

# BDNF Val66Met is Associated with Pre-existing but not with Paclitaxel-induced Peripheral Neuropathy in an Israeli Cohort of Breast Cancer Patients

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**ABSTRACT:** **Background:** Brain-derived neurotrophic factor (BDNF) is a neuronal growth factor that is important for the development, maintenance, and repair of the peripheral nervous system. The *BDNF* gene commonly carries a single nucleotide polymorphism (Val66Met-SNP), which affects the cellular distribution and activity-dependent secretion of BDNF in neuronal cells.

**Objectives:** To check the association of BDNF Val66Met-SNP as a predisposition that enhances the development of chemotherapy-induced peripheral neuropathy in an Israeli cohort of patients with breast cancer who were treated with paclitaxel.

**Methods:** Peripheral neuropathy symptoms were assessed and graded at baseline, before beginning treatment, and during the treatment protocol in 35 patients, using the reduced version of the Total Neuropathy Score (TNSr). Allelic discrimination of BDNF polymorphism was determined in the patients' peripheral blood by established polymerase chain reaction and Sanger sequencing.

**Results:** We found Val/Val in 20 patients (57.14%), Val/Met in 15 patients (42.86%), and Met/Met in none of the patients (0%). Baseline TNSr scores were higher in Met-BDNF patients compared to Val-BDNF patients. The maximal TNSr scores that developed during the follow-up in Met-BDNF patients were higher than in Val-BDNF patients. However, exclusion of patients with pre-existing peripheral neuropathy from the analysis resulted in equivalent maximal TNSr scores in Met-BDNF and Val-BDNF patients.

**Conclusions:** These observations suggest that BDNF Val66met-SNP has no detectable effect on the peripheral neuropathy that is induced by paclitaxel. The significance of BDNF Val66Met-SNP in pre-existing peripheral neuropathy-related conditions, such as diabetes, should be further investigated.

IMAJ 2018; 20: 746–748

**KEY WORDS:** brain-derived neurotrophic factor (BDNF), chemotherapy-induced peripheral neuropathy (CIPN), paclitaxel, pre-existing peripheral neuropathy

Paclitaxel is a commonly used chemotherapeutic agent that is known to be associated with chemotherapy-induced peripheral neuropathy (CIPN) [1]. Interference with microtubule axonal transport in normal neuronal cells is part of the mechanism of the neuronal damage underlying paclitaxel-induced peripheral neuropathy (PIPNe) [2]. However, it is not completely known why some patients are more vulnerable to PIPNe than others.

Brain derived neurotrophic factor (BDNF) is a member of a family of neuronal growth factors that are important for the development [3], maintenance [4], and repair of the central and peripheral nervous systems [5]. The human BDNF gene carries a frequent non-conservative single nucleotide polymorphism (SNP) that results in valine to methionine substitution at codon 66 (Val66Met). This SNP was reported to cause a deficit in the cellular distribution and activity-regulated secretion of BDNF in neuronal cