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The 196G/A (val66met) polymorphism of the BDNF gene is significantly associated with binge eating behavior in women with bulimia nervosa or binge eating disorder

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Abstract

The brain-derived neurotrophic factor (BDNF) is involved not only in promoting neuronal outgrowth and differentiation, synaptic connectivity and neuronal repair, but also in modulating eating behavior. Since genetic factors likely contribute to the biological vulnerability to bulimia nervosa (BN) and binge eating disorder (BED), we investigated whether the functional 196G/A single nucleotide polymorphism (SNP) of the BDNF gene was associated to BN and/or BED or to some phenotypic aspects of the disordered eating. Two hundred and ten Caucasian women (126 with BN, 84 with BED and 121 healthy controls) participated into the study. No significant differences were found in the frequencies of the 196G/A variants of the BDNF gene among patients with BN or BED and healthy controls. In both BN and BED groups, subjects carrying the 196A/A genotype exhibited a weekly frequency of bingeing and a severity of binge eating (as assessed by the Bulimia Investigation Test Edinburgh) significantly higher than those with the 196A/G and 196G/G genotypes. These results suggest that the 196G/A SNP of the human BDNF gene does not contribute to the genetic susceptibility to BN and BED, but may predispose those patients to a more severe binge eating behavior.

Keywords: Bulimia nervosa; Binge eating disorder; BDNF; Gene polymorphisms

The brain-derived neurotrophic factor (BDNF) is known not only to promote neuronal outgrowth and differentiation, synaptic connectivity and neuronal repair [10,11], but also to modulate eating behavior. Indeed, BDNF and its tyrosine kinase receptor are expressed in various hypothalamic nuclei implicated in the regulation of food intake [8], and both central and peripheral administration of BDNF decrease food ingestion, increase energy expenditure and ameliorate hyperinsulinaemia and hyperglycemia in diabetic *db*/*db* mice [13,15,20]. Moreover, heterozygous mice with one functional BDNF allele and mice in which the BDNF gene has been deleted in excitatory brain neurons display a massive ingestion of food, which mimics the human bingeing behavior, gaining body weight (BW) up to becoming obese [8,12,17]. Collectively, these findings suggest that BDNF signaling in the brain is likely involved in regulating energy homeostasis, and suggest that alterations in its function or expression pattern could represent susceptibility factors to disordered eating, especially binge eating.

There are two eating disorders characterized by severe binge eating. The first is bulimia nervosa (BN) in which recurrent episodes of binge eating are punctuated by compensatory behaviors aiming to prevent BW gain. The second condition, included in appendix B of DSM-IV, is binge eating disorder (BED), which is characterized by uncontrolled overeating, as in BN, without compensatory behaviors; as consequence of the massive ingestion of calories during bingeing, people with BED generally incur a frank obesity. There is substantial evidence that genetic factors contribute to the biological vulnerability to eating disorders [6]. Therefore, functional variants of the BDNF gene could play a role in the genetic susceptibility to BN and/or BED or

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may predispose affected subjects to some phenotypic aspects of the disordered eating.

A functional single nucleotide polymorphism (SNP) of the BDNF gene was first described by Egan et al. [3], who demonstrated that a valine (G196) to methionine (A196) substitution in the 5′ pro-region of the human BDNF protein affects the intracellular trafficking and activity-dependent secretion of BDNF. A significant association between the 196G/A BDNF polymorphism and BN has been claimed by some authors, but not confirmed by others [5,9,16] while, to the best of our knowledge, the association between the 196G/A SNP of the BDNF gene and BED has never been assessed. Therefore, we designed a case-control study aiming to explore the frequencies of the 196G/A SNP of the BDNF gene in women with BN or BED and healthy controls. Moreover, we investigated whether the 196G/A SNP of BDNF gene was associated with phenotypic characteristics of BN and BED, especially with binge eating.

Two hundred ten female Caucasian patients consecutively attending the outpatient unit of the Eating Disorder Center of the Department of Psychiatry of the University of Naples were enrolled into the study. One hundred and twenty-six of them met the DSM-IV diagnosis of BN purging subtype while 84 met the DSM-IV diagnosis of BED, as confirmed by the Structured Clinical Interview for DSM IV-Patient Edition (SCID-IP) [4]. They underwent the following clinical assessments: (1) Axis I comorbid psychiatric disorders were investigated using the SCID-I-P; (2) eating-related psychopathology was assessed by means of the Bulimia Investigation Test Edinburgh (BITE) [7]; (3) historical and clinical data concerning eating-related symptomatology were collected by an *ad hoc* non-diagnostic structured clinical interview, that habitually we use in our clinical practice to get as much information possible on patients' eating attitudes. The weekly frequency of binge eating was derived by the specific item of the BITE, which is a self-reported assessment scale evaluating both the presence of bulimic symptoms and their severity over the 4 weeks before the clinical assessment.

