levels immediately following stress. In terms of the "neurotrophin hypothesis of depression," antidepressants, but not anxiolytic drugs, can ameliorate the symptoms of depression and prevent stress-related recurrence of depression.

The present study has several limitations. First, the sample was restricted to a small number of healthy adult male volunteers. It is possible that the responses to mental stress in female, depressed, or elderly patients could differ widely from those of healthy, younger men. Second, the present study evaluated only the immediate effects of low-dose administration of the drugs on plasma BDNF levels. Third, a 5-min simulator task may be inadequate for assessing mental stress. Although AWWL scores showed that this task induced mental stress, there is a possibility of a type 1 error because of the small sample size. Therefore, future studies using a larger number of subjects with repeated drug administration over a range of doses need to be conducted for conclusions to be drawn regarding the effects on plasma BDNF level. From the AWWL score, we regarded the car-following task as a mental stress condition, although there is no significant difference in plasma cortisol levels before and after the car-following task (data not shown). Then it is necessary to examine how the duration of the DS task influences plasma BDNF levels in more detail. Fourth, the degree of stress associated with the DS task needs to be examined by measuring changes in other stress-related variables (e.g., heartbeat and skin electrical resistance). Fifth, we did not examine plasma BDNF level change at 4 h post-dosing without DS task to elucidate whether drug treatments without DS task could affect plasma BDNF levels. Finally, we evaluated only 4-h time point for DS task when plasma concentration of paroxetine reaches its maximum. Because three drugs have different pharmacokinetic and pharmacodynamic profiles, we need to examine plasma BDNF level change at a time when plasma concentrations of diazepam and tandospirone reach their maximum in future study.

Our findings should be interpreted with following caveat. The treatments for depression, such as antidepressants (Shimizu *et al.*, 2003), electroconvulsive therapy (Okamoto *et al.*, 2008), and sleep deprivation (Gorgulu and Caliyurt, 2009) increase expression of BDNF. Although this is suggesting that there is an etiological link between the development of depression and BDNF, scientific studies have found that numerous brain areas show altered activity in depressed patients (Krishnan and Nestler, 2008), and it has not been possible to determine a single cause of depression.

In conclusion, diazepam, tandospirone, and paroxetine could have different effects on plasma BDNF levels under mental stress after 4 h post-dosing. Furthermore, antidepressants, unlike anxiolytics, might have immediate positive effects on the mental stress response.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

We sincerely thank the volunteers for participating in our study and Kazumi Sasada, Kunihiro Kohmura, and Maeri Yamamoto for their help. This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Ministry of Health, Labor and Welfare of Japan, the Nagoya University Science Foundation, The Academic Frontier Project for Private Universities, Comparative Cognitive Science Institutes, Meijo University, The General Insurance Association of Japan, Conference for Expressway-related Social Contribution Activities (CERSCA), and ZENKYOREN.

REFERENCES

aan het Rot M, Mathew SJ, Charney DS. 2009. Neurobiological mechanisms in major depressive disorder. *Cmaj* **180**: 305–313.

Caspi A, Sugden K, Moffitt TE, *et al.* 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* **301**: 386–389.

Charmey DS. 2004. Psychobiological mechanisms of resilience and vulnerability: implications for successful adaptation to extreme stress. *Am J Psychiatry* **161**: 195–216.

Doyle GD, Laher M, Kelly JG, Byrne MM, Clarkson A, Zussman BD. 1989. The pharmacokinetics of paroxetine in renal impairment. *Acta Psychiatr Scand Suppl* **350**: 89–90.

Duman RS, Monteggia LM. 2006. A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* **59**: 1116–1127.

Ghose K. 1989. The pharmacokinetics of paroxetine in elderly depressed patients. *Acta Psychiatr Scand Suppl* **350**: 87–88.

Gorgulu Y, Caliyurt O. 2009. Rapid antidepressant effects of sleep deprivation therapy correlates with serum BDNF changes in major depression. *Brain Res Bull* **80**: 158–162.

Haga S, Mizukami N. 1996. Japanese version of NASA Task Load Index: sensitivity of its workload score to difficulty of three different laboratory tasks. *Jpn J Ergonom* **32**: 71–79.

Iwamoto K, Takahashi M, Nakamura Y, *et al.* 2008. The effects of acute treatment with paroxetine, amitriptyline, and placebo on driving performance and cognitive function in healthy Japanese subjects: a double-blind crossover trial. *Hum Psychopharmacol* **23**: 399–407.

Karege F, Schwald M, Cisse M. 2002. Postnatal developmental profile of brain-derived neurotrophic factor in rat brain and platelets. *Neurosci Lett* **328**: 261–264.

Krishnan V, Nestler EJ. 2008. The molecular neurobiology of depression. *Nature* **455**: 894–902.

Lee HY, Kim YK. 2008. Plasma brain-derived neurotrophic factor as a peripheral marker for the action mechanism of antidepressants. *Neuropsychobiology* **57**: 194–199.

Martinowich K, Lu B. 2008. Interaction between BDNF and serotonin: role in mood disorders. *Neuropsychopharmacology* **33**: 73–83.

Martinowich K, Manji H, Lu B. 2007. New insights into BDNF function in depression and anxiety. *Nat Neurosci* **10**: 1089–1093.

Mitoma M, Yoshimura R, Sugita A, *et al.* 2008. Stress at work alters serum brain-derived neurotrophic factor (BDNF) levels and plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in healthy volunteers: BDNF and MHPG as possible biological markers of mental stress? *Prog Neuropsychopharmacol Biol Psychiatry* **32**: 679–685.

Miyake S, Kumashiro M. 1993. Subjective mental workload assessment technique—an introduction to NASA-TLX and SWAT and a proposal of simple scoring methods. *Jpn J Ergonom* **29**: 399–408.

Okamoto T, Yoshimura R, Ikenouchi-Sugita A, *et al.* 2008. Efficacy of electroconvulsive therapy is associated with changing blood levels of homovanillic acid and brain-derived neurotrophic factor (BDNF) in refractory depressed patients: a pilot study. *Prog Neuro-Psychopharmacol Biol Psychiatry* **32**: 1185–1190.

Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. 1998. Transport of brain-derived neurotrophic factor across the blood–brain barrier. *Neuropharmacology* **37**: 1553–1561.

Radka SF, Holst PA, Fritsche M, Altar CA. 1996. Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. *Brain Res* **709**: 122–301.

Sen S, Duman R, Sanacora G. 2008. Serum brain-derived neurotrophic factor, depression, and antidepressant medications: meta-analyses and implications. *Biol Psychiatry* **64**: 527–532.

Shimizu E, Hashimoto K, Okamura N, *et al.* 2003. Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. *Biol Psychiatry* **54**: 70–75.

Takahashi M, Iwamoto K, Kawamura Y, *et al.* 2010. The effects of acute treatment with tandospirone, diazepam, and placebo on driving performance and cognitive function in healthy volunteers. *Hum Psychopharmacol* **25**: 260–267.

Uchiyama Y, Ebe K, Kozato A, Okada T, Sadato N. 2003. The neural substrates of driving at a safe distance: a functional MRI study. *Neurosci Lett* **352**: 199–202.

Watanabe K, Hashimoto E, Ukai W, *et al.* 2010. Effect of antidepressants on brain-derived neurotrophic factor (BDNF) release from platelets in the rats. *Prog Neuropsychopharmacol Biol Psychiatry* **34**: 1450–1454.

Zafra F, Castren E, Thoenen H, Lindholm D. 1991. Interplay between glutamate and gamma-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. *Proc Natl Acad Sci USA* **88**: 10037–10041.

Hum. Psychopharmacol Clin Exp 2012; **27**: 329–333.
DOI: 10.1002/hup

Journal of Affective Disorders 142 (2012) 315–322

Contents lists available at SciVerse ScienceDirect

Journal of Affective Disorders

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

Preliminary communication

GTP cyclohydrolase 1 gene haplotypes as predictors of SSRI response in Japanese patients with major depressive disorder

Taro Kishi [a,b,*], Hiroshi Ichinose [c], Reiji Yoshimura [d], Yasuhisa Fukuo [b], Tsuyoshi Kitajima [b], Toshiya Inada [e], Hiroshi Kunugi [f], Tadafumi Kato [g], Takeo Yoshikawa [h], Hiroshi Ujike [i], Giovanna M. Musso [a], Wakako Umene-Nakano [d], Jun Nakamura [d], Norio Ozaki [j], Nakao Iwata [b]

[a] Department of Psychiatry Research, The Zucker Hillside Hospital, Glen Oaks, NY 11004, USA
[b] Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan
[c] Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, Japan
[d] Department of Psychiatry, University of Occupational and Environmental Health, Kitakyushu, Fukuoka 807-8555, Japan
[e] Seiwa Hospital, Institute of Neuropsychiatry, Tokyo 162-0851, Japan
[f] National Center of Neurology and Psychiatry, Mental Disorder Research, National Institute of Neuroscience, Tokyo 187-8502, Japan
[g] Laboratory for Molecular Dynamics of Mental Disorders, RIKEN Brain Science Institute, Saitama 351-0198, Japan
[h] Laboratory for Molecular Psychiatry, RIKEN Brain Science Institute, Saitama 351-0198, Japan
[i] Department of Neuropsychiatry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, Okayama 700-8558, Japan
[j] Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya 466-8850, Japan

ARTICLE INFO

Article history:
Received 29 March 2012
Accepted 1 May 2012
Available online 6 July 2012

Keywords:
Tetrahydrobiopterin (BH4)
GTP cyclohydrolase gene (*GCH1*)
Major depressive disorder
Bipolar disorder
Case-control study
Pharmacogenetic study

ABSTRACT

Background: Tetrahydrobiopterin (BH4) plays an important role in the biosynthesis of serotonin, melatonin and catecholamines, all of which are implicated in the pathophysiology of mood disorders (MDs), including major depressive disorder (MDD) and bipolar disorder (BP). Production of BH4 is regulated by GTP cyclohydrolase transcription and activity. Thus, we considered the GTP cyclohydrolase gene (*GCH1*) to be a good candidate gene in the pathophysiology of MDs and of the serotonin selective reuptake inhibitors (SSRIs) response in MDD, and conducted a case-control study utilizing three SNPs (rs8007267, rs3783641 and rs841) and moderate sample sizes (405 MDD patients, including 262 patients treated by SSRIs, 1022 BP patients and 1805 controls).
Method: A multiple logistic regression analysis was carried out to compare the frequencies of each SNP genotype for the target phenotype across patients and controls in several genetic models, while adjusting for possible confounding factors. A clinical response was defined as a decrease of more than 50% from the baseline score on the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks.
Result: No associations between three SNPs in *GCH1* and MDD or BP were observed; however, *GCH1* was associated with SSRI therapeutic response in MDD in all the marker's haplotype analysis (Global P value = 0.0379).
Conclusions: Results suggest that *GCH1* may predict response to SSRI in MDD in the Japanese population. Nevertheless, a replication study using larger samples may be required for conclusive results, since our sample size was small.

1. Introduction

The pathophysiology of mood disorders (MDs), including major depressive disorder (MDD) and bipolar disorder (BP) are still unknown; however, considerable evidence has accumulated implicating multiple system pathogenesis in mood disorders, including abnormalities of monoamine as well as other neurotransmitter systems (Dhir and Kulkarni, 2011; Dunlop and Nemeroff, 2007; Salvadore et al., 2010). It has been suggested that MDs may be caused by abnormalities in the biochemistry of one or more neural transmission systems or by neurotransmitter disruptions that are secondary to other biological, environmental, and/or psychological causes (Dhir and Kulkarni, 2011; Dunlop and Nemeroff, 2007; Salvadore et al., 2010).

Tetrahydrobiopterin (BH4) is an essential cofactor for tyrosine hydroxylase (TH), tryptophan hydroxylase (TPH), nitric oxide synthase, phenylalanine hydroxylase and alkylglycerol

* Corresponding author at: Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi 470-1192, Japan. Tel.: +81 562 93 9250; fax: +81 562 93 1831.
E-mail address: taroh@fujita-hu.ac.jp (T. Kishi).

0165-0327/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jad.2012.05.004

monooxygenase (Thony et al., 2000; McLeod et al., 2001). TH and TPH are the rate-limiting enzyme in the biosynthesis of catecholamine and serotonin, respectively (Thony et al., 2000). There is substantial evidence for the relationship between abnormalities in these neurotransmitters and the pathophysiology of MDs (see detailed discussion in the following reviews:) (Dunlop and Nemeroff, 2007; Russo et al., 2009). Several studies reported an association between depressive symptoms and BH4 deficiency (Abou-Saleh et al., 1995; Blair et al., 1984; Bottiglieri et al., 1992; Coppen et al., 1989; Hashimoto et al., 1990), and between hypomania and increased levels of BH4 (Hashimoto et al., 1990). Based on these associations, MDs are believed to have dysfunctions in several neurotransmitter systems in the central nervous system; this may also lead to the notion that BH4 has some effects on mood regulation (Sumi-Ichinose et al., 2001).

