

## Research Article

# Adenosine Deaminase (ADA1) G22A Allele and Sleep-Related Movement

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**Abstract**

**Background:** Adenosine deaminase (ADA1) G22A polymorphism has been linked to variations in sleep depth and vigilance, however not yet studied in relation to clinical sleep problems. This exploratory study evaluated the association between the ADA G22A allele and sleep disorders.

**Methods:** 144 patients presenting for sleep medicine evaluation including overnight polysomnography underwent genetic analysis to determine ADA G22A allele status. Clinical data were obtained by sleep questionnaires, detailed medical history, and polysomnography. Wilcoxon-rank sum tests and independent t-tests were used to assess group differences according to ADA genotype.

**Results:** The frequency of the heterozygous ADA G22A allele was 6.9%. ADA G22A allele carriers more often described symptoms of acting out dreams ( $p=0.008$ ), unusual behaviors during sleep ( $p=0.057$ ), and restless legs ( $p=0.016$ ).

**Conclusions:** ADA G22A allele carriers were found to exhibit more symptoms of sleep movement disorder than patients without the polymorphism. Additional studies are needed to evaluate the strength of this association.

## INTRODUCTION

The common adenosine Deaminase isoenzyme variant (ADA1) G22A polymorphism (c.22G>A, rs73598374) leads to a decreased rate of conversion of adenosine to inosine in erythrocytes and lymphocytes by approximately 20-35%, possibly leading to a buildup of adenosine within the localized milieu [1-3]. Research studies in sleep revealed that the ADA G22A genotype is associated with fewer awakenings throughout the night, and a higher duration of slow wave sleep (SWS), as compared to the normal ADA G22G genotype [4-6]. This exploratory study was designed to evaluate if ADA G22A allele status in a group of patients presenting for baseline overnight sleep studies at an academic Sleep Medicine program was associated with the presence of sleep-related disorders in this population.

## MATERIALS AND METHODS

Written informed consent was obtained in accordance with the Weill Cornell Medical College Institutional Review Board (IRB) guidelines. All patients presenting for an outpatient clinic

visit within 90 days of undergoing a baseline attended overnight polysomnography were offered participation in this study, irrespective of the underlying diagnosis. Patients with split night or CPAP titration studies at baseline were excluded. Venipuncture was performed by a licensed phlebotomist after consent was obtained. Data from standardized medical history and clinical sleep questionnaires as well as polysomnographic analyses were collected.

### Medical and sleep history

Data regarding previous and current medical and sleep problems, including social history and medication use, were acquired during a detailed clinical visit. Standardized questionnaires were filled prior to the baseline polysomnography, and included the Epworth Sleepiness Scale (ESS), Berlin, Cataplexy, and STOP BANG questionnaires, along with questions based on the four-item diagnostic criterion for restless legs syndrome (RLS), and detailed medical history and review of systems questionnaire [7-11]. Additionally, patients were

asked the following question: "Do you have unusual behaviors during sleep?" for which they could answer yes/no. The main sleep disorder clinical diagnosis was documented by the sleep specialist for all patients during this follow-up visit, based on clinical and polysomnographic data interpretation.

### Polysomnography

Baseline polysomnographic data were scored by a single sleep research technician according to American Academy of Sleep Medicine rules [12]. Recorded channels included electroencephalogram, electro-oculogram, submental and anterior tibial is electromyogram, electrocardiogram, inductive plethysmography, body position, nasal cannula/pressure transducer, and oximetry. Sleep stages, respiratory events and limb movements were scored according to AASM guidelines [12]. Sleep was classified as stages 1, 2, 3 and REM sleep. Sleep duration was reported in minutes as total sleep time (TST), and sleep efficiency (SE) was scored as total sleep time over total time in bed. Respiratory events were classified as apneas or hypopneas, and indexed by the total sleep time, generating the apnea-hypopnea index (AHI). The mean and lowest oxygen saturation values were also analyzed, as well as the percentage of sleep time spent at saturation levels below 90%. The oxygen desaturation index (ODI) was calculated based on the hourly drops of oxygen saturation ( $\text{SaO}_2$ )  $\geq 4\%$  during sleep and indexed by the total sleep time, using a beat to beat oximetry recording. Periodic limb movements (PLM) were reported as a PLM index and PLM arousal index.

