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European Neuropsychopharmacology 15 (2005) 51–55

EUROPEAN NEURO-PSYCHOPHARMACOLOGY

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No association of clock gene T3111C polymorphism and affective disorders

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Received 25 November 2003; accepted 13 May 2004

Abstract

CLOCK was hypothesised to be related to susceptibility of affective disorders. To test subsamples of affectively disordered patients, we examined age of onset (AoO), numbers of episodes and melancholic type of clinical manifestation. Using PCR and RFLP, we investigated in patients with unipolar depression and bipolar disorder (BP) whether the CLOCK T3111C SNP is associated with affective disorders ($n = 102$) compared to healthy controls ($n = 103$). No differences were found either in genotype or allele frequency distributions of T3111C polymorphism between patients compared to healthy controls ($p > 0.2$). No deviations from Hardy–Weinberg Equilibrium (HWE) were detected either in patients, or healthy controls. Results suggest that there is no association between the T3111C SNP and affective disorders in general. Data of our sample replicate prior findings of Desan et al. [Am. J. Med. Genet. 12 (2000) 418]. Subsamples of patients with high numbers of affective episodes did show some deviations in genotypes ($p = 0.0585$).

Keywords: Clock gene; T3111C Polymorphism; Circadian rhythms; Affective disorders

1. Introduction

Malfunctions in circadian rhythms in psychiatric disorders are well documented (Hawkins and Mendels, 1966). Not only deviations in sleep rhythms and architecture from interrupted sleep and early awakening to insomnia, but also variation of mood can occur even in the course of one single day. In the DSM-IV (American Psychiatric Association, 1994) criteria for the specification of a current depressive episode "with melancholic features", three out of six mainly somatic items have to be confirmed to qualify for this subtype. As biologists long before have recognised circadian clocks to be an integral aspect in physiology in flora and fauna, their deviations in seasonal and daily timescales have been observed. Phenomena like hibernation, sleep–wake cycle, day activity and plasma levels of hormones were studied in non-vertebrates and vertebrates and mammals. Insights especially from drosophila and recent advances in molecular genetics revealed key features in understanding the physiology of the evolutionary rather invariant genetic background of the elements regulating circadian timekeeping mechanisms in humans (Edery, 2000).

To date, in mammals the three central genes for organising circadian rhythms are clock (clk), period (per) and timeless (tim). Expression analysis confirmed the highest levels for clock in the suprachiasmatic nucleus (SCN) and the eye in order to postulate the SCN as the central pacemaker for peripheral clocks in setting and resetting the internal clock (Edery, 2000; Hardin, 2000; Turek and Kolker, 2001). Based on findings in fauna and flora on per, tim and clk several authors such as Bunney and Bunney (2000), Mitterauer (2000) and Copinschi et al. (2000) conclude that in interaction with genes and environment, these genes have an important impact on circadian rhythmicity. In addition, they hypothesise that the circadian

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doi:10.1016/j.euroneuro.2004.05.004

pacemaker consisting of these three genes might play an important role in the development and course of affective disorders and psychiatric disorders in general.

In 2001, two deviations from sleep–wake cycle in humans and their associations with clock genes were described. Delayed sleep phase syndrome (DSPS) was found to be associated with an h(uman)per3-polymorphism (Ebisawa et al., 2001). Toh et al. (2001) showed causal links in familial advanced sleep phase syndrome (FASPS) and an hper2-mutation.

Moreover, several biological findings support the effect of deviations in the circadian rhythms in pathogenesis of mood disorders and vice versa: Kupfer and Foster (1972) found a decrease in latency to REM sleep, while alterations in light–dark schedules are known to be effective in the treatment of depression (Gillin, 1983). Zobel et al. (1999) used repeated dexamethasone suppression and combined Corticotropin-Releasing-Hormone-dexamethasone tests for prediction of the course of mood disorders to support deviations from cortisole excretion and corticosteroid hypothesis in major depression (Holsboer et al., 1982; Holsboer, 2000).

