



# Brain-Derived Neurotrophic Factor Val66Met Polymorphism in Context of Executive Functions and Working Memory in Obese Patients

Maciej Bieliński<sup>1,†</sup>, Natalia Lesiewska<sup>1</sup>, Marcin Jaracz<sup>1</sup>, Marta Tomaszewska<sup>1</sup>, Marcin Sikora<sup>2</sup>, Artur Mieczkowski<sup>1</sup>, Roman Junik<sup>3</sup>, Anna Kamińska<sup>3</sup>, Andrzej Tretyn<sup>4</sup>, Alina Borkowska<sup>1</sup>

### ABSTRACT

Neurotrophins are crucial in the regulation of many processes, such as nerve cells development and neuroplasticity and brain-derived neurotrophic factor (BDNF) is the major one. Researchers still scrutinize the role of BDNF Val66Met polymorphism in eating disorders and cognition, however, literature present inconsistent findings regarding this topic. Most of all, the exact mechanism of how exactly BDNF influences cognitive functioning in obesity is unknown. In our study, we try to elucidate the association of BDNF Val66Met polymorphism with executive functions and working memory on large obese population.

375 obese patients underwent biometric analyses and neuropsychological assessment. The Wisconsin Card Sorting Test (WCST) was used to assess cognitive functions. Genetic polymorphism in BDNF was evaluated in peripheral blood samples.

Results indicated that both BDNF Val66Met polymorphism and gender showed significant correlations with BMI, WCST\_P and WCST\_CLR. Females showed higher BMI values than men,, however males scored higher in WCST\_P and WCST\_CLR. Val/Val carriers presented worse results of WCST and BMI. Kendall's partial rank correlation tests determined that both genotype and BMI showed significant correlations with WCST\_CLR, WCST\_CC and WCST\_1st, in which BMI correlation was stronger. Analysis of mild and morbid obesity showed, that severe obese younger subjects presented worse WCST\_P, WCST\_NP, and WCST\_CLR. Significant interaction effects for WCST parameters were found for gender, age, BMI and BDNF Val66Met. Importantly, in this study none Met/Met carriers were observed.

The Val66Met BDNF polymorphism indirectly influenced the poorer cognitive functioning observed in the obese subpopulation with Val/Val genotype, rather than Val/Met genotype. A higher BMI was associated with the Val allele, suggesting that obesity may have deteriorated the activity of the prefrontal cortex. According to results, BDNF Val66Met polymorphism seems to contribute to greater obesity in our study. It is greater BMI, rather than genotype, which caused worse executive functioning and working memory.

### Keywords

Val66Met BDNF, Neurotrophins, Obesity

<sup>1</sup>Chair and Department of Clinical Neuropsychology, Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Poland

<sup>2</sup>Chair of Plant Physiology and Biotechnology, Nicolaus Copernicus University in Toruń, Poland

<sup>3</sup>Department of Endocrinology and Diabetology, Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Poland

<sup>4</sup>Department of Biotechnology, Nicolaus Copernicus University in Toruń, Poland

<sup>†</sup>Author for correspondence: Maciej Bieliński, Chair and Department of Clinical Neuropsychology, Nicolaus Copernicus University in Toruń, Collegium Medicum in Bydgoszcz, Poland, Tel/Fax:+48 52 585 37 03; email: bielinskim@gmail.com

---

**Introduction**

Neurotrophins play a major role in the physiology of central nervous system (CNS). Among them, brain-derived neurotrophic factor (BDNF) and its TrkB receptor are the most widespread in the maturing and adult brains of all mammals [1,2]. BDNF is distributed throughout the whole CNS, and it is abundant in such regions as hippocampus, hypothalamus, cerebral cortex and neuronal pathways e.g. frontal-striatal circuit [3-5]. BDNF takes part in many processes which involve proper brain physiology and functioning; it participates in neuronal survival (proliferation, differentiation, maturation and apoptosis) and initiates synaptic transmission [6,7].

