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Short communication

Family-based study of brain-derived neurotrophic factor (*BDNF*) gene polymorphism in alcohol dependence

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Abstract:
Brain-derived neurotrophic factor (BDNF) belongs to a family of proteins related to the nerve growth factor family, which are responsible for the proliferation, survival and differentiation of neurons. BDNF is thought to be involved in the pathogenesis of bipolar disorder, schizophrenia, eating disorders and addiction. We hypothesize that a functionally relevant polymorphism of the *BDNF* gene promoter may be associated with the pathogenesis of alcohol dependence.
We performed an association study of 141 families with alcohol dependence. One hundred and thirty-eight healthy control subjects were matched based on ethnicity and gender. An association between the *BDNF* Val66Met gene polymorphism and alcoholism was not found.

Key words:
BDNF, gene, alcohol dependence, TDT (transmission disequilibrium test), trios, family

Abbreviations: BDNF – brain-derived neurotrophic factor, ICD – International Classification of Diseases, SSAGA – semi-structured assessment on genetics in alcoholism, TDT – transmission disequilibrium test

Introduction

Neurotrophins are critical to the development and maintenance of the mammalian central nervous system. Among them is brain-cderived neurotrophic factor (BDNF), whose synthesis and release is regulated by glutamate receptor activation; dysregulation of this process may underlie neurodegenerative and psychiatric disorders [4].

Therefore, the *BDNF* gene is a candidate for molecular genetics studies of mood disorders, schizophrenia, eating disorders and addiction [9, 11].

At the molecular and neurobiochemical level, the neurotrophic factors BDNF and neurotrophin 3 are involved in developmental alternations. These factors

are responsible for prenatal neuronal differentiation and development as well as postnatal plasticity. Such knowledge has led to the neurothrophin hypothesis of schizophrenic psychoses [10]; decreased BDNF concentration has been found in both cortical areas and hippocampus of schizophrenic patients [1].

Alcoholism is a heterogeneous disorder with an estimated heritability of 40–60% [6]. Recent studies have indicated that BDNF may also underlie alcohol dependence, especially in ethanol-induced neurodegeneration; the BDNF promoter is differentially regulated during various states of intoxication and stress/anxiety. Numerous publications suggest that BDNF can modulate and potentiate reward pathways [2, 7, 8].

From studies of knockout animals, BDNF has been implicated in both alcohol preference and aggressive behavior. Disturbances in this system cause impulsiveness, and elevated levels of impulsivity is thought to be a fundamental feature of alcoholism [3].

In this study, we tested whether there was an association between the G196A (Val66Met: rs 6265) polymorphism and alcoholism in a family-based model.

Materials and Methods

A group of 141 Caucasian families with no history of ICD-10 psychiatric disorders other than alcohol or nicotine dependence (125 males aged 34 ± 9 and 16, females aged 40 ± 8) were chosen for this study. The mean age of the fathers was 62 ± 11 years old and that of the mothers was 59 ± 10 years old. Twenty-seven percent of the alcohol-dependent subjects had at least one alcoholic parent.

The control subjects consisted of 138 unrelated healthy individuals that were matched based on ethnicity and gender (119 males, 19 females; mean age 39 ± 16 years old). Presence of mental disorders was determined using the Prime MD questionnaire (Primary Care Evaluation of Mental Disorders). All subjects were recruited from the northwest region of Poland. A family history of alcoholism was assessed during a structured interview based on the Polish version of the SSAGA (Semi-Structured Assessment on Genetics in Alcoholism). The protocols were approved by the local Ethics Committee of Pomeranian Medical University, and each participant provided a written informed consent.

Genotyping: DNA was extracted from the venous blood using a salting method. The G196A (Val66Met) *BDNF* gene polymorphism was examined using the PCR-RFLP method. The following primers were used: F: 5' GAG GCTTGACATCATTGGCT 3'; R: 5' CGTGTACAAGTC TGC GTC CT 3'. DNA fragments were amplified in an Eppendorf thermal cycler and then digested overnight with the Eco72I restriction endonuclease (MBI Fermentas). The digestion products were separated on a 2% agarose gel and visualized by ethidium bromide staining. Band size was determined using the O'Range Ruler 50 bp DNA Ladder (MBI Fermentas). The undigested PCR product was 113 bp (allele A). Allele G was comprised of digested bands of 78 and 35 bp.

Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS), version 9. Differences between sample groups were analyzed using a χ^2 test and considered significant if the type 1 error was less than 5%. The Transmission Disequilibrium Test (TDT) and Hardy-Weinberg equilibrium were calculated using the SAS software. The odds ratios and 95% confidence intervals were also calculated.

Results

In the present study, an association between BDNF genetic variants and alcohol dependence was tested. An association between alcohol dependence and the occurrence of the *BDNF* genetic polymorphism was not found (Tab. 1). Furthermore, TDT analysis did not show association of the *BDNF* gene with the development of alcoholism (Tab. 2).

Discussion and Conclusion

An association between the *BDNF* G196A gene polymorphism and alcoholism was not found.

Our data are in agreement with previous association studies [5, 11], in which an association between this polymorphism and alcoholism was not found in

Tab. 1. Comparison of genotypes and the occurence of the Val66Met (G/A) *BDNF* polymorphism in patients with alcohol dependence with healthy control subjects (CON)

Group	n	Genotypes						P *vs.* CON	Alleles				P *vs.* CON
		G/G		G/A		A/A			G		A		
		N	%	n	%	n	%		n	%	n	%	
Alcoholics total	138	91	66	46	33	1	1	0.285	228	83	48	17	**0.74** HWE 0.06
CON	153	107	70	42	27	4	3		256	84	50	16	HWE 0.95 OR 1.078

Tab. 2. Data describing the number of informative families, fathers and mothers, and the transmission of alleles to the affected offspring

Genes polymorphisms	BDNF
Number of informative families	61
Number of informative both parents	16
Number of informative fathers	40
Number of informative mothers	37
Transmission of alleles	44 G (57%) 33 A (43%)
χ^2	1.571
p value	0.2101

Taiwanese and Japanese patients. The Val66Met *BDNF* gene polymorphism is associated with disease severity (predisposition to violence and/or withdrawal complications, including delirium) and the efficacy of therapy, rather than with the disease *per se* [5, 12]. Thus, the *BDNF* gene polymorphism may modify the alcoholism phenotype.

The present study is limited by a relatively small sample size; however, the sample size and the power estimation were sufficient to exclude false negative results. Although a single *BDNF* polymorphism was analyzed, this study was conducted using an ethnically homogenous population.

In conclusion, our data suggest that the *BDNF* Val66Met polymorphism does not significantly affect alcohol dependence.

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