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Clock genes in human alcohol abuse and comorbid conditions

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ABSTRACT

Alcohol-use disorders are often comorbid conditions with mood and anxiety disorders. Clinical studies have demonstrated that there are abnormalities in circadian rhythms and clocks in patients with alcohol-use disorders. Circadian clock gene variants are therefore a fruitful target of interest. Concerning alcohol use, the current findings give support, but are preliminary to, the associations of *ARNTL (BMAL1)* rs6486120 with alcohol consumption, *ARNTL2* rs7958822 and *ARNTL2* rs4964057 with alcohol abuse, and *PER1* rs3027172 and *PER2* rs56013859 with alcohol dependence. Furthermore, it is of interest that *CLOCK* rs2412646 and *CLOCK* rs11240 associate with alcohol-use disorders only if comorbid with depressive disorders. The mechanistic basis of these associations and the intracellular actions for the encoded proteins in question remain to be elucidated in order to have the first insight of the potential small-molecule options for treatment of alcohol-use disorders.

Introduction

The global transitions of day and night provide the basis for assessment of the time of day, and the length of night relative to that of day yields information on season. Circadian clocks are universal, subject to natural selection, and driven by signals emerging inside the organism and those from the habitat in order to match the solar day and to reset their phase relative to the local time. Circadian clocks do not only passively measure the length of day, or that of night, but also actively generate and maintain the routine variation in a range of functions of the organism. Their accuracy depends on individual response characteristics of these clocks, on which the knowledge of time and the state of health have a vital influence. Not only internal or societal stimuli, but also physical time-givers that act through the circadian clocks can modify complex behaviors such as moving, eating, drinking, and sleeping in animals, humans being no exception (for review, see Takahashi, Hong, Ko, & McDearmon, 2008).

Circadian data have pointed at the circadian clocks as *a* key, please note that I say not *the* key, first, to bipolar disorder, thereafter to seasonal affective disorder, and finally, to depressive disorder (for review, see Partonen, 2012a). Genetic variants in the core, or canonical, circadian clock genes have been analyzed in a range of phenotypes that display or are suggested to display abnormal circadian rhythms (for reviews, see Barnard & Nolan, 2008; Menet & Rosbash, 2011). Depending on the definition for the core circadian clock genes, their number varies from study to study, but usually it is around 20.

It has become clear that drinking and alcohol intake specifically influence the functions of the circadian body clocks (Spanagel, Rosenwasser, Schumann, & Sarkar, 2005). Findings on the human circadian clock genes from molecular genetic studies of alcohol-use disorders with their common comorbid conditions, i.e., anxiety and depressive disorders (Pirkola et al., 2005), are reviewed here. My literature search covered all the publications on the topic that have been entered into the PubMed database (www.ncbi.nlm.nih.gov/pubmed) up to date and the references therein.

The main findings are presented in Table 1. The names of the genes are given according to the Human Genome Organisation (HUGO) Gene Nomenclature Committee (HGNC) database (see the list of abbreviations). In the following, I review in detail the current data-based findings and give my view on the relevance of circadian clocks to alcohol use.

To start with, concerning the methods for genetic investigation, an obvious strength of genome-wide association studies is their

Abbreviations: ARNTL, aryl hydrocarbon receptor nuclear translocator-like [also known as BMAL1]; ARNTL2, aryl hydrocarbon receptor nuclear translocator-like 2; BMAL1, brain and muscle ARNT [aryl hydrocarbon receptor nuclear translocator]-like 1; CLOCK, circadian locomotor output cycles kaput homolog (mouse); CRY1, cryptochrome 1 (photolyase-like); CRY2, cryptochrome 2 (photolyase-like); NPAS2, neuronal PAS [Period – Aryl hydrocarbon receptor nuclear translocator – Single-minded] domain protein 2; MAOA, monoamine oxidase A; NR1D1, nuclear receptor subfamily 1, group D, member 1; PER1, period homolog 1 (Drosophila); PER2, period homolog 2 (Drosophila); PER3, period homolog 3 (Drosophila); SIRT1, sirtuin 1; TIMELESS, timeless homolog (Drosophila).

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Table 1
The associations of alcohol use with circadian clock gene variants.

Condition	Gene	SNP	Risk allele	Reference
Alcohol abuse	ARNTL2	rs7958822	G allele	Kovanen et al., 2010.
	ARNTL2	rs4964057	T allele	Kovanen et al., 2010.
Alcohol dependence	PER2	rs56013859	A allele	Spanagel, Pendyala, et al., 2005
	PER1	rs3027172	C allele	Dong et al., 2011.
Comorbid alcohol-use disorder	CLOCK	rs2412646	C allele	Sjöholm, Kovanen, et al., 2010
	CLOCK	rs11240	G allele	Sjöholm, Kovanen, et al., 2010
Alcohol consumption	ARNTL	rs6486120	T allele	Kovanen et al., 2010.
	NPAS2	#NPAS2.5	G allele	Gamble et al., 2011.
	PER2	rs56013859	A allele	Comasco et al., 2010.
	PER2	rs56013859	A allele	Blomeyer et al., 2013.

unbiased approach, but the lack of hypothesis-driven analysis and the incomplete gene coverage are their disadvantages. Results from genome-wide association studies are often conflicting, and subsequent replication studies usually fail to confirm the earlier findings. Instead of reducing the uncertainty, they tend to produce a list of novel chromosomal regions of interest, and generate a big number of hits outside the genes rather than identify potential candidate genes. The false discovery rates may be rather high in these efforts.

