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Case control study of *ANKK1 Taq 1A* polymorphism in patients with alcohol dependence classified according to Lesch's typology*

Badania asocjacyjne polimorfizmu *Taq1A genu ANKK1* u pacjentów z zespołem zależności alkoholowej według typologii Lescha

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Summary

Objective: The aim of this study was to examine the association between the *Taq 1A* polymorphism of the *ANKK1* gene in homogeneous subgroups of patients with alcohol dependence syndrome divided according to Lesch's typology.

Material/Methods: DNA was provided from alcohol-dependent (AD) patients (n = 373) and healthy control subjects (n = 168), all of Polish descent. The history of alcoholism was obtained using the Polish version of the SSAGA (Semi-Structured Assessment for the Genetics of Alcoholism). Samples were genotyped using the PCR method.

Results: We found no association between alcohol dependence and *ANKK1 Taq 1A* polymorphism.

Conclusions: Lesch's typology is a clinical consequence of the disease, and its phenotypic description is too complex for simple genetic analysis.

Keywords: **Alcohol dependence • ANKK1 gene • Taq1A polymorphism • Lesch's typology**

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The widespread prevalence of alcohol dependence and alcohol-related mental and physical disorders poses a major global health, social and economic challenge. New forms of prevention and treatment are implemented to address the new threats [28,29].

Depending on the family history of alcohol dependence syndrome, previous personal psychopathology, and hypothetical neurobiological background [16], four evolutionary types of alcohol-dependent subjects (ADS) were established. According to Lesch's typology (LT), type I ADS (the 'allergy' model) suffer from severe alcohol withdrawal syndrome, probably associated with dopamine deficits, and tend to use alcohol to reduce withdrawal symptoms. Patients of type II (conflict resolution and anxiety model) use alcohol as self-medication because of its anxiolytic effect. In ADS of type III, the main characteristic is an affective disorder, and thus alcohol is used as an antidepressant by these subjects. Type IV patients (alcohol drinking as adaptation) show premorbid cerebral defects, behavioral disorders, and a high social burden [16].

Background

An association between the *Taq1 A* polymorphism and alcohol dependence was first reported in 1990 [4]. The TaqIA polymorphism has been associated with DRD2 gene expression. The function of the mesolimbic dopaminergic system was postulated to arouse rewarding and uncontrolled drinking which may lead to AD. AD subjects have fewer striatal D2 receptors than controls [12]. Studies show that individuals carrying at least one A1 allele appear to have up to 40% fewer striatal DRD2 receptors than do those carrying the A2/A2 allele [20,25]. The A1 allele is considered a risk factor for alcohol dependence [10,19,24]. A meta-analysis performed by Munafo [18] found a small effect of the *Taq1A* polymorphism A1 allele on risk of AD. Comings in 1999 [8] found that heterozygotes (A1/A2) of the *Taq1A* polymorphism are more likely to exhibit the phenotype than either homozygotes (A1/A1 or A2/A2). The association between AD and the *Taq1A* polymorphism remains controversial [10,13].

Methods

this study included a group of 373 Caucasian subjects, with no history of psychiatric disorders other than alcohol or nicotine dependence as classified by ICD-10. According to Lesch's typology, 106 AD subjects were of type I, 87 patients were of type II, 92 patients were of type III, and 88 patients were of type IV. The control group comprised 168 unrelated individuals matched for ethnicity and gender, and excluded for mental disorders using the Primary Care Evaluation of Mental Disorders (Prime MD) questionnaire. Recruitment and study of each patient were carried out by authorized personnel of the Department of Psychiatry, Pomeranian Medical University. The study protocol was approved by the Ethical Committee of Pomeranian Medical University of Szczecin (KB-0012/103/11). All participants gave written informed consent.

Genomic DNA was extracted from venous blood samples using a salting out method.

Genotyping for *ANKK1 TaqI A* polymorphism

The genotyping of the *TaqI A* polymorphism was performed by the PCR-RLFP method. The following primers were used F: 5′CTT GCC CTC TAG GAA GGA CAT 3′, R: 5′ACC TTC CTG AGT GTC ATC AAC C 3′. The amplification of DNA fragments was performed in a PTC-200 (MJ Research) thermal cycler. The 15 µl amplification mixture contained 250 ng of genomic DNA, 0.45 µM of each primer, 0.17 mM of each dNTP, 1.5 mM $MgCl_2$, 75 mM Tris-HCl, 20 mM $(NH_4)_2$ SO_4, 0.01% Tween, 0.15% DMSO, and 0.5 U of Taq DNA polymerase (MBI Fermentas). The cycling conditions were: initial denaturation 94°C for 3 min followed by 35 cycles at 94°C for 30 s, annealing temperature 60°C for 1 min and 72°C for 1 min, and final elongation at 72°C for 7 min. A volume of 5 µl of each PCR product was then digested overnight in a total volume of 10 µl at 37°C with 0.5 U Taq I restriction endonuclease (MBI Fermentas). Amplification products were separated by electrophoresis on Metaphor agarose gel. The repeats were visualized by ethidium bromide staining. The uncut PCR product size was 310 bp (allele A1). Allele A2 comprised the cut bands of 180 and 130 bp.

Statistical analysis

Frequencies of genotypes and alleles in patients with ADS and the control group were compared by Pearson's chi-square test, using the computer program IBM SPSS Statistics 20.

Results

Tables 1 to 5 present an association analysis with regard to the allele genotypes. No statistical significance was observed within the entire group and specific subgroups according to Lesch's typology.

Discussion

In our study we did not find any differences regarding ANKK1 Taq1A in the subtypes according to Lesch's typology. This may indicate that Lesch's typology as a phenotypic description of the course of alcoholism is far too complex for simple genetic analysis. Alcohol typologies are a consequence of different clinical pictures of the disease. However, there are reports with regard to genetic studies into this complex disease entity. Some of them confirm relationships, while others do not confirm such relationships.

