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 pos-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, supposingly, with higher adenosine levels is less frequent among schizophrenic patients.

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

Resumo

***Objetivo**: A adenosina pode ter um papel importante na fisiopatologia da esquizofrenia, uma vez que modula a liberação de vários neurotransmissores, tais como glutamato, dopamina, serotonina e acetilcolina, diminui a atividade neuronal por hiperpolarização pós-sináptica e inibe a atividade dopaminérgica. A adenosina desaminase participa do metabolismo das purinas pela conversão de adenosina em inosina. O mais frequente polimorfismo funcional da adenosina desaminase (22G→A) (ADA1*2) exibe uma diminuição de 20-30% da atividade funcional em indivíduos com genótipo G/A quando comparados com indivíduos com o genótipo G/G. O objetivo deste estudo foi avaliar o polimorfismo 22G→A (ADA1*2) em pacientes esquizofrênicos e em controles saudáveis. **Método**: Os genótipos da ADA 22G→A foram identificados através de uma estratégia de PCR alelo-específica em 152 pacientes esquizofrênicos e 111 controles saudáveis. **Resultados**: Foi observada uma diminuição significativa na frequência do genótipo G/A em pacientes esquizofrênicos (7 – 4,6%) em relação ao grupo controle (13 – 11,7%, p = 0,032, OR = 2,6). **Conclusão**: Estes resultados sugerem que o genótipo G/A associado com baixa atividade de adenosina desaminase, e potencialmente com níveis aumentados de adenosina, é menos frequente entre pacientes esquizofrênicos.*

***Descritores**: Polimorfismo de fragmento de restrição; Adenosina; Adenosina deaminase; Esquizofrenia; Frequência do gene*

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[2] and antipsychotic-like actions.[3]

A2aR and D2R are co-localized in GABAergic striatopallidal neurons and form functional heteromeric complexes, with opposing actions via coupling with G proteins.[3] Activation of A2aR decreases the affinity for D2 receptor antagonists[3] and A2aR knockout mice present increased aggressiveness, anxiety and hypoalgesia[4] and reduced behavioral effects with amphetamine and cocaine administration.[5] For these reasons, it has been suggested that low adenosine activity is involved in schizophrenia.[1]

Adenosine deaminase (ADA) participates in purine metabolism by converting either adenosine or 2'-deoxyadenosine into inosine or 2'-deoxyinosine, respectively. Further metabolization of these deaminated nucleosides leads to hypoxantine, which can be either transformed into uric acid by xanthine oxidase or salvaged into mononucleotides by hypoxanthine-guanine phosphoribosyl-transferase. Beside its classical intracellular localization, ADA is an ectoenzyme (Ecto-ADA) on the surface of many cell types, including neurons,[6] 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.[7] Individuals with the G/A genotype exhibit 20-30% lower enzymatic activity in erythrocytes and leucocytes than individuals with the G/G genotype.[8] This genotype has been associated to autism,[9] mild mental retardation[10] and higher duration and intensity of deep sleep in healthy subjects,[11] 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%.[12,13]

To our knowledge, ADA genotypes have not yet been investigated in schizophrenia. In this paper, we evaluated the ADA polymorphism 22G→A (ADA1*2) in schizophrenic patients and healthy controls.

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.

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. HotStarTaq DNA polymerase (Qiagen) and allele-specific primers were used. ADA primers were as follows: forward-G, 5' –CCC AGA CGC CCG CCT TCG-3'; forward-A, 5' –CCC AGA CGC CCG CCT TCA- 3'; reverse, 5' –GAA CTC GCC TGC AGG AGC C- 3' (annealing temperature, 62ºC; 1.5nMMgCl$_2$; 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.oege.org/software/hwe-mr-calc.shtml.

Results

The sample consisted of 152 schizophrenic patients (108 males, mean age of 36.7 ± 10.8 years old; 80.6% Caucasian, 8.6% African-American, 11% mixed) and 111 healthy individuals (71 males, mean age and standard deviation equal to of 44.4 ± 15.5 years old, 79.6% Caucasian, 6.1% African-American, 14.3% mixed) defined as the control group. No significant difference was found in terms of the distribution of ethnic groups or gender ($p > 0.10$).

Table 1 shows the distribution of ADA genotypes in schizophrenic patients and healthy individuals. In the group of schizophrenic patients, there was a lower frequency of the G/A genotype compared to that of the control group ($p = 0.032$, odds ratio 0.364; IC 95% 0.140 – 0.945). The A/A genotype was not found.

The Hardy-Weinberg equilibrium calculations for the ADA1*2 allelic variations showed equilibrium in both controls ($\chi^2 = 0.43$) and patients ($\chi^2 = 0.08$, $p = 0.77$).

Discussion

The frequency of alleles and G/A genotype in the control group was similar to the values reported by other researchers.[11] Our

Table 1 - ADA G22A (ADA1*2) genotype distributions in healthy controls and individuals with schizophrenia

Genotype	Healthy controls	Schizophrenic patients
GG	98 (88.3%)	145 (95.4%)
GA	13 (11.7%)	7 (4.6%)*
AA	0	0
Total	111	152

** 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 antagonists.[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 supposingly 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 genoptype 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.[10] 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

Writing group member	Employment	Research grant[1]	Other research grant or medical continuous education[2]	Speaker's honoraria	Ownership interest	Consultant/ Advisory board	Other[3]
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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.

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