GTP cyclohydrolase I (GCH1) is the rate-limiting enzyme in the biosynthesis of BH4. GCH1 gene (*GCH1*) is known as the susceptibility gene of Segawa disease, which is also called dopa responsive dystonia (Ichinose et al., 1994). Treatment with L-dopa/carbidopa leads to significant improvement of motor symptoms in the majority of patients with Segawa's disease. Patients with Segawa disease reported by Hahn and colleagues exhibited depressive and anxiety symptoms. Kealey and colleagues reported that *GCH1* was associated with BP in the Irish population (Kealey et al., 2005). Recently, McHugh and colleagues reported that GTP-cyclohydrolase I feedback regulator (GFRP) gene, which mediates feedback inhibition of GCH1 activity by BH4, was associated with the serotonin selective reuptake inhibitors (SSRIs), which are antidepressant, response in MDD patients in the New Zealand population (McHugh et al., 2011). Sepiapterin reductase gene, which catalyzes the final step in BH4 synthesis, was associated with BP and the SSRI response in MDs (McHugh et al., 2009). Based on these findings, *GCH1* was selected as a candidate gene for involvement in the pathogenesis of MDs. It was hypothesized that *GCH1* would be related to the SSRIs response in MDD, since MDs and SSRI response may have some shared mechanisms with respect to neural transmission systems. *GCH1* (OMIM * 2643, 6 exons in this genomic region spanning 61.620 bp), is located on 14q22.1–q22.2. There are no reported genetic association analyses between *GCH1* and MDs in the Japanese population. Therefore, a case-control study with Japanese MDs samples was conducted and an association analysis between *GCH1* and the efficacy of SSRI treatment in Japanese patients with MDD was performed.

2. Materials and methods

2.1. Subjects

Subjects were 405 MDD patients (191 males and 214 females; mean age ± standard deviation 48.8 ± 16.2 years), 1022 BP patients (506 males and 516 females; mean age ± standard deviation 49.9 ± 14.2 years) and 1805 healthy control individuals (888 males and 917 females; 43.7 ± 15.0 years). Among the 262 MDD patients treated with SSRIs, 184 were assessed with the Mini-International Neuropsychiatric Interview (MINI) and the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D) and 78 were assessed only with the SIGH-D. All 1022 BP patients were diagnosed by unstructured interviews, based on DSM-IV criteria with the consensus of at least two experienced psychiatrists. Among the 1805 healthy control individuals, 1628 controls were screened for DSM-IV psychiatric diagnoses by unstructured interviews with the consensus of at least two experienced psychiatrists, 46 healthy controls underwent the MINI, and 131 healthy controls underwent the Structured Clinical Interview for DSM-IV disorders (SCID-1). None of the subjects had comorbid DSM-IV Axis-I disorders or severe medical complications, including liver cirrhosis, renal failure, heart failure.

The study was described to subjects and written informed consent was obtained from each participant. Most of the subjects in this study sample were also previous participants of the Collaborative Study of Mood Disorder consortium. Seven laboratories (National Institute of Neuroscience, two laboratories of RIKEN Brain Science Institute, Kohnodai Hospital, Teikyo University, Okayama University and Fujita Health University) provided the BP and healthy control samples. This study was approved by the ethics committees of all participating institutes. This study was also approved by the Ethics Committee at Fujita Health University, Nagoya University School of Medicine and University of Occupational and Environmental Health.

2.2. Procedures of the pharmacogenetic study

Fluvoxamine was taken two or three times a day and sertraline and paroxetine were taken one or two times a day for eight weeks. Fluvoxamine, sertraline and paroxetine was increased gradually to a maximum of 150 mg, 100 mg and 40 mg, respectively, depending on the patient's condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study.

The 262 MDD patients included in this study had scores of 12 or higher on the 17 items of the SIGH-D (Peveler and Kendrick, 2005). We defined a clinical response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than 7 at 8 weeks. The clinical characteristics of patients in this study, classified according to these definitions, can be seen in Table 1.

2.3. SNP selection and LD Evaluation

Zhang et al. (2007) reported that rs841, which is located in 3′ UTR in *GCH1*, affects expression of *GCH1*. Tegeder et al. (2006) also reported that among individuals with the C-A-T haplotype in rs10483639-rs3783641-rs8007267, GCH1 mRNA levels were lower than among individuals with other haplotypes (Tegeder et al., 2006). Therefore, we evaluated LD between rs841, rs10483639, rs3783641 and rs8007267 utilizing the HapMap database (release##27, Feb 10, http://www.hapmap.org, population: Japanese Tokyo,) and HAPLOVIEW software (Barrett et al., 2005). Between rs841 and rs10483639 was in absolute LD ($r^2 = 1.00$). The other pairs provide negative evidence for LD ($r^2 < 0.8$). Therefore, we selected rs8007267, rs3783641 and rs841for the following association analysis.

2.4. SNP Genotyping

We used TaqMan assays (ABI: Applied Biosystems, Inc., Foster City, CA,) for all SNPs. Three tagging SNPs (rs8007267, rs3783641 and rs841) were genotyped using the TaqMan allelic discrimination assay and the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). TaqMan SNP Genotyping Assays C___1545138_10, C__25800745_10 and C___9866639_10 were used for the three SNPs (rs8007267, rs3783641, and rs841), respectively (http://www.appliedbiosystems.com/absite/us/en/home.html). One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. The plates were heated for 2 min at 50 °C and 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s and 58 °C for 1 min. Please refer to ABI for the primer sequence. Detailed information is available upon request.

Table 1
Clinical characteristics of the patients in both definition groups for the pharmacogenetics study.

	N			Age (mean ± SD)	Baseline SIGH-D (mean ± SD)	Number of previous episode (mean ± SD)	SSRI dose at 8 weeks (mg/day) (mean ± SD)[c]	Patients permitted with anxiolytics/ hypnotics, n (%)
	Total	Male	Female					
Overall	262	118	144	48.2 ± 16.4	20.7 ± 5.22	1.79 ± 0.789	118 ± 41.9	119 (45.4)
Clinical response group[a]								
Responders	146	71	75	48.3 ± 15.8	21.4 ± 5.37	1.79 ± 0.755	117 ± 39.8	68 (30.0)
Non-responders	116	47	69	48.1 ± 17.1	19.8 ± 4.90	1.79 ± 0.842	120 ± 44.5	51 (19.5)
P-value	0.189			0.926	**0.0134**	0.993	0.597	0.673
Clinical remission group[b]								
Remitters	102	51	51	48.1 ± 16.1	19.8 ± 4.57	1.71 ± 0.694	112 ± 42.3	42 (16.0)
Non-remitters	160	67	93	48.2 ± 16.6	21.3 ± 5.53	1.85 ± 0.847	122 ± 41.3	77 (29.4)
P-value	0.198			0.930	**0.0165**	0.208	0.0900	0.270

[a] Clinical response was defined as a 50% or greater decrease in the baseline SIGH-D score.
[b] Clinical remission was defined as a final SIGH-D score of less than 7.
[c] Imipramine equivalent.

2.5. Statistical analysis

Genotype deviation from the Hardy–Weinberg equilibrium (HWE) was evaluated by chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan). A logistic regression analysis was performed to compare the frequencies of each SNP genotype for the target phenotype across patients and healthy controls in allele, recessive, dominant and co-dominant models. In addition, age at time of recruitment and gender were controlled for in the logistic regression analyses in order to adjust the results for potential confounding effects, since age at time of recruitment in healthy controls was significantly lower compared to MDD and BP groups ($P_{MDD} < 0.0001$ and $P_{BP} < 0.0001$). In the pharmacogenic study, individual *t*-test and chi-square tests were used to compare means and categorical proportions (responders or non-responders and remitters or nonremitters), respectively. Among the clinical characteristics of patients in this pharmacogenetic study, in the case of either responder or nonresponder and remitter or nonremitter, significant differences with total SIGH-D score at the baseline were detected ($P_{response}=0.0134$ and $P_{remission}=0.0165$) (Table 1). Therefore, a logistic regression was carried out to analyze the possible correlations between response or remission, SSRI treatment, and several clinical factors. In these analyses, response classification was set as the dependent variable, and gender, age at the time of recruitment, SSRIs dose at eight weeks (imipramine-equivalent), SIGH-D total score at the baseline, and each SNP genotype were set as the independent variables. The statistical package JMP for Windows was used for logistic regression analysis (JMP 5.0. 1J, SAS Japan Inc, Tokyo, Japan). Bonferroni's correction was also used to control for a potential inflation of the type I error rate in the single marker association analysis. The following Bonferroni correction was employed for the multiple comparison tests: 12 tests for each sample set in each genotype model analysis (3 examined SNPs in *GCH1* and four genotype models (the allele, recessive, dominant and co-dominant model)). Haplotype-wise association analysis was conducted with a likelihood ratio test utilizing the COCAPHASE2.403 program (Dudbridge, 2003). The permutation test option as provided in the haplotype-wise analysis was used to avoid spurious results and to correct for multiple testing. Permutation test correction was performed using 10,000 iterations (random permutations). For the individual haplotype association analysis, three sets of multiple tests (Bonferroni's correction) for rs8007267–rs378364–rs841 (maximum number of haplotypes) were employed for each sample group in haplotype analysis. The following assumptions in the CaTS power calculator analysis (Skol et al., 2006) were used: Prevalence: 0.2, 0.01 and 0.2 for MDD, BP and SSRI response in MDD, respectively, User-defined statistical threshold: 0.0167 (3 SNPs examined in this study. Bonferroni's correction was used to control for a potential inflation of the type I error rate). The significance level for all statistical tests was $p < 0.05$.

3. Results

3.1. Case control study

The LD structure as determined from our MDD, BP and control sample can be seen in Fig. 1. The LD in the healthy control samples was similar to that of MDD and BP. Genotype frequencies of all SNPs were in HWE (Table 2). No associations between the three SNPs in *GCH1* and BP or MDD in any of the genotype models (Table 3) or the haplotype analysis (Table 4) were detected.

3.2. Pharmacogenic study

Among the clinical characteristics of patients in this pharmacogenetic study, in the case of either responder or nonresponder and remitter or nonremitter, significant differences with total SIGH-D score at the baseline were detected ($P_{response}=0.0134$ and $P_{remission}=0.0165$) (Table 1). The LD structure as determined from MDD samples in both definition groups can be seen in Fig. 2. The LD is almost similar to each clinical definition group. Genotype frequencies of all SNPs were in HWE (Table 5). SNPs rs841, rs3783641 and rs8007267 were associated with the SSRI therapeutic response in MDD in the dominant model ($P_{rs841}=0.0325$, $P_{rs3783641}=0.0370$ and $P_{rs8007267}=0.0325$) (Table 6). However, these significance did not persist with the Bonferroni correction ($P_{rs841}=0.390$, $P_{rs3783641}=0.444$ and $P_{rs8007267}=0.390$) (Table 6). Results show that *GCH1* was associated with the SSRI response in MDD in all three markers of the haplotype-wise analysis (Global $P_{all\ markers}=0.0379$) (Table 7). The individual haplotype analysis is shown in Table 7. Haplotype analysis to investigate both responders and non-responders indicated several common haplotypes as follows: rs8007267–rs378364–rs841: C-T-C, C-T-T, and T-A-T (Table 7). The C-T-C haplotype was less prevalent in non-responders compared with responders ($P_{C\text{-}T\text{-}C}=0.0107$). Moreover, the

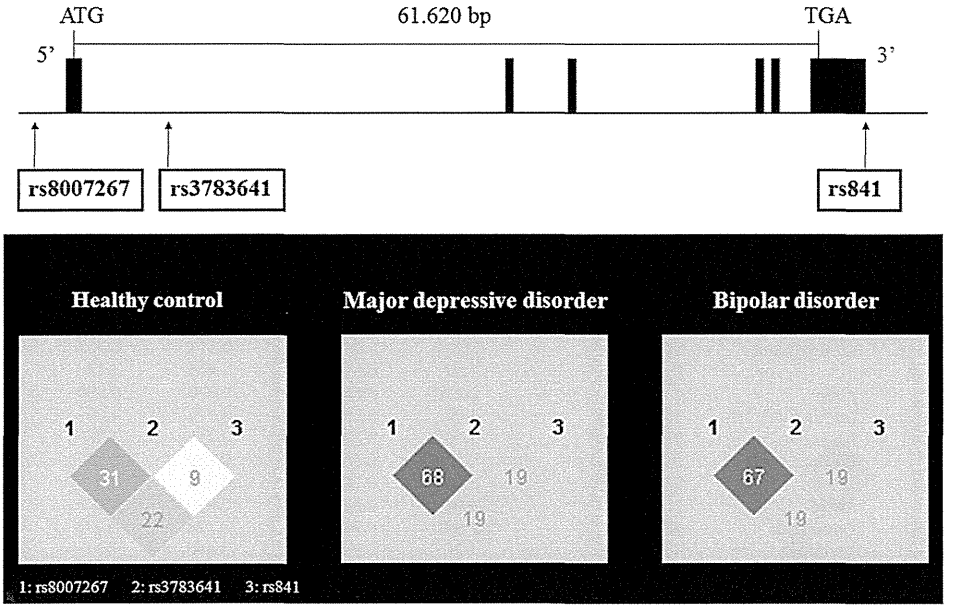

Fig. 1. Gene Structure of *GCH1* and LD Evaluation Constructed Three SNPs in *GCH1* in Major Depressive Disorder, Bipolar Disorder and Healthy Controls. ATG is the start codon and TGA is the stop codon. Vertical bar represent exon. Three examined SNPs are represented by black boxes. Color scheme is based on r^2 value. Other information can be seen at the Haploview website. LDs of major depressive disorder, bipolar disorder and healthy controls are almost same. (For interpretation of the references to color in this figure legend, the reader is reffered to the web version of this article.)

