Contribution of adenosine related genes to the risk of depression with disturbed sleep

Natalia Gassa, Hanna M. Ollila, Siddheshwar Utge, Timo Partonen, Erkki Kronholm, Sami Pirkola, Johanna Suhonen, Kaisa Silander, Tarja Porkka-Heiskanen, Tiina Paunio

Department of Physiology, Institute of Biomedicine, University of Helsinki, Helsinki, Finland
Public Health Genomics Unit and Institute for Molecular Medicine FIMM, National Institute for Health and Welfare, Helsinki, Finland
Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland
Department of Mental Health and Substance Abuse Services, National Institute for Health and Welfare, Helsinki, Finland
Department of Chronic Disease Prevention, Population Studies Unit, National Institute for Health and Welfare, Turku, Finland

Background: Most patients with major depression report problems in their sleep: insomnia, early morning awakenings and fatigue correlating with poor sleep quality. One of the key substances regulating sleep is adenosine. We hypothesized that variations in polymorphic sites of adenosine related genes may predispose to depression with sleep disturbances.

Methods: We selected 117 single nucleotide polymorphisms from 13 genes and analyzed their association with depression and specific sleep problems (early morning awakenings and fatigue). Data were collected as part of the Health 2000 Study based on Finnish population and included 1423 adult subjects.

Results: Our major finding herein was, among women, the association of SLC29A3 polymorphism rs12256138 with depressive disorder (p=0.0004, odds ratio=0.68, 95% CI 0.55–0.84, p<0.05 after Bonferroni correction for multiple testing). Only one gene showing any evidence for association was common to women and men (ADA).

Limitations: Relatively small size of the case samples.

Conclusions: Our results suggest that compromised adenosine transport due to variation in nucleoside transporter gene SLC29A3 in women, could predispose to depression, and could suggest new directions in treatment research. The shortage of overlapping genes between the genders indicates that the genetics of mood regulation may vary between the sexes.

© 2010 Elsevier B.V. All rights reserved.

Keywords: Depression
Sleep disturbances
Adenosine
Genes
Polymorphisms

1. Introduction

Major depression is one of the most frequent forms of psychiatric disorders estimated to become the second most prevalent illness by 2020 (Viinamaki et al., 2009). Several twin studies demonstrate that heritability of depression as defined by Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) is approximately 25–29% for males.
and 42–49% for females (Kendler et al., 2006; Orstavik et al., 2007). The majority of depressed patients have sleep disturbances, including reduced amount of slow wave sleep, increased rapid eye movement (REM) sleep amount and shortened REM sleep latency (Berger et al., 2003; Mendlewicz, 2009). Also, early morning awakenings are characteristic of depression, especially in its severe, “melancholic” type (Kiloh and Garside, 1963). Another core symptom of depression is fatigue, which correlates with sleep quality and daytime sleepiness in depressed patients (Ferentinos et al., 2009).

One of the major substances regulating sleep is the neuromodulator adenosine (Basheer et al., 2004; Landolt, 2008; Porkka-Heiskanen et al., 1997). Adenosine defines sleep intensity (index of sleep depth) and maintains sleep via adenosine A1 and A2A receptors (Bjorness et al., 2009; Retey et al., 2005; Urade et al., 2003). Adenosine serves as a building block for adenosine triphosphate, and under increased energy demand when ATP is used for energy production, the levels of adenosine increase as a result of ATP metabolism (Porkka-Heiskanen et al., 1997). As fatigue and lack of energy are one of the core characteristics of depression (American Psychiatric Association, 1994), it has been suggested that metabolic depression defined as a decrease in standard metabolic rates, might partially explain these symptoms (Tsiouris, 2005). It could be hypothesized that this downregulation of energy production might partially result from impaired synthesis of adenosine which, on the other hand, may lead to disturbed sleep and exacerbate the mentioned symptoms.

Adenosine is formed intracellularly from adenosine monophosphate (AMP) by cytosolic 5′-nucleotidase and is metabolised either to AMP by adenosine kinase (ADK), or to inosine by adenosine deaminase (ADA), or to S-adenosylhomocysteine by S-adenosylhomocysteine hydrolase (SAHH) (Fig. 1). Extracellularly, adenosine is produced from AMP by 5′-ectonucleotidase or is released by astrocytes (Halassa et al., 2009). Since adenosine is a hydrophilic compound, it is transported through plasma membrane and intracellular membranes by specialized transporters, which comprise sodium-driven concentrative and equilibrative nucleoside transporters (CNTs and ENTs, respectively). CNTs move adenosine into the cell using inward Na+ gradient provided by Na+–K+–ATPase (Gray et al., 2004), whereas ENTs regulate adenosine extracellular/intracellular concentration by transporting it according to concentration gradient (Young et al., 2008). Interestingly, humans with excessive levels of adenosine due to a functional polymorphism in ADA, have increased amount of slow wave sleep and reduced amount of nocturnal awakenings (Retey et al., 2005).

