Effects of the Adenosine Deaminase Polymorphism and Caffeine Intake on Sleep Parameters in a Large Population Sample

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Study Objectives: To evaluate the association between the adenosine deaminase polymorphism, sleep architecture, and caffeine consumption.
Design: Genetic association study.
Setting: NA
Patients or Participants: 958 participants who underwent polysomnography and genotyping.
Interventions: NA
Measurements and Results: Individuals carrying the A allele who consumed caffeine in the day prior to polysomnography demonstrated higher sleep efficiency and REM sleep percentage, after adjustment for potential confounders. No effect was observed in the absence of caffeine.
Conclusions: Our data support the role of the *ADA* G22A polymorphism in sleep, and demonstrate for the first time that caffeine may act as a modulator of its functional effects.
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INTRODUCTION

The signaling molecule adenosine has been strongly implicated in the regulation of sleep homeostasis. During sleep-wakefulness cycles, studies have shown that adenosine levels vary in certain areas of the brain, most notably in the basal forebrain.[1,2] Increased sleep need coupled with prolonged wakefulness results in compensatory changes that are due in part to regulation of adenosine metabolism.[3]

Caffeine, a widely consumed stimulant, is known to induce vigilance and wakefulness by blocking adenosine receptors, which results in inhibition of endogenous adenosine activity.[1] Several lines of recent evidence confirm that the activation of adenosine receptors A_1 and A_{2A}, as well as the regulation of adenosine production and degradation, are essential for sleep induction and proper control of the sleep-wakefulness cycle.[1]

Adenosine deaminase (ADA), an enzyme responsible for the clearance of extracellular adenosine,[4] contains a polymorphism within exon 1 that has been identified as a G to A transition at nucleotide 22 (*ADA* G22A; rs73598374) that leads to the substitution of asparagine for aspartic acid at the codon 8 of the gene.[3] A previous study revealed that in erythrocytes and lymphocytes of individuals with the GA genotype, ADA activity decreases by 20% to 30%,[5] which highlights the importance of this polymorphism as a potential marker in the regulation of adenosine-dependent sleep homeostasis. Indeed, the G22A polymorphism has been associated with slow wave sleep duration and intensity as well as fewer reports of awakenings in a sample of healthy adults.[3] Interestingly, after this first report, no other study has attempted to replicate these findings in a larger sample.

The aims of this study were to evaluate the association between the *ADA* G22A polymorphism and sleep measurements as well as the relationship between the *ADA* G22A polymorphism with caffeine intake in a large population-based sample from Sao Paulo City, Brazil.

METHODS

Subjects

This study was conducted with participants of the Sao Paulo Epidemiologic Sleep Study, a large population-based survey from Sao Paulo City, Brazil.[6] The sample was composed of 1042 individuals who completed questionnaires involving socioeconomic, demographic, and lifestyle factors. In addition, polysomnographic recordings were performed. The participants were also asked how many cups/glasses of caffeine-containing drinks they consumed during the day prior to the polysomnography. Moreover, the volunteers answered questionnaires regarding habitual caffeine and alcohol consumption, use of sleeping or awaking promoting drugs and other medications, sleep-wake habits, and subjective sleep perception,[7] as described in Santos-Silva, et al.[6] The study protocol was approved by the Research Ethics Committee of the Universidade Federal de Sao Paulo (CEP 0593/06), and it was registered with ClinicalTrials.gov (number: NCT00596713). All volunteers read and signed the informed consent form.

Polysomnography

Full-night polysomnography was performed using a digital system (EMBLA S7000, Embla Systems, Inc., Broomfield,

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Table 1—Comparisons of polysomnographic sleep parameters between *ADA* G22A polymorphism genotype groups in the whole sample and split by caffeine consumption status

Whole Sample (N = 958)

	GG			GA+AA			
Sleep Parameters	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**P**
Lights off time (hh:mm)	874	12:02AM	00:46	84	12:08AM	00:46	0.28
Lights on time (hh:mm)	874	07:01AM	01:04	84	07:07AM	01:07	0.56
Sleep latency (min)	874	16.8	21.7	84	14.6	17.2	0.36
REM sleep latency (min)	874	101.4	54.9	84	94.9	44.9	0.73
Total sleep time (min)	874	342.1	76.4	84	350.8	70.0	0.20
Sleep efficiency (%)	874	81.7	12.6	84	84.0	12.8	0.04
Stage 1 (%)	874	4.6	3.2	84	4.5	3.7	0.63
Stage 2 (%)	874	54.7	9.3	84	54.9	8.0	0.98
Stages 3 and 4 (%)	874	21.9	8.1	84	20.7	6.6	0.25
REM sleep (%)	874	18.8	6.5	84	19.9	6.6	0.09
Minutes awake	874	60.2	45.3	84	53.6	50.3	0.05
Arousals per hour	874	14.8	11.0	84	14.8	11.0	0.97

