GENETICS

Circadian Clock Gene Polymorphisms in Alcohol Use Disorders and Alcohol Consumption

Leena Kovanen\textsuperscript{1,2,*}, Sirkku T. Saarikoski\textsuperscript{2}, Jari Haukka\textsuperscript{1}, Sami Pirkola\textsuperscript{1,4}, Arpo Aromaa\textsuperscript{3}, Jouko Lönnqvist\textsuperscript{1,5}
and Timo Partonen\textsuperscript{1}

\textsuperscript{1}Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, Finland, \textsuperscript{2}Department of Alcohol, Drugs and Addiction, National Institute for Health and Welfare, Helsinki, Finland, \textsuperscript{3}Division of Welfare and Health Policies, National Institute for Health and Welfare, Helsinki, Finland, \textsuperscript{4}Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland and \textsuperscript{5}Department of Psychiatry, University of Helsinki, Helsinki, Finland

*Corresponding author: Tel: +358 20 610 8128; Fax: +358 20 610 7191; E-mail: leena.kovanen@thl.fi

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Abstract — Aims: Circadian clock genes are involved in the development of drug-induced behaviors and regulate neurotransmission pathways in addiction. Our aim was to study whether circadian clock gene polymorphisms predispose to alcohol dependence or abuse or other alcohol-related characteristics. Methods: The study sample comprised of 512 individuals having alcohol dependence or alcohol abuse (according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV)) and their 511 age- and sex-matched controls. This population-based sample was drawn from a cohort \((n = 7413)\), representative of the Finnish general population aged 30 and over. Altogether 42 single-nucleotide polymorphisms of 19 genes related to the circadian pacemaker system were genotyped. Results: \(\text{ARNTL} \) rs6486120 \(T^\text{c} \) allele status \((P = 0.0007, q = 0.17)\), \(\text{ADCYAP1} \) rs2856966 \(GG \) genotype \((P = 0.0006, q = 0.17)\) and \(\text{VIP} \) CC haplotype \((\text{rs}3823082 – \text{rs}688136) \) \((P = 0.0006)\) were suggestively associated with alcohol consumption in socially drinking controls. \(\text{ARNTL2} \) GT haplotype \((\text{rs}7958822 – \text{rs}4964057) \) \((P = 0.0013)\) associated suggestively with alcohol abuse diagnosis \((P = 0.0013)\). Earlier findings on the associations of \(\text{DRD2} \) and \(\text{NPY} \) with alcohol dependence were supported. \(\text{DRD2/ANKK1 Taq1A1} \) \((P = 0.04)\) and \(\text{NPY} \) Pro7 decreased \((P = 0.01)\) the risk of alcohol dependence. Conclusions: \(\text{ARNTL}, \text{ARNTL2}, \text{VIP} \) and \(\text{ADCYAP1} \) were indicated as having influence on alcohol use or abuse. The role of \(\text{DRD2} \) and \(\text{NPY} \) on alcohol dependence was also supported.

INTRODUCTION

Alcohol consumption is disruptive to circadian functions (reviewed by Spanagel et al., 2005b). Pathways implicated in the development of alcohol dependence are linked to the circadian clock. The principal circadian clock in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus influences cells located in regions of importance to addiction such as dorsomedial hypothalamus, lateral hypothalamic area and ventral tegmental area (Sakurai, 2007; Aston-Jones et al., 2008). Abnormalities in the stimuli processed by the principal circadian clock may not only disrupt their integration with emotional states (Lamont et al., 2005) but also sensitize to stressors (Gonzalez and Aston-Jones, 2008) that are known to predispose to addiction (Cleck and Blendy, 2008). Circadian clock genes are involved in the development of drug-induced behaviors and regulate neurotransmission pathways having a role in addiction (McClung et al., 2005; Hampp et al., 2008; Perreau-Lenz and Spanagel, 2008). On the other hand, drugs of abuse induce specific expression changes in circadian clock genes in the brain (reviewed by Perreau-Lenz and Spanagel, 2008).

The molecular circadian clock consists of several clock proteins, such as aryl hydrocarbon receptor nuclear translocator-like (\(\text{ARNTL} \) or \(\text{BMAL1} \)), \(\text{ARNTL2} \) or \(\text{BMAL2} \)), clock (\(\text{CLOCK} \)), neuronal PAS domain protein 2 (\(\text{NPAS2} \)), periods (\(\text{PER1}, \text{PER2} \)) and cryptochromes (\(\text{CRY1}, \text{CRY2} \)), that interact with each other in the regulatory transcriptional and translation-translation feedback loops. In the primary feedback loop, \(\text{CLOCK} \) (Vitaterna et al., 1994) or \(\text{NPAS2} \) (Zhou et al., 1998) pairs up with \(\text{ARNTL} \) or \(\text{ARNTL2} \) (Ikeda and Nomura, 1997; Ikeda et al., 2000) and activates transcription of \(\text{PER} \) and \(\text{CRY} \) proteins, which dimerize (Dardente et al., 2007) and inhibit \(\text{CLOCK} \)/\(\text{NPAS2} – \text{ARNTL}/\text{ARNTL2} \)-mediated transcription. \(\text{PER} \) proteins dimerize also with timeless (\(\text{TIMELESS} \)) to inhibit transcription (Sangoram et al., 1998). The \(\text{CLOCK} /\text{NPAS2} – \text{ARNTL}/\text{ARNTL2} \) dimers activate, in addition to core clock genes, the transcription of groups of other target genes, thereby modulating the expression of clock-controlled genes involved in the sleep–wake cycle, locomotor activity, endocrine rhythms, body temperature, cardiovascular rhythms and hepatic metabolism such as farnesyl-diphosphate farnesyltransferase 1 (\(\text{FDFT1} \)) (Acimovic et al., 2008). Nuclear receptors together with their co-activators (e.g. nuclear receptor co-activator 3, \(\text{NCOA3} \) or \(\text{ACTR} \)) (Asher and Schibler, 2006) and repressors are needed for control of the subsequent cycles of the feedback loops (Ko and Takahashi, 2006).

Environmental time-givers synchronize the SCN with the external 24-h cycles. The main entraining signal is the daily light–dark rotation, and its information is transmitted from the retina of the eye directly to the SCN via the retinohypothalamic tract. This process is mediated by a number of proteins, e.g. opsin 4 (\(\text{OPN4} \)) (Ukai et al., 2007), adenylyl cyclase-activating polypeptide 1 (\(\text{ADCYAP1} \) or \(\text{PACAP} \)) (Hannibal, 2006) and phospholipase C, beta 4 (\(\text{PLCB4} \)) (Park et al., 2003). One of the main chemical constituents of light-activated SCN cells is vasoactive intestinal peptide (\(\text{VIP} \)) (Piggins and Cutler, 2003). Its receptor, vasoactive intestinal peptide receptor 2 (\(\text{VIPR2} \)), appears to play a role within the SCN, as mice lacking the receptor have disrupted circadian rhythms and impaired synchronization to the light–dark transitions (Hughes and Piggins, 2008). The SCN sends neural and chemical signals to other brain areas and peripheral tissues to regulate output pathways that are responsible for the proper timing of physiological functions. The sleep–wake cycle is one of the output pathways. This cycle is further regulated by genes that are involved in the process of sleep such as adenosine deaminase (\(\text{ADA} \)) and acyl-coenzyme A dehydrogenase, C-2 to C-3 short...
chain (ACADS), the latter being indicated in rapid eye movement sleep that is under control of the circadian pacemaker system (Retey et al., 2005; Tafti et al., 2003).

Genes involved in the circadian pacemaker system include some traditional candidate genes of alcohol dependence such as neuropeptide Y (NPY) and dopamine receptor D2 (DRD2). Modifications to the NPYergic functions modify ethanol intake (Badia-Elder et al., 2007). In the clockwork, the non-photic information to the SCN is communicated through NPY (Cheng et al., 2004) and modulated by ADcyAP1 (Cheng et al., 2006) containing projections. Dopamine is centrally involved in the rewarding effects of drugs of abuse (reviewed by Nestler, 2005). In the clockwork, light exposure activates dopaminergic signals in the retina of the eye (Brainard and Morgan, 1987), and the signals that are mediated by DRD2 enhance the transcriptional capacity of CLOCK–ARNTL dimers in control of physiological pathways that react to light exposure (Yujnovsky et al., 2006).

Genetic linkage between circadian clocks and ethanol preference is suggested by studies on mice and rats selectively bred for ethanol preference that demonstrate alterations in the circadian pacemaker system (reviewed by Spanagel et al., 2005b). Little is known of specific clock genes or their protein products in relation to alcohol dependence and consumption. The strongest evidence concerns period genes (Perreau-Lenz and Spanagel, 2008). Chronic ethanol consumption alters the circadian rhythm of Per2 mRNA levels in the rat SCN (Chen et al., 2004). On the other hand, Per2 mutant mice voluntarily consume more alcohol than wild-type mice (Spanagel et al., 2005a). There is also evidence for the genetic association of circadian clock gene variants with drug abuse. In humans, a genetic variation of the PER2 gene SNP 10870 (Spanagel et al., 2005a, no rs number available) has been associated with the risk for high alcohol intake (>300 g/day) in alcohol-dependent patients (Spanagel et al., 2005a) In addition to PER genes, also CLOCK, ARNTL and NPAS2 have been linked to other drugs of abuse (reviewed by Perreau-Lenz and Spanagel, 2008), and the latter two have been associated with seasonal affective disorder (Johansson et al., 2003; Partonen et al., 2007) in which the risk of high alcohol use is increased and segregates in families (reviewed by Sher, 2004). The influence of polymorphisms of CLOCK rs1801260 (3111 C/T), PER1 2548 G/A and PER2 rs934945 (Gly1244Glu, C/T) on cocaine dependence and cocaine-induced paranoia has been studied but no association was found (Malison et al., 2006).

Based on these earlier data, we wanted to study whether variations in genes that are related to the circadian pacemaker system predispose to alcohol dependence, abuse or higher alcohol consumption.

MATERIALS AND METHODS

Participants

This study was part of a nationwide health interview and examination survey, Health 2000, which is described in detail elsewhere (http://www.terveys2000.fi/indexe.html). The ethical approval of the survey has been accepted by the ethics committees of the National Public Health Institute and the Helsinki and Uusimaa Hospital District, and all participants provided a written informed consent. Of the 7415 participants, 5480 took part in a health status examination and a diagnostic mental health interview, Munich-Composite International Diagnostic Interview (M-CID) (Wittchen et al., 1998) that is a valid and reliable instrument for the assessment of alcohol use, mood and anxiety disorders, yielding the diagnosis according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). These participants completed a self-report on seasonal variations in mood and behavior and gave venous blood samples for DNA extraction.

The participants of the current study included cases with a diagnosis of alcohol dependence or alcohol abuse (n = 512) and their matched (by sex and age) controls having no mental disorder (n = 511). There were 99 women in both cases and controls. Of the 512 cases, 414 had a diagnosis of alcohol dependence, and 89 had a diagnosis of alcohol abuse. As part of the assessment, participants (417 of the cases and 414 of the controls) answered a question asking how many glasses they needed to drink to start feeling the effects of alcohol (the first five times they ever drank alcohol). For socially drinking controls (n = 423), the absolute amount of alcohol consumption (g/kg/week) was assessed. In addition, this group was also analyzed by dividing it into high (>280 g/week for men and >190 g/week for women, n = 38) and low (n = 385) levels of alcohol consumption.

SNP selection and genotyping

We focused on the analysis of known canonical circadian clock genes. In addition, we were interested in genes that are related to the circadian pacemaker system along pathways located upstream or downstream of the SCN (Ukai et al., 2007; Yujnovsky et al., 2006) and genes that are linked to the circadian clock through the process of sleep (Retey et al., 2005; Tafti et al., 2003). The genes and polymorphisms were selected on the basis of prior published associations with substance use or mental health disorders and/or based on the potential for functional relevance, e.g. single-nucleotide polymorphisms (SNPs) resulting in amino acid changes. In addition to this candidate SNP approach, we selected HapMap tag-SNPs from the core clock genes (ARNTL, ARNTL2 and CLOCK) in order to increase coverage. Overall, 42 SNPs for 19 genes (ARNTL, ARNTL2, CLOCK, CRY2, NPAS2, PER2, TIMELESS, VIP, VIPR2, PLCB4, NPY, NCOA3, OPN4, GLO1, FDF1, DRD2, ADcyAP1, ADA and ACADS) were selected.

Genomic DNA was isolated from white blood according to standard procedures. SNPs were genotyped with a fluorogenic 5′ nucleotide assay method (TaQMan™) with pre-designed primer-probe kits (TaQMan Pre-Designed SNP Genotyping Assays, Applied Biosystems, Foster City, CA, USA) using Applied Biosystems 7300 Real Time PCR System according to manufacturers’ instructions. For ADA rs73598374 (Asp8Asn, G/A (Retey et al., 2005)), PER2 10870 (Spanagel et al., 2005a) and FDFT1 rs11549147, Custom TaqMan SNP Genotyping Assays were used (Supplementary Table 1). All laboratory procedures were carried out blind to the case/control status. All the samples were successfully genotyped. About 5% of samples were re-genotyped to confirm that the genotyping results had no error. Genotyping of the first 200 samples indicated that the following four SNPs were not polymorphic in our Finnish study population and therefore were excluded from further analysis: ARNTL2 rs35878285, CRY2 rs2863712, NCOA3 rs2230783 and PER2 S662G T/C. NCOA3...
rs6094752, with a minor allele frequency of <5%, was excluded from the analyses. Finally, data for statistical analyses were derived for 37 SNPs in 512 cases and 511 controls.

Statistical analysis
Two-sided statistical analyses of the data were performed using the PLINK 1.05 software (http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell et al., 2007). Additive, dominant and recessive models were analyzed, controlling for age and sex, using linear and logistic regression models for quantitative and dichotomous traits, respectively. For the dichotomous trait results, odds ratios (OR) with their 95% confidence intervals (CIs) were computed. For replication of previous findings, P-values < 0.05 were considered significant. To correct the SNP analyses for multiple testing across all the tests, R software (http://www.r-project.org/) was used to calculate the false discovery rate (FDR q-values) (Storey, 2003). q-Values < 0.05 were considered significant and 0.05 < q < 0.20 indicative of significance, i.e. nominally significant but suggestive by FDR criteria. Similar studies to ours have applied a q-value threshold of <0.2 (Lu et al., 2008; Smith et al., 2007; Smith L et al., 2008; Smith NL et al., 2008). The Haploview software (Barrett et al., 2005) was used to calculate the linkage disequilibrium (LD) estimates. The formed haplotype blocks (Supplementary Fig. 1) were analyzed by using the PLINK software, with the sliding window approach, regression models, and controlling for age and sex. The Genetic power calculator (Purcell et al., 2003) was used for power analysis.

RESULTS
The genotype and allele frequencies and Hardy–Weinberg equilibrium (HWE) estimates in cases and controls are shown in Table 1. All the SNPs were in HWE in the 512 cases and 511 controls. The alleles are presented in the same orientation as in HapMap unless other nomenclature is well-established, as it is for DRD2 rs1800497 (A1/A2) and NPY rs16139 (Leu7-Pro). The associations with P < 0.05 are listed in Table 2. The haplotypes in the formed blocks (Supplementary Fig. 1), with P-values higher than for the individual SNPs within the haplotype (Supplementary Table 2), are not discussed. The power to detect allele frequency differences was ≥0.8 for an OR of 1.7 or greater when allele frequency was ≥0.05.

Diagnosis-based phenotype
The frequently analyzed SNP, known as DRD2 Taq1A (rs1800497), was originally associated with the DRD2 gene but was later found to be located within exon 8 of the adjacent ANKK1 gene. Here, the SNP was associated with alcohol dependence or abuse (n = 512) and with alcohol dependence alone (n = 414 excluding alcohol abuse), the A1 allele being predisposing in the additive (P = 0.048, OR = 1.24, 95% CI = 1.00–1.53 and P = 0.038, OR = 1.27, 95% CI = 1.01–1.59, respectively) and recessive models (P = 0.013, OR = 2.22, 95% CI = 1.18–4.15 and P = 0.010, OR = 2.35, 95% CI = 1.23–4.48, respectively). Similarly, NPY rs16139 (Leu7Pro) was associated with alcohol dependence or abuse (n = 512) in the additive model (P = 0.038, OR = 0.69, 95% CI = 0.48–0.98) and alcohol dependence alone (n = 414) in additive and dominant models (P = 0.010, OR = 0.59, 95% CI = 0.40–0.88 and P = 0.020, OR = 0.61, 95% CI = 0.40–0.92, respectively), the Pro7 allele being protective. ARNTL2 GT haplotype (Block1, rs7958822–rs4964057) associated with the increased risk of alcohol dependence or abuse (P = 0.03, OR = 1.31) and with abuse alone (P = 0.0013, OR = 1.96).

Consumption related phenotype
In socially drinking controls (n = 423), ADCYAP1 or PACAP rs2856966 was suggestively associated with alcohol consumption (g/kg/week), the GG genotype being predisposing in the recessive model (P = 0.0060, Beta = 2.10, 95% CI = 0.91–3.29, q = 0.17). In the dominant model, ARNTL rs486120 was suggestively associated with consumption in controls (g/kg/week), the T’ allelic status (TT and GT genotypes) being predisposing (P = 0.00067, Beta = 0.60, 95% CI = 0.26–0.94, q = 0.17). VIP CC haplotype (rs3823082–rs688136) suggestively associated with the increased risk to consume more alcohol (P = 0.00057, Beta = 0.57).

First-effect phenotype
In controls, DRD2 rs6277 was suggestively associated with a higher number of drinks needed (the first five times ever drinking) to obtain an effect, the TT genotype being predisposing in the recessive model (P = 0.00038, Beta = 0.51, 95% CI = 0.23–0.78, q = 0.17). No significant associations were found for the first-effect in those having alcohol dependence or abuse.

DISCUSSION
In the current study, we analyzed cases with alcohol dependence or abuse and their matched controls from a big cohort representative of the Finnish general population. The diagnoses of mental and behavioral disorders were assessed according to DSM-IV using a structured diagnostic interview. Versatile phenotype data available from the nationwide health examination survey allowed us to analyze alcohol-related phenotypes on a broad scale.