Table 2
Genotype distribution in tagging SNPs in *GCH1* in each phenotype for the case-control study.

SNP[a]	Phenotype[b]	MAF[c]	N	Genotype distribution[d]			Hardy–Weinberg equilibrium
				M/M	M/m	m/m	*P*-value
rs8007267	Control	0.236	1805	1048	662	95	0.470
C > T	BP	0.221	1022	624	344	54	0.465
	MDD	0.206	405	255	133	17	0.948
rs3783641	Control	0.216	1805	1115	600	90	0.426
T > A	BP	0.214	1022	632	342	48	0.842
	MDD	0.216	405	254	127	24	0.135
rs841	Control	0.405	1805	643	861	301	0.657
C > T	BP	0.405	1022	370	477	175	0.318
	MDD	0.412	405	144	188	73	0.396

[a] Major allele > minor allele.
[b] BP: Bipolar disorder, MDD: Major depressive disorder.
[c] MAF: minor allele frequency.
[d] M: major allele, m: minor allele.

association of this individual haplotype with the SSRI response in MDD remained even after Bonferroni correction ($P_{C\text{-}T\text{-}C}=0.0321$) (Table 7). On the other hand, no association between the three SNPs in *GCH1* and the SSRI remission in MDD was found in any of the genotype models and haplotype analysis (Tables 6 and 7).

In the power analysis for *GCH1* under a multiplicative model of inheritance, more than 80% power was calculated for the detection of association when the genotype relative risk was set at 1.23 and 1.27 in MDD, 1.20 and 1.24 in BP, and 1.58 and 1.63 in MDD SSRI response.

4. Discussion

The association analysis between *GCH1* and SSRI response in MDD indicated that *GCH1* was associated with SSRI therapeutic response in Japanese patients with MDD. This suggests that *GCH1* may predict response to SSRI in MDD in the Japanese population. On the other hand, no associations between *GCH1* and Japanese BP and MDD patients in any of the genotype models and haplotype analysis were detected. However, because our samples are rather small to detect the susceptibility genes for common complex disease such as BP and MDD (Committee, 2009), the possibility of statistical error in the present results exists. To overcome this limitation, it will be necessary to conduct further study utilizing larger samples in the future.

A significant association was found between the C-T-C in rs8007267–rs378364–rs841 and SSRI response in Japanese MDD patients in the haplotype analysis. In the single marker association analysis, SNPs rs8007267, rs378364 and rs841 were marginally associated with SSRI response in the dominant model. Based on these results, perhaps some of the association between these haplotypes in *GCH1* and SSRI response in MDD in this study might be reflected in the genotype on rs8007267, rs378364 and rs841, reciprocally and/or independently. SNPs rs8007267, rs378364 and rs841 are located in the 5′ flanking region, intron1 and 3′ flanking region, respectively, in *GCH1*. Since mutation screening was not performed in the present study, further investigation will be

Table 3
The case-control association analysis of three SNPs in *GCH1*.

SNP[a]	Phenotype[b]	Genetic model	Allele or genotype	Unadjusted analyses[c]			Adjusted analyses[c,d]		
				OR	95% CI	*P*	OR	95% CI	*P*
rs8007267	BP	Allele	C	0.919	0.807–1.05	0.202	0.919	0.807–1.63	0.202
C > T		Dominant	CC	1.13	0.968–1.32	0.120	1.07	0.909–1.26	0.408
		Recessive	CC+CT	0.996	0.709–1.41	0.981	1.01	0.706–1.45	0.972
		Co-dominant	TT	1.13	0.861–1.49	0.383	1.08	0.809–1.43	0.618
			CC	1.03	0.646–1.62	0.902	1.00	0.618–1.62	0.985
	MDD	Allele	C	0.841	0.698–1.01	0.0684	0.841	0.698–1.01	0.0684
		Dominant	CC	0.814	0.651–1.02	0.0702	0.814	0.644–1.03	0.0823
		Recessive	CC+CT	0.789	0.450–1.30	0.378	0.768	0.428–1.30	0.348
		Co-dominant	TT	0.717	0.469–1.66	0.111	0.707	0.454–1.07	0.111
			CC	1.33	0.678–2.80	0.433	1.37	0.681–3.00	0.401
rs3783641	BP	Allele	T	0.990	0.867–1.13	0.876	0.990	0.867–1.13	0.876
T > A		Dominant	TT	1.00	0.856–1.17	0.972	1.00	0.850–1.19	0.961
		Recessive	TT+TA	1.06	0.747–1.54	0.732	1.06	0.732–1.54	0.767
		Co-dominant	AA	0.919	0.563–1.47	0.729	0.927	0.559–1.52	0.766
			TT	1.04	0.785–1.38	0.798	1.03	0.773–1.39	0.819
	MDD	Allele	T	1.00	0.831–1.20	0.999	1.00	0.831–1.20	0.999
		Dominant	TT	0.961	0.768–1.20	0.724	0.938	0.742–1.18	0.591
		Recessive	TT+TA	1.20	0.740–1.88	0.440	1.17	0.706–1.86	0.530
		Co-dominant	AA	0.772	0.424–1.47	0.413	0.797	0.428–1.56	0.490
			TT	1.06	0.721–1.53	0.769	1.02	0.684–1.49	0.932
rs841	BP	Allele	C	1.00	0.897–1.12	0.982	1.00	0.897–1.12	0.982
C > T		Dominant	CC	1.03	0.874–1.20	0.757	1.03	0.867–1.21	0.763
		Recessive	CC+CT	0.955	0.780–1.17	0.661	0.959	0.774–1.19	0.701
		Co-dominant	TT	1.06	0.805–1.39	0.682	1.05	0.789–1.40	0.721
			CC	1.01	0.811–1.28	0.905	1.02	0.802–1.29	0.897
	MDD	Allele	C	1.03	0.882–1.20	0.711	1.03	0.882–1.20	0.711
		Dominant	CC	1.00	0.802–1.26	0.980	0.933	0.740–1.18	0.562
		Recessive	CC+CT	1.10	0.824–1.45	0.513	1.10	0.816–1.47	0.519
		Co-dominant	TT	0.884	0.612–1.30	0.523	0.887	0.603–1.32	0.548
			CC	1.04	0.759–1.42	0.821	0.953	0.690–1.32	0.771

[a] Major allele > minor allele.
[b] BP: Bipolar disorder, MDD: Major depressive disorder.
[c] OR: Odds ratio, CI: Confidence interval.
[d] Analyses adjusted for sex and age.

Table 4
Haplotype-wise analysis of three SNPs in *GCH1* for the pharmacogenetics study.

Phenotype	Global *P*-value	Common haplotypes rs8007267-rs378364-rs841	Phenotype	Individual haplotype frequency	OR	95% CI	Individual *P*-value
Bipolar disorder	0.605	CTC	Control	0.601	1.00	1.00–1.00	0.623
			BP	0.592			
		CTT	Control	0.227	1.03	0.869–1.15	0.822
			BP	0.231			
		TAT	Control	0.172	1.06	0.893–1.08	0.899
			BP	0.177			
Major depressive disorder	0.823	CTC	Control	0.601	1.00	1.00–1.00	0.602
			MDD	0.590			
		CTT	Control	0.227	1.07	0.871–1.31	0.545
			MDD	0.238			
		TAT	Control	0.172	1.02	0.811–1.28	0.998
			MDD	0.172			

necessary. Tegeder et al. (2006) reported that among individuals with C-A-T haplotype in rs10483639, rs3783641 and rs8007267, GCH1 mRNA was lower compared to individuals with other haplotypes (Tegeder et al., 2006). According to the HapMap database, LD between rs10483639, rs3783641 and rs8007267 are significantly different in each population (Supplementary Fig. 1). In addition, because there are not any studies that have investigated an association between C-T-C in rs8007267-rs378364-rs841 and biologically functional effect in GCH1 to date, further study is required to confirm this utilizing both Japanese and other population samples.

Several studies reported that depressive symptoms may be linked to a deficiency of BH4 (Abou-Saleh et al., 1995; Blair et al., 1984; Bottiglieri et al., 1992; Coppen et al., 1989; Hashimoto et al., 1990). On the other hand, Miura and colleagues reported that, in male ddY mice, fluvoxamine inhibited BH4 in the hippocampus (Miura et al., 2004) and in the meso-prefrontal cortex (Miura et al., 2005). These results appear inconsistent when considering the relationship between BH4 and the pathophysiology of MDD and pharmacological mechanism of antidepressants in MDD patients. Further investigation will be needed to clarify this question using larger cohort samples of antidepressants clinical trial.

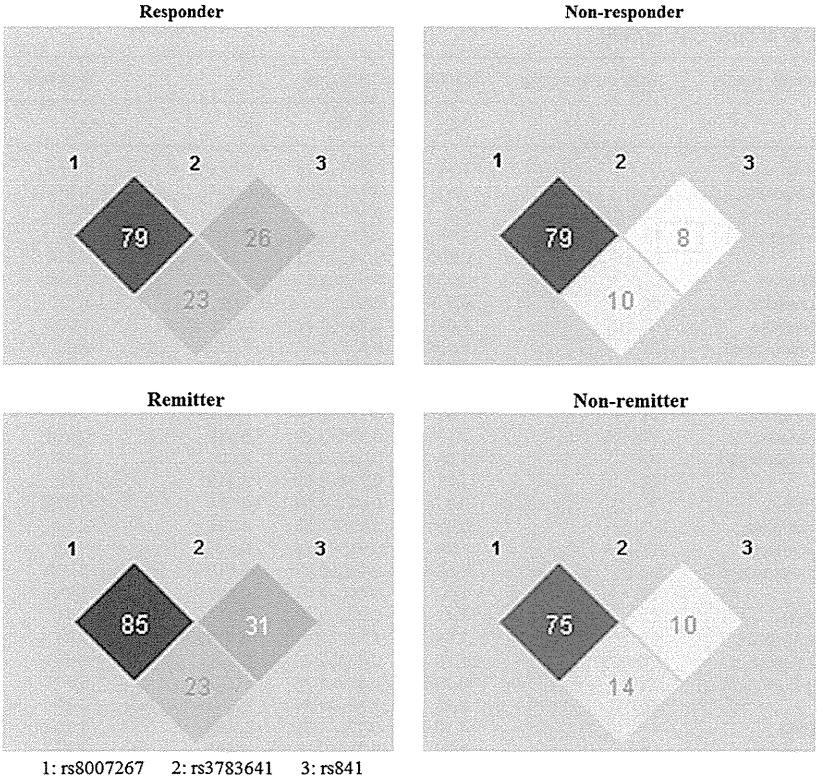

Fig. 2. LD Evaluation Constructed Three SNPs in *GCH1* in Responders, Non-responders, Remitters and Non-remitters. Color scheme is based on r^2 value. Other information can be seen at the Haploview website. LDs of responders, non-responders, remitters and non-remitters are almost same. (For interpretation of the references to color in this figure legend, the reader is reffered to the web version of this article.)

Table 5
Genotype distribution in three SNPs in *GCH1* in each phenotype for the pharmacogenetics study.

SNP[a]	Phenotype	MAF[b]	N	Genotype distribution[c]			Hardy–Weinberg equilibrium *P*-value
				M/M	M/m	m/m	
rs8007267	Responder	0.164	146	104	36	6	0.216
C > T	Non-responder	0.228	116	70	39	7	0.618
	Remitter	0.196	102	67	30	5	0.498
	Non-remitter	0.191	160	107	45	8	0.263
rs3783641	Responder	0.168	146	103	37	6	0.263
T > A	Non-responder	0.233	116	69	40	7	0.710
	Remitter	0.201	102	66	31	5	0.588
	Non-remitter	0.200	160	106	44	10	0.0753
rs841	Responder	0.370	146	62	60	24	0.153
C > T	Non-responder	0.453	116	33	61	22	0.509
	Remitter	0.393	102	40	44	18	0.337
	Non-remitter	0.416	160	55	77	28	0.907

[a] Major allele > minor allele.
[b] MAF: minor allele frequency.
[c] M: major allele, m: minor allele.

