GABA-A receptor genes do not play a role in genetics of Lesch's typology in Caucasian subjects

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Submitted: 4 February 2011
Accepted: 24 July 2011

Arch Med Sci 2012; 8, 2: 357-361
DOI: 10.5114/aoms.2012.28565

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Abstract

Introduction: Lesch's typology differentiates alcoholics into different treatment response subgroups. The effects of ethanol are mediated, to an important extent, via the GABA-ergic system.
Material and methods: We have evaluated the linkage disequilibrium patterns and haplotype frequencies of GABRG1 and GABRA2 genes in 133 alcoholics divided according to Lesch's typology and in 145 matched controls.
Results: Besides several relationships at a threshold of statistical significance, we found no significant differences in the haplotype distribution of these genes between alcoholics and controls.
Conclusions: Lesch's typology may not be related with the genotype of alcoholics – at least in terms of genes with an established role in the development of dependency.

Key words: alcohol dependence, GABA, genetics, Lesch's typology.

Introduction

Alcohol dependence (AD) is a highly prevalent disorder that is associated with serious morbidity and mortality. The development of alcohol dependence is the result of an interaction between environmental and hereditary factors. According to twin, adoption and family studies, the heritability of alcoholism ranges between 50% and 60% [1].

While studies have found that genetic factors account for a substantial amount of variance in alcohol consumption, there is inconsistent evidence on which polymorphisms are related to alcohol use and serious alcohol problems.

Lesch *et al.* [2] distinguished four evolutionary types of alcoholics, depending on the family history of alcoholism, previous personal psychopathology, and hypothetical neurobiological background. According to Lesch's typology (LT), type I alcoholics (the so-called model of 'allergy') suffer from heavy alcohol withdrawal syndrome, probably associated with dopamine deficits, and tend to use alcohol to weaken withdrawal symptoms. Patients of type II (model of anxiety of conflict) use alcohol as self-medication because of its anxiolytic effect. In alcoholics of type III, the main characteristic is of 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, behavioural disorders, and a high social burden [2].

Currently performed studies deal with the possible genetic background of alcoholism amongst LT subjects. The glutamine gene is one of the candidate genes tested in this context. GABA-ergic neurotransmission is supposed to play a central role in the behavioural effects of alcohol, particularly sedation, tolerance, loss of motor coordination, and withdrawal [3]. Previous studies have implicated GABA-A receptor genes with AD, alcohol use patterns, and levels of response to alcohol [4].

Animal studies may provide clues to the potential mechanism by which GABRA2 influences the risk for alcohol dependence. Research in animals selectively manipulated for the GABA α1, α2, α3, α5 receptor subtypes suggests that the α2 subtype selectively mediates the anxiolytic properties of diazepam [5]. Baseline anxiety, rates of anxiety disorders, and responses to anxiolytic agents, such as benzodiazepines or alcohol, or other substance dependence-related phenotypes, were revealed to differ among individuals as a function of the GABRA2 haplotypes [6].

Genome wide linkage scans implicate a region of chromosome 4p12 harbouring a cluster of four genes encoding GABA-A receptor subunits as a potential genetic background of LT [7, 8]. Also another cluster of GABA-A receptor genes (GABRA1, GABRA6, GABRB2 and GABRG2) on chromosome 5q was revealed to be significantly associated with drinking behaviours, such as the level of response to alcohol, history of blackouts, age of first drunkenness, and alcohol dependence [4, 9].

Importantly, a study by Covault *et al*. [10] suggests that markers in the 5' region of the GABRAG1 gene, which encodes the GABA-A receptor γ-1 submit, are in linkage disequilibrium (LD) with markers found in the GABRAA2 gene adjacent to the GABRAG1 gene and also located in chromosome 4p. Moreover, markers in the 5' region of the GABRAG1 gene show associations with AD in 2 samples of individuals of European ancestry [10].

