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ORIGINAL PAPER

CLOCK may Predict the Response to Fluvoxamine Treatment in Japanese Major Depressive Disorder Patients

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Abstract Recent studies have shown that selective serotonin reuptake inhibitors (SSRIs) have circadian properties, suggesting that the antidepressive action of SSRIs may also be attributable to circadian mechanisms. Another study reported an association between clock gene (*CLOCK*) and improvements in insomnia symptoms from SSRIs treatment. Therefore, we examined the association between *CLOCK* and the efficacy of fluvoxamine treatment in 121 patients with Japanese major depressive disorder (MDD). The MDD patients in this study had scores of 12 or higher on the 17 items of the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). We defined a therapeutic response as a decrease of more than a 50% in baseline SIGH-D within 8 weeks, and clinical remission as a SIGH-D score of less than seven at 8 weeks. We selected three tagging SNPs in *CLOCK* for the subsequent statistical association analysis. We detected a significant association between rs3736544, a synonymous polymorphism in exon 20, and the fluvoxamine therapeutic response in MDD in the allele/genotype-wise analyses. In addition, remission with fluvoxamine was also significantly associated with rs3736544. These associations remained significant after Bonferroni correction. Moreover, haplotype analysis findings supported these significant associations, which appeared to be due mainly to rs3736544, in the fluvoxamine therapeutic remission. Our results indicate that *CLOCK* genotype may be a predictor of fluvoxamine treatment response in Japanese MDD. However, our sample size was small, and a replication study using larger samples may be required for conclusive results.

Keywords Major depressive disorder · *CLOCK* · Tagging SNPs · Fluvoxamine · SSRIs

Introduction

Major depressive disorder (MDD) patients commonly present not only abnormalities in sleep–wake rhythms but also disruptions in biological circadian rhythms. Therefore, disruptions in circadian rhythms have been suggested to be involved in the pathogenesis of MDD (Barnard and Nolan 2008; Kishi et al. 2008a, 2008b). All psychotropic drugs act on the systems of neurotransmitters such as dopamine and serotonin in the brain (Barnard and Nolan 2008), and recently these neurotransmitter systems have been reported to have reciprocal interactions with circadian rhythms (Monteleone and Maj 2008).

Selective serotonin reuptake inhibitors (SSRIs) such as fluvoxamine, which are major therapeutic agents for MDD, inhibit serotonin transport in the presynaptic neuron, and increase the extracellular serotonin level. This mechanism is believed to relieve depressive symptoms (Peveler and

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Kendrick 2005). On the other hand, many animal and in vitro studies have shown that serotonin directly affects circadian rhythms (Monteleone and Maj 2008), and SSRIs have also been reported to have circadian properties. SSRIs have a phase shifting effect in neural firing in the rat suprachiasmatic nucleus (Sprouse et al. 2006), and change the expression of clock genes in the striatum and hippocampus of mice (Uz et al. 2005), suggesting that the antidepressive action of SSRIs may also be attributable to circadian mechanisms. Therefore, we considered that clock genes might be therapeutic targets for SSRIs.

The clock gene (*CLOCK*, OMIM *601851, 25 exons in this genomic region spanning 115.138 kb), located on 4q12, is one of the major components of the cellular clock gene mechanism. It is known to be associated with human circadian preference (morningness/eveningness) (Katzenberg et al. 1998; Mishima et al. 2005). Several clinical subgroup analyses have shown a significant association between an SNP (rs1801260: T3111C) in *CLOCK* and sleep disregulation in mood disorders including MDD and bipolar disorder (BP) (Serretti et al. 2003) and a higher recurrence rate in BP (Benedetti et al. 2003). In addition, Serretti and colleagues reported an association between T3111C and improved insomnia from fluvoxamine or paroxetine treatment (Serretti et al. 2005). However, three genetic studies, including our previous study, reported no association between *CLOCK* and MDD (Bailer et al. 2005; Desan et al. 2000; Kishi et al. 2008a). Thus, there is disagreement in the results of these studies as to treatment response and the pathophysiology of MDD (Gratacos et al. 2008).

In this study, we examined the association between *CLOCK* and the efficacy of fluvoxamine treatment in Japanese MDD patients. To do this, we applied the recently recommended strategy of "gene-based" association analysis (Neale and Sham 2004).

Materials and Methods

Subjects

The subjects were 121 MDD patients (60 males and 61 females: mean age ± standard deviation (SD) 44.5 ± 16.5 years). All subjects were unrelated to each other, ethnically Japanese, and lived in the central area of Japan. The patients were diagnosed according to DSM-IV criteria with consensus of at least two experienced psychiatrists on the basis of a review of medical records. Fluvoxamine was taken two or three times a day for 8 weeks. The initial total dose was 50–100 mg per day, and the dosage was then increased gradually to a maximum of 150 mg, depending on the patients' condition. Patients with insomnia and severe anxiety were prescribed benzodiazepine drugs, but no other psychotropic drugs were permitted during the study. The study was described to subjects and written informed consent was obtained from each. This study was approved by the Ethics Committee at Fujita Health University.