The human BDNF gene is located on chromosome 11p14.1 [8]. The most recent investigations examining BDNF gene polymorphisms frequently feature a single nucleotide polymorphism (SNP) Val66Met. This polymorphism is located on 66 codon of proBDNF and involves the conversion of valine to methionine in the pro-region of the 5' end of the gene. This impairs intracellular trafficking and packaging of pro-BDNF and is associated with defective BDNF depolarization-induced secretion and thus lower BDNF serum concentration [9-11].

Building on BDNF concentration in CNS, Val66Met polymorphism may contribute to the development of eating disorders and cognition impairment. In context of obesity, many studies show the influence of BDNF on food intake, feeding behavior, body mass index (BMI) and the pathogenesis of eating disorders [12-18]. However, the exact mechanism by which BDNF affects appetite, and hence contribute to development of obesity is still unknown [19]. Moreover, it is questionable which BDNF allele (Val or Met) is responsible for greater weight gain.

Literature also presents mixed results regarding the link between BDNF gene polymorphism and executive functions and working memory. In some studies Met allele is considered to deteriorate cognitive functioning, while other studies do not present any significant associations between BDNF Val66Met and cognition [9,20-22].

To elucidate abovementioned uncertainties, the aim of our research was: to assess executive function performance in obese population, determine what factor is mainly responsible for

the worse scores in WCST, and how BDNF Val66Met influences the severity of obesity.

---

**Materials and Methods****■ Participants**

A total of 375 adult Caucasian patients of Polish nationality were enrolled in this study, including 233 women and 142 men, after excluding secondary reasons of obesity. The mean age of all participants was 54 (range, 20–76) years old; the mean age for women was 52 (20–75) years old, and for men 56 (42–67) years old. The exclusion criteria were individuals with a severe somatic or psychiatric disorder included in the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, any neurological abnormality, or any addiction to drugs or alcohol. All participants were informed about the aims of this study and gave their written informed consent. Permission for the study was obtained from the Bioethical Commission of the NCU, Collegium Medium in Bydgoszcz (No. 533/2008) and the study conforms to recognized standards of Declaration of Helsinki.

**■ Clinical assessments and measures**

All participants underwent clinical evaluation, which comprised a physical examination, medical history, metric measurements of body weight and height, and a neuropsychological assessment. For biometric analyses, adiposity was evaluated using BMI, which was calculated as a ratio between weight (kg) and height squared (m<sup>2</sup>). Primary obesity was defined as a BMI > 30. Secondary causes of obesity were excluded based on medical history, physical examination, and biochemical results, including cortisol, prolactin, and thyroid-stimulating hormone levels.

The computerized version of the WCST with instructions in Polish was used to evaluate the function of the frontal lobe. The following psychometric parameters were measured: (1) percentage of perseverative errors (WCST\_P); (2) percentage of non-perseverative errors (WCST\_NP); (3) number of correctly completed categories (WCST\_CC); (4) number of trials required to finish the first category (WCST\_1st); (5) percentage of correct responses occurring in runs of three or more (WCST\_CLR).

**■ Genotyping**

Genomic DNA was extracted from 7-10 mL of peripheral blood using the method of Lahiri and Schnabel (1993) [23]. Blood

was collected and mixed with 0.5 mL of 0.5 ethylene diamine tetraacetic acid, frozen in liquid nitrogen, and stored at  $-80^{\circ}\text{C}$  prior to extraction. *BDNF* genotypes were determined by polymerase chain reaction (PCR). The following primers were used: *BDNF* forward, 5'-TTCTCCCTACAGTTCCACCAG-3'; *BDNF* reverse, 5'-GTTTCCCTTCTGGTCATGGA-3'. The expanded fragment was 963 bp long and covered sector 27619182-27620144, where polymorphism Val/Met was in position 27619916. PCR products were subjected to restriction digestion (2 h/37°C) using the enzyme Eco72I (Fermentas). Products with the CACGTG sequence corresponding to the 66/V allele were digested with 228 and 735 bp fragments. Products with the CACATG sequence corresponding to the 66 /Met allele retained their length and were not subject to cutting.

■ Statistical analysis

A Shapiro–Wilk test revealed that the distribution of variables was not normal; therefore, nonparametric tests were used in subsequent analyses. The statistical significance of the differences was calculated using Mann–Whitney *U* tests. The Spearman rank correlation test was used to determine correlations between variables. Kendall’s partial rank correlation coefficient method was used to determine significance in cases of complex variables. Analysis of covariance

(ANCOVA) was performed to assess interaction effects. Statistical 12.5 was used to conduct statistical analyses, and the software program Utility Programs for Analysis of Genetic Linkage was utilized to test the goodness of fit to the Hardy–Weinberg equilibrium. A  $\chi^2$  value of 35 (*p* value<0.0001) indicated that the study population was not in Hardy–Weinberg equilibrium.

Results

The study population consisted of 375 individuals with a diagnosis of simple obesity. The median age was 45.2 (SD, 9.5) years in the study group and 40.4 (SD, 12.9) years in the control group. There were 233 women and 142 men in the study group. The results of the WCST and basic demographic factors are presented in **Table 1**. Significant differences were found between women and men in BMI and two WCST parameters, namely, in the number of perseverative errors and the conceptual level responses. Men scored significantly higher on WCST\_P and WCST\_CLR. Women were characterized by having BMIs significantly higher than those of the men.

The distribution of the BDNF polymorphism is shown in **Table 2**. A notable finding was that the study population consisted of no Met/Met carriers.

The BMI results and WCST findings according to BDNF polymorphism are presented in **Table 2**.

**Table 1: Demographic factors and results on the Wisconsin Card Sorting Test in study participants.**

	Female n = 233	Male n = 142	p	Cohen’s d	Partial tau Kendall	
					Gender	Genotype
Age	52.0 (40.0–69.0)	56.0 (39.0–67.0)	0.81	0.02		
BMI	37.2 (32.4–43.9)	35.3 (30.8–44.0)	0.04	0.08	-0.42 p = 5.8E-35	0.067 p = 0.025
WCST_P	12.0 (7.0–19.0)	9.0 (6.0–19.0)	0.004	0.03	-0.152 p = 0.00001	-0.0116 p = 0.0007
WCST_NP	14.0 (9.0–23.0)	16.0 (9.0–19.0)	0.21	0.15		
WCST_CLR	64.0 (42.0–76.0)	73.0 (52.0–76.0)	0.02	0.23	0.1256 p = 0.0002	0.0895 p = 0.009
WCST_CC	6.0 (2.0–6.0)	6.0 (3.0–6.0)	0.76	0.09		
WCST_1 <sup>st</sup>	18.0 (11.0–35.0)	12.0 (11.0–31.0)	0.17	0.01		

Notes: Values are expressed as median (25%-75%) or as number of patients (n).Significance of differences between sexes was determined by the Mann–Whitney **U** test. Kendall’s partial rank correlation coefficient method indicates the significance of the individual factors. Size effect was measured by Cohen’s d method. Bold values indicate statistical significance.

**Abbreviations:** BMI, body mass index; WCST\_P, percentage of perseverative errors; WCST\_NP, percentage of nonperseverative errors; WCST\_CLR, percentage of conceptual responses; WCST\_CC, level of category completed; WCST\_1st, number of cards needed to complete first category.

**Table 2: Body mass index and results on the Wisconsin Card Sorting test in different BDNF Val66Met polymorphism group.**

	Val/Met n = 196	Val/Val n = 179	P	Cohen's d	Partial tau Kendall	
					BMI	Genotype
Gender	116♀/80♂	117♀/62♂	0.22	-		
BMI	32.4 (30.8–40.1)	42.7 (35.5–52.1)	9.69E-17	0.64		
WCST_P	10.0 (5.0–19.0)	12.0 (9.0–19.0)	0.0002	0.24	-0.14; p = 00006	0.02; p = 0.39
WCST_NP	16.0 (7.0–19.0)	14.0 (10.0–23.0)	0.08	0.12		
WCST_CLR	72.0 (52.0–85.0)	67.0 (42.0–76.0)	0.01	0.21	0.08; p = 0.02	-0.07; p = 0.048
WCST_CC	6.0 (4.0–6.0)	6.0 (2.0–6.0)	0.0007	0.47	0.17; p = 3.7E-07	0.07; p = 0.03
WCST_1st	12.0 (11.0–27.0)	14.0 (11.0–69.0)	0.002	0.55	-0.16; p = 0.00002	-0.1; p = 0.002

Notes: Values are expressed as median (25%-75%) or as number of patients (n).Significance of differences between genotypes was determined by the Mann–Whitney U test. Kendall's partial rank correlation coefficient method indicates the significance of the individual factors. Size effect was measured by Cohen's d method. Bold values indicate statistical significance.

