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Family-based and case–control association studies of glutamate receptor GRIK3 Ser310Ala polymorphism in Polish patients and families with alcohol dependence

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Abstract

The aim of this study was to evaluate the role of the GRIK3 functional polymorphism (Ser310Ala) in the pathogenesis of alcoholism. This polymorphism was investigated in two types of studies: (1) the association study in a whole group of alcoholics (116 patients fulfilling ICD-10 alcohol dependence (AD) criteria and 255 controls, Polish descent) and homogenous overlapping subgroups of patients with: a history of delirium tremens and/or alcohol seizures, early age of onset of alcoholism (AOO < 26 years), a co-occurrence of dissocial personality disorder, a history of familial alcoholism; (2) the family-based study (using Transmission Disequilibrium Test (TDT) in 100 Polish families with alcohol dependence). The history of alcoholism was obtained using SSAGA (Polish version). GRIK3 functional polymorphism was determined using PCR. TDT revealed an adequate transmission of both alleles to the affected offspring in the whole group of alcohol families (29 × Ser, 24 × Ala; $\chi^2 = 0.472$; d.f. = 1; $p = 0.492$) and in the homogenous subgroups of families. No significant associations between any of the above mentioned alcohol phenotypes and Ser310 allele were observed (the whole AD group: $p = 0.66$ AD with delirium and/or seizures: $p = 0.521$; early onset AD: $p = 0.868$; AD with familial history of alcoholism: $p = 0.798$ and AD with dissocial personality disorder: $p = 0.618$). These findings do not seem to support the hypothesis of the role of this polymorphism in the pathogenesis of alcoholism.

Keywords: Alcohol dependence; Genetics; TDT; Case–control study; Kainate receptor

Alcoholism is a heterogeneous disorder with an estimated heritability of 40–60% [10].

The glutaminergic neurotransmission plays an essential role in the pathogenesis of alcohol dependence since *N*-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors (KA-R), located in the limbic system, basal ganglia and in the cortex have a fundamental role in synaptic plasticity and influence cognitive processes like learning and memory which are crucial in the development of dependences [3]. Alcohol has two opposite effects on glutamate receptor ion channel complexes: acute exposure to alcohol inhibits ion flow through these receptor-channel complexes, whereas chronic exposure up-regulates the number of these receptors and thereby increases ion flow. An acute withdrawal from alcohol results in hyperexcitability and seizures in the presence of up-regulated channels, thereby making postsynaptic neurons vulnerable to excitotoxic damage [4]. Out of many glutamate receptors, the ones most commonly suggested to be involved in alcoholism are ionotropic subtypes, NMDA, AMPA, and kainate receptors. Both NMDA and AMPA subtypes were extensively studied, but relatively little is known about the role of kainate subtype of glutamate receptor in alcohol dependence [12,18,23,24].

Hofman and Tabakoff [6] reported that barbiturates have a greater inhibitory effect at the kainate subtype of glutamate receptor than at the NMDA receptor in contrast to alcohol.

According to Carta et al. [2] an activation of KA-R enhances interneuron firing, which significantly increases spontaneous inhibitory postsynaptic currents in pyramidal neurons but

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ethanol (EtOH) potently inhibits this KA-R-mediated effect only at low concentrations.

Up regulation of glutamatergic neurotransmission resulting from chronic ethanol intoxication may cause a hyperexcitable state during alcohol withdrawal which may lead to seizures and delirium tremens [13].

Kainate receptors, which are a subtype of glutamate ionotropic receptors, are composed of both low affinity subunits GRIK1, GRIK2, GRIK3, and high affinity subunits GRIK4 and GRIK5 [17].

This study focuses on investigating GRIK3 gene polymorphism (T928G).The T/G (thymine/guanine) variation leads to a serine/alanine change in position 310 in the extracellular N-terminus of the protein.

The T allele is more frequently associated with lower expression levels whereas the G allele was expressed at higher rates [11,17]. This polymorphism affects the primary structure of the human ionotropic glutamate receptor subunit suggesting, that Ser 310 is less stable than Ala 310 form [1,17]. The aim of the present study was to evaluate the role of functional polymorphism (Ser310Ala) of GRIK3 gene in the pathogenesis of alcoholism since Preuss et al. [14] reported a significant relationship between a history of delirium tremens and Ser3l0 allele. To our knowledge this is the first family designed study of the above mentioned polymorphism in alcohol dependence.