A group of 121 normal weight Caucasian healthy women was also recruited. They were mentally healthy as assessed by the SCID-I non-patient edition [4]. Information on their eating habits was collected by a clinical interview.

In each subject, the body mass index (BMI) was calculated as the ratio between BW (kg) and height (m^2). All of the subjects provided written informed consent to participate into the study after a complete description of the study procedure. The study was approved by the local ethics committee. Experiments were conducted in accordance with the Declaration of Helsinki.

From all subjects, 20 ml of venous blood was drawn into EDTA vacuum tubes and immediately frozen at −20 °C till the extraction of genomic DNA from nucleated white blood cells.

The A196G polymorphism was analyzed by SNaPshot mini-sequencing method as recommended by the manufacturer (Applied Biosystem, Foster City, CA, USA). In particular, the polymorphic region of BDNF gene was amplified by polymerase chain reaction (PCR) using primers and conditions as described by Ribasés et al. [16]. Successively, the amplification product was purified using a Montage PCR plate (Millipore, Bedford, USA). For the SNaPshot extension reaction, the specific primer (5′-TCATTGGCTGACACTTTCGAACAC-3′) was added to 3 μl purified PCR product and mixed with 2.5 μl of ready reaction premix and 3 μl of water. This mixture was placed in the thermal cycler and underwent 25 cycles of 96 °C/10 s, 50 °C/5 s and 60 °C/30 s. When completed, 1 μl of Shrimp Alkaline Phosphatase (SAP; USB, Ohio, USA) was added and incubated for 60 min. Prior to loading onto the Automated Sequencer ABI PRISM 3100 Applied Biosystem, 10 μl formamide was added to 1 μl of reaction mixture and samples were heated to 95 °C for 5 min.

Statistical analyses were performed using the BMDP statistic software package [1]. The Pearson χ^2 test was performed to compare genotype and allele frequencies between patients and healthy controls and between patients grouped according to different phenotypic criteria. Moreover, in order to test whether differences in phenotypic variables among the groups were influenced by 196G/A BDNF polymorphism, two-way analyses of variance (ANOVA) were performed with diagnosis and 196G/A BDNF genotype variants as independent variables. Post-hoc one-way ANOVAs and Tukey's tests were performed to assess statistical differences among the various genotypes in each group. Since analyzed variables were not available for some of our subjects, different degrees of freedom occurred in the various analyses.

The distribution of the 196G/A genotypes did not differ significantly from that expected according to the Hardy–Weinberg equilibrium in both BED patients ($\chi^2 = 0.45$; d.f. = 1, P = NS) and healthy controls ($\chi^2 = 0.49$; d.f. = 1, P = NS), whereas it deviated significantly from the Hardy–Weinberg equilibrium in BN subjects ($\chi^2 = 8.273$; d.f. = 1, $P < 0.01$). Differences in genotype and allele distributions between each patient group or all the patients as a whole and normal controls were not statistically significant (Table 1).

Clinical and demographic characteristics of the study groups according to 196G/A genotype are shown in Table 2. Sta-

Table 1
Genotypes and allele frequencies of BDNF 196G/A polymorphism in patients with bulimia nervosa (BN) or binge eating disorder (BED) and control subjects

	Genotypes			P^*	Alleles		P^*
	GG	GA	AA		G	A	
Control women (N = 121)	75 (61.9%)	40 (33.0%)	6 (4.9%)		190 (78.5%)	52 (21.4%)	
Women with BN (N = 126)	79 (62.7%)	34 (26.9%)	13 (10.3%)	0.7	192 (76.2%)	60 (23.8%)	0.5
Women with BED (N = 84)	56 (66.6 %)	24 (28.5%)	4 (4.9%)	0.2	136 (80.91%)	32 (19.0%)	0.5
Total women with eating disorders (N = 210)	135 (64.2%)	58 (27.6%)	17 (8.0%)	0.3	328 (78.0%)	92 (21.9%)	0.9

* Pearson χ^2 test vs. control women.