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ALLELIC DISTRIBUTION OF BDNF VAL66MET POLYMORPHISM IN HEALTHY ROMANIAN VOLUNTEERS

Romana Vulturar[1,2#], Adina Chiș[1,2#], Melinda Hambrich[3], Beatrice Kelemen[4], Loredana Ungureanu[5], Andrei C. Miu[2*]

[1]*Discipline of Cell and Molecular Biology, Department of Molecular Sciences, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[2]*Cognitive Neuroscience Laboratory, Department of Psychology, Babeș-Bolyai University, Cluj-Napoca, Romania*

[3]*Discipline of Medical Psychology, Department of Neurosciences, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[4]*Molecular Biology Center, Interdisciplinary Research Institute on Bio-Nano-Sciences, Babeș-Bolyai University, Cluj-Napoca, Romania*

[5]*Department of Dermatology, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[#]*Equally contributed authors*

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Abstract

Population stratification of functional gene polymorphisms is a potential confounding factor in genetic association studies. The Val66Met (rs6265) single-nucleotide polymorphism in the brain-derived neurotrophic factor gene (*BDNF*) exhibits one of the highest variabilities in terms of allelic distribution between populations. The present study reports the distribution of BDNF Val66Met alleles in a sample of healthy volunteers (N = 1124) selected from the Romanian population. Frequencies were 80.74% for the Val allele and 19.26% for the Met allele. The data from this study extends efforts to map the allelic distribution of BDNF Val66Met in populations around the world and emphasizes that population stratification should be controlled for in future studies that report phenotypic associations in samples from different populations.

Keywords

• Gene candidate • Gene polymorphism • Population stratification

Introduction

Numerous genetic association studies have investigated relations amongst various functional polymorphisms found in candidate genes involved in the development and functioning of the nervous system [for review, see 1, 2]. Population stratification (a difference in the allelic distribution of certain gene polymorphisms between populations) accounts for one source of variation in the results of these studies [3]. Large-scale efforts to map gene polymorphisms in multiple human populations (such as the 1000 Genomes Project [4] and the International HapMap Project [5]) are currently underway. While they have described genetic heterogeneity between samples with different ancestry, they have not provided detailed information on differences in the distribution of gene alleles between people of the same ancestry (e.g., European), but from different populations.

A single-nucleotide polymorphism (SNP) (rs6265 in the Database of Single Nucleotide Polymorphisms) [6], which involves a guanine to adenine (G>A) substitution at nucleotide 196 in the brain-derived neurotrophic factor (*BDNF*) gene (11p13), has been extensively investigated in relation to behavior [e.g., 7]. This SNP results in a valine (Val) to methionine (Met) substitution at codon 66 in the pro-domain of the BDNF precursor protein (proBDNF), hence its common name, BDNF Val66Met. The Met66 variant of proBDNF interferes with the regulated or activity-dependent secretion of mature BDNF in neurons [8, 9], and has been associated with differences in hippocampal activity and memory performance [9], as well as psychiatric conditions such as bipolar disorder [10] and schizophrenia [11]. However, replication studies and meta-analyses have reported mixed results and it was suggested that some of the inconsistencies may be related to genetic differences between populations at the *BDNF* Val66Met locus [12-15].

The frequency of the Met allele has a wide range of values: from 0.55% in Sub-Saharan Africans to 19.9% in Europeans and 43.6% in Asian groups [13]. Moreover, it differs between European populations, varying from 6.7% in groups from the Russian Caucasus to 34.6% in groups from the Orkney Islands, UK [13]. These and similar results from the 1000 Genomes Project [16] and other large studies [17, 18] indicate that population differentiation at the BDNF Val66Met locus is in the top 1% genome-wide and emphasize that these differences should be taken into account when studying phenotypic associations in different populations. This is especially applicable in

* *E-mail: andreimiu@psychology.ro*

meta-analyses, which aim to combine data from multiple studies [15]. In this study, we describe the previously uninvestigated distribution of *BDNF* Val66Met in a Romanian sample of healthy volunteers.