Table 1. Frequency of genotypes and alleles of the polymorphism of the ANKK1 Taq1A gene (rs1800497) in patients with alcohol dependence syndrome (ADS) and in controls

Group	n	Genotypes			p	Alleles		p
		A1/A1 n (%)	A1/A2 n (%)	A2/A2 n (%)		A1 n (%)	A2 n(%)	
ADS patients	373	15 (0.04)	111 (0.30)	247 (0.66)	0.863	141 (0.19)	605 (0.81)	0.953
Control group	168	8 (0.05)	47 (0.28)	113 (0.67)		63 (0.19)	273 (0.81)	

Table 2. Frequency of genotypes and alleles of the polymorphism of the *ANKK1 Taq1A* gene (rs1800497) in patients with alcohol dependence syndrome (ADS) with Lesch's type I and in controls

Group	n	Genotypes			p	Alleles		p
		A1/A1 n (%)	A1/A2 n (%)	A2/A2 n (%)		A1 n (%)	A2 n(%)	
ADS – type I	106	2 (0.02)	29 (0.27)	75 (0.71)	0.450	33 (0.16)	179 (0.84)	0.340
Control group	168	8 (0.05)	47 (0.28)	113 (0.67)		63 (0.19)	273 (0.81)	

Table 3. Frequency of genotypes and alleles of the polymorphism of the *ANKK1 Taq1A* gene (rs1800497) in patients with alcohol dependence syndrome (ADS) with Lesch's type II and in controls

Group	n	Genotypes			p	Alleles		p
		A1/A1 n (%)	A1/A2 n (%)	A2/A2 n (%)		A1 n (%)	A2 n(%)	
ADS type II	87	2 (0.02)	26 (0.30)	59 (0.68)	0.618	30 (0.17)	144 (0.83)	0.676
Control group	168	8 (0.05)	47 (0.28)	113 (0.67)		63 (0.19)	273 (0.81)	

Table 4. Frequency of genotypes and alleles of the polymorphism of the *ANKK1 Taq1A* gene (rs1800497) in patients with alcohol dependence syndrome (ADS) with Lesch's type III and in controls

Group	n	Genotypes			p	Alleles		p
		A1/A1 n (%)	A1/A2 n (%)	A2/A2 n (%)		A1 n (%)	A2 n(%)	
ADS type III	92	7 (0.07)	30 (0.33)	55 (0.60)	0.411	44 (0.24)	140 (0.76)	0.164
Control group	168	8 (0.05)	47 (0.28)	113 (0.67)		63 (0.19)	273 (0.81)	

Table 5. Frequency of genotypes and alleles of the polymorphism of the *ANKK1 Taq1A* gene (rs1800497) in patients with alcohol dependence syndrome (ADS) with Lesch's type IV and in controls

Group	n	Genotypes			p	Alleles		p
		A1/A1 n (%)	A1/A2 n (%)	A2/A2 n (%)		A1 n (%)	A2 n(%)	
ADS type IV	88	4 (0.05)	26 (0.29)	58 (0.66)	0.965	34 (0.19)	142 (0.81)	0.876
Control group	168	8 (0.05)	47 (0.28)	113 (0.67)		63 (0.19)	273 (0.81)	

The results of several recent studies on the association between alcohol dependence and the *TaqI A* polymorphism are conflicting. Some studies have found an association [1,4,7,21,23], while in others no association was found [5,9,11,15,17,22]. Literature reviews also have not been consistent [1,25]. Studies using transmission disequilibrium or the affected family-based association test did not find an association either [3,24].

Previous reports provide evidence for biological mechanisms and their relation to Lesch's typology, although this classification is a clinical component.

As shown in previous studies, various neurobiological and also neuroendocrinological mechanisms seem to be of special importance in specific patient groups. An association between prolactin serum levels and craving, particularly in patients of Lesch's type I, was reported by Hillemacher [14]. Bönsch found an elevated frequency of MTHFR 677 C/T and 393 C/A polymorphisms within the group of Lesch type I patients, as well as a small group of type IV, which could be associated with elevated homocysteine and a higher risk of experiencing withdrawal seizures [6]. Lesch type I patients with a history of alcohol withdrawal seizures revealed higher homocysteine levels on admission [2]. A significant correlation between the number of preceding detoxifications and the extent of craving, particularly for Lesch type I patients, could be observed, rendering these subgroups at a higher risk of complications in withdrawal due to kindling effects [13]. Additionally, it has been shown that alterations of glutamic acid during detoxification differ between Lesch's subtypes, with higher glutamic acid levels in type I, characterized by a gradual increase in response to drinking based on a vulnerability related to the NMDA system [29].

However, other studies equally aimed at associating biological markers, such as polymorphisms of dopamine- and serotonin-related genes, to the Lesch typology failed to show an association [26].

Conclusions

In our study we did not find any differences regarding *ANKK1 Taq1A* in the subtypes according to Lesch's typology. This might indicate that Lesch's typology as a phenotypic description of the course of alcoholism is far too complex for simple genetic analysis. Alcohol typologies are a consequence of different clinical pictures of the disease. Empirically derived typologies typically identify 2 to 5 meaningful subtypes. Despite the differences in study samples and methods, several consistent types have been found: early onset, externalizing, affective, and antisocial personality disorder. Multidimensional typologies did not differentiate

alcoholism by symptom profile, only by severity. Existing typologies are typically derived from samples of alcohol-dependent persons only. Lesch's typology is a clinical consequence of the disease, and its phenotypic description is too complex for simple genetic analysis. Further studies should be performed taking into account alcohol typologies which include dissocial personality.

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