Impact of genetic variations in *ADORA2A* gene on depression and symptoms: a cross-sectional population-based study

Sílvia Oliveira^{1,2} · Ana Paula Ardais^{1,3} · Clarissa Ribeiro Bastos¹ · Marta Gazal¹ · Karen Jansen¹ · Luciano de Mattos Souza¹ · Ricardo Azevedo da Silva¹ · Manuella Pinto Kaster⁴ · Diogo Rizzato Lara⁵ · Gabriele Ghisleni¹

Received: 17 July 2018 / Accepted: 12 November 2018 / Published online: 3 December 2018
© Springer Nature B.V. 2018

Abstract

Genetic variants involved in adenosine metabolism and its receptors were associated with increased risk for psychiatric disorders, including anxiety, depression, and schizophrenia. Here, we examined an association between a single nucleotide polymorphism in *A_{2A}* receptor gene (*ADORA2A*, rs2298383 SNP) with current depressive episode and symptom profile. A total of 1253 individuals from a cross-sectional population-based study were analyzed by the Mini International Neuropsychiatric Interview 5.0. Our data showed that the TT genotype of *ADORA2A* rs2298383 SNP was associated with reduced risk for depression when compared to the CC/CT genotypes ($p = 0.020$). This association remained significant after adjusting for confounding variables such as smoking, gender, socioeconomic class, and ethnicity (OR = 0.631 (95% CI 0.425–0.937); $p = 0.022$). Regarding the symptoms associated with depression, we evaluated the impact of the *ADORA2A* SNP in the occurrence of sad/discouraged mood, anhedonia, appetite changes, sleep disturbances, motion changes, energy loss, feelings of worthless or guilty, difficulty in concentrating, and presence of bad thoughts. Notably, the TT genotype was independently associated with reduced sleep disturbances (OR = 0.438 (95% CI 0.258–0.743); $p = 0.002$) and less difficulty in concentrating (OR = 0.534 (95% CI 0.316–0.901); $p = 0.019$). The cross-sectional design cannot evaluate the cause-effect relationship and did not evaluate the functional consequences of this polymorphism. Our data support an important role for *ADORA2A* rs2298383 SNP in clinical heterogeneity associated with depression. The presence of the TT genotype was associated with decrease risk for current depression and disturbances in sleep and attention, two of the most common symptoms associated with this disorder.

Keywords Depression · Adenosine receptor · Polymorphism · Depressive symptoms

Introduction

Depression is a leading cause of disability and is estimated to become the second most prevalent illness worldwide by 2020 [1, 2]. The etiology of this condition is complex and multifactorial, involving environmental, genetic, and psychosocial factors [3]. Genetic variability plays an important role in depression, and studies suggest a heritability of 25–29% for males and 42–49% for females. However, multiple genes with small effects seem to contribute to the clinical characteristics and progression of this disorder [4, 5].

According to the Diagnostic and Statistical Manual for Mental Disorders 5 (DSM-5), an episode of depression is characterized by the presence of depressed mood and/or anhedonia and other symptoms including changes in appetite or weight; sleep; fluctuations in general activity and energy; feelings of hopelessness, worthlessness, or guilt; diminished

✉ Ana Paula Ardais
ardais@gmail.com

¹ Department of Life and Health Sciences, Catholic University of Pelotas, Pelotas, Rio Grande do Sul, Brazil

² Biology Laboratory, University of Campanha Region, Bagé, Rio Grande do Sul, Brazil

³ Laboratório de Neurociências Clínicas, Programa de Pós-Graduação em Saúde e Comportamento, Universidade Católica de Pelotas (UCPel), Rua Gonçalves Chaves 373, sala 324C, Pelotas, RS 96015-560, Brazil

⁴ Department of Biochemistry, Federal University of Santa Catarina, Florianópolis, Santa Catarina, Brazil

⁵ Department of Cellular and Molecular Biology, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil

concentration; and suicide ideation [6]. Thus, depression is currently defined only on the basis of behavioral modifications found in patients, and the heterogeneity in clinical presentation might suggest the involvement of different neurochemical and genetic substrates [7]. Hence, there is a major socioeconomical pressure in order for the development of new and more effective strategies to identify, classify, and manage this condition.

In this context, the adenosinergic system appears as a neuromodulatory system able to control the release of neurotransmitters and hormones involved in the pathophysiology of depression, including the serotonergic and dopaminergic systems and the hypothalamic-pituitary-adrenal axis [8–10]. There is clinical evidence suggesting that the consumption of caffeine (a non-selective adenosine receptor antagonist) and caffeinated beverages improves the effect of classical antidepressant and is inversely correlated with the appearance of depressive symptoms [11–17]. In attempt to clarify the involvement of adenosinergic system in depression, preclinical studies have suggested that inhibition of adenosine A2A receptors using pharmacological or genetic approaches produced antidepressant-like effects in animal models of depression and improved the social behavior, a fundamental element affected in many psychiatric disorders [18–27]. Moreover, a recent finding also showed that increased levels and functionality of the A2A receptor induced by early-life hyperthermic seizures might induce depressive-like behavior in adult rats [28].

More recently, another line of evidence emerged, suggesting a possible influence of genetic variations in genes involved in adenosine metabolism and adenosine receptors in psychiatric conditions. In fact, a SNP in the nucleoside transporter gene, *SLC29A3*, was reported to compromise adenosine transport and predispose women to depression [29]. Besides that, polymorphisms in *ADORA2A*, the gene that encodes A2A receptors, have been associated with several psychiatric conditions, such as schizophrenia, psychosis, anxiety, and phobia [30–37]. Besides that, risk genotypes for *ADORA2A*, associated to anxiety and panic disorder, were also associated with increased binding and availability of adenosine A1 receptor in image studies in the human brain, which might suggest an imbalance between inhibitory and facilitatory regulations of adenosinergic system [38]. Thus, considering the clinical and preclinical findings that point to the involvement of the adenosinergic system in depression and related psychiatric disorders, it is important to evaluate the association of the *ADORA2A* rs2298383 SNP in depression and its impact on specific symptoms associated with this disorder. Our results might potentially contribute to the characterization of disease heterogeneity and identification of susceptible and vulnerable individuals.

Methodology

Study design and participants

This is a cross-sectional population-based study of people aged 18 to 35. The sample consisted of 1253 participants living in the urban Pelotas, Southern Brazil. Sample selection was performed by clusters (June 2011 to May 2013), considering a population of 97,000 people in that age range in the current census of 448 sectors in the city, provided by the Brazilian Institute of Geography and Statistics (IBGE). In order to ensure the necessary sample inclusion, 86 census-based sectors were systematically drawn. After identification, the subjects signed the informed consent and answered to a sociodemographic questionnaire. In order to ascertain current and lifetime psychiatric disorders, trained psychologists used a structured diagnostic interview, the Mini International Neuropsychiatric Interview according to DSM-IV criteria (M.I.N.I. 5.0, Brazilian version/DSM IV, Medical Outcome Systems Inc., Jacksonville, FL, USA). For this study, we considered individuals in current episode of depression. The study was approved by the Ethics Committee of our University (2010/15).

Genotyping assay and functional analysis

Blood samples were obtained by venipuncture after the clinical interview. Total DNA was extracted from peripheral blood leukocytes using a standardized salting-out procedure [39]. Subsequently, the total DNA has been quantified by spectrophotometry and stored at -20°C until molecular analysis. *ADORA2A* rs2298383 (C/T) SNP was genotyped using primers and probes contained in the 40x Human Custom TaqMan Genotyping Assay (Life Technologies, Foster City, CA, USA). The reactions were conducted in a 96-well plate, in a total 5- μl reaction volume using 2 ng of genomic DNA, TaqMan Genotyping Master Mix 1x (Applied Biosystems), and Custom TaqMan Genotyping Assay 1x, as determined by the manufacturer. Then, plates were positioned in a real-time PCR thermal cycler (7500 Fast Real PCR System; Applied Biosystems) and heated for 10 min at 95°C , followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. Fluorescence data files from each plate were analyzed using automated allele-calling software (SDS 2.0.1; Applied Biosystems). GTEx dataset (Release V7) was used to performed eQTL analysis to examine the effects of SNPs on mRNA expression (<https://gtexportal.org>).

Data analysis

The questionnaires were typed and coded directly into the EpiInfo6 program at the interview time, and statistical analysis was performed using the statistical package SPSS 23.0 for

Windows. The genotypic and allelic frequencies were estimated by direct counting of the alleles, and the Hardy-Weinberg equilibrium was tested by the chi-square test. Comparisons between groups were performed by Student's *t* test and chi-square test. A measure of magnitude of effect was calculated by logistic regression and presented as odds ratio (OR) and 95% confidence interval. Logistic regression analysis was adjusted for smoking, gender, socioeconomic class, and ethnicity. Values of $p \leq 0.05$ are considered statistically significant.

Results

Our study encompassed 1253 participants, out of which 228 were in current episode of depression. The prevalence of depression in the sample was higher in women (70.6%; $p < 0.001$), non-Caucasian (30.7%; $p = 0.01$), smokers (38.9%; $p < 0.001$), and low-income individuals (33.5%; $p < 0.001$) (Table 1). When we analyzed the participants according to the genotype, we identified that the TT genotype of the rs2298383 SNP presents a reduced frequency in individuals with current depression episode (16.2%) compared with that in control (23.5%; $p = 0.02$) (Table 2).

In Table 3, sociodemographic and clinical characteristics according to *ADORA2A* rs2298383 SNP genotypes demonstrated no significant differences were found for gender ($p = 0.190$), age (0.104), socioeconomic class ($p = 0.219$), and smoking ($p = 0.459$). However, there was a significant difference in ethnicity, with a higher prevalence of the T allele in Caucasian individuals compared to CC genotype ($p < 0.0001$) (Table 3). The genotype distribution of rs2298383 SNP was in agreement with the predicted by the Hardy-Weinberg equilibrium ($\chi^2 = 0.16$; $p = 0.689$). To evaluate the functionality of the SNP rs2298383, we queried this SNP for alteration in gene expression in the GTEx dataset and found that rs2298383-TT was associated with increased mRNA expression of *ADORA2A* ($p = 0.00013$; Fig. 1a) in whole blood tissue. The gene eQTL Visualizer plot is shown in Fig. 1b. However, this SNP was not identified as an eQTL for *ADORA2A* in other tissues.

Table 1 Sociodemographic and clinical characteristics of the sample according to the current depression episode

Variables	Current depression episode		
	No	Yes	<i>p</i> value
Female sex (%)	52.0	70.6	< 0.001*
Age (years)	25.62 ± 5.45	26.04 ± 5.45	0.286
Caucasian ethnicity (%)	77.5	69.3	0.0100*
Low socioeconomic class (%)	18.1	33.5	< 0.001*
Smokers (%)	18.4	38.9	< 0.001*

Data are presented as mean ± standard deviation or %

**p* values were computed using chi-square test or Student's *t* test, as appropriated

Table 2 Genotype distribution of *ADORA2A* rs2298383 SNP according to current depression episode

	Current depression		
	No	Yes	<i>p</i> value
Genotype (rs2298383 SNP)			
CC	308 (30.0%)	70 (30.7%)	0.040*
CT	477 (46.5%)	121 (53.1%)	
TT	241 (23.5%)	37 (16.2%)	
Genotypes (recessive model)			
CC/CT	785 (76.5%)	191 (83.8%)	0.020*
TT	241 (23.5%)	37 (16.2%)	
Genotypes (dominant model)			
CC	308 (30.0%)	70 (30.7%)	0.902
CT/TT	718 (70.0%)	158 (69.3%)	

Data are presented as *n* (%)

**p* values were computed using the chi-square test

Table 4 depicted the specific symptom profile of the population according to genotype distribution for the rs2298383 SNP. Our results showed that individuals carrying TT genotype presented reduced sleep disturbances (48.6%) and less difficulty in concentration and decision-making (39.2%), when compared to the C allele carriers for both symptoms (68.2% and 55.1%, respectively). Logistic regression analysis adjusted for smoking, gender, socioeconomic class, and ethnicity showed that the polymorphism in the *ADORA2A* gene remains associated with a reduced risk for depression, revealing an independent effect of the polymorphism with the disorder (OR = 0.631 (95% CI 0.425–0.937); $p = 0.022$). The C allele of rs2298383 remained independently associated with sleep disturbances (OR = 0.438 (95% CI 0.258–0.743); $p = 0.002$) and difficulty in concentration and decision-making (OR = 0.534 (95% CI 0.316–0.901); $p = 0.019$) after adjustment for confounding variables such as smoking, gender, socioeconomic class, and ethnicity (Table 5).

Discussion

In the present study, we investigated the impact of *ADORA2A* rs2298383 SNP in current depression and associated symptom profile. We found a reduced risk of the TT genetic variant for depression. In addition, this genotype was also associated with protection against sleep dysfunction and attentional impairment, two symptoms frequently observed in patients with depression.

The *ADORA2A* locus is located on human chromosome 22 and contains a set of SNPs. The most studied SNP in the *ADORA2A* gene, rs5751876, is the synonymous exonic exchange Tyr361Tyr (formerly designated as 1976C/T or 1083C/T). This SNP has been observed as risk factor for panic

Table 3 Sociodemographic and clinical characteristics of sample according to *ADORA2A* rs2298383 SNP genotype

	Genotype (rs2298383 SNP)			<i>p</i> value
	CC	CT	TT	
Female sex (%)	51.9	55.9	58.8	0.190
Age (years)	25.1 ± 5.9	25.9 ± 5.1	25.8 ± 5.3	0.104
Low socioeconomic class (%)	24.3	20.4	17.3	0.219
Caucasian ethnicity (%)	69.0	77.5 ^a	82.3 ^a	< 0.001*
Smokers (%)	19.9	23.2	22.7	0.459

Data are presented as mean ± standard deviation or %

**p* values were computed using the chi-square test or one-way ANOVA test followed by Bonferroni's post hoc test, as appropriated

^a Differences between groups

disorder, blood-injury phobia [34], autism spectrum disorders [33], and caffeine- or amphetamine-induced anxiety [32, 36, 40, 41]. However, a study including 192 individuals failed to find an association between *ADORA2A* rs5751876 SNP and major depression or bipolar disorder [42]. Since the rs5751876 SNP is a synonymous exchange with no obvious functional impact on the protein, other polymorphisms in strong linkage disequilibrium are probably responsible for the underlying functional variation. In fact, the rs5751862, rs2298383, and rs3761422 SNPs were associated with different anxiety-related personality scores [35]. In the present study, we evaluated the rs2298383, which is in high linkage disequilibrium with the rs5751876 SNP and presents functional potential [35]. The rs2298383 SNP is located in putative promoter region upstream of the newly identified exon 1 variants [43] and serves as tagging SNPs according to the HapMap database. In addition, rs2298383 has a position within a predicted regulatory element suggested to display high regulatory potential (UCSC database) computed from alignments of human, chimpanzee, macaque, mouse, rat, dog, and cow [44, 45].

However, the functional impact of this SNP on A2A receptors was not determined yet. A recent finding has pointed out that other *ADORA2A* SNPs in strong linkage disequilibrium with rs2298383 are associated with increased binding and availability of A1 receptors in encephalic regions related to emotion, which might contribute to their effects on anxiety and panic disorder [38]. Increased A1 receptor binding was also detected in the hippocampus of two different rat models of stress, using unpredictable chronic mild stress or chronic restraint stress, demonstrating that A1 receptor regulation is involved on depressive-like behavior [46]. Beyond that, in silico analyses (<http://pupasuite.bioinfo.cipf.es/>) indicated that the rs2298383 SNP occurs within a triplex-forming oligonucleotide (TFO) target sequence which is involved in gene expression regulation [47]. Therefore, TFO binding to the C allele (identified as the risk allele) potentially leads to transcription inhibition and thus drastically decreased amounts of A2A receptor molecules. On the other hand, in TT individuals, the TFO sequence is disrupted, leading to a normal expression of the A2A receptors. Moreover, functional analysis of rs2298383 using eQTL database showed that the

Fig. 1 eQTL analysis of rs2298383. **a** Boxplots from GTEx data showing the effect of rs2298383 genotypes on *ADORA2A* expression in whole blood tissue. Numbers below each boxplot indicate the sample size of each genotype. **b** Gene eQTL Visualizer plot showing significant eQTL for *ADORA2A* in whole blood tissue. An eQTL appears as a circle and

rectangle box on the heat map. The color and size of the circle represent the effect size and *p* value of the eQTL results, respectively. Additionally, the closer a SNP to the transcription end site (TES), the darker the box is. Numbers in black ovals indicate sample sizes. The TSS is drawn as a flag and the direction of the flag indicates the transcription direction

Table 4 Symptom profile in sample according to *ADORA2A* rs2298383 SNP genotype

Symptoms	Genotype (rs2298383 SNP)		<i>p</i> value
	CC/CT	TT	
Sad and discouraged mood			
Yes	257 (26.3%)	71 (25.5%)	0.858
Anhedonia			
Yes	196 (20.1%)	44 (15.8%)	0.134
Appetite changes			
Yes	174 (59.6%)	47 (63.5%)	0.629
Sleep disturbances			
Yes	199 (68.2%)	36 (48.6%)	0.003*
Emotion changes			
Yes	160 (54.8%)	41 (55.4%)	1.000
Tiredness and loss of energy			
Yes	188 (64.4%)	42 (56.8%)	0.281
Feelings of worthless or guilty			
Yes	127 (43.5%)	28 (37.8%)	0.455
Difficulty in concentrating			
Yes	161 (55.1%)	29 (39.2%)	0.020*
Bad thoughts			
Yes	111 (38.0%)	21 (28.4%)	0.160

Data are presented as *n* (%)

**p* values were computed by the chi-square test comparing depressive symptoms with different genotypes

rs2298383-TT genotype increases the *ADORA2A* expression in whole blood tissue. In this scenario, our results showed reduced risk of rs2298383-TT genotype for depression suggesting the relevant role of *ADORA2A* gene expression as well as an imbalance between inhibitory and facilitatory regulations of adenosinergic system in mood regulation.

Interestingly, our results also demonstrated an association between the TT genotype of the rs2298383 SNP and protection against sleep dysfunction. Adenosine is well-known endogenous sleep-promoting substance, and extracellular concentrations of adenosine in most brain areas are decreased during sleep compared to those during wakefulness [48]. These high levels of adenosine interact with both A1 and A2A receptors, which play a crucial role in the modulation of wakefulness and sleep cycle, and pharmacological studies showed that A2A receptor agonists produced significant increases in the total amounts of non-REM and REM sleep [49].

A dysregulation in sleep patterns is highly associated with many psychiatric disorders, including major depression, and more than 70% of all depressed patients report difficulties in onset or in maintenance of sleep [50–52]. In a study including 1423 subjects, a variation in the nucleoside transporter gene (*SLC29A3*) was reported to compromise adenosine transport and predispose women with sleep disturbances to depression [29]. Depression may also be observed as an effect of sleep

Table 5 Logistic regression analysis of *ADORA2A* rs2298383 SNP and clinical covariates in current depression episode and specific associated symptoms

Polymorphism	Current depression		Sleep disturbances		Difficulty in concentrating	
	Unadjusted OR (95% CI)/ <i>p</i>	Adjusted OR (95% CI)/ <i>p</i>	Unadjusted OR (95% CI)/ <i>p</i>	Adjusted OR (95% CI)/ <i>p</i>	Unadjusted OR (95% CI)/ <i>p</i>	Adjusted OR (95% CI)/ <i>p</i>
Recessive model						
CC/CT	1	1	1	1	1	1
TT	0.631 (0.431–0.923)/0.018*	0.631 (0.425–0.937)/0.022*	0.443 (0.264–0.743)/0.002*	0.438 (0.258–0.745)/0.002*	0.524 (0.312–0.883)/0.015*	0.533 (0.316–0.901)/0.019*

Data are presented as odds ratios (OR and 95% CI) for unadjusted and adjusted analysis (gender, ethnicity, socioeconomic class and smoking)

**p* values were computed by logistic regression

disorders, which highlights an important relationship of reciprocity between them. Thus, we can speculate that impairment in A2A receptor expression and function in individuals carrying the C allele of the rs2298383 SNP might be associated with predisposition to sleep dysfunction and, consequently, depressive episodes.