Methods

Participants

The present study is based on a sample of N = 1124 healthy volunteers (848 women, 276 men) who were high school or college students (age: M = 20.14; SD = 1.12 years). They were all Caucasians living in Romania. None of the participants reported neuropsychiatric, endocrine or other chronic conditions. Prior to study participation, written informed consent was obtained from each volunteer.

Genotyping

DNA was extracted from buccal epithelial cells using the MasterPure Complete DNA & RNA Purification Kit (Epicentre, Madison, USA) and kept at -20°C.

BDNF Val66Met genotyping was performed using the tetra primer amplified refractory mutation system (ARMS)-PCR method [19]. This is a high throughput method, which provides rapid and sensitive *BDNF* Val66Met genotyping [19]. PCR amplifications were carried out in a 25 µl reaction volume containing 50 ng of genomic DNA template, 12.5 µl PCR mastermix (2x) and all the four primers [P1 (forward): 5′-CCT ACA GTT CCA CCA GGT GAG AAG AGT G-3′; P2 (reverse): 5′- TCA TGG ACA TGT TTG CAG CAT CTA GGT A-3′; P3 (G allele specific): 5′-CTG GTC CTC ATC CAA CAG CTC TTC TAT AAC-3′and P4 (A allele specific): 5′-ATC ATT GGC TGA CAC TTT CGA ACC CA-3′]. The first set of primers (P1 and P2) amplify the 401 bp region containing the SNP of interest, whereas the second set (P3 and P4) of primers are allele specific and account for the G>A substitution. The PCR amplification was carried out in a T100 Thermal Cycler (BioRad Laboratories, Mississauga, Canada) with an initial denaturation temperature of 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, 62.5°C for 60 s and 72°C for 60 s and a final extension step of 5 min at 72°C. Stained with Midori Green Advanced (NIPPON Genetics EUROPE GmbH, Duren, Germany) and visualized in 3% agarose gel, the PCR products indicated successful amplification of all three genotypes (i.e., Val/Val, Val/Met, Met/Met), with Val and Met allele-specific bands at 253 and 201 bp, respectively (Fig. 1).

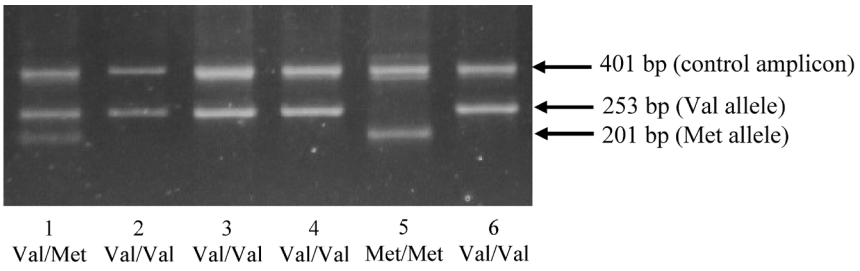

Figure 1. Illustration of BDNF Val66Met genotyping using tetra primer amplified refractory mutation system (ARMS)-PCR visualized on a 3% polyacrylamide gel.

Statistical analysis

Genotype and allele frequencies were determined. In line with previous studies [e.g., 17], allele frequencies were inspected for potential sex-related differences. Departures from Hardy-Weinberg equilibrium were also examined [20]. All statistical analyses involved χ^2 tests run in SPSS.

Results

The distribution of BDNF Val66Met genotypes was as follows: 736 Val/Val; 343 Val/Met; and 45 Met/Met. Allele frequencies were 80.74% for Val and 19.26% for Met. Genotypes were in Hardy-Weinberg equilibrium: $\chi^2[1] = 0.4$, $p > 0.05$. Genotype distributions were similar in men and women: $\chi^2[2] = 1.27$, $p > 0.05$ (Table 1).