Therefore, it is hypothesized that certain polymorphisms of the GABRAG1 gene will be associated with lower levels of response to alcohol, heavier drinking patterns, and more alcohol-related problems in a group of hazardous drinkers [10].

Table I. Linkage disequilibrium of the GABRG1 loci in AD subjects (n = 133) and the controls (n = 145)

	gab1	gab2	gab3
gab1 D'		0.99	0.84
gab2 D'			0.91

gab1 – polymorphism rs 1391166 A/T, gab2 – polymorphism rs 1497577 A/T, gab 3 – polymorphism rs 10034075 A/G

To understand the reported associations, we sought to understand the linkage disequilibrium (LD) patterns and haplotype structures of GABRG1 and GABRA2 in Polish alcoholics depending on their classification to certain phenotypes of Lesch's typology.

Material and methods

This study included a group of 133 Caucasian subjects, with no history of psychiatric disorders other than alcohol or nicotine dependence as classified by ICD-10. According to Lesch's typology, 39 AD subjects were of type I, 33 patients of type II, 41 patients of type III, and 20 patients of type IV. The control group comprised 145 unrelated individuals matched for ethnicity and gender, and excluded for mental disorders using the Primary Care Evaluation of Mental Disorders (Prime MD) questionnaire. All subjects were recruited in the North West region of Poland. Alcohol use and family history of alcoholism were assessed by means of a structured interview, based on the Polish version of Semi-Structured Assessment on Genetics in Alcoholism (SSAGA).

The study protocol was approved by the local Ethic Committee of Pomeranian Medical University and all the participants provided their written informed consent.

Genomic DNA was extracted from venous blood samples using a salting out method. SNP rs1391166 A/T, rs1497577 A/T, and rs10034075 A/G polymorphisms of GABRG1 and GABRA2 polymorphisms rs2119767 A/T, rs16859354 G/T, rs13152740 A/G, rs279836 A/T, rs279826 A/G, rs279844 A/T, rs3775282 C/T, rs279871 A/G, rs17537141 C/T, rs529826 C/T, and rs572227 A/G were analysed. Single nucleotide polymorphism (SNP) genotyping was carried out using the Centaurus (Nanogen) platform [11].

Statistical analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 9. Differences between controls and alcoholics were tested using the χ^2 test and considered significant if the type 1 error was less than 5% using SPSS. The Hardy Weinberg equilibrium was calculated using the SAS computer program for Windows. Odds ratios and confidence intervals at 95% were also calculated. Haplotype frequencies and linkage disequilibrium were calculated using R with Bioconductor packages haplo.stats and genetics.

Results

GABRG1

In the group of alcoholics and controls, high values of D' were observed between gab1 (rs 1391166) and gab2 (rs 1497577) polymorphisms as well as between gab1 and gab3 (rs 10034075) or gab2 and gab3 polymorphisms (Table I). Alcoholics and non-

Table II. Linkage disequilibrium of GABRGA2 loci in AD subjects (n = 133) and the controls (n = 145)

	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11
g1 D'	0.93	0.90	0.05	0.32	0.34	0.99	0.02	0.99	0.05	0.06
g2 D'		0.93	0.05	0.07	0.12	0.89	0.05	0.91	0.03	0.02
g3 D'			0.34	0.26	0.22	0.93	0.39	0.96	0.36	0.32
g4 D'				0.99	0.97	0.93	0.96	0.99	0.97	0.87
g5 D'					0.99	0.92	0.95	0.99	0.97	0.85
g6 D'						0.92	0.95	0.99	0.97	0.85
g7 D'							0.93	0.99	0.94	0.86
g8 D'								0.99	0.99	0.96
g9 D'									0.99	0.99
g10 D'										0.96

GABRGA2 polymorphisms: g1 – rs2119767 A/T, g2 – rs16859354 G/T, g3 – rs13152740 A/G, g4 – rs279836 A/T, g5 – rs279826 A/G, g6 – rs279844 A/T, g7 – rs3775282 C/T, g8 – rs279871 A/G, g9 – rs17537141 C/T, g10 – rs529826 C/T, g11 – rs572227 A/G

alcoholics did not differ significantly in terms of 3-SNP GABRG1 haplotype frequencies. No significant differences in GABRG1 haplotype distributions were also observed between subgroups of alcoholics selected based on Lesch's typology and non-drinking controls.

