patients even if they were treated with the drug at different doses. This means a small individual variation in drug metabolism, suggesting milnacipran's efficacy in the clinical setting.

The results of this study showed that milnacipran may be effective for the treatment of orofacial pain according to the significant decrease in the VAS score after treatment, as demonstrated by our previous study (Ito *et al.*, 2010). Patients with more severe painful symptoms appear to have a better pain-relieving effect of milnacipfan. However, when treating orofacial pain, that is, pain in the oral cavity, oral adverse effects such as dry mouth may be a major impediment to therapy. A meta-analysis that compared the antidepressive effect and safety of milnacipran, an SSRI, and a TCA (Montogomery *et al.*, 1996) showed that the incidence of dry mouth was much lower for milnacipran (7.9%) than for a TCA (37.3%) but higher than for an SSRI (3.8%). These results suggest that SSRIs may be more preferable than milnacipran in terms of adverse events associated with salivary secretion. On the other hand, no dry mouth was reported during this study, although this adverse event was reported in 8 (32%) of 30 patients with orofacial pain treated with amitriptyline (Ikawa *et al.*, 2006). The lower initial dose of milnacipran (15 mg/day) and subsequent gradual dose increase might have contributed to the prevention of dry mouth during the study treatment. A meta-analysis (Mico *et al.*, 2006) showed that the pain-relief effect of SNRIs were less significant than that of TCAs but superior to that of SSRIs. In addition, the incidence of dry mouth is about 5% for both milnacipran and SSRIs. Milnacipran is thus a reasonable option for the treatment of orofacial pain.

CONCLUSION

The results of this study suggest the therapeutic range of the plasma milnacipran level for its pain-relief effect after treatment with the drug for chronic orofacial pain, whereas no significant relationship was noted between the plasma milnacipran level and its antidepressive effect. The pain-relief effect of milnacipran treatment was favorable showing only a modest incidence of adverse events such as dry mouth. These findings suggest milnacipran's efficacy for the treatment of chronic orofacial pain in the clinical setting and may also help us understand the exact pathophysiology of BMS, AO, and other chronic pain. Further studies are necessary to investigate the relationship between the therapeutic response and individual predisposition to or other psychiatric/social factors of orofacial pain. We will continue clinical research in this field in a larger sample size.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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PLOS ONE

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

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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.

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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).

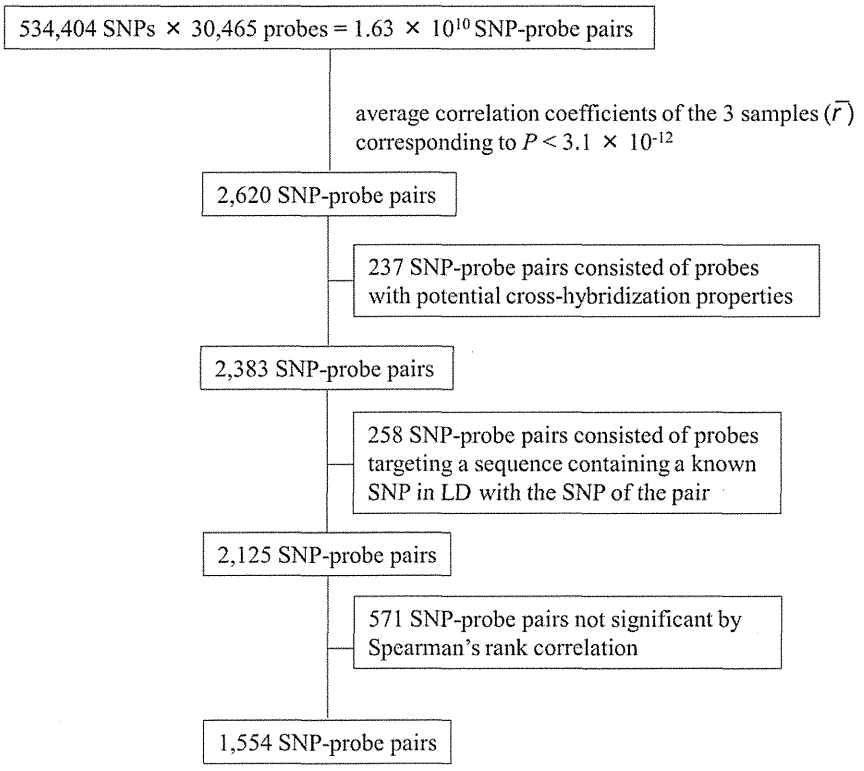

Figure 1. Procedure for selecting significant SNP-probe pairs. The procedure for selecting significant SNP-probe pairs is shown. SNP-probe pairs with a high likelihood of cross-hybridization and SNP-in-probe effects were excluded to exclude false positive results. The SNPs of the remaining 1,554 SNP-probe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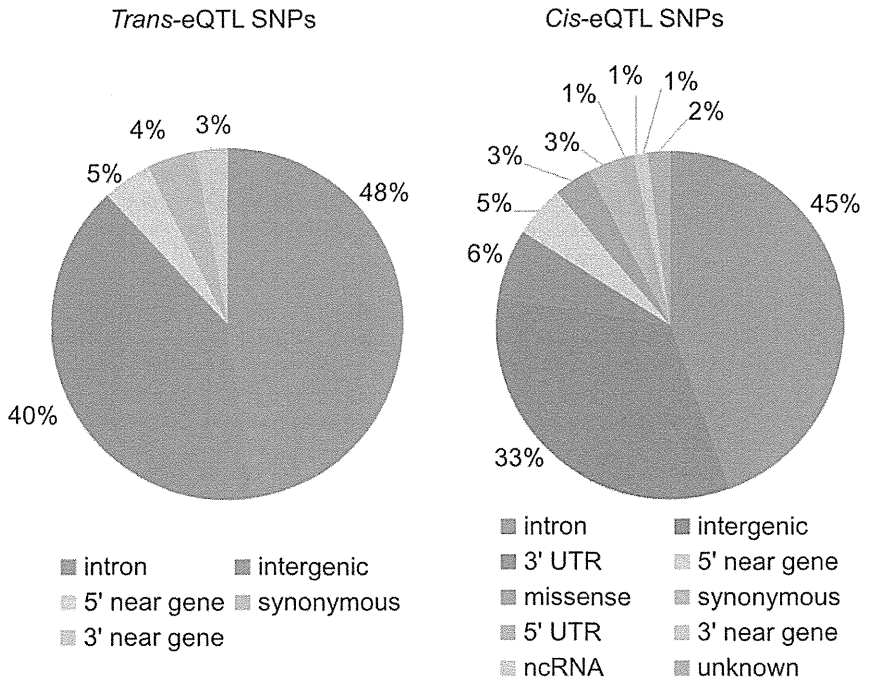

Figure 2. Functional types of the eQTL SNPs. The percentage of SNP types is shown for *cis*- and *trans*- eQTL SNPs.
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Since several of the probes targeted the same gene, the total number of genes identified was 107. As shown in Figure 2, the majority 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).
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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)

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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.

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Increased cerebrospinal fluid interleukin-6 levels in patients with schizophrenia and those with major depressive disorder

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ABSTRACT

Elevated peripheral levels of interleukin-6 (IL-6) are common findings in schizophrenia and depression. However, previous studies that measured cerebrospinal fluid (CSF) IL-6 levels in these disorders reported controversial results. The present study examined whether CSF IL-6 levels are altered in patients with schizophrenia and those with depression. Lumbar punctures were performed in 32 patients with schizophrenia, 30 with major depressive disorder (MDD), and 35 healthy controls. Serum samples were simultaneously collected from all subjects in the patient groups and from 32 of the control group. CSF and serum IL-6 levels were determined by enzyme-linked immunosorbent assay. Both the patients with schizophrenia and MDD had significantly higher CSF IL-6 levels compared to the controls (schizophrenia: $P = 0.0027$; MDD: $P = 0.012$). IL-6 levels were significantly higher in the CSF than in the serum. No significant correlation was observed between CSF and serum IL-6 levels. The present findings suggest that IL-6 of central origin is associated with the pathophysiology of schizophrenia and MDD, although confounding effect of smoking status can not be entirely excluded.