A few points of caution should be noted in interpreting the present results. First, the study sample presents with several limitations. Given the relatively small sample size and limited power, the *GCH1* association with SSRI response may be due to type I error. Similarly, the negative findings in the MDD and BP groups may be due to type II error. Also, participants were not screened for Axis II disorders, which may also represent potential confounding factors. Furthermore, the healthy control group was significantly younger than the patient groups; perhaps some of the healthy control participants may develop MDD or BP in the future. In fact, the incidence of major depression is as high as 5% or more (Bowden, 2001). A part of our subjects did not undergo structured interviews, and it is reported that MDD patients who are not diagnosed by structured interview may develop bipolar disorder in the future (Kishi et al., 2010). Second, we did not carry out a mutation scan of *GCH1*. Considering the magnitude of the *GCH1* and the limited sample sizes available, only the three functional effect variant SNPs were analyzed. Third, although plasma levels were assessed in the administration of sertraline and paroxetine, they were not assessed in fluvoxamine. However, these effects should be minimal because no correlation between plasma SSRI concentration and clinical response has been reported (Kasper et al., 1993). Fourth, the present analyses included only study-completers and not those individuals who

Table 6
The pharmacogenetic association study of three SNPs in *GCH1*.

SNP[a]	Phenotype	Genetic model	Allele or genotype	Unadjusted analyses[b]			Adjusted analyses[b,c]		
				OR	95% CI	P^d	OR	95% CI	P^d
rs8007267	Response	Allele	C	1.51	0.974–2.33	0.0648	1.51	0.974–2.33	0.0648
C > T		Dominant	CC	1.63	0.972–2.74	0.0647	1.84	1.05–3.24	**0.0325** (0.390)
		Recessive	CC+CT	1.50	0.484–4.78	0.479	1.25	0.373–4.18	0.711
		Co-dominant	TT	1.98	0.816–4.89	0.130	2.03	0.784–5.26	0.140
			CC	0.660	0.140–2.99	0.586	0.884	0.175–4.48	0.879
	Remission	Allele	C	0.966	0.619–1.51	0.877	0.966	0.619–1.51	0.877
		Dominant	CC	0.948	0.562	1.61	1.09	0.619–1.95	0.759
		Recessive	CC+CT	1.02	0.331–3.46	0.972	1.00	0.305–3.55	0.994
		Co-dominant	TT	0.960	0.393–2.45	0.930	1.09	0.423–2.92	0.855
			CC	0.957	0.187–4.33	0.955	1.02	0.188–5.06	0.978
rs3783641	Response	Allele	T	1.43	0.934–2.20	0.0989	1.43	0.934–2.20	0.0989
T > A		Dominant	TT	1.63	0.977–2.74	0.0619	1.81	1.04–3.17	**0.0370** (0.444)
		Recessive	TT+TA	1.11	0.378–3.18	0.848	0.914	0.287–2.77	0.874
		Co-dominant	AA	1.00	0.244–4.22	1.00	1.35	0.304–6.38	0.696
			TT	1.71	0.724–3.99	0.215	1.72	0.683–4.26	0.239
	Remission	Allele	T	0.994	0.641–1.54	0.978	0.994	0.641–1.54	0.978
		Dominant	TT	0.934	0.555–1.58	0.798	1.10	0.624–1.95	0.747
		Recessive	TT+TA	1.29	0.445–4.26	0.648	1.31	0.432–4.45	0.640
		Co-dominant	AA	0.687	0.140–2.87	0.619	0.705	0.127–3.13	0.654
			TT	1.07	0.450–2.67	0.887	1.25	0.505–3.28	0.632
rs841	Response	Allele	C	1.41	0.992–2.00	0.0555	1.41	0.992–2.00	0.0555
C > T		Dominant	CC	1.86	1.11–3.14	**0.0197** (0.236)	1.84	1.06–3.23	**0.0325** (0.390)
		Recessive	CC+CT	1.19	0.626–2.25	0.594	1.06	0.528–2.09	0.878
		Co-dominant	TT	0.746	0.317–1.76	0.501	0.877	0.351–2.22	0.779
			CC	2.21	1.08–4.60	**0.0313** (0.376)	2.10	0.972–4.58	0.0609
	Remission	Allele	C	1.10	0.770–1.58	0.594	1.10	0.770–1.58	0.594
		Dominant	CC	1.23	0.735–2.06	0.427	1.15	0.661–2.00	0.615
		Recessive	CC+CT	0.990	0.519–1.93	0.976	0.977	0.490–1.98	0.947
		Co-dominantTT	TT	0.996	0.410–2.36	0.993	1.02	0.396–2.56	0.966
			CC	1.28	0.622–2.61	0.506	1.17	0.544–2.53	0.682

[a] Major allele > minor allele.
[b] OR: Odds ratio, CI: Confidence interval.
[c] Analyses adjusted for sex, age, HAMD scores at baseline and SSRI dose at 8 weeks.
[d] Corrected *P* value are in parentheses (We employed 12 Bonferroni multiple tests.).

Table 7
Haplotype-wise analysis of tagging SNPs in *GCH1* for the pharmacogenetics study.

Clinical definition groups	Global *P*-value[a]	Common haplotypes rs8007267–rs378364–rs841	Phenotype	Individual haplotype frequency	OR	95% CI	Individual *P*-value[a,b]
Response	**0.0379**	CTC	Responder	0.636	1.00	1.00	**0.0107 (0.0321)**
			Non-responder	0.520			
		CTT	Responder	0.221	1.58	1.022–2.44	0.111
			Non-responder	0.284			
		TAT	Responder	0.143	1.67	1.01–2.77	0.126
			Non-responder	0.196			
Remission	0.351	CTC	Remitter	0.609	1.00	1.00	0.397
			Non-remitter	0.570			
		CTT	Remitter	0.214	1.36	0.867–2.12	0.154
			Non-remitter	0.271			
		TAT	Remitter	0.177	0.956	0.577–1.58	0.592
			Non-remitter	0.159			

[a] Bold numbers represent significant *P*-value.
[b] Corrected *P* value are in parentheses (We employed 3 Bonferroni multiple tests.).

dropped out of the study. Since the focus was on SSRI response in MDD patients who were able to take tolerate SSRIs without side effects during the treatment protocol, drop-out patients due to side effects were not observed in this study. Finally, demographic data gathered from participants in the present study were limited, thereby neglecting potentially important confounding variables (e.g., education, income, etc.).

In conclusion, our results suggest that *GCH1* may predict response to SSRI in MDD in the Japanese population. However, because our samples are small, it will be important to replicate and confirm these findings in other independent studies utilizing larger samples.

Conflict of interest
No Conflict declared.

Role of funding source
Nothing declared.

Acknowledgments
We thank Ms. M. Miyata, and Ms. S. Ishihara for their technical support. This work was supported in part by research grants from the Japan Ministry of Education, Culture, Sports, Science and Technology, the Ministry of Health, Labor and Welfare, and the Health Sciences Foundation (Research on Health Sciences focusing on Drug Innovation). Dr. Kishi is a postdoctoral fellow for research

abroad, and is additionally supported by the Japan Research Foundation for Clinical Pharmacology and the Ministry of Education.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2012.05.004.

References

Abou-Saleh, M.T., Anderson, D.N., Collins, J., Hughes, K., Cattell, R.J., Hamon, C.G., Blair, J.A., 1995. The role of pterins in depression and the effects of antidepressive therapy. Biological Psychiatry 38, 458–463.

Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.

Blair, J.A., Barford, P.A., Morar, C., Pheasant, A.E., Hamon, C.G., Whitburn, S.B., Leeming, R.J., Reynolds, G.P., Coppen, A., 1984. Tetrahydrobiopterin metabolism in depression. Lancet 2, 163.

Bottiglieri, T., Hyland, K., Laundy, M., Godfrey, P., Carney, M.W., Toone, B.K., Reynolds, E.H., 1992. Folate deficiency, biopterin and monoamine metabolism in depression. Psychological Medicine 22, 871–876.

Bowden, C.L., 2001. Strategies to reduce misdiagnosis of bipolar depression. Psychiatry Services 52, 51–55.

Committee, P.G.C.S, 2009. A framework for interpreting genome-wide association studies of psychiatric disorders. Molecular Psychiatry 14 (10–17).

Coppen, A., Swade, C., Jones, S.A., Armstrong, R.A., Blair, J.A., Leeming, R.J., 1989. Depression and tetrahydrobiopterin: the folate connection. Journal Affective Disorder 16, 103–107.

Dhir, A., Kulkarni, S.K., 2011. Nitric oxide and major depression. Nitric Oxide 24, 125–131.

Dudbridge, F., 2003. Pedigree disequilibrium tests for multilocus haplotypes. Genetic Epidemiology 25, 115–121.

Dunlop, B.W., Nemeroff, C.B., 2007. The role of dopamine in the pathophysiology of depression. Archives of General Psychiatry 64, 327–337.

Hashimoto, R., Ozaki, N., Ohta, T., Kasahara, Y., Kaneda, N., Nagatsu, T., 1990. The plasma tetrahydrobiopterin levels in patients with affective disorders. Biological Psychiatry 28, 526–528.

Ichinose, H., Ohye, T., Takahashi, E., Seki, N., Hori, T., Segawa, M., Nomura, Y., Endo, K., Tanaka, H., Tsuji, S., et al., 1994. Hereditary progressive dystonia with marked diurnal fluctuation caused by mutations in the GTP cyclohydrolase I gene. Nature Genetics 8, 236–242.

Kasper, S., Dotsch, M., Kick, H., Vieira, A., Moller, H.J., 1993. Plasma concentrations of fluvoxamine and maprotiline in major depression: implications on therapeutic efficacy and side effects. European Neuropsychopharmacology 3, 13–21.

Kealey, C., Roche, S., Claffey, E., McKeon, P., 2005. Linkage and candidate gene analysis of 14q22–24 in bipolar disorder: support for GCHI as a novel susceptibility gene. America Journal of Medical Genomics B Neuropsychiatry Genetics 136B, 75–80.

Kishi, T., Yoshimura, R., Kitajima, T., Okochi, T., Okumura, T., Tsunoka, T., Yamanouchi, Y., Kinoshita, Y., Kawashima, K., Fukuo, Y., Naitoh, H., Umene-Nakano, W., Inada, T., Nakamura, J., Ozaki, N., Iwata, N., 2010. SIRT1 gene is associated with major depressive disorder in the Japanese population. Journal Affective Disorder 126, 167–173.

McHugh, P.C., Joyce, P.R., Deng, X., Kennedy, M.A., 2011. A polymorphism of the GTP-cyclohydrolase I feedback regulator gene alters transcriptional activity and may affect response to SSRI antidepressants. Pharmacogenomics Journal 11, 207–213.

McHugh, P.C., Joyce, P.R., Kennedy, M.R., 2009. Polymorphisms of sepiapterin reductase gene alter promoter activity and may influence risk of bipolar disorder. Pharmacogenetics Genomics 19, 330–337.

McLeod, T.M., Lopez-Figueroa, A.L., Lopez-Figueroa, M.O., 2001. Nitric oxide, stress, and depression. Psychopharmacology Bulletin 35, 24–41.

Miura, H., Qiao, H., Kitagami, T., Ohta, T., 2004. Fluvoxamine, a selective serotonin reuptake inhibitor, suppresses tetrahydrobiopterin in the mouse hippocampus. Neuropharmacology 46, 340–348.

Miura, H., Qiao, H., Kitagami, T., Ohta, T., Ozaki, N., 2005. Fluvoxamine, a selective serotonin reuptake inhibitor, suppresses tetrahydrobiopterin levels and dopamine as well as serotonin turnover in the mesoprefrontal system of mice. Psychopharmacology (Berlin) 177, 307–314.

Peveler, R., Kendrick, T., 2005. Selective serotonin reuptake inhibitors: THREAD trial may show way forward. British Medical Journal 330, 420–421.

Russo, S., Kema, I.P., Bosker, F., Haavik, J., Korf, J., 2009. Tryptophan as an evolutionarily conserved signal to brain serotonin: molecular evidence and psychiatric implications. World Journal of Biological Psychiatry 10, 258–268.

Salvadore, G., Quiroz, J.A., Machado-Vieira, R., Henter, I.D., Manji, H.K., Zarate Jr., C.A., 2010. The neurobiology of the switch process in bipolar disorder: a review. Journal of Clinical Psychiatry 71, 1488–1501.

Skol, A.D., Scott, L.J., Abecasis, G.R., Boehnke, M., 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nature Genetics 38, 209–213.

Sumi-Ichinose, C., Urano, F., Kuroda, R., Ohye, T., Kojima, M., Tazawa, M., Shiraishi, H., Hagino, Y., Nagatsu, T., Nomura, T., Ichinose, H., 2001. Catecholamines and serotonin are differently regulated by tetrahydrobiopterin. A study from 6-pyruvoyltetrahydropterin synthase knockout mice. Journal of Biological Chemistry 276, 41150–41160.

Tegeder, I., Costigan, M., Griffin, R.S., Abele, A., Belfer, I., Schmidt, H., Ehnert, C., Nejim, J., Marian, C., Scholz, J., Wu, T., Allchorne, A., Diatchenko, L., Binshtok, A.M., Goldman, D., Adolph, J., Sama, S., Atlas, S.J., Carlezon, W.A., Parsegian, A., Lotsch, J., Fillingim, R.B., Maixner, W., Geisslinger, G., Max, M.B., Woolf, C.J., 2006. GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. Nature Medicine 12, 1269–1277.

Thony, B., Auerbach, G., Blau, N., 2000. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochemical Journal 347 (Pt 1), 1–16.