**Abbreviations:** BDNF, Brain Derived Neurotrophic Factor; BMI, body mass index; WCST\_P, percentage of perseverative errors; WCST\_NP, percentage of nonperseverative errors; WCST\_CLR, percentage of conceptual responses; WCST\_CC, level of category completed; WCST\_1st, number of cards needed to complete first category.

The Val/Val carriers had significantly higher BMIs and worse results for most of the WCST parameters examined.

Using Kendall's partial rank correlation coefficient technique, we found that both genotype and gender showed significant correlations with BMI, WCST\_P, and WCST\_NP (Table 1).

Kendall's partial rank correlation tests also determined that both genotype and BMI, after their mutual correlation had been partialled out, showed significant correlations with WCST\_CLR, WCST\_CC, and WCST\_1st (Table 2). The correlations involving BMI were statistically stronger than genotype for the results of the cognitive task.

An analysis was also performed when the group was divided into those with mild (n=227) and morbid (n=148) obesity (Table 3). Patients with severe obesity were characterized by younger age and worse outcomes in terms of WCST parameters: number of perseverative and non-perseverative errors as well as conceptual level response.

The Spearman rank correlation test indicated that BMI was significantly correlated with worse WCST scores (Table 4) as follows: WCST\_P, WCST\_NP, WCST\_CC and WCST\_1st. whereas age was associated with worse

performance in the WCST\_P, WCST\_NP and WCST\_CLR.

Significant interaction effects for WCST parameters were found for gender, age, BMI and investigated BDNF polymorphism (Table 5).

### Discussion

Neurodevelopmental theories are currently popular among researchers exploring the etiopathogenesis of mental illnesses. This interesting research stream is ideally suited to issues related to BDNF, a leading neurotrophin responsible for growth, maturation, and differentiation of neurons. The role of BDNF in eating disorders appears to be complex. On the one hand, BDNF in the brain acts as a satiety factor<sup>19</sup>; on the other hand, BDNF gene expression is dependent on leptin and cholecystokinin, both of which act on satiety and hunger.

However, some publications question the association between BDNF and obesity [24,25]. The study of 339 adult healthy Caucasians did not present any co-relations between BDNF Val66Met and possible weight gain. Results demonstrated that smoking was a significant factor which affected BMI [24].

Other studies show significant correlations of the Val66Met BDNF polymorphism in participants

**Table 3: Demographic factors and Wisconsin Card Sorting Test results in mild and morbid obesity groups.**

	Mild obesity N=227	Morbid obesity N=148	P	Cohen's d
Gender	143(63%)/ 84(37%)	90(60,1%)/ 58(39,9%)	0,67	-
BMI	32,4 (30,8 – 35,5)	49,6 (43,4 – 56,5)	0,00E-01	8,6
Age	62,0 (52,0 – 70,0)	43,0 (38,5 – 68,0)	2,04,68E-21	0,23
BDNF (n,%) Val/Val//Val/Met	84(37%)/ 143(63%)	95(64,2%)/ 53(35,8%)	2,69E-07	-
WCST_P	10,0 (6,0 – 19,0)	12,0 (8,0-19,0)	0,025	0,18
WCST_NP	12,0 (7,0 – 19,0)	16,0 (10,0-24,0)	0,002	1,27
WCST_CLR	73,0 (52,0 – 81,0)	62,0 (42,0 – 75,0)	0,0001	2,8
WCST_CC	6,0 (2,0 – 6,0)	6,0 (3,0 – 6,0)	0,25	17,7
WCST_1st	21,0 (11,0 – 37,0)	12,0 (11,0 – 25,0)	0,06	0,39

Notes: Values are expressed as median (25%-75%) or as number of patients (n).Significance of differences between genotypes was determined by the Mann–Whitney **U** test. Size effect was measured by Cohen's d method. Bold values indicate statistical significance.