1. Introduction

Elevated serum or plasma levels of interleukin-6 (IL-6) are common findings in schizophrenia (Potvin et al., 2008; Sasayama et al., 2011) and depression (Howren et al., 2009; Liu et al., 2012). Although the source of the elevated blood IL-6 remains to be elucidated, such evidence suggests immune alterations in the peripheral tissues of these disorders.

IL-6 is not only synthesized in immune cells of the peripheral blood but is also produced in the central nervous system (CNS) by astrocytes and microglia. According to the recent microglia hypothesis of schizophrenia (Monji et al., 2009), activated microglia release pro-inflammatory cytokines and free radicals, causing neuronal degeneration, white matter abnormalities, and decreased neurogenesis associated with the pathophysiology of schizophrenia. In previous studies of patients with depression (Hamidi et al., 2004; Ongur et al., 1998), loss of glial elements in mood-relevant brain regions, such as amygdala and subgenual prefrontal cortex, has been observed. Such findings suggest that the effect of cytokines and central inflammatory processes on glia may play a role in the etiology of depression. These hypothetical models of immune pathophysiology underline the importance of the assessment of CNS levels of IL-6 in schizophrenia and depression. Some previous studies have shown that CSF IL-6 levels may not significantly correlate with peripheral IL-6 levels (Lindqvist et al., 2009; Stenlof et al., 2003). Therefore, measurement in the cerebrospinal fluid (CSF) is necessary for the direct assessment of CNS-derived IL-6.

A few studies have measured IL-6 levels in the CSF in patients with schizophrenia (Barak et al., 1995; Garver et al., 2003) and depression (Carpenter et al., 2004; Levine et al., 1999; Lindqvist et al., 2009; Martinez et al., 2012; Stubner et al., 1999). However, the findings are inconsistent across studies. Barak et al. (1995) reported no significant difference in CSF IL-6 levels between schizophrenic patients and healthy controls, while Garver et al. (2003) found significantly higher CSF IL-6 levels in a subtype of schizophrenia. As for depressed patients, CSF IL-6 levels were found to be decreased (Levine et al., 1999; Stubner et al., 1999), unaltered (Carpenter et al., 2004; Martinez et al., 2012), or elevated (Lindqvist

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et al., 2009) compared to healthy controls. However, findings among previous studies measuring CSF IL-6 levels in schizophrenia and depression should be interpreted with caution due to the small numbers of subjects.

1.1. Aims of the study

The aims of the present study were to examine whether CSF IL-6 levels were altered in patients with schizophrenia and those with depression. From the inflammatory hypotheses of these disorders (Maes, 2011; Miller et al., 2009; Monji et al., 2009), we hypothesized that the central IL-6 levels would be increased in the patient groups compared to the healthy controls.

2. Material and methods

2.1. Subjects

Lumbar punctures were performed in 32 patients with schizophrenia, 30 patients with major depressive disorder (MDD), and 35 healthy controls. The mean age and sex ratio were matched across the three groups. Most subjects of the patient groups were on antipsychotic and/or antidepressant treatment. Simultaneously with the lumbar punctures, serum samples were also collected from all subjects in the patient groups and from 32 of the control group. Table 1 shows the demographic and clinical characteristics of the participants. All subjects were biologically unrelated Japanese who were recruited from the outpatient clinic of the National Center Hospital, National Center of Neurology and Psychiatry, Kodaira, 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, if they met the criteria for substance abuse or dependence, or mental retardation, if they were currently taking anti-inflammatory medication, or if they suffered from any inflammatory, infectious, or systemic immune diseases, based on self-reports, at the time of assessment. 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. Laboratory methods

CSF was drawn between 1000 h and 1600 h from the L4–L5 or L3–L4 interspace, with the subject in the left decubitus position. The samples were immediately transferred on ice, centrifuged at 4000 × *g*, aliquoted and stored at −80 °C until they were assayed. Serum samples were collected immediately before the lumbar punctures. All the samples were collected during the period of 2010–2011. CSF and serum levels of IL-6 were determined by a commercially available immunoassay kit (Quantikine, R&D systems, Inc., Minneapolis) according to manufacturer's instructions. The mean minimum detectable dose of the kit was 0.039 pg/ml. The within and between-run coefficients of variance of the assay were less than 10%.

2.3. Clinical measures

Schizophrenic symptoms and depressive symptoms 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) and the Japanese version of the GRID Hamilton Depression Rating Scale, 21-item version (HAMD-21) (Hamilton, 1967), which have both been demonstrated to show good inter-rater reliability (Igarashi et al., 1998; Tabuse et al., 2007). Daily doses of antipsychotics in patients with schizophrenia and antidepressants in patients with MDD were converted to chlorpromazine and imipramine equivalent doses, respectively, using published guidelines (Inagaki et al., 1999).

2.4. Statistical analysis

Difference in gender distribution between groups was analyzed by χ^2 analysis. Clinical characteristics between groups were compared using analysis of variance. Because CSF and serum IL-6 levels were not normally distributed, difference between diagnostic groups was assessed using Kruskal–Wallis test, and thereafter pairwise Mann–Whitney *U* tests for *post hoc* comparisons. Relationship between IL-6 levels and clinical measures were assessed using Spearman's rank correlation coefficients (ρ). Serum and CSF samples were compared using Spearman's rank correlation and Wilcoxon's signed rank test. All statistical tests were two tailed and statistical significance was considered when $P < 0.05$. Bonferroni correction was applied for the *post hoc* pairwise Mann–Whitney *U* tests between the three diagnostic groups

Table 1
Demographic and clinical characteristics.

	Controls ($N = 35$)	Schizophrenia ($N = 32$)	MDD ($N = 30$)	Analysis
Age [years]	41.3 (16.4)	40.8 (8.8)	42.7 (8.2)	$F = 0.21$, $P = 0.81$
Gender [M/F]	21/14	20/12	19/11	$\chi^2 = 0.08$, $P = 0.96$
Age at onset [years]		25.0 (8.0)	33.6 (13.3)	
Illness duration [years]		16.2 (7.9)	8.8 (8.9)	
BMI	23.4 (4.0)	24.2 (5.1)	23.1 (4.3)	$F = 0.47$, $P = 0.63$
%Smokers	11.4	50.0	46.7	$\chi^2 = 13.5$, $P < 0.01$
CP equivalent dose [mg/day]	0.0 (0.0)	803.5 (583.0)	83.7 (175.2)	$F = 50.2$, $P < 0.01$
IMI equivalent dose [mg/day]	0.0 (0.0)	15.6 (48.7)	164.3 (128.6)	$F = 43.7$, $P < 0.01$
PANSS positive scores		13.2 (5.1)		
PANSS negative scores		14.5 (5.5)		
HAMD-21 scores			13.3 (9.8)	
Time of day of sampling [h]	1340 (0139)	1327 (0129)	1309 (0141)	$F = 0.42$, $P = 0.66$
Number of days between sample collection and IL-6 assay	308 (140)	292 (144)	293 (150)	$F = 0.13$, $P = 0.88$

Values are shown as mean (standard deviation).
MDD: major depressive disorder; BMI: body mass index; CP: chlorpromazine; IMI: imipramine; PANSS: Positive and Negative Syndrome Scale; HAMD-21: 21 item Hamilton Rating Scale for Depression.

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(significance criteria of $P < 0.017$). Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

As shown in Table 1, no significant difference was found between diagnostic groups in mean age, gender distribution, or body mass index (BMI). The prevalence of smoking was higher in the patients groups compared to controls. Fig. 1 shows the CSF and serum IL-6 levels in each diagnostic group. All samples analyzed were well above the lower detection limit of 0.039 pg/ml. The difference in serum IL-6 levels between the groups was not statistically significant ($\chi^2 = 1.8$, d$f = 2$, $P = 0.40$); however, CSF IL-6 levels differed significantly across the groups ($\chi^2 = 10.7$, d$f = 2$, $P = 0.0049$). *Post hoc* pairwise Mann–Whitney-*U* test showed that both the patients with schizophrenia and MDD had significantly higher CSF IL-6 levels compared to the controls (schizophrenia: $U = 321$, $P = 0.0027$; MDD: $U = 334$, $P = 0.012$).