Zhang, L., Rao, F., Zhang, K., Khandrika, S., Das, M., Vaingankar, S.M., Bao, X., Rana, B.K., Smith, D.W., Wessel, J., Salem, R.M., Rodriguez-Flores, J.L., Mahata, S.K., Schork, N.J., Ziegler, M.G., O'Connor, D.T., 2007. Discovery of common human genetic variants of GTP cyclohydrolase 1 (GCH1) governing nitric oxide, autonomic activity, and cardiovascular risk. Journal of Clinical Investigations 117, 2658–2671.

Effectiveness of Duloxetine for the Treatment of Chronic Nonorganic Orofacial Pain

Wataru Nagashima, MD, Hiroyuki Kimura, MD, PhD,* Mikiko Ito, DDS, PhD,† Tatsuya Tokura, MD,* Munetaka Arao, DDS, PhD,† Branko Aleksic, MD, PhD,* Keizo Yoshida, MD, PhD,‡ Kenichi Kurita, DDS, PhD,† and Norio Ozaki, MD, PhD**

Objective: We examined the pain-relieving effect of duloxetine on chronic nonorganic orofacial pain (burning mouth syndrome and atypical odontalgia), considering the influence of baseline depressive symptoms.

Methods: In this study of 12 weeks, duloxetine was administered in a fixed-flexible dose of 20 to 40 mg/d to 41 patients with burning mouth syndrome and/or atypical odontalgia. Pain was evaluated using the visual analog scale (VAS) at baseline and at 2, 4, 6, 8, 10, and 12 weeks of treatment. Depressive symptoms were assessed using the Hamilton Depression Rating Scale at baseline and at 12 weeks of treatment.

Results: We analyzed the data from 29 patients who completed the study. The VAS score at 12 weeks of treatment was significantly lower than that at baseline. The time course of the VAS scores revealed its significant decrease from 2 weeks of treatment compared to the baseline score. To investigate the influence of baseline depressive symptoms on the pain-relieving effect of duloxetine, the subjects were divided into 2 groups based on the Hamilton Depression Rating Scale score on initial consultation: groups with (≥8) and without (≤7) depressive symptoms. Two-way repeated-measures analysis of variance revealed no significant interaction between time and initial presence or absence of depression. An additional intent-to-treat last-observation-carried-forward analysis including dropped-out patients revealed a similar result.

Conclusion: Duloxetine significantly relieved chronic nonorganic orofacial pain. Its pain-relieving effect appeared from 2 weeks of treatment. Furthermore, the pain-relieving effects of duloxetine similarly appeared regardless of the presence or absence of baseline depressive symptoms.

Key Words: burning mouth syndrome, atypical odontalgia, chronic pain, duloxetine, serotonin-noradrenaline reuptake inhibitor

(*Clin Neuropharm* 2012;35: 00–00)

The incidence of chronic pain is estimated to be approximately 20%.[1] Insufficient pain control may have significant influence on their jobs/daily activities.[1]

Chronic nonorganic orofacial pain is classified into 2 types: burning mouth syndrome (BMS) and atypical odontalgia (AO). Burning mouth syndrome is an idiopathic pain or burning sensation in clinically normal oral mucosa with no causative medical or dental factors. The incidence ranges from 0.7% to 15%.[2] Burning mouth syndrome is frequent in postmenopausal women.[2] Atypical odontalgia refers to tooth pain without pathological abnormalities on clinical/x-ray examination results. Pain involves the region of face, neck, and shoulders in some cases. A study reported that AO occurred in 3% to 6% of patients undergoing dental treatment.[3] Atypical odontalgia is particularly frequent in women in their 40s.[3] The pathogeneses of BMS and AO remain to be clarified.[3] Painful symptoms of BMS and AO often persist, leading to mental problems such as depression.[2,3]

Tricyclic antidepressants (TCAs) are recommended as a first-line treatment of chronic pain.[4] However, TCAs may cause various adverse effects such as dry mouth, constipation, and sleepiness through anticholinergic, anti-α1, and antihistaminic actions, raising issues such as cardiac toxicity[5] and the influence on the cognitive function.[6]

Duloxetine is a serotonin/noradrenaline reuptake inhibitor (SNRI). Its affinity for muscarinic, α1 adrenergic, and histamine H1 receptors is weaker than that of TCAs.[7] Duloxetine is primarily administered to treat major depressive disorder (MDD). With respect to chronic pain, clinical trials and meta-analyses have demonstrated the analgesic efficacy of duloxetine for fibromyalgia[8–10] and painful diabetic neuropathy (PDN).[10–12]

Antidepressants are also used for chronic orofacial pain.[13–15] We reported the pain-relieving effect of milnacipran, an SNRI, on BMS and AO[13,16] in 2010 and 2012. On the other hand, only one case report has suggested a favorable effect of duloxetine, another SNRI, on BMS.[17]

Based on the aforementioned background, in this study, we investigated the effects of duloxetine on chronic nonorganic orofacial pain.

The close relationship has been reported between pain and depression.[18] Pain is associated with the deterioration of depressive symptoms. Conversely, concomitant depression deteriorates the state of pain.[18] In this study, we tried to evaluate depressive symptoms and investigate their influence on pain-relieving effects.

MATERIALS AND METHODS

The subjects were 114 patients who consulted the Liaison Psychiatry Medical Group Outpatient Clinic, Department of Oral Surgery, Aichi-Gakuin Dental School Hospital, between April 2010 and September 2011. Of 82 patients who had a diagnosis of BMS/AO by dentists and with pain disorder based on the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (*DSM-IV-TR*)[19] by psychiatrists, we analyzed 41 patients from whom we obtained written informed consent regarding study participation. Psychiatric diagnoses were made using the Structured Clinical Interview for *DSM-IV* Axis I Disorders. To diagnose pain disorder, it is necessary to evaluate and exclude organic disease directly related to pain by dentists. In the 41 patients, dentists confirmed the absence of organic abnormalities such as topical inflammation based on clinical findings and radiological/hematological

*Department of Psychiatry, Nagoya University Graduate School of Medicine, Nagoya, Japan; †Department of Oral and Maxillofacial Surgery, School of Dentistry, Aichi-Gakuin University, Nagoya, Japan; and ‡Health Care Promotion Division, Denso Corporation, Kariya, Japan.

Conflicts of Interest and Source of Funding: The authors have no conflicts of interest to declare.

Address correspondence and reprint requests to Hiroyuki Kimura, MD, PhD, Department of Psychiatry, Nagoya University, Graduate School of Medicine, 65 Tsurumai Showa, Nagoya, Aichi 466-8550, Japan; E-mail: kimurahi@med.nagoya-u.ac.jp

DOI: 10.1097/WNF.0b013e31827453fa

results if necessary, leading to a diagnosis of pain disorder. In all patients, the disease duration exceeded 6 months. They were regarded as having "chronic pain" based on the definition of pain duration in the "pain disorder" section of the *DSM-IV-TR*.

The exclusion criteria were as follows: (1) a diagnosis of MDD on initial consultation, (2) a history of schizophrenia or other psychotic disorders, or obvious current psychotic symptoms, (3) clinically overt dementia, (4) any serious somatic disorder, (5) previous use of duloxetine, and (6) use of any psychotropic agents within 2 weeks before study participation. In the situation of (6), patients were allowed to enroll in the study if they discontinued drugs for 2 weeks. During this period, only the administration of alprazolam (up to 1.2 mg/d) and brotizolam (up to 0.5 mg/d) were allowed if necessary to relieve withdrawal symptoms.

The initial dose of duloxetine is 20 mg once daily. Two weeks or more after the start of administration, the dose was gradually increased to a maximum of 40 mg once daily, considering symptom changes and adverse effects. This is the recommended regimen for MDD in Japan. Only alprazolam (up to 1.2 mg/d) or brotizolam (up to 0.5 mg/d) could be combined with duloxetine as needed for anxiety and insomnia that might occur early after administration of duloxetine. The use of analgesic was not allowed during the study. Actually, all of the subjects realized that the use of analgesics was not effective on their pain, and none of the subjects requested the medication of analgesics during the study.

Pain was assessed using the visual analog scale (VAS) at baseline and at 2, 4, 6, 8, 10, and 12 weeks of treatment. Depressive symptoms were assessed using the structured interview guide for Hamilton Depression Rating Scale (HDRS) at baseline and at 12 weeks of treatment. The evaluation of pain (using the VAS) and that of depressive symptoms (using the HDRS) were conducted by the attending physicians. To eliminate the onset of acute topical inflammation and oral diseases, dentists confirmed the absence of topical abnormalities through an inquiry/examination at every consultation.

This study was approved by the Ethical Committee of the School of Dentistry, Aichi-Gakuin University, and the Ethics Review Committee of Nagoya University School of Medicine.

STATISTICAL ANALYSIS

The data are expressed as mean (SD). To compare the VAS score at baseline with that at the completion of the study treatment, we used the paired t test. To analyze the time course of VAS scores, one-way repeated-measures analysis of variance (ANOVA) was used. Contrasts were used to enable comparisons of the scores at each time point, when significant results were observed in the aforementioned analysis.

To examine the influence of baseline depressive symptoms on the time course of VAS scores, the patients were divided into 2 groups based on the initial HDRS score: groups with (initial HDRS score $\geq$8) and without (initial HDRS score $\leq$7) depressive symptoms; and analysis was performed using 2-way repeated-measures ANOVA, regarding the depressive status and time as factors. To investigate the influence of the initial HDRS score (not with respect to the groups but as a continuous volume) on the time course of VAS scores, repeated-measures analysis of covariance (ANCOVA)[20] was conducted using the initial HDRS score as a covariate. $P < 0.05$ was regarded as significant.

Differences between enrolled and completed patients were analyzed with the use of the unpaired t test or $\chi 2$ test, where appropriate.

For these analyses, we used StatView version 5.0 (SAS Institute, Inc, Cary, NC) and SuperANOVA version 1.11 (Abacus Concepts, Inc, Berkeley, Calif) software. Power analysis was performed with the use of G*Power 3.[21]

RESULTS

Baseline characteristics are shown in Table 1. There was no significant difference between enrolled and completed patients with regard to age ($P = 0.89$), sex ($P = 0.81$), dental diagnosis ($P = 0.75$), duration of illness ($P = 0.92$), previous antidepressant use ($P = 0.26$), and initial HDRS scores ($P = 0.97$). Twenty-nine of the enrolled 41 patients completed the 12-week study. The mean (SD) final dose of duloxetine was 35.9 (8.2) mg/d. The reasons for dropping out included adverse effects in 6 patients, insufficient efficacy in 1 (the patient refused to continue medication), and others in 5 patient (returning home, family's objection). Thirteen patients had received antidepressants before this study. They used milnacipran (n = 11) or fluvoxamine (n = 2) for treatment of BMS/AO (13 patients) and concomitant anxiety disorder (1 patient), and they did not have good pain-relieving effect of these antidepressants.

When analyzing the 29 patients, the mean (SD) VAS scores at baseline and after 12 weeks were 55.4 (25.9) and 26.4 (22.5), respectively, showing a significant decrease (paired t test: $t = 6.64$, $P < 0.0001$). After 12 weeks, the proportions of patients having 30% and 50% pain-relieving effect compared with baseline were 79.3% (23/29) and 51.7% (15/29), respectively.

When reviewing the time course of VAS scores in the 29 patients, repeated-measures ANOVA revealed a significant result ($df = 6$, $F = 17.22$, $P < 0.0001$), indicating that the mean scores at each time point were not all equivalent (Fig. 1). Contrast analysis indicated that the VAS score significantly decreased from 2 weeks after the baseline (Fig. 1). The analysis excluding 13 patients having taken antidepressants before entry showed a similar result; repeated-measures ANOVA revealed a significant result ($df = 6$, $F = 10.58$, $P < 0.0001$), and the VAS score significantly decreased from 2 weeks after the start of this study (figure not shown).

In consideration of the number of dropped-out patients, the aforementioned repeated-measures ANOVA was performed for all of the enrolled 41 patients in an intent-to-treat last observation carried forward (ITT-LOCF) method. The analysis revealed a significant result ($df = 6$, $F = 20.47$, $P < 0.0001$), and contrast analysis indicated the significant decrease of the VAS score

TABLE 1. Patients' Characteristics, Diagnosis, and Initial HDRS scores

	Enrolled (n = 41)	Completed (n = 29)
Age, yrs*	61.2 (12.8)	60.8 (11.1)
Sex, n (%)		
Male	8 (19.5)	5 (17.2)
Female	33 (80.5)	24 (82.7)
Dental diagnosis, n (%)		
BMS	23 (56.1)	15 (51.7)
AO	17 (41.5)	13 (44.8)
BMS/AO	1 (2.4)	1 (3.4)
Duration of illness, mo*	37.5 (61.5)	39.1 (70.7)
Previous antidepressant use, n (%)	13 (31.7)	13 (44.8)
Initial HDRS scores*	6.9 (5.1)	6.9 (5.2)

*Mean (SD).

