Cry1 and *Tef* gene polymorphisms are associated with Major Depressive Disorder in the Chinese population

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Abstract

Introduction—Accumulating evidences indicate that circadian abnormalities lead to sleep disorder, neurodegenerative diseases and depression. We have reported that the polymorphisms of a clock-related gene, *Tef*, contributed to the risk of sleep disturbances and depression in Parkinson disease. The objective of the present study was to examine whether the three clock genes we previous studied are associated with major depressive disorder (MDD) in the Chinese population.

Methods—105 subjects with MDD and 485 control subjects participated in this case-control study. Demographics, Mini-mental Status Examination (MMSE), and Hamilton rating scale for depression (HAMD) were obtained in all subjects. Genotypes of SNPs (single nucleotide polymorphism) of *Cry1* rs2287161, *Cry2* rs10838524 and *Tef* rs738499 was screened by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

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Results—MDD cases had a significantly higher frequency carrying the C allele and CC genotype in *Cry1* rs2287161 and the T allele and TT genotype in *Tef* rs738499 than controls.

Limitations—The sample size of MDD group was relatively small.

Conclusions—The polymorphisms of *Cry1* rs2287161 and *Tef* rs738499 are associated to MDD.

Keywords

Major Depressive Disorder; Circadian rhythm; Clock genes; *Cry1*; *Tef*

Introduction

Circadian effects on depression are presented in four areas. First, the physiological and behavioural abnormalities in depression are associated with circadian dysfunction, such as abnormal temperature rhythms, disturbed sleep, disregulated hormonal secretion, and a repetitive pattern of diurnal mood cycle (Bunney and Potkin, 2008). Second, chronobiological interventions on patients with depression, such as sleep deprivation, light therapy, or combining with sleep phase advance, can dramatically improve their depressive symptoms within hours (Wu and Bunney, 1990; Fritzsche et al., 2001; Dallaspezia and Benedetti, 2011). Third, the effects of traditional antidepressants such as fluoxetine and lithium, might exert their effects via regulating circadian-related mechanisms (Sprouse et al., 2006; Abe et al., 2000). Fourth, agomelatine, a melatonergic agonist, is developed on the basis of resynchronising the circadian rhythms, and agomelatine has been demonstrated to be effective to treat depression (Zajecka et al., 2010; Kasper et al., 2010). Based on the above, the relationship between circadian rhythm and depression is of great interests.

The circadian rhythm is maintained by the clock genes networks consisting of a series of auto-regulatory transcription-translation loops with positive and negative feedbacks to core clock genes and their proteins (Takahashi et al., 2008). Several core clock genes have been characterized including the cryptochrome genes (*Cry1*, *2*), the period genes (*Per1*, *2*, *3*), and the genes *Clock* (*Npas2*) and *Bmal1*. Some other clock-related genes are also involved in the regulation of circadian rhythmicity, such as *Tef*, *Vip*, *AANAT*, *Sirl1*, *Prock2*, *Timeless*, and so on. A few studies have reported that several clock gene polymorphisms were associated with certain subtypes of depression including seasonal affective disorder (SAD), major depressive disorder (MDD) and bipolar disorder (BPD). Soria et al. (2010a) reported that *Cry1* rs2287161 and *Npas2* rs11123857 were associated with MDD, and *Clock* rs10462028 and *Vip* rs17083008 were associated with BPD. Soria et al. (2010b) also presented evidence of the association of genetic polymorphism in the *AANAT* gene with susceptibility to MDD. Lavebratt et al. (2010) found the *Cry2* rs10838524was significantly associated with SAD; and a recent study by Kovanen et al. (2013) demonstrated several SNPs including rs10838524 to be related to dysthymia. *Tef* rs738499 was suggested to be a risk factor to unipolar major depression (Kripke et al., 2009), and in a replication study (Kripke et al., 2010), *Tef* rs738499 was also associated with the depressive symptoms in the sleep clinic patients. *Sirt1* (Kishi et al., 2010a) and *Prock2* (Kishi et al., 2010b) genes were reported to associate with MDD in Japanese population. These reported risk clock gene polymorphisms

have individually studied to be associated with depression; however, no replication studies have been conducted.

