Lower frequency of the low activity adenosine deaminase allelic variant (ADA1*2) in schizophrenic patients

Diminuição da frequência da variante alélica de baixa atividade da adenosina desaminase (ADA1*2) em pacientes esquizofrênicos

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Abstract

Objective: Adenosine may play a role in the pathophysiology of schizophrenia, since it modulates the release of several neurotransmitters such as glutamate, dopamine, serotonin and acetylcholine, decreases neuronal activity by post-synaptic hyperpolarization and inhibits dopaminergic activity. Adenosine deaminase participates in purine metabolism by converting adenosine into inosine. The most frequent functional polymorphism of adenosine deaminase (22G→A) (ADA1*2) exhibits 20-30% lower enzymatic activity in individuals with the G/A genotype than individuals with the G/G genotype. The aim of this study was to evaluate the ADA polymorphism 22G→A (ADA1*2) in schizophrenic patients and healthy controls.

Method: The genotypes of the ADA 22G→A were identified with allele-specific PCR strategy in 152 schizophrenic patients and 111 healthy individuals.

Results: A significant decrease in the frequency of the G/A genotype was seen in schizophrenic patients (7/152 – 4.6%) relative to controls (13/111 – 11.7%, p = 0.032, OR = 2.6).

Conclusion: These results suggest that the G/A genotype associated with low adenosine deaminase activity and, supposedly, with higher adenosine levels is less frequent among schizophrenic patients.

Descriptors: Polymorphism, restriction fragment length; Adenosine; Adenosine deaminase; Schizophrenia; Gene frequency

Introduction

Adenosinergic activity may play a role in schizophrenia, especially because adenosine modulates most neurotransmitter systems.1 The neuromodulator adenosine acts on A1, A2a, A2b and A3 receptors. Activation of adenosine A1R inhibits the release of several neurotransmitters, such as glutamate, dopamine, serotonin and acetylcholine, and decreases neuronal activity by post-synaptic hyperpolarization.2 Adenosine is considered an endogenous anticonvulsant and neuroprotective agent.

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Accordingly, pre-clinical studies show that administration of A1R agonists exerts anticonvulsant, neuroprotective, anxiolytic, sedative and antipsychotic-like actions. A2aR and D2R are co-localized in GABAergic striatopallidal neurons and form functional heteromeric complexes, with opposing actions via coupling with G proteins. Activation of A2aR decreases the affinity for D2 receptor antagonists and A2aR knockout mice present increased aggressiveness, anxiety and hypalgesia and reduced behavioral effects with amphetamine and cocaine administration. For these reasons, it has been suggested that low adenosine activity is involved in schizophrenia.

Adenosine deaminase (ADA) participates in purine metabolism by converting either adenosine or 2′-deoxyadenosine into inosine or 2′-deoxyinosine, respectively. Further metabolism of these deaminated nucleosides leads to hypoxanthine, which can be either transformed into uric acid by xanthine oxidase or salvaged into mononucleotides by hypoxanthine-guanine phosphoribosyltransferase. Beside its classical intracellular localization, ADA is an ectoenzyme (Ecto-ADA) on the surface of many cell types, including neurons, where it behaves as a cell adhesion molecule with an important role in the regulation of neuronal growth and plasticity processes. The most frequent functional polymorphism of ADA is caused by a G-to-A transition at nucleotide 22 (coding DNA 22G→A). This transition leads to the substitution of asparagine for aspartic acid at codon 8 (protein Asp8Asn) of the ADA protein. Individuals with the G/A genotype exhibit 20-30% lower enzymatic activity in erythrocytes and leucocytes than individuals with the G/G genotype. This genotype has been associated to autism, mild mental retardation and higher duration and intensity of deep sleep in healthy subjects, reinforcing the functional consequences of this polymorphism on adenosine-mediated neuromodulation. The following genotype frequencies are expected to occur in a healthy Caucasian population: G/G 88-92%; G/A 8-12%; A/A < 1%. Activation of A2aR decreases the affinity for D2 receptor antagonists and A2aR knockout mice present increased aggressiveness, anxiety and hypalgesia and reduced behavioral effects with amphetamine and cocaine administration. For these reasons, it has been suggested that low adenosine activity is involved in schizophrenia.

Genomic DNA was extracted from 300μL blood samples using the “Perfect gDNA Blood Mini” (Ependorf) or “Wizard® Genomic Purification Kit” (Promega). The genotypes of the ADA 22G→A were analyzed in 100ng of DNA with allele-specific PCR. HotStarTag DNA polymerase (Qiagen) and allele-specific primers were used. ADA primers were as follows: forward-G, 5′ –CCC AGA CGC CGG CCT TC-3′; forward-A, 5′ – CCC AGA CGC CGG CCT TC-3′; reverse, 5′ –GAA CTC GCC TGC AGG AGC C- 3′ (annealing temperature, 62°C; 1.5mM MgCl₂; 1 x Q-solution) (adapted of Rétey et al., 2005). The amplification product of 152 pb was visualized with Gel Red in U.V. illumination.

