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Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Brief report

Combination of polymorphic variants in serotonin transporter and monoamine oxidase-A genes may influence the risk for early-onset alcoholism

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ARTICLE INFO

Article history:

Received 28 November 2011

Received in revised form

7 March 2012

Accepted 23 April 2012

Keywords:

Serotonin transporter

Monoamine-oxidase

Alcoholism subtypes

ABSTRACT

The combinatory effect of polymorphisms in serotonin transporter and monoamine oxidase-A genes on the aetiopathogenesis of alcoholism was investigated in a sample of 714 individuals. Increased frequency of subjects having three 'suspected' genotypes (5-HTTLPR-LL, STin2-1010 and MAO-A 3-repeat allele) was found among type-2 alcoholic patients ($P=0.0189$). Results highlight serotonergic/genetic contribution to early-onset alcoholism.

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1. Introduction

Alcohol dependence is a chronic and recurrent psychiatric disorder, influenced by genetic and environmental factors. Treatment of alcoholism is mostly ineffective due to the great clinical heterogeneity of alcoholic patients, but their classification into more homogeneous groups offers a chance to deal with this problem (Johnson, 2008; Leggio et al., 2009). Several typological concepts of alcoholism have been suggested based on the possibility that dysfunction of different neurotransmitter systems underlies a particular alcoholic subtype (Cloninger et al., 1996; Gardini et al., 2009; Pombo and Lesch, 2009). One of the most applied classifications is Cloninger's typology which initially proposed two major alcoholic subtypes: type-1 alcoholism, characterised by a later age of onset and neurotic features, has only moderate genetic contribution and is more environmentally conditioned; type-2 alcoholism is a less common and more severe form of disorder, characterised by early-onset and high impulsive/antisocial behaviour, and it has been associated with deficient serotonin (5-hydroxytryptamine, 5HT) transmission

5HT transporter (5HTT) and 5HT-catabolising enzyme, monoamine oxidase-A (MAO-A), are synaptic proteins considered to be the major regulators of serotonergic neurotransmission and have been shown to mutually affect each other at the gene level

(Murphy et al., 2003). In 5HTT gene two common polymorphic regions have been identified (Murphy et al., 2004): promoter polymorphism (5-HTTLPR) consisting of long (L) and short (S) alleles which differ in 44 bp insertion/deletion sequence and intronic polymorphism (STin2) consisting of three allelic variants with 9, 10 or 12 copies of 17-bp repeating element. Both polymorphisms influence transcriptional efficiency of 5HTT gene, with L and 12-repeat allele having stronger enhancer-like properties. MAO-A gene contains functional polymorphism in the 5'-upstream region (uVNTR) differing in the copy number of the 30-bp repeat sequence (Sabol et al., 1998).

Polymorphisms in 5HTT and MAO genes were frequently examined in relation to alcoholism (McHugh et al., 2010; Sari et al., 2011), but without unequivocal findings. Due to the possibility that serotonergic deficit is more pronounced in early-onset alcoholism (Cloninger et al., 1996; Storkovik et al., 2008), differentiating patients according to their alcoholic phenotype may help to delineate mentioned associations. The aim of this study was to investigate 5HTT and MAO-A polymorphisms in different subtypes of alcoholism. Our second aim was to test their possible combinatory effect on the risk for alcohol dependence.

2. Subjects and methods

Male alcoholic inpatients ($N=356$) who met Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) criteria for substance dependence were diagnosed type-1

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($N=236$) or type-2 ($N=120$) alcoholism according to Cloninger's criteria (Cloninger et al., 1996). Controls (358 males) were recruited from blood donors whose healthy status, including alcohol drinking habits, was checked by a written questionnaire. All participants gave written informed consent. The study was approved by local Ethics Committee.

Polymorphisms of 5HTT and MAO-A genes were genotyped as described previously (Filic et al., 2005; Hranilovic et al., 2000; Mokrovic et al., 2008).

Additional data about subjects, genotyping and statistic analyses are given in Supplementary material 1.

3. Results

Distributions of genotype/allele frequencies of 5-HTTLPR polymorphism showed no significant differences between controls and alcohol-dependent subjects, although somewhat higher frequency of genotype LL in type-2 alcoholics was observed. Regarding STin2 polymorphism, no differences were observed between controls and type-1 patients, while type-2 alcoholics and controls significantly differed in the genotype distributions ($P=0.0326$), with genotype 1010 being more frequent in patients ($P=0.0134$; odds ratio (OR)=1.98; 95% confidence interval (CI) 1.1–3.34). Testing of linkage disequilibrium between 5-HTTLPR and STin2 polymorphic loci demonstrated highly significant association of allele L and 10 ($P < 0.001$, all groups). Overall haplotype distributions did not differ between groups, but estimated frequency of haplotype L10 was significantly increased in type-2 subjects, as compared to controls ($P=0.0201$; OR=1.46; 95%CI 1.06–2.01). Distributions of MAO-A uVNTR genotypes/alleles among the groups were not statistically different, but frequency of allele 3 tended to increase in type-2 alcoholics.

Based on the observations that type-2 alcoholic patients showed significantly higher, or tendency towards higher, frequency of 1010, LL and 3-repeat genotypes, we further compared control and patient groups for concurrent presence of 'suspected' genotype variants. Subjects categories were: absence of suspected genotypes (-/-/-), presence of one (+/-/-), two (+/+/-) or three suspected genotypes (+/+/+). A significant difference in distribution of these categories was found between type-2 alcoholics and controls ($\chi^2=9.958$, $P=0.0189$). Chi-square test for trend showed significant dose-effect of the suspected genotypes on the individual's susceptibility to type-2 alcoholism ($\chi^2=4.517$, $P=0.0336$), with the patient group containing 77% more individuals possessing two of the suspected genotypes and 130% more individuals possessing three of them, as compared to the control (Fig. 1). More data are given in Supplementary material 2.

4. Discussion

Functional polymorphisms in genes encoding serotonergic synaptic proteins are frequently studied in alcoholism, but without unequivocal results so far. Majority of these studies have not differentiated between alcoholic subtypes, although it has long been suggested that different subtypes of alcoholism may have distinct neurobiological basis (Cloninger et al., 1996; Gardini et al., 2009; Johnson, 2008; Leggio et al., 2009). In our study, encompassing patients subgrouped according to Cloninger's typology, 5-HTTLPR and MAO-A uVNTR polymorphisms did not display a great individual impact on alcoholism as a whole, but STin2 variant was significantly associated with type-2 alcoholism, which confirms our previous results (Mokrovic et al., 2008).

We and others have shown that 5-HTTLPR and STin2 variants of 5HTT gene act in concert to modulate 5HTT gene expression (Ali

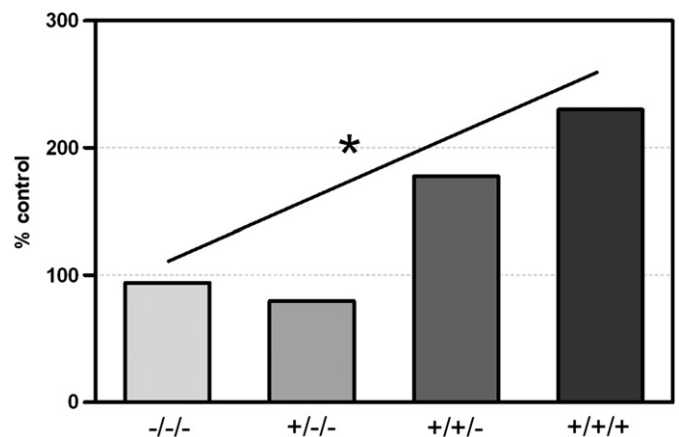


Fig. 1. Co-occurrence of "suspected" genotypes of 5-HTTLPR (LL), STin2 (1010) and MAO-A uVNTR (3 repeat) polymorphisms in patients with type-2 alcoholism expressed relatively to control subjects. -/-/-: absence of suspected genotypes; +/-/-: presence of one suspected genotype; +/+/-: presence of two suspected genotypes (1010 and 3 or LL and 1010 or LL and 3); +/+/+: presence of three suspected genotypes. * $p=0.0336$ (chi-square test for trend).

et al., 2010; Hranilovic et al., 2004); however, they have been mostly considered separately in the genetic studies of alcoholism. Here, we have found an increased estimated frequency of haplotype L10 in type-2 alcoholic patients, suggesting that this combination of 5HTTLPR and STin2 alleles may account for susceptibility to early-onset alcoholism. Although alleles L and 10 are generally considered to exert opposite individual effects on 5HTT gene expression, it was recently demonstrated that their combination is associated with the lower transcriptional activity as compared to the combination of L and 12 alleles (Ali et al., 2010). It should be also noted that although allele S is typically considered the risk allele for various psychological disorders, including alcoholism, recent comprehensive review by Glenn (2011) suggested a role of the L-allele in psychological conditions such as aggression and risk-taking behaviour, which both represent important traits in type-2 alcoholism; hence, our finding of somewhat higher frequency of LL genotype in type-2 alcoholics is not very unexpected.

As the second aim of this study, we tested the combined effect of 5HTT and MAO-A polymorphisms. Genotypes LL, 1010 and 3-repeat were considered as suspected, since they showed significantly (or tendency towards) increased frequencies in type-2 alcoholism, as compared to controls. Significant difference between controls and type-2 alcoholics in the distribution of the genotype categories (as defined in the Results section) as well as observed dose-effect of suspected genotypes (Fig. 1) suggest that 5-HTTLPR-LL, STin2-1010 and MAO-A 3-repeat genotypes may have additive effects on the genetic load for severe alcoholism, supporting the concept of stronger serotonergic contribution in Cloninger's type-2 alcoholism. In conclusion, our results confirmed association of 5HTT STin2 polymorphism with early-onset, type-2 alcoholism and suggest, for the first time, the combined effect of suspected genotypes of 5HTT and MAO-A genes on the individuals' predisposition to the severe alcoholic subtype. The main strength of our study is patients' classification, while the main limitations include possible population stratification and relatively modest statistical power. Therefore, replication studies are needed to verify these results.

Acknowledgements

The study was funded by Croatian Ministry of Science, Education and Sport, grants No.098-1081870-2395 (to JS) and 098-1081870-2397 (to LCS).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.psychres.2012.04.031>.

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