No significant correlation between CSF and serum IL-6 levels was observed for each diagnostic group. Spearman's rank correlation coefficients and the 95% confidence intervals (95% CI) were as follows: controls, $\rho = 0.18$ (95% CI: −0.18–0.55); schizophrenia, $\rho = 0.23$ (−0.13–0.59); MDD, $\rho = 0.19$ (−0.18–0.57); and all groups combined, $\rho = 0.20$ (−0.006–0.41). IL-6 levels were significantly higher in the CSF than in the serum ($Z = 4.04$, $P < 0.0001$). When analyzed separately in each diagnostic group, the difference between CSF and serum IL-6 levels reached statistical significance in only patients with schizophrenia (schizophrenia: $Z = 3.54$, $P = 0.0004$; MDD: $Z = 1.74$, $P = 0.082$; controls: $Z = 1.82$, $P = 0.068$).

Next, we examined the influence of clinical factors on CSF IL-6 levels (Table 2). CSF IL-6 levels of the schizophrenic patients did not significantly correlate with the antipsychotic dose ($\rho = 0.12$, $P > 0.1$) or with the PANSS scores (positive symptoms: $\rho = 0.065$, $P > 0.1$; negative symptoms: $\rho = 0.12$, $P > 0.1$). Similarly, CSF IL-6 levels of the patients with MDD did not significantly correlate with the antidepressant dose ($\rho = 0.044$, $P > 0.1$) or with the HAMD-21 score ($\rho = -0.036$, $P > 0.1$). Because smoking prevalence was significantly different between controls and patient groups, we also compared CSF IL-6 levels in only nonsmokers to avoid the confounding effects of smoking. When only nonsmokers were compared, patients with schizophrenia had significantly higher CSF IL-6 levels compared to the controls ($U = 158$, $P = 0.04$), but the difference between MDD patients and controls did not reach statistical significance ($U = 194$, $P = 0.22$). No significant correlation with CSF IL-6 levels was observed for time of day of sampling or number of days between sample collection and IL-6 assay. Furthermore, no significant difference in CSF IL-6 levels of those sampled before and after noon was observed for each diagnostic group.

4. Discussion

The results showed that CSF IL-6 levels were higher in patients with schizophrenia and those with MDD than in healthy controls. The present findings further support the evidence for the role of IL-6 in the pathogenesis of these disorders. No significant increase in serum IL-6 levels of patients with schizophrenia or MDD was obtained. However, this does not contradict with previous findings, because the effect size reported in previous meta-analyses (Howren et al., 2009; Potvin et al., 2008) requires a sample more than twice as large as ours to reach 80% power to detect the difference at the

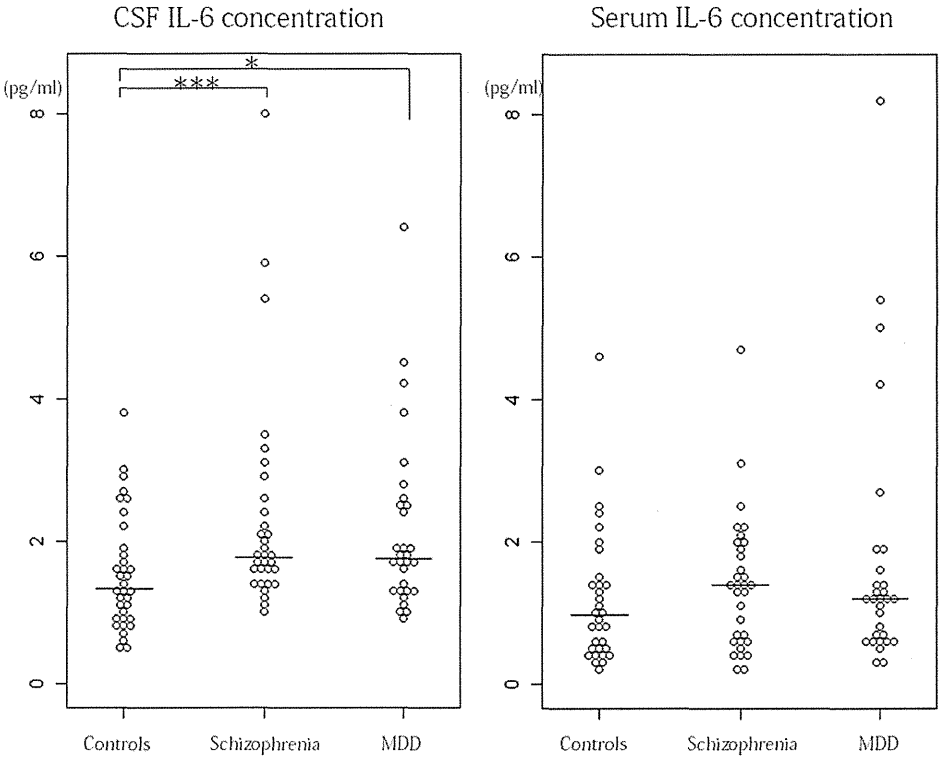

Fig. 1. CSF and serum IL-6 levels in patients with schizophrenia, those with major depressive disorder, and healthy controls CSF IL-6 levels of both the patients with schizophrenia and those with MDD were significantly higher compared to that of the healthy controls. The horizontal lines indicate the median value of each group. *$P < 0.05$, ***$P < 0.005$ (Mann–Whitney *U* test) n.s.: no significant difference; MDD: major depressive disorder; CSF: cerebrospinal fluid; IL-6: interleukin-6.

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Table 2
Association between cerebrospinal fluid IL-6 levels and clinical factors.

		Controls	Schizophrenia	MDD
Spearman's correlation coefficients between CSF IL-6 levels and clinical factors				
Age [years]		$\rho = 0.18$	$\rho = 0.36$[a]	$\rho = 0.062$
Age at onset [years]			$\rho = 0.41$[a]	$\rho = -0.057$
Illness duration [years]			$\rho = 0.079$	$\rho = 0.067$
BMI		$\rho = 0.36$[a]	$\rho = 0.27$	$\rho = 0.11$
CP equivalent dose [mg/day]			$\rho = 0.12$	$\rho = -0.28$
IMI equivalent dose [mg/day]			$\rho = 0.12$	$\rho = 0.044$
PANSS positive scores			$\rho = 0.065$	
PANSS negative scores			$\rho = 0.12$	
HAMD-21 scores				$\rho = -0.036$
Time of day of sampling [h]		$\rho = 0.088$	$\rho = 0.023$	$\rho = -0.11$
Number of days between sample collection and IL-6 assay		$\rho = -0.23$	$\rho = 0.066$	$\rho = -0.17$
Mean (standard deviation) CSF IL-6 levels [pg/ml]				
Gender	Men	1.70 (0.78)	2.57 (1.61)	2.37 (1.37)
	Women	1.30 (0.78)	1.92 (1.29)	1.75 (0.83)
Smoking status	Smokers	1.44 (0.80)	2.06 (0.68)	2.60 (1.48)
	Nonsmokers	1.55 (0.81)	2.60 (2.03)	1.74 (0.80)

MDD: major depressive disorder; CSF: cerebrospinal fluid; BMI: body mass index; CP: chlorpromazine; IMI: imipramine; PANSS: Positive and Negative Syndrome Scale; HAMD-21: 21 item Hamilton Rating Scale for Depression.
[a] $P < 0.05$.

5% significance level (calculated by G*Power 3.1.3 (Faul et al., 2007)). It is of note that significant difference in CSF IL-6 levels was obtained with the present sample, suggesting that the effect size may be larger for CSF than for serum.

No significant correlation was observed between CSF and serum IL-6 levels. Although there is a possibility that a larger sample may yield a significant correlation, the correlation coefficient is likely to be lower than the upper limit of the 95% confidence interval (i.e. $\rho = 0.41$) obtained in the present study. Furthermore, IL-6 levels were higher in the CSF compared to the serum, especially for schizophrenic patients. Thus, the increased CSF IL-6 levels in patients with schizophrenia and MDD are unlikely to be explained by the diffusion from the peripheral circulation. These findings suggest that IL-6 of central origin is associated with the pathophysiology of these disorders.

Increased CSF IL-6 levels in both patients with schizophrenia and those with MDD suggest that inflammatory mediators may be commonly involved in the pathogenesis of these disorders. Although a plethora of studies examining peripheral cytokine levels also support the hypothesis that inflammation plays a role in these disorders, a unique cytokine profile capable of distinguishing these two disorders has not been described. There is a possibility that common underlying pathogenic mechanisms may be involved in schizophrenia and MDD.

A number of studies indicate involvement of abnormal neurogenesis in the pathophysiology of MDD (Leonard and Maes, 2012) as well as schizophrenia (Balu and Coyle, 2011). Monje et al. (2003) have shown that inflammation can inhibit neurogenesis and that IL-6 is implicated as a potential regulator of hippocampal neurogenesis in neuroinflammation. Therefore, increased microglial production of IL-6 may be a common etiological risk factor for schizophrenia and MDD. Another common potential etiological factor of these two disorders may be the changes in kynurenine metabolism. The increased kynurenine induces increased production of kynurenic acid in schizophrenia and quinolinic acid in depression, which may result in an imbalance in glutamatergic neurotransmission. Raison et al. (2010) have shown that the changes in kynurenine metabolism are linked to central cytokine responses. Thus, the increased central IL-6 observed in the present study is in line with the role of kynurenine pathway on the pathophysiology of schizophrenia (Muller et al., 2011) and MDD (Myint et al., 2007, 2012).

Not all individuals with depression or schizophrenia exhibit high levels of CSF IL-6 levels. Therefore, it is likely that inflammation is involved in the pathogenesis of a subgroup of patients. We could not identify any major clinical features specific to those with high CSF IL-6 levels. The positive correlation observed between CSF IL-6 levels and age at onset in patients with schizophrenia suggests that inflammatory mechanism may be more likely to be associated with late-onset schizophrenia; however, the sample size was too small to draw definitive conclusion regarding the association with particular clinical features.

Some previous studies failed to find significant change of CSF IL-6 levels in patients with schizophrenia (Barak et al., 1995) or those with MDD (Carpenter et al., 2004; Martinez et al., 2012). Because the sample sizes were smaller than that in the present study, insufficient statistical power may have precluded detection of statistically significant differences in these studies. Some other studies have yielded results consistent with the present findings. Garver et al. (2003) reported increased CSF IL-6 levels in schizophrenic patients who subsequently responded to antipsychotic treatment. Lindqvist et al. (2009) reported that CSF IL-6 levels in patients with MDD after a suicide attempt were higher compared to healthy controls. In contrast to our findings, one previous study of patients with geriatric depression (Stubner et al., 1999) and another of patients with acute severe depression (Levine et al., 1999) have shown that CSF IL-6 levels were lower in depressed subjects compared to controls. Since the majority of the patients in our study were middle-aged and were in the chronic stage of illness, the influence of the patients' age and the illness stage may have resulted in a different outcome. Further studies are necessary to clarify how the clinical characteristics of the disease affect IL-6 levels.

The major limitation of the present study was the uncontrolled medication. The results showed that neither the chlorpromazine equivalent dose in schizophrenic patients nor the imipramine equivalent dose in MDD patients significantly correlated with CSF IL-6 levels. However, the effects of medication could not be adequately assessed due to the variability in types and doses. Evidence shows that both antipsychotic and antidepressant treatment decrease peripheral IL-6 levels (Hiles et al., 2012; Miller et al., 2011). If similar effects occur in the CSF, the increase in CSF IL-6 levels would be more prominent in untreated patients than observed in the medicated patients in the present study. The present study provides evidence that IL-6 levels of central origin may be increased in patients receiving treatment in the real-world setting. However, the possible confounding effects of medications must be addressed in future studies including medication-free patients. Different smoking prevalence between patients and controls may

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have also influenced the findings of the present study. The results of comparison in only non-smoking subjects indicate that CSF IL-6 levels are increased in patients with schizophrenia regardless of the smoking status. However, a larger number of non-smoking subjects are needed to confirm the results for MDD patients. Another limitation was that the time of day of sampling was not consistent across subjects. Although no significant association was found between CSF IL-6 levels and the sampling time of day, larger sample size is necessary to clarify the influence of sampling time on IL-6 levels. However, because the average time of day of sampling was similar between diagnostic groups, it is unlikely that the sampling time of day had a major impact on the overall results of the present study. Finally, the cross-sectional design of the study did not allow determination of whether the increased IL-6 levels preceded or resulted from illness onset. The lack of significant correlation with PANSS or HAMD-21 scores suggests that IL-6 levels are not greatly influenced by the severity of the symptoms. However, further studies with a longitudinal design are required to investigate how CSF IL-6 levels change during the course of the disease.

In conclusion, CSF IL-6 levels were significantly increased in patients with schizophrenia and those with MDD. No significant correlation was observed between CSF and serum IL-6 levels. The present findings suggest that IL-6 of central origin is associated with the pathophysiology of these disorders.

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Contributors

D.S., K.H., C.W., and H.K. designed the study and D.S. wrote the draft of the manuscript. D.S., H.H., T.T., K.H., M.O., S.Y., 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. D.S., K.H., H.H., T.T., and M.O. collected plasma and cerebrospinal fluid samples. D.S. measured the IL-6 levels and 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.

Conflict of interest

The authors report no conflicts of interest.

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Research report

Relationship of temperament and character with cortisol reactivity to the combined dexamethasone/CRH test in depressed outpatients

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ABSTRACT

Background: Evidence shows that depression is associated with hypothalamic–pituitary–adrenal (HPA) axis hyperactivation, although such findings are not entirely unequivocal. In contrast, various psychiatric conditions, including atypical depression, are associated with hypocortisolism. Another line of research has demonstrated that personality is associated with HPA axis alteration. It is thus hypothesized that different personality pathology in depression would be associated with distinct cortisol reactivity.

Methods: Eighty-seven outpatients with DSM-IV major depressive disorder were recruited. Personality was assessed by the temperament and character inventory (TCI). HPA axis reactivity was measured by the combined dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test. According to our previous studies, two subgroups were considered based on their cortisol responses to the DEX/CRH test: incomplete-suppressors whose cortisol response was exaggerated and enhanced-suppressors whose cortisol response was blunted.

Results: The analysis of covariance, controlling for age, gender and symptom severity, revealed that incomplete-suppressors scored significantly higher on cooperativeness than enhanced-suppressors ($p=0.002$). A multivariate stepwise logistic regression analysis predicting the cortisol suppression pattern from the seven TCI dimensions, controlling for age, gender and symptom severity, revealed that lower cooperativeness ($p=0.001$) and higher reward dependence ($p=0.018$) were significant predictors toward enhanced suppression.

Limitations: The neuroendocrine challenge test was administered only once, based on a simple test protocol.

Conclusions: Our findings suggest that (personality-related) subtypes of depression might be differentiated based on the different pattern of cortisol reactivity. Future studies are warranted to further characterize the HPA axis alteration in relation to various subtypes of depression.

1. Introduction

Depression imposes a great burden on afflicted individuals and society, while its pathophysiology remains elusive. One of the repeatedly reported biological abnormalities in depression is the alteration in the hypothalamic–pituitary–adrenal (HPA) axis function (Holsboer, 2000; Kunugi et al., 2010). To quantify the dysregulation of HPA axis, the dexamethasone suppression test (DST) has been enthusiastically studied since Carroll et al. (1981) introduced it as a biological marker for the diagnosis of "melancholia". In serial DST studies, cortisol levels were shown to be increased in depressed patients (e.g., Carroll, 1982). However, it has subsequently become clear that its sensitivity to differentiate depressed patients from healthy controls is not very high (Arana et al., 1985; Braddock, 1986), and elevated cortisol levels were also observed in non-clinical populations under various stressful conditions (Ceulemans et al., 1985; Mellsop et al., 1985). The DST thus failed to fulfill the initial promise as a diagnostic tool for depression.

The dexamethasone (DEX)/corticotropin-releasing hormone (CRH) test, which was developed by Holsboer et al. (1987), Heuser et al. (1994a) in an attempt to enhance the sensitivity of

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the DST, is an integrated challenge test for HPA axis function that combines DEX-pretreatment with CRH administration on the following day; thus, it is essentially a DST followed by CRH challenge. The merit of this combined test is that at the moment of CRH infusion the HPA axis is downregulated due to negative feedback induced by the DEX. A number of independent studies have confirmed that sensitivity of the DEX/CRH test for depression is relatively high (Kunugi et al., 2004; Kunugi et al., 2006; Watson et al., 2006; Ising et al., 2007). However, such findings are not necessarily conclusive because other studies have reported rather low sensitivity of this test for depression, i.e., no more than around 20–30% (e.g., Ising et al., 2005; Nickel et al., 2003; Schüle et al., 2009). Moreover, recent studies using this test have shown that depressed patients exhibit similar (Oshima et al., 2000; Watson et al., 2002; Gervasoni et al., 2004; Van Den Eede et al., 2006), or even attenuated (Rydmark et al., 2006; Veen et al., 2009; Wahlberg et al., 2009) cortisol responses as compared to healthy controls. In these studies, patients had either of the following characteristics: outpatients (Oshima et al., 2000; Gervasoni et al., 2004; Van Den Eede et al., 2006; Carpenter et al., 2009), chronically depressed patients (Watson et al., 2002), depressed patients with psychiatric comorbidity (Veen et al., 2009) or long-term sick-leave patients (Rydmark et al., 2006; Wahlberg et al., 2009). Inconsistent findings across the DEX/CRH studies may therefore result from the heterogeneity of depression, rather than from the limited sensitivity of this neuroendocrine challenge test. A similar interpretation has also been proposed for the original DST (e.g., Fink, 2005).

A promising marker for such phenotypic heterogeneity of depression would be personality traits, given that personality profile of depressed patients is different from that of healthy controls (Enns and Cox, 1997; Bagby et al., 2008) and such profile varies even within depressed patients depending on diagnostic (sub)categories, e.g., melancholic vs. atypical depression (Joyce et al., 2004; Chopra et al., 2005) and bipolar vs. unipolar depression (Bagby et al., 1996; Mendlowicz et al., 2005; Akiskal et al., 2006; Sasayama et al., 2011).

Apart from depression, several lines of research have demonstrated that personality impacts on HPA axis function as measured by the DEX/CRH test. In a non-clinical population, Tyrka and her colleagues have found that low novelty seeking of the Cloninger's temperament dimension (Cloninger et al., 1991), particularly when combined with high harm avoidance, is associated with exaggerated cortisol responses to the DEX/CRH test (Tyrka et al., 2006, 2008). Using another well-established measure of personality, McCleery and Goodwin (2001) observed a relationship between higher neuroticism and blunted cortisol response to this pharmacological challenge test, whereas Zobel et al. (2004) found the opposite relation, i.e., higher neuroticism and greater cortisol response. More specific personality characteristics have also been examined in relation to HPA axis reactivity as measured by the DEX/CRH test. For instance, we reported that non-clinical schizotypal personality to be associated with blunted cortisol response to this test (Hori et al., 2011a). Furthermore, Rinne et al. (2002) observed exaggerated cortisol responses to the DEX/CRH test in female subjects with borderline personality disorder who had a history of sustained childhood abuse. These findings not only suggest that the DEX/CRH test can serve as a useful tool to probe the altered HPA axis function in relation to a wide variety of personality traits but also point to the importance of taking into account blunted cortisol reactivity as well as exaggerated reactivity. Indeed, it is now widely recognized that hypocortisolism, in addition to hypercortisolism, represents impaired HPA axis regulation (Raison and Miller, 2003), which is reflected by the fact that the former has been associated with various stress-related psychopathologies including posttraumatic stress disorder, fibromyalgia, chronic fatigue syndrome and atypical depression (Heim et al., 2000; Gold and Chrousos, 2002; Fries et al., 2005).

In this context, the present study aimed to explore the relationship between personality traits as assessed by the temperament and character inventory (TCI) (Cloninger et al., 1993) and cortisol reactivity to the DEX/CRH test in depressed outpatients. To this end, we first dimensionally examined this relationship and then compared the personality traits between patients who exhibited exaggerated cortisol reactivity and those who did blunted reactivity. We hypothesized that these two extreme ends of cortisol reactivity would be associated with different temperament/character traits.

2. Methods

2.1. Participants

Eighty-seven depressed outpatients (age range: 21–69; 46 women) were recruited from the outpatient clinic of the National Center of Neurology and Psychiatry (NCNP) Hospital, Tokyo, Japan, or through advertisements in free local magazines and our website announcement. Most of the patients recruited via advertisements or website announcement were regularly attending to a nearby hospital or clinic located in the same geographical area, i.e., the western part of Tokyo. Consensus diagnoses were made based on clinical interviews, observations and case notes by at least two experienced psychiatrists. For those patients under treatment at the NCNP Hospital, the diagnosis was confirmed using the Structured Clinical Interview for DSM-IV Axis I disorders (First et al., 1997). For the remaining patients under treatment at a nearby hospital/clinic, the diagnosis made by his/her attending doctor was confirmed by the Mini-International Neuropsychiatric Interview (Sheehan et al., 1998; Otsubo et al., 2005) by a trained research psychiatrist. All met the DSM-IV criteria (American Psychiatric Association, 1994) for major depressive disorder (MDD). Patients who were in remission, as defined by the total score on the Hamilton Depression Rating Scale 21-item version (HAMD-21) (Hamilton, 1967) of less than 8, were excluded from the study. Of the total 87 MDD patients, 13 were diagnosed as having comorbid dysthymic disorder. Patients with bipolar disorders were not enrolled as they are shown to have a different personality profile from that of MDD patients (Bagby et al., 1996; Mendlowicz et al., 2005; Akiskal et al., 2006; Sasayama et al., 2011). Patients who were taking carbamazepine were also excluded from the study since it induces dexamethasone metabolism (Privitera et al., 1982). Additional exclusion criteria for study participation were as follows: having a prior medical history of central nervous system disease or severe head injury, having a history of substance abuse/dependence, taking corticosteroids, antihypertensives or oral contraceptives, and being on hormone replacement therapy. The present experiment on our participants was conducted in accordance with the Declaration of Helsinki. After the nature of the study procedures had been fully explained, written informed consent was obtained from all participants. The study was approved by the ethics committee of the NCNP, Tokyo, Japan.

2.2. DEX/CRH test procedure and presentation for neuroendocrine data

The DEX/CRH test was administered to all participants according to a simple test protocol (Kunugi et al., 2006), which was modified from the original protocol of Heuser et al. (1994a). This simple protocol was described in our recent reports (Hori et al., 2010, 2011a, b). Briefly, participants took 1.5 mg of DEX (Banyu Pharmaceutical Corporation, Tokyo, Japan) orally at 2300 h.

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On the next day, a vein was cannulated at 1430 h to collect blood at 1500 h and 1600 h. Human CRH (100 μg) (hCRH 'Mitsubishi', Mitsubishi Pharma Corporation, Tokyo, Japan) was administered intravenously at 1500 h, immediately after the first blood collection. Plasma concentrations of cortisol were measured by radioimmunoassay at SRL Corporation (Tokyo, Japan). The detection limit for cortisol was 27.59 nmol/l (=1.0 μg/dl). Cortisol values under the detection limit were treated as 0 nmol/l. Outcome measures of this neuroendocrine test were "DST-Cort" (i.e., the concentration of cortisol [nmol/l] at 1500 h) and "DEX/CRH-Cort" (i.e., the concentration of cortisol at 1600 h). To dissect the extent to which the subject's HPA axis responded to the CRH challenge, the magnitude of change from DST-Cort to DEX/CRH-Cort, namely ΔCort, was calculated for each subject.