Data Collection

The MDD patients in this study had scores of 12 or higher on the 17 items of the Structured Interview Guide for Hamilton Rating Scale for Depression (SIGH-D). Patients with this moderate range of severity tend to respond to antidepressants (Saito et al. 2006). We defined a therapeutic response as a decrease of more than 50% in baseline SIGH-D within 8 weeks, and a clinical remission as a SIGH-D score of less than 7 at 8 weeks. Detailed information on data collection was described in a previous paper (Saito et al. 2006). The clinical characteristics of the patients in this study, classified according to these definitions, can be seen in Table 1.

SNPs Selection and Linkage Disequilibrium (LD) Evaluation

We selected three "tagging SNPs" (rs3736544: synonymous polymorphism in exon 20, rs1801260: 3′ untranslated region (UTR) in exon 23, rs3749474: 3′ UTR in exon 23) in *CLOCK*. Detailed information can be seen in our previous paper (Kishi et al. 2008a).

SNPs Genotyping

We used TaqMan assays (Applied Biosystems, Inc., Foster City, CA,) for all SNPs. Detailed information can be seen in our previous paper (Kishi et al. 2008a).

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).

Marker-trait association analysis was used to evaluate allele- and genotype-wise associations with the chi-square test (SAS/Genetics, release 8.2, SAS Japan Inc, Tokyo, Japan), and haplotype-wise association analysis was done with a likelihood ratio test using the COCAPHASE 2.403 program (Dudbridge 2003). Bonferroni's correction was used to control inflation of the type I error rate. Power calculation was performed using the Genetic Power Calculator (Purcell et al. 2003).

The significance level for all statistical tests was 0.05.

Table 1 Clinical characteristics of the patients in both definition groups

	N			Age (mean ± SD)	Baseline SIGH-D (avg ± SD)	Fluvoxamine dose at 8 weeks (mg/day) (avg ± SD)	Number of previous episode (avg ± SD)
	Total	Male	Female				
Overall	121	60	61	44.5 ± 16.5	20.2 ± 5.88	122 ± 40.9	1.39 ± 0.658
Clinical response group[a]							
Responders	60	31	29	44.4 ± 16.3	21.5 ± 6.19	118 ± 41.1	1.36 ± 0.574
Nonresponders	61	29	32	44.3 ± 17.3	18.8 ± 5.28	125 ± 40.7	1.43 ± 0.774
P-value	0.645			0.819	0.0145	0.391	0.480
Clinical remission group[b]							
Remitters	45	22	23	43.7 ± 15.9	19.6 ± 5.06	113 ± 43.9	1.37 ± 0.598
Nonremitters	76	38	38	45.1 ± 17.1	20.5 ± 6.34	127 ± 38.2	1.41 ± 0.715
P-value	0.722			0.750	0.750	0.101	0.856

[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 seven

Results

The LD structures of *CLOCK* from the HapMap database were described in our previous paper (Kishi et al. 2008a). Among the clinical characteristics of the patients in this study, only one significant difference with total SIGH-D score was detected at the baseline in relation to fluvoxamine therapeutic response (*P*-value = 0.0145) (Table 1). Genotype frequencies of all SNPs were in HWE. We detected a significant association between rs3736544 and the fluvoxamine therapeutic response in MDD in the allele/genotype-wise analysis (Table 2). In addition, remission with fluvoxamine was significantly associated with rs3736544 (Table 2). Moreover, the significance of these associations remained after Bonferroni correction (Table 2). We also found an association between rs3749474 and the fluvoxamine therapeutic response in MDD in the genotype-wise analysis (*P*-value: 0.0251) (Table 2). However, this might have resulted from type I error due to multiple testing (*P*-value: 0.0752 after Bonferroni's correction) (Table 2). The haplotype-wise analysis provided evidence for a significant association that appears to be due mainly to rs3736544 in fluvoxamine therapeutic remission (Table 3).

Table 2 Association analysis of tagging SNPs in *CLOCK*

SNP[a]	Phenotype	MAF	*N*	Genotype distribution[b]			*P*-value[d]			Corrected *P*-value[d,e]	
				M/M	M/m	m/m	HWE[c]	Genotype	Allele	Genotype	Allele
rs3736544 G > T	Responders	0.267	60	30	28	2	0.135				
	Nonresponders	0.115	61	48	12	1	0.804	**0.00434**	**0.00261**	**0.00130**	**0.00738**
	Remission	0.289	45	21	22	2	0.203				
	Nonremission	0.132	76	57	18	1	0.751	**0.00651**	**0.00257**	**0.0195**	**0.00771**
rs1801260 T > C	Responders	0.133	60	46	12	2	0.297				
	Nonresponders	0.189	61	39	21	1	0.328	0.187	0.243		
	Remission	0.156	45	33	10	2	0.301				
	Nonremission	0.164	76	52	23	1	0.378	0.390	0.855		
rs3749474 T > C	Responders	0.417	60	19	32	9	0.452				
	Nonresponders	0.336	61	27	27	7	0.949	0.358	0.196		
	Remission	0.467	45	12	24	9	0.632				
	Nonremission	0.322	76	34	35	7	0.637	0.0734	**0.0251**		0.0752