Since impaired sleep is one core symptom of depression (Mendlewicz, 2009), and adenosine is one of the best known sleep factors (Basheer et al., 2004; Landolt, 2008; Porkka-Heiskanen et al., 1997), we hypothesized that variations in polymorphic sites of adenosine related genes could predispose humans to depression with sleep disturbances. To test our hypotheses we selected 117 variants from thirteen genes encoding adenosine receptors, transporters and metabolic enzymes (Fig. 1) and analyzed their association with depression and specific problems in sleep (early morning awakenings and fatigue) in healthy and depressed individuals.

2. Methods

2.1. Study sample

Our depression case–control study sample was selected from the population-based Health 2000 cohort, a Finnish population-based survey performed in 2000. Details of the survey have been previously described (http://www.terveys2000.fi/indexe.html). The sample consisted of 1423 unrelated individuals with age range of 30–88 years from 80 regions of Finland (Table 1). The health status of all subjects was evaluated by interview conducted at home and health examination at the local health care center. Depression, i.e. major depressive disorder, current (past 12 months),
was diagnosed according to the DSM-IV definitions and criteria for psychiatric disorders by using the research version of the Composite International Diagnostic Interview (Pirkola et al., 2005). Early morning awakenings and fatigue without relation to depression were assessed with a questionnaire (see Supplementary 1 in the Appendix for details).

The depression group comprised 258 women (mean age 49 years) and 125 men (mean age 48 years). In the depression group 109/258 women and 61/125 men had also early morning awakenings. The corresponding numbers for fatigue were 194 and 103. The control group included 557 women (mean age 46 years) and 483 men (mean age 45 years) with no depression according to the Composite International Diagnostic Interview. Of these, 554 women and 483 men had no signs of early morning awakenings, and 449 women and 409 men had no signs of fatigue.

The study protocol was approved by the Ethics Committee of the Helsinki and Uusimaa Hospital District, and a signed informed consent was obtained from all participants.

### 2.2. Genotyping methods

Genomic DNA was isolated from peripheral blood leukocytes using a standard EDTA extraction procedure (Blin and Stafford, 1976). We studied the following candidate genes encoding adenosine transporters, receptors and metabolism enzymes: adenosine deaminase (ADA), adenosine kinase (ADK), cytosolic 5′-nucleotidase (NT5C1B), 5′-ectonucleotidase (NT5E), S-adenosylhomocysteine hydrolase (AHCY), solute carrier family 29 (nucleoside transporters), member 1, ENT1 (SLC29A1), solute carrier family 29, member 2, ENT2 (SLC29A2), solute carrier family 29, member 3, ENT3 (SLC29A3), solute carrier family 29, member 4, ENT4 (SLC29A4), solute carrier family 28 (sodium-coupled nucleoside transporter), member 1, CNT1 (SLC28A1), solute carrier family 28, member 2, CNT2 (SLC28A2), adenosine receptor type A1 (ADORA1), adenosine receptor type 2A (ADORA2A). We selected haplotype-tagging single nucleotide polymorphisms (SNPs) based on HapMap CEU data set (International HapMap Consortium, 2005), using cut-off value for minor allele frequency 0.05 and for multimarker tagging coefficient of determination ($r^2$) 0.8.

We genotyped SNPs with Sequenom’s MassARRAY technology (Sequenom Inc., San Diego, CA, USA), based on single base extension and iPLEX Gold chemistry, using the protocol recommended by the manufacturer. We manually reviewed genotype clusters and removed genotypes of poor quality. We further calculated genotyping success rate, minor allele frequencies and Hardy–Weinberg equilibrium $p$-values by using PLINK software package web-based version 1.06 (Purcell et al., 2007). We omitted SNPs with genotyping success rate < 96%, minor allele frequencies < 1% (calculated in control individuals), or Hardy–Weinberg equilibrium $p$-value < 0.001 (calculated in control individuals). Out of 117 selected SNPs, 102 passed the requirements (Supplementary 2 in the Appendix).

### 2.3. Statistical analysis

We used PLINK software to compare allele frequencies between cases with depression and controls with chi-square tests. We compared the following groups: (1) all depressed patients against all controls, (2) depressed patients with early morning awakenings against controls without early morning awakenings, and (3) depressed patients with fatigue against controls without fatigue. We analyzed the genders separately, since women and men have highly varying prevalence of depression and probably different pathogenic mechanisms. For SNPs which gave association of $p < 0.05$, we performed analysis for SNPs interaction with gender using PLINK software.

Associations were corrected for multiple testing by using PLINK software, and $p$-values were adjusted by Bonferroni approach as the most stringent test for controlling false-positive results.