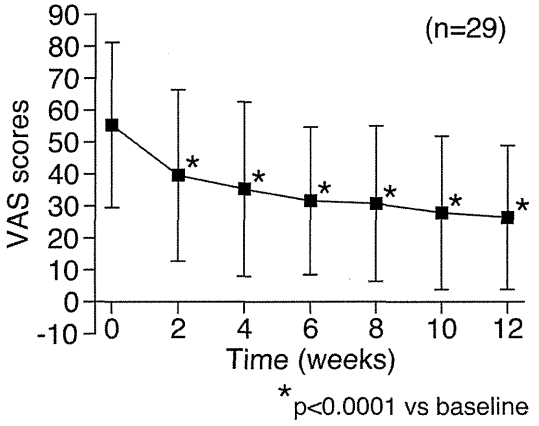

FIGURE 1. The time course of VAS scores in 29 patients who completed this study. Each point represents the mean (SD) score. One-way repeated-measures ANOVA revealed a significant result (df = 6, F = 17.22, $P < 0.0001$), indicating that the mean scores at each point were not all equivalent. Contrast analysis indicated that the VAS score significantly decreased from 2 weeks after the start of this study.

from 2 weeks after the start of this study (Fig. 2). Both the results of per-protocol and ITT-LOCF analysis support an early and significant pain-relieving effect of duloxetine.

To investigate the influence of baseline depressive symptoms on pain-reducing effects, the 29 completed patients were divided into 2 groups based on the initial HDRS score: groups with (initial HDRS score ≥8) and without (initial HDRS score ≤7) depressive symptoms, and the time course of VAS scores was compared between the 2 groups. Two-way repeated-measures ANOVA revealed no significant interaction between time and initial presence or absence of depression (df = 6, F = 0.58, $P = 0.75$). The VAS score similarly decreased regardless of the presence or absence of baseline depressive symptoms (Fig. 3). In addition, to investigate the influence of baseline depressive symptoms (not with respect to the groups but as a continuous volume) on pain-relieving effects, repeated-measures ANCOVA was conducted using the initial HDRS score as a covariate. Repeated-measures ANCOVA revealed no significant interaction between time and initial HDRS score interaction (F = 1.44, df = 6, $P = 0.20$). The VAS score similarly decreased regardless of the initial HDRS scores.

In the one-way repeated-measures ANOVA, to analyze the time course of VAS scores for the per-protocol analysis (n = 29), this study had a power of 0.20 to detect a small effect, 0.88 to detect a medium effect, and 0.99 to detect a large effect. For the ITT-LOCF analysis (n = 41), this study had a power of 0.25 to detect a small effect, 0.95 to detect a medium effect, and 0.99 to detect a large effect. In the power analysis, effect size conventions were determined according to Cohen[22] as follows: small effect size, 0.10; medium effect size, 0.25; and large effect size, 0.40 ($\alpha = 0.05$).

Adverse effects of duloxetine are shown in Table 2. Of the 41 enrolled patients, the adverse effect of nausea was most frequently observed in 7 patients (17.0%).

DISCUSSION

The results showed that duloxetine significantly relieved chronic nonorganic orofacial pain. Its pain-relieving effect appeared from 2 weeks of treatment.

Among various antidepressants, TCAs are commonly used for chronic pain. However, recently, SNRIs have also been recommended.[4] According to a review in which the analgesic effects of antidepressants on neuropathic pain were compared using the number needed to treat, the numbers needed to treat TCAs, SNRIs, and SSRIs were 3.1, 5.5, and 6.8, respectively[23]; SNRIs were superior in therapeutic efficacy to SSRIs, although not comparable with TCAs. Both the serotonin and noradrenaline nervous systems play an important role in pain control in the descending pain inhibitory system.[24] Iyengar et al[25] demonstrated that duloxetine significantly attenuated late-phase paw-licking behavior in the formalin model in rats and its attenuating effect was more potent than venlafaxine, milnacipran, and amitriptyline. A meta-analysis regarding the therapeutic effects of duloxetine, milnacipran, and pregabalin in patients with

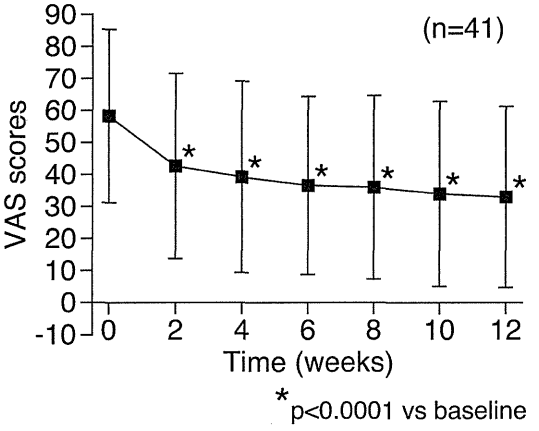

FIGURE 2. The time course of VAS scores in all of the enrolled 41 patients. Each point represents the mean (SD) score. One-way repeated-measures ANOVA was performed for all the enrolled 41 patients in an ITT-LOCF method. The analysis revealed a significant result (df = 6, F = 20.47, $P < 0.0001$), and contrast analysis indicated the significant decrease of the VAS score from 2 weeks after the start of this study.

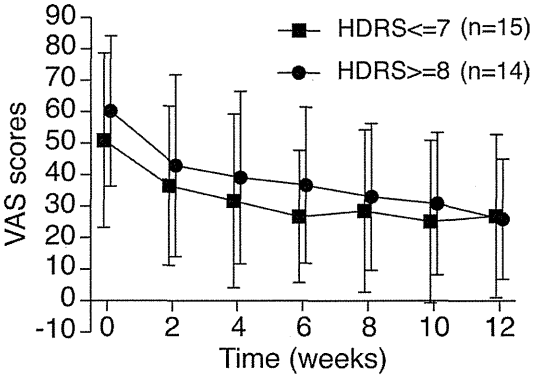

FIGURE 3. The time course of VAS scores with respect to the presence or absence of depressive symptoms at the start of this study (depressive patients, HDRS ≥8; nondepressive patients, HDRS ≤7). Each point represents the mean (SD) score. Two-way repeated-measures ANOVA revealed no significant interaction between time and initial presence or absence of depression (df = 6, F = 0.58, $P = 0.75$).

TABLE 2. Adverse Events With Duloxetine; N = 41

Adverse Event	Number of Patients (%)
Nausea	7 (17.0)
Constipation	5 (12.2)
Somnolence	4 (9.6)
Fatigue	3 (7.3)
Dry mouth	3 (7.3)
Diarrhea	2 (4.9)
Urinary retention	2 (4.9)
Insomnia	1 (2.4)
Vomit	1 (2.4)
Dizziness	1 (2.4)

fibromyalgia also demonstrated that duloxetine was most effective.[26] These studies support the therapeutic effects of duloxetine on chronic nonorganic orofacial pain observed in this study.

Chronic pain is closely associated with depressive states and depression. According to Ohayon and Schatzberg,[27] 27.6% of persons with at least one depressive symptom (sadness, depression, hopelessness, loss of interest, or lack of pleasure) complained of pain symptoms. In addition, 43.4% of patients with a diagnosis of MDD complained of pain symptoms.[27] Another study reported that antidepressant therapy reduced depression and pain symptoms in depression patients with pain symptoms (headache, shoulder pain, and back pain).[28] Based on these results, it is controversial whether the pain-reducing effects obtained in this study were independent or accompanied antidepressant effects. In this study, pain subsided through similar time course regardless of the presence or absence of baseline depressive symptoms. Initial HDRS scores also did not affect the time course of manifestation of the pain-relieving effect. Even in patients without baseline depressive symptoms, the pain-relieving effect appeared as observed in those with depressive symptoms, suggesting that effect of duloxetine is independent of antidepressant actions. This is consistent with the results of our previous study with milnacipran (SNRI).[13] A review regarding the analgesic effects of duloxetine on fibromyalgia and PDN also indicated the direct effects of duloxetine rather than depression improvement–related secondary analgesic effects.[29]

In this study, 6 patients (14.6%) dropped out owing to adverse effects. According to several studies regarding the safety and tolerability after duloxetine administration to patients with chronic pain (fibromyalgia[30] and PDN[31]), the adverse effect–related dropout rate ranges from 12.1% to 21.1%, which is consistent with the results of this study. In this study, duloxetine administration was increased from 20 mg/d carefully. However, if a lower-dose form (10 mg) were available, it might have further decreased the incidence of adverse effects and dropout cases.

The reported incidence of duloxetine-related dry mouth is 15%.[32] In this study, the incidence of duloxetine-related dry mouth was lower (3 patients [7.3%]). In a study involving the administration of amitriptyline (≥10 mg/d) to 30 patients with chronic orofacial pain, dry mouth appeared in 8 patients (26.7%) at high rates.[14] Considering the oral sanitary state–maintaining actions of saliva, TCA administration–induced dry mouth may become a stress-related factor for patients with oral disorders. Therefore, duloxetine may be more useful than TCAs in patients with oral disorders, considering its effects on pain and adverse effects.

There are several limitations to be considered when interpreting the results of our study. The evaluation of pain and depressive symptoms was conducted by the attending physicians, not the independent investigators. This situation made it impossible to completely avoid bias in evaluating pain and depressive symptoms. The effects of duloxetine on pain were examined before and after administration. However, to evaluate its analgesic effects, a randomized, double-blind, placebo-controlled trial should be conducted. The subjects of this study included patients without depressive symptoms and those with depressive symptoms not enough to fulfill the *DSM-IV-TR* criteria for MDD. The purpose of this selection was to accurately evaluate the effect of duloxetine on BMS/AO under its own pathophysiology, without that of comorbid disorders. However, pain and depression frequently coexists in clinical practice; therefore, it must also be investigated whether the pain-reducing effects are obtained in BMS/AO patients with MDD. Finally, the use of pain-specific multidimensional scales such as the short-form McGill Pain Questionnaire[33] would be helpful in a future study to fully evaluate diverse characteristics of chronic pain.

CONCLUSION

Duloxetine administration to patients with chronic nonorganic orofacial pain for 12 weeks significantly relieved pain. Furthermore, its effects began to appear 2 weeks after the start of administration. Similar pain-reducing effects were achieved regardless of the presence or absence and grade of baseline depressive symptoms.

REFERENCES

1. Reid KJ, Harker J, Bala MM, et al. Epidemiology of chronic non-cancer pain in Europe: narrative review of prevalence, pain treatments and pain impact. *Curr Med Res Opin* 2011;27:449–462.
2. Zakrzewska JM, Forssell H, Glenny AM. Interventions for the treatment of burning mouth syndrome. *Cochrane Database Syst Rev* 2005;(1):CD002779. DOI:10.1002/14651858.CD002779.pub2.
3. Melis M, Lobo SL, Cenevis C, et al. Atypical odontalgia: a review of the literature. *Headache* 2003;43:1060–1074.
4. Kroenke K, Krebs EE, Bair MJ. Pharmacotherapy of chronic pain: a synthesis of recommendations from systematic reviews. *Gen Hosp Psychiatry* 2009;31:206–219.
5. Ray WA, Meredith S, Thapa PB, et al. Cyclic antidepressants and the risk of sudden cardiac death. *Clin Pharmacol Ther* 2004;75:234–241.
6. Hindmarch I, Rigney U, Stanley N, et al. Pharmacodynamics of milnacipran in young and elderly volunteers. *Br J Clin Pharmacol* 2000;49:118–125.
7. Bymaster FP, Dreshfield-Ahmad LJ, Threlkeld PG, et al. Comparative affinity of duloxetine and venlafaxine for serotonin and norepinephrine transporters in vitro and in vivo, human serotonin receptor subtypes, and other neuronal receptors. *Neuropsychopharmacology* 2001; 25:871–880.
8. Arnold LM, Rosen A, Pritchett YL, et al. A randomized, double-blind, placebo-controlled trial of duloxetine in the treatment of women with fibromyalgia with or without major depressive disorder. *Pain* 2005; 119:5–15.
9. Häuser W, Bernardy K, Uçeyler N, et al. Treatment of fibromyalgia syndrome with antidepressants: a meta-analysis. *JAMA* 2009; 301:198–209.
10. Lunn MP, Hughes RA, Wiffen PJ. Duloxetine for treating painful neuropathy or chronic pain. *Cochrane Database Syst Rev* 2009;(4):CD007115. DOI:10.1002/14651858.CD007115.pub2.
11. Goldstein DJ, Lu Y, Detke MJ, et al. Duloxetine vs. placebo in patients with painful diabetic neuropathy. *Pain* 2005;116:109–118.

