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No association of clock gene T3111C polymorphism and affective disorders

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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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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, $p<0.05$.

Table 2a
Demographics of patients with affective disorder and healthy controls

	Total *n* (Counts, 100%)	Male (absolute/relative%)		Female (absolute/relative%)		Age of onset[a] (mean/ ± S.D.)		Age[b] (mean/ ± S.D.)	
Patients	102	39	38.24	63	61.76	32.31	13.98	44.06	14.28
Controls[c,d]	103	34	33.01	60	58.25	–	–	32.75	9.63

[a] $p = 0.3249$ (age of onset in patients vs. age in controls).
[b] $p = 3.4467 \times 10^{-9}$ (age in patients vs. controls).
[c] Age of onset not applicable.
[d] For nine individuals, no gender available.

and outpatients at University Hospital for Psychiatry, Department of General Psychiatry, in Vienna, Austria. All test subjects gave their consent to participate after having received appropriate information and signing informed consent according to ethical protocols. Mean age was 44.06 years (S.D. ± 14.28) in patients and 32.75 years (S.D. ± 9.63) in controls (Table 2a). Comparing age of onset (AoO) in patients with affective disorders ($mean_{AoO} = 32.31$ years (S.D. ± 13.98) to mean age in controls, no difference was present (Kruskal–Wallis rank sum test; $p > 0.3249$).

Based on these data collections by clinical interview and M.I.N.I. or SCID-I, we counted the number of depressed, hypomanic and manic episodes, established the age of onset of the first affective episode and set by SCID-I-specifyer the subtype "melancholic depression" if a current depressive episode was present in the patient ($n = 61$) (Table 2b). Specifications of former episodes of depression were unknown and were not counted in these data. As suggested by Sullivan et al. (2000), we tested a high number of affective episodes (more than five episodes), a low age of first onset of the affective disorder (39 years and younger) and presence of a high number of somatic symptoms ("melancholic subtype") for an association with the genetic polymorphism.

2.2. Laboratory methods

According to Desan et al. (2000), we isolated genomic DNA from 5 ml EDTA blood samples using the Nucleon BACC3 Kit (Amersham Biosciences). The polymorphism T3111C of the CLOCK gene was determined according to the protocol of Desan et al. (2000): By using 100 ng genomic DNA and primers CLOCK F (5′ -TCC AGC AGT TTC ATG AGA TGC-3′) and CLOCK R (5′ -GAG GTC ATT TCA TAG CTG AGC-3′), a 221-bp fragment was PCR-amplified. The T3111C polymorphism was identified by restriction of the 221-bp PCR-fragment with the restriction enzyme *Bsp*1286I. C-Allele was cut by *Bsp*1286I (generates two fragments: 95 and 126 bp), T-Allele was not cut (221-bp fragment). After incubation with *Bsp*1286I, the resulting DNA fragments were separated by agarose gel electrophoresis, stained with ethidium bromide and photographed.

2.3. Statistical analyses

Tests comparing the location of quantitative variables between groups (age vs. diagnosis, etc.) were performed using the nonparametric Kruskal–Wallis rank sum test. Tests for independence in contingency tables of qualitative variables (genotype vs. diagnosis, etc.) were performed using Fisher's exact test. Hardy–Weinberg equilibria were tested using the a chi-squared goodness-of-fit test. All computations have been done using the statistical computing environment R version 1.6.1 (http://www.R-project.org).

3. Results

A total of 102 patients with affective disorders and 103 healthy controls were investigated. Genotypes and allele frequencies are summarized in Table 3.

Table 2b
Comparison between subgroups of patients with affective disorders: absolute and relative frequencies of genotypes and allele frequencies of clock gene T3111C polymorphism

	T/T (absolute/relative%)		T/C (absolute/relative%)		C/C (absolute/relative%)		T (absolute/relative%)		C (absolute/relative%)	
AoO < 40 years[a]	33	55.0	23	38.33	4	6.67	89	74.17	31	25.83
AoO ≥ 40 years[a]	22	52.38	16	38.10	4	9.52	60	71.43	24	28.57
Episodes ≤ 5[b]	27	52.94	23	45.10	1	1.96	77	75.49	25	24.51
Episodes > 5[b]	28	54.90	16	31.37	7	13.73	72	70.59	30	29.41
mel subtype no[c]	18	43.90	20	48.78	3	7.32	56	68.29	26	31.71
mel subtype yes[c]	37	60.66	19	31.15	5	8.19	93	76.23	29	23.77
Female[d]	33	52.38	26	41.27	4	6.35	92	73.02	34	26.98
Male[d]	22	56.41	13	33.33	4	10.26	57	73.08	21	26.92

All calculations with Fisher's exact test (two-sided).
[a] Age of Onset (AoO): genotypes ($p = 0.4854$), allele frequencies ($p = 0.8223$).
[b] Number of episodes: genotypes ($p = 0.0585$), allele frequencies ($p = 0.6956$).
[c] Melancholic (mel) Subtype: genotypes ($p = 0.1615$), allele frequencies ($p = 0.41$).
[d] Gender: genotypes ($p = 0.5747$), allele frequencies ($p = 1$).