**Abbreviations:** BDNF, Brain Derived Neurotrophic Factor; BMI, body mass index; WCST\_P, percentage of perseverative errors; WCST\_NP, percentage of nonperseverative errors; WCST\_CLR, percentage of conceptual responses; WCST\_CC, level of category completed; WCST\_1st, number of cards needed to complete first category.

**Table 4: Age and BMI R-Spearman correlations with WCST parameters.**

	WCST_P	WCST_NP	WCST_CLR	WCST_CC	WCST_1st
Age	-0,116 (p=0,02)	0,148 (p=0,004)	-0,157 (p=0,002)	0,014 (p=0,78)	0,070 (p=0,17)
BMI	-0,126 (p=0,01)	0,128 (p=0,01)	-0,02 (p=0,70)	-0,179 (p=0,0004)	0,275 (p<0,0001)

**Abbreviations:** BMI, body mass index; WCST\_P, percentage of perseverative errors; WCST\_NP, percentage of nonperseverative errors; WCST\_CLR, percentage of conceptual responses; WCST\_CC, level of category completed; WCST\_1st, number of cards needed to complete first category.

diagnosed with simple obesity, but exact effects of BDNF concentration on appetite and weight currently remain uncertain.

According to Beckers et al., carriers of the methionine allele have higher BMI, as well as increased risk of developing obesity [17]. The methionine allele is also associated with a greater risk of developing eating disorders, such as bulimia nervosa and binge-eating [26]. Also decreased BDNF levels have been reported in women with anorexia nervosa [14]. Some studies prove the connection between BDNF depletion and greater BMI, by demonstrating lower food intake and greater energy expenditure after administrating exogenous BDNF into the brain [27-29]. Kerner et al. studied mice having one functional copy of the BDNF gene and found greater weight gain in these mice than in the wild-type population. Significantly higher body

weights were observed in 50% of the males and 27% of the females [4].

Some studies show that greater BDNF concentration may be associated with obesity. In the study on Monteleone et al. increased BDNF levels have been observed in the serum of obese women compared with those in healthy women. The level of BDNF has been positively correlated with BMI and weight [14]. Many studies show the correlation between Val homozygotes and greater BMI in comparison to Met carriers in both adults and children in many ethnicities [13,18,30,31]. Those results are consistent with our research.

The results on the WCST for participants who were homozygous for Val were significantly worse than those for participants who were heterozygous for Val/Met. The poorer results for the individuals homozygous for Val were

**Table 5: Analyses of unidimensional interaction effects for WCST parameters.**

		WCST_P	WCST_NP	WCST_CLR	WCST_CC	WCST_1st
Gender	SS	22,6	36,3	44,7	5,6	24,7
	F	4,8	9,8	8,8	3,1	6,9
	p	3,6E-12	00E-01	7,49E-11	0,002	3,33E-16
Age	SS	26656	42332	52950	5215	29679
	F	5,9	12,5	12,4	2,8	8,8
	p	1,1E-15	0,00E-01	0,00E-01	0,004	0,00E-01
BMI	SS	14109	18542	17374	2311	10255
	F	9,6	16,1	8,6	3,2	7,4
	p	0,00E-01	0,00E-01	0,00E-01	0,001	0,00E-01
BDNF	SS	38	42	47	11,9	29
	F	10	11	9	7	8
	p	0,00E-01	0,00E-01	0,00E-01	3,42E-08	0,00E-01

**Notes:** One-dimensional ANOVA F-test based on SS. Bold values indicate statistical significance.

**Abbreviations:** BMI, body mass index; WCST\_P, percentage of perseverative errors; WCST\_NP, percentage of nonperseverative errors; WCST\_CLR, percentage of conceptual responses; WCST\_CC, level of category completed; WCST\_1st, number of cards needed to complete first category; ANOVA, analysis of variance; SS, sum of squares.

unexpected because better cognitive performance has been widely associated in previous studies with the Val allele, for example, in children [32] the elderly [33] and patients with Parkinson disease [34].

The main mechanism for the effect of BDNF on cognitive performance is the promotion of enhanced synaptic potentials. Long-term potentiation, as a form of synaptic plasticity, is associated with a cell model of long-term memory. Extended potentiation occurs during the monosynaptic conduction of high frequency stimulation and increases the efficiency of synaptic transmission [35-37].

It is generally thought that the Met allele is associated with poor performance on cognitive tests. Worse outcome in episodic memory may be due to abnormal neuronal activation of the hippocampus caused by lower BDNF secretion in Met allele carriers [9]; in a study on obese population Met carriers performed worse than non-carriers in WCST and made more perseverative errors. Furthermore, MRI studies displayed differences in cortex thickness between obese and non-obese Met carriers, showing that the thinnest frontal cortex areas pertained to obese subjects. This strongly suggests the connection between obesity, BDNF Val66Met and executive functions [21].

However, in some researches higher BDNF concentration correlates with worse cognitive performance. Study examining 64 people with bipolar disorder, displayed significantly more non-perseverative errors in the group homozygous for Val allele [20]. Cunha et al. showed that moderate BDNF overexpression

in rats may lead to impaired learning in spatial and instrumental memory tasks. Authors suggest that the excess of mature BDNF may lead to inhibition of synaptic circuitry involved in learning [38].

In the present study, we conducted further analyses using Kendall’s partial rank correlation coefficient method and determined that it was likely that higher body weight was associated with the poorer cognitive performance in the group homozygous for Val allele. Compared with the Val/Val group, the group heterozygous for Val/Met had a lower median BMI and demonstrated better results on the WCST test parameters, particularly in terms of response rates consistent with logical concepts, indicating better use of new information and experience, as well as better control of thinking during the test. Excess body weight has long been considered a modifiable risk factor for cognitive dysfunction [39]. For example, more than 30 years ago, significant and extensive dysfunction of the prefrontal cortex was observed in extremely obese populations [40].

**Table 3** showed that patients with morbid obesity despite younger age demonstrated worse scores in WCST\_P, WCST\_NP and WCST\_CRL in comparison to the mild obese group. Obese population demonstrated altered executive functions with underlying dysfunction of frontal-subcortical circuit [41]. It may result in impaired decision making, response inhibition and cognitive flexibility [42,43]. Moreover, obesity affects the structure of the frontal lobe; obesity correlates with lowered thickness of cortical and subcortical areas. Abovementioned may be

the explanation of worse executive functioning in such patients despite younger age, compared to mild obese group. The study of Marqués-Iturria et al. presented the co-relation between lower cortical thickness and obesity, as well as performing more perseverative errors in WCST test. Our study corroborates with those findings, as our subjects - with greater BMI - made more perseverative errors as well [21].

Negative correlation between BMI and executive functions increases with age. In our study, younger group, but more severely obese, showed worse executive functioning. The possible explanation might be, that greater weight affected the performance more than advanced age; especially that age-range does not differ significantly between those two groups [41].

In conclusion, our results give new insight into the connection between BDNF concentration, obesity and cognitive performance on large sample. The Val66Met BDNF polymorphism in the indirect manner effected the poorer cognitive functioning observed in obese individuals who were homozygous for Val, rather than heterozygous for Val/Met. The Val allele was associated with higher BMI, suggesting

that excessive weight may have impaired the activity of the prefrontal cortex. We propose that by affecting weight BDNF Val66Met may contribute to worse cognitive functioning in obese participants. Our findings attach importance to the role of BDNF in cognition and eating disorders especially that the latter has become a serious health problem worldwide. Therefore better understanding of processes involved in eating disorders is crucial to develop new therapeutic options for patients.

### Limitations

The main limitation of our study is the lack of a population homozygous for Met allele, which made it difficult to draw further specific conclusions. Second, our study does not include control non-obese group, so it may make it difficult to interpret the relationship between variables properly.