A comparison between cases and controls in terms of genotype frequency was performed, and the statistical significance of the associations was tested using the chi-square test. Odds ratio with 95% confidence intervals were also calculated to estimate the strength of the association between adenosine deaminase genotype and schizophrenia and its statistical significance. The Hardy-Weinberg equilibrium was calculated at the website http://www.oego.org/software/hwe-mr-calc.shtml.

Subjects and method
The study protocol and all experimental procedures were approved by the national ethic committee in research on human subjects (CONEP: 7589). All subjects received a full explanation of the procedures and written informed consents were obtained. We determined diagnoses by using best-estimate procedures based on the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). Controls who denied having experienced psychotic symptoms and been subjected to psychiatric treatment were selected from a pool of non-psychiatric patients and staff in a general hospital. All subjects were from the Rio Grande do Sul state. Subjects were asked to define their ethnicity as either Caucasian, African-American or mixed. In order to be included in the sample, this self-definition had to agree with that made by the investigator.

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<th>Table 1 - ADA G22A (ADA1*2) genotype distributions in healthy controls and individuals with schizophrenia</th>
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<td>Genotype</td>
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* p = 0.032. Odds Ratio: 0.364 (95% confidence interval: 0.140 – 0.945)
results showed that the G/A genotype with low activity was less
frequent in schizophrenic patients (GG 95.4% and GA 4.6%)
than in the general population (GG 88.3% and GA 11.7%).
Interestingly, serum adenosine deaminase activity is increased in
medicated schizophrenic patients,14 but it remains to be established
if this increase in related to the phenotype or to medication.

Several indirect findings are suggestive of adenosine dysfunction
or more specifically, of a reduction of its role in schizophrenia.1
The adenosine A1 and A2A receptor antagonists theophylline
and caffeine decrease P50 sensory gating in normal volunteers,
mimicking the findings in schizophrenic patients,15 and
caffeine exacerbates symptoms of schizophrenia.3 Moreover, the
psychostimulant effects of caffeine are blocked by D2 receptor
agonists.3 In animal models of schizophrenia, A1 and A2aR
agonists prevent behavioral, as well as neurophysiological (EEG and
prepulse inhibition) alterations induced by NMDA antagonists.16
Moreover, the xanthine oxidase inhibitor allopurinol, which may
increase adenosine levels,17 was effective as add-on treatment of
schizophrenia.18 Taken together, these findings are in line with
reduced adenosinergic activity in schizophrenia and also with
our results, according to which the low activity ADA genotype
in schizophrenic patients is less frequent since this genotype is
supposedly associated with higher adenosine levels.

Stubbs and et al.19 reported decreased ADA serum activity in
children with autism compared to normal controls, individuals
with cerebral palsy and subjects with intellectual impairment
(F-test 0.02 when compared with other group). A higher
frequency of the A (*2) allele was observed in a study with 118
Italian autistic children compared with 126 healthy controls
(by genotype Asp/Asn p < 0.0001; by allele Asn frequency
p < 0.00001).9 However, Zoruglu et al. measured activities of
erythrocyte free radical scavenging enzymes, including ADA in
children with autism and sex- and age-matched controls and
found no differences in ADA activity (p = 0.52).20 In contrast to
the previous genetic studies, a recent study found no significant
increase in the frequency of the A (ADA*2) allele in cases from
North America.21 Thus, the role of ADA in autism remains
controversial. The low-activity G/A genotype has been associated
with mild mental retardation showing genotype frequency with
p < 0.05 and odds ratio 2.16.19 These are relevant findings
considering that cognitive problems and autistic symptoms are
part of the schizophrenia syndrome.

Previous studies indicate that adenosine plays a direct role in
human sleep homeostasis,22 with the G/A genotype associated
with better sleep. Individuals with the G/A genotype (n = 13) reported
fewer awakenings at night than individuals with the G/G genotype
(n = 106).11 Moreover, individuals with G/A genotype showed
almost twice the amount of deep, stage-4 sleep and roughly 30min
more slow-wave sleep within the 8-h sleep period when compared
with the G/G genotype.11 This suggests that the ADA 22G→A
polymorphism modulates not only the duration of slow-wave
sleep but also the intensity of sleep.11 Unmedicated schizophrenic
patients had longer sleep onset latency, slept less and had lower
sleep efficiency.23 Overall, these results are in accordance with the
proposed reduced adenosine activity in schizophrenia.

In conclusion, for the first time, our data suggests that the G/A
genotype associated with low ADA activity and, supposingly,
with higher adenosine levels is less frequent among schizophrenic
patients. However, this finding needs to be replicated in larger
samples. This finding is in line with the hypothesis of lower
adenosinergic activity in schizophrenia, but replication using
independent and larger samples is needed.

Disclosures

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<th>Writing group member</th>
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* Modest
** Significant
*** Significant: Amounts given to the author’s institution or to a colleague for research in which the author has participation, not directly to the author.

Note: PUCRS = Pontifícia Universidade Católica do Rio Grande do Sul; UFRGS = Universidade Federal do Rio Grande do Sul; CNPq = Conselho Nacional de Pesquisa e Desenvolvimento; FINEP = Financiadora de Estudos e Projetos.

For more information, see Instructions for Authors.

References