Individuals having the DRD2 Taq1A A1/A1 (rs1800497) genotype were over-represented among those with alcohol dependence or abuse, as well as among those with dependence only. These individuals had over a 2-fold risk of alcohol dependence as compared with those having the A2’ allelic status. Inconsistent literature has accumulated on the association of Taq1A with alcohol dependence since the first report (Blum et al., 1991; Blum et al., 1990). Two recent meta-analyses confirm the association of the A1 allele with the increased risk of alcohol dependence (Munafo et al., 2007; Smith L et al., 2008; Smith NL et al., 2008). They report a minor but significant association with alcohol dependence in the dominant (OR = 1.38, 95% CI = 1.20–1.58), recessive (OR = 1.22 95%, CI = 1.05–1.43) (Smith L et al., 2008; Smith NL et al., 2008) and additive (OR = 1.21, 95% CI = 1.13–1.30) (Munafo et al., 2007) models. However, a true effect might be more modest due to a publication bias (Munafo et al., 2007). DRD2 levels are lower in alcohol-dependent individuals than in controls (Hietala et al., 1994; Volkov et al., 2002), and Drd2 over-expression was associated with lower levels of self-administration in an animal study (Thanos et al., 2001). Historically, DRD2 Taq1A (rs1800497) has been assigned to DRD2 gene...
<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Groupa</th>
<th>Alleles (1/2)</th>
<th>Genotype counts (%)</th>
<th>Allele counts (%)</th>
<th>( P ) (HWE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NPAS2</strong></td>
<td>rs10206927</td>
<td>Cases</td>
<td>G/C</td>
<td>362 (70.7) 132 (25.8) 18 (3.5)</td>
<td>856 (83.6) 168 (16.4)</td>
<td>0.195</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td></td>
<td>347 (67.9) 150 (29.4) 14 (2.7)</td>
<td>843 (82.6) 178 (17.4)</td>
<td>0.759</td>
</tr>
<tr>
<td><strong>NPAS2</strong></td>
<td>rs6725296</td>
<td>Cases</td>
<td>G/A</td>
<td>393 (76.8) 110 (21.5)  9 (1.8)</td>
<td>896 (87.5) 128 (12.5)</td>
<td>0.685</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Controls</td>
<td></td>
<td>390 (76.3) 116 (22.7)  5 (1)</td>
<td>896 (87.7) 126 (12.3)</td>
<td>0.311</td>
</tr>
<tr>
<td><strong>NPAS2</strong></td>
<td>rs11673746</td>
<td>Cases</td>
<td>C/T</td>
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<td>717 (70) 307 (30)</td>
<td>0.207</td>
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<td>Controls</td>
<td></td>
<td>249 (48.7) 208 (40.7) 54 (10.6)</td>
<td>706 (69.1) 316 (30.9)</td>
<td>0.3</td>
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<td><strong>NPAS2</strong></td>
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<td>C/T</td>
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<td>839 (81.9) 185 (18.1)</td>
<td>0.459</td>
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<td>Controls</td>
<td></td>
<td>352 (68.9) 143 (28)  16 (3.1)</td>
<td>847 (82.9) 175 (17.1)</td>
<td>0.755</td>
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<tr>
<td><strong>NPAS2</strong></td>
<td>rs2305160</td>
<td>Cases</td>
<td>G/A</td>
<td>275 (53.7) 195 (38.1) 42 (8.2)</td>
<td>745 (72.8) 279 (27.2)</td>
<td>0.373</td>
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<td>Controls</td>
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<td>252 (49.3) 218 (42.7) 41 (8)</td>
<td>722 (70.6) 300 (29.4)</td>
<td>0.594</td>
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<tr>
<td><strong>PER2</strong></td>
<td>rs934945</td>
<td>Cases</td>
<td>C/T</td>
<td>409 (79.9) 99 (19.3)  4 (0.8)</td>
<td>917 (89.6) 107 (10.4)</td>
<td>0.636</td>
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<td></td>
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<td>10870</td>
<td>Cases</td>
<td>A/G</td>
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<td>857 (83.7) 167 (16.3)</td>
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<tr>
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<td>Controls</td>
<td></td>
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<td>0.539</td>
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<tr>
<td><strong>PER2</strong></td>
<td>rs2304672</td>
<td>Cases</td>
<td>G/C</td>
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<td>851 (83.1) 173 (16.9)</td>
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<td>Controls</td>
<td></td>
<td>357 (69.9) 141 (27.6) 13 (2.5)</td>
<td>855 (83.7) 167 (16.3)</td>
<td>1</td>
</tr>
</tbody>
</table>
| **PER2**  | S662G, T/C | All    | T−            | 183 (100) 0 0 0 | 366 (100) 0 0 | (Continued)
whereas more recent data have indicated that this SNP is actually located within the coding region of *ANKK1* gene (reviewed by Ponce et al., 2009). These two genes overlap and are transcribed from the opposite directions. Proximity of the two genes may be non- incidental and reflect functional relationship. *Tag*1A could associate with dopaminergic phenotypes by being in LD with other SNPs in *DRD2* or by reflecting functional variations of the *ANKK1* that are implicated in dopaminergic transmission (reviewed by Ponce et al., 2009).

With respect to alcohol consumption, studies have paradoxically demonstrated the opposite association to that reported for alcohol dependence: the *A1A1* genotype has been associated with lower levels of alcohol consumption in social drinkers (Hallikainen et al., 2003; Munafò et al., 2005). In our study, this polymorphism was not associated with alcohol consumption in social drinkers.

We analyzed another *DRD2* polymorphism, rs6277 (*C957T*), which is located in the coding region of the *DRD2* gene. It does not change amino acids, but it affects mRNA stability and translation, and changes considerably dopamine-induced up-regulation of *DRD2* expression (Duan et al., 2003). With respect to this polymorphism, in controls, a nominally significant association was observed with a higher number of drinks needed to obtain an effect during the first five times ever drinking. Individuals having the *TT* genotype appeared to have lower alcohol sensitivity, as they needed significantly more drinks to feel the effects of alcohol compared with those having the *CT* allelic status. Sensitivity to alcohol is an important endophenotype related to alcohol dependence risk, because low sensitivity to alcohol predicts the increased risk of developing alcohol dependence (Heath et al., 1999). In a previous study, the *T* allele was also reported to increase the risk of alcohol dependence (Hill et al., 2008). In our study, no significant relationship was observed between this polymorphism and the diagnosed alcohol dependence.