### ADA Genotype Testing

Genomic DNA was extracted from 3 mL fresh ethylenediaminetetraacetic acid (EDTA) blood using the Qiagen Blood DNA Extraction kit (Qiagen, Valencia, CA). Presence of the ADA G22A allele was detected by allele-specific PCR on an Eppendorf Mastercycler (Eppendorf Scientific, Inc. Westbury, NY) [13,14]. Platinum Taq DNA Polymerase High Fidelity primers were used for gene amplification [15]. Total DNAs (250 ng) were used for PCR in a 50ul volume reaction with 1mM  $\text{MgSO}_4$ . Platinum Taq DNA Polymerase High Fidelity (Invitrogen, cat#11304-011) and the following primers were used: forward primer, 5'- *gcccgcccggttaagaagagc* -3' (sense) and 5'- *ggtcaagtcaggggcagaagcaga* -3' (antisense). The condition for PCR was as follows: 94 °C for 2 minutes, 36 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 68°C for 40 seconds, 68°C at 5 minutes for extension, then hold at 4°C. Sequencing was performed at Life Sciences Core Laboratories Center, Cornell University (Ithaca, NY) by the Sanger chain-termination method with Applied Bio systems Automated 3730XL DNA Analyzer (Applied Bio systems Inc., Foster City, CA) [16,17]. Genetic analysis of the ADA G22A genotype was performed after enrolment in the study was completed in order to avoid any bias. De-identified data was kept on a secure, password-protected CLIMS database.

Associations between the ADA G22A allele and various sleep variables from subjective and objective sources were assessed using SAS® version 9.3 (SAS Institute, Cary, NC). For categorical variables, frequencies were reported and significance between groups was assessed using Chi Square tests. For continuous variables, means and standard deviations (SD) were calculated and independent t-tests or Wilcoxon Rank Sum tests (for variables with skewed distributions) were used to compare groups.

Significance was defined as  $p \leq 0.05$ . Additionally, the association between PLM indices and sleep movements was also stratified by G22A allele status using two-way ANOVA with interaction.

## RESULTS

### Demographics

A total of 152 adult patients were enrolled in the study. Eight patients were excluded due to difficulties in scheduling or performing the blood draw. Data from the remaining 144 patients were analyzed. The final sample was predominately White (71.5%) and included 100 men (69.4%, ADA G22A 7.0%) and 44 women (30.6%, ADA G22A 6.8%,  $p = 0.954$ ). The overall ADA G22A allele frequency was 6.9%. There were no significant differences in age or BMI between groups ( $p=0.451$ ,  $p=0.704$ ), as shown in (Table 1). The presence of underlying heart disease was significantly higher in the ADA G22A allele carriers as compared to ADA G22G allele carriers (35.0 vs. 9.7 %,  $p=0.006$ ). Conversely, a history of pulmonary disease was not reported by any of the ADA G22A allele carriers (0 vs. 16.9 %,  $p=0.045$ ). There were no significant differences in the reported frequencies of hypertension, hypercholesterolemia, diabetes, or mood disorders between groups, as shown in (Table 1).

### Polysomnography data

There were no significant differences in sleep duration, architecture or respiratory parameters between groups, as shown in (Table 2). The mean AHI was similar between groups ( $45.9 \pm 35.1$  vs.  $36.4 \pm 32.9$  events/h in the ADA G22A and ADA G22G group, respectively,  $p=0.264$ ). The indices of PLM and PLM arousals were also similar ( $p=0.705$ ,  $p=0.618$ , respectively). Interaction between polymorphism and sleep movement variables was not significantly associated with PLM indices

