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Regular Article

Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery: Validation of the Japanese version

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Aim: This preliminary study was performed to test the reliability and validity of the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) Consensus Cognitive Battery (MCCB), developed by the National Institute of Mental Health MATRICS initiative, as an assessment tool in a Japanese-language version (MCCB-J).

Methods: The subjects for the present study were 37 patients with schizophrenia. Each subject gave written informed consent to participate in the research. In order to examine the validity of the MCCB-J, the correlation between the MCCB-J and the Japanese-language version of the Brief Assessment of Cognition in Schizophrenia (BACS) was determined.

Results: Cronbach's alpha for the MCCB-J was 0.72. The MCCB-J composite score was significantly correlated with all subtests of the MCCB-J. There was a significant correlation between the MCCB-J and the BACS composite score.

Conclusion: This preliminary study indicates that the MCCB-J has good psychometric properties and validity.

Key words: cognitive function, Japanese version, Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery, psychometrics, schizophrenia.

THE ASSESSMENT OF cognitive function is an important step in evaluating patients with schizophrenia because of the extensive evidence that cognitive impairment is a core feature of schizophrenia,[1,2] as well as a key determinant of functional outcome.[3,4]

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Cognitive domains that show differential impairment in schizophrenia include attention (vigilance), executive function, long-term and learning memory, working memory, and verbal fluency.[3–7] The long-term and learning memory and attention, in particular, have been shown to predict functional outcome better than positive and negative symptoms.[3,4]

For assessing cognitive function in schizophrenia, an extensive neuropsychological test battery (NTB), usually consisting of 8–12 tests tapping each of several tests for the putative neurocognitive domains,

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has been employed. These NTB are, however, time-consuming, technically difficult, and costly. Moreover, differences in NTB chosen for various studies make a direct comparison across studies difficult. Thus, the availability of a quick and efficient tool for measuring cognition in patients with schizophrenia would be extremely useful for clinicians in considering an indication of rehabilitation, as well as for researchers implementing clinical trials to assess cognitive improvement. In this regard, we introduced the Brief Assessment of Cognition in Schizophrenia (BACS),[8] and prepared the Japanese-language version (BACS-J).[9]

Meanwhile, the National Institute of Mental Health Measurement and Treatment Research to Improve Cognition in Schizophrenia (NIMH-MATRICS) initiative, created to stimulate the development of cognition-enhancing drugs for schizophrenia,[10] developed a consensus cognitive battery for clinical trials of cognition-enhancing treatments for schizophrenia through a broadly based scientific evaluation of measures: the MATRICS Consensus Cognitive Battery (MCCB). The MCCB has been recommended as the standard battery for clinical trials of cognition-enhancing interventions for schizophrenia by the US Food and Drug Administration (FDA). Therefore, for its clinical application, we prepared the Japanese-language version (MCCB-J). The aim of this study was to test psychometric properties and validity of the MCCB-J for the evaluation of cognitive function in schizophrenia.

METHODS

Translation of the MCCB

In order to have an approved translation of the MCCB, the following translation methodology was used: two independent forward translations, reconciliation, two independent back translations, and pilot testing on five people with schizophrenia. The author of the scale checked the back-translation. In addition, we have adjusted two sub-tests (Letter–Number Span [LNS] and Mayer–Salovey–Caruso Emotional Intelligence Test: Managing Emotions [MSCEIT]) to Japanese language/culture.

Subjects

Thirty-seven patients with chronic schizophrenia were recruited for the present study. The diagnosis was based on DSM-IV criteria for schizophrenia,[11] a detailed clinical interview, and review of the prior records. In the subjects, hepatic and renal functions were normal, and subjects were excluded if they presented with any organic central nervous system disorder, significant substance abuse, or mental retardation. The study was approved by the relevant ethics committees and was performed in accordance with the Declaration of Helsinki II. The ethics committees of each institution were as follows: the Ethics Committee of University of Tokushima, the Ethics Committee of Okayama University Graduate School of Medicine, the Committee on Medical Ethics of Toyama Medical and Pharmaceutical University, and the Ethics Committee of the Tottori University Faculty of Medicine. The patients who gave informed consent to the research participated in this study. Table 1 shows the demographic characteristics of the subjects.

Table 1. Demographic information ($n = 37$)

Age (years)	38.4 (11.2)
Sex (F/M)	14/23
Education (years)	13.3 (2.0)
BPRS (total)	41.0 (7.1)

Data are given as mean (SD).
BPRS, Brief Psychiatric Rating Scale.

Twenty of the 37 patients were being treated with a single second-generation antipsychotic medication (aripiprazole, $n = 3$; blonanserin, $n = 2$; olanzapine, $n = 7$; perospirone, $n = 1$; quetiapine, $n = 1$; risperidone, $n = 6$), four with a single first-generation antipsychotic medication (haloperidol, $n = 3$; zotepine, $n = 1$), nine with a combination of antipsychotic drugs, and two without antipsychotic medications. The medications for two of 37 patients were unknown, because a site could not obtain the information.

Assessment procedures

The MCCB-J comprises 10 tasks that measure speed of processing (BACS Symbol-coding [BACS SC]; Category Fluency Animal Naming [Fluency]; Trail-making Test Part A [TMT-A]), attention/vigilance (Continuous Performance Test-Identical Pairs), working memory (Wechsler Memory Scale-Third Edition Spatial Span; LNS), verbal learning (Hopkins Verbal Learning Test-Revised), visual learning (Brief

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Visuospatial Memory Test-Revised), reasoning and problem solving (Neuropsychological Assessment Battery Mazes), social cognition (MSCEIT). A composite score was calculated by averaging all z-scores of the 10 subtests from the MCCB-J.

In order to examine the validity of the MCCB-J, the correlation between the MCCB-J and the BACS-J was determined. The BACS-J takes approximately 30 min, and is devised for easy administration and scoring by non-psychologists. It is specifically designed to measure treatment-related improvements. The BACS-J has high test–retest reliability,[9] and is as sensitive to cognitive dysfunction in schizophrenia as standard 2.5-h NTB. The BACS-J includes brief assessments of verbal memory (Verbal Memory), working memory (Digit Sequencing), motor speed (Token Motor), verbal fluency (Verbal Fluency), attention and processing speed (Symbol-coding), and executive function (Tower of London), and its composite scores are calculated by averaging all z-scores of the six subtests from the BACS-J. The 18-item Brief Psychiatric Rating Scale (BPRS,[12] 1–7 score) was completed by trained psychiatrists along with the MCCB -J and BACS-J.

Statistical analysis

The data analyses were conducted using JMP-8.0.2.J for Mac software (SAS Institute Inc., Cary, NC, USA). Descriptive statistics were used to report the patients in terms of sociodemographic and clinical data. Cronbach's alpha[13] was used to determine the internal consistency of the MCCB-J and BACS-J. The relations among the MCCB-J and BACS-J measures were determined by calculating Pearson correlations among the scores. To test the construct validity, Pearson correlation coefficients (r) were used between the MCCB-J and BACS-J scores. The factor structure of the scores was determined by performing a principal component analysis with varimax rotation. The level of significance was set at $P < 0.05$ (two-tailed).

RESULTS

Table 2 lists the means and standard deviations for all of the measures from the MCCB-J and BACS-J. The MCCB-J required a mean of 80.0 min (SD = 13.8) to complete, while the BACS-J required 35.4 min (1.9).