GABRA2

The values of D' ranged from 0.02 to 0.99 in this study population (Table II). Similar to GABRG1, no significant differences in 11-SNP GABRA2 haplotype frequencies were observed in the population of alcoholics compared to non-alcoholics. No significant differences in terms of GABRA2 haplotype distributions were also observed between Lesch I, II or IV alcoholics and the controls. In a subgroup of Lesch III alcoholics, the frequency of T-G-A-A-G-T-C-G-C-C-A haplotype was higher compared to non-alcoholics, but this difference was at a threshold of statistical significance (p = 0.04508).

GABRG1 and GABRA2

LD analysis for GABRG1 and GABRA2 showed high variability of D' values, which ranged from 0.01 to 0.99 (Table III). The groups of alcoholics and non-alcoholics did not differ in terms of 14-SNP GABRA2 and GABRG1 haplotype frequencies. Although T-T-G-A-G-T-T-A-T-T-G-T-T-G haplotype was absent in the controls and observed in some alcoholics, even this difference was only at the significance threshold (p = 0.02104).

Discussion

Sub-typing of alcohol dependence has become an important issue as studies have proposed different neurobiological mechanisms in regards to alcoholism in recent years. It was shown that alcohol dependence reflects a wide range of different phenotypes, including psychological, social, and neurobiological factors. Different methods of sub-typing have been proposed over the last few decades, one of them being Lesch's typology of alcohol dependence [12].

Results from a genome-wide linkage scan by the Collaborative Study on the Genetics of Alcoholism showed a suggestive linkage to alcohol dependence in the region of the GABA receptor gene cluster on chromosome 4p13-p12. This region contains the GABRG1, GABRA2, GABRA4, and GABRB1 genes, encoding γ-1, α-2, α-4, and β-1 subunits of the $GABA_A$ receptor, respectively. Association studies on some of these loci have indicated that a genetic predisposition to alcohol dependence is related to polymorphic variation at GABRA2 or near this gene [6, 13-15].

Ittiwut *et al*. [16] focused on two adjacent $GABA_A$ receptor subunit genes, including one which has been associated with risk for alcohol dependence in several previous studies. To define the linkage disequilibrium in the chromosomal region extensively, more SNPs covering the GABRG1 gene located telomerically to GABRA2 on chromosome 4p were studied. These studies concluded that this is in fact the case, and associations observed with GABRA2 might be attributable to functional genetic variations at the GABRG1 locus, or that there may be disease-related variants at both loci. This may facilitate our understanding of the reported associations between GABRA2 polymorphisms and AD and permit a more informed search for the functional variant or variants underlying this association – a search that must now extend into the intergenic region and the GABRG1 locus [16].

Covault *et al*. [10] extended their findings on associations between alcohol dependence and the chromosome 4 $GABA_A$ gene cluster by examining the intergenic extent of the GABRA2 3' region hap-

Table III. Linkage disequilibrium (LD) of the GABRA2 and GABRG1 loci in AD subjects (n = 133) and the controls (n = 145)

	g2	g3	g4	g5	g6	g7	g8	g9	g10	g11	g12	g13	g14
g1 D'	0.93	0.89	0.03	0.30	0.35	0.99	0.01	0.99	0.03	0.04	0.35	0.05	0.04
g2 D'		0.93	0.04	0.08	0.13	0.88	0.03	0.9	0.01	0.04	0.11	0.03	0.12
g3 D'			0.36	0.28	0.24	0.93	0.41	0.96	0.38	0.34	0.26	0.25	0.17
g4 D'				0.99	0.97	0.93	0.96	0.99	0.97	0.87	0.51	0.48	0.53
g5 D'					0.99	0.91	0.95	0.99	0.96	0.85	0.58	0.38	0.35
g6 D'						0.92	0.95	0.99	0.97	0.85	0.57	0.35	0.33
g7 D'							0.93	0.99	0.93	0.86	0.57	0.45	0.4
g8 D'								0.99	0.99	0.96	0.58	0.53	0.52
g9 D'									0.99	0.99	0.63	0.72	0.55
g10 D'										0.96	0.56	0.51	0.53
g11 D'											0.53	0.49	0.46
g12 D'												0.99	0.81
g13 D'													0.93