Animal studies demonstrate that NPY has a role in control of alcohol consumption (Badia-Elder et al., 2007). The literature concerning the associations of NPY rs16139 (*Leu7Pro*) with alcohol dependence and alcohol consumption is inconsistent. Pro7 (*C*) has been linked to an increased risk of alcohol dependence (Lappalainen et al., 2002) and with alcohol consumption (Kauhanen et al., 2000). On the other hand, Pro7 has also been associated with the decreased risk of either type 1 or type 2 alcoholism (Ilveskoski et al., 2001). In line with the latter study, a nonsignificant (*P* = 0.053) overrepresentation of the Pro7 allele was observed among the controls in another study (Mottagui-Tabar et al., 2005). In a study combining data from the Finnish and Swedish populations, there was no association of the *NPY* polymorphism with alcohol dependence in the pooled or separate study materials, but a slight underrepresentation of the Pro7 allele in a subgroup of pooled Swedish and Finnish late-onset cases with alcoholism (Zhu et al., 2003). In addition, there is a study reporting no relationship between this SNP and alcohol dependence or alcohol intake per day in patients with alcohol dependence (Zill et al., 2008). Our findings support the protective effect of Pro7 in alcohol dependence or abuse thus being in line with several earlier studies (Ilveskoski et al., 2001; Mottagui-Tabar et al., 2005; Zhu et al., 2003), but in contrast with the earlier Finnish study (Lappalainen et al., 2002). However, in the study by Lappalainen et al., the allelic frequencies in cases were similar to those observed in other studies but exceptionally low in controls.

We found no association of *PER2* with alcohol consumption (g/kg/week) or misuse (high, >280 g/week for men and >190 g/week for women, vs. low alcohol intake) among social drinkers. Previously, *PER2* gene polymorphism (SNP 10870) has been associated with high alcohol intake (≥300 g/day) in alcohol-dependent patients with *G* allele being protective (Spanagel et al., 2005a). Moreover, *G* carriers have been shown to have reduced alcohol consumption in the presence of several and frequent sleep problems (Comasco et al., 2010). Alcohol-dependent individuals comprise a heterogeneous group in regard to their drinking patterns (Epstein et al., 2004). In fact some of the individuals diagnosed with alcohol dependence drink relatively little (Dawson, 2000). Accumulating ill health as well as social and mental problems that are related to alcohol dependence may result in drinking cessation or reduction (Dawson, 2000; Shaper, 1990; Dawson et al., 2007). In line with this, in our study 31 out of 401 currently drinking cases had used health or social services due to their drinking problem during the past 12 months. Due to these confounding factors, we did not in principle analyze the polymorphisms in relation to consumption levels in the group of alcohol dependence or abuse. However, to be better able to compare our
results with those obtained by Spanagel et al., we made an exception and analyzed the PER2 (SNP 10870) polymorphism in relation to alcohol consumption among alcohol-dependent cases but found no association. These two studies represent very different study designs, i.e. alcohol-dependent individuals admitted for an inpatient withdrawal therapy with very high levels of alcohol consumption (≥300 g/day in the high alcohol intake group) in the Spanagel study vs. our general population-based study including no individual with such high consumption level. Consequently, the possibility that the association is limited to alcoholics with very high alcohol consumption or to alcohol consumption in the presence of several and frequent sleep problems remains to be elucidated. The stretch of DNA in which this SNP is located is exceptionally rich in hypothetical allelic transcription factor binding site changes for SP1, MYB and NFKB1. This SNP may be an intrinsic transcriptional enhancer element, whose allelic constitution may thereby influence PER2 levels. If this holds,
PER2 may display an association with alcohol use only in the most severe cases of alcohol dependence, together with sleep problems or in such cases in which there are abnormalities in the cell-division cycle control, in addition to those in the circadian regulation. Furthermore, PER2 is involved in CLOCK–ARNTL2-mediated transactivation (Sasaki et al., 2009). ARNTL2 can dimerize with CLOCK to activate a group of target genes, and there is a functional partnership between ARNTL2 and PER2 that might bridge social phobia and alcohol use (Zimmermann et al., 2003) to end in alcohol dependence. Earlier, in a sample of 321 individuals with anxiety disorder and 653 matched controls derived from this cohort of the Finnish general population, ARNTL2 was demonstrated to associate with social phobia (Sipila et al., 2010). In our current study, we found ARNTL2 GT haplotype (rs7958822–rs4964057) to suggestively associate with the increased risk of alcohol dependence or abuse.