Several clinical studies established a direct correlation between circadian dysfunction and neurodegenerative disease (Kondratova and Kondratov, 2012), we studied three of the above genetic polymorphisms in patients with Parkinson disease recently. As result, we found that *Tef* rs738499 was related to sleep disturbances (Hua et al., 2012a) and depression in Parkinson disease (Hua et al., 2012b), but no association was found as for *Cry1* rs2287161 or *Cry2* rs10838524 and depression in Parkinson disease. It is uncertain whether *Tef* rs738499 is specific to Parkinson disease or is associated to primary mood disorder. Therefore, we conducted a case-control study for an association analysis between *Cry1* rs2287161, *Cry2* rs10838524 and *Tef* rs738499 and MDD in the Chinese population.

Methods

Study subjects

We studied 105 unrelated MDD patients and 485 unrelated healthy controls. MDD patients were recruited from the psychiatry ward of the Affiliated Brain Hospital of Nanjing Medical University between January 2010 and October 2010. They were diagnosed through a structured interview for the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; APA.2000) by two experienced psychiatrist. Depression severity was evaluated with the 24-item Hamilton rating scale for depression (HAMD) (Hamilton, 1967). We excluded MDD patients with an HAMD score < 20, primary sleep disorders, sever sleep disturbances, major medical illnesses, other psychiatric disorders, a history of substance abuse, and cognitive impairment determined by Mini-Mental State Examination (MMSE) (Folstein et al., 1975) adjusted by age and education levels (Crum et al., 1993). Control subjects free of severe medical diseases or any history of neurological or psychiatric disorders were recruited from a community-based population. Control subjects with a HAMD score 8, primary sleep disorders, sever sleep disturbances, psychiatric disorders, and cognitive impairment was excluded from the study. The study was approved by the Ethics Committee of the Nanjing Medical University. Informed consent was obtained in each participant.

Genotyping

Genomic DNA was extracted from citric acid-anticoagulated peripheral blood using a DNA extract kit (Tiangen Beijing) according to the manufacturer's recommendations. The genotypes of *Cry1* rs2287161, *Cry2* rs10838524, and *Tef* rs738499 were screened by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The digested products were loaded onto a 2% agarose gel and ran at 80V for 1 hour. The separated bands were visualized by ethidium bromide under the ultraviolet lamp. To confirm the results obtained by PCR-RFLP, three samples of each genotype in SNPs (single nucleotide polymorphisms) were randomly selected for DNA sequencing. Detailed information can be seen in our previous papers (Hua et al., 2012a) (Hua et al., 2012b).

Statistical analysis

Statistical analysis was performed with SPSS for windows version 18.0. Differences between groups were calculated with the t-test for continuous data and a chi-square test for categorical data. The Hardy–Weinberg equilibrium (HWE) of SNPs was calculated with the SHEsis program (Shi and He, 2005). The distributions of allelic and genotypic frequency of SNPs in MDD were compared with controls by chi-square test or the Fisher's exact test with the SHEsis program, respectively. Allelic association analysis was performed with the SHEsis program as well. Association analysis of genotypes of SNPs between MDD and controls was based on Binary Logistic Regression analysis. Because there was no significant difference in age, gender, and MMSE scores between MDD cases and the controls, these variables were not been entered into the logistic regressions. During the regression analysis, each SNP was modeled as a dichotomous variable with the reported risk genotype chosen as a category, and the other two genotypes combined as the reference category. The correlation between variables and HAMD scores and three item sleep questions of the scale (early, middle, and end-of-sleep awakening) were analyzed using Spearman's rank correlation. A linear regression was generated to determine the extent to which the SNPs were associated with the HAMD scores and three item sleep questions of the scale. Significant p-value for the regression analysis was set to 0.05.