**Table 1:** Demographic characteristics and clinical diagnoses of ADA G22A and ADA G22G patients.

	ADA G22A (N=20) mean $\pm$ SD	ADA G22G (N=124) mean $\pm$ SD	P
Age (years)	53.5 $\pm$ 19.1	50.6 $\pm$ 15.3	0.451
BMI (kg/m <sup>2</sup> )	30.3 $\pm$ 7.5	29.6 $\pm$ 7.1	0.704
<b>Ethnicity %*</b>			
White	65.0	72.5	0.486
Other	35.0	27.4	0.486
<b>Gender %*</b>			
Males (n=100)	7.0	93.0	0.954
Females (n=44)	6.8	93.2	0.954
<b>Underling Medical Conditions %*</b>			
Lung disease	0	16.9	<b>0.045</b>
Heart Disease	35.0	9.7	<b>0.006</b>
Hypertension	55.0	37.1	0.129
Hypercholesterolemia	50.0	33.1	0.142
Diabetes	20.0	11.3	0.280
Mood Disorders	30.0	36.3	0.585

ADA adenosine deaminase, SD standard deviation, BMI Body Mass Index; \*Percentages indicate prevalence within that category

( $p=0.351$  and  $p=0.271$ , PLM arousal index and total PLM index, respectively). An association between stage 3 slow wave sleep and ADA G22A allele status was significant among females (mean slow wave sleep duration 21.6 vs. 6.26 ( $p=0.001$ ), but not males (mean slow wave sleep duration 4.0 vs. 4.7, ( $P\leq 0.999$ ). The overall interaction was significant ( $p=0.001$ ).

## Sleep problems

Movement-related activity during sleep was more frequently reported by the ADA G22A allele carriers, and consisted of reporting "acting out dreams" ( $p=0.008$ ), having "unusual behaviors during sleep" ( $p=0.056$ ), and reporting that they "kick their legs at night prior to or during sleep," as compared to ADA G22G allele carriers ( $p=0.010$ ). The ADA G22A allele carriers were significantly more likely to have at least one of the sleep movement variables ( $p=0.037$ ), as shown in (Table 3).

## DISCUSSION

In this group of predominately White participants (71.5%), the prevalence of ADA G22A allele was 6.9%. This rate is similar

to the reported frequency in Caucasian populations. (4-6, 18, 19) A novel association between ADA G22A allele status and sleep related movement was identified, by increased reports of acting out dreams, unusual behaviors during sleep, and kicking legs at night.

Adenosine has known involvement in sleep and movement, though the ADA G22A polymorphism has not been studied in the context of sleep related movement. Polysomnography data and clinical questionnaire results from this study support the hypothesis that the ADA G22A polymorphism (rs73598374) is implicated in sleep related movement.

Adenosine and dopamine receptors interact in the striatum through heterodimerization of their receptors to control movement, and adenosine has additionally been found to independently affect locomotion.(20, 21). Decreased iron stores in the brain are thought to contribute to the pathogenesis of RLS by affecting adenosine  $A_{2A}$  receptors in the striatum.(22, 23)Recent research has highlighted adenosine's role in the mechanism of movement disorders; however, detailed studies haven't yet evaluated the sleep-related movement manifestations of the ADA G22A polymorphism. Adenosine and dopamine receptors are co-localized in the brain and their interaction has been documented in Parkinson's and Huntington's disease.(24) It is possible that the finding of increased movements in sleep seen in ADA G22A allele carriers might be related to the interaction of these receptors, especially if their interaction is affected by increased adenosine due to decreased ADA enzymatic function. Furthermore, genetic analysis of a Caucasian family isolated sleepwalking to chromosome 20q12-q13.12, which encompasses the ADA1 gene locus 20q12.13.(25)The results of this study, along with the sleepwalking linkage area findings, corroborate the need for further genetic exploration of this complex area of sleep and movement disorders.

Recent studies have linked the ADA G22A polymorphism with increased duration and intensity of slow wave sleep, which could contribute to parasomnia-related movements; however, this study does not report a significant association between ADA G22A allele status and greater duration of slow wave sleep. Secondary analysis in this study showed increased slow wave sleep in the female ADA G22A allele carriers.