Vitaterna et al. (1994) found and mapped Clock in mouse for the first time. King et al. (1997) revealed the A→T transversion at the third base position of the 5′ -splice donor site of intron 19 of this gene and proved that this transversion causes skipping of exon 19 resulting in the mutant phenotype in mouse. In humans, following these experiments, Steeves et al. (1999) discovered the highest expression of CLOCK mRNA in the SCN and cerebellum and 2 SNPs in a non-coding sequence flanking the CLOCK open reading frame. The SNP T3111C (SNP ID: rs1801260; http://www.ncbi.nlm.nih.gov/SNP]) is located at position 3111 of the CLOCK mRNA 3′ untranslated region (GenBank accession number AF011568) (Steeves et al., 1999).

In humans, Katzenberg et al. (1998) suggested one or two 3111C alleles of CLOCK T3111C SNP to be linked to a delay in preferred timing for activity or sleep episodes. Desan et al. (2000) explored possible associations between the T3111C SNP and the occurrence of affective disorders (cf. Table 1): In a sample of 143 white European Americans (EA) with major depressive disorder, they found no evidence that this SNP at the clock locus influences the risk for major depressive disorder ($p>0.61$). Besides, they found a significant difference in allele frequencies between EA ($n=137$) and African American (AA; $n=58$) control subjects ($p<0.05$), with the 3111C allele being less frequent in AAs. There were no significant deviations from Hardy–Weinberg Equilibrium (HWE) in all three samples (EA depressed, EA and AA controls).

Although there is a lack of studies based on animal models on the function of clock T3111C polymorphism in affective disorders, several suppositions evolved from epidemiology supporting the impact of the circadian timekeeping system in psychiatric disorders. Recently, Sullivan et al. (2000) emphasised epidemiological findings which underline clinical features such as recurrence, age of onset and symptom patterns to be useful to identify susceptibility loci for major depressive disorder (MDD). This might be a first step to close the gap between genetics and clinical phenomena.

According to the findings of Desan et al. (2000), we hypothesised as our primary hypothesis that first, there is no association between the occurrence of affective disorders (unipolar, bipolar) and the genotype (C/C, C/T, T/T) of polymorphism T3111C of CLOCK gene and second, no association between the occurrence of an affective disorder (unipolar, bipolar) and the allele frequencies of polymorphism T3111C of CLOCK gene.

Considering suggestions made by Sullivan et al. (2000), we tested possible associations of clinical factors such as a high number of affective episodes, a young age of first appearance of the disorder and a high number of somatic symptoms (“melancholic subtype”) to be associated with biological factors, in our case with the genetic polymorphism.

2. Experimental procedures

2.1. Clinical methods

Diagnostic evaluation of the experimental sample ($n=102$; male: 38.24%, female: 61.76%) and healthy controls ($n=103$; male: 33.01%, female: 58.25, no gender available: 8.74%) and used a comprehensive anamnesis, psychiatric exploration and structured interviews using DSM-IV criteria (German versions of M.I.N.I. version 5.0.0 (Ackenheil et al., 1999) or SCID-I (Wittchen et al., 1997)) (Table 2a).

Consensus diagnoses of two psychiatrists were established. Healthy controls were not to show psychiatric disorders in present or past and had no psychiatric history in first-degree relatives. All patients and healthy controls were white European Caucasian and patients recruited from in-

Table 1
Results of Desan et al. (2000): patients with major depressive disorder and healthy controls

	T/T (absolute/relative%)		T/C (absolute/relative%)		C/C (absolute/relative%)		T (relative%)	C (relative %)
Patients[a]	75	52.45	59	41.26	9	6.29	73.1	26.9
Controls EA[b]	68	49.63	58	42.34	11	8.03	70.8	29.2
Controls AA 2[b]	38	65.52	18	31.03	2	3.45	81.0	19.0

[a] European (Caucasian) American (EA) sample of patients compared to controls (EA, AA), $p>0.05$.
[b] African American (AA) controls compared to EA controls, $p<0.05$.