Two new rare variants in the circadian "clock" gene may influence sleep pattern

To the Editor:

Circadian rhythms of biological processes are important features in most living organism and have been conserved during evolution. These rhythms are controlled by endogenous self-sustaining oscillators, which represent the core of the so-called biological clock.[1] The principal function of this structure is synchronizing biological activities with external cues, in particular with light stimuli. In mammals, this role is also suggested by the localization of the circadian clock in the bilaterally paired suprachiasmatic nuclei (SCN) of hypothalamus.

At cell level, the circadian clock system is linked to the expression of different genes also called "clock genes." Among these genes, the Circadian Locomotor Output Cycles Kaput one (*CLOCK*) is the first essential component of the mammalian biological clock and encodes a protein that acts as transcriptional activator. At the molecular level, Clock protein interacts with different macromolecules within an autoregulatory transcriptional-translational feedback loop. These positive and negative effects on gene expression are thought to underlie circadian rhythms (see reviews[2,3]). In humans, clock genes play a central role in generating and regulating circadian rhythms and it has been hypothesized that this genetic system could be involved into the well-known biorhythms dysfunctions of mood disorders,[4] and even that polymorphisms in these genes could be associated with circadian and seasonal mood changes and linked symptoms.[5] Moreover, Katzenberg et al.[6] suggested a possible association between a 3' polymorphism in *CLOCK* gene (T3111C) and eveningness in healthy subjects. Recently, our research group reported a possible relationship between the abovementioned polymorphism and sleep disturbances[7,8] or recurrency rate,[9] in mood disorders.

In order to investigate the possible effect of *CLOCK* gene on sleep disorders in relation to mood disorders, a sample of 1113 subjects (479 major depressive disorder, 558 bipolar disorder, and 76 healthy volunteers) was completely sequenced for the 3'UTR region. Our sample was composed of subjects with Italian antecedents for at least two generations; the Italian ethnic origins assured, first of all, a substantial genetic homogeneity and, therefore, the absence of stratification bias in the sample.[10]

We reported two new rare Single Nucleotide Polymorphisms (SNPs), downstream the known T3111C polymorphism. The first SNPs is a G→T transversion at nucleotide 3117, while the second one is an A→G transition at nucleotide 3125 (Gene Bank accession no. AF011568).

Each of the two new rare variants was found only in two affected subjects and not in healthy controls. The first subject, carrying a G3117T transversion is heterozygous for the new rare variant (GT), such as the other patient carrying the A3125G (AG).

Intriguingly, the subjects bearing the two new SNPs, besides showing a classical depressive symptomatology in the index episode, showed a peculiar pattern of night sleep, characterized by alternative phases of good sleep and total insomnia, within few days. In both cases, we could hypothesize a link between the new rare variants and peculiar sleep disturbances, observed during the depressive episode; in fact, single nucleotide rare variant (3117 G/T and 3125 A/G) could be responsible for alteration in CLOCK protein translation. Many literature data confirmed that sequence modification in the 3' UTR region often affect mRNA stability and half-life.[11,12]

The vast majority of eukaryotic mRNAs carry a 3' poly(A) tail of up to 200 adenosine residues in length, which protect the RNA chain from degradation by 5' to 3' or 3' to 5' exonucleases, or both. The mRNA stability is also influenced by specific internal sequence elements, which were also found in the 3'UTR region of different mRNAs[13*]. There are several mechanisms that seem to be involved in eukaryotic mRNA decay; the major mRNA decay pathway consists of shortening of the poly(A) tail.[14] Several sequence elements seem to promote mRNA degradation via poly(A) shortening: this is the case of specific sequences, within the mammalian c-fos 3'UTR, containing an AU-rich element (ARE).[15] Also in yeast, sequences within the 3'UTR of the MFA2 mRNA[13] were shown to promote poly(A) shortening. Other mechanisms, involving 3'UTR region seem to be responsible for mRNA stability (see review[16]).

Moreover, the low frequency of these new rare variants (1 allele among 2226, for each new SNP) could underline the functional importance of this region, whose integrity is probably needed to obtain a physiological level of transcriptional activity and, therefore, the existence of circadian rhythms.

Interestingly, the two new rare variants are localized only at 8-bp distance each other and near to the 3111 (T/C) polymorphism. We hypothesized this region was genetically instable, even if the discovered rare variants allowed however a sufficient degree of translational stability in order to generate the sleep-wake rhythm.

In order to understand the exact role of this genetically instable consensus sequence we screened a specific online database (http://www.gene-regulation.com/pub/databases.html#transfac); both the DNA alterations are localized in a clue region for some transcriptional factors binding. Particularly, the G3117T variant should make the DNA able to bind the c-fos factor,[17] the alpha and beta glucocorticoid receptors,[18] and the YY1nuclear factor[19]; whereas the A3125G substitution may interfere with binding of multiple transcriptional factors (for example, CREB).[20]

To better understand the likely relationship between sleep disorders and depressive syndrome, we planned a polysonnographic analysis on subjects carrying new rare variants, but unfortunately they did not consent. Without this new approach, by now we were not able to verify if the molecular alterations could be effectively responsible for particular patterns of sleep disturbances. Nevertheless, our previous studies indicate that in some depressed patients sleep abnormalities may be partly independent from mood disorder itself,[8] but could be related to a particular genetic pattern; these promising results prompt us to pursue this research way, even if evidences obtained so far need to be confirmed by complementary approaches.

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