Head-up tilt induced syncope and adenosine A$_{2A}$ receptor gene polymorphism

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Aims
High adenosine plasma levels and high expression of adenosine A$_{2A}$ receptors are observed in patients with unexplained syncope and a positive head-up tilt test (HUT). This study aimed to evaluate the single nucleotide polymorphism (SNP) (c.1364 T$\rightarrow$C) which is the most commonly found polymorphism in the A$_{2A}$ receptor gene, in patients with unexplained syncope undergoing HUT.

Methods and results
One hundred and five patients with unexplained syncope who underwent HUT were included. Fifty-two had a positive test. Receptor genotype determinations were performed in patients and in 121 healthy subjects. Genotype (TT, CC, TC) was determined from DNA leucocytes. The distribution of the polymorphism showed significant ($P < 0.0001$) difference when the results of HUT were analysed. Fifty-two per cent of patients with a positive HUT had a CC genotype and 34.6% a TC genotype, whereas 13.2% of the patients with a negative HUT had a CC genotype and 71.7% a TC genotype. Patients with a CC genotype had a higher incidence of spontaneous syncopal episodes.

Conclusion
In patients with unexplained syncope, a significant association between high incidence of syncopal episodes, positive HUT, and the presence of the CC variant in the adenosine A$_{2A}$ receptor gene was elicited.

Keywords
Adenosine  •  Syncope  •  A2A adenosine receptors  •  Genes

Introduction
Unexplained syncope is a common clinical problem that can alter the quality of life of affected patients. Head-up tilt testing (HUT) is an established and widely used tool for the evaluation of patients with unexplained syncope.$^{1}$ Exogenous adenosine or ATP has been used in the provocation of syncope during tilt testing in susceptible patients.$^{2,4}$ On the other hand, recent studies support the concept that endogenous adenosine may be a possible mediator in a subset of syncope patients suspected to be of neurocardiogenic origin. This is based on higher baseline plasma adenosine levels (APLs) observed during positive HUT together with a positive correlation between rising plasma adenosine concentration and the rapidity of onset of tilt-induced syncope.$^{5,6}$ It is well established that adenosine effect on blood vessel tone and on sinoatrial node occurs via activation of four subtypes of membrane receptors (A$_{1}$, A$_{2A}$, A$_{2B}$, and A$_{3}$).$^{7}$ Stimulation of A$_{1}$ and A$_{3}$ receptors is associated with cardio-protection and ischaemic preconditioning,$^{8,9}$ and activation of the adenosine A$_{2A}$ receptors results in vasodilatation.$^{10,11}$ The role of adenosine A$_{2A}$ receptors in the control of heart rate and blood pressure may be essential.$^{12,13}$ Moreover, these receptors are expressed in an area of the brainstem strongly implicated in the baroreflex function,$^{14}$ and the role of the baroreflex among the various mechanisms of neurocardiogenic syncope has been emphasized.$^{15}$ It has been also shown in patients with unexplained syncope that an over expression of adenosine A$_{2A}$ receptors was observed in patients with a positive HUT.$^{9}$ The goal of this prospective study was to
investigate the distribution frequency of the single nucleotide polymorphism (SNP) c.1364 T>C in the adenosine A2A receptor gene in patients with an unexplained syncope and a positive HUT.