As our hypotheses was that the two extreme ends of cortisol values (i.e., both exaggerated and blunted cortisol reactivity) would be each related to unique personality profiles, in the main analysis we adopted a categorical division of participants according to a priori defined cut-off values of cortisol. Considering the marked gender difference in cortisol reactivity to the DEX/CRH challenge, the cut-off values were separately defined for men and women, using our database of healthy controls. Recruitment methods for these control subjects were described previously (Hori et al., 2010). For men, "incomplete-suppressors" were defined as those individuals whose DEX/CRH-Cort level was equal to or more than 137.95 nmol/l (=5.0 μg/dl) and "enhanced-suppressors" were defined as those individuals whose DEX/CRH-Cort level was less than 27.59 nmol/l (=1.0 μg/dl), based on our previous studies (Hori et al., 2010, 2011a, b). These cut-off values resulted in 21.3% incomplete-suppressors and 19.7% enhanced-suppressors out of the total sample of healthy men. We then applied these percentages for incomplete- and enhanced-suppressors in healthy men to the cortisol data of healthy women in order to make the percentages identical between men and women. Consequently, cortisol levels to define incomplete- and enhanced-suppressors in women were "equal to or more than 273.14 nmol/l (=9.9 μg/dl)" and "less than 49.66 nmol/l (=1.8 μg/dl)", respectively, and these cut-off values yielded 21.0% and 19.6% healthy women who fell into the respective suppressor categories. Finally, these cut-off values separately defined for healthy men and women were applied to the present sample of 87 MDD patients, which yielded 11 incomplete- and 11 enhanced-suppressors in men and 13 incomplete- and 8 enhanced-suppressors in women. The remaining patients were considered to be "moderate-suppressors" and excluded from the categorical analyses comparing incomplete- vs. enhanced-suppressors.

2.3. Personality assessment

Personality was assessed in all subjects using the TCI. The TCI (Cloninger et al., 1993) is a 240-item (including 14 items which are not analyzed) self-report questionnaire; each item requires a true/false answer. The term temperament refers to automatic emotional reactions to subjective experiences that may be genetically transmitted and therefore stable over time. Four dimensions of temperament are distinguished: novelty seeking, harm avoidance, reward dependence, and persistence. Novelty seeking, harm avoidance, and reward dependence have been assumed to relate to dopaminergic, serotonergic, and noradrenergic neurotransmission, respectively (Cloninger, 1987). The term character refers to concepts pertaining to the individual, focusing on personal differences in intentions, decisions and values. Three dimensions of character are distinguished: self-directedness, cooperativeness, and self-transcendence. The reliability and validity of the original American version of the TCI in general community dwellers as well as in psychiatric patients have been established (Cloninger et al., 1993; Svrakic et al., 1993). The Japanese version of the TCI translated and validated by Kijima et al. (1996, 2000) was used in the present study.

2.4. Symptom assessment

Severity of the depressive symptoms was assessed with the HAMD-21 interview at the time of the neuroendocrine testing. In addition to the total score of the HAMD-21, we used the following four factors that had been identified in a previous study where a factor analytic technique (using the principal axis factoring method with oblique rotation) had been applied to the scores on the 17-item version of HAMD in depressed outpatients (Pancheri et al., 2002), in order to further examine the possible association between different symptom dimensions and cortisol reactivity. The four factors were "somatic anxiety" (consisting of early insomnia, middle insomnia, late insomnia, somatic anxiety, general somatic symptoms, and hypochondria), "psychic anxiety" (guilt, agitation, psychic anxiety, and insight), "core depressive symptoms" (depressed mood, work and interests, and retardation), and "anorexia" (gastrointestinal symptoms) (Pancheri et al., 2002).

Subjectively perceived symptoms during one week preceding the neuroendocrine test was assessed via the Hopkins Symptom Checklist (HSCL, Derogatis et al., 1974). The HSCL is a self-report questionnaire consisting of 58 (or 54) items which are scored on five underlying symptom dimensions, i.e., somatization, obsessive-compulsive, interpersonal sensitivity, anxiety, and depression symptoms. A validated Japanese version of the HSCL comprising 54 items (Nakano, 2005) was used in the present study. In this questionnaire, subjects were instructed to rate each item based on the distress perceived during the previous week, using a four-point scale of frequency, with "not-at-all" being scored 1, "occasionally", 2, "sometimes", 3, and "frequently", 4.

2.5. Statistical analyses

Averages are reported as means ± standard deviation (SD). Non-parametric tests were used to examine the association of DST-Cort with other variables, given that this cortisol index fell under the detection limit in a substantial portion of subjects and thus the data did not satisfy the assumptions for parametrical testing. In contrast, DEX/CRH-Cort and ΔCort were examined using parametric tests. The *t*-test or Mann–Whitney *U*-test was used to examine differences between two groups. Categorical variables were compared using the χ^2 test or Fisher's exact test where appropriate. Partial correlation analysis, controlling for confounders that were defined below, was performed to examine correlations of DEX/CRH-Cort and ΔCort with other variables, while Spearman's ρ was used to examine correlations for DST-Cort. The analysis of covariance (ANCOVA) was performed to examine differences between groups, controlling for confounders. Post-hoc pairwise comparisons were made with Bonferroni correction, when applicable. Since age, gender and depressive symptoms have been reported to significantly influence cortisol levels (e.g., Heuser et al., 1994b; Künzel et al., 2003; Kunugi et al., 2006) and TCI scores (e.g., Brändström et al., 2001; Miettunen et al., 2007; Spittlehouse et al., 2010), these three variables were considered as potential confounders regardless of the present data.

A multivariate forward stepwise logistic regression model based on the likelihood ratio test, with inclusion and exclusion p value thresholds of 0.05 and 0.1, respectively, was used to test the effects of TCI results, in addition to age, gender and HAMD-21 total score, on the DEX/CRH suppression pattern. Nagelkerke R^2 was used to estimate the proportion of explained variance in the

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model. The Hosmer–Lemeshow goodness-of-fit test was used to evaluate the fit of the logistic model to our data, with p value greater than 0.05 indicating an acceptable fit.

Statistical significance was set at two-tailed $p < 0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS Japan, Tokyo).

3. Results

3.1. Demographic/clinical characteristics of patients

Age was not significantly correlated with any of the three cortisol indices (all $p > 0.1$). Females showed significantly higher DEX/CRH-Cort ($t=2.51$, df=85, $p=0.014$) and ΔCort ($t=2.44$, df=85, $p=0.017$), but not DST-Cort (Mann–Whitney $U=901.5$, $p=0.71$), than males.

Demographic and clinical characteristics of patients stratified by the suppression status (i.e., incomplete- vs. enhanced-suppressors) are shown in Table 1. No significant differences were seen between incomplete- and enhanced-suppressors in any of the variables examined. The mean HAMD-21 total score was around 15, indicating that the subjects were mildly to moderately depressed (Table 1).

3.2. Relationships between symptoms and DEX/CRH test results

DST-Cort was not significantly correlated with depressive symptoms as indexed by the HAMD-21 total score and four factor scores (all $p > 0.2$ by Spearman's ρ) or with distress scores as indicated by the five HSCL dimensions (all $p > 0.4$ by Spearman's ρ). Similarly, DEX/CRH-Cort and ΔCort were not significantly correlated with depressive symptoms as indexed by the HAMD-21 total score and four factor scores (all $p > 0.3$ by partial correlation analysis controlling for age and gender) or with distress scores as indicated by the five HSCL dimensions (all $p > 0.1$ by partial correlation analysis controlling for age and gender).

As shown in Table 1, no significant differences were observed between incomplete- vs. enhanced-suppressors in any of the symptom dimensions as assessed by the HAM-D and the HSCL, using the ANCOVA controlling for age and gender.

3.3. Relationships between TCI scores and DEX/CRH test results

Results of the three cortisol indices for the three suppressor groups, stratified by gender, are presented in Table 2.