No caffeine consumption during the day before the PSG (N = 262)

	GG			GA+AA			
Sleep Parameters	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**P**
Lights off time (hh:mm)	240	12:02AM	00:47	22	12:07AM	00:42	0.90
Lights on time (hh:mm)	240	06:57AM	01:06	22	07:08AM	01:21	0.77
Sleep latency (min)	240	15.7	20.4	22	20.0	21.7	0.17
REM sleep latency (min)	240	102.9	55.8	22	99.4	52.7	0.83
Total sleep time (min)	240	340.0	77.7	22	340.9	77.9	0.78
Sleep efficiency (%)	240	82.0	13.0	22	81.5	15.2	0.90
Stage 1 (%)	240	4.4	2.8	22	4.7	5.1	0.73
Stage 2 (%)	240	55.0	9.9	22	55.5	6.7	0.57
Stages 3 and 4 (%)	240	21.9	8.7	22	21.6	7.1	0.80
REM sleep (%)	240	18.6	6.6	22	18.1	7.3	0.98
Minutes awake	240	59.3	47.5	22	60.8	67.1	0.69
Arousals per hour	240	15.0	11.9	22	11.9	6.7	0.39

Caffeine consumption during the day before the PSG (N = 670)

	GG			GA+AA			
Sleep Parameters	**N**	**Mean**	**SD**	**N**	**Mean**	**SD**	**P**
Lights off time (hh:mm)	612	12:01AM	00:46	58	12:03AM	00:45	0.60
Lights on time (hh:mm)	612	07:03AM	01:04	58	07:01AM	00:55	0.99
Sleep latency (min)	612	17.4	22.5	58	12.4	15.3	0.03
REM sleep latency (min)	612	101.1	55.1	58	93.7	43.2	0.62
Total sleep time (min)	612	343.6	75.4	58	354.1	66.9	0.19
Sleep efficiency (%)	612	81.5	12.5	58	84.9	12.1	0.01
Stage 1 (%)	612	4.6	3.4	58	4.4	3.1	0.60
Stage 2 (%)	612	54.6	9.0	58	54.5	8.6	0.57
Stages 3 and 4 (%)	612	21.8	7.9	58	20.3	6.2	0.19
REM sleep (%)	612	19.0	6.4	58	20.8	6.4	0.02
Minutes awake	612	61.0	44.6	58	51.0	43.8	0.04
Arousals per hour	612	14.8	10.6	58	16.6	12.1	0.30

Results were compared using Mann-Whitney test; PSG, polysomnography; SD, standard deviation.

CO, USA) at the sleep laboratory. Physiological variables were monitored continuously and recordings were visually scored according to Rechtschaffen and Kales standardized criteria.[8] Sleep-related events were scored according to the American Academy of Sleep Medicine Manual for the Scoring of Sleep and Associated Events.[9] The full methodology with further details was previously published.[6]

Polymorphism Detection

Genomic DNA was obtained from peripheral lymphocytes using a standard salting-out protocol. Genotyping of the *ADA* G22A polymorphism was performed using an Allele-Specific PCR Assay Protocol, in which methodology have been previously described.[10] The assay uses 2-tailed allele-specific primers, a common reverse primer, and 2 different fluorescently labeled universal primers in a single well reaction. Submicroliter PCR reactions were carried out with Array Tape instrumentation (Douglas Scientific, Alexandria, MN, USA), and allele calls were generated based on clustering of fluorescent signals. Internal quality of genotype data was assessed by typing 10% blind samples in duplicate. The concordance was > 99%. In addition, genetic ancestry proportions in our sample were obtained using a set of 31 ancestry informative markers as previously described.[11]

Statistical Analysis

Genotypic and allelic frequencies, deviation from Hardy-Weinberg Equilibrium, as well as the association between the *ADA* G22A polymorphism and the presence of sleep disturbances or subjective sleep perception were evaluated using a χ^2 test or Fisher exact test, when appropriate. Polysomnography sleep parameters were compared between genotype groups using Mann-Whitney test. Correlations between quantitative variables were investigated using Spearman correlation coefficient (ρ). A general linear model with Bonferroni correction for multiple comparisons was applied to the association between sleep parameters and *ADA* genotype groups, adjusted for potential confounders. Statistical analyses were performed using SPSS 15.0 (Chicago, IL, USA).