In addition, in the present study, we observed that TT genotype of the rs2298383 SNP was also associated with a reduced impairment in concentration, another important symptom frequently observed in patients with depression. Attention and concentration are behavioral substrates particularly sensitive to the modulation by adenosine. Pharmacological manipulation of adenosine receptors plays a crucial role for concentration and decision-making [53, 54]. In fact, blockade of A2A receptors by caffeine and other A2A receptor antagonists is effective in preventing attentional and memory deficits in an animal model of attention deficit/hyperactivity disorder [55–57]. Thus, despite the well-established beneficial role of A2A receptor antagonists in attention, in our study, individuals carrying the C allele, associated with reduced expression of the A2A receptors, were more prompt to deficits in attention and depression. A functional evaluation of the A2A receptor in these individuals is still required, but we can speculate the normal levels of A2A receptors are important to attentional regulation.

Since the ability of the adenosinergic signaling to regulate sleep function, attention, and mood, our results highlight a relevant aspect of genetic variants in the *ADORA2A* in depression. Our study has some limitation, especially the fact that the functional consequences of these polymorphisms to expression and function of A2A receptors were not determined yet. In addition, with our cross-sectional design, we cannot evaluate a causal effect of impairments in sleep and attention and susceptibility to depression or the consumption of caffeine in individuals with different genotypes and consequences to mood regulation. In this context, further clinical studies should be developed to better comprehend the role of *ADORA2A* genes in depression. Future studies will be needed to address some of the limitations inherent in our cross-sectional design and to fill the gaps about the functional impact of this SNP on depressive disorder.

Authors' contributions All authors mentioned in the paper have significantly contributed to the research. RAS, KJ, LDMS, and DRL conceived and supervised the clinical evaluation. MPK and GG and supervised the collection and processing of biological samples. CRB and SO performed the DNA extraction and genotyping. SO, APA, GG, and MPK performed the statistical analysis and wrote the article. All authors approved the final manuscript.

Funding information This study was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), and Programa de Apoio a Núcleos de Excelência-Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (PRONEX-FAPERGS 08/2009 - Pronex 10/0055-0). RAS, KJ, LDMS, and MPK are CNPq Research Fellows. APA and CRB receive a fellowship from CAPES.

Compliance with ethical standards

Conflicts of interest Silvia Oliveira declares no conflict of interest.

Ana Paula Ardais declares no conflict of interest.

Clarissa Ribeiro Bastos declares no conflict of interest.

Marta Gazal declares no conflict of interest.

Karen Jansen declares no conflict of interest.

Luciano de Mattos Souza declares no conflict of interest.

Ricardo Azevedo da Silva declares no conflict of interest.

Manuella Pinto Kaster declares no conflict of interest.

Diogo Rizzato Lara declares no conflict of interest.

Gabriele Ghisleni declares no conflict of interest.

Ethical approval The study was approved by the Ethical Committee of the Catholic University of Pelotas, Brazil (protocol number 2010/15), and all participants signed the informed consent.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Sobocki P, Jönsson B, Angst J, Rehnberg C (2006) Cost of depression in Europe. *J Ment Health Policy Econ* 9:87–98
2. Viinamäki H, Heiskanen T, Lehto SM, Niskanen L, Koivumaa-Honkanen H, Tolmunen T, Honkalampi K, Saharinen T, Haatainen K, Hintikka J (2009) Association of depressive symptoms and metabolic syndrome in men. *Acta Psychiatr Scand* 120: 23–29. <https://doi.org/10.1111/j.1600-0447.2008.01333.x>
3. Schroeter ML, Abdul-Khaliq H, Acher J, Steiner J, Blasig IE, Mueller K (2010) Mood disorders are glial disorders: evidence from in vivo studies. *Cardiovasc Psychiatry Neurol* 780645. <https://doi.org/10.1155/2010/780645>
4. Kendler KS, Myers JO, Gardner C (2006) Caffeine intake, toxicity and dependence and lifetime risk for psychiatric and substance use disorders: an epidemiologic and co-twin control analysis. *Psychol Med* 36:1717–1725. <https://doi.org/10.1017/S0033291706008622>
5. Orstavik RE, Kendler KS, Czajkowski N, Tambs K, Reichborn-Kjennerud T (2007) Genetic and environmental contributions to depressive personality disorder in a population-based sample of Norwegian twins. *J Affect Disord* 99:181–189. <https://doi.org/10.1016/j.jad.2006.09.011>
6. American Psychiatric Association (2013) Diagnostic and statistical manual of mental disorders. American Psychiatric Association, Washington DC
7. Insel TR (2014) The NIMH Research Domain Criteria (RDoC) Project: precision medicine for psychiatry. *Am J Psychiatry* 171: 395–397. <https://doi.org/10.1176/appi.ajp.2014.14020138>
8. Scaccianoce S, Navarra D, Sciallo AD, Angelucci L, Endröczy E (1989) Adenosine and pituitary–adrenocortical axis activity in the rat. *Neuroendocrinology* 50:464–468. <https://doi.org/10.1159/000125264>
9. Okada M, Nutt DJ, Murakami T, Zhu G, Kamata A, Kawata Y, Kaneko S (2001) Adenosine receptor subtypes modulate two major functional pathways for hippocampal serotonin release. *J Neurosci* 21:628–640
10. Pechlivanova DM, Tchekalarova JD, Alova LH, Petkov VV, Nikolov RP, Yakimova KS (2012) Effect of long-term caffeine administration on depressive-like behavior in rats exposed to chronic unpredictable stress. *Behav Pharmacol* 23:339–347. <https://doi.org/10.1097/FBP.0b013e3283564dd9>