Table 2. Mean (SD) of MCCB-J and BACS-J-tests

Tests	Raw scores
MCCB-J	
Trail-making Test Part A (time in s)	38.4 (15.5)
BACS Symbol-coding	49.6 (11.8)
Hopkins Verbal Learning Test-Revised	21.7 (4.8)
Wechsler Memory Scale-Third Edition Spatial Span	15.7 (3.6)
Letter–Number Span	10.7 (3.2)
Neuropsychological Assessment Battery Mazes	17.1 (7.0)
Brief Visuospatial Memory Test-Revised	22.0 (6.9)
Category Fluency Animal Naming	17.8 (5.2)
Mayer–Salovey–Caruso Emotional Intelligence Test Managing Emotions (branch score)	80.8 (8.5)
Continuous Performance Test-Identical Pairs (d-prime)	2.6 (0.6)
BACS-J	
Verbal Memory	37.2 (11.6)
Digit Sequencing	17.6 (4.6)
Token Motor Task	68.4 (18.7)
Verbal Fluency	38.3 (9.9)
Symbol-coding Task	51.3 (13.2)
Tower of London	16.6 (3.9)

Data are given as mean (SD).
BACS-J, Brief Assessment of Cognition in Schizophrenia Japanese-language version, MCCB-J, Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery Japanese-language version.

Reliability

Internal consistency

Cronbach's alpha was 0.72 for the MCCB-J, and 0.78 for the BACS-J.

Intercorrelations between MCCB-J/BACS-J subtests and composite score

Table 3 presents the correlations among the MCCB-J subtests and composite score. The MCCB-J composite score was significantly correlated with all primary MCCB-J measures. In addition, Table 4 presents the correlations among the BACS-J subtests and composite score, and the BACS-J composite score was also significantly correlated with all primary BACS-J measures.

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Table 3. Pearson correlations among MCCB-J measures

	TMT-A	BACS SC	HVLT-R	WMS-III SS	LNS	NAB Mazes	BVMT-R	Fluency	MSCEIT ME	CPT-IP
Composite Score	−0.62****	0.67****	0.71****	0.55***	0.75****	0.67****	0.73****	0.51**	0.39*	0.75****
CPT-IP	−0.34*	0.52**	0.37*	0.39*	0.61****	0.37*	0.38*	0.39*	0.33*	–
MSCEIT ME	−0.11	0.05	0.26	0.01	0.34*	−0.01	0.29	0.15	–	–
Fluency	−0.22	0.15	0.29	0.15	0.26	0.22	0.40*	–	–	–
BVMT-R	−0.38*	0.37*	0.53***	0.33*	0.55***	0.46**	–	–	–	–
NAB Mazes	−0.60****	0.43**	0.44**	0.34*	0.35*	–	–	–	–	–
LNS	−0.20	0.47**	0.56***	0.46**	–	–	–	–	–	–
WMS-III SS	−0.17	0.38*	0.32	–	–	–	–	–	–	–
HVLT-R	−0.40*	0.35*	–	–	–	–	–	–	–	–
BACS SC	−0.57***	–	–	–	–	–	–	–	–	–

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.
BACS SC, BACS Symbol-coding; BVMT-R, Brief Visuospatial Memory Test-Revised; CPT-IP, Continuous Performance Test-Identical Pairs; Fluency, Category Fluency Animal Naming; HVLT-R, Hopkins Verbal Learning Test-Revised; LNS, Letter-Number Span; MCCB-J, Measurement and Treatment Research to Improve Cognition in Schizophrenia Consensus Cognitive Battery Japanese-language version.; MSCEIT ME, Mayer-Salovey-Caruso Emotional Intelligence Test Managing Emotions; NAB Mazes, Neuropsychological Assessment Battery Mazes; TMT-A, Trail-making Test Part A; WMS-III SS, Wechsler Memory Scale-Third Edition Spatial Span.

Validity

Correlations between MCCB-J and BACS-J measures

Table 5 shows the Pearson correlations between corresponding subtests from the MCCB-J and BACS-J. Because the BACS-J includes only four of the seven cognitive domains nominated by the MCCB, we examined correlations of corresponding subtests in only these four domains. The MCCB-J subtests in working memory, verbal learning, and reasoning and problem-solving were significantly correlated with relevant subtests. In speed of processing, BACS SC was significantly correlated with all three relevant BACS subtests, MCCB Fluency was correlated with BACS Verbal Fluency, and TMT-A with Symbol-coding Task. In addition, the MCCB-J composite score was significantly correlated with BACS-J composite score.

Factor analysis for MCCB-J/BACS-J subtests

Tables 6 and 7 show the factor loadings for the MCCB-J and BACS-J, respectively. For both MCCB-J and BACS-J, a factor accounts for more 40% of the

Table 4. Pearson correlations among BACS-J measures

	Verbal Memory	Digit Sequencing	Token Motor Task	Verbal Fluency	Symbol-coding Task	Tower of London
Composite Score	0.76****	0.75****	0.58***	0.63****	0.66****	0.80****
Tower of London	0.53***	0.60***	0.35*	0.42**	0.42*	–
Symbol-coding Task	0.37*	0.46**	0.33*	0.41*	–	–
Verbal Fluency	0.40*	0.44**	0.12	–	–	–
Token Motor Task	0.39*	0.17	–	–	–	–
Digit Sequencing	0.43**	–	–	–	–	–

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.
BACS-J, Brief Assessment of Cognition in Schizophrenia Japanese-language version.

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Table 5. Pearson correlations between the MCCB-J and BACS-J for the same cognitive domains

Cognitive domains	MCCB-J	BACS-J	r
Speed of processing	BACS SC	Symbol-coding Task	0.87****
		Verbal Fluency	0.42**
		Token Motor Task	0.37*
	Fluency	Symbol-coding Task	0.14
		Verbal Fluency	0.63****
		Token Motor Task	0.19
	TMT-A	Symbol-coding Task	−0.50**
		Verbal Fluency	−0.24
		Token Motor Task	−0.25
Attention/Vigilance	CPT-IP	–	–
Working memory	WMS-III SS	Digit Sequencing	0.57***
	LNS	Digit Sequencing	0.66****
Verbal learning	HVLT-R	Verbal Memory	0.67****
Visual learning	BVMT-R	–	–
Reasoning and problem-solving	NAB Mazes	Tower of London	0.38*
Social cognition	MSCEIT ME	–	–
Composite score			0.76****

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$.

BACS-J, Brief Assessment of Cognition in Schizophrenia Japanese-language version; BACS SC, BACS Symbol-coding; BVMT-R, Brief Visuospatial Memory Test-Revised; CPT-IP, Continuous Performance Test-Identical Pairs; Fluency, Category Fluency Animal Naming; HVLT-R, Hopkins Verbal Learning Test-Revised; LNS, Letter–Number Span; MCCB-J, MATRICS Consensus Cognitive Battery Japanese-language version; MSCEIT ME, Mayer–Salovey–Caruso Emotional Intelligence Test Managing Emotions; NAB Mazes, Neuropsychological Assessment Battery Mazes; TMT-A, Trail-making Test Part A; WMS-III SS, Wechsler Memory Scale-Third Edition Spatial Span.

total variance, and thus a single-factor solution was indicated.

DISCUSSION

Overall, the results of this study indicate that the MCCB-J is a useful tool to evaluate cognitive function in schizophrenia. In addition, some of the psychometric properties of the BACS-J are replicated. More importantly, this was the first study to compare the MCCB and BACS directly.

The MCCB-J required a mean of 80.0 min to complete, whereas the BACS-J required a mean of 35.4 min. This was easily predicted, as the MCCB-J covered much more cognitive domains than the BACS-J.

As for internal consistency, Cronbach's alpha was 0.72 for the MCCB-J, and 0.78 for the BACS-J, which is in the acceptable range for internal consistency (>0.60). In addition, the intercorrelations between MCCB-J/BACS-J subtests and composite score indicate that the MCCB-J and BACS-J are reliable.