In the case–control part of the study 116 (99 male, 17 female) patients, mean age 35 ± 9, fulfilling International Classification of Diseases (ICD-10) alcohol dependence (AD) criteria were investigated. In the family-based study 100 families, Caucasians, with no history of psychiatric disorders of axis I of ICD-10 other than alcohol or tobacco dependence were investigated. Alcohol dependent offspring were: 88 males aged 34 ± 9 and 12 females aged 40 ± 8. Mean alcohol consumption was: 224 ± 108 g/day, mean age at onset was: 25 ± 7 years; 64% of them had an early onset of alcoholism (AOO < 26 years), 51% of the patients fulfilled the criteria of heavy drinkers, 33% had at least one alcoholic parent, 79% were type I alcoholics according to Clonninger. Alcohol and family history was assessed by means of a structured interview, based on SSAGA (Semi-Structured Assessment for the Genetics of Alcoholism) [5]. Alcohol-dependence status of the parents was assessed in personal examination using Michigan Alcohol Screening Test (MAST), Alcohol Use Disorders Identification Test (AUDIT) and Research Diagnostic Criteria (RDC) [20]. The control group consisted of 255 controls, Polish descent (139 males, 116 females), with excluded psychiatric disorders using Prime MD questionnaire (Primary Care Evaluation of Mental Disorders) [21], mean age 35 ± 14. The study protocol was approved by the Ethical Committee of Pomeranian Medical University of Szczecin. The overlapping subgroups were formed with respect to more homogeneous etiology of alcohol dependence according to the following criteria: (1) history of delirium tremens and/or seizures during withdrawal; (2) alcoholics characterized by early age at onset, i.e. under 26 years of age; (3) alcoholics with dissocial personality; (4) alcoholics with positive familial history of alcoholism.

The above mentioned subtypes of alcoholism were selected according to the data suggesting strong genetic basis of these disturbances [8,9].

Genomic deoxyribonucleic acid (DNA) was isolated from the whole blood according to standard procedures. GRIK3 Ser310Ala (T928G) polymorphism (GenBank accession number U16127) was detected by polymerase chain reaction (PCR) amplification, restriction enzyme digestion (SmaI – Fermentas), electrophoresis on 2% agarose gels and visualization by ethidium bromide [1]. The Statistical Package for the Social Sciences (SPSS) computer program (PC version for Windows, release 9 in English) was used for the statistical analysis of the data. Transmission Disequilibrium Test (TDT) was used to test for linkage in the possible presence of association [15]. The families' data from heterozygous parents were calculated using χ^2 [4]. The differences between the controls and the alcoholics were tested by χ^2-test and considered significant if type 1 error was less than 5% using SPSS. The Hardy–Weinberg equilibrium was calculated using SAS computer program for Windows [16].

The alleles and genotypes distribution of the investigated polymorphism did not differ significantly between the whole group of alcoholics, the created homogenous subgroups and the controls (Table 1). Also, while comparing gender divided alcoholics and controls no associations were found. TDT revealed an adequate transmission of both alleles to the affected offspring in the whole group and in the homogenous subgroups. The number of informative parents and transmitted alleles is shown in Table 2.

No significant results were obtained when the association was measured separately for the subgroup with delirium tremens and in the subgroup with alcohol seizures.

For the arbitrally chosen relative risk of 1.5 the obtained level of statistical power was 0.32 for the whole group of alcoholics. The relative risk of 2.22 would be the level at which the statistical power exceeds 0.8 for the whole group of patients. For the homogenous subgroups the relative risk should achieve values as shown in Table 1 [22].

According to the literature data about the role of glutamate receptors in excitation and inhibition processes, we tested the hypothesis about the influence of GRIK3 functional polymorphism on alcoholism etiology and on the development of alcoholism complications. As reported by Preuss et al. [14], a significant relationship between a history of delirium tremens and Ser3l0 allele was detected; no significant results were obtained for alcohol withdrawal-related seizures.

A genetically homogenous, well characterized group of alcoholics and their families was investigated. The investigations in the case–control group did not confirm the results obtained by Preuss et al. [14].

In comparison to the German clinical sample our alcoholics had a lower mean age of onset (29.5 ± 9 versus 25 ± 7) and had a lower daily alcohol intake g/day (331.65 ± 191 versus 224 ± 108).

Because frequencies of these alleles may vary considerably in different populations, the conflicting results could be due to population stratification bias. The Transmission Disequilibrium Test can help to avoid this problem. For example, TDT method