11. Lucas M, Mirzaei F, Pan A, Okereke OI, Willett WC, O'Reilly EJ, Koenen K, Ascherio (2011) Coffee, caffeine, and risk of depression among women. *Arch Intern Med* 171:1571–1578. <https://doi.org/10.1001/archinternmed.2011.393>
12. Omagari K, Sakaki M, Tsujimoto Y, Shioyama Y, Iwanaga A, Ishimoto M, Yamaguchi A, Masuzumi M, Kawase M, Ichimura M, Yoshitake T, Miyahara Y (2014) Coffee consumption is inversely associated with depressive status in Japanese patients with type 2 diabetes. *J Clin Biochem Nutr* 55:135–142. <https://doi.org/10.3164/jcbs.14-30>
13. Pham NM, Nanri A, Kurotani K, Kuwahara K, Kume A, Sato M, Hayabuchi H, Mizoue T (2014) Green tea and coffee consumption is inversely associated with depressive symptoms in a Japanese working population. *Public Health Nutr* 17:625–633. <https://doi.org/10.1017/s1368980013000360>
14. Guo X, Park Y, Freedman ND, Sinha R, Hollenbeck AR, Blair A, Chen H (2014) Sweetened beverages, coffee, and tea and depression risk among older US adults. *PLoS One* 9:e94715. <https://doi.org/10.1371/journal.pone.0094715>
15. Wang L, Shen X, Wu Y, Zhang D (2016) Coffee and caffeine consumption and depression: a meta-analysis of observational studies. *Aust N Z J Psychiatry* 50:228–242. <https://doi.org/10.1177/0004867415603131>
16. Liu QS, Deng R, Fan Y, Li K, Meng F, Li X, Liu R (2017) Low dose of caffeine enhances the efficacy of antidepressants in major depressive disorder and the underlying neural substrates. *Mol Nutr Food Res* 61:1600910. <https://doi.org/10.1002/mnfr.201600910>
17. Kim J, Kim J (2018) Green tea, coffee, and caffeine consumption are inversely associated with self-report lifetime depression in the Korean population. *Nutrients* 10. <https://doi.org/10.3390/nu10091201>
18. El Yacoubi M, Ledent C, Parmentier M, Bertorelli R, Ongini E, Costentin J, Vaugeois JM (2001) Adenosine A_{2A} receptor antagonists are potential antidepressants: evidence based on pharmacology and A_{2A} receptor knockout mice. *Br J Pharmacol* 134:68–77. <https://doi.org/10.1038/sj.bjp.0704240>
19. El Yacoubi M, Costentin J, Vaugeois JM (2003) Adenosine A_{2A} receptors and depression. *Neurology* 61:S82–S87
20. Cunha RA, Ferré S, Vaugeois JM, Chen JF (2008) Potential therapeutic interest of adenosine A_{2A} receptors in psychiatric disorders. *Curr Pharm Des* 14:1512–1524
21. Hodgson RA, Bertorelli R, Varty GB, Lachowicz JE, Forlani A, Fredduzzi S, Cohen-Williams ME, Higgins GA, Impagnatiello F, Nicolussi E, Parra LE, Foster C, Zhai Y, Neustadt BR, Stamford AW, Parker EM, Reggiani A, Hunter JC (2009) Characterization of the potent and highly selective A_{2A} receptor antagonists preladenant and SCH 412348 [7-[2-[4-(2,4-difluorophenyl)-1-piperazinyl]ethyl]-2-(2-furanyl)7Hpyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine] in rodent models of movement disorders and depression. *J Pharmacol Exp Ther* 330:294–303. <https://doi.org/10.1124/jpet.108.149617>
22. Batalha VL, Pego JM, Fontinha BM, Costenla AR, Valadas JS, Baqi Y, Radjainia H, Müller CE, Sebastião AM, Lopes LV (2013) Adenosine A_(2A) receptor blockade reverts hippocampal stress-induced deficits and restores corticosterone circadian oscillation. *Mol Psychiatry* 18:320–331. <https://doi.org/10.1038/mp.2012.8>
23. Yamada K, Kobayashi M, Mori A, Jenner P, Kanda T (2013) Antidepressant-like activity of the adenosine A_(2A) receptor antagonist, istradefylline (KW-6002), in the forced swim test and the tail suspension test in rodents. *Pharmacol Biochem Behav* 114–115:23–30. <https://doi.org/10.1016/j.pbb.2013.10.022>
24. Kaster MP, Machado NJ, Silva HB, Nunes A, Ardaís AP, Santana M, Baqi Y, Müller CE, Rodrigues AL, Porciúncula LO, Chen JF, Tomé ÁR, Agostinho P, Canas PM, Cunha RA (2015) Caffeine acts through neuronal adenosine A_{2A} receptors to prevent mood and memory dysfunction triggered by chronic stress. *Proc Natl Acad Sci U S A* 112:7833–7838. <https://doi.org/10.1073/pnas.1423088112>