GABRGA2 and GABRG1 polymorphisms: g1 – rs2119767 (GABRA2) A/T, g2 – rs16859354 (GABRA2) G/T, g3 – rs13152740 (GABRA2) A/G, g4 – rs279836 (GABRA2) A/T, g5 – rs279826 (GABRA2) A/G, g6 – rs279844 (GABRA2) A/T, g7 – rs3775282 (GABRA2) C/T, g8 – rs279871 (GABRA2) A/G, g9 – rs17537141 (GABRA2) C/T, g10 – rs529826 (GABRA2) C/T, g11 – rs572227 (GABRA2) A/G, g12 – rs1391166 (GABRG1) A/T, g13 – rs1497577 (GABRG1) A/T, g14 – rs10034075 (GABRG1) A/G

lotype block associated with alcohol dependence and by examining markers in adjacent haplotype block in the 5' region of GABRG1. They observed a stronger association between GABRG1 upstream markers in the GABRA2 haplotype block [10].

The key to fully understanding the aetiology of alcohol dependence, however, is to associate these aforementioned genetic findings with behavioural patterns of AD, including Lesch's typology of phenotypes. This typology is important since it is widely used in Europe and especially in German-speaking countries and integrates biological, social, and psychological factors into the classification system [2, 17]. Our previous study focused on genetic variants of the dopamine transporter DAT, D2 receptor (DRD2), serotonin transporter (5HTT), and catechol-O-methyltransferase (COMT) in different subgroups of Lesch's typology. We have found no evidence for the role of these gene polymorphisms in Lesch type 1 and 2 subjects [18].

In this study, we focussed on GABA-ergic neurotransmission and the impact of GABA receptors on different subgroups of Lesch's typology. We investigated the genetics of Lesch's typology and the GABRG1 and GABRA2 genes. From a neurobiological standpoint, genetic variations in the GABRG1 locus may modulate susceptibility to AD through mechanisms of reward and/or drug response. In the rat brain, the λ-1 subunit of the $GABA_A$ receptor, encoded by the GABRG1 gene, is expressed selectively in only a few regions such as the amygdale, striatum, and substantia nigra [19]. These regions in turn are often implicated in the addiction and reward mechanisms [1].

Interestingly, this study showed no significant differences in GABRG1 and GABRA2 haplotype distributions between the subgroups of alcoholics with various Lesch's phenotypes and the controls with no AD. Consequently, in view of this study's findings and our previous experiments [18], Lesch's typology may not be related to the genotype of AD subjects – at least in terms of genes with an already established role in the development of dependency. It should be remembered that alcoholism is a complex disease with strong environmental determinants. Since Lesch's typology is based mostly on environmental criteria, this may be the reason that no genetic background of this classification has been found thus far. Perhaps other typologies of AD, e.g. Cloninger, Babor, or Early/Late onset [20], would be associated with GABA-A polymorphisms. On the other hand, alcoholism is considered a multigenetic disease [21] and some genes other than GABA-A may still exist undiscovered and determine the phenotype of AD subjects.

In conclusion, larger – possibly multicentre – studies are needed to finally uncover the role of GABA-A genes in determining Lesch's phenotypes and other phenotypic traits in AD subjects. Moreover, other candidate genes with a possible impact on the phenotype of alcohol-dependent individuals should be sought.

Acknowledgments

This study was supported by a grant (No. NN 402 189335) from the Ministry of Science and Education.

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