Pearson correlations between corresponding subtests from the MCCB-J and BACS-J provide evidence of good construct validity for all four domains, speed of processing, working memory, verbal learning, and reasoning and problem-solving. In addition, the MCCB-J composite score was significantly and strongly correlated with BACS-J composite score.

Data from the factor analyses show, for both MCCB-J and BACS-J, that a factor accounts for more than 40% of the total variance. According to guidelines regarding the proportion of explained variance,[14] a single-factor solution is the only appropriate solution when any factor accounts for more than 40% of the total variance, regardless of the size of additional factors. Presence of a single factor underlying the baseline cognitive data in both batteries may indicate that a single composite index is appropriate.

The definitive validation of the MCCB-J will require further study. First, further comparisons need to be made for temporal stability. Second, the

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Table 6. Factor loadings of MCCB-J measures

	Component		
	1	2	3
TMT-A	**−0.88**	−0.01	−0.16
BACS SC	0.59	0.51	0.03
HVLT-R	0.40	0.37	0.49
WMS-III SS	0.13	**0.84**	−0.06
LNS	0.12	**0.74**	0.47
NAB Mazes	**0.78**	0.28	0.08
BVMT-R	0.41	0.34	0.56
Fluency	0.27	0.01	**0.58**
MSCEIT ME	−0.17	0.05	**0.79**
CPT	0.26	0.58	0.46
Eigenvalue	4.2	1.2	0.9
Percentage of variance	42.5	12.9	9.8

BACS SC, BACS Symbol-coding; BVMT-R, Brief Visuospatial Memory Test-Revised; CPT-IP, Continuous Performance Test-Identical Pairs; Fluency, Category Fluency Animal Naming; HVLT-R, Hopkins Verbal Learning Test-Revised; LNS, Letter–Number Span; MCCB-J, MATRICS Consensus Cognitive Battery (MCCB) Japanese-language version; MSCEIT ME, Mayer–Salovey–Caruso Emotional Intelligence Test Managing Emotions; NAB Mazes, Neuropsychological Assessment Battery Mazes; TMT-A, Trail-making Test Part A; WMS-III SS, Wechsler Memory Scale-Third Edition Spatial Span.

MCCB-J will need to be assessed in various populations of patients with schizophrenia. It is also important to determine whether the MCCB-J is sensitive to cognitive changes during clinical trials.

Table 7. Factor loadings of BACS-J measures

	Component	
	1	2
Verbal Memory	0.55	0.53
Digit Sequencing	**0.81**	0.10
Token Motor Task	0.04	**0.94**
Verbal Fluency	**0.79**	−0.02
Symbol-coding Task	**0.61**	0.36
Tower of London	**0.71**	0.38
Eigenvalue	3.0	0.9
Percentage of variance	50.2	15.7

BACS-J, Brief Assessment of Cognition in Schizophrenia Japanese-language version.

Conclusion

The present study indicates that the MCCB-J is a promising tool for assessing the major constructs of cognition that have been found to be most impaired and strongly correlated with outcome in patients with schizophrenia.

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None of the authors has any conflict of interest to declare.

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SHORT COMMUNICATION

Microarray analysis of global gene expression in leukocytes following lithium treatment

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Objectives To elucidate the molecular effects of lithium, we studied global gene expression changes induced by lithium in leukocytes from healthy subjects.
Methods Eight healthy male subjects participated in this study. Lithium was prescribed for weeks to reach a therapeutic serum concentration. Leukocyte counts and serum lithium concentrations were determined at baseline (before medication), after 1 and 2 weeks of medication and at 2 weeks after stopping medication. Gene expression profiling was performed at each time point using Agilent G4112F Whole Human Genome arrays (The Agilent Technologies, Santa Clara, CA, USA). Expression of some candidate genes was also assessed by real-time polymerase chain reaction (PCR).
Results Gene ontology analysis revealed that the cellular and immune responses to stimulus and stress indeed played a major role in the cellular response to lithium treatment. Pathway analysis revealed that the interleukin 6 pathway, the inhibitor of differentiation pathway, and the methane metabolism pathway were regulated by lithium. Using real-time PCR, we also confirmed that five candidate genes in these pathways were significantly changed, including suppressor of cytokine signaling 3 and myeloperoxidase.
Conclusions Our investigation suggests that the molecular action of lithium is mediated in part by its effects on the cellular and immune response to stimulus and stress followed by the interleukin 6, inhibitor of differentiation, and methane metabolism pathways. Copyright © 2014 John Wiley & Sons, Ltd.

KEY WORDS—lithium; leukocyte; gene expression; interleukin 6

INTRODUCTION

Lithium is typically prescribed as a mood stabilizer for bipolar disorder and as an augmentation agent for refractory depression; however, the exact molecular mechanisms remain unknown (reviewed in Serretti and Drago, 2010). Several studies have recently demonstrated altered messenger RNA (mRNA) expression in the peripheral leukocytes of patients with mood disorders, and their application as biological markers has been suggested (reviewed in Iga *et al.*, 2008). There are various advantages associated with leukocyte gene expression biomarkers. For example, mRNA is stable for long-term storage, and using DNA microarrays, numerous genes can be examined quickly and simultaneously with only a small amount of blood. Furthermore, the test is simple to repeat during disease progression. By examining the effects of lithium on gene expression in healthy subjects, we were able to investigate the precise action of lithium without relevant confounding factors, such as disease pathophysiology and other medications (patients are often prescribed several medications simultaneously). Thus, we used microarray analysis and real-time PCR to analyze global gene expression changes in the leukocytes of healthy subjects undergoing 2 weeks of lithium treatment. Our aim was to perform a comprehensive analysis of changes induced by lithium treatment, which may reveal new targets for the molecular mechanisms underlying lithium action.

METHODS

Lithium treatment for healthy subjects

The healthy subjects chosen for this study were eight men with no present Diagnostic and Statistical Manual of Mental Disorders-IV axis I psychiatric disorders. Additional information about lithium treatment

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parameters and subjects is detailed in our earlier paper (Kikuchi *et al.*, 2011) and in Table 1. All individuals provided signed informed consent, which was approved by the university ethics committee.

Tissue processing, RNA purification, and preparation for microarray analysis

Total RNA was extracted from peripheral leukocytes of whole blood samples using the PAXgene Blood RNA tube (Qiagen, Tokyo, Japan) and the PAXgene Blood RNA kit (Qiagen, Tokyo, Japan). RNA concentration and its integrity were analyzed with an Agilent 2100 Caliper LabChip Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). RNA integrity numbers (RIN) of all samples were above 7.0 (cutoff RIN), and the mean RIN was 7.8. For Agilent G4112F Whole Human Genome array preparation and analysis, we followed the protocols provided by Agilent Technologies. We used two micrograms of RNA from each of the leukocytes from eight subjects per group for microarray experiments, because it has been reported that pooled RNA samples on microarrays showed comparable quality criteria when compared with individual RNA samples (Port *et al.*, 2012). All microarray methods and results may be found at the Gene Expression Omnibus Web site (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE44987.

Quantitative real-time PCR

Transcripts from 17 different genes were quantified by real-time PCR. In these experiments, we did not use pooled RNA samples but analyzed individual RNA specimens from each sample. Two micrograms of total RNA was used for complementary DNA synthesis by random (N6) primers and Quantiscript Reverse Transcriptase (Qiagen, Tokyo, Japan) after assessing RNA quality and quantity with NanoDrop (NanoDrop Technologies, DE, USA). Quantitative real-time PCR was performed with the ABI 7500 Fast System (Applied Biosystems, Foster City, CA, USA). NORMFINDER, a statistical application, was used to evaluate the stability of reference genes (Andersen *et al.*, 2004). glyceraldehyde-3-phosphate dehydrogenase showed the best stability score and was used as reference gene in our study. Measurements of each gene expression were conducted in triplicate. Primers for each gene are summarized in Table 2.