25. López-Cruz L, Carbó-Gas M, Pardo M, Bayarri P, Valverde O, Ledent C, Salamone JD, Correa M (2017) Adenosine A_{2A} receptor deletion affects social behaviors and anxiety in mice: involvement of anterior cingulate cortex and amygdala. *Behav Brain Res* 321:8–17. <https://doi.org/10.1016/j.bbr.2016.12.020>
26. López-Cruz L, Salamone JD, Correa M (2018) Caffeine and selective adenosine receptor antagonists as new therapeutic tools for the motivational symptoms of depression. *Front Pharmacol* 9:526. <https://doi.org/10.3389/fphar.2018.00526>
27. Padilla KM, Quintanar-Setephano A, López-Vallejo F, Berumen LC, Miledi R, García-Alcocer G (2018) Behavioral changes induced through adenosine A_{2A} receptor ligands in a rat depression model induced by olfactory bulbectomy. *Brain Behav* 8:e00952. <https://doi.org/10.1002/brb3.952>
28. Crespo M, León-Navarro DA, Martín M (2018) Early-life hyperthermic seizures upregulate adenosine A_{2A} receptors in the cortex and promote depressive-like behavior in adult rats. *Epilepsy Behav* 86:173–178. <https://doi.org/10.1016/j.yebeh.2018.06.048>
29. Gass N, Ollila HM, Utge S, Partonen T, Kronholm E, Pirkola S, Suhonen J, Silander K, Porkka-Heiskanen T, Paunio T (2010) Contribution of adenosine related genes to the risk of depression with disturbed sleep. *J Affect Disord* 126:134–139. <https://doi.org/10.1016/j.jad.2010.03.009>
30. Deckert J, Nöthen MM, Rietschel M, Wildenauer D, Bondy B, Ertl MA, Knapp M, Schofield PR, Albus M, Maier W, Propping P (1996) Human adenosine A_{2A} receptor (A_{2A}R) gene: systematic mutation screening in patients with schizophrenia. *J Neural Transm* 103:1447–1455. <https://doi.org/10.1007/BF01271259>
31. Hong CJ, Liu HC, Liu TY, Liao DL, Tsai SJ (2005) Association studies of the adenosine A_{2A} receptor (1976T>C) genetic polymorphism in Parkinson's disease and schizophrenia. *J Neural Transm* 112:1503–1510. <https://doi.org/10.1007/s00702-005-0286-4>
32. Childs E, Hohoff C, Deckert J, Xu K, Badner J, de Wit H (2008) Association between ADORA_{2A} and DRD₂ polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacology* 33:2791–2800. <https://doi.org/10.1038/npp.2008.17>
33. Freitag CM, Agelopoulos K, Huy E, Rothermundt M, Krakowitzky P, Meyer J, Deckert J, von Gontard A, Hohoff C (2010) Adenosine A_(2A) receptor gene (ADORA_{2A}) variants may increase autistic symptoms and anxiety in autism spectrum disorder. *Eur Child Adolesc Psychiatry* 19:67–74. <https://doi.org/10.1007/s00787-009-0043-6>
34. Hohoff C, Domschke K, Schwarte K, Spellmeyer G, Vögele C, Hetzel G, Deckert J, Gerlach AL (2009) Sympathetic activity relates to adenosine A_(2A) receptor gene variation in blood-injury phobia. *J Neural Transm* 116:659–662. <https://doi.org/10.1007/s00702-008-0089-5>
35. Hohoff C, Mullings EL, Heatherley SV, Freitag CM, Neumann LC, Domschke K, Krakowitzky P, Rothermundt M, Keck ME, Erhardt A, Unschuld PG, Jacob C, Fritze J, Bandelow B, Maier W, Holsboer F, Rogers PJ, Deckert J (2010) Adenosine A_{2A} receptor gene: evidence for association of risk variants with panic disorder and anxious personality. *J Psychiatry Res* 44:930–937. <https://doi.org/10.1016/j.jpsychires.2010.02.006>
36. Rogers PJ, Hohoff C, Heatherley SV, Mullings EL, Maxfield PJ, Evershed RP, Deckert J, Nutt DJ (2010) Association of the anxiogenic and alerting effects of caffeine with ADORA_{2A} and ADORA₁ polymorphisms and habitual level of caffeine consumption. *Neuropsychopharmacology* 35:1973–1983. <https://doi.org/10.1038/npp.2010.71>
37. Kobayashi H, Ujike H, Iwata N, Inada T, Yamada M, Sekine Y, Uchimura N, Iyo M, Ozaki N, Itokawa M, Sora I (2010) The adenosine A_{2A} receptor is associated with methamphetamine