RESULTS

Valid *ADA* G22A polymorphism genotyping was obtained for a total of 958 individuals (537 women and 421 men; mean age 42.56 ± 14.41 years). Frequencies were 91.2% for GG, 8.5% for GA, and 0.3% for AA genotypes. No deviation from Hardy-Weinberg equilibrium was found ($P > 0.05$). Due to the low frequency of the AA genotype in the sample, the GA and AA genotype carriers were grouped together in the analyses. No association between *ADA* G22A polymorphism and sleep disorders such as obstructive sleep apnea syndrome, restless legs syndrome, or insomnia was found ($P > 0.05$). Furthermore, no significant differences were observed among genotypes in respect to sex, age, habitual caffeine and alcohol consumption, use of sleeping or wake promoting drugs and other medications, sleep-wake habits, and subjective sleep perception ($P > 0.05$). Regarding the objective polysomnographic measurements, A allele carriers (GA+AA genotypes) showed higher sleep efficiency percentage (83.99 ± 12.82) than non-carriers (81.71 ± 12.59; $P = 0.04$; Table 1).

To assess whether caffeine intake can influence the effect of the *ADA* G22A polymorphism on sleep parameters, the participants were asked how many cups/glasses of caffeine-containing drinks they consumed during the day prior to the polysomnography. Accordingly, the sample was subdivided into individuals who consumed at least one cup and individuals who did not consume any. Interestingly, among those who consumed caffeine, A allele carriers showed lower sleep latency in minutes (12.41 ± 15.26 versus 17.40 ± 22.51 for non-carriers; P = 0.03; Table 1), higher sleep efficiency percentage (84.93 ± 12.12 versus 81.52 ± 12.45 for non-carriers; P = 0.01), higher REM sleep percentage (20.77 ± 6.37 versus 18.95 ± 6.41 for non-carriers; P = 0.02), and fewer minutes awake (51.04 ± 43.85 versus 61.04 ± 44.62 for non carriers; P = 0.04). Among those who did not consume caffeine, no such difference was found (P > 0.05).

A general linear model was applied to verify the effect of possible factors and covariates that might confound the associations of the *ADA* G22A polymorphism among individuals who consumed caffeine. After adjustment for age, sex, and European ancestry, the associations with higher sleep efficiency (P = 0.03) and REM sleep percentage remained significant (P = 0.03; Table 2), suggesting an independent effect of the *ADA* G22A polymorphism on these sleep parameters.

Table 2—General linear model applied to individuals who consumed caffeine during the day prior to the polysomnographic recordings adjusted by age, sex, and European ancestry

Variable	Mean Difference[a]	95% CI for Difference Lower	95% CI for Difference Upper	P[b]
Sleep efficiency (%)	3.50	0.27	6.72	0.03
Sleep latency (minutes)	-5.00	-11.00	1.00	0.10
REM sleep (%)	1.91	0.16	3.67	0.03
Minutes awake	-10.28	-21.88	1.31	0.08

95% CI, 95% confidence interval; [a]Mean difference between ADA G22A polymorphism A allele carriers (GA+AA genotypes) and non-carriers (GG genotype); [b]P-value adjusted for age, sex and European ancestry in a general linear model corrected for multiple comparisons.

DISCUSSION

This study reports novel associations between the *ADA* G22A polymorphism and sleep parameters in a large population-based sample. ADA is considered an important enzyme involved in homeostatic regulation of sleep, and its pharmacological manipulation has been shown to determine extracellular adenosine levels in the basal forebrain and changes in sleep pattern.[1] Recently, Rétey et al.[3] demonstrated the effect of the *ADA* G22A polymorphism on sleep architecture and self-reported awakenings. More specifically, GA genotype carriers reported fewer awakenings at night than GG homozygous individuals, suggesting an association of the A allele with deeper sleep.[3] Indeed, our results show that A allele carriers have more efficient sleep when compared to individuals with the GG genotype. It can be speculated that in the first group, lower enzymatic activity may reduce the clearance of extracellular adenosine, promoting adenosine accumulation in sleep regulatory areas and a more pronounced homeostatic drive. However, in contrast with our study, Rétey and colleagues demonstrated that GA genotype carriers show higher percentage of slow wave sleep as well as higher slow wave amplitude than GG homozygous individuals.[3] Differences in the study protocol and design may account for these divergent results. In the report by Rétey et al., subjects were asked to abstain from caffeine two weeks before the study and objective sleep measurements were evaluated in a fewer number of participants (N = 7 for GG and GA genotypes each). In the present investigation, polysomnographic recordings were obtained from all participants (N = 874 for GG genotype and N = 84 for GA+AA genotypes) and due to the epidemiological nature of our study, the participants' caffeine consumption could not be controlled. Therefore, the great divergence in caffeine intake by individuals in the two studies, as well as the vast interindividual variability observed in the population-based sample, may help explain the lack of association of the A allele with deeper sleep in our findings. Moreover, it is important to note that one limitation of our study is the lack of data regarding the quantitative analysis of sleep electroencephalogram, which could have brought additional results regarding the ADA G22A polymorphism and sleep microstructure.