12. Wernicke JF, Pritchett YL, D'Souza DN, et al. A randomized controlled trial of duloxetine in diabetic peripheral neuropathic pain. *Neurology* 2006;67:1411–1420.
13. Ito M, Kimura H, Yoshida K, et al. Effectiveness of milnacipran for the treatment of chronic pain in the orofacial region. *Clin Neuropharmacol* 2010;33:79–83.
14. Ikawa M, Yamada K, Ikeuchi S. Efficacy of amitriptyline for treatment of somatoform pain disorder in the orofacial region: a case series. *J Orofac Pain* 2006;20:234–240.
15. Pinto A, Sollecito TP, DeRossi SS. Burning mouth syndrome. 1A retrospective analysis of clinical characteristics and treatment outcomes. *N Y State Dent J* 2003;69:18–24.
16. Kimura H, Yoshida K, Ito M, et al. Plasma levels of milnacipran and its effectiveness for the treatment of chronic pain in the orofacial region. *Hum Psychopharmacol Clin Exp* 2012;27:322–328.
17. Mignogna MD, Adamo D, Schiavone V, et al. Burning mouth syndrome responsive to duloxetine: a case report. *Pain Med* 2011; 12:466–469.
18. Bair MJ, Robinson RL, Katon W, et al. Depression and pain comorbidity: a literature review. *Arch Intern Med* 2003;163: 2433–2445.
19. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision (DSM -IV-TR).* Washington DC: APA; 2000.
20. Urdan TC. Repeated-measures analysis of variance. In: *Statistics in Plain English*. 2nd ed. Mahwah: Lawrence Erlbaum Associates; 2005:129–144.
21. Faul F, Erdfelder E, Lang AG, et al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007;39:175–191.
22. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*, 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.
23. Mico JA, Ardid D, Berrocoso E, et al. Antidepressants and pain. *Trends Pharmacol Sci* 2006;27:348–354.
24. Millan MJ. Descending control of pain. *Prog Neurobiol* 2002;66: 355–474.
25. Iyengar S, Webster AA, Hemrick-Luecke SK, et al. Efficacy of duloxetine, a potent and balanced serotonin-norepinephrine reuptake inhibitor in persistent pain models in rats. *J Pharmacol Exp Ther* 2004;311:576–584.
26. Häuser W, Petzke F, Sommer C. Comparative efficacy and harms of duloxetine, milnacipran, and pregabalin in fibromyalgia syndrome. *J Pain* 2010;11:505–521.
27. Ohayon MM, Schatzberg AF. Using chronic pain to predict depressive morbidity in the general population. *Arch Gen Psychiatry* 2003; 60:39–47.
28. Raskin J, Wiltse CG, Siegal A, et al. Efficacy of duloxetine on cognition, depression, and pain in elderly patients with major depressive disorder: an 8-week, double-blind, placebo-controlled trial. *Am J Psychiatry* 2007;164:900–909.
29. Wright A, Luedtke KE, Vandenberg C. Duloxetine in the treatment of chronic pain due to fibromyalgia and diabetic neuropathy. *J Pain Res* 2010;4:1–10.
30. Choy EH, Mease PJ, Kajdasz DK, et al. Safety and tolerability of duloxetine in the treatment of patients with fibromyalgia: pooled analysis of data from five clinical trials. *Clin Rheumatol* 2009;28:1035–1044.
31. Hall JA, Wang F, Oakes TM, et al. Safety and tolerability of duloxetine in the acute management of diabetic peripheral neuropathic pain: analysis of pooled data from three placebo-controlled clinical trials. *Expert Opin Drug Saf* 2010;9:525–537.
32. Bellingham GA, Peng PW. Duloxetine: a review of its pharmacology and use in chronic pain management. *Reg Anesth Pain Med* 2010; 35:294–303.
33. Melzack R. The short-form McGill Pain Questionnaire. *Pain* 1987;30:191–197.

HUMAN PSYCHOPHARMACOLOGY
Hum. Psychopharmacol Clin Exp 2012; **27**: 322–328.
Published online in Wiley Online Library
(wileyonlinelibrary.com) **DOI**: 10.1002/hup.2230

Plasma levels of milnacipran and its effectiveness for the treatment of chronic pain in the orofacial region

Hiroyuki Kimura[1]*, Keizo Yoshida[2], Mikiko Ito[3], Tatsuya Tokura[1], Wataru Nagashima[1], Kenichi Kurita[3] and Norio Ozaki[1]

[1]*Department of Psychiatry, Nagoya University Graduate School of Medicine, Japan*
[2]*Health Care Promotion Division, DENSO CORPORATION, Japan*
[3]*Department of Oral and Maxillofacial Surgery, School of Dentistry, Aichi-Gakuin University, Japan*

Objectives This study was performed to assess the relationship between plasma levels of milnacipran and its analgesic/antidepressive effect in patients with chronic orofacial pain treated with this drug.
Methods A total of 44 patients took milnacipran for 12 weeks. Patients were assessed for their pain and depressive symptoms using the visual analog scale (VAS) and Hamilton Depression Rating Scale, respectively. The plasma milnacipran level was also assessed at week 12.
Results Forty patients completed study treatment and were included in the analysis. In these patients, the VAS score at week 12 significantly decreased from the baseline score ($t=5.15$, $p<0.0001$). The dose of milnacipran was positively correlated in a linear manner with the plasma level of the drug ($Y=44.86+0.33X$, $r=0.54$, $R^2=0.29$, $p=0.0004$). A quadratic regression curve was plotted between the percentage of decrease in the VAS score and plasma milnacipran level ($Y=27.39+0.76X-0.008X^2$, $p=0.048$, $r=0.40$, $R^2=0.16$). On the other hand, no significant relationship was noted between the percentage of decrease in the Hamilton Depression Rating Scale score and plasma milnacipran level.
Conclusion The analgesic effect of milnacipran was suppressed in the presence of the plasma level of the drug outside the therapeutic range, whereas its antidepressant effect was not affected by its plasma level. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS—chronic pain; pain disorder; milnacipran; SNRI; liaison psychiatry; blood concentration

INTRODUCTION

Pain treatment has recently attracted attention from different angles because of the association between pain and considerable social loss ([No authors listed] 2010). The use of antidepressants for pain relief, which is now one of the general treatment options, originated from the incidental discovery of their analgesic effect in 1960 (Paoli *et al.*, 1960). The pain-relief mechanism of these drugs is considered to be associated with the action of 5-hydroxytryptamine and norepinephrine that leads to an increased intrinsic analgesic mechanism through the descending pain suppression pathway in the brain and spinal cord. The pain-relief effect of antidepressant therapy has been demonstrated by the results of a first randomized controlled trial of this field conducted in patients with chronic headache in 1964 (Lance and Curran, 1964) and several subsequent studies (Max *et al.*, 1988; Sindrup *et al.*, 1990; Sindrup *et al.*, 1991; Rasmussen *et al.*, 2004; Atkinson *et al.*, 2007). Antidepressants have been used most commonly in patients with diabetic peripheral neuropathy, followed by chronic back pain, fibromyalgia, and others. The relationship between the plasma antidepressant level and its pain-relief effect was first reported by a study using imipramine for pain in 1984 (Kvinesdal *et al.*, 1984). Several other studies also investigated such a relationship for tricyclic antidepressants (TCAs; Max *et al.*, 1988; Sindrup *et al.*, 1990; Rasmussen *et al.*, 2004) and paroxetine, a selective serotonin reuptake inhibitor (SSRI; Sindrup *et al.*, 1991). Many of them have shown an increased analgesic effect in a concentration-dependent manner, whereas some studies using amitriptyline (Watson, 1984; Watson *et al.*, 1982) and desipramine (Atkinson *et al.*, 2007) suggested the therapeutic range of the plasma antidepressant level. To date, no studies have been conducted to assess such correlation using

*Correspondence to: H. Kimura, MD, PhD, Department of Psychiatry, Nagoya University, Graduate School of Medicine, 65 Tsurumai, Showa, Nagoya, Aichi 466-8550, Japan. Tel: +81 52 7442282; Fax: +81 52 7442293. E-mail: kimurahi@med.nagoya-u.ac.jp

Received 27 September 2011
Accepted 15 March 2012

serotonin norepinephrine reuptake inhibitors (SNRIs), including milnacipran.

Both burning mouth syndrome (BMS) and atypical odontalgia (AO) are chronic orofacial pain of unknown etiology with no organic findings and observed commonly in middle-aged women. BMS is an idiopathic pain or burning sensation in clinically normal oral mucosa with no causative medical or dental factors. In 1920, Engman (1920) first reported this disease as a burning tongue noted in nine patients with tongue cancer concerns. Many studies have demonstrated the prevalence of BMS in middle-aged women with altered hormone levels or some psychological disorders. However, there is still controversy in the pathophysiology of BMS (Scada *et al.*, 2003). AO is tooth pain in patients with no established dental abnormalities in clinical or radiological findings. The pain may be extended to the face, neck, or shoulders (Melis *et al.*, 2003). This condition was first reported by McElin and Horton, 1947 (1947). AO is most commonly reported in women in their forties although it can be observed in people of all ages except for children (Melis *et al.*, 2003). The precise pathological mechanism of AO is also unknown. Therefore, no consensus has been reached regarding the diagnosis and treatment of these diseases (Scada *et al.*, 2003; Melis *et al.*, 2003). Prolonged cases are often encountered, and patients are likely to suffer from anxiety or depression resulting from refractory pain (Scada *et al.*, 2003; Melis *et al.*, 2003).

Tricyclic antidepressants have already been demonstrated to be more effective for chronic pain than other antidepressants. However, it is also well recognized that adverse effects associated with the use of these drugs are of clinical concern. In particular, dry mouth may result in the discontinuation of treatment in patients with oral symptoms. Compared with TCAs, SNRIs such as milnacipran are less associated with adverse effects, and several studies have conducted treatment for chronic pain using this class of drugs (Toyofuku, 2003; Kamata *et al.*, 2004; Vitton *et al.*, 2004; Gendreau *et al.*, 2005; Ito *et al.*, 2007; Sugimoto, 2011).

In 2010, we first reported the efficacy of milnacipran for chronic orofacial pain with or without the presence of depressive symptoms (Ito *et al.*, 2010). This study was conducted to determine the correlation between the plasma milnacipran level and its pain-relief effect for the first time in a larger sample size. In addition, we investigated the relationship between the plasma level of the drug and its antidepressive effect, for which few studies have been conducted (Higuchi *et al.*, 2003).

PATIENTS AND METHODS

A total of 421 patients first visited the outpatient service of the liaison team in Aichi Gakuin University Dental Hospital (Nagoya, Aichi, Japan) between 2005 September and 2010 March. Of them, 152 patients were diagnosed with pain disorders by both expert dentists and expert psychiatrists using semi-structured interview based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision. Forty-four of these patients who did not meet any of the exclusion criteria described below were finally enrolled in the study after they gave written consent to participate in the study. All these patients had been suffering from pain for more than 6 months. They were diagnosed with chronic pain according to the definition of pain duration described in the pain disorders section of Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision.

A diagnosis of BMS or AO was established in 44 patients after obtaining clinical dental findings and hematological/radiological results as necessary to confirm no local inflammation and other organic abnormalities. Three of the 44 patients also had a diagnosis of major depressive disorder (MDD).

The exclusion criteria were (i) a previous diagnosis of schizophrenia or other psychotic disorders, or current obvious psychotic symptoms such as hallucination and delusion seen at the time of the initial hospital visit; (ii) clinically overt dementia; (iii) use of any psychotropic agent within 2 weeks prior to study participation (patients taking psychotropic agents were allowed to be enrolled in the study if they discontinued the drug for 2 weeks after they agreed to do so), (iv) any serious somatic disorder; and (v) previous use of milnacipran prior to study participation.

Milnacipran was administered at an initial dose of 15 mg/day, followed by a gradual dose increase up to 100 mg/day while monitoring the patient's symptoms and development of adverse events. Patients took the initial daily dose of milnacipran (15 mg) once in the evening every day. After dose increase, the drug was administered in two divided doses to be taken after lunch and before bedtime. Only alprazolam (up to 1.2 mg/day) and brotizolam (up to 0.5 mg/day) as required were allowed to be used concomitantly with milnacipran during the study. Any regularly prescribed psychotropic agents were discontinued before the study treatment. No non-steroidal anti-inflammatory drugs and other pain-relief medications were used during the study.

Pain and depressive symptoms were assessed using the visual analog scale (VAS) and the Structured Interview Guide for the Hamilton Depression Rating

Hum. Psychopharmacol Clin Exp 2012; **27**: 322–328.
DOI: 10.1002/hup

Scale (HDRS), respectively, at baseline and 1, 2, 4, 6, 8, 10, and 12 weeks of study treatment. Patients consulted a dentist at every hospital visit to exclude any new acute local inflammation or oral diseases as well as any local abnormalities. Blood samples were obtained approximately 12 h after the final dose at week 12 of the study. The blood milnacipran level was assessed using high-performance liquid chromatography, as described by Higuchi *et al.* (2003).

The conduct of this study was approved by the Ethical Committee of the School of Dentistry, Aichi Gakuin University, and the Ethics Review Committee of Nagoya University School of Medicine.

STATISTICAL ANALYSIS

Statistical data are expressed as mean ± standard deviation. A paired *t*-test was used for comparison of the VAS score at baseline and that at the completion of study treatment. Linear or quadratic regression was used for analysis of the relationship between daily milnacipran dose and plasma milnacipran level, as well as that between plasma milnacipran level and VAS or HDRS scores. Two-way repeated-measures analysis of variance was used for analysis of the relationship between the initial pain severity and pain-relieving effect of milnacipran. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Forty (90.9%) of the 44 patients included in the study completed the 12-week study treatment. The remaining four patients discontinued the study owing to the development of nausea (two patients) and headache (two patients). The 40 patients who completed the study included 5 men and 35 women, and their mean age was 58.3 ± 12.9 years, ranging from 22 to 76 years. They had been suffering from pain between 6 and 146 months with a mean duration of disease of 30 ± 5.8 months. Twenty-eight patients were diagnosed with BMS, and 12 had AO (Table 1). The mean dose of milnacipran at the time of study completion was 65.8 ± 22.8 mg/day.