- dependence/psychosis in the Japanese population. *Behav Brain Funct* 6:50. <https://doi.org/10.1186/1744-9081-6-50>
38. Hohoff C, Garibotto V, Elmenhorst D, Baffa A, Kroll T, Hoffmann A, Schwarte K, Zhang W, Arolt V, Deckert J, Bauer A (2014) Association of adenosine receptor gene polymorphisms and in vivo adenosine A1 receptor binding in the human brain. *Neuropsychopharmacology* 39:2989–2999. <https://doi.org/10.1038/npp.2014.150>
 39. Lahiri DK, Numberger Jr JI (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* 19:5444
 40. Alsene K, Deckert J, Sand P, de Wit H (2003) Association between A2A receptor gene polymorphisms and caffeine-induced anxiety. *Neuropsychopharmacology* 28:1694–1702
 41. Hohoff C, McDonald JM, Baune BT, Cook EH, Deckert J, de Wit H (2005) Interindividual variation in anxiety response to amphetamine: possible role for adenosine A2A receptor gene variants. *Am J Med Genet B Neuropsychiatr Genet* 139B:42–44. <https://doi.org/10.1002/ajmg.b.30228>
 42. Tsai SJ, Hong CJ, Hou SJ, Yen FC (2006) Association study of adenosine A2a receptor (1976C>T) genetic polymorphism and mood disorders and age of onset. *Psychiatr Genet* 16:185. <https://doi.org/10.1097/01.ypg.0000218627.26622.eb>
 43. Yu L, Frith MC, Suzuki Y, Peterfreund RA, Gearan T, Sugano S, Schwarzschild MA, Weng Z, Fink JS, Chen JF (2004) Characterization of genomic organization of the adenosine A2A receptor gene by molecular and bioinformatics analyses. *Brain Res* 1000:156–173. <https://doi.org/10.1016/j.brainres.2003.11.072>
 44. Kolbe D, Taylor J, Elnitski L, Eswara P, Li J, Miller W, Hardison R, Chiaromonte F (2004) Regulatory potential scores from genome-wide three-way alignments of human, mouse, and rat. *Genome Res* 14:700–707. <https://doi.org/10.1101/gr.1976004>
 45. King DC, Taylor J, Elnitski L, Chiaromonte F, Miller W, Hardison RC (2005) Evaluation of regulatory potential and conservation scores for detecting cis-regulatory modules in aligned mammalian genome sequences. *Genome Res* 15:1051–1060. <https://doi.org/10.1101/gr.3642605>
 46. Crema LM, Pettenuzzo LF, Schlabit M, Diehl L, Hoppe J, Mestriner R, Laureano D, Salbego C, Dalmaz C, Vendite D (2013) The effect of unpredictable chronic mild stress on depressive-like behavior and on hippocampal A₁ and striatal A_{2A} adenosine receptors. *Physiol Behav* 109:1–7. <https://doi.org/10.1016/j.physbeh.2012.11.001>
 47. Conde L, Vaquerizas JM, Dopazo H, Arbiza L, Reumers J, Rousseau F, Schymkowitz J, Dopazo J (2006) PupaSuite: finding functional single nucleotide polymorphisms for large-scale genotyping purposes. *Nucleic Acids Res* 34:W621–W625. <https://doi.org/10.1093/nar/gkl071>
 48. Moreau JL, Huber G (1999) Central adenosine A(2A) receptors: an overview. *Brain Res Brain Res Rev* 31:65–82
 49. Satoh S, Matsumura H, Hayaishi O (1998) Involvement of adenosine A2A receptor in sleep promotion. *Eur J Pharmacol* 351:155–162
 50. Wulff K, Gatti S, Wettstein JG, Foster RG (2010) Sleep and circadian rhythm disruption in psychiatric and neurodegenerative disease. *Nat Rev Neurosci* 11:589–599. <https://doi.org/10.1038/nrn2868>
 51. Jagannath A, Peirson SN, Foster RG (2013) Sleep and circadian rhythm disruption in neuropsychiatric illness. *Curr Opin Neurobiol* 23:888–894. <https://doi.org/10.1016/j.conb.2013.03.008>
 52. Spiegelhalter K, Regen W, Nanovska S, Baglioni C, Riemann D (2013) Comorbid sleep disorders in neuropsychiatric disorders across the life cycle. *Curr Psychiatry Rep* 15:364. <https://doi.org/10.1007/s11920-013-0364-5>
 53. Pardo M, Lopez-Cruz L, Valverde O, Ledent C, Baqi Y, Müller CE, Salamone JD, Correa M (2012) Adenosine A2A receptor antagonism and genetic deletion attenuate the effects of dopamine D2 antagonism on effort-based decision making in mice. *Neuropharmacology* 62:2068–2077. <https://doi.org/10.1016/j.neuropharm.2011.12.033>
 54. Yohn SE, Thompson C, Randall PA, Lee CA, Müller CE, Baqi Y, Correa M, Salamone JD (2015) The VMAT-2 inhibitor tetrabenazine alters effort-related decision making as measured by the T-maze barrier choice task: reversal with the adenosine A2A antagonist MSX-3 and the catecholamine uptake blocker bupropion. *Psychopharmacology* 232:1313–1323. <https://doi.org/10.1007/s00213-014-3766-0>
 55. Takahashi RN, Pamplona FA, Prediger RD (2008) Adenosine receptor antagonists for cognitive dysfunction: a review of animal studies. *Front Biosci* 13:2614–2632
 56. Prediger RD, Pamplona FA, Fernandes D, Takahashi RN (2005) Caffeine improves spatial learning deficits in an animal model of attention deficit hyperactivity disorder (ADHD)—the spontaneously hypertensive rat (SHR). *Int J Neuropsychopharmacol* 8:583–594. <https://doi.org/10.1017/S1461145705005341>
 57. Pandolfo P, Machado NJ, Köfalvi A, Takahashi RN, Cunha RA (2013) Caffeine regulates frontocortico-striatal dopamine transporter density and improves attention and cognitive deficits in an animal model of attention deficit hyperactivity disorder. *Eur Neuropsychopharmacol* 23:317–328. <https://doi.org/10.1016/j.euroneuro.2012.04.011>