Of the canonical circadian clock genes, ARNTL rs6486120 was nominally indicated in alcohol consumption in socially drinking controls: individuals having the T' allelic status (TT or GT genotype) were more likely to consume greater amounts of alcohol as compared with those having the GG genotype, even though there was no significant association with any diagnosis of alcohol use disorder. ARNTL gene polymorphisms have previously been associated with bipolar disorder (Mansour et al., 2006; Nievergelt et al., 2006), and the role of ARNTL in drug-seeking behavior is supported by the observation that repeated cocaine self-administration up-regulates the expression of ARNTL in the dorsal striatum in rats (Lynch et al., 2008).

ADCYAP1-deficient mice have deficits in responses of the circadian pacemaker system to light exposure (Colwell et al., 2004). Interestingly, both ADCYAP1 and VIP have been reported to modulate the development of tolerance to morphine and ethanol in mice (Szabo et al., 1998). Furthermore, neonatal alcohol exposure induces decreases in the density of VIP containing neurons that contribute to disturbances of circadian clock-driven behavior in rats (Farnell et al., 2009). In the current study, a non-synonymous SNP of ADCYAP1 rs2856966 (Asp54Gly) had potential relevance to alcohol consumption (g/kg/week), the GG genotype being predisposing to higher levels of consumption in the recessive model in socially drinking controls. Moreover, our study indicated that VIP might also be involved in human consumption as well, since VIP CC haplotype (rs3823082–rs688136) was suggestively associated with higher level of alcohol consumption in socially drinking controls.

Our study bears several strengths. It is a population-based and nationwide study. The study population is representative of the general population aged over 30 living in Finland, and its sample derives from the population that has reduced genetic and environmental heterogeneity. Hence, these data can be generalized directly to concern the whole adult general population of Finland or any population having similar living conditions and similar genetic background. Since the study sample derives from an epidemiological cohort, it is well characterized for somatic and psychiatric conditions. We had extensive data on the phenotype and valid assessments of conditions on our focus. The SNPs were selected for their potential role in the function of a gene, which improves the possibility that the genotype seen here contributes to the phenotype although experimental analysis is needed for verification. On the other hand, there are limitations that are related to candidate gene association studies in general, such as sample sizes. Due to its origin, the sample sizes of specific disorder categories remain rather small. We controlled for the number of statistical tests, adjusted the level of significance and calculated the FDR q-values. However, the new findings were only suggestive by false discovery criteria, and replication of these nominally significant findings in independent study samples is the most practical way to increase the probability of a true association.

CONCLUSION

To conclude, the variants of the core circadian clock genes studied herein seem not to have a major effect on alcohol dependence. However, we report suggestive associations of ARNTL (rs6486120), ADCYAP1 (rs2856966) and VIP (haplotype rs3823082+rs688136) with alcohol consumption, DRD2 (rs6277) with alcohol sensitivity and ARNTL2 (haplotype rs7958822+rs4964057) with alcohol abuse. Our findings are reasonable, because these genes have a role in the circadian pacemaker system. Light exposure activates dopaminergic signals in the retina of the eye (Brainard and Morgan, 1987), and those signals that are mediated by the DRD2 receptors enhance the capacity of CLOCK–ARNTL transcriptional complex in reactions to light exposure (Yujnovsky et al., 2006). ADCYAP1 is a neurotransmitter of the retinohypothalamic tract, the direct input pathway from the retina to the SCN, whereas VIP participates in maintaining oscillations within and sending signals from the SCN. In the SCN, ARNTL is one of the core clock genes that control the synchronized output. Our results also support the earlier findings that DRD2/ANKK1 Taq1A1 predisposes to alcohol dependence and NPY Pro7 protects against it.

SUPPLEMENTARY DATA

Supplemental material is available at Alcohol and Alcoholism online.

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