Discussion

In light of a high level of population stratification (including heterogeneity amongst European populations [13]) it is important to control for this potential confound in genetic association studies. Taking this into consideration, the present study described for the first time, the distribution of BDNF Val66Met in a sample of healthy Romanian volunteers.

As expected, the distribution of BDNF Val66Met alleles in this Romanian sample was similar to those reported in most other European populations. The frequency of the Met allele was 19.26% in the present sample, similar to previous estimates in healthy volunteers: 19.9% across small samples from several European populations [13]; 19.5% in a large Croatian sample [17]; 19% and 21% in two German samples [18]; and 18.8% in a sample of European Americans [21]. The present results extend the description of *BDNF* Val66Met distribution in European populations and support the perspective that European ancestry is associated with Met allele frequencies that are higher than in African populations and lower than in Asian populations. The frequency of the Met allele is, to a certain extent, also variable between European populations. The Orcadian population in the Orkney Islands is at the high end and the Adygei population in the Russian Caucasus at the low end of the distribution [13] while Romanian people and most Western (i.e., Germany; France; Italy) [13, 18] and Central European populations (i.e., Croatians) [17] show similar frequencies of the Met allele, ranging between 19-21%.

The differences in the distribution of BDNF Val66Met alleles between populations may be attributed to the evolutionary selection of an allele associated with increased survival fitness and to founder effects (i.e., limited genetic variation due to a small number of population founders) [22]. While it is beyond the aim of this study to explore these mechanisms, several relevant observations from the literature are worth mentioning. First, the differences across African, European and Asian populations seem to be driven by the Met allele, with a higher frequency occurring outside of Africa [13]. The Met allele is present though rare in people of native African ancestry (e.g., Yoruba from Ibadan, Nigeria; Luhya in Webuye, Kenya),

Table 1. Frequency of BDNF Val66Met genotypes and alleles according to sex.

	Absolute frequencies, N (and relative frequencies, %) of genotypes			Absolute frequencies, N (and relative frequencies, %) of alleles	
	Val/Val	Val/Met	Met/Met	Val	Met
Women (N = 848)	551 (64.98)	260 (30.66)	37 (4.36)	1362 (80.3)	334 (19.7)
Men (N = 276)	185 (67.03)	83 (30.07)	8 (2.9)	453 (82.07)	99 (17.93)

which suggests that it arose in evolution before early humans migrated out of Africa [16]. The fact that the Met allele was not selected out in evolution and its frequency is actually higher in populations outside Africa suggests that the Met allele may have pleiotropic functions, some detrimental and some beneficial [13]. Second, a haplotype carrying the Val allele shows more extensive linkage disequilibrium compared to other chromosome 11 haplotypes from the HapMap European data, suggesting that this haplotype and the Val allele were positively selected [13]. Therefore, selection may have been operating on both *BDNF* Val66Met alleles in European populations, at different times in evolution.

This study has at least three limitations. First, it is based on a convenience sample that was neither randomized, nor large enough to be representative of the Romanian population. Second, the presence of mental disorders, endocrine and other chronic conditions that may be associated with *BDNF* Val66Met genotype was ascertained by self-report rather than objective clinical evaluation or medical history. However, all participants were high school or college students and while this status does not exclude the possibility of chronic conditions, it suggests that the level of functioning of these participants was high. Third, the majority of participants were women and this may have biased the analyses on sex differences. Due to these limitations, the present results should be generalized with caution.

In conclusion, the high population stratification of *BDNF* Val66Met may explain some of the inconsistent phenotypic associations reported in various populations around the world. The present results extend efforts to map the allelic distribution of *BDNF* Val66Met in populations around the world. In line with previous research, results of this study emphasize that future studies that report data from different populations (e.g., meta-analyses) should investigate whether population stratification moderates the relation between *BDNF* Val66Met and behavior.

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