Table 3
Patients with affective disorders and healthy controls: absolute and relative frequencies of genotypes and allele frequencies of clock gene T3111C polymorphism

	T/T (absolute/relative%)		T/C (absolute/relative%)		C/C (absolute/relative%)		T (absolute/relative%)		C (absolute/relative%)	
Patients	55	53.92	39	38.24	8	7.84	149	73.04	55	26.96
Controls	54	52.43	46	44.66	3	2.91	154	74.76	52	25.24

Fisher's exact test (two sided) comparing genotypes ($p=0.2387$) and allele frequency ($p=0.7363$).

There was no significant deviation from Hardy–Weinberg Equilibrium (HWE) in the samples of patients and healthy controls (χ^2, $p>0.05$, $df=1$). There were no significant differences between patients with affective disorder and controls concerning distribution of genotypes and allele frequencies ($p>0.2$).

In addition, associations were investigated in patients between allele frequencies and genotypes on one hand and number of affective episodes, melancholic depressive subtype, age of onset and gender on the other hand each (Table 2b).

Within all these groups, no significant difference was found concerning genotypes or allele frequencies (all $p>0.05$): While in clinical parameters, "melancholic subtype" of depression ($p>0.1615$) and "age of onset" of the psychiatric disorder ($p>0.4854$), no association is evident, a trend for an association of a high number of episodes and genotypes was found ($p=0.0585$). Calculation on mean age in the subgroup of ≤ 5 affective episodes (mean age = 42.76 years, S.D. $\pm$ 14.07) and >5 (mean age = 45.33 years, S.D. $\pm$ 14.50) did not show a difference ($p=0.35$).

4. Discussion

In recent years, several investigations considering chronobiological approaches to psychiatric disorders have been published. Associations and causal links with several disorders such as chronic sleep disorders, bipolar disorder (BP) and unipolar major depressive disorder (MDD) are currently active areas of research. Despite a lack of studies based on animal models on the function of clock T3111C SNP in affective disorders, several suppositions evolved from epidemiology supporting the impact of the circadian timekeeping system in psychiatric disorders. In our study, no association between T3111C SNP of CLOCK gene and affective disorder was found. This is in agreement with prior findings of Desan et al. (2000) and his allele frequencies for depressed Caucasians (cf. Table 1).

This supports the hypothesis that there is no evidence that T3111C SNP rises vulnerability for affective disorders, all genotypes were in HWE as well in patients as in controls. Similar findings were reported by Johannson et al. (2003), who found no significant difference between patients with seasonal affective disorder (SAD) and controls.

Our additional tests concerning T3111C SNP and age of onset, number of affective episodes and melancholic subtype did not show significant associations either. Noticeable, comparison between patients with ≤ 5 affective episodes and patients with more than 5 affective episodes just failed to reach level of significance ($p=0.0585$). Calculations with larger samples have to be performed to give more conclusive answers on the question in how far clinical features might be influenced by genes of the circadian timekeeping system.

Still, our sample shows ethnic homogeneity in patients and healthy controls, since they all were of Caucasian origin and recruited in Vienna, Austria. Thus, replication of significant differences in allele frequency between European American (EA) and African American (AA) control subjects as found by Desan et al. (2000) with the 3111C allele being less frequent in AAs could not be performed in our sample. Noteworthy, they did not perform calculations on gender differences of allele frequencies.

As mentioned above, there is strong support for biological and clinical features such as atypical sleep in patients with alterations in genes of the time keeping system. Despite our replication of the results of Desan et al. (2000), other findings in the time keeping system still should encourage other research groups to investigate genes for organising circadian rhythms as candidate genes in psychiatry. As mentioned by other authors, patients with seasonal affective disorder (SAD) might be an interesting group for association studies in clock genes. In future investigations, it also might be a useful strategy to form subgroups with sleep abnormalities, for example, early awakening, hypersomnia, etc., in patients with psychiatric disorders. Johannson et al. (2003) suggest that two circadian-clock-related polymorphisms—NPAS2 471 Leu/Ser and Period3 647 Val/Gly—might be implicated in further research in SAD and diurnal preference. One might conclude that the objective to find one single gene responsible for complex relations of chronobiological shifts in psychiatric disorders is at risk to fail. However, considering a more detailed and sophisticated approach to phenotypes and genotypes as well, this might provide more clearly associations between genes and behaviour in humans.

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