**Methods**

**Patients selection**

One hundred and twenty height patients with unexplained syncope were referred to our institution for tilt testing from March 2003 to December 2007. The inclusion criteria were: (i) patients experienced two or more episodes of syncope or pre-syncope within the preceding year; (ii) The cause of syncope was unexplained despite a complete clinical work-up and was assumed to be neurocardiogenic by the referring physician; (iii) Head-up tilt was indicated for the first time and no patient had previously had an adenosine or an ATP test. Exclusion criteria were (i) the presence of orthostatic hypotension, anaemia, or endocrinological dysfunctions such as diabetes, hypoglycaemia, or thyroid dysfunction; (ii) the presence of sinus bradycardia <50 b.p.m., or bundle branch block on the electrocardiogram; (iii) the presence of abnormal electrophysiological study, implanted pacemakers, aortic stenosis, hypertrophic cardiomyopathy, pulmonary hypertension, subclavian steal syndrome, or drug induced syncope. Patients should be drug-free and were instructed to avoid coffee and tea intake 48 h prior to the study. Twenty-three of the 128 patients (18%) were not included in the study: 14 patients because they were on beta-blockers at the time of the test, 5 patients had orthostatic hypotension, 3 had a positive carotid massage subsequent to the HUT reproducing their symptoms, and one patient who developed for the first time, before tilting, a paroxysmal atrial fibrillation episode. Ultimately, 105 patients were included in the analysis. The control group consisted of healthy volunteers, members of the medical and technical staff of our institution, consecutively included during the study period. None of them had a past story of fainting or syncope. They were on no medication and were as instructed, to avoid coffee and tea for 48 h before blood sampling.

The investigation was conducted according to the principles outlined in the Declaration of Helsinki. All patients and control subjects gave a written informed consent to participate to the study. The study protocol was approved by the Ethical Committee for Human Research of the Centre Hospitalo-Universitaire de Marseille, Université de la Méditerranée.

**Study protocol**

The protocol used for performing the HUT test was consistent with the Recommendations of the European Society of Cardiology Task Force report. In order to avoid false-positive diagnosis, no pharmacological provocation was allowed. Tilt testing was performed in a quiet room at 21°C equipped for cardio-pulmonary resuscitation. An intravenous catheter was inserted before the onset of the test for safety reasons and blood sampling. Patients were instructed to lie down on a tilt table for 30 min. The table was then tilted within 20 s to 60° for 45 min. The heart rate was monitored using continuous recording of six electrocardiographic leads and blood pressure was measured manually by the same operator every 2 min.

**Collection of blood samples**

Samples were collected and treated as previously described. In brief, venous blood was withdrawn together with an iced cold stop solution in order to prevent adenosine degradation and uptake. Samples were collected: (i) just before tilting (baseline), (ii) immediately after tilting the table in order to have information on adenosine release during tilting (mentioned as ‘Tilt’ in the tables), and (iii) either during syncope, immediately after resetting the table in the horizontal position (positive test) or after 45 min of tilting (negative test). The blood samples for ADORA2A genotype determination were withdrawn just before tilting.

**Definition**

A positive test was defined as the occurrence of syncope or pre-syncope in association with bradycardia (at least 20% decrease in heart rate) and/or hypotension (systolic blood pressure <80 mm Hg). Syncope was defined as a transient loss of consciousness, and pre-syncope as premonitory signs and symptoms of imminent syncope (e.g. severe light-headedness, transient hearing loss, blurring of vision, or severe weakness).

**Adenosine plasma levels**

Adenosine plasma measurement has been previously described. After samples deproteinization, adenosine was identified and quantified using HPLC (Hewlett Packard 1100). The absence of xanthine derivatives was checked on each chromatogram. Technicians blind to the tilt-test response performed all the biological measurements.

**Genotyping of the ADORA2A polymorphism**

The T/C polymorphism located in codon 361 (rs5751876) was determined after genomic DNA extraction from blood cells and PCR amplification. The amplification was performed using forward primer F2 5’CTGAGCCGGAGCCCAATGGGTA3’ and reverse primer R2 5’CTCCCCAAGTGACTGGTCAG3’. The primer F2 was modified to introduce a Rsa1 restriction site when C replaced T at nucleotide 1364 in the sequence (NM_000675 GenBank) that resulted in the cleavage of the 256 bp ampiclon into 22 bp and 234 bp fragments.

**Statistical analysis**

Statistical analyses were performed using the SPSS software for Windows 13.1. As the TT variant is not common (10–15%;16,19,20 the recruitment of a minimum of 100 patients was necessary to include 10–15 patients with the TT genotype.