Of the 40 patients who completed the study, 4 and 3 reported nausea and constipation during the study period, respectively. These events had resolved after treatment with domperidone for nausea and a bowel-stimulating laxative for constipation.

Visual analog scale scores at baseline and week 12 of the study treatment are shown in Figure 1. The VAS score at week 12 of the study (27.4 ± 23.1) significantly decreased from the baseline score (47.5 ± 27.1) in the 40 patients who completed the study (paired *t*-test, $t = 5.15$, $p < 0.0001$).

The dose of milnacipran was positively correlated in a linear manner with the plasma level of the drug ($Y = 46.11 + 0.30X$, $R^2 = 0.27$, $p = 0.0006$, $n = 40$, Figure 2). An analysis of correlation between the percentage of decrease in the VAS score and plasma milnacipran level revealed a quadratic regression curve that indicates smaller percentages of VAS score decrease in the presence of the plasma level of the drug outside the therapeutic range ($Y = 27.39 + 0.76X - 0.008X^2$, $p = 0.048$, $r = 0.40$, $R^2 = 0.16$, $n = 40$, Figure 3). On the other hand, no significant linear regression ($p = 0.84$) or quadratic regression curve ($p = 0.83$) was noted between the percentage of decrease in the HDRS score and plasma milnacipran level (Figure 4). An analysis for 20 patients who had an HDRS score of 8 points or more (i.e., overt depressive symptoms) at baseline also showed no significant linear regression ($p = 0.09$) or quadratic regression curve ($p = 0.25$, data not shown). An analysis of correlation between the final HDRS score and plasma milnacipran level revealed no significant linear regression ($p = 0.22$) or quadratic regression curve ($p = 0.47$, data now shown).

To investigate the effect of initial pain severity on the pain-relieving effect of milnacipran, we divided the patients into two groups on the basis of their initial VAS scores of ≥50 ($n = 17$) and <50 ($n = 23$) in accordance with the recent study (Ko *et al.*, 2011), which investigated the efficacy of clonazepam for BMS. Two-way repeated-measures analysis of variance revealed a significant Time × Initial pain severity interaction ($df = 7$, $F = 10.25$, $p < 0.0001$, data not shown), indicating a better pain-relieving effect in patients with more severe initial painful symptoms.

DISCUSSION

This study was performed to first investigate the relationship between the plasma milnacipran level and its analgesic effect for chronic orofacial pain in a larger sample size than that used in our previous study conducted in 2010 (Ito *et al.*, 2010). The results suggested the therapeutic range of the plasma milnacipran level for its pain-relief effect, as indicated by the findings showing the suppression of pain relief in the presence of the plasma level of the drug outside this therapeutic range.

A number of studies that investigated such a relationship for TCA (Sindrup *et al.*,1990; Rasmussen *et al.*, 2004) and paroxetine, SSRI (Sindrup *et al.*, 1991), have shown an increased analgesic effect in a concentration-dependent manner. On the other hand, some studies

DOI: 10.1002/hup

Table 1. Patient characteristics and diagnosis

Patient no.	Age (y)	Sex	Duration of illness (months)	Milnacipran dose at week 12 (mg)	Stressful occurrence	Dental diagnose	DSM-IV-TR
1	64	F	16	50	Retirement	AO	PD
2	69	F	18	50	None	BMS	PD
3	55	F	18	40	Dental prosthesis	BMS	PD
4	43	F	6	75	Trouble with person	BMS	PD
5	70	F	6	75	Dental prosthesis	BMS	PD
6	70	F	7	75	Root canal treatment	AO	PD
7	57	F	14	45	Root canal treatment	AO	PD
8	26	F	22	100	Overwork	AO	PD
9	53	F	120	100	Losing husband	BMS	PD
10	40	F	96	50	Domestic violence	AO	PD
11	22	M	18	100	Overwork	AO	PD
12	59	F	6	75	Changing post	BMS	PD
13	49	F	30	75	Stomach trouble	BMS	PD
14	74	F	8	100	None	AO	PD
15	71	F	6	100	Trouble with person	BMS	PD
16	65	M	8	75	Retirement	BMS	PD, MDD
17	74	F	24	100	Trouble with person	BMS	PD
18	70	M	120	100	Losing mother	BMS	PD, MDD
19	62	F	12	75	Moving	BMS	PD
20	64	F	7	50	Dental prosthesis	BMS	PD
21	62	F	24	30	Dental prosthesis	BMS	PD
22	65	F	20	50	Retirement	BMS	PD, MDD
23	55	F	24	40	Root canal treatment	AO	PD
24	63	F	9	50	Extraction a tooth	AO	PD
25	60	F	7	100	Dental prosthesis	BMS	PD
26	65	F	21	65	Dental prosthesis	BMS	PD
27	67	F	120	50	Trouble with family	BMS	PD
28	49	F	42	40	Trouble with family	BMS	PD
29	67	F	20	50	Trouble with husband	AO	PD
30	60	F	7	50	None	BMS	PD
31	55	F	7	75	hearing loss	BMS	PD
32	76	F	24	50	Dental prosthesis	BMS	PD
33	44	F	9	75	None	BMS	PD
34	65	F	19	50	Root canal treatment	AO	PD
35	33	M	36	40	None	BMS	PD
36	56	M	47	100	Dental prosthesis	BMS	PD
37	41	F	12	75	Oral ulcer	BMS	PD
38	54	F	42	30	Dental prosthesis	BMS	PD
39	66	F	146	50	Root canal treatment	BMS	PD
40	72	F	12	50	Dental prosthesis	BMS	PD

DSM-IV-TR, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision; AO, atypical odontalgia; BMS, burning mouth syndrome (BMS); PD, pain disorder; MDD, major depressive disorder.

using TCAs including amitriptyline (Watson, 1984; Watson *et al.*, 1982) and desipramine (Atkinson *et al.*, 2007) suggested the therapeutic range of the plasma antidepressant level. However, these studies provided no hypothesis explaining the decreased pain-relief effect in the presence of the elevated plasma drug level. A study that investigated the antidepressive effect of TCAs proposed a hypothesis regarding the decreased antidepressive effect in higher plasma drug levels (Kragh-Sørensen *et al.*, 1973). That is, TCAs exert the antidepressive effect by increasing the monoamine level in the synaptic cleft to promote neurotransmission through their reuptake-inhibiting effect; however, an excessive plasma drug level may trigger significant expression of antagonistic action in various receptors, offsetting the neurotransmission-promoting effect through reuptake inhibition (Kragh-Sørensen *et al.*, 1973). This hypothesis may also hold true for the relationship between the plasma TCA level and its analgesic effect but cannot be applied to the plasma level of milnacipran, which has no receptor-antagonistic effect. It is difficult to precisely explain the mechanism of the aforementioned therapeutic range seen in the analgesic action of milnacipran on the basis of its pharmacological profile currently available. Dose increase is a common practice in the clinical setting when no sufficient therapeutic response is observed; however, it may be rather an adverse effect to increase the dose injudiciously if there is a therapeutic range during therapy. Further investigation is necessary to clarify the relationship between the plasma level of SNRIs and their pain-relief effect. On the other hand, no significant correlation

DOI: 10.1002/hup

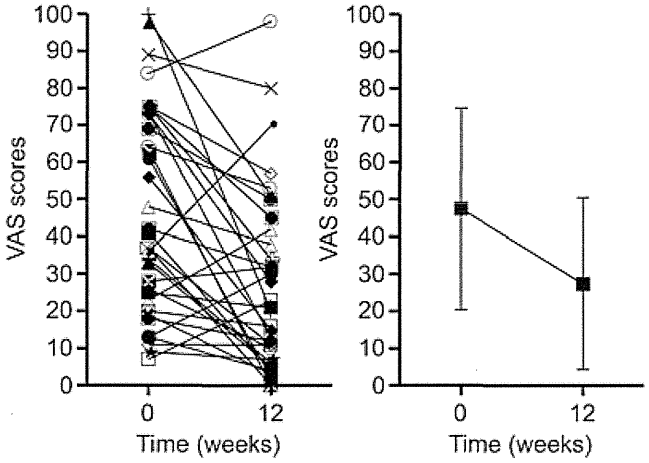

Figure 1. Visual analog scale score at week 12 of the study (27.4 ± 23.1) significantly decreased from the baseline score (47.5 ± 27.1) in the 40 patients who completed the study (paired *t*-test, $t = 5.15$, $p < 0.0001$)

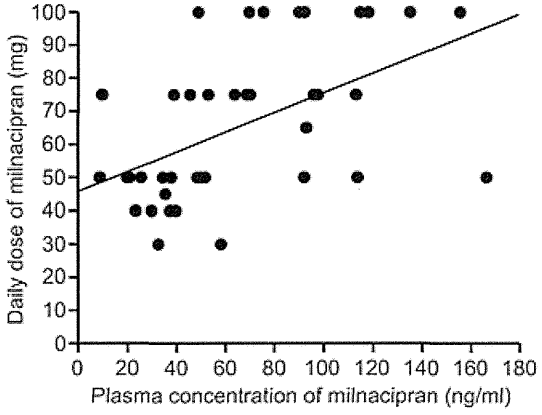

Figure 2. Dose of milnacipran was positively correlated in a linear manner with the plasma level of the drug ($Y = 46.11 + 0.30X$, $R^2 = 0.27$, $p = 0.0006$, $n = 40$)

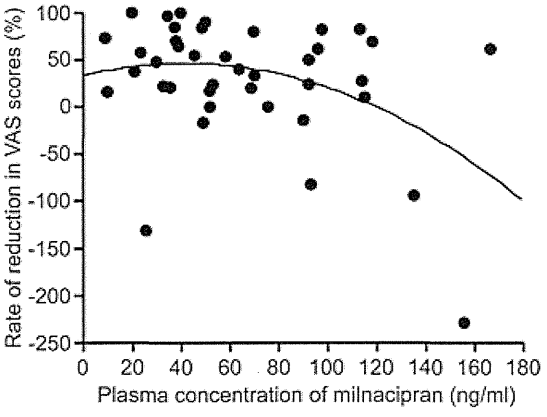

Figure 3. An analysis of correlation between the percentage of decrease in the visual analog scale score and plasma milnacipran level revealed a quadratic regression curve that indicates smaller percentages of visual analog scale score decrease in the presence of the plasma level of the drug outside the therapeutic range ($Y = 27.39 + 0.76X - 0.008X^2$, $p = 0.048$, $R^2 = 0.16$, $n = 40$)

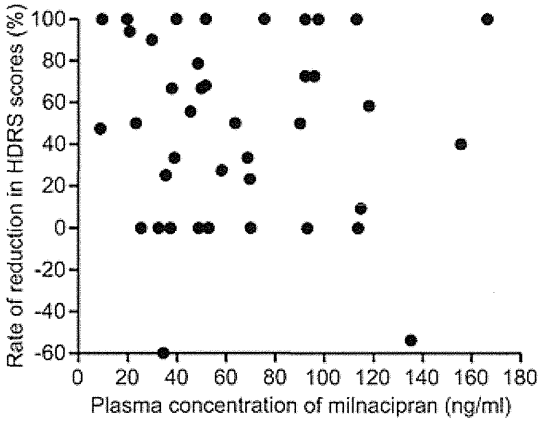

Figure 4. No significant linear regression ($p = 0.84$) or quadratic regression curve ($p = 0.83$) was noted between the percentage of decrease in the Hamilton Depression Rating Scale score and plasma milnacipran level ($n = 40$)

was noted between the plasma milnacipran level and its antidepressive effect. Although patients who participated in this study had different degrees of depressive symptoms, including only three patients with a diagnosis of MDD, this finding is consistent with that reported by Higuchi *et al.* (2003) for MDD patients.

This study was conducted in a fixed-flexible dosing design, which caused limitation of a possible bias that the plasma milnacipran level increased by increasing the dose of the drug in patients less responsive to analgesic therapy. Another limitation is the use of an antidepressant in patients with a lower HDRS score representing the absence of MDD. The results of this study demonstrated the therapeutic range in the pain-relief effect of milnacipran while revealing no significant correlation between the plasma drug level and its antidepressive effect. This suggests that there may be different mechanisms of action of the drug in the treatment of pain and depression, in addition to their pathophysiological difference.

As mentioned earlier, patients who participated in this study had different degrees of depressive symptoms, and the antidepressive effect in these patients might not be fully assessed. This is probably the third limitation of this study; however, the subanalysis of patients with an HDRS score of 8 points or more also showed no significant relationship between the plasma level of milnacipran and its antidepressive effect, indicating the robustness of this finding. The initial pharmacological action of antidepressants is, both for pain-relief and depression treatment, inhibition of the reuptake of serotonin and norepinephrine. However, the ultimate mechanism of action may be different between the analgesic and antidepressive effects of antidepressants.

A significant linear regression was plotted between the dose of milnacipran and plasma drug level for all