Statistical analysis

The transcripts represented on the array together with quantitative data were analyzed using the GENESPRING GX 11.5.1 (Agilent Technologies, Palo Alto, CA, USA). Data were normalized against the 75th percentile for per-chip normalization.

For the Gene Ontology (GO) analysis, the significance of the association between the dataset and the pathway was measured using two methods. First, only changes equal to or greater than plus/minus two-fold at any time point (80 upregulated and 54 downregulated genes) were included in GO analysis. Second, Fisher's exact test was used to calculate a p-value determining the probability that the association between the genes and the GO may be explained by chance alone. GO annotations were obtained from the Gene Ontology website (http://www.geneontology.org/).

For pathway analysis, the significance of the association between the dataset and the pathway was measured using two methods: First, only changes equal or greater than positive/negative two-fold were included for the pathway analysis. Second, Fisher's exact test was used to calculate a p-value determining the probability that the association between the genes in the dataset and the pathway may be explained by chance alone. Pathway annotations were obtained from WikiPathways (http://www.wikipathways.org/index.php/WikiPathways).

For real-time PCR analysis, statistical calculations were carried out using the SPSS Statistical Software Package 11.5 (SPSS, Tokyo, Japan). Changes during lithium treatment were calculated with the Wilcoxon matched-pairs signed-rank test. The criterion for significance was set at $p < 0.05$ for all tests.

Table 1. Lithium concentration and leukocyte count

Parameter	Baseline	1 week	2 weeks	Post-2 weeks
Lithium dose (mg/day)	None	1200 ± 0.0	1375 ± 225	None
Lithium conc (mEq/l)	None	0.55 ± 0.23	0.73 ± 0.27	None
Leukocyte count (cells/μl)	5513 ± 1382	6538 ± 1016*	6400 ± 1021*	5438 ± 835
Granulocytes (%)	50.4 ± 8.7	53.6 ± 10.3	47.3 ± 9.4	49.3 ± 7.3
Lymphocytes (%)	39.1 ± 9.1	34.4 ± 10.4	39.3 ± 10.1	38.6 ± 7.6
Monocytes (%)	6.5 ± 2.1	5.6 ± 2.4	7.1 ± 2.6	7.2 ± 2.7

Leukocyte counts were significantly increased at 1 and 2 weeks (Wilcoxon matched-pairs signed-rank test) compared with baseline and were decreased at post-2 weeks compared with 1 and 2 weeks. Mean percentages of the leukocyte fraction were not significantly altered.
*$p < 0.05$

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Table 2. List of primers used in real-time PCR experiments

Name	Gene symbol	Forward primer	Reverse primer
Interleukin 6	IL6	CCATCTCACAGTTTCATTGGTG	GCCAATTGAAACAGCTACAAAAG
Suppressor of cytokine signaling 3	SOCS3	CAAGGACGGAGACTTCGATTC	GAAACTTGCTGTGGGTGAC
FBJ murine osteosarcoma viral oncogene homolog	FOS	TTGTGAAGACCATGACAGGAG	CCATCTTATTCCTTTCCCTTCGG
Jun proto-oncogene	JUN	AGCCCAAACTAACCTCACG	TGCTCTGTTTCAGGATCTTGG
Myeloperoxidase	MPO	TCTGAACATGCAGCGCAG	CATCAGTTTCCTCGCCAATTTC
cAMP responsive element binding protein 1	CREB1	GGCAGACAGTTCAAGTCCATG	CGCTTTTGGGAATCAGTTACAC
Tumor necrosis factor	TNF	ACTTTGGAGTGATCGGCC	GCTTGAGGGTTTGCTACAAC
Microtubule-associated protein tau	MAPT	GACAGAGTCCAGTCGAAGATTG	GCGACTTGTACACGATCTCC
Signal transducer and activator of transcription 3	STAT3	TGCAGAAAACTCTCACGGAC	TGTTGACGGGTCTGAAGTTG
Radical S-adenosyl methionine domain containing 2	RSAD2	AGAATACCTGGGCAAGTTGG	GTCACAGGAGATAGCGAGAATG
Ubiquitin specific peptidase 28	USP28	GAGTTAAGGAGCCCAGTCAAG	CAGTAGACTCAAAGCAATGGC
Zinc finger protein 507	ZNF507	GTGTCCAGGAAGGGAATAAGTC	CACATACCCACACAGAGAGC
Heat shock protein 90 kDa alpha (cytosolic), class A member 1	HSP90AA1	GTCTGTGAAGGATCTGGTCATC	CAGCAGTAGGGTCATCTTCATC
Apolipoprotein L, 6	APOL6	AGAAAGTGAGGCTGGTGTTG	CAGATCTCCGTCTTGTAGCTC
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	CAGCCTCAAGATCATCAGCA	TGTGGTCATGAGTCCTTCCA
Beta-actin	ACTB	GGAATTATGTGTCTACCCTCGC	TCCTGTCTTTGCGTTCCTG
C-abl oncogene 1	ABL1	CTCAGATCTCGTCAGCCATG	ATCAGCTACCTTCACCAAGTG

cAMP, cyclic adenosine monophosphate.

RESULTS

Leukocyte counts in lithium-treated healthy subjects

As reported in a previous paper (Kikuchi *et al.* 2011), the mean leukocyte count in lithium-treated healthy subjects was significantly increased, but the leukocyte fraction was not significantly altered during lithium treatment (Table 2). This indicates that the change in leukocyte gene expression was not influenced by changes in the leukocyte fraction.

Microarray gene expression

In total, 44 000 genes were present on the Agilent G4112F Whole Human Genome arrays at all four time points, that is, baseline, 1, 2, and 2 weeks after the end of lithium treatment (post-2 weeks). A threshold of two-fold at any time point was used as a minimum expression change from baseline. Lithium treatment for 2 weeks led to upregulation of 80 genes (0.18% of all detected genes) and downregulation of 54 genes (0.12% of all detected genes) (Figure 1). All upregulated or downregulated genes are shown in Supplemental Table 1. All microarray results may be found at the Gene Expression Omnibus Web site (http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE44987.

Gene ontology analysis

The top five GO categories for upregulated genes include response to stimulus (GO:0050896|GO:0051869), cellular response to stimulus (GO:0051716), response to stress (GO:0006950), immune system process (GO:0002376), and cell surface receptor signaling pathway (GO:0007166) (Figure 2). The top five GO categories for downregulated genes include response to

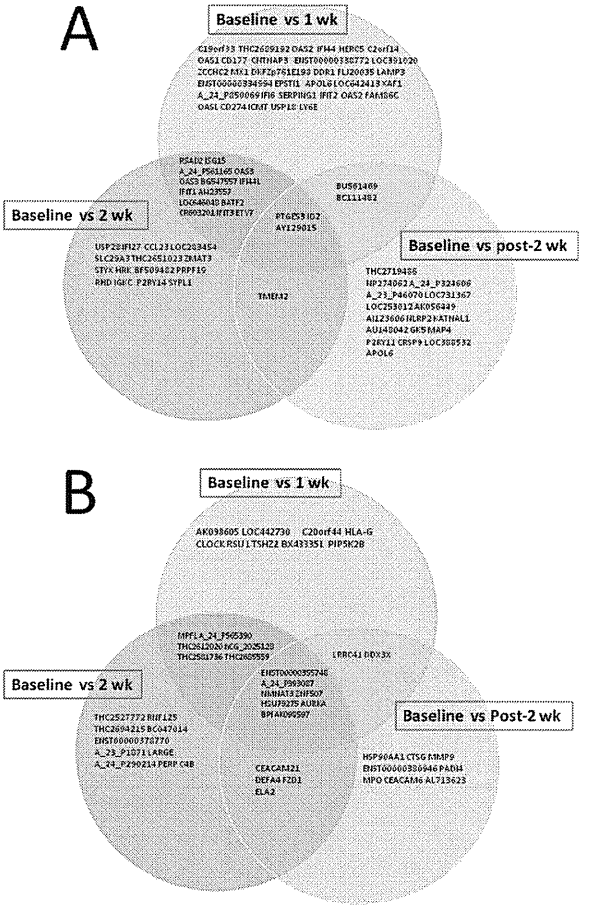

Figure 1. Venn diagram of genes regulated by lithium. Only those genes that showed at least two-fold upregulation (A) or downregulation (B) in comparison with baseline are illustrated. A total of 80 and 54 genes were upregulated and downregulated, respectively, by lithium treatment at any time point