Results

There was no significant difference in age, gender, and MMSE scores between the two groups. The demographic and clinical characteristics of the study subjects were shown in Table 1. A significantly higher frequency of the C allele in *Cry1* rs2287161 were found in MDD cases than in controls (OR=1.75, 95% CI: 1.12~2.71, p=0.012). Similarly the MDD cases had a higher frequency of the T allele in *Tef* rs738499 than controls (OR=2.22, 95% CI: 1.42~3.46, p<0.001) (Table 2). MDD cases are 1.75 times more likely to carry C allele in *Cry1* rs2287161 and 2.22 times more likely to carry T allele in *Tef* rs738499 than controls (Table 2).

The distribution of genotypes for each SNP was fitting with HWE in each group (Table 2). Carriers of the CC genotype in *Cry1* rs2287161 were 1.91 times more likely to suffer from MDD (OR=1.91, 95% CI: 1.16~3.14, p=0.010). Carriers of the TT genotype in *Tef* rs738499 were associated with a 2.66 times risk of MDD (OR=2.66, 95% CI: 1.60~4.41, p<0.001). There was no significant association of *Cry2* rs10838524 with MDD. No additive effects among SNPs existed.

Spearman's rank correlation showed that a higher HAMD score was associated with the CC genotype in *Cry1 rs2287161* (p<0.01), and the TT genotype in *Tef rs738499* (p<0.01) and MMSE scores (p<0.01); there was no correlation between the HAMD score and gender, age, or *Cry2 rs10838524*. The same results were obtained from item analysis of the three sleep questions of the scale. Stepwise linear regression adjusted by MMSE scores showed that MMSE scores, the genotype of *Cry1 rs2287161*, and the genotype of *Tef rs738499* contributed to 93.5% of the variance in the HAMD scores, the genotype of *Cry1 rs2287161* itself accounted for 1.7% (p<0.001), and the genotype of *Tef rs738499* accounted for 2.0% (p<0.001); MMSE scores and the genotype of *Tef rs738499* contributed to 51.5% of the

variance in the early-sleep (*p*<0.001), the genotype of *Tef rs738499* accounted for 2.1% (*p*<0.001); MMES scores contributed to 47.1% of the variance in the middle-sleep (*p*<0.001), no SNP was significant; MMES scores and the genotype of *Cry1 rs2287161* contributed to 49.1% of the variance in the end-of-sleep awakening, and the genotype of *Cry1 rs2287161* accounted for 2.9% (*p*<0.001).

Discussion

The main findings in the present study were the associations of *Cry1* rs2287161 and *Tef* rs738499 polymorphisms with MDD. We did not find any significant association between *Cry2* rs10838524 and MDD. Therefore, these findings suggested that *Cry1* rs2287161 and *Tef* rs738499 could play a role in the pathogenesis of MDD. Meanwhile, the association with *Tef* indicated that it was not only a risk factor to depressive symptoms in Parkinson's disease but also a risk factor for MDD. From the linear regression, we conclude that *Cry1 rs2287161* and *Tef* rs738499 were still associated with HAMD scores excluding the effect of cognition. The item analysis of the three sleep questions of the scale indicated that *Tef rs738499* was more likely to act through circadian phase delay and *Cry1 rs2287161* was more likely to act through circadian phase advance. Polymorphisms of two circadian-related genes could contribute to MDD via different mechanisms.

How these genetic polymorphisms affect circadian gene expression and thus contribute to MDD is not entirely clear. *Cry1* is essential to the clock gene regulation, for the role of suppressing the transcription of *Clock/Bmal1* and activating the transcription of *Per* (Weger et al., 2011). The SNP rs2287161 is located at 3′downstream of polyadenylation site of *Cry1* gene, which may regulate the gene expression (Soria et al., 2010a). The alteration of *Cry1* expression in the cells may disrupt the normal circadian rhythm. Tef plays an important role in photoperiodic responses (Dardente et al., 2010), seasonal timing in mammals (Gachon et al., 2004a), and the metabolism of neurotransmitters, such as dopamine and serotonin (Gachon et al., 2004b). Meanwhile, rs738499 has been reported (Kripke et al., 2009) to have the highest linkage with rs599609, which was found to be correlated with *Tef* expression (Stranger et al., 2007). The polymorphism of *Tef* rs738499 may relate to a disturbed circadian rhythm and dysfunctional neurotransmission, leading to the predisposition of MDD.

The three studied SNPs have not been reported associated with MDD in multiple public GWAS databases including Genetic Association Database (http://geneticassociationdb.nih.gov/cgi-bin/index.cgi); GWAS Catalog by National Human Genome Research Institute (http://www.genome.gov/page.cfm?pageid=26525384#download); GWAS Central supported by European GEN2PHEN project (http://www.gwascentral.org/index). It will also be interest to find if these SNPs play disease causative roles in MDD in more GWAS studies.

To our knowledge, this is the first study to replicate the association between clock genes variants and MDD. There might be some limits relevant to this study. First, the sample size of the current study is relatively small, therefore, studies with a lager sample size are needed to examine the current results. Second, considering the link between sleep and circadian

rhythms, we excluded subjects with primary sleep disorders and severe sleep disturbances, however, we didn't assess their sleep quality using a quantitative scale. Therefore, disturbed sleep in MDD may play a role on the current association. However, sufficient evidence suggested that the activity of the circadian clock in the regulation of mood is far beyond the circadian control of sleep (Bunney and Potkin, 2008; Kondratova and Kondratov, 2012). On the other hand, the mechanism of sleep disturbances in MDD is an inseparable part of the pathophysiology of MDD (Lopresti et al., 2013). Thus, from the results of the present study and the previous studies, we conclude that *Cry1* rs2287161 and *Tef* rs738499 are associated with the susceptibility of MDD. Further studies on the effects of genetic polymorphisms and the gene expression are important to elucidate the molecular mechanisms underlying the genetic susceptibility to MDD.

Acknowledgments

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Table 1

The demographic and clinical characteristics of the study subjects

	MDD n (105)	Control n (485)
Age (years mean±SD)	65.0±7.4	65.5±5.7
Gender (males)	57.10%	49.90%
HAMD scores	31.5±5.4	2.7±1.7
MMSE scores	26.5±2.5	28.1±2.0

Abbreviations: MDD, Major Depressive Disorder; HAMD, Hamilton rating scale for depression; MMSE, Mini-Mental State Examination.

Table 2

Allelic and genotypic frequency and association analysis of SNPs

Gene	MDD			Control
SNPs	Number (percentage)	OR (95% CI)	*p*-value	Number (percentage)
Cry1 rs2287161				
CC	80 (0.762)	**1.91 (1.16~3.14)**[a]	**0.010**	314 (0.647)
CG	24 (0.229)			150 (0.309)
GG	1 (0.010)			21 (0.043)
C	184 (0.876)	**1.75 (1.12~2.71)**[b]	**0.012**	778 (0.802)
G	26 (0.124)			192 (0.198)
Cry2 rs10838524				
AA	93 (0.886)	1.65 (0.85~3.21)[c]	0.138	407 (0.839)
AG	12 (0.114)			78 (0.161)
A	198 (0.943)	1.44 (0.77~2.70)[d]	0.249	892 (0.920)
G	12 (0.057)			78 (0.080)
Tef rs738499				
TT	82 (0.781)	**2.66 (1.60~4.41)**[e]	**<0.001**	293 (0.604)
TG	21 (0.200)			160 (0.330)
GG	2 (0.019)			32 (0.066)
T	185 (0.881)	**2.22 (1.42~3.46)**[f]	**<0.001**	746 (0.769)
G	25 (0.119)			224 (0.231)

Abbreviations: OR, Odds Ratio; 95% CI, 95% confidence intervals; MDD, Major Depressive Disorder;

[a]CC/(CG+GG);

[b]C/G;

[c]AA/AG;

[d]A/G;

[e]TT/(TG+GG);

[f]T/G.