The interpretation of polysomnographic data in this study is limited by the fact that spectral analyses were not performed. In the previously published reports, women made up a larger proportion of the samples (48% and 58%, respectively), versus 30.6% in this study.(5, 6) If considering a gender-specific effect of this polymorphism on sleep architecture, the higher percentage of women in previous studies may have skewed the overall data.

While exploratory, these findings deserve further evaluation, especially using larger samples determined by power analysis and specifically evaluating movement disorders and potential patho physiological pathways involving this polymorphism in RLS and REM behavior disorder. Several limitations of this study need to be considered, including the small sample size relative to genetic studies and the fact that it is difficult to match the polymorphism group for analyses. The fact that the entire population was sampled from the sleep clinic likely added an intrinsic bias restricting the generalizability of these results. The

**Table 2:** Sleep related characteristics and polysomnography data of ADA G22A and ADA G22G patients.

	ADA G22A (N=20) mean $\pm$ SD	ADA G22G (N=124) mean $\pm$ SD	P
TST (min)	238.1 $\pm$ 151.1	269.2 $\pm$ 133.1	0.338
SE (%)	76.1 $\pm$ 14	73.8 $\pm$ 17.3	0.566
Stage 1 (%TST)	19.9 $\pm$ 22	18.5 $\pm$ 13.8	0.447
Stage 2 (%TST)	61.7 $\pm$ 19.8	64.6 $\pm$ 14.5	0.437
Stage 3 (%TST)	9.3 $\pm$ 16.8	5.2 $\pm$ 7.8	0.773
REM (%TST)	9.1 $\pm$ 9.9	11.8 $\pm$ 8.6	0.208
AHI (events/h)	45.9 $\pm$ 35.1	36.4 $\pm$ 33	0.264
ODI (events/h)	31.2 $\pm$ 27.4	27.0 $\pm$ 28.3	0.464
Lowest SaO <sub>2</sub> (%Hgb)	80.4 $\pm$ 9.7	81.9 $\pm$ 8.5	0.467
Mean SaO <sub>2</sub> (%Hgb)	91.3 $\pm$ 6.2	92.6 $\pm$ 3.8	0.572
SaO <sub>2</sub> <90% (min)	17.9 $\pm$ 23.5	12.6 $\pm$ 20.4	0.347
PLM Index (events/ hr)	16.0 $\pm$ 33.0	8.7 $\pm$ 16.5	0.705
PLM Arousal Index (events/ hr)	0.4 $\pm$ 1.1	1.4 $\pm$ 5.9	0.618

TST total sleep time, SE sleep efficiency (TST/ Total Time in Bed), REM rapid eye movement, AHI apnea hypopnea index, SaO<sub>2</sub> oxygen desaturation, PLM periodic limb movement

**Table 3:** Frequency of self-reported sleep-related symptoms of ADA G22A and ADA G22G patients.

	ADA G22A (%)	ADA G22G (%)	P
Acting out Dreams (n=105)	41.2	13.8	<b>0.008</b>
Unusual Behaviors during Sleep (n=114)	44.4	22.7	<b>0.057</b>
Kicking Legs prior to Sleep (n=123)	52.6	25.0	<b>0.016</b>
Sleep Movement (n=88)‡	65.0	39.8	<b>0.038</b>

\* Percentage of subjects who self-reported this symptom; ‡Answering one of the sleep movement variables

results support a possible association between the ADA G22A polymorphism and movements during sleep, without delineating which specific sleep related movement was present, nor did this study identify a mechanism that explains this association. Nonetheless, the unique findings identified by this study may shed light into the physiological and pathophysiological control of sleep and other disorders. The results from this exploratory study may provoke further exploration of this polymorphism and its sleep movement related phenotypes.

## CONCLUSION

This study reports the possibility of a novel association between the ADA G22A allele and sleep related movement. This is the first description of such associations with the ADA G22A allele and these findings deserve further investigation.

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