Quantitative variables are reported as mean ± standard deviation (SD) or median and interquartile range (IQR), and qualitative variables as numbers and percentages. Differences in the distribution of patient’s characteristics by tilt-test results and ADORA2A genotypes were tested using the Mann-Whitney non-parametric test (continuous variables) or using the χ² or, where appropriate, the Fisher’s exact test (categorical variables). Two-sided tests were performed and a P-value <0.05 was considered as significant. A Bonferroni correction procedure was then used to account for false positive results and to accurately determine which differences in the distribution were significant across ADORA2A genotypes. To evaluate the presence of a significant statistical difference, and to ensure an overall type I error rate of 5%, an adjusted P < 0.05/3 = 0.016 was considered significant.

To determine the independent relationship between genotype and positive HUT, a logistic regression multivariable analysis was systematically adjusted for the main predictive factors, based on epidemiological knowledge17,18,21,22 (i.e. age, sex, number of syncopal episodes per patient, presence of vasovagal symptoms, presence of triggering factors). The number of variables was voluntary limited in order to have an appropriate ratio between the number of variables and the number of events. For each continuous variable, the linearity assumption was assessed by plotting the unadjusted relationship between variables and logit probability of HUT. The model’s goodness-of-fit was accurate across ADORA 2A genotypes. To evaluate the presence of a significant statistical difference, and to ensure an overall type I error rate of 5%, an adjusted P < 0.05/3 = 0.016 was considered significant.

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assessed by the Hosmer–Lemeshow test (larger \(P\)-value means better fit or reliability), and predictive accuracy was assessed by the area under the receiver-operating characteristic curve.

Linkage analyses were not performed in this study because c 1364 T>C is a silent polymorphism and no assumption concerning the mode of inheritance, dominant or recessive, is therefore necessary.

### Results

#### Demographics and symptoms

One hundred and five patients referred for unexplained syncope and HUT were included in the study. Their mean age was 49.05 ± 1.8 years (range 13–76). The control group included 121 healthy volunteers: 71 males and 50 females with a mean age of 48.2 ± 1.23 years (range 32–69). No difference was observed between the control group and the syncope patients, neither between the patients with a positive or with a negative HUT with respect to age, sex, height, baseline heart rate, and blood pressure. The HUT was positive in 52 patients and negative in 53 patients. The clinical characteristics of the patients are summarized in Table 1. In the group of patients with a positive HUT test, syncope occurred in 46 and pre-syncope in 6 after a mean of 30 ± 1.8 min (range 4–45). Upon syncope, systolic blood pressure dropped by 51 ± 2% and heart rate by 33 ± 3.5% (\(P < 0.0001\)). Regarding the history of symptoms, the time since first episode and the frequency of spontaneous syncope or pre-syncope events per month were similar in both groups. However, the numbers of syncope and pre-syncope episodes per patient were higher in patients with a positive HUT test as shown in Table 1.

#### Adenosine A2A receptor gene polymorphism

The global distribution of genotype frequency was not significantly different (\(P = 0.80\)) in the 105 syncope patients and in the 121 control subjects: TT = 15 (14.3%) vs. 21 (17.3%); CC = 34 (32.4%) vs. 39 (32.2%), and TC = 56 (53.3%) vs. 61 (50.5%), respectively (Figure 1). As seen in Table 2, the patients with the CC variant were significantly younger (45.6 ± 15.6 years) than those with the TC variant (54.3 ± 18 years). The TC variant was found more commonly in men than in women. The distribution
of the genotype was in accordance with the Hardy–Weinberg laws of equilibrium (P = 0.29). Differences in the distribution of genotype frequencies were found in the patients with a negative HUT when compared with those with a positive HUT: 7 CC (13.2%) vs. 27 (52%) (P < 0.0001) and 38 TC (71.7%) vs. 18 (34.6%), respectively, (P < 0.0001). These differences were detected with a statistical power >95%. The TT variant distribution was similar in the two groups: 8 (15.1%) vs. 7 (13.4%). The number of syncopal episodes per patient was significantly higher in patients with the CC variant when compared with those with the TC variant (P = 0.001) (Table 2). On the other hand, the number of syncopes and pre-syncopes per month, the time since first episode, the number of patients with situational symptoms and triggering factors were not different. Table 3 shows that CC genotype was associated with positive HUT, after adjustment for age, sex, the number of syncopal episodes per patient, the presence of vasovagal symptoms, and the presence of triggering factors. The model was reliable (Hosmer–Lemeshow test: P = 0.93) and accurate (area under the curve = 0.82; 95% confidence interval: 0.74–0.90; P ≤ 0.001).

**Adenosine plasma levels**

Baseline APLs were comparable in patients with a negative HUT and in those of the control group (Table 4). Patients with a positive test had significantly higher baseline APLs than those with a negative HUT. In patients with a negative HUT, APLs remained stable after 45 min of tilting (Table 4). Finally, APLs were higher in patients with CC variant compared with TC (Table 2).

### Table 2 Clinical characteristics of the patients, adenosine levels, and tilt-test response according to the genotype of the A2A receptor

<table>
<thead>
<tr>
<th>Genotype</th>
<th>TT (n = 15)</th>
<th>CC (n = 34)</th>
<th>TC (n = 56)</th>
<th>P-value&lt;sup&gt;b&lt;/sup&gt; (CC vs. TC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40.5 ± 20.4</td>
<td>45.6 ± 15.6</td>
<td>54.3 ± 18.0</td>
<td>0.01</td>
</tr>
<tr>
<td>History of syncopal episode</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since first episode (months)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3 (1–24)</td>
<td>7 (3–24)</td>
<td>6 (1–24)</td>
<td>0.39</td>
</tr>
<tr>
<td>Syncopes and pre-syncopes per month&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1 (0.4–3)</td>
<td>1.3 (0.7–2.5)</td>
<td>1 (0.5–1.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>Syncopal episodes per patient&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2 (1–3)</td>
<td>3.5 (1.7–6)</td>
<td>1.5 (1–3)</td>
<td>0.001</td>
</tr>
<tr>
<td>Pre-syncopal episodes per patient&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2 (0–8)</td>
<td>4.5 (0–18.5)</td>
<td>3 (0–6)</td>
<td>0.22</td>
</tr>
<tr>
<td>Number of patients with pre-syncopal episodes (%)</td>
<td>10 (66.7)</td>
<td>23 (67.6)</td>
<td>37 (66.1)</td>
<td>0.88</td>
</tr>
<tr>
<td>Number of patients with situational symptoms (%)</td>
<td>5 (33.3)</td>
<td>10 (29.4)</td>
<td>19 (33.9)</td>
<td>0.66</td>
</tr>
<tr>
<td>Number of patients with triggering factors (%)</td>
<td>5 (33.3)</td>
<td>17 (50.0)</td>
<td>20 (35.7)</td>
<td>0.18</td>
</tr>
<tr>
<td>Number of patients with vasovagal symptoms (%)</td>
<td>9 (60.0)</td>
<td>24 (70.6)</td>
<td>23 (41.1)</td>
<td>0.007</td>
</tr>
<tr>
<td>Adenosine plasma levels (μM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.8 ± 1.8</td>
<td>1.9 ± 1.6</td>
<td>1.2 ± 1.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Tilt&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.4 ± 2.4</td>
<td>2.1 ± 1.7</td>
<td>1.3 ± 1.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Syncopal episodes (52 positive tests)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.7 ± 1.7</td>
<td>2.5 ± 1.4</td>
<td>2.9 ± 1.4</td>
<td>0.20</td>
</tr>
<tr>
<td>End of test (45th min) (53 negative tests)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.3 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.53</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive HUTT (%)</td>
<td>7 (46.7)</td>
<td>27 (79.4)</td>
<td>18 (32.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Odds ratio were systematically adjusted for age, sex, number of syncopal episodes, and in those of the control group (Table 4). Patients with a positive test had significantly higher baseline APLs than those with a negative HUT. In patients with a negative HUT, APLs remained stable after 45 min of tilting (Table 4). Finally, APLs were higher in patients with CC variant compared with TC (Table 2).