Because caffeine intake may alter the effect of the *ADA* G22A polymorphism on sleep measurements, the sample was subdivided into individuals who consumed caffeine during the day before the polysomnography and individuals who did not. In the presence of caffeine consumption only, higher sleep efficiency and REM sleep percentage were found in A allele carriers compared to non-carriers, after adjustment for age, sex, and European ancestry. No significant difference was observed in individuals who did not consume caffeine during the day prior to the polysomnography.

Interestingly, a previous study showed that acute caffeine administration increased ATP and ADP hydrolysis in rat striatal and hippocampal synaptosomes, respectively, suggesting a compensatory mechanism that increases extracellular adenosine availability in response to the antagonistic action of caffeine.[12] Taken together, these results suggest that individuals who consumed caffeine during the day before the polysomnography might be affected by this compensatory mechanism. As a result, increased adenosine levels mediated by caffeine administration together with reduced ADA enzymatic activity in A allele carriers may have increased the adenosine's homeostatic sleep drive, which could have resulted in the different sleep pattern found among these individuals.

In conclusion, our data support an important role for the *ADA* G22A polymorphism in sleep architecture and maintenance and demonstrate for the first time that caffeine, a widely consumed stimulant, may act as a modulator of the functional effects of the *ADA* G22A polymorphism. Thus, a better understanding of the mechanisms involved in the regulation of the adenosinergic system as well as the characterization of the interindividual variability in sleep pattern in response to caffeine may be helpful to the development of therapeutic targets for treatment of sleep disturbances.

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DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

REFERENCES

1. Landolt HP. Sleep homeostasis: a role for adenosine in humans? Biochem Pharmacol 2008;75:2070-9.
2. Christie MA, Bolortuya Y, Chen LC, McKenna JT, McCarley RW, Strecker RE. Microdialysis elevation of adenosine in the basal forebrain produces vigilance impairments in the rat psychomotor vigilance task. Sleep 2008;31:1393-8.
3. Retey JV, Adam M, Honegger E, et al. A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. Proc Natl Acad Sci U S A 2005;102:15676-81.
4. Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. Adenosine and brain function. Int Rev Neurobiol 2005;63:191-270.
5. Battistuzzi G, Iudicone P, Santolamazza P, Petrucci R. Activity of adenosine deaminase allelic forms in intact erythrocytes and in lymphocytes. Ann Hum Genet 1981;45(Pt 1):15-9.
6. Santos-Silva R, Tufik S, Conway SG, Taddei JA, Bittencourt LR. Sao Paulo Epidemiologic Sleep Study: rationale, design, sampling, and procedures. Sleep Med 2009;10:679-85.
7. Braz S, Neumann B, Tufik S. Evaluation of sleep disorders: elaboration and validation of a questionnaire. Revista ABP-APL 1987;9:9-14.
8. Rechtschaffen A, Kales A. Manual of standardized terminology: techniques and scoring system for sleep stages of human subjects. Los Angeles: Brain Information Service/Brain Research Institute, 1968.
9. Iber C, Ancoli-Israel S, Chesson Jr A, Quan S. The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications. Westchester, IL: American Academy of Sleep Medicine, 2007.
10. Myakishev MV, Khripin Y, Hu S, Hamer DH. High-throughput SNP genotyping by allele-specific PCR with universal energy-transfer-labeled primers. Genome Res 2001;11:163-9.
11. Guindalini C, Colugnati FA, Pellegrino R, Santos-Silva R, Bittencourt LR, Tufik S. Influence of genetic ancestry on the risk for obstructive sleep apnoea syndrome. Eur Respir J 2010 Mar 11. [Epub ahead of print]
12. da Silva RS, Bruno AN, Battastini AM, Sarkis JJ, Lara DR, Bonan CD. Acute caffeine treatment increases extracellular nucleotide hydrolysis from rat striatal and hippocampal synaptosomes. Neurochem Res 2003;28:1249-54.