To analyze the linkage disequilibrium structure of SNPs within the genes we used Haploview software (version 4.1) (Barrett et al., 2005). Furthermore, haplotype analysis with two- and three-SNP sliding window approach was performed using PLINK software in gene that survived multiple testing correction.

### 3. Results

The results for association analysis with depression, early morning awakenings and fatigue, are presented in Tables 2 and 3, for women and men respectively.

In women genes which gave some evidence for association with depression and disturbed sleep from pointwise analysis ($p < 0.05$) included SLC29A1, SLC29A3, SLC28A1, NT5E, ADK, ADA and ADORA1 (Table 2), and in men – SLC28A1, SLC29A2, ADK and ADA (Table 3).

Our major finding herein was, among women, the associations of SLC29A3 polymorphism with depressive disorder, depression with early morning awakenings, and depression with fatigue (rs12256138, $p = 0.0004$, OR = 0.68 for depression; $p = 0.0033$, OR = 0.64 for depression with early morning awakening; $p = 0.0046$, OR = 0.71 for depression with fatigue). The association of rs12256138 with depression remained significant after applying Bonferroni multiple testing correction (corrected $p = 0.0472$). All five SLC29A3 polymorphisms associated with depression are in the same linkage disequilibrium block (rs12256138/rs780659 $D^\prime$ = 0.996, $r^2$ = 0.629; rs12256138/rs780662 $D^\prime$ = 1.000, $r^2$ = 0.113; rs12767108/rs2487067 $D^\prime$ = 0.992, $r^2$ = 0.174; rs780659/rs780662 $D^\prime$ = 0.955, $r^2$ = 0.083).
Since several variants of SLC29A3 showed evidence for association, we performed haplotype analysis by sliding window approach in females. The two-SNP haplotype 'A-C' of rs2066210 and rs12256138 increased risk for depression \( (p = 0.0005, \text{OR} = 0.60) \), whereas two haplotypes, 'T-A' (rs12256138 and rs780659) and 'A-G' (rs780659 and rs780662), were protective \( (p = 0.0006, \text{OR} = 0.40) \). In coherence with analysis on two-SNP haplotypes, we also identified a protective haplotype 'T-A-G' spanning the SNPs rs12256138, rs780659, and rs780662, which failed to show any protective effect. In men, the strongest association with depression was obtained for SLC28A1 \( (p = 0.0130, \text{OR} = 1.42) \) for depression; \( p = 0.0065, \text{OR} = 1.67 \) for depression with early morning awakening; \( p = 0.0069, \text{OR} = 1.53 \) for depression with fatigue). However, these findings did not remain significant when multiple testing was taken into account.

Two distinctive variants from ADA associated significantly with depression in the two genders (rs6031682 for women and rs452159 for men).

Analysis of the two above mentioned strongest findings for women and men for gene interaction with gender demonstrated that they were gender-specific (rs12256138 \( p = 0.0154 \), rs11853372 \( p = 0.0178 \) for depression, \( p = 0.0115 \) for depression with early morning awakening, and \( p = 0.0113 \) for depression with fatigue).

### 4. Discussion

Our main finding is the association of SLC29A3 polymorphism rs12256138 with depressive disorder in women. This association with depression remained significant after applying correction for multiple testing. Furthermore, the finding was replicated in women, from an independent set of Health 2000 data, who did not have a diagnosis of depression but reported early morning awakenings (269 cases, 550 controls, \( p = 0.0433, \text{OR} 0.81 \), unpublished data). Altogether, these data imply that compromised adenosine transport could contribute to signs of depression and early morning awakenings. However, until the effect of the gene variant on actual function of the gene is verified, this implication remains speculative.