Candidate-gene association studies may on the one hand suffer from an isolated scope, but on the other hand gain by the full coverage of the gene of interest. If the results from a candidate–gene association study do survive after statistical correction for multiple tests calculated for analysis, they may elucidate the genetic association in a reliable way and open an avenue to study the mechanism and pathogenesis in more detail.

Concerning the study designs, the case–control assessment of population-based samples provides the advantage of having a representative reference for comparison. Ideally, the phenotype of interest needs to be assessed with the same method for both patients and controls. The patient-only studies clearly suffer from the lack of such reference, but they can provide detailed information on the clinical picture, course of illness, and treatment response that, however, may not be specific to the disorder.

Alcohol-use disorders

Circadian clock gene variants in alcohol-use disorders have been analyzed thus far in samples derived from one nationwide study, being representative of the general adult population (Kovanen et al., 2010; Sjöholm, Kovanen, et al., 2010).

Kovanen et al. (2010) analyzed 42 single-nucleotide polymorphisms (SNPs) of 19 genes in 512 cases with alcohol-use disorder and 511 controls from a population-based sample, and found no association with alcohol-use disorder. However, the best findings of indicative significance included the associations of alcohol abuse with *ARNTL2* rs7958822 and with *ARNTL2* rs4964057 (Kovanen et al., 2010).

Sjöholm, Kovanen, et al. (2010) analyzed 32 SNPs of 19 genes in 446 cases with alcohol-use disorder and 517 controls from a population-based sample, and found that none were significantly associated with alcohol-use disorder. However, their report suggested nominal associations for *CLOCK* rs2412646 and *CLOCK* rs11240 among the 76 cases with a comorbid alcohol-use disorder and depressive disorder (Sjöholm, Kovanen, et al., 2010). Here, it is of note that the *CLOCK* gene was not at all associated with alcohol-use disorder only or with depressive disorder only.

Interestingly, the *CLOCK* gene was among the best candidates to contribute to multiple substance dependence, i.e., cocaine or opioid or alcohol dependence. It was found that the chromosomal region has a significant linkage in a sample of 355 families (with 806 individuals), as ascertained through affected sibling pairs (Yang et al., 2012). No SNPs of *CLOCK* were specifically analyzed for the report, but the results point out a role for CLOCK in the pathogenesis of such comorbid conditions where alcohol-use disorders are a component.

Concerning the *PER2* gene in particular, the data are conflicting, as patient-only studies have produced mixed results. At the start, the so-called Spanagel-Albrecht SNP (rs56013859, or SNP #10870 as it was labeled in the beginning) received much attention, after it was demonstrated, using a hospital-based sample of 215 patients with alcohol dependence, that the A allele contributed to the high (>300 g per day versus < 300 g per day) alcohol use (Spanagel, Pendyala, et al., 2005). However, in a sample of 110 women with alcohol dependence who were recruited from a long-term in-patient treatment facility, there was no association of *PER2* rs56013859 with alcohol dependence. Rather, the G allele was over-represented among these patients, as compared to the adolescent female controls (Comasco et al., 2010).

Moreover, *PER2* rs56013859 was not associated with alcohol-use disorder or alcohol use in 512 subjects with alcohol-use disorder and 511 controls from a population-based sample (Kovanen et al., 2010). Furthermore, in a study of skin fibroblast cultures derived from 19 patients with alcohol-use disorders and 13 healthy controls, presence of *PER2* rs56013859 in the cells was not associated with the diagnosis (McCarthy, Fernandes, Kranzler, Covault, & Welsh, 2013). In this study, *PER2* rs56013859 was not associated with any of the circadian rhythm parameters as measured in fibroblasts (McCarthy et al., 2013), in agreement with no association of *PER2* rs56013859 with the behavioral trait of diurnal preference (Johansson et al., 2003).

However, as the number of alcohol-dependence criteria that were met increased, the circadian period (in the study by McCarthy et al., 2013) decreased. The finding agrees with the results from Hätönen and colleagues (Hätönen, Forsblom, Kieseppä, Lönnqvist, & Partonen, 2008) that patients with comorbid alcohol-use disorder and bipolar disorder were more of the morning type (morning larks) as compared with patients with bipolar disorder only, and therefore suggests that the circadian body clock may contribute to an altered trajectory of alcohol-use disorders.

Beyond *PER2*, alcohol dependence was associated with *PER1* rs3027172, with the C allele being the risk allele. This was found in a hospital-based sample of 1006 patients with alcohol dependence, to which 1178 individuals recruited as a comparison sample for genetic studies of several neuropsychiatric phenotypes were referred as controls (Dong et al., 2011).

In addition to these studies of the genetic variants, the messenger RNA levels of 6 genes, as assessed in peripheral blood mononuclear cells at 9:00 AM, have been analyzed from a sample of 22 patients with alcohol dependence (Huang et al., 2010). These results yielded strikingly lower expression levels of *CLOCK*, *CRY1*, *CRY2*, *ARNTL,* and *PER2*, in this order, among the patients as compared with 12 healthy controls, as if the circadian body clock were to have been knocked-down. Only *PER1* was not affected to the extent of the others.

Alcohol consumption

Among the best findings of indicative significance from a population-based sample of 511 controls, there is the association of *ARNTL* rs6486120 with alcohol use, as assessed in grams per