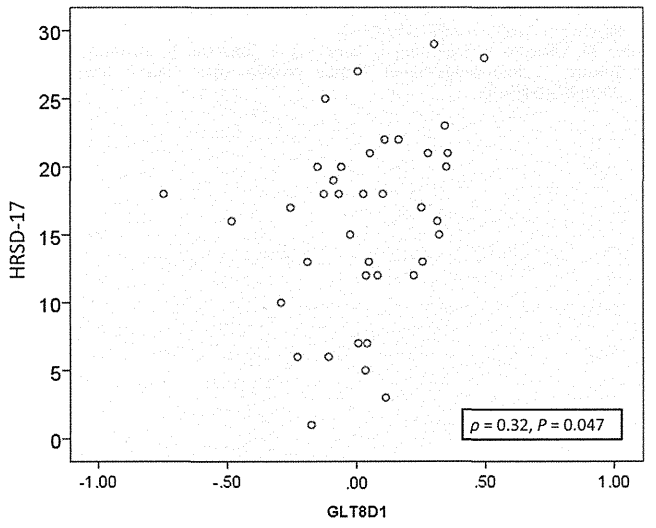

Fig. 2. Association between gene expression levels of *GLT8D1* and depression severity. The expression levels of *GLT8D1* of patients with MDD were significantly positively correlated with the Hamilton rating scale for depression, 17 item version (HRSD-17) score ($\rho = 0.32$, $P = 0.047$).

We found no significant difference in the expression levels of *ITIH4* between patients with MDD and healthy controls. However, the small number of participants limited the power to detect the difference. The G allele of rs2535629 was associated with decreased expression levels of *ITIH4*. Therefore, it is conceivable that the expression levels of *ITIH4* are lower in patients with MDD than in controls, albeit with smaller effect size than for *GLT8D1*. ITIH4 plays an important role as an anti-inflammatory protein (Kashyap et al., 2009; Pineiro et al., 1999). It can be speculated that genetically determined decreased expression of ITIH4 may contribute to confer risk of MDD, which has been shown to be associated with inflammation (Krishnadas and Cavanagh, 2012; Sasayama et al., 2013a). However, a separate study is required to examine whether the alteration of *ITIH4* expression is actually a causal factor of MDD.

One of the limitations of the study was that the gene expression levels were examined only in patients with MDD and controls. The role of rs2535629 on the etiology may differ between schizophrenia and MDD. Therefore, future studies should examine the gene expression levels in patients with schizophrenia as well as in other psychiatric disorders to elucidate the mechanism of the shared genetic effects of rs2535629 on psychiatric disorders. Another limitation of the present study was the uncontrolled medication in MDD patients who participated in the microarray analysis. The effects of medication on gene expression levels could not be assessed due to the variability in types and doses. The effects of psychotropic medications must be addressed in future studies.

In conclusions, the present study showed for the first time the association of rs2535629 with schizophrenia and MDD in a Japanese population. The expression levels of *GLT8D1* were significantly higher in patients with MDD than in healthy controls. Our findings suggest that rs2535629 may influence the susceptibility to psychiatric disorders by affecting the expression levels of *GLT8D1*.

Role of funding source

This study was supported by Health and Labor Sciences Research Grants, the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Understanding of molecular and environmental bases for brain health) and Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP (H.K.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Contributors

D.S. and H.K. designed the study and D.S. wrote the draft of the manuscript. D.S., H.H., T.T., M.T., K.H., M.O., and H.K. screened the study participants using the Mini International Neuropsychiatric Interview (M.I.N.I.) and diagnosed the patients according to the DSM-IV criteria. N.Y. and S.N. performed the microarray analysis. D.S., S.N., and H.K. undertook the statistical analysis. H.K. supervised the data analysis and writing of the paper. T.H. also supervised the writing of the paper and gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

Acknowledgments

The authors would like to thank the participants for taking part in the study.

References

American Psychiatric Association. DSM-IV: diagnostic and statistical manual of mental disorders. 4th ed. Washington (DC): American Psychiatric Press; 1994.

Cross-Disorder Group of the Psychiatric Genomics Consortium. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet 2013;381:1371–9.

Dixon AL, Liang L, Moffatt MF, Chen W, Heath S, Wong KC, et al. A genome-wide association study of global gene expression. Nature Genetics 2007;39:1202–7.

Hamilton M. Development of a rating scale for primary depressive illness. British Journal of Social and Clinical Psychology 1967;6:278–96.

Hamshere ML, Walters JT, Smith R, Richards AL, Green E, Grozeva D, et al. Genome-wide significant associations in schizophrenia to ITIH3/4, CACNA1C and SDCCAG8, and extensive replication of associations reported by the schizophrenia PGC. Molecular Psychiatry 2013;18:708–12.

Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 2008;24:2938–9.

Kashyap RS, Nayak AR, Deshpande PS, Kabra D, Purohit HJ, Taori GM, et al. Inter-alpha-trypsin inhibitor heavy chain 4 is a novel marker of acute ischemic stroke. Clinica Chimica Acta 2009;402:160–3.

Krishnadas R, Cavanagh J. Depression: an inflammatory illness? Journal of Neurology, Neurosurgery, and Psychiatry 2012;83:495–502.

Otsubo T, Tanaka K, Koda R, Shinoda J, Sano N, Tanaka S, et al. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry and Clinical Neurosciences 2005;59:517–26.

Pineiro M, Alava MA, Gonzalez-Ramon N, Osada J, Lasierra P, Larrad L, et al. ITIH4 serum concentration increases during acute-phase processes in human patients and is up-regulated by interleukin-6 in hepatocarcinoma HepG2 cells. Biochemical and Biophysical Research Communication 1999;263:224–9.

Psychiatric GWAS Consortium Bipolar Disorder Working Group. Large-scale genome-wide association analysis of bipolar disorder identifies a new susceptibility locus near ODZ4. Nature Genetics 2011;43:977–83.

Purcell S, Cherny SS, Sham PC. Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2003;19:149–50.

Ripke S, Wray NR, Lewis CM, Hamilton SP, Weissman MM, Breen G, et al. A mega-analysis of genome-wide association studies for major depressive disorder. Molecular Psychiatry 2013;18:497–511.

Sasayama D, Hattori K, Wakabayashi C, Teraishi T, Hori H, Ota M, et al. Increased cerebrospinal fluid interleukin-6 levels in patients with schizophrenia and those with major depressive disorder. Journal of Psychiatric Research 2013a;47:401–6.

Sasayama D, Hori H, Nakamura S, Miyata R, Teraishi T, Hattori K, et al. Identification of single nucleotide polymorphisms regulating peripheral blood mRNA expression with genome-wide significance: an eQTL study in the Japanese population. PLoS One 2013b;8:e54967.

Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium. Genome-wide association study identifies five new schizophrenia loci. Nature Genetics 2011;43:969–76.

Scott LJ, Muglia P, Kong XQ, Guan W, Flickinger M, Upmanyu R, et al. Genome-wide association and meta-analysis of bipolar disorder in individuals of European ancestry. Proceedings of the National Academy of Sciences of the United States of America 2009;106:7501–6.

Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. Journal of Clinical Psychiatry 1998;59(Suppl. 20):22–33 [quiz 34–57].

Tabuse H, Kalali A, Azuma H, Ozaki N, Iwata N, Naitoh H, et al. The new GRID Hamilton Rating Scale for Depression demonstrates excellent inter-rater reliability for inexperienced and experienced raters before and after training. Psychiatry Research 2007;153:61–7.

Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, et al. Towards a knowledge-based human protein atlas. Nature Biotechnology 2010;28:1248–50.

Journal of Psychiatric Research 49 (2014) 37–42

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Benzodiazepines, benzodiazepine-like drugs, and typical antipsychotics impair manual dexterity in patients with schizophrenia

Daimei Sasayama [a,b], Hiroaki Hori [a], Toshiya Teraishi [a], Kotaro Hattori [a], Miho Ota [a], Junko Matsuo [a], Yukiko Kinoshita [a], Mitsutoshi Okazaki [c], Kunimasa Arima [c], Naoji Amano [b], Teruhiko Higuchi [d], Hiroshi Kunugi [a,*]

[a] Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, 4-1-1, Ogawahigashi, Kodaira, Tokyo 187-8502, Japan
[b] Department of Psychiatry, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan
[c] Department of Psychiatry, National Center Hospital, National Center of Neurology and Psychiatry, Kodaira 187-8502, Japan
[d] National Center of Neurology and Psychiatry, Kodaira 187-8502, Japan

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form
3 October 2013
Accepted 29 October 2013

Keywords:
Schizophrenia
Dexterity
Benzodiazepines
Antipsychotics

ABSTRACT

Impaired dexterity is a major psychomotor deficit reported in patients with schizophrenia. In the present study, the Purdue pegboard test was used to compare the manual dexterity in patients with schizophrenia and healthy controls. We also examined the influence of antipsychotics, benzodiazepines, and benzodiazepine-like drugs on manual dexterity. Subjects were 93 patients with schizophrenia and 93 healthy controls, matched for sex and age distributions. Control subjects scored significantly higher on all scores of Purdue pegboard than patients with schizophrenia. Age, PANSS negative symptom scale, typical antipsychotic dose, and use of benzodiazepines and/or benzodiazepine-like drugs were negatively correlated with the pegboard scores in patients with schizophrenia. The present results indicate that patients with schizophrenia have impaired gross and fine fingertip dexterity compared to healthy controls. The use of typical antipsychotics and benzodiazepines and/or benzodiazepine-like drugs, but not atypical antipsychotics, had significant negative impact on dexterity in patients with schizophrenia. Psychiatrists should be aware that some psychotropic medications may enhance the disability caused by the impairment of dexterity in patients with schizophrenia.

1. Introduction

Impaired motor function is a major neurocognitive phenotype reported in patients with schizophrenia (Jahn et al., 2006). One of the most prominent motor dysfunctions in patients with schizophrenia may be the deficit in fine motor skills. Previous studies have shown impaired dexterity not only in patients with schizophrenia (Midorikawa et al., 2008; Sponheim et al., 2010) but also in individuals at risk for schizophrenia (Gschwandtner et al., 2006). Recent evidence suggests that the impairment of fine motor dexterity may also be an endophenotype of bipolar disorder (Langenecker et al., 2010; Sasayama et al., 2012) and, thus, may be one of the several neurocognitive deficits shared by patients with schizophrenia and those with bipolar disorder (Szoke et al., 2008).

Fine motor skills are also influenced by the use of antipsychotic drugs (Sasayama et al., 2012; Sponheim et al., 2010) and benzodiazepines (McManus et al., 1983; Morgan et al., 1984). Benzodiazepine-like drugs—a class of drugs with similar pharmacodynamics and different chemical structures to benzodiazepines—are generally well tolerated compared to benzodiazepines. However, these newer agents also have a similar adverse effect profile as benzodiazepines (McKean and Vella-Brincat, 2011; Zammit, 2009). Excess use of such psychotropic medications may exacerbate the impaired fine motor skills of patients with schizophrenia. Understanding these risks is essential in selecting optimal treatment strategies. However, only few studies have documented the adverse effects of benzodiazepine treatment in schizophrenia (Dold et al., 2012). Furthermore, to our knowledge, no previous studies have shown the influence of benzodiazepines and benzodiazepine-like drugs on dexterity in patients with schizophrenia. Studies have also shown that the cognitive benefits of atypical antipsychotics are superior to those of typical antipsychotics, with the former class of drugs having fewer extrapyramidal

* Corresponding author. Tel.: +81 42 341 2712x5132; fax: +81 42 346 1744.
E-mail address: hkunugi@ncnp.go.jp (H. Kunugi).

0022-3956/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.jpsychires.2013.10.019

side effects (Meltzer, 2013). These side effects as well as the sedative effects of antipsychotics may be the cause of impaired dexterity. However, no studies have considered separately the effect of typical and atypical antipsychotics on dexterity.

Purdue pegboard test (Tiffin and Asher, 1948) has been used to examine manual dexterity of patients with psychiatric disorders including schizophrenia (Wobrock et al., 2013), major depressive disorder, bipolar disorder (Sasayama et al., 2012), and schizotypy (Hori et al., 2012). In the present study, the Purdue pegboard test was used to assess the manual dexterity in patients with schizophrenia and healthy controls. The influence of antipsychotics, benzodiazepines, and benzodiazepine-like drugs on manual dexterity was examined. Because grip strength is known to decrease with sedation caused by a benzodiazepine-receptor agonist (Huang et al., 2012; Tomita et al., 2013), we also measured the handgrip force to assess the sedative effects induced by the prescribed drugs.

2. Materials and methods

2.1. Subjects

Subjects were 93 patients with schizophrenia and 93 healthy controls, matched for sex and age distributions. Participants were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan or through advertisements in local free magazines, website announcement, notices posted in the hospital, flyers, and word of mouth. Only self-reported right-handed subjects were included in the study. Consensus diagnoses by at least two research psychiatrists were made according to the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia for enrollment in the study. Healthy participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by a research psychiatrist, and only those who demonstrated no history of psychiatric illness or contact to psychiatric services were enrolled as healthy controls. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse. All subjects were biologically unrelated Japanese individuals who resided in the Western part of Tokyo. Written informed consent was obtained from all subjects prior to their inclusion in the study and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

Table 1
Clinical characteristics.

	Healthy controls	Patients with schizophrenia	Statistics
	($N = 93$)	($N = 93$)	
Sex (male/female)	55/38	55/38	$\chi^2 = 0$, $P = 1.0$
Average age (years)	40.59 ± 11.5	40.25 ± 11.4	$t = 0.21$, $P = 0.84$
Age at onset (years)		24.1 ± 8.9	
Treatment duration (years)		14.8 ± 10.0	
PANSS scores			
Positive symptom scale		13.2 ± 5.0	
Negative symptom scale		15.6 ± 6.0	
General symptom scale		28.2 ± 7.2	
CP equivalent dose			
Typical antipsychotics (mg)		238.2 ± 326.9	
Atypical antipsychotics (mg)		290.0 ± 441.0	
All antipsychotics combined (mg)		528.2 ± 496.5	
Use of BZDs and/or BZD-like drugs (no current use/ current use)		30/63	
Handgrip force (kg)	34.7 ± 9.8	30.54 ± 9.4	$t = 2.97$, $P = 0.003$

PANSS: positive and negative syndrome scale, CP: chlorpromazine, BZD: benzodiazepine.
The values are shown as mean ± standard deviation.

2.2. Measures

2.2.1. Purdue pegboard test

Participants were administered the Purdue pegboard test (Model 32030, manufactured by Lafayette Instrument Company, IN, USA). The pegboard contains two vertical arrays of 25 holes in which pegs are placed one hand at a time and then with both hands simultaneously under timed conditions (30 s per trial). Scores for these measures (right, left, and both hands subtests) were derived for each trial according to how many pegs were placed within the time limit. The sum of the right, left, and both hands subtest scores (R + L + B) was used as the representation of gross dexterity of the fingers, hands, and arms. Fine fingertip dexterity was assessed by the assembly subtest, which involves using both hands alternately to construct assemblies consisting of a pin, a washer, a collar and another washer. This subtest requires participants to complete as many assemblies as possible within 60 s. The total number of pieces assembled was recorded as the score of the assembly subtest. Subjects did not take psychotropic medications for at least an hour prior to the pegboard test.

2.2.2. Handgrip force

Handgrip force was measured using a digital handgrip dynamometer (T.K.K.5401; Takei Co., Tokyo, Japan) to record the muscle strength of the right hand (*i.e.* the dominant hand). Participants were instructed to exert maximum grip force while standing upright, keeping their active arm stretched down vertically close to the body. The average of two trials was defined as the maximal handgrip force.

2.2.3. Clinical measures

Clinical symptoms of patients with schizophrenia were assessed by an experienced research psychiatrist using the Japanese version of the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987; Yamada et al., 1991), which has been demonstrated to show good inter-rater reliability (Igarashi et al., 1998). Daily doses of antipsychotics were converted to chlorpromazine equivalent dose, using published guidelines (Inagaki et al., 1999). We classified imidazopyridines, pyrazolopyrimidines, cyclopyrrolones, and thienodiazepines as benzodiazepine-like drugs.

2.3. Statistical analysis

Differences of clinical data between groups were evaluated by the chi-squared test for categorical variables and Student's *t* test for continuous variables. Correlations between continuous values were assessed using Pearson's correlation coefficient. Associations of the pegboard scores with clinical characteristics were assessed in patients with schizophrenia using stepwise linear regression model (entry criteria $P < 0.05$, removal criteria $P > 0.1$) including the following candidate independent variables: age, sex, PANSS positive symptoms scale, PANSS negative symptoms scale, PANSS general symptom scale, typical antipsychotic dose, atypical antipsychotic dose, and use of benzodiazepines and/or

benzodiazepine-like drugs. Handgrip force was not included in the stepwise model because of high collinearity with sex and age. Partial correlation controlling for sex and age was used to assess the relationship between handgrip force and pegboard scores. Analyses were performed using IBM SPSS Statistics Version 21 (IBM SPSS, Tokyo, Japan). All statistical tests were two-tailed, and $P < 0.05$ indicated statistical significance.

3. Results

Table 1 shows the clinical characteristics of the subjects. The subjects of the patient group included 53 patients with paranoid type, 7 with disorganized type, 1 with catatonic type, 16 with undifferentiated type, and 16 with residual type. Benzodiazepines and/or benzodiazepine-like drugs were prescribed in 63 patients with schizophrenia. Typical and atypical antipsychotics were prescribed in 55 and 54 patients, respectively, and 8 patients were antipsychotic-free. Typical antipsychotics included chlorpromazine, fluphenazine, haloperidol, levomepromazine, propericiazine, sulpiride, sultopride, and zotepine. Atypical antipsychotics included aripiprazole, blonaserine, olanzapine, perospirone, quetiapine, and risperidone. Benzodiazepines included bromazepam, clonazepam, estazolam, flunitrazepam, flurazepam, haloxazolam, loflazepate, lorazepam, nitrazepam, rilmazafone, and triazolam. Benzodiazepine-like drugs included brotizolam, etizolam, and zolpidem. No significant difference in any of the PANSS subscales was observed between current users and non-current users of benzodiazepines and/or benzodiazepine-like drugs. None of the PANSS subscales showed significant correlation with the chlorpromazine equivalent of typical, atypical, or total antipsychotic doses.

Control subjects scored significantly higher on all scores of Purdue pegboard than patients with schizophrenia (right hand: $t = 4.08$, $P < 0.0001$; left hand: $t = 5.09$, $P < 0.0001$; both hands: $t = 3.70$, $P = 0.0003$; R + L + B: $t = 4.76$, $P < 0.0001$, assembly: $t = 5.17$, $P < 0.0001$). Fig. 1 shows the Purdue pegboard scores of R + L + B and assembly in patients with schizophrenia and controls.

Simple Pearson's correlation analyses in patients with schizophrenia showed that PANSS negative and general symptom scale, and age, were significantly negatively correlated with both R + L + B and assembly scores. Fig. 2 shows the relationship between antipsychotic dose and Purdue pegboard scores in patients with schizophrenia. For those prescribed typical antipsychotic medication, the daily dose of typical antipsychotics was significantly correlated with both R + L + B and assembly scores. Conversely, for those prescribed atypical antipsychotic medication, no significant correlation was found between daily dose of atypical antipsychotic and R + L + B or assembly score. Patients currently using benzodiazepines and/or benzodiazepine-like drugs scored significantly lower on R + L + B and assembly scores than those not currently using benzodiazepines and/or benzodiazepine-like drugs (Fig. 3).

No significant difference in the handgrip force was observed between patients currently prescribed benzodiazepines and/or benzodiazepine-like drugs and those who were not ($t = 0.32$, $P = 0.75$). For those prescribed antipsychotics, handgrip force was not significantly correlated with typical antipsychotic dose, atypical antipsychotic dose, or total antipsychotic dose (typical ($N = 55$): $r = 0.082$, $P = 0.55$; atypical ($N = 54$): $r = 0.11$, $P = 0.41$; total ($N = 85$): $r = 0.13$, $P = 0.23$). Partial correlation analysis controlling for sex and age showed that handgrip force of patients with schizophrenia was significantly positively correlated with the pegboard scores (R + L + B: partial correlation coefficient $r_p = 0.31$, $P = 0.003$; assembly: $r_p = 0.26$, $P = 0.013$). In contrast, the handgrip force of healthy controls showed no significant correlation with the pegboard scores (R + L + B: $r_p = 0.097$, $P = 0.36$; assembly: $r_p = 0.20$, $P = 0.054$).

Table 2 shows the results of the stepwise linear regression analyses in patients with schizophrenia with R + L + B or assembly scores as the dependent variable. Age, sex, PANSS positive symptoms scale, PANSS negative symptoms scale, PANSS general symptom scale, typical antipsychotic dose, atypical antipsychotic dose, and use of benzodiazepines and/or benzodiazepine-like drugs were included as predictor variables. Age, PANSS negative symptom scale, typical antipsychotic dose, and use of benzodiazepines and/or benzodiazepine-like drugs were significantly negatively correlated with both R + L + B and assembly scores.

Twenty patients were free of typical antipsychotics and benzodiazepines and/or benzodiazepine-like drugs. Neither R + L + B nor assembly scores of these patients was significantly different from that of healthy controls (R + L + B: patients 38.8 ± 6.14 (mean ± standard deviation) and controls 38.6 ± 4.5, $t = 0.18$, $P = 0.86$; assembly: patients 33.6 ± 8.1 and controls 34.5 ± 6.4, $t = 0.56$, $P = 0.58$).