### Table 2

<table>
<thead>
<tr>
<th>CHR</th>
<th>SNP</th>
<th>Gene name</th>
<th>D, men</th>
<th>OR</th>
<th>L95</th>
<th>U95</th>
<th>D, women</th>
<th>OR</th>
<th>L95</th>
<th>U95</th>
<th>D+EMA, men</th>
<th>OR</th>
<th>L95</th>
<th>U95</th>
<th>D+FAT, men</th>
<th>OR</th>
<th>L95</th>
<th>U95</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>rs4980345</td>
<td>SLC28A1</td>
<td>0.0980</td>
<td>0.47</td>
<td>0.20</td>
<td>0.12</td>
<td>0.0016</td>
<td>0.16</td>
<td>0.00</td>
<td>none</td>
<td>0.068</td>
<td>0.35</td>
<td>0.12</td>
<td>0.99</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>rs4721567</td>
<td>ADA</td>
<td>0.0653</td>
<td>0.74</td>
<td>0.54</td>
<td>1.01</td>
<td>0.0441</td>
<td>0.62</td>
<td>0.41</td>
<td>0.96</td>
<td>0.0903</td>
<td>0.74</td>
<td>0.53</td>
<td>1.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>rs1290199</td>
<td>SLC28A1</td>
<td>0.0465</td>
<td>1.34</td>
<td>1.01</td>
<td>1.79</td>
<td>0.1094</td>
<td>1.30</td>
<td>0.94</td>
<td>2.02</td>
<td>0.0638</td>
<td>1.35</td>
<td>0.99</td>
<td>1.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>rs452159</td>
<td>ADA</td>
<td>0.2712</td>
<td>0.83</td>
<td>0.59</td>
<td>1.16</td>
<td>0.0573</td>
<td>0.63</td>
<td>0.38</td>
<td>1.03</td>
<td>0.0165</td>
<td>0.63</td>
<td>0.43</td>
<td>0.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Number of men with minor/minor allele genotype (AA) is 2 out of 538 (cases and controls for D+EMA phenotype).
SLC29A3 encodes equilibrative nucleoside transporter ENT3. The gene is expressed in multiple tissues, and the protein localizes intracellularly and may be involved in nucleoside transport from the lysosomes (Baldwin et al., 2005). Moreover, SLC29A1 encoding ENT1 transporter yielded suggestive evidence for association in women with depression accompanied by early morning awakenings, although those findings did not remain significant after correction for multiple testing in the present study. Similar to ENT3, ENT1 is localized in multiple tissues (Podgorska et al., 2005). Studies on knock out animals indicate that ENT1 is involved in alcohol consumption behavior (Choi et al. et al., 2004). Interestingly, the same allelic variant of SLC29A1 (minor allele T from rs6905285) associated with alcohol abuse phenotype in women (33 cases, 782 controls) from the Health 2000 dataset (p = 0.0304, OR = 1.75, unpublished data). This observation suggests that adenosine levels might be a common substrate for the pathogenesis in depression and alcohol abuse.

In men, we observed that SLC28A1 suggestively associated with depression, particularly with depression and early morning awakenings. SLC28A1 encodes CNT1 transporter, which is responsible for high-affinity transport of adenosine and mediates adenosine homeostasis by regulating its influx into cells (Gray et al., 2004).

There is an increasing evidence of role for adenosine in mood disorders. Adenosine affects glutamate and dopamine release which are the key neurotransmitters involved in mood regulation (Boison et al., 2009). Depressed patients have increased cortical glutamate levels (Sanacora et al., 2004). We assume that adenosine might be partly involved in this phenomenon via its signaling through A1 receptors (Dunwiddie and Masino, 2001). Adenosine is an important regulator of sleep and sleep quality (Basheer et al., 2004; Landolt, 2008; Porkka-Heiskanen et al., 1997), and a number of earlier studies have shown that poor sleep presents a risk for depression in the adult population (Ford and Kamarow, 1989; Paffenbarger et al., 1994). Consequently, one might speculate that the current finding of association of SLC29A3 to depressive disorder could reflect its involvement in sleep regulation. Alternatively, the effect of SLC29A3 could also be mediated via energy metabolism, as adenosine is constituent of ATP molecule, and it has been hypothesized that down-regulation of energy production takes place in major depression (Tsiouris, 2005).

One important finding of our study is that men and women showed different sets of genes in association with disease. Analysis of interaction with gender revealed that our most significant findings were specific for gender. The only overlapping gene was ADA in depression with fatigue. There are several possible reasons for this pattern. First, the pathogenesis of depression might have a different cascade in men and women, as suggested by studies in twins (Kendler et al., 2006). Second, adenosine related genes might be differentially expressed between the genders, thus some polymorphisms might not affect the phenotype.

The study was limited by the relatively low sample size. However, since the role of adenosine in sleep regulation is well established, we logically assumed that adenosine related genes might contribute specifically to disturbed sleep, and our results support this view. Also by analysing separately cases with sleep disturbances, we diminished genetic heterogeneity of the sample.

To conclude, the main finding of our study is that variation in nucleoside transporter genes, particularly SLC29A3, may predispose to depression. The finding will need to be replicated in an independent sample. Also polymorphisms should be explored with respect to the functional effect they produce on the protein to interpret more clearly our results and make an affirmative conclusion about the role of adenosine in the development of the depressive disorder.

Role of funding source
The study was supported by grants from Center for International Mobility, European Union (LSHM-CT-2005-518189) and Helsinki University Central Hospital (EVO, TYH6254). The funding sponsors had no further role in study design; in the collection, analysis and interpretation of data; in the writing of report; and in the decision to submit the paper for publication.

Conflict of interest
None declared.

Acknowledgments
We thank all the Finnish volunteers who participated in the Health 2000 study. The study was supported by grants from the Center for International Mobility, European Union (LSHM-CT-2005-518189) and Helsinki University Central Hospital (EVO, TYH6254).

Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jad.2010.03.009.

References