### Table 3 Independent predictors of head-up tilt-testing response (multivariate logistic regression)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Adjusted odds ratio&lt;sup&gt;b&lt;/sup&gt;</th>
<th>95% confidence intervals</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1.4</td>
<td>0.3–5.4</td>
<td>0.66</td>
</tr>
<tr>
<td>CC</td>
<td>5.1</td>
<td>1.7–15.4</td>
<td>0.004</td>
</tr>
</tbody>
</table>

<sup>b</sup>Odds ratio were systematically adjusted for age, sex, number of syncopal episodes per patient, presence of vasovagal symptoms, presence of triggering factors. Hosmer–Lemeshow test: P = 0.93.

### Discussion

The main findings of this study were threefold: (i) in patients with unexplained syncope, an over-representation of the genotype CC variant in the adenosine A<sub>2A</sub> receptor gene was observed in the group with a positive HUT when compared with those with a negative HUT. Conversely, when the HUT was negative, the variant TC was more common; (ii) a higher number of spontaneous syncopal episodes per patient was observed in the
group of CC genotype; (iii) the general distribution frequency of genotypes did not differ in the syncope patients from that of the control group.

The mechanisms of cardiogenic syncope have not yet been unravelled. Previous studies have suggested that endogenous adenosine may play a role in a subset of patients with unexplained syncope.\(^5\) In brief, baseline APLs were found to be significantly higher in patients with a positive HUT than in those with a negative HUT or in those of healthy volunteers serving as a control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) The mechanisms of cardiogenic syncope have not yet been unravelled. Previous studies have suggested that endogenous adenosine may play a role in a subset of patients with unexplained syncope.\(^5\) In brief, baseline APLs were found to be significantly higher in patients with a positive HUT than in those with a negative HUT or in those of healthy volunteers serving as a control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) A positive correlation was also found in those with a positive HUT when compared with the control group.\(^5\) The expression of A\(_{2A}\) receptor at the membrane level was found to be increased in patients with positive HUT.\(^6\)

The SNP (c.1364 T>C) is the most commonly found polymorphism in the A\(_{2A}\) receptor gene in the general population.\(^16,19\) This study is to our knowledge the first to investigate the SNP (c. 1364 T>C) in patients with unexplained syncope. Despite the absence of a significant difference in SNP distribution frequency between the syncope patient group and the control group, the study showed differences between patients with a positive HUT when compared with those with a negative HUT, suggesting a significant association between c. T1364 T>C polymorphism and positive HUT. Consequently, the question arises whether the presence of the CC variant in the adenosine A\(_{2A}\) receptors is related to the susceptibility to develop syncope. Our results clearly identify a subset of patients with unexplained syncope, high basal APLs and with high incidence of the CC variant in the A\(_{2A}\) adenosine receptor gene. Substances, which are able to modulate the A\(_{2A}\) receptors, deserve a trial in this subset of patients.

The group of positive HUT is also the group in which baseline APLs are high and clinically the group in which the frequency of spontaneous syncope attacks per patient is the highest. Such findings support the hypothesis that an adenosinergic mechanism may play a role in this subset of patients.

**Limitations of the study**

In the present report, we found a significant association between c. T1364 T>C polymorphism and positive HUT. However, because a ‘silent’ SNP do not produce altered coding sequence and therefore are not expected to change the function of the protein receptor, the question arises whether such a silent mutation could modify the interaction between adenosineA\(_{2A}\) receptors and adenosine in the susceptible patients. However, it was recently shown that a ‘silent’ polymorphism in MDR1 gene (a transmembrane protein without any relationship with adenosine receptors) changes substrate specificity by altering the folding, the insertion, and the quantity of protein expressed at the cell membrane.\(^23\) It is possible that such a mechanism could be involved in adenosine A\(_{2A}\) receptors. The fact that the polymorphism in adenosine A\(_{2A}\) receptors gene is associated with habitual caffeine consumption\(^24\) supports this hypothesis since caffeine is a well-known ligand for adenosine A\(_{2A}\) receptors.

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**Conflict of interest:** none declared.

**References**