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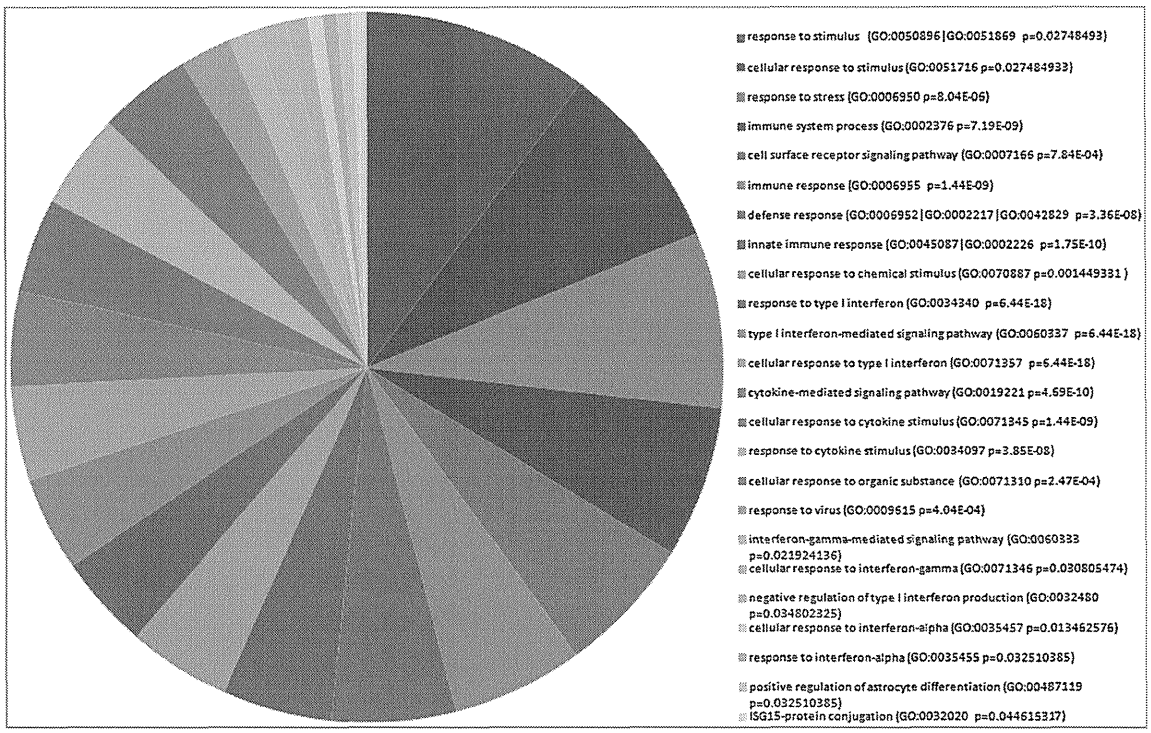

Figure 2. Gene Ontology (GO) analysis of 80 genes upregulated by lithium. Pie chart shows the percentage of upregulated genes, which were categorized on the basis of their involvement in biological processes. *p*-values were adjusted for multiple testing

bacterium (GO:0009617|GO:0009618|GO:0009680), modification of morphology or physiology of other organisms involved in symbiotic interactions (GO:0035821), cell killing (GO:0001906), response to fungus (GO:0009620|GO:0009621), and killing of cells of other organisms (GO:0031640|GO:0001908) (Figure 3).

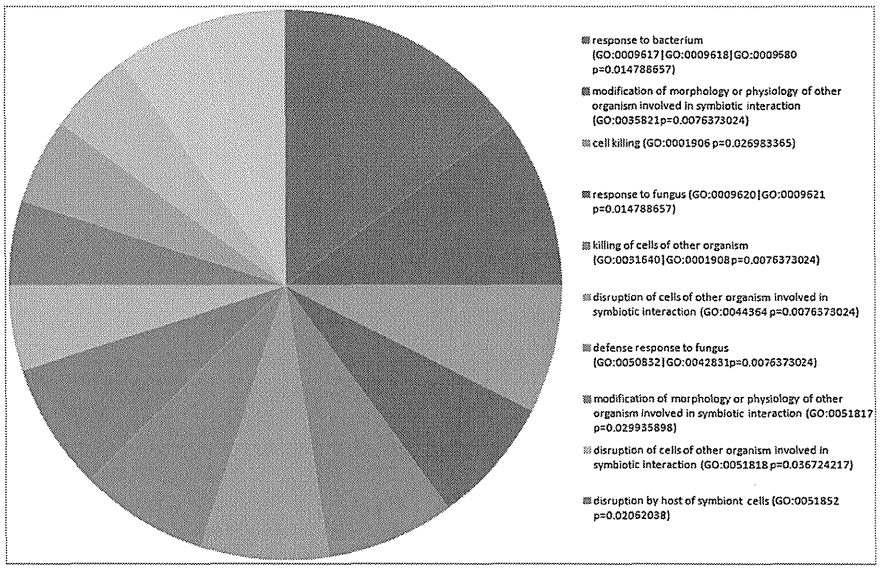

Figure 3. Gene Ontology (GO) analysis of 54 genes downregulated by lithium. Pie chart shows the percentage of downregulated genes, which were categorized on the basis of their involvement in biological processes. *p*-values were adjusted for multiple testing

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Pathway analysis

Of the genes that showed changes greater than positive/negative two-fold from baseline versus 1, 2, and post-2 weeks, the three pathways that emerged were the interleukin 6 (IL6) pathway, the inhibitor of differentiation pathway, and the methane metabolism pathway (Figure 4). All pathways were significantly altered by lithium treatment after adjusting for multiple testing ($p < 0.05$).

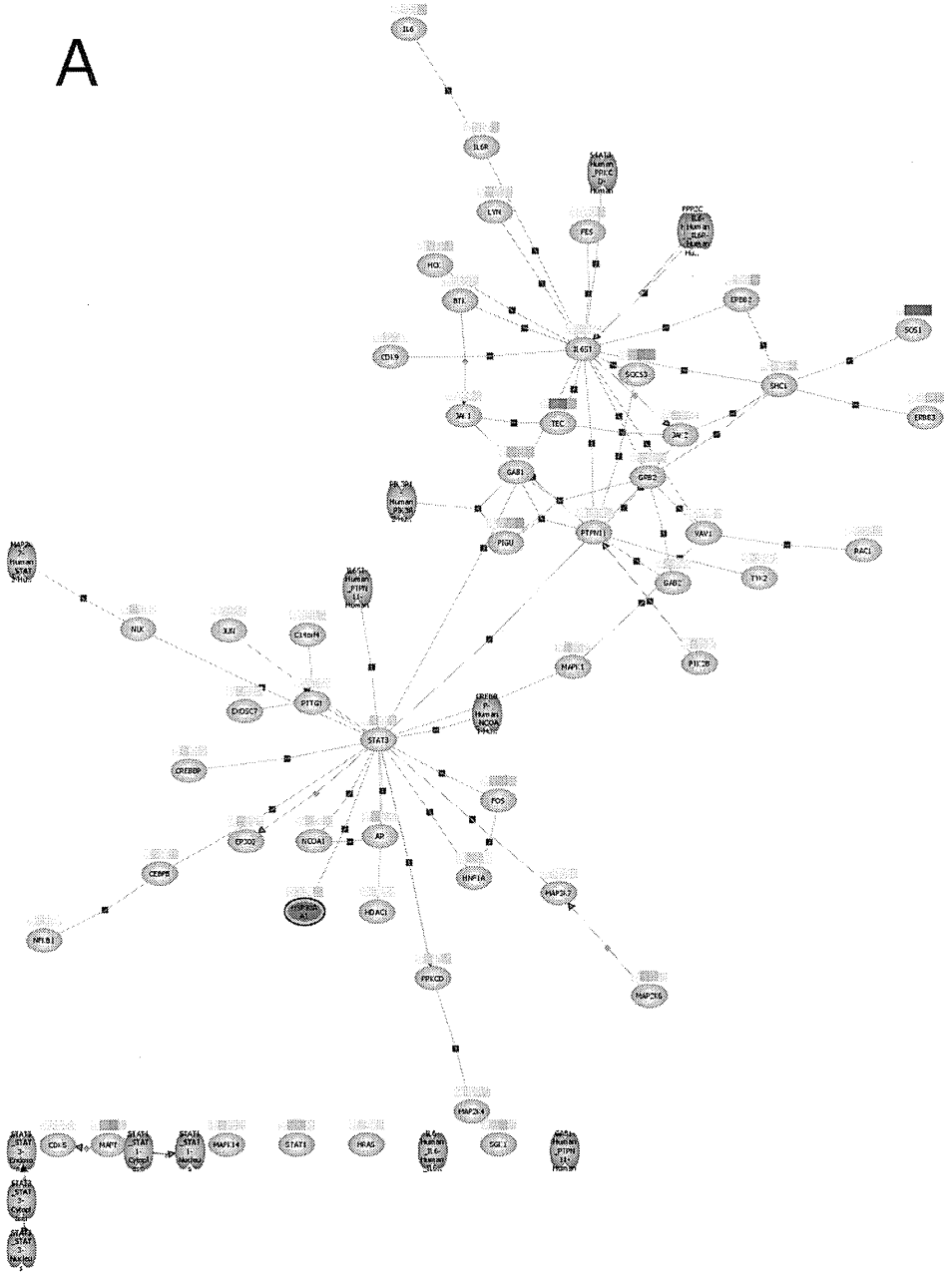

Figure 4. (A) Diagram of the interleukin 6 (IL6) pathway, which had the most significant association with expression level changes induced by lithium treatment. (B) Diagram of the ID pathway. (C) Diagram of the methane metabolism pathway. All pathways were significantly changed by lithium treatment after adjusting for multiple testing ($p < 0.05$). Expression levels at each time point are indicated by column color over each gene symbol. Red columns were upregulated, whereas green columns were downregulated. Yellow columns were detected by microarray analysis, but their expression was unaffected

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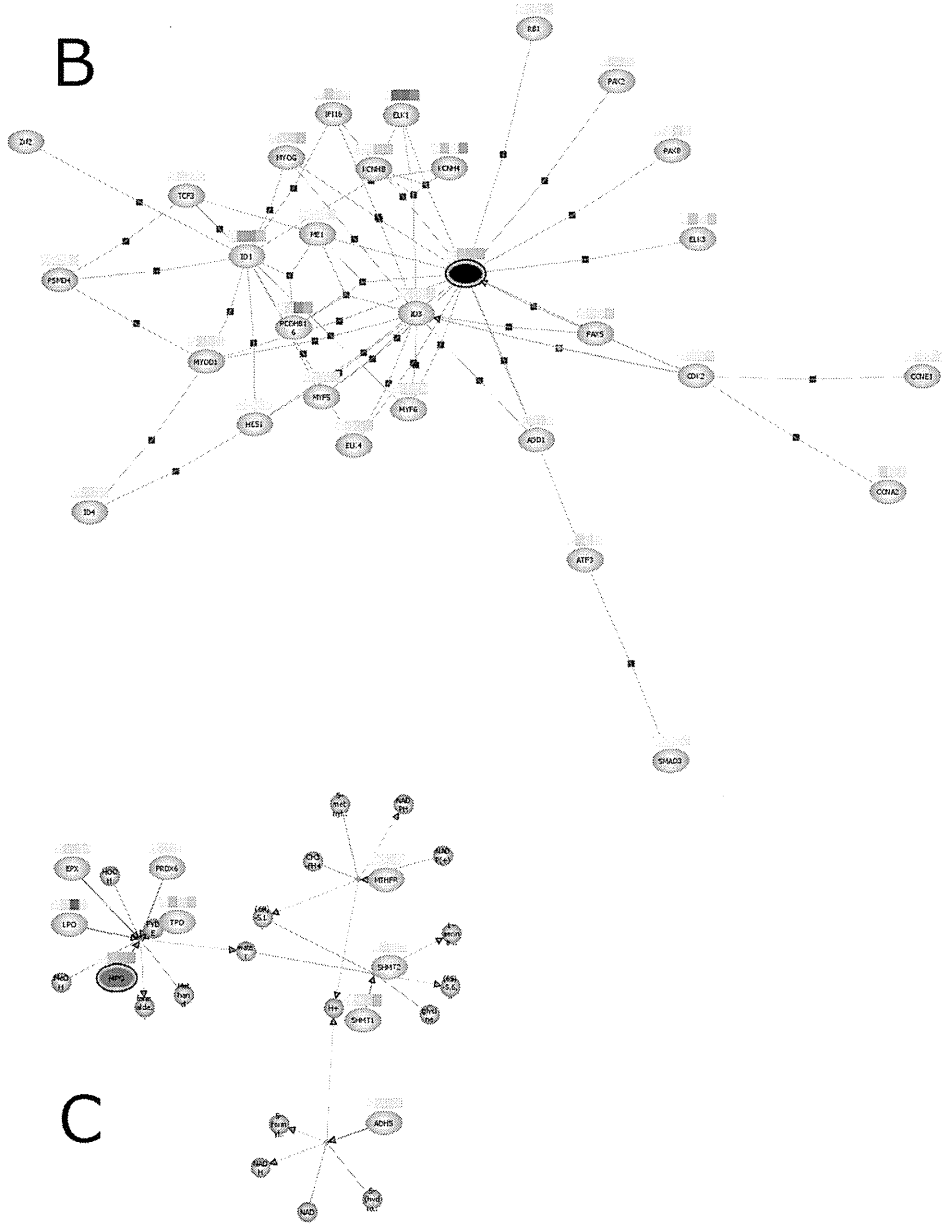

Figure 4. Continued.

Validation of the microarray data by real-time PCR

We confirmed the expression of five genes (RSAD2, USP28, ZNF507, HSP90AA1, and APOL6) in the top five upregulated and downregulated genes by real-time PCR. For statistical comparisons between PCR and Agilent data, linear regression with $r^2 = 0.39$ and $p < 0.014$ was performed (Figure 5). Nine candidate genes from the three pathways reportedly associated with the effects of lithium were also selected, and significant changes in the six genes were reconfirmed by real-time PCR. The six genes included one gene that was upregulated in the IL6 pathway (FBJ murine osteosarcoma viral oncogene homolog [FOS]) and four genes that were downregulated in the IL6 pathway (IL6, suppressor of cytokine signaling 3

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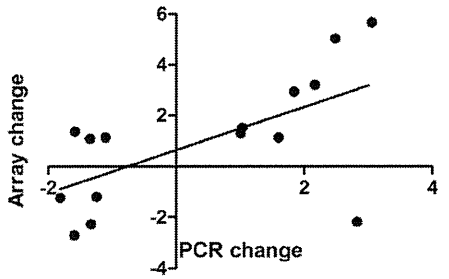

Figure 5. Five gene expression changes (RSAD2, USP28, ZNF507, HSP90AA1, and APOL6) at each time point in the top five upregulated and downregulated genes in the Agilent data could be quantified by PCR. For statistical comparison between PCR and Agilent data, linear regression with $r^2 = 0.39$ and $p < 0.014$ was calculated

[SOCS3], and jun proto-oncogene [JUN]) and in the methane metabolism pathway (myeloperoxidase [MPO]) (Table 3).

DISCUSSION

On GO analysis, categories for upregulated genes included response to stimulus, response to stress, immune system process, and response to cytokines (Figure 2), while downregulated genes included response to bacterium or fungus, cell killing, and neutrophil-mediated cytotoxicity (Figure 3). Taken together, the results indicate that the genes involved in the response to stimulus, stress, and cytokines were increased, while the genes involved in the response to infection and cell killing were decreased by lithium treatment. Our data suggest a promising role for lithium in the regulation of inflammation and cell death (Dai *et al.*, 2012, Green and Nolan, 2012, Matsebatlela *et al.*, 2012).

Table 3. Mean expression levels of nine candidate genes in the three pathways

	Baseline	1 week	2 weeks	Post-2 weeks
SOCS3	1.00	0.98	0.66*	0.75
IL6	1.00	1.07	1.06	0.78*
MPO	1.00	0.77*	0.93	0.96
CREB1	1.00	1.12	1.01	0.81*
FOS	1.00	1.64	1.79*	0.87
JUN	1.00	0.72	0.65*	0.73
TNF	1.00	1.14	1.01	1.04
MAPT	1.00	1.26	1.06	1.18
STAT3	1.00	0.91	0.92	0.93

SOCS3, Suppressor of cytokine signaling 3; IL6, interleukin 6; MPO, myeloperoxidase; CREB1, cyclic adenosine monophosphate responsive element binding protein 1; FOS, FBJ murine osteosarcoma viral oncogene homolog; JUN, jun proto-oncogene; TNF, tumor necrosis factor; MAPT, microtubule-associated protein tau; STAT3, signal transducer and activator of transcription 3.
*$p < 0.05$ Wilcoxon matched-paired signed-rank test (vs. baseline).

On pathway analysis, the IL6 pathway was associated with the action of lithium. There is evidence indicating an association between lithium, IL6, and bipolar disorder. For example, IL6 gene expression in the monocytes of bipolar patients was significantly increased (Padmos *et al.*, 2008). Serum IL6 protein levels were significantly increased in selective serotonin re-uptake inhibitors-refractory depression (O'Brien *et al.*, 2007, Yoshimura *et al.*, 2009), which is often treated with lithium augmentation. The tumor-necrosis-factor-mediated IL6 induction was further enhanced by lithium (Vandevoorde 1992). Following lithium treatment, SOCS3 in the IL6 pathway showed marked downregulation. SOCS3 is expressed by immune cells and cells in the central nervous system (CNS) (microglia and astrocytes) that have the potential to regulate numerous CNS disease states (reviewed in Baker *et al.*, 2009). Our results suggest that lithium has neuroprotective effects by decreasing both IL6 and SOCS3 expressions in immune cells. Very recently, we found that drug-naive major depressive disorder (MDD) patients showed significantly lower SOCS3 mRNA levels when compared with healthy subjects, while SOCS3 mRNA levels were significantly increased to healthy control levels after successful treatment with antidepressants (unpublished data). Because SOCS3 expression is stimulated by cytokine signaling and SOCS3 acts as a negative feedback regulator to prevent overstimulation of cytokine-responsive cells, the decrease in SOCS3 mRNA expression in the leukocytes of MDD patients may be associated with the unsuccessful prevention of over-activated inflammation. Successful treatment with antidepressants may enhance the function of SOCS3 and recover the abnormal immune response in MDD. Thus, SOCS3 mRNA levels in leukocytes may be available as a biomarker for the diagnosis of mood disorders and responsiveness to lithium and antidepressant. FOS and JUN gene expressions in the IL6 pathway were also significantly altered. FOS and JUN protein homodimer or heterodimer assemble in the activator protein 1 (AP-1) transcription factor. Interestingly, AP-1 proteins are also known to be involved in the molecular action of lithium (Jope and Bijur, 2002).

In the CNS, inhibitor of DNA binding (ID) proteins are positive regulators of neural cell proliferation, are required for neural cell cycle progression, and also play a role in the timing of oligodendroglial differentiation (Tzeng 2003). Lithium is known to increase adult hippocampal neurogenesis (Boku *et al.*, 2010). Although we did not reconfirm significant changes in ID gene expression by real-time PCR, ID proteins may play important roles in lithium-induced cell growth and differentiation.

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Significant changes were observed in the methane metabolism pathway, which include candidate genes for mood disorders. It was reported that MPO is a useful biomarker of immune activation in MDD and by promoting inflammation, may be involved in the pathogenesis of MDD (Vaccarino *et al.*, 2008). Our results suggest that lithium exerts clinical effects by decreasing MPO activity and immune activation. Thyroid peroxidase is an important enzyme in thyroid hormone synthesis, and it is reported that a high prevalence of autoimmunity for this enzyme was confirmed in patients with bipolar disorder (Padmos *et al.*, 2004). Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme for homocysteine metabolism. Homocysteine is known to be associated with geriatric depression (Almeida *et al.*, 2008), and MTHFR C677T polymorphism, which impairs homocysteine metabolism, is over-represented among depressive patients (Coppen and Bolander-Gouaille, 2005).

Although this is the first study to examine the global gene expression effects of lithium on peripheral leukocytes, Sun *et al.* (2004) found that chronic lithium treatment at a therapeutically relevant concentration decreased the expression of seven genes (alpha 1B-adrenoceptor, acetylcholine receptor protein alpha chain precursor, cyclic adenosine monophosphate-dependent 3′,5′-cyclic phosphordiesterase4D, substance-P receptor, somatostatin receptor type 2, nuclear factor kappa-B DNA binding subunit, and ras-related protein) in lymphoblasts from lithium responders (Sun *et al.*, 2004). Unfortunately, none of these seven genes are listed in our results. These discrepancies may be due to differences in the tissue sampled and/or the pathophysiology of bipolar disorder. Further research is needed to identify the lithium response markers using leukocytes from patients.

The limitations of this study include the low rate of validation. Although most of the validated genes showed similar changes as in the microarray results, we were only able to validate significant changes in five of the 17 selected genes. In addition, four of 15 data points in the top five upregulated and downregulated genes showed changes in the opposite direction from microarray to PCR. These differences may be due to differences in sample preparation, sensitivity of the two techniques, and use of different statistical analysis methods. Setting the threshold for minimum change as two-fold on microarray analysis may ignore important genes with expression changes of less than two-fold.

CONCLUSION

We show that lithium treatment significantly affects leukocyte gene expression. GO analysis revealed the promising effects of lithium in the regulation of inflammation and cell death. Pathway analysis suggests that the molecular action of lithium is mediated by the IL6 pathway, the ID pathway, and the methane metabolism pathway. Some genes in these pathways, such as SOCS3 and MPO, were also validated by real-time PCR.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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Meta-analyses of Blood Homocysteine Levels for Gender and Genetic Association Studies of the *MTHFR* C677T Polymorphism in Schizophrenia

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Previous studies suggest that elevated blood homocysteine levels and the methylenetetrahydrofolate reductase (*MTHFR*) C677T polymorphism are risk factors for schizophrenia. However, the effects of gender and *MTHFR* C677T genotypes on blood homocysteine levels in schizophrenia have not been consistent. We first investigated whether plasma total homocysteine levels were higher in patients with schizophrenia than in controls with stratification by gender and by the *MTHFR* C677T genotypes in a large cohort (N = 1379). Second, we conducted a meta-analysis of association studies between blood homocysteine levels and schizophrenia separately by gender (N = 4714). Third, we performed a case-control association study between the *MTHFR* C677T polymorphism and schizophrenia (N = 4998) and conducted a meta-analysis of genetic association studies based on Japanese subjects (N = 10 378). Finally, we assessed the effect of plasma total homocysteine levels on schizophrenia by a mendelian randomization approach. The ANCOVA after adjustment for age demonstrated a significant effect of diagnosis on the plasma total homocysteine levels in all strata, and the subsequent meta-analysis for gender demonstrated elevated blood homocysteine levels in both male and female patients with schizophrenia although antipsychotic medication might influence the outcome. The meta-analysis of the Japanese genetic association studies demonstrated a significant association between the *MTHFR* C677T polymorphism and schizophrenia. The mendelian randomization analysis in the Japanese populations yielded an OR of 1.15 for schizophrenia per 1-SD increase in plasma total homocysteine. Our study suggests that increased plasma total homocysteine levels may be associated with an increased risk of schizophrenia.

Key words: mendelian randomization/SNP/Japanese/plasma homocysteine

Introduction

Schizophrenia is a devastating psychiatric disorder with a median lifetime prevalence rate of 0.7%–0.8%.[1] Accumulating evidence has shown that alterations in 1-carbon metabolism might play an important role in the pathogenesis of schizophrenia.[2,3] A number of studies have been conducted to evaluate the association between blood homocysteine levels and schizophrenia. The majority of these studies have demonstrated elevated blood homocysteine levels in patients with schizophrenia compared with controls.[4–24] However, several studies have reported no significant diagnostic differences in the blood homocysteine levels between the 2 groups.[25–31]

To date, 1 study has examined an association between blood homocysteine levels and schizophrenia by conducting a meta-analysis of 8 case-control studies (a total number of 812 cases with schizophrenia and 2113 control subjects) and demonstrated that a 5 μmol/l increase in homocysteine concentration was associated with a higher risk of schizophrenia (OR = 1.7; 95% CI = 1.27–2.29).[32]

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However, this meta-analysis was performed without consideration of the effect of gender. Higher blood homocysteine levels in men than in women have been reported,[33] and the results of the previous association studies between blood homocysteine levels and schizophrenia with stratification by gender are inconclusive. In some studies, elevated blood homocysteine levels were observed in only male patients with schizophrenia and not in female patients,[5,6,10,19] whereas other studies have demonstrated that both male and female patients with schizophrenia had increased blood homocysteine levels.[11,17]

Blood homocysteine levels are also influenced by genetic variations.[34,35] Among these variants, 1 common functional single nucleotide polymorphism (SNP) of the methylenetetrahydrofolate reductase (*MTHFR*) gene, C677T (rs1801133), has been investigated well. The *MTHFR* C677T polymorphism results in amino acid substitution (Ala222Val) and causes a reduction of enzyme activity and higher homocysteine levels.[36] The results of the previous association studies between blood homocysteine levels and schizophrenia with stratification by C677T genotypes are inconclusive. A significant diagnostic difference in blood homocysteine levels has been found only in the subjects carrying the CT genotype or only in the subjects carrying the TT genotype.[7,18] However, Feng et al[16] showed a significant diagnostic difference for both CT and TT genotype carriers.

Many genetic case-control association studies between the *MTHFR* C677T polymorphism and schizophrenia have been performed in various populations, and the results of these association studies are not consistent. Only 1 study of the Japanese population reported a significant association between the *MTHFR* C677T polymorphism and schizophrenia, while the other 3 studies of the Japanese population have not replicated this positive finding.[37–40] However, several meta-analyses of association studies have revealed a significant association between this SNP and schizophrenia.[39,41–47]

In this study, we first investigated whether plasma total homocysteine levels were higher in patients with schizophrenia than in nonpsychiatric controls with stratification by gender and by the *MTHFR* C677T genotypes in a large cohort (N = 1379). Second, we conducted a meta-analysis of association studies between the blood homocysteine levels and schizophrenia separately by gender to evaluate a precise estimation of the association (N = 4714). Third, we performed a case-control association study between the *MTHFR* C677T polymorphism and schizophrenia (N = 4998) and carried out a meta-analysis of genetic association studies of this SNP with schizophrenia based on Japanese subjects to determine whether the *MTHFR* C677T polymorphism was genetically implicated in schizophrenia in the Japanese population (N = 10 378). Finally, we assessed the effect of plasma total homocysteine levels on schizophrenia by a mendelian randomization (MR) approach, a useful tool for assessing causal associations in observational data.[48,49]

Methods

Subjects of the Association Study Between the Plasma Total Homocysteine Levels and Schizophrenia

Three hundred and eighty-one patients with schizophrenia (225 men, mean age: 58.2 ± 9.3 y; 156 women, mean age: 59.4 ± 9.7 y) were recruited from Tokushima University Hospital in Japan. The diagnosis of schizophrenia was made according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria by at least 2 expert psychiatrists on the basis of extensive clinical interviews and a review of medical records. None of the patients had any psychiatric comorbidity or cardiovascular diseases. All patients were treated with various antipsychotic drugs. The mean chlorpromazine equivalent dose was 689.6 ± 581.3 mg/d. Nine hundred and ninety-eight control subjects (331 men, mean age: 38.3 ± 12 y; 667 women, mean age: 43.0 ± 11.9 y) were selected from volunteers who were recruited from hospital staff, students, and company employees documented to be free from psychiatric problems and past histories of mental illness. All subjects who participated in this study were of Japanese origin. All subjects signed written informed consent approved by the institutional ethics committees of the University of Tokushima Graduate School. Of 1379 subjects used in this association study, 1357 with genomic DNA (377 patients and 980 controls) were used in the next genetic association study.

Subjects of the Association Study Between the MTHFR C677T Polymorphism and Schizophrenia

Two case-control sets were used: the Tokushima sample set (A southern island of Japan) and the Osaka sample set (Midwestern Japan). Both sets have been described in previous studies.[50,51] For the Tokushima sample set, 1149 patients with schizophrenia (676 males, 473 females, mean age: 54.6 ± 14.9 y) were recruited from the Tokushima and Kochi University Hospitals in Japan. The diagnosis of schizophrenia was made according to DSM-IV criteria. A total of 2742 control subjects (1230 males, 1512 females, mean age: 38.8 ± 12.6 y) were selected from volunteers. For the Osaka sample set, 621 patients with schizophrenia (302 males, 319 females, mean age: 46.5 ± 15.8 y) were recruited from Osaka University Hospital in Japan. The diagnosis of schizophrenia was made according to DSM-IV criteria. A total of 486 control subjects (231 males, 255 females, mean age: 35.0 ± 12.7 y) were selected from volunteers. All subjects signed written informed consent approved by the institutional ethics committees of the University of Tokushima Graduate School, Kochi Medical School, and University of Osaka Graduate School.

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