4. Discussion

Gross dexterity assessed by R + L + B score and fine fingertip dexterity assessed by assembly score were both impaired by the use of typical antipsychotics and benzodiazepines and/or

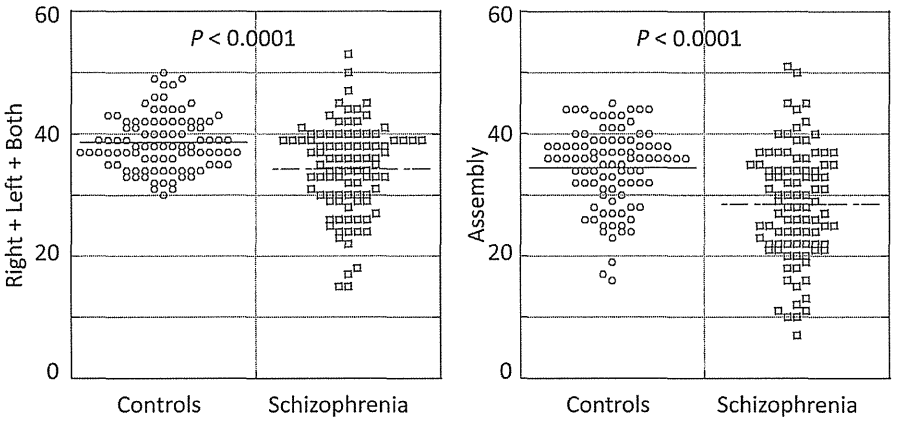

Fig. 1. Comparison of Purdue pegboard scores between patients with schizophrenia and controls. R + L + B and assembly scores of patients with schizophrenia and controls are shown. Patients with schizophrenia scored significantly lower on both scores than controls (R + L + B: $t = 4.76$, $P < 0.0001$, assembly: $t = 5.17$, $P < 0.0001$).

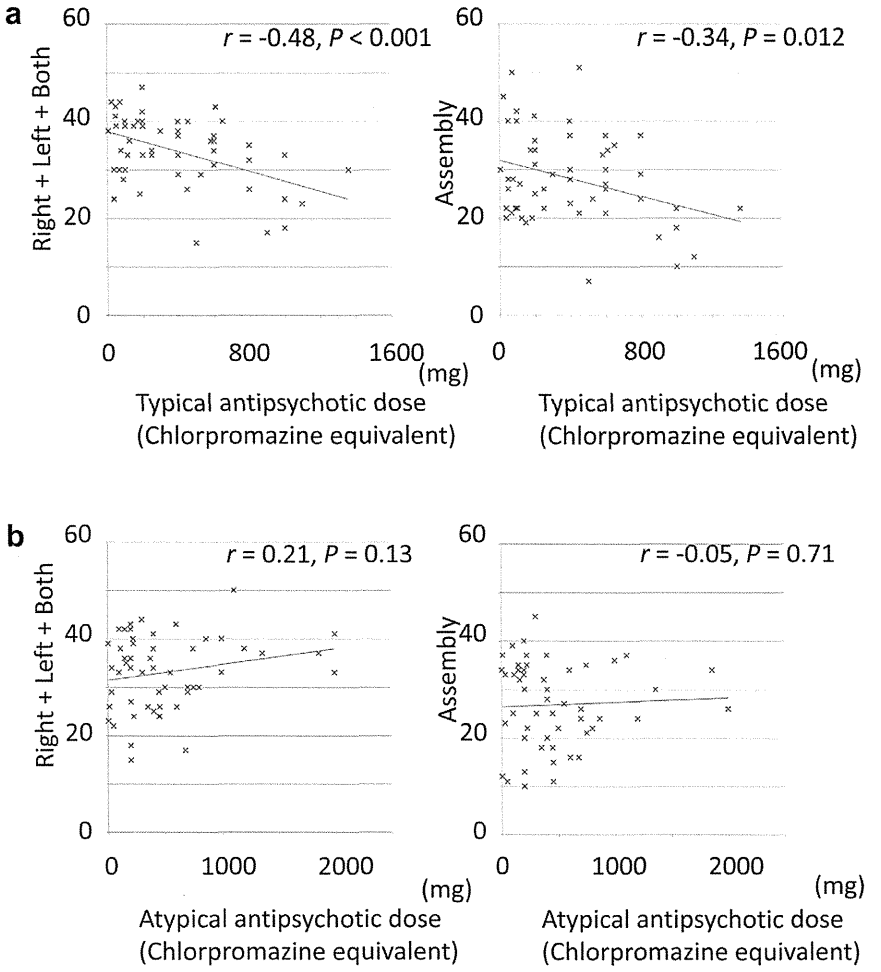

Fig. 2. a: Relationship between typical antipsychotic dose and Purdue pegboard scores in patients with schizophrenia. 2b: Relationship between atypical antipsychotic dose and Purdue pegboard scores in patients with schizophrenia. The associations between chlorpromazine equivalent of antipsychotic dose and the Purdue pegboard scores are shown for patients prescribed the medication. Typical but not atypical antipsychotic dose was significantly correlated with Purdue pegboard scores.

benzodiazepine-like drugs but not by the use of atypical antipsychotics. Patients with schizophrenia scored lower on both R + L + B and assembly compared to healthy controls. However, no significant difference in the Purdue pegboard scores was found between healthy controls and patients not prescribed typical antipsychotics or benzodiazepines and/or benzodiazepine-like drugs.

Previous studies have also reported association between antipsychotic use and lowered dexterity. Sponheim et al. (2010) showed that impairment of fine motor dexterity measured by Grooved Pegboard was correlated with chlorpromazine equivalent antipsychotic dose in patients with schizophrenia. Another study also showed that antipsychotic medication had significantly

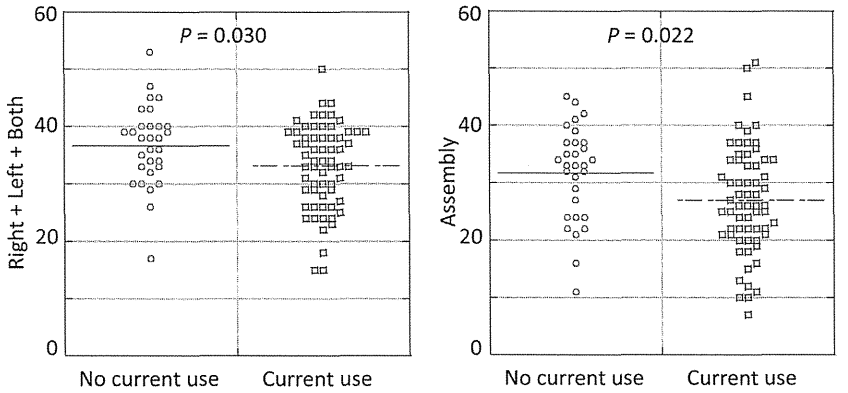

Fig. 3. Comparison of Purdue pegboard scores of patients with schizophrenia between benzodiazepine and/or benzodiazepine-like drug users and non-users. R + L + B and assembly scores of patients with schizophrenia were higher for current users of benzodiazepines and/or benzodiazepine-like drugs than for non-users.

Table 2
The results of the stepwise regression analyses.

	Unstandardized coefficient (B)	SE	Standardized coefficient (β)	t	P	Collinearity statistics	
						Tolerance	VIF
Right + left + both							
Constant	44.534	3.692		12.063	<0.001		
Typical antipsychotic dose	−0.006	0.002	−0.283	−3.207	0.002	0.961	1.040
Age	−0.161	0.057	−0.250	−2.827	0.006	0.960	1.041
PANSS negative symptom scale	−0.289	0.108	−0.235	−2.669	0.009	0.963	1.039
Sex	3.126	1.330	0.210	2.351	0.021	0.939	1.065
Use of BZDs and/or BZD-like drugs	−3.084	1.382	−0.197	−2.232	0.028	0.961	1.040
Assembly							
Constant	49.918	3.725		13.402	<0.001		
Age	−0.282	0.073	−0.349	−3.886	<0.001	0.986	1.014
PANSS negative symptom scale	−0.390	0.140	−0.253	−2.793	0.006	0.967	1.034
Typical antipsychotic dose	−0.006	0.003	−0.203	−2.262	0.026	0.980	1.020
Use of BZDs and/or BZD-like drugs	−3.855	1.772	−0.196	−2.175	0.032	0.976	1.024

Age, sex, PANSS positive symptoms scale, PANSS negative symptoms scale, PANSS general symptom scale, typical antipsychotic dose, atypical antipsychotic dose, and use of benzodiazepines and/or benzodiazepine-like drugs were included as predictor variables.
Antipsychotic dose was converted to chlorpromazine equivalents.
Use of BZDs and/orBZD-like drugs was coded as 0 = "no current use" and 1 = "current use".
Sex was coded as 1 = "men" and 2 = "women".
SE: standard error, VIF: variance inflation factor, PANSS: positive and negative syndrome scale, BZD: benzodiazepine.

negative influence on dexterity in patients with bipolar disorder (Sasayama et al., 2012). To our knowledge, this is the first study to consider separately the influence of typical and atypical antipsychotics on dexterity. Our findings suggest that atypical antipsychotics have less influence, if any, on dexterity in patients with schizophrenia. The major difference between the typical and atypical antipsychotics is the incidence of extrapyramidal side effects (Leucht et al., 1999). Therefore, typical antipsychotics are more likely to cause tremors, which may be one of the causes of impaired manual dexterity.

Another possible cause of impaired dexterity may be the sedative effects of antipsychotic, benzodiazepine, and benzodiazepine-like drugs. Previous studies have shown that sedation caused by injection of midazolam, a benzodiazepine-receptor agonist, resulted in decreased grip strength, which recovered following administration of flumazenil, a benzodiazepine-receptor antagonist (Huang et al., 2012; Tomita et al., 2013). Therefore, we attempted to assess the sedation level by measuring the handgrip force. However, the results of the handgrip force test in the present study showed no association of the grip strength with benzodiazepine and/or benzodiazepine-like drug use or with antipsychotic dose. A possible explanation for this observation might be that the sedation levels caused by daily psychotropic medications were too low to induce detectable handgrip force decrease. Alternatively, failure to control for the type and timing of medications may have reduced the ability to detect a significant association. The handgrip force was significantly positively correlated with the pegboard scores in the patient group. A possibility remains that sedation due to medication may have been the common factor responsible for reduced hand strength and impaired dexterity. Future studies should consider separately the influence of individual antipsychotic, benzodiazepine, and benzodiazepine-like agents on sedation and dexterity.

The present study is the first, to our knowledge, to show that benzodiazepines and benzodiazepine-like drugs have negative influence on dexterity in patients with schizophrenia. This finding is likely to have a large clinical impact, because a recent meta-analysis by Dold et al. (2013) has raised questions about the use of benzodiazepines in patients with schizophrenia. Their results showed no evidence for medium- or long-term efficacy of benzodiazepine augmentation of antipsychotics. The patients prescribed benzodiazepines and/or benzodiazepine-like drugs in the present study were mostly on chronic use of these medications. Previous studies showed that psychomotor effects of benzodiazepines are seen not only in acute users (Loring et al., 2012; McManus et al., 1983) but also in long-term users (Gorenstein et al., 1994). Use of benzodiazepines in schizophrenia is recommended only as a short term adjunct therapy to neuroleptics (Dold et al., 2012), and therefore, long-term use should be considered with particular caution. The benefit of using benzodiazepines and/or benzodiazepine-like drugs must be carefully weighed against potential adverse effects including loss of dexterity, which may reduce quality of life.

Some limitations must be considered when interpreting the results of this study. First, the cross-sectional design did not allow any definitive conclusions as to whether the medications were the direct cause of impaired dexterity. Secondly, due to the small number of subjects, we could not consider separately the influence of individual antipsychotic, benzodiazepine, and benzodiazepine-like agents on dexterity. The effects each drug has on dexterity should be examined in future studies. Thirdly, the small number of medication-free patients did not allow us to adequately compare non-medicated patients and healthy controls. Our results showed no significant difference in dexterity between controls and patients not prescribed typical antipsychotics or benzodiazepines and/or benzodiazepine-like drugs, suggesting that these medications may be the main cause of the impaired dexterity in patients with schizophrenia. However, this finding must be confirmed in larger studies.

In conclusion, the present results indicate that patients with schizophrenia have impaired gross and fine fingertip dexterity compared to healthy controls. The use of typical antipsychotics and benzodiazepines and/or benzodiazepine-like drugs, but not atypical antipsychotics, had significant negative impact on dexterity in patients with schizophrenia. Psychiatrists should be aware that some psychotropic medications may enhance the disability caused by the impairment of dexterity in patients with schizophrenia.

Contributors

D.S. and H.K. designed the study and D.S. wrote the draft of the manuscript. D.S., H.H., T.T., K.H., M.Ota., M.Okazaki., A.K., and H.K. screened the study participants using the Mini International Neuropsychiatric Interview (M.I.N.I.) and diagnosed the patients according to the DSM-IV criteria. M.J. and Y.K. administered the

Purdue pegboard test. D.S. and H.K. undertook the statistical analysis. H.K. supervised the data analysis and writing of the paper. K.A., T.H., and N.A. also supervised the writing of the paper and gave critical comments on the manuscript. All authors contributed to and have approved the final manuscript.

Role of funding source

This study was supported by Health and Labour Sciences Research Grants (Comprehensive Research on Disability, Health, and Welfare) and Intramural Research Grant for Neurological and Psychiatric Disorders of NCNP (H.K.). They had no further role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Conflict of interest

The authors report no conflicts of interest.

Acknowledgements

The authors would like to thank the participants for taking part in the study.

References

American Psychiatric Association. DSM-IV: Diagnostic and statistical manual of mental disorders. 4th ed. Washington D.C.: American Psychiatric Press; 1994.
Dold M, Li C, Gillies D, Leucht S. Benzodiazepine augmentation of antipsychotic drugs in schizophrenia: a meta-analysis and Cochrane review of randomized controlled trials. Eur Neuropsychopharmacol 2013;23:1023–33.
Dold M, Li C, Tardy M, Khorsand V, Gillies D, Leucht S. Benzodiazepines for schizophrenia. Cochrane Database Syst Rev 2012;11:CD006391.
Gorenstein C, Bernik MA, Pompeia S. Differential acute psychomotor and cognitive effects of diazepam on long-term benzodiazepine users. Int Clin Psychopharmacol 1994;9:145–53.
Gschwandtner U, Pfluger M, Aston J, Borgwardt S, Drewe M, Stieglitz RD, et al. Fine motor function and neuropsychological deficits in individuals at risk for schizophrenia. Eur Arch Psychiatry Clin Neurosci 2006;256:201–6.
Hori H, Teraishi T, Sasayama D, Hattori K, Ota M, Matsuo J, et al. Schizotypal trait in healthy women is associated with a shift away from dextrality on a spatial motor control task, but not on a force control task. Psychiatry Res 2012;200:629–34.
Huang MY, Matsuura N, Kaneko Y, Ichinohe T. Midazolam increases bite force during intravenous sedation. J Oral Maxillofac Surg 2012;70:e458–463.
Igarashi Y, Hayashi N, Yamashina M, Otsuka N, Kuroki N, Anzai N, et al. Interrater reliability of the Japanese version of the Positive and Negative Syndrome Scale and the appraisal of its training effect. Psychiatry Clin Neurosci 1998;52:467–70.
Inagaki A, Inada T, Fujii Y, Yagi G, editors. Equivalent dose of psychotropics. Tokyo: Seiwa Shoten; 1999.
Jahn T, Hubmann W, Karr M, Mohr F, Schlenker R, Heidenreich T, et al. Motoric neurological soft signs and psychopathological symptoms in schizophrenic psychoses. Psychiatry Res 2006;142:191–9.
Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 1987;13:261–76.
Langenecker SA, Saunders EF, Kade AM, Ransom MT, McInnis MG. Intermediate: cognitive phenotypes in bipolar disorder. J Affect Disord 2010;122:285–93.
Leucht S, Pitschel-Walz G, Abraham D, Kissling W. Efficacy and extrapyramidal side-effects of the new antipsychotics olanzapine, quetiapine, risperidone, and sertindole compared to conventional antipsychotics and placebo. A meta-analysis of randomized controlled trials. Schizophr Res 1999;35:51–68.
Loring DW, Marino SE, Parfitt D, Finney GR, Meador KJ. Acute lorazepam effects on neurocognitive performance. Epilepsy Behav 2012;25:329–33.
McKean A, Vella-Brincat J. Ten-year dispensing trends of hypnotics in New Zealand. N Z Med J 2011;124:108–10.
McManus IC, Ankier SI, Norfolk J, Phillips M, Priest RG. Effects on psychological performance of the benzodiazepine, loprazolam, alone and with alcohol. Br J Clin Pharmacol 1983;16:291–300.
Meltzer HY. Update on typical and atypical antipsychotic drugs. Annu Rev Med 2013;64:393–406.
Midorikawa A, Hashimoto R, Noguchi H, Saitoh O, Kunugi H, Nakamura K. Impairment of motor dexterity in schizophrenia assessed by a novel finger movement test. Psychiatry Res 2008;159:281–9.
Morgan K, Adam K, Oswald I. Effect of loprazolam and of triazolam on psychological functions. Psychopharmacol (Berl) 1984;82:386–8.
Otsubo T, Tanaka K, Koda R, Shinoda J, Sano N, Tanaka S, et al. Reliability and validity of Japanese version of the Mini-International Neuropsychiatric Interview. Psychiatry Clin Neurosci 2005;59:517–26.
Sasayama D, Hori H, Teraishi T, Hattori K, Ota M, Matsuo J, et al. More severe impairment of manual dexterity in bipolar disorder compared to unipolar major depression. J Affect Disord 2012;136:1047–52.
Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998;59(Suppl. 20):22–33. quiz 34–57.
Sponheim SR, Jung RE, Seidman LJ, Mesholam-Gately RI, Manoach DS, O'Leary DS, et al. Cognitive deficits in recent-onset and chronic schizophrenia. J Psychiatr Res 2010;44:421–8.
Szoke A, Meary A, Trandafir A, Bellivier F, Roy I, Schurhoff F, et al. Executive deficits in psychotic and bipolar disorders – implications for our understanding of schizoaffective disorder. Eur Psychiatry 2008;23:20–5.
Tiffin J, Asher EJ. The Purdue pegboard; norms and studies of reliability and validity. J Appl Psychol 1948;32:234–47.
Tomita S, Matsuura N, Ichinohe T. The combined effects of midazolam and propofol sedation on muscle power. Anaesthesia 2013;68:478–83.
Wobrock T, Falkai P, Schneider-Axmann T, Hasan A, Galderisi S, Davidson M, et al. Comorbid substance abuse in first-episode schizophrenia: effects on cognition and psychopathology in the EUFEST study. Schizophr Res 2013;147:132–9.
Yamada H, Masui K, Kikuimoto K. The Japanese version of the positive and negative syndrome scale (PANSS) rating manual. Tokyo: Seiwa; 1991.
Zammit G. Comparative tolerability of newer agents for insomnia. Drug Saf 2009;32:735–48.

PLOS ONE

Identification of Single Nucleotide Polymorphisms Regulating Peripheral Blood mRNA Expression with Genome-Wide Significance: An eQTL Study in the Japanese Population

Daimei Sasayama[1,2], Hiroaki Hori[1], Seiji Nakamura[3], Ryo Miyata[3], Toshiya Teraishi[1], Kotaro Hattori[1], Miho Ota[1], Noriko Yamamoto[1], Teruhiko Higuchi[4], Naoji Amano[2], Hiroshi Kunugi[1,5]*

1 Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Ogawahigashi, Kodaira, Tokyo, Japan, **2** Department of Psychiatry, Shinshu University School of Medicine, Matsumoto, Nagano, Japan, **3** DNA Chip Research Inc., Yokohama, Kanagawa, Japan, **4** National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan, **5** Core Research of Evolutional Science and Technology, Japan Science and Technology Agency, Chiyoda-ku, Tokyo, Japan

Abstract

Several recent studies have reported that expression quantitative trait loci (eQTLs) may affect gene expression in a cell-dependent manner. In the current study, a genome-wide eQTL analysis was performed in whole blood samples collected from 76 Japanese subjects. RNA microarray analysis was performed for 3 independent sample groups that were genotyped in a genome-wide scan. The correlations between the genotypes of 534,404 autosomal single nucleotide polymorphisms (SNPs) and the expression levels of 30,465 probes were examined for each sample group. The SNP-probe pairs with combined correlation coefficients of all 3 sample groups corresponding to $P<3.1\times10^{-12}$ (i.e., Bonferroni-corrected $P<0.05$) were considered significant. SNP-probe pairs with a high likelihood of cross-hybridization and SNP-in-probe effects were excluded to avoid false positive results. We identified 102 *cis*-acting and 5 *trans*-acting eQTL regions. The *cis*-eQTL regions were widely distributed both upstream and downstream of the gene, as well as within the gene. The eQTL SNPs identified were examined for their influence on the expression levels in lymphoblastoid cell lines by using a public database. The results showed that genetic variants affecting expression levels in whole blood may have different effects on gene expression in lymphoblastoid cell lines. Further studies are required to clarify how SNPs function in affecting the expression levels in whole blood as well as in other tissues.

Citation: Sasayama D, Hori H, Nakamura S, Miyata R, Teraishi T, et al. (2013) Identification of Single Nucleotide Polymorphisms Regulating Peripheral Blood mRNA Expression with Genome-Wide Significance: An eQTL Study in the Japanese Population. PLoS ONE 8(1): e54967. doi:10.1371/journal.pone.0054967

Editor: Toshi Shioda, Massachusetts General Hospital, United States of America

Received August 16, 2012; **Accepted** December 18, 2012; **Published** January 24, 2013

Funding: This study was supported by Health and Labor Sciences Research Grants, the Strategic Research Program for Brain Sciences by the Ministry of Education, Culture, Sports, Science and Technology of Japan (Understanding of molecular and environmental bases for brain health), Core Research of Evolutional Science & Technology by the Japan Science and Technology Agency, and an Intramural Research Grant for Neurological and Psychiatric Disorders of the National Center of Neurology and Psychiatry (H.K.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Two of the authors are employed by the DNA Chip Research Inc and assisted in performing the analyses of microarray expression levels. Apart from this, the DNA Chip Research Inc had no role in study design, data collection, decision to publish, or preparation of the manuscript. The affiliation to this company does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: hkunugi@ncnp.go.jp

Introduction

Advances in high-throughput genotyping and gene expression platforms have enabled genome-wide analysis of gene expression quantitative trait loci (eQTLs), allowing investigation of both *cis* and *trans* effects. Previous eQTL studies have examined the association between genetic variants and gene expression levels in various biological samples, including human whole blood [1,2], lymphocytes [3], the liver [4], and, primarily, in lymphoblastoid cell lines [5,6]. Recently developed web tools such as SNPexp [7] and Genevar [8] have enabled analysis of the correlation between SNP genotypes in HapMap genotype data and genome-wide expression levels in lymphoblastoid cell lines. Development of such tools in other cell types is also anticipated, as a substantial fraction of eQTLs are cell type-specific [9,10,11,12].

Despite these advances, several challenges still remain in the field of genome-wide eQTL research. The large number of gene expression traits and genomic loci requires enormous calculations, raising issues of computer efficiency and statistical power. Another challenge is the varying genetic backgrounds in study populations, which may be one of the causes of the poor reproducibility observed across studies. Furthermore, confounding variables, such as the time of day at which sampling was performed, may also affect gene expression patterns in peripheral blood [13]. In addition, microarray probes may contain one or more SNPs in the target sequence. These probes may cause hybridization differences due to sequence polymorphisms present in the mRNA region, resulting in the occurrence of false positive results [14]. Other probes may undergo cross-hybridization, also resulting in false positive results for *trans*-eQTLs. The large number of probes and

SNPs cause difficulties in accounting for these confounding and influencing variables. A limited number of studies have overcome these methodological issues; therefore, further accumulation of data is required. Specifically, genome-wide eQTL data for Asian population is scarce [15].

Gene expression in whole blood could function as biomarkers for several disease conditions such as diabetes [16] and attention deficit hyperactivity disorder [17]. Elucidation of the genetic basis affecting such gene expression may be important in uncovering the etiological factors and pathophysiology of the diseases. Taking the aforementioned issues into consideration, we have examined the correlations between the genotypes of every SNP from a genome-wide scan and the expression levels of genes in the whole blood of Japanese individuals. To avoid the influence of batch effects, which is often ignored in eQTL studies, microarray data collected in different batches were first analyzed separately and then integrated. After strict corrections for multiple testing and exclusion of potential false-positive eQTLs, we investigated whether the SNPs found to have an effect on the expression levels in whole blood also influenced the expression levels in lymphoblastoid cell lines. Public data from the HapMap project of SNP genotypes and gene expression levels in lymphoblastoid cell lines were used for the analysis.

Materials and Methods

Genomic DNA was collected from 24 subjects (13 men and 11 women, mean age [SD] = 39.9 [7.6] years) in sample group 1, 24 subjects in sample group 2 (12 men and 12 women, 34.1 [11.5] years), and 28 subjects (14 men and 14 women, 41.4 [11.8] years) in sample group 3. The blood samples of each of the 3 sample groups were collected at different times and the microarray data of each sample group were obtained separately. Approximately half of the subjects suffered from depressive disorder (11, 12, and 16 subjects in sample groups 1, 2, and 3, respectively), but all were physically healthy and without clinically significant systemic disease (e.g., malignant disease, diabetes mellitus, hypertension, renal failure, or endocrine disorders). Subjects were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry Hospital, Tokyo, Japan, through advertisements in free local information magazines or through our website announcement. All the subjects were biologically unrelated Japanese individuals who resided in the same geographical area (western Tokyo). The study protocol was approved by the ethics committee at the National Center of Neurology and Psychiatry, Japan. Written informed consent was obtained from every subject after the study was explained to them.

Venous blood was collected between 1100 and 1200 h in PAXgene tubes (Qiagen, Valencia) from each subject and was incubated at room temperature for 24 h for RNA stabilization. RNA was extracted from whole blood according to the manufacturer's guidelines by using the PAXgene Blood RNA System Kit (PreAnalytix GmbH, Hombrechtikon, Switzerland). The RNA was quantified by optical density readings at A260 nm by using the NanoDrop ND-1000 (Thermo Scientific, Rockford). Gene expression analysis was performed using Agilent Human Genome 4×44 K arrays (Agilent Technologies, Santa Clara). Raw signal data for each of the 3 independent sample groups were analyzed separately by the GeneSpring GX software (Agilent Technologies). Data were filtered according to the expression level for quality control to eliminate genes that were below the 20th percentile threshold. The expression value of each gene was normalized to the median expression value of all genes in each chip. A total of 30,465 probes were included in the analysis.

Genomic DNA was obtained from venous blood samples. Genotyping was performed by Riken Genesis (Yokohama, Japan) using the Illumina HumanOmni1-Quad BeadChip (Illumina, Inc., San Diego). A total of 713,495 autosomal SNPs were assessed for quality using the PLINK v1.07 software [18]. All SNPs with a call rate below 95%, a deviation from Hardy-Weinberg equilibrium at an error level of $P<0.001$, or a minor allele frequency of less than 10% were excluded. The remaining 534,404 SNPs were used for further analysis. RNA expression and DNA genotype data are available at NCBI's Gene Expression Omnibus under accession number GSE42488.

Since RNA expression arrays of the 3 sample groups were performed at different times, the correlation between the genotype and expression levels was calculated separately in each sample group to avoid the influences of batch effects. The Pearson's correlation coefficient (r) between the genotype (coded as 0, 1, or 2) and gene expression level was calculated for each of the 1.63×10^{10} SNP-expression probe pairs in the 3 sample groups. The correlation coefficients of the 3 sample groups were averaged according to the following equation [19]:

$$\bar{r}=e^{2\bar{z}}-1/e^{2\bar{z}}+1$$

where $\bar{z}=\Sigma_i\ [1/2\times\ln\{(1+r_i)/(1-r_i)\}\times n_i]/\Sigma_i\ n_i$

n_i = the number of individuals in sample group i.

r_i = the correlation coefficient between the genotype and expression level in sample group i.

To minimize the possibility of false positives, the SNP-expression probe pairs with $\bar{r}$ corresponding to a Bonferroni-corrected P value of <0.05 (i.e., uncorrected $P<0.05/[30{,}465\times534{,}404]=3.1\times10^{-12}$) were also examined using Spearman's rank correlation in a similar method as described above. The SNP-probe pairs with Bonferroni-corrected P value of the average Spearman's rank correlation <0.05 (i.e., uncorrected $P<3.1\times10^{-12}$) were considered significant.

To determine the potential for cross-hybridization of the probes, a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed against the human genome by using the online Ensembl database. Probes with greater than 50% homology with other genomic regions were excluded.

Sequence polymorphisms in the mRNA region targeted by the microarray expression probes may cause hybridization differences due to SNP-in-probe effects. Therefore, SNP-probe pairs were excluded from the analysis if the 60-mer probe was mapped to a genomic location that contained a known SNP showing linkage disequilibrium (LD; $r^2>0.1$) with the SNP of the SNP-probe pair.

We also examined whether the eQTL SNPs affecting the expression levels in whole blood also influence expression levels in lymphoblastoid cell lines. The SNPexp [7] software was used to retrieve public data from the HapMap project (release 23) of SNP genotypes and the gene expression levels in lymphoblastoid cell lines of 45 Japanese subjects. Pearson's correlation coefficients were used to assess the influence of SNPs on expression levels in lymphoblastoid cell lines.

Results

Identification of eQTLs

The procedure used for SNP-probe pair selection (Figure 1) generated 1,554 pairs, which are listed in Table S1. These SNP-probe pairs consisted of 1,153 SNPs, defined as eQTL SNPs, and 185 probes. For 122 of these 185 probes, we could identify the corresponding gene from the HapMap database (Release 28).

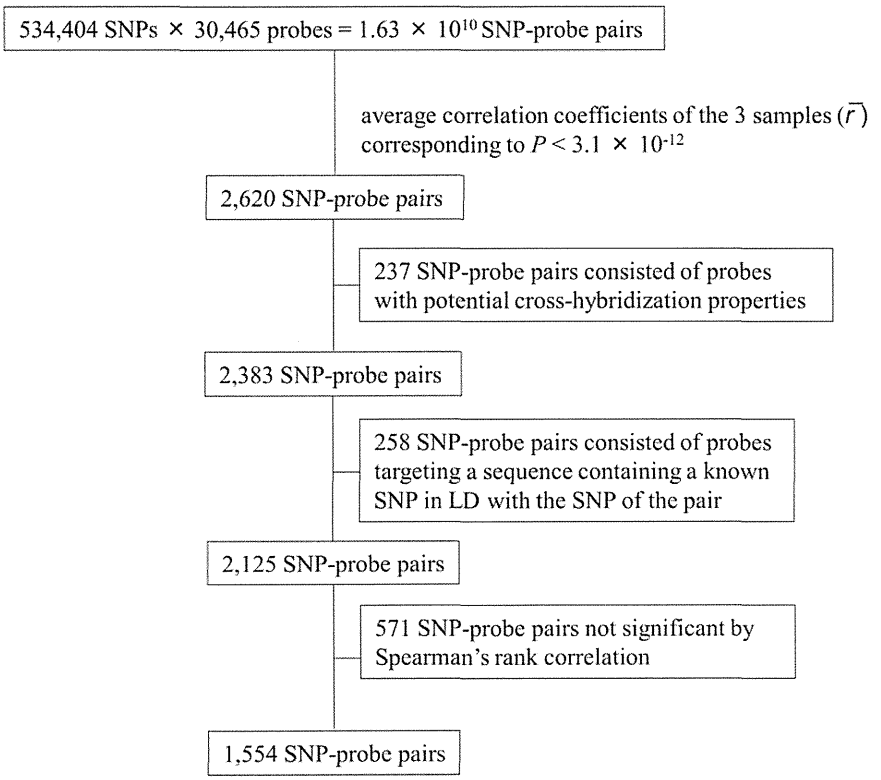

Figure 1. Procedure for selecting significant SNP-probe pairs. The procedure for selecting significant SNP-probe pairs is shown. SNP-probe pairs with a high likelihood of cross-hybridization and SNP-in-probe effects were excluded to exclude false positive results. The SNPs of the remaining 1,554 SNP-probe pairs were considered as eQTL SNPs.
doi:10.1371/journal.pone.0054967.g001

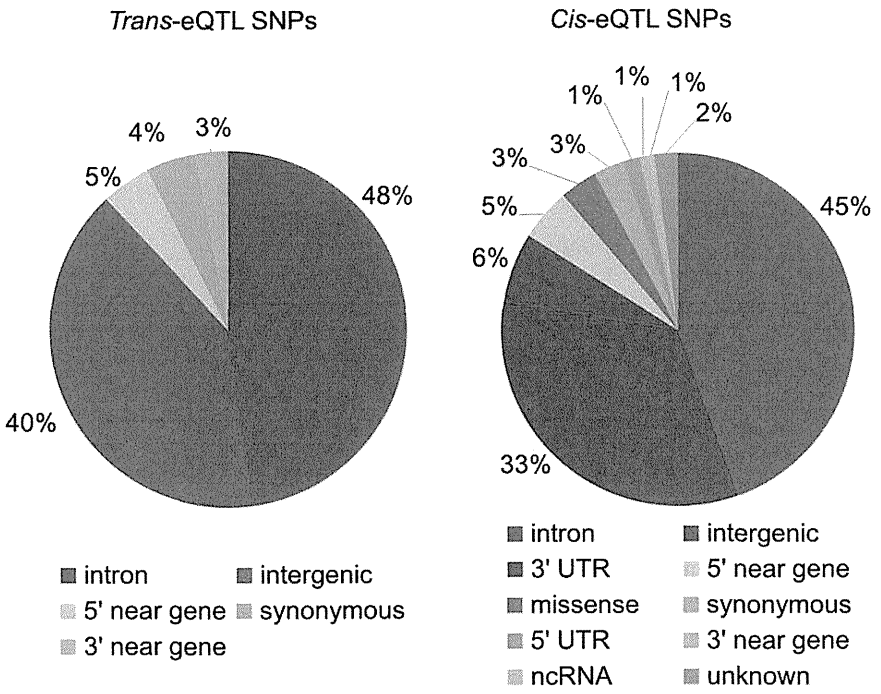

Figure 2. Functional types of the eQTL SNPs. The percentage of SNP types is shown for *cis*- and *trans*- eQTL SNPs.
doi:10.1371/journal.pone.0054967.g002

Since several of the probes targeted the same gene, the total number of genes identified was 107. As shown in Figure 2, the majority of the eQTL SNPs were located in intronic (45% and 48% for *cis*- and *trans*-eQTL SNPs, respectively) or intergenic (33% and 40% for *cis*- and *trans*-eQTL SNPs, respectively) regions.

Table S2 shows the names and properties of the 107 genes whose expression levels in whole blood were affected by SNPs. The SNPs affecting expression levels of the same gene were primarily in high LD with each other. Furthermore, investigation of combined Chinese and Japanese (CHB+JPT) panels from the 1000 Genomes Pilot 1 SNP data set and the HapMap release 22 data set showed a greater number of SNPs in high LD ($r^2>0.8$) with the eQTL SNPs identified in the current study. Since the high intermarker correlations cause difficulties in determining which SNP is responsible for the regulation of gene expression, we defined the eQTL region of a gene as the genomic range in which the SNPs in LD ($r^2>0.8$) with the eQTL SNPs of the gene are located. LD was determined by SNAP [20] using the population panel CHB+JPT from the 1000 Genomes Pilot 1 SNP data set and the HapMap release 22 data set.

Locational Relationships between the eQTL and the Gene

Regarding the locational relationships between the eQTL and the gene, 102 of the eQTLs were *cis*-acting (within 1 Mb upstream or downstream of the gene), and 5 were *trans*-acting, of which 4 were located on a different chromosome from the gene that they influenced. When the genome was divided into 3 segments (i.e., upstream, intragenic, and downstream), 69 *cis*-acting eQTL regions covered multiple segments that included the intragenic segments, 13 were confined to upstream segments, 7 were confined to downstream segments, and 13 were confined to intragenic segments.

Comparison of Results with Previously Reported Whole Blood eQTLs

We compared our results with those of the study by Fehrmann et al. [21], which performed a genome-wide eQTL analysis on 289,044 SNPs in whole blood expression data of 1,469 unrelated individuals from the United Kingdom and the Netherlands. The genotyping platform which they used (Illumina HumanHap300 platform) included only 24% of the 534,404 SNPs analyzed in the current study and 15% of the 1,153 eQTL SNPs identified in the current study. Therefore, 85% of the eQTL SNPs identified in the current study had not been identified by Fehrmann et al. [21], because they were not included in the Illumina HumanHap300 platform. On the other hand, 84% of the eQTL SNPs identified in the current study which were included in the Illumina HumanHap300 platform had also been identified as eQTL SNPs in their study. The high replication rate supports the robustness of our findings.

Influence on Expression Levels in Lymphoblastoid Cell Lines

Next, we examined whether the eQTL SNPs affecting the expression levels in whole blood also influence expression levels in lymphoblastoid cell lines. We selected representative SNPs in eQTL regions and examined their effects on the expression of the corresponding gene in lymphoblastoid cell lines. The SNPs that showed the strongest correlation with the expression levels in whole blood for each eQTL region were selected for examination of the possible effects on expression levels in lymphoblastoid cell lines. If there were any additional eQTL SNPs in the same region that were not in LD with the selected SNP ($r^2<0.1$), then one of the SNPs with the strongest correlation with the expression levels in whole blood was also selected. In the eQTL regions for *MICA*, *MICB*, *HLA-DRB5*, *HLA-DQB1*, and *HLA-DQA2*, 2 representative SNPs, which were not in significant LD with each other ($r^2<0.1$), were selected. For other genes, the eQTL SNPs in the same eQTL region were in LD with each other ($r^2>0.1$); therefore, 1 representative SNP was selected for each region. If the genotype data of the selected SNP were not available in the HapMap data, the SNP within the same eQTL region having the next strongest correlation with the expression levels in whole blood was selected.

Genotype and expression levels in lymphoblastoid cell lines were retrieved from public data for 45 Japanese individuals for 88 (86 *cis* and 2 *trans*) of the 112 representative SNPs. The average number of individuals with applicable data for genotype and the expression levels of lymphoblastoid cell lines in the 88 retrieved SNP-gene pairs was 43.8. The Pearson's correlation coefficients between the eQTL SNPs and the expression levels of the corresponding genes in lymphoblastoid cell lines were calculated and have been shown in Table S3. A positive correlation coefficient indicates that the SNP has a similar effect on expression levels in whole blood and lymphoblastoid cell lines. Of the 86 *cis*-eQTL SNPs, 34 showed a significantly positive correlation, whereas 13 showed a significantly negative correlation with the expression levels of lymphoblastoid cell lines (FDR-corrected, $P<0.05$). None of the *trans*-eQTL SNPs identified in the current study significantly affected expression levels in lymphoblastoid cell lines.

Functional Properties of the eQTL SNPs

We examined whether the regulatory effects of eQTL SNPs were caused by mutations in transcription factor-binding sites (TFBSs), splicing-affecting sites, or microRNA (miRNA)-binding sites. The proportion of SNPs in LD ($r^2>0.8$) with a SNP predicted to be located on such sites was compared between the 37 eQTL SNPs affecting expression levels in both whole blood and lymphoblastoid cell lines; 49 eQTL SNPs affecting only whole blood expression levels; and 5,681 non-eQTL SNPs located within 100 kB of the 107 genes that were regulated by the eQTL SNPs identified in the current study. A web-based tool (FuncPred; http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm) was used to predict the functional properties of the SNPs. As shown in Table 1, eQTL SNPs were more likely to be in LD with SNPs located on TFBSs, splicing-affecting sites, and miRNA-binding sites.

Cis-only Analysis

The small-effect eQTL SNPs are likely to have remained undetected in the present study due to the strict correction procedures for multiple testing. In order to reduce the number of unreported *cis*-eQTL SNPs, we also performed *cis*-only analysis by examining only SNPs 1 Mb upstream or downstream of the targeted gene. A total of 955,370 SNP-probe pairs were examined, and those with an average Pearson's correlation ($\bar{r}$) of the 3 sample groups corresponding to $P<5.23\times10^{-9}$ (i.e., Bonferroni-corrected $P<0.05$) were considered significant. As shown in Table S4, the *cis*-only analysis resulted in 3,883 SNP-probe pairs consisting of 3,161 SNPs and 347 probes.

The Influence of Depressive Disorder on Gene Expression Regulation

In order to investigate whether depressive disorder was a major confounding factor for gene expression regulation, we calculated the Spearman's correlation coefficients separately in depressed and non-depressed subjects. All the 1,554 SNP-probe pairs identified

Table 1. Percentage of SNPs that are in linkage disequilibrium ($r^2>0.8$) with a SNP predicted to be located on TFBS, splicing-affecting site, or miRNA binding site.

	TFBS	Splicing	miRNA binding site
eQTL SNPs affecting expression levels in both whole blood and LCLs (37 SNPs)	73.7% ‡	42.1% ‡	44.7% ‡
eQTL SNPs affecting expression levels in only whole blood (49 SNPs)	58.8% ‡	43.1%‡	29.4% †
non-eQTL SNPs (5,681 SNPs)	34.8%	17.3%	14.1%

The following abbreviations are used: TFBS, transcription factor binding site; miRNA, micro RNA; LCL, lymphoblastoid cell line.
†$P<0.01$,
‡$P<0.001$: Significantly higher compared to non-eQTL SNPs (χ^2 test).
doi:10.1371/journal.pone.0054967.t001

as eQTL in the present study achieved high correlations for both depressed and non-depressed subjects (average Spearman's correlation of the 3 sample groups $\overline{\rho}>0.4$, FDR-corrected $P<0.01$ in non-depressed subjects and $\overline{\rho}>0.5$, FDR-corrected $P<0.005$ in depressed subjects for all 1,554 SNP-probe pairs).

Discussion

To our knowledge, this is the first genome-wide eQTL study in Asian subjects that examined the association of SNPs with expression levels in whole blood. The genome-wide investigation uncovered 1,153 SNPs affecting gene expression levels in human whole blood. Although the number of eQTL regions identified in the current study was relatively small, the likelihood of false positives is low because of the strict correction procedures for multiple testing and exclusion of SNPs with potential cross-hybridization or SNP-in-probe effects.

Since SNPs in strong LD with a SNP directly responsible for regulating gene expression levels are also correlated with gene expression levels, it is difficult to determine which SNP is the causative one. We assumed that the genetic regulatory locus would be included in the eQTL region, defined as the genomic range in which the SNPs in LD ($r^2>0.8$) with the eQTL SNPs identified in our study are found. Although the numerous SNPs in LD with each other hindered the identification of the responsible SNP, the locations of the eQTL regions indicated that eQTLs are widely distributed both upstream and downstream of the gene, as well as within the gene.

The current study showed that several of the SNPs affecting the expression levels of a gene in whole blood also influenced the expression levels of the same gene in lymphoblastoid cell lines. A recent study by Powell et al. [22] has shown that the genetic control mechanisms of gene expression in whole blood and lymphoblastoid cell lines are largely independent. Despite the evidence of low genetic correlation of regulatory variation averaged across the genome, our results suggest that a subset of eQTLs commonly affect expression levels in whole blood and lymphoblastoid cell lines. Conversely, our findings suggest that some of the whole blood eQTL SNPs do not regulate expression levels in lymphoblastoid cell lines. This is in line with a previous study that reported that 69–80% of the identified regulatory variants operated in a cell type-specific manner [9]. Compared to SNPs affecting only expression levels in whole blood, higher, although not statistically significant, proportion of SNPs affecting expression levels in both whole blood and lymphoblastoid cell lines were in LD with SNPs located on TFBSs and miRNA-binding sites. The finding suggests that these functional properties affect expression levels across multiple cell types.

Intriguingly, 13 of the 88 eQTL SNPs in whole blood were observed to have opposite effects on expression levels in whole blood and lymphoblastoid cell lines. Dimas et al. [9] compared gene expression variation in fibroblasts, lymphoblastoid cell lines, and T cells and reported that the same directional effect in each cell type was observed for eQTLs shared between multiple cell types. However, 2 recently published studies reported that some eQTL SNPs have opposite allelic effects on gene expression in the liver, adipose tissue, skeletal muscle [10], or in B cells and monocytes [11]. Our findings also suggest the possibility that some SNPs may exert opposite effects on gene expression in different cell types. However, an alternative explanation may be that the eQTL SNPs identified may function to alter the splicing of the mRNA. Since the gene expression microarray platform used in the previous eQTL study examining LCL expression levels in Japanese subjects was different from ours, the different probes may have detected different splicing variants, resulting in seemingly opposite allelic effects. A comparison using the same platform would be necessary to uncover cell-specific effects on expression levels.

The strength of the current study is that a relatively homogeneous Japanese population was used, which may have minimized the effects of differential genetic backgrounds. The major limitation of the current study is that the conservative corrections for multiple testing may have missed a large proportions of eQTL SNPs. Increasing power allows better detection of weaker and more distantly located *cis*-regulatory elements [23]. Greater than 82% of the significant eQTL-probe pairs identified in the current study had $P<3.1\times10^{-13}$, which far exceeded the predetermined significance level ($P<3.1\times10^{-12}$). Our findings should not be generalized to more weakly associated eQTLs since they may have different regulatory mechanisms. Another limitation is that approximately half of the samples were collected from patients with a depressive disorder. However, analyzing healthy and depressive subjects separately also resulted in achieving high correlations (FDR-corrected $P<0.01$) for all the 1,554 SNP-probe pairs identified in the current study. Therefore, it is unlikely that depressive disorder has a major impact on gene expression regulation of the identified eQTL SNPs. Further investigation on the influence of depressive symptoms on gene expression levels is underway using a larger sample size.

In summary, we have presented the results on genome-wide investigations of SNPs affecting the expression levels in whole blood. Both *cis*-acting and *trans*-acting eQTL SNPs were identified for a total of 107 genes. The eQTL regions were widely distributed upstream, downstream, and within the gene sequence. The findings of this study are valuable if gene expression levels in whole blood are used as biomarkers for disease conditions. Gene expression levels and their connection with disease-associated SNPs may lead to a better understanding of genetic predisposition to disease and may be used to predict disease susceptibility. Further studies are required to clarify how SNPs function in

affecting the expression levels in whole blood as well as in other tissues.

Supporting Information

Table S1 Significant SNP-probe pairs. The SNP-probe pair selection procedure generated 1,554 significant pairs, consisted of 1,153 SNPs, defined as eQTL SNPs, and 185 probes.
(XLSX)

Table S2 Genes whose expression levels in whole blood are affected by SNPs. The names and properties of the 107 genes whose expression levels in whole blood were affected by SNPs are shown.
(XLSX)

Table S3 The Pearson's correlation coefficients between the eQTL SNPs and the expression levels of the corresponding genes in lymphoblastoid cell lines. A positive correlation coefficient indicates that the SNP has a similar effect on expression levels in whole blood and lymphoblastoid cell lines. Of the 86 *cis*-eQTL SNPs, 34 showed a significantly positive correlation, whereas 13 showed a significantly negative correlation with the expression levels of lymphoblastoid cell lines (FDR-corrected, $P<0.05$).
(XLSX)

Table S4 The results of the *cis*-only analysis. The *cis*-only analysis resulted in 3,883 SNP-probe pairs consisting of 3,161 SNPs and 347 probes.
(XLSX)

Acknowledgments

The authors would like to thank the participants for taking part in the study.

Author Contributions

Conceived and designed the experiments: DS HH HK. Performed the experiments: DS SN RM NY. Analyzed the data: DS HH SN RM TT KH MO. Contributed reagents/materials/analysis tools: DS HH TT KH MO NY HK. Wrote the paper: DS TH NA HK.

References

1. Emilsson V, Thorleifsson G, Zhang B, Leonardson AS, Zink F, et al. (2008) Genetics of gene expression and its effect on disease. Nature 452: 423–428.
2. Mehta D, Heim K, Herder C, Carstensen M, Eckstein G, et al. (2012) Impact of common regulatory single-nucleotide variants on gene expression profiles in whole blood. Eur J Hum Genet.
3. Goring HH, Curran JE, Johnson MP, Dyer TD, Charlesworth J, et al. (2007) Discovery of expression QTLs using large-scale transcriptional profiling in human lymphocytes. Nat Genet 39: 1208–1216.
4. Innocenti F, Cooper GM, Stanaway IB, Gamazon ER, Smith JD, et al. (2011) Identification, replication, and functional fine-mapping of expression quantitative trait loci in primary human liver tissue. PLoS Genet 7: e1002078.
5. Stranger BE, Forrest MS, Clark AG, Minichiello MJ, Deutsch S, et al. (2005) Genome-wide associations of gene expression variation in humans. PLoS Genet 1: e78.
6. Veyrieras JB, Kudaravalli S, Kim SY, Dermitzakis ET, Gilad Y, et al. (2008) High-resolution mapping of expression-QTLs yields insight into human gene regulation. PLoS Genet 4: e1000214.
7. Holm K, Melum E, Franke A, Karlsen TH (2010) SNPexp - A web tool for calculating and visualizing correlation between HapMap genotypes and gene expression levels. BMC Bioinformatics 11: 600.
8. Yang TP, Beazley C, Montgomery SB, Dimas AS, Gutierrez-Arcelus M, et al. (2010) Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. Bioinformatics 26: 2474–2476.
9. Dimas AS, Deutsch S, Stranger BE, Montgomery SB, Borel C, et al. (2009) Common regulatory variation impacts gene expression in a cell type-dependent manner. Science 325: 1246–1250.
10. Fu J, Wolfs MG, Deelen P, Westra HJ, Fehrmann RS, et al. (2012) Unraveling the regulatory mechanisms underlying tissue-dependent genetic variation of gene expression. PLoS Genet 8: e1002431.
11. Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, et al. (2012) Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. Nat Genet 44: 502–510.
12. Ding J, Gudjonsson JE, Liang L, Stuart PE, Li Y, et al. (2010) Gene expression in skin and lymphoblastoid cells: Refined statistical method reveals extensive overlap in cis-eQTL signals. Am J Hum Genet 87: 779–789.
13. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, et al. (2003) Individuality and variation in gene expression patterns in human blood. Proc Natl Acad Sci U S A 100: 1896–1901.
14. Alberts R, Terpstra P, Li Y, Breitling R, Nap JP, et al. (2007) Sequence polymorphisms cause many false cis eQTLs. PLoS One 2: e622.
15. Stranger BE, Forrest MS, Dunning M, Ingle CE, Beazley C, et al. (2007) Relative impact of nucleotide and copy number variation on gene expression phenotypes. Science 315: 848–853.
16. Berisha SZ, Serre D, Schauer P, Kashyap SR, Smith JD (2011) Changes in whole blood gene expression in obese subjects with type 2 diabetes following bariatric surgery: a pilot study. PLoS One 6: e16729.
17. Taurines R, Grunblatt E, Schecklmann M, Schwenck C, Albantakis L, et al. (2011) Altered mRNA expression of monoaminergic candidate genes in the blood of children with attention deficit hyperactivity disorder and autism spectrum disorder. World J Biol Psychiatry 12 Suppl 1: 104–108.
18. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, et al. (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81: 559–575.
19. Faller AJ (1981) An Average Correlation Coefficient. Journal of Applied Metereology 20: 203–205.
20. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, et al. (2008) SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics 24: 2938–2939.
21. Fehrmann RS, Jansen RC, Veldink JH, Westra HJ, Arends D, et al. (2011) Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. PLoS Genet 7: e1002197.
22. Powell JE, Henders AK, McRae AF, Wright MJ, Martin NG, et al. (2012) Genetic control of gene expression in whole blood and lymphoblastoid cell lines is largely independent. Genome Res 22: 456–466.
23. Dobrin R, Greenawalt DM, Hu G, Kemp DM, Kaplan LM, et al. (2011) Dissecting cis regulation of gene expression in human metabolic tissues. PLoS One 6: e23480.

Kubota-Sakashita et al. Molecular Brain 2014, 7:5
http://www.molecularbrain.com/content/7/1/5

Molecular Brain

RESEARCH Open Access

A role of *ADAR2* and RNA editing of glutamate receptors in mood disorders and schizophrenia

Mie Kubota-Sakashita[1†], Kazuya Iwamoto[1,2†], Miki Bundo[1,2] and Tadafumi Kato[1*]

Abstract

Background: Pre-mRNAs of 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)-propanoic acid (AMPA)/kainate glutamate receptors undergo post-transcriptional modification known as RNA editing that is mediated by adenosine deaminase acting on RNA type 2 (*ADAR2*). This modification alters the amino acid sequence and function of the receptor. Glutamatergic signaling has been suggested to have a role in mood disorders and schizophrenia, but it is unknown whether altered RNA editing of AMPA/kainate receptors has pathophysiological significance in these mental disorders. In this study, we found that *ADAR2* expression tended to be decreased in the postmortem brains of patients with schizophrenia and bipolar disorder.

Results: Decreased *ADAR2* expression was significantly correlated with decreased editing of the R/G sites of AMPA receptors. In heterozygous *Adar2* knockout mice (*Adar2*$^{+/-}$ mice), editing of the R/G sites of AMPA receptors was decreased. *Adar2*$^{+/-}$ mice showed a tendency of increased activity in the open-field test and a tendency of resistance to immobility in the forced swimming test. They also showed enhanced amphetamine-induced hyperactivity. There was no significant difference in amphetamine-induced hyperactivity between *Adar2*$^{+/-}$ and wild type mice after the treatment with an AMPA/kainate receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline.

Conclusions: These findings collectively suggest that altered RNA editing efficiency of AMPA receptors due to down-regulation of *ADAR2* has a possible role in the pathophysiology of mental disorders.

Keywords: RNA editing, Adenosine deaminase acting on RNA type 2, AMPA/kainate receptors, Serotonin 2C receptor

Background

Mood disorders and schizophrenia are major psychiatric diseases that cause severe psychosocial impairment. Because many antidepressant and antipsychotic drugs act on the serotonin transporter and serotonin receptors, the serotonin has been implicated as having a role in these diseases [1]. However, among the drugs acting on glutamate receptors, phencyclidine causes schizophrenia-like psychosis [2] and ketamine has rapid antidepressive efficacy [3]. These findings suggest a possible role of altered glutamatergic neurotransmission in mood disorders and schizophrenia [1].

To date, numerous studies using genome analysis, positron emission tomography, and postmortem brain analysis have revealed possible roles of serotonergic and glutamatergic pathways in schizophrenia and mood disorders [1]. However, the detailed molecular mechanisms of these diseases have yet to be totally elucidated. Among the receptors in these pathways, pre-mRNAs of the serotonin 2C receptor (*HTR2C*) and two classes of ionotropic glutamate receptors, 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)-propanoic acid (AMPA) and kainate receptors, undergo RNA editing [4,5].

RNA editing is a post-transcriptional modification of pre-mRNA, which is mediated by adenosine deaminases acting on RNA (ADAR) enzymes. Research has especially focused on adenosine-to-inosine (A-to-I) editing of *HTR2C* and AMPA/kainate receptors by ADAR2 [6]. *HTR2C* undergoes editing at five sites (from A to E), which results in amino acid changes and causes functional alteration. Among the editing sites of AMPA/kainate receptors, two sites (Q/R and R/G) that result in amino acid changes and have functional significance, have been relatively well studied. The Q/R site is almost 100% edited in *GRIA2*, and loss of editing at this site causes enhanced

* Correspondence: kato@brain.riken.jp
†Equal contributors
[1]Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan
Full list of author information is available at the end of the article

Ca^{2+} permeability, resulting in cellular dysfunction [6]. The R/G site is not fully edited, which changes the kinetics of desensitization [7].

Numerous studies have investigated altered RNA editing of *HTR2C* in postmortem brains of patients with mental disorders, but the findings have not been consistent. While several studies did not show any specific alteration of RNA editing in mental disorders [8-11], other studies showed disease-specific alteration, such as increased D site editing in depression and increased A site editing in suicide completers [12], decreased B site editing in schizophrenia [13], and increased E site and decreased D site editing in depression [14]. Among these studies, increased A site editing in suicide completers has been shown to be relatively consistent [9,10,12,15]. A recent study using a next-generation sequencer [11] showed no robust alteration of RNA editing of *HTR2C* in schizophrenia and depression, except that a trend of decreased editing at the C, D, and E sites in nonsuicidal depression was found. However, a trend of increased A site editing in depressive patients that died by suicide is compatible with previous studies.

The number of studies regarding the RNA editing of AMPA/kainate receptors is relatively small. While several studies showed no alteration in schizophrenia [16,17] and bipolar disorder [17], a recent study revealed altered editing of the I/V site of *GRIK2* in bipolar disorder [6]. The role of RNA editing of glutamate receptors has drawn attention because the glutamatergic hypothesis of mood disorders has recently been established [18].

As already mentioned, there is no consensus on what kind of alteration of RNA editing is characteristic of schizophrenia and mood disorders. Possible reasons for such discordance include the effect of confounding factors in postmortem brain studies, such as medication and premortem or postmortem changes, and complex interactions between the cause of death (such as suicide) and mental disorders. Recently, Lyddon and colleagues argued that there are two factors involved in the altered editing of *HTR2C* in mood disorders; one is decreased *ADAR2* expression associated with decreased RNA editing of *HTR2C*, and the other is increased A site editing in suicide [15].

Collectively, it is difficult to elucidate how RNA editing is related to mental disorders by postmortem brain analysis alone. To understand the pathophysiological significance of altered *ADAR2* expression and RNA editing in mental disorders, integration of human postmortem brain analysis and animal model studies is crucial. In this study, we investigated the possible roles of altered *ADAR2* expression and RNA editing of AMPA/kainate glutamate receptors through an analysis of these factors in human postmortem brains. Molecular, behavioral, and pharmacological analyses of heterozygous *Adar2* knockout mice (*Adar2*$^{+/-}$ mice) were also conducted to elucidate the roles of RNA editing in schizophrenia and mood disorders.

Results

Overview

We analyzed the gene expression levels of the *ADAR2* and RNA editing status of AMPA/kainite glutamate receptors in two sets of postmortem human brain samples donated by the Stanley Medical Research Institute, Array Collection and Neuropathology Consortium (Table 1). To elucidate the molecular neurobehavioral consequence of reduced *ADAR2* expression, we analyzed the RNA editing status of *Htr2c* and AMPA/kainate glutamate receptors in *Adar2*$^{+/-}$ mice; the behavior of the mice was analyzed by a comprehensive battery of behavioral tests. To further understand the molecular basis of the hyperactivity of the mice, pharmacological experiments using amphetamine and the selective AMPA receptor antagonist, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline (NBQX) were performed.

ADAR2 expression in human postmortem brains

In the Array Collection samples, there was a significant difference in the *CFL1* (*cofilin 1*)-normalized *ADAR2* expression level between diagnoses [one-way analysis of variance

Table 1 Subjects for RNA editing and expression analysis of ADARs

	Bipolar disorder	Schizophrenia	Control	Depression
Array Collection (all samples)				
n	32	35	34	NA
Sex (F:M)	17:15	9:26	9:25	
Age[a)]	45.6 ± 11.0	42.6 ± 8.5	44.1 ± 7.7	
PMI[b)]	36.3 ± 17.9	31.4 ± 15.5	29.6 ± 13.0	
Brain pH[c)]	6.43 ± 0.30[g)]	6.47 ± 0.24[h)]	6.60 ± 0.27	
Array Collection (pH-adjusted)				
n	19	24	29	NA
Sex (F:M)	11:8	9:15	6:23	
Age[d)]	46.1 ± 9.9	42.6 ± 8.5	44.6 ± 7.7	
PMI[e)]	39.9 ± 20.2	35.0 ± 14.8	30.2 ± 12.5	
Brain pH[f)]	6.63 ± 0.15	6.61 ± 0.14	6.69 ± 0.17	
Neuropathology Consortium Samples[i)]				
n	11	13	14	11
Sex (F:M)	3:8	5:8	5:9	5:6
Age	39.4 ± 12.4	43.5 ± 13.6	49.0 ± 10.4	46.3 ± 10.5
PMI	31.5 ± 15.5	33.0 ± 14.9	22.6 ± 9.2	27.0 ± 11.9
Brain pH	6.25 ± 0.20	6.15 ± 0.25	6.30 ± 0.21	6.18 ± 0.24

NA, not available.
[a)]One way ANOVA, $F = 0.94$, $P = 0.39$, [b)]$F = 1.61$, $P = 0.20$, [c)]$F = 3.5$, $P = 0.031$.
[d)]One-way ANOVA, $F = 0.89$, $P = 0.41$, [e)]One-way ANOVA, $F = 2.2$, $P = 0.11$.
[f)]One-way ANOVA, $F = 1.7$, $P = 0.18$, [g)]$t = -2.4$, $P = 0.017$ to control.
[h)]$t = 2.0$, $P = 0.042$ to control.
[i)]No significant difference among 4 groups were found for age, PMI, brain pH by One-way ANOVA.

(ANOVA), $P < 0.05$]. Patients with schizophrenia showed significantly lower *ADAR2* expressions in both Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH)*- and *CFL1*-normalized data ($P < 0.05$). Patients with bipolar disorder also showed a nonsignificant trend in the same direction, and it was close to significance ($P = 0.05$) for the *CFL1*-normalized *ADAR2* expression level (Table 2). There was a significant correlation between brain pH and *ADAR2* expression (*GAPDH*-normalized *ADAR2*: $r = 0.28$, $P = 0.001$; *CFL1*-normalized *ADAR2*: $r = 0.222$, $P < 0.05$). Because low pH in brain affected the measurement of postmortem brain gene expression [19], we selected samples with high brain pH (pH 6.4 or higher). This threshold was determined in our previous gene expression study [20]. We found that the results were similar after selection of high pH samples (Table 2). A similar nonsignificant trend for decreased *ADAR2* expression was found in the Neuropathology Consortium samples (Table 2). There was no significant difference of *ADAR1* expression between diagnoses (Table 2).

RNA editing changes in *Adar2*$^{+/-}$ mice

The initial report showed that homozygous *Adar2* knockout is lethal in mice due to seizures caused by a marked decrease of the Q/R site of *Gria2*, which results in enhanced Ca^{2+} permeability of the AMPA receptors [21]. *Adar2*$^{+/-}$ mice showed less prominent alteration of RNA editing and were viable. Thus, *Adar2*$^{+/-}$ might be a better model for mental disorders.

We first characterized the effect of *Adar2*$^{+/-}$ on RNA editing status. Examination of *Htr2c* editing revealed that the E and C sites were not altered, whereas the A, B, and D sites had significantly decreased editing efficiency, except in the cerebellum, where only the D site showed significant alteration (Figure 1a). The alteration of the A and B sites was relatively modest (10% or less), whereas the change in the D site was larger (up to 20%).

With regard to the AMPA/kainate receptors, the Q/R site of *Gria2* was almost fully edited (Figure 1b). Editing of the Q/R sites of *Grik1* and *Grik2* was slightly decreased. There are two alternative spliced isoforms of flip and flop types that have different desensitization kinetics. Because the R/G site is located just before this alternative spliced exon [7], the R/G sites were separately examined for two alternative spliced isoforms. We found that RNA editing of the R/G site was generally decreased in both the flip and flop isoforms for all receptors investigated (*Gria2*,

Table 2 Expression levels of ADARs in postmortem brains of patients with mental disorders

Array collection samples				
	All samples (normalized by *GAPDH*)		All samples (normalized by *CFL1*)	
Diagnosis	*ADAR1*	*ADAR2*	*ADAR1*	*ADAR2*
Bipolar disorder	0.0219 ± 0.0090	0.0025 ± 0.0011	0.0397 ± 0.0163	0.0046 ± 0.0018[b)]
Schizophrenia	0.0216 ± 0.0086	0.0024 ± 0.0008[a)]	0.0393 ± 0.0192	0.0043 ± 0.0015[c)]
Control	0.0232 ± 0.0063	0.0029 ± 0.0009	0.0477 ± 0.0380	0.0054 ± 0.0015
One-way ANOVA	NS	$F = 2.11$, $P = 0.120$	NS	$F = 3.86$, $P = 0.024$
	pH-adjusted (normalized by *GAPDH*)		pH-adjusted (normalized by *CFL1*)	
Diagnosis	*ADAR1*	*ADAR2*	*ADAR1*	*ADAR2*
Bipolar disorder	0.0028 ± 0.0010	0.0244 ± 0.0096	0.0050 ± 0.0016	0.0442 ± 0.0180
Schizophrenia	0.0025 ± 0.0008[d)]	0.0231 ± 0.0088	0.0043 ± 0.0013[e)]	0.0401 ± 0.0149
Control	0.0031 ± 0.0008	0.0238 ± 0.0054	0.0054 ± 0.0014	0.0420 ± 0.0080
One-way ANOVA	$F = 3.09$, $P = 0.052$	NS	$F = 4.37$, $P = 0.016$	NS
Neuropathology Consortium Samples				
	Normalized by *GAPDH*		Normalized by *CFL1*	
Diagnosis	*ADAR1*	*ADAR2*	*ADAR1*	*ADAR2*
Bipolar disorder	0.0025 ± 0.0016[f)]	0.0110 ± 0.0071	0.0122 ± 0.0097	0.0026 ± 0.0017[g)]
Depression	0.0030 ± 0.0012	0.0107 ± 0.0036	0.0043 ± 0.0013[e)]	0.0039 ± 0.0012
Schizophrenia	0.0029 ± 0.0006	0.0107 ± 0.0045	0.0151 ± 0.0051	0.0039 ± 0.0010
Control	0.0033 ± 0.0008	0.0119 ± 0.0054	0.0133 ± 0.0062	0.0037 ± 0.0010
One-way ANOVA	NS	NS	NS	*$F = 5.6$, $P = 0.008$*

Values are given in ± mean SD.
[a)] $t = 2.22$, $P = 0.029$ to control, [b)] $t = -1.92$, $P = 0.05$ to control.
[c)] $t = 2.90$, $P = 0.005$ to control, [d)] $t = -2.65$, $P = 0.01$, [e)] $t = -3.12$, $P = 0.003$.
[f)] $t = -1.70$, $P = 0.09$ to control, [g)] $t = -2.0$, $P = 0.06$ to control.
NS: not significant.
For one of 13 subjects with schizophrenia in Neuropathology Consortium, data was missing.