[a] major allele > minor allele

[b] M: major allele, m: minor allele

[c] HWE: Hardy–Weinberg equilibrium

[d] Bold numbers represent significant *P*-value

[e] Caluculated by Bonferroni's correction

Table 3 Haplotype-wise analysis of tagging SNPs in *CLOCK*

Common haplotypes rs3736544-rs1801260-rs3749474	Phenotype	Individual haplotype frequency	Individual *P*-value[a]	Phenotype	Global *P*-value[a]
GTT	Responders	0.600	0.173		
	Nonresponders	0.686		Responders	0.436
	Remission	0.548	**0.0191**	Nonresponders	
	Nonremission	0.703		Remission	**0.015**
GCC	Responders	0.146	0.401	Nonremission	
	Nonresponders	0.188			
	Remission	0.167	1.00		
	Nonremission	0.167			
TTC	Responders	0.255	**0.0137**		
	Nonresponders	0.125			
	Remission	0.286	**0.00417**		
	Nonremission	0.130			

[a] Bold numbers represent significant *P*-value

Discussion

In this study, we detected a significant association between rs3736544 in *CLOCK*, which is a synonymous polymorphism in exon 20, and the fluvoxamine therapeutic response and remission in the allele/genotype-wise analysis. This significance remained after Bonferroni correction. Haplotype analysis indicated three common haplotypes (rs3736544-rs1801260-rs3749474: GTT, GCC and TTC). Among them, the TTC haplotype was less prevalent in subjects with a fluvoxamine therapeutic response ($P = 0.0137$) and was associated with remission on fluvoxamine ($P = 0.00417$). The GTT haplotype was also significantly associated with remission on fluvoxamine ($P = 0.0191$). In a recent study, we selected six tagging SNPs among 106 SNPs covering all of *CLOCK*, including 5′-flanking regions about 2 kb upstream (5′) from the initial exon and about 5 kb downstream (3′) from the last exon (HapMap database contig number chr4: 55990340.. 56108588), with the criteria of an r^2 threshold greater than 0.8 in "pair-wise tagging only" mode using the Tagger program. LD structures of *CLOCK* from the HapMap database were described in our previous paper (Kishi et al. 2008a). However, the LD structure of *CLOCK* in our sample was very tight except for rs1801260 and rs3749474 (Kishi et al. 2008a). Also, the LD structures of MDD samples treated with fluvoxamine and control samples were almost the same (Kishi et al. 2008a). As these results show, rs3736544 covers a wide and important region including the exons and the promoter region in *CLOCK*. Therefore, it is possible that rs3736544 influences biological function in the brain. In previous genetic analyses of *CLOCK*, only T3111C (rs1801260) was selected. T3111C (rs1801260) has been detected at position 3111 in the *CLOCK* mRNA 3′ untranslated region, and was reported to be associated with a substantial delay in preferred timing for activity and sleep in a human study (Katzenberg et al. 1998). As for function, T3111C (rs1801260) has been speculated to affect mRNA (Katzenberg et al. 1998); however, one study with luciferase reported no significant effect on mRNA translatability from T3111C (Robilliard et al. 2002). We found an association of rs3736544 but not T3111C (rs1801260) with treatment outcome in this study. These findings suggest that functional analyses for other regions of the *CLOCK* should be performed in future studies.

A subgroup analysis has shown a significant association between an SNP (rs1801260: T3111C) in *CLOCK* and sleep disregulation in mood disorders (Serretti et al. 2003). Because benzodiazepine drugs are surely effective for insomnia and severe anxiety in MDD patients, which might mask the sleep disruption in MDD due to circadian abnormality, the analysis which takes the usage of benzodiazepines into account may also need to be carried out in the future. Because we had only a few MDD fluvoxamine treatment samples without benzodiazepine drugs, and we wanted to avoid statistical error, we did not perform such an analysis among these samples. Another subgroup analysis has shown a higher recurrence rate in BP in relation to T3111C (Benedetti et al. 2003), but we lacked data on recurrence in our sample, so we could not perform such analysis.

Our recent study found no association between *CLOCK* and MDD in the Japanese population (Kishi et al. 2008a). Thus, there is disagreement in the results among studies as to the treatment response and the pathophysiology of MDD (Gratacos et al. 2008).

A few points of caution should be noted in interpreting our results. First, it will be necessary to investigate the possibility that rs3736544 reflects biological function,

which we did not do in the present study. Second, we did not include a mutation scan to detect rare variants with functional effects. However, it is difficult to evaluate the association of such extremely rare variants (e.g., minor allele frequencies less than 0.01) from the viewpoint of power. Third, our sample sizes were small. A replication study using larger samples may be required for conclusive results.

In conclusion, our results indicate that *CLOCK* may be associated with fluvoxamine treatment outcome in Japanese MDD.

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