### Acknowledgement

*This research was supported by a grant from the Polish Ministry of Science and Higher Education (Grant No. NN 402053136).*

### References

- Barde YA, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. *EMBO. J* 1(5), 549-553 (1982).
- Murer MG, Yan Q, Raisman-Vozari R. Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Prog. Neurobiol* 63(1), 71-124 (2001).
- Maisonpierre PC, Belluscio L, Friedman B, et al. NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. *Neuron* 5(4), 501-509 (1990).
- Kernie SG, Liebl DJ, Parada LF. BDNF regulates behaviour and locomotor activity in mice. *EMBO. J* 19(6), 1290-1300 (2000).
- Baroni A, Castellanos FX. Neuroanatomic and cognitive abnormalities in attention-deficit/hyperactivity disorder in the era of 'high definition' neuroimaging. *Curr. Opin. Neurobiol* 30, 1-8 (2015).
- Lu B, Nagappan G, Lu Y. BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb. Exp. Pharmacol* 220, 223-50 (2014).
- Bramham CR, Panja D. BDNF regulation of synaptic structure, function, and plasticity. *Neuropharmacology* 76 Pt C, 601-602 (2014).
- Hanson IM, Seawright A, van Heyningen V. The human BDNF gene maps between FSHB and HVBS1 at the boundary of 11p13-p14. *Genomics* 13(4), 1331-1333 (1992).
- Egan MF, Kojima M, Callicott JH, et al. The BDNF Val66Met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112(2), 257-269 (2003).
- Su H, Tao J, Zhang J, et al. The Effects of BDNF Val66Met Gene Polymorphism on Serum BDNF and Cognitive Function in Methamphetamine-Dependent Patients and Normal Controls: A Case-Control Study. *J. Clin. Psychopharmacol* 35(5), 517-524 (2015).
- Chen ZY, Patel PD, Sant G, et al. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J. Neurosci*, 24(18), 4401-4411 (2004).
- Rosas-Vargas H, Martínez-Ezquerro JD, Bienvenu T. Brain-derived neurotrophic factor, food intake regulation, and obesity. *Arch. Med. Res* 42(6), 482-494 (2011).
- Gunstad J, Schofield P, Paul RH, et al. BDNF Val66Met polymorphism is associated with body mass index in healthy adults. *Neuropsychobiology* 53(3), 153-156 (2006).
- Monteleone P, Tortorella A, Martiadis V, et al. Opposite changes in the serum brain-derived neurotrophic factor in anorexia nervosa and obesity. *Psychosom. Med* 66(5), 744-748 (2004).
- Ribasés M, Gratacòs M, Armengol L, et al. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol. Psychiatry* 8(8), 745-751 (2003).
- Skledar M, Nikolac M, Dodig-Curkovic K, et al. Association between brain-derived neurotrophic factor Val66Met and obesity in children and adolescents. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 36(1), 136-140 (2012).
- Beckers S, Peeters A, Zegers D, et al. Association of the BDNF Val66Met variation with obesity in women. *Mol. Genet. Metab* 95(1-2), 110-112 (2008).
- Ma XY, Qiu WQ, Smith CE, et al. Association between BDNF rs6265 and obesity in the Boston Puerto Rican Health Study. *J. Obes* 102942 (2012).
- Unger TJ, Calderon GA, Bradley LC, et al. Selective deletion of Bdnf in the ventromedial and dorsomedial hypothalamus of adult mice results in hyperphagic behavior and obesity. *J. Neurosci*, 27(52), 14265-14274 (2007).

20. Tramontina JF, Yates D, Magalhães PV *et al.* Brain-derived neurotrophic factor gene val66met polymorphism and executive functioning in patients with bipolar disorder. *Rev. Bras. Psiquiatr* 31(2), 136-140 (2009).
21. Marqués-Iturria I, Garolera M, Pueyo R, *et al.* The interaction effect between BDNF val66met polymorphism and obesity on executive functions and frontal structure. *Am. J. Med. Genet. B Neuropsychiatr. Genet* 165B(3), 245-253 (2014).
22. Mandelman SD, Grigorenko EL. BDNF Val66Met and cognition: all, none, or some? A meta-analysis of the genetic association. *Genes. Brain. Behav* 11(2), 127-136 (2012).
23. Lahiri DK, Schnabel B. DNA isolation by a rapid method from human blood samples: effects of MgCl<sub>2</sub>, EDTA, storage time, and temperature on DNA yield and quality. *Biochem. Genet* 31(7-8), 321-328 (1993).
24. Nikolac Perkovic M, Mustapic M, Pavlovic M, *et al.* Lack of association between brain-derived neurotrophic factor Val66Met polymorphism and body mass index change over time in healthy adults. *Neurosci. Lett* 545, 127-131 (2013).
25. Zhao XY, Zhang MX, Cheng H, *et al.* [Risk of obesity-related gene polymorphism on the incidence and durative of childhood obesity]. *Zhonghua. Liu. Xing. Bing. Xue. Za. Zhi*, 34(6), 560-565 (2013).
26. Gratacòs M, González JR, Mercader JM, *et al.* Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. *Biol. Psychiatry* 61(7), 911-922 (2007).
27. Lapchak PA, Hefti F. BDNF and NGF treatment in lesioned rats: effects on cholinergic function and weight gain. *Neuroreport* 3(5), 405-408 (1992).
28. Wang C, Bomberg E, Billington CJ, *et al.* Brain-derived neurotrophic factor (BDNF) in the hypothalamic ventromedial nucleus increases energy expenditure. *Brain. Res* 1336, 66-77 (2010).
29. Wang C, Bomberg E, Billington C, *et al.* Brain-derived neurotrophic factor in the hypothalamic paraventricular nucleus reduces energy intake. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 293(3), R1003-12 (2007).
30. Xi B, Wang C, Wu L, *et al.* Influence of physical inactivity on associations between single nucleotide polymorphisms and genetic predisposition to childhood obesity. *Am. J. Epidemiol* 173(11), 1256-1262 (2011).
31. Timpano K.R., Schmidt N.B., Wheaton M.G., *et al.* Consideration of the BDNF gene in relation to two phenotypes: hoarding and obesity. *J. Abnorm. Psychol* 120(3), 700-707 (2011).
32. Jasińska KK, Molfese PJ, Kornilov SA, *et al.* The BDNF Val66Met Polymorphism Influences Reading Ability and Patterns of Neural Activation in Children. *PLoS. One* 11(8), e0157449 (2016).
33. Canivet A, Albinet CT, André N, *et al.* Effects of BDNF polymorphism and physical activity on episodic memory in the elderly: a cross sectional study. *Eur. Rev. Aging. Phys. Act* 12, 15 (2015).
34. Altmann V, Schumacher-Schuh AF, Rieck M, *et al.* Val66Met BDNF polymorphism is associated with Parkinson's disease cognitive impairment. *Neurosci. Lett* 615, 88-91 (2016).
35. Bliss TV & Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361(6407), 31-39 (1993).
36. Bliss T.V. & Gardner-Medwin A.R. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaesthetized rabbit following stimulation of the perforant path. *J. Physiol* 232(2), 357-374 (1973).
37. Xie H, Leung KL, Chen L, *et al.* Brain-derived neurotrophic factor rescues and prevents chronic intermittent hypoxia-induced impairment of hippocampal long-term synaptic plasticity. *Neurobiol. Dis* 40(1), 155-162 (2010).
38. Cunha C, Angelucci A, D'Antoni A, *et al.* Brain-derived neurotrophic factor (BDNF) overexpression in the forebrain results in learning and memory impairments. *Neurobiol. Dis* 33(3), 358-368 (2009).
39. Han C, Jo SA, Seo JA, *et al.* Adiposity parameters and cognitive function in the elderly: application of "Jolly Fat" hypothesis to cognition. *Arch. Gerontol. Geriatr* 49(2), e133-8 (2009).
40. Chelune G., Ortega D., Linton J. *et al.* Personality and cognitive findings among patients electing gastroplasty for morbid obesity. *Int. J. Eat. Disord* 5, 701-712 (1986).
41. Stanek KM, Strain G, Devlin M *et al.* Body mass index and neurocognitive functioning across the adult lifespan. *Neuropsychology* 27(2), 141-151 (2013).
42. Siervo M, Arnold R, Wells JC, *et al.* Intentional weight loss in overweight and obese individuals and cognitive function: a systematic review and meta-analysis. *Obes. Rev*, 12(11), 968-983 (2011).
43. Smith E, Hay P, Campbell L, *et al.* A review of the association between obesity and cognitive function across the lifespan: implications for novel approaches to prevention and treatment. *Obes. Rev* 12(9), 740-755 (2011).