3.3.1. Correlations between TCI scores and cortisol measures among the patients

No significant correlations were observed between the seven TCI dimensions and DST-Cort (all $p > 0.1$ by Spearman's ρ). Partial correlation analysis, controlling for age, gender and HAMD-21 total score, revealed that cooperativeness was correlated significantly with DEX/CRH-Cort ($r=0.23$, df=82, $p=0.03$) (Fig. 1a) and marginally significantly with ΔCort ($r=0.21$, df=82, $p=0.06$) (Fig. 1b); while no significant correlations were seen between the other six TCI dimensions and these two cortisol indices.

3.3.2. Comparison of TCI scores between incomplete- vs. enhanced-suppressors

Fig. 2 shows comparisons of the seven TCI dimensions between incomplete- and enhanced-suppressors. The ANCOVA, controlling

Table 1
Demographic/clinical characteristics and symptom dimensions of the sample stratified by the suppression pattern to the DEX/CRH test.

Variable	Total sample ($n=87$)	Incomplete-suppressors[a] ($n=24$)	Enhanced-suppressors[b] ($n=19$)	Analysis (incomplete-vs. enhanced-suppressors)		
				Statistic	d.f.	p
Age, years: mean ± SD	40.1 ± 10.7	40.0 ± 9.9	37.1 ± 11.1	$t=0.92$	41	0.36
Gender, female: n (%)	46 (52.9)	13 (54.2)	8 (42.1)	$\chi^2=0.62$	1	0.43
Comorbid dysthymic disorder: Yes, n (%)	13 (14.9)	3 (12.5)	3 (15.8)	Fisher's exact test		1
Family history of any psychiatric disorder: Yes, n (%)	35 (40.2)	13 (54.2)	6 (31.6)	$\chi^2=2.19$	1	0.14
Lifetime hospitalization to psychiatric ward: Yes, n (%)	19 (21.8)	8 (33.3)	2 (10.5)	Fisher's exact test		0.14
Lifetime electroconvulsive therapy: Yes, n (%)	1 (1.1)	1 (4.2)	0 (0.0)	Fisher's exact test		1
Medication, n (%)						
Antipsychotic	27 (31.0)	11 (45.8)	4 (21.1)	Fisher's exact test		0.12
Antidepressant	75 (86.2)	22 (91.7)	17 (89.5)	Fisher's exact test		1
Lithium	9 (10.3)	5 (20.8)	0 (0.0)	Fisher's exact test		0.06
Benzodiazepine	63 (72.4)	20 (83.3)	12 (63.2)	Fisher's exact test		0.17
HAMD-21 total score: mean ± SD	15.0 ± 5.6	15.6 ± 5.9	15.2 ± 5.2	$F=0.21$[c]	1,39	0.65
Somatic anxiety: mean ± SD	4.1 ± 2.3	4.2 ± 2.8	4.2 ± 2.4	$F=0.02$[c]	1,39	0.89
Psychic anxiety: mean ± SD	1.8 ± 1.2	1.5 ± 0.9	2.1 ± 1.2	$F=1.73$[c]	1,39	0.20
Core depressive symptoms: mean ± SD	4.6 ± 1.9	4.9 ± 1.8	4.6 ± 1.9	$F=0.54$[c]	1,39	0.47
Anorexia: mean ± SD	0.8 ± 1.2	0.8 ± 1.0	0.8 ± 1.0	$F=0.01$[c]	1,39	0.94
Hopkins Symptom Checklist						
Somatization: mean ± SD	29.1 ± 7.1	29.5 ± 6.1	28.6 ± 5.9	$F=0.27$[c]	1,39	0.60
Obsessive-compulsive: mean ± SD	25.3 ± 5.6	26.3 ± 5.7	25.8 ± 5.0	$F=0.10$[c]	1,39	0.75
Interpersonal sensitivity: mean ± SD	22.8 ± 5.7	23.5 ± 5.6	23.1 ± 5.8	$F=0.18$[c]	1,39	0.67
Anxiety: mean ± SD	16.6 ± 4.7	17.1 ± 4.7	17.0 ± 4.8	$F=0.20$[c]	1,39	0.65
Depression: mean ± SD	32.6 ± 7.1	32.9 ± 6.6	33.6 ± 6.4	$F=0.09$[c]	1,39	0.77

Abbreviations: DEX/CRH test, dexamethasone/corticotropin-releasing hormone test; HAMD-21, 21-item version of the Hamilton Depression Rating Scale.
Notes:

[a] Defined as DEX/CRH-Cort ≥ 137.95 (nmol/l) for men and DEX/CRH-Cort ≥ 273.14 for women.
[b] Defined as DEX/CRH-Cort < 27.59 (i.e., under the detection limit) for men and DEX/CRH-Cort < 49.66 for women.
[c] Analysis of covariance, controlling for age and gender.

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Table 2
Plasma cortisol concentrations (nmol/l) [mean ± SD (range)] for the three groups based on the suppression pattern, stratified by gender.

	Incomplete-suppressors[d]		Moderate-suppressors[e]		Enhanced-suppressors[f]	
	Men (n=11)	Women (n=13)	Men (n=19)	Women (n=25)	Men (n=11)	Women (n=8)
DST-Cort[a]	32.9 ± 24.1 (0.0–66.2)	59.0 ± 94.7 (0.0–364.2)	26.9 ± 23.4 (0.0–80.0)	19.1 ± 22.6 (0.0–80.0)	14.8 ± 17.5 (0.0–44.1)	18.3 ± 20.2 (0.0–46.9)
DEX/CRH-Cort[b]	290.4 ± 125.4 (140.7–485.6)	375.4 ± 62.4 (303.5–502.1)	63.2 ± 28.8 (30.3–118.6)	129.7 ± 60.4 (57.9–264.9)	0.0 ± 0.0 (0.0–0.0)	25.5 ± 21.3 (0.0–44.1)
ΔCort[c]	257.6 ± 128.2 (104.8–485.6)	316.4 ± 109.7 (24.8–455.2)	36.3 ± 40.9 (−13.8–118.6)	110.6 ± 58.8 (13.8–264.9)	−14.8 ± 17.5 (−44.1–0.0)	7.2 ± 13.0 (−2.8–35.9)

Notes:
[a] The concentration of cortisol (nmol/l) at 1500 h (i.e., immediately before the CRH challenge).
[b] The concentration of cortisol (nmol/l) at 1600 h (i.e., 1 h after the CRH challenge).
[c] Defined as "DEX/CRH-Cort minus DST-Cort".
[d] Defined as DEX/CRH-Cort ≥ 137.95 for men and DEX/CRH-Cort ≥ 273.14 for women.
[e] Defined as 27.59 ≤ DEX/CRH-Cort < 137.95 for men and 49.66 ≤ DEX/CRH-Cort < 273.14 for women.
[f] Defined as DEX/CRH-Cort < 27.59 (i.e., under the detection limit) for men and DEX/CRH-Cort < 49.66 for women.

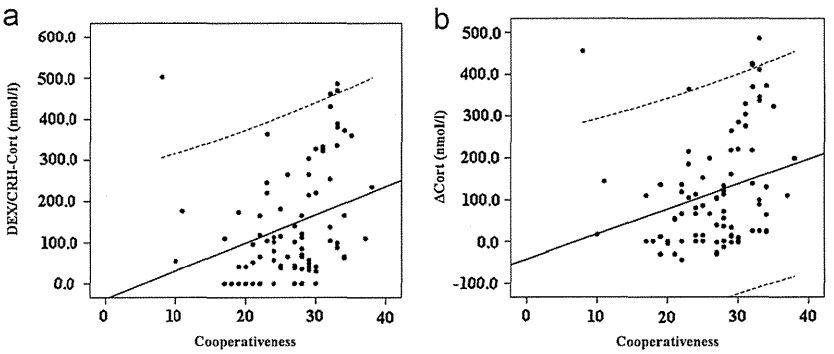

Fig. 1. Scatterplot showing the correlation between the cooperativeness score of the temperament and character inventory and cortisol measures including DEX/CRH-Cort (a) and ΔCort (b). The solid line and broken lines represent regression line and 95% prediction interval, respectively.

for age, gender and HAMD-21 total score, revealed that these two groups significantly differed on cooperativeness; Incomplete-suppressors scored higher on this dimension than enhanced-suppressors [$F(1,38)=11.7$, $p=0.002$]. No significant differences were found between the two suppressor groups in any of the other six TCI dimensions (all $p>0.2$). In order to further explore which subscales pertaining to the cooperativeness dimension contributed to this result, an additional ANCOVA was conducted on the five subscales of cooperativeness (i.e., social acceptance, empathy, helpfulness, compassion, and pure-hearted conscience), controlling for age, gender and HAM-D total score. This analysis revealed that incomplete-suppressors scored significantly higher on social acceptance [$F(1,38)=13.9$, $p<0.001$] and compassion [$F(1,38)=19.2$, $p<0.001$] than enhanced-suppressors, while no significant differences were found for the other three subscales (all $p>0.1$).

3.3.3. *Prediction of the DEX/CRH suppression pattern from TCI results*

The forward stepwise logistic regression analysis to predict the cortisol suppression pattern by the seven TCI dimensions, in addition to age, gender and HAM-D total score, revealed that cooperativeness and reward dependence were significant predictors; lower cooperativeness and higher reward dependence were associated with enhanced suppression of cortisol (Table 3). Of note, the goodness-of-fit improved considerably in the second step relative to the first step, indicating that not only cooperativeness but reward dependence played an important role in predicting the suppression pattern.

The fact that reward dependence was a significant predictor for the suppressor group, albeit not significantly different between the two suppressor groups, raised a possibility that there could be an interactive relationship between reward dependence and cooperativeness for the reactive cortisol measures. To scrutinize this interaction, a composite variable, "RD&CO", was created by dichotomizing reward dependence and cooperativeness scores based on median split. Since the incomplete suppression was associated with lower reward dependence and higher cooperativeness, three groups were considered: patients with both lower reward dependence and higher cooperativeness ($n=22$), those with both higher reward dependence and lower cooperativeness ($n=13$) and the remaining patients with either lower reward dependence or higher cooperativeness but not both ($n=52$). As illustrated in Fig. 3, patients with both low reward dependence and high cooperativeness showed the highest DEX/CRH-Cort and ΔCort values, those with both high reward dependence and low cooperativeness showed the lowest values, and in-between the remaing patients. The ANCOVA comparing DEX/CRH-Cort and ΔCort between the three RD&CO groups with age, gender and HAM-D total score as covariates revealed a significant main effect of group for both DEX/CRH-Cort [$F(2,81)=4.18$, $p=0.019$] and ΔCort [$F(2,81)=4.26$, $p=0.017$]. Post-hoc pairwise comparisons with Bonferroni correction revealed that patients with both high reward dependence and low cooperativeness showed significantly lower DEX/CRH-Cort (estimated mean difference: 135.6, 95% confidence interval: 20.8-250.5, $p=0.015$) and ΔCort (estimated mean difference: 130.0, 95% confidence interval: 20.0-240.0, $p=0.015$) than those with both low reward dependence and high cooperativeness.

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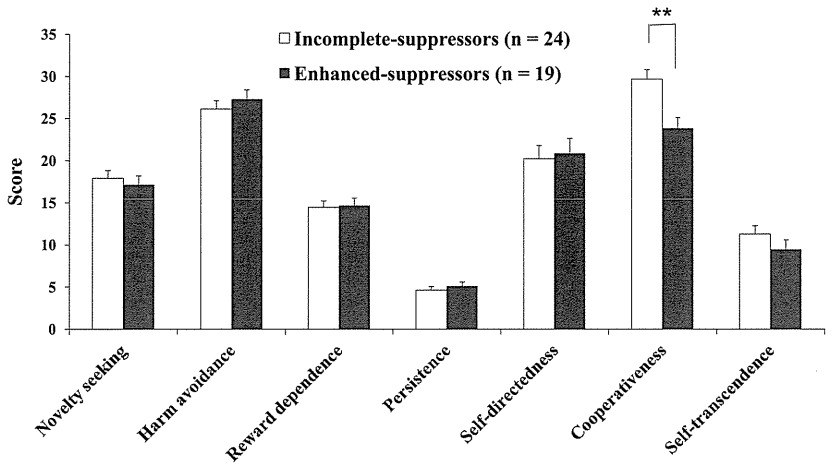

Fig. 2. Estimated mean scores of the seven dimensions of the temperament and character inventory, adjusted for age, gender and symptom severity as assessed by the Hamilton Depression Rating Scale 21-item version, for incomplete-suppressors and enhanced-suppressors. $^{**}p=0.002$ (by the analysis of covariance controlling for age, gender and symptom severity as assessed by the Hamilton Depression Rating Scale 21-item version). Error bars represent standard errors of the mean.

Table 3
Forward stepwise logistic regression analysis predicting DEX/CRH suppression pattern from the 7 dimensions of the temperament and character inventory, in addition to age, gender and depressive symptoms.

Step/variable	Nagelkerke R^2	Hosmer–Lemeshow p	B	Odds ratio	95% confidence interval	p
Step 1	**0.29**	**0.12**				
Cooperativeness			−0.20	0.82	0.71–0.94	0.006
Constant			5.2	176.4		0.010
Step 2	**0.46**	**0.69**				
Cooperativeness			−0.37	0.69	0.55–0.87	0.001
Reward dependence			0.41	1.5	1.07–2.12	0.018
Constant			3.5	31.6		0.089

Abbreviation: DEX/CRH test, dexamethasone/corticotropin-releasing hormone test.
Note: Odds ratio is the exponentiation of the B coefficient.

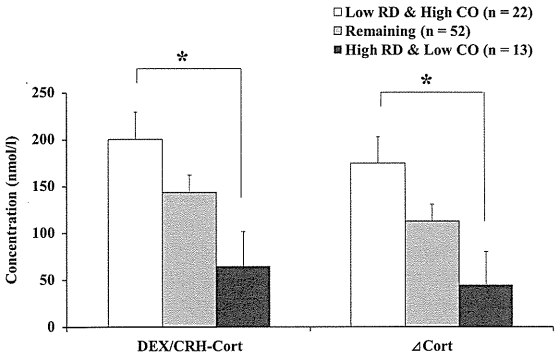

Fig. 3. Cortisol levels in response to the DEX/CRH test according to the composite RD/CO variable. RD and CO refer to reward dependence and cooperativeness, respectively. $^{*}p=0.015$ for both (by the analysis of covariance controlling for age, gender and symptom severity as assessed by the Hamilton Depression Rating Scale 21-item version, with post-hoc pairwise comparisons with Bonferroni correction). Error bars represent standard errors of the mean.

4. Discussion

The present study is the first attempt to examine the relationship between personality and cortisol reactivity as measured by the DEX/CRH test in depression, focusing on hypocortisolism. Main findings of this study were that (1) incomplete-suppressors scored significantly higher on cooperativeness than enhanced-suppressors and (2) the enhanced suppression of cortisol was significantly predicted by a unique pattern of personality traits, i.e., the combination of lower cooperativeness and higher reward dependence. These results were not confounded by age, gender or symptom severity.

To begin with, we should note that 28% (24/87) and 22% (19/87) of our patients with MDD fell into the categories of incomplete- and enhanced-suppression, respectively, by using the cutoff values for healthy adults where the highest 21% and lowest 20% were classified as the respective suppressor groups. This result indicates that the overall cortisol responses to the DEX/CRH test in patients with MDD were not very different from those in healthy controls, being in line with previous DEX/CRH studies in depressed outpatients (Oshima et al., 2000; Gervasoni et al., 2004; Van Den Eede et al., 2006; Carpenter et al., 2009), and further that cortisol reactivity varies widely within MDD, i.e., a considerable proportion of outpatients with MDD exhibit blunted cortisol reactivity. Hence, we propose that enhanced suppression, or hypocortisolism, in addition to hypercortisolism, needs to be taken into account when investigating HPA axis (dys)function in relation to depression. Supporting this, a recent study by Herbert et al. (2012) showed a significant quadratic (U-shaped) relationship between levels of morning salivary cortisol and the probability of depression onset during follow-up.

The main purpose of this study was to characterize the two extreme ends of cortisol reactivity using a set of personality dimensions. In the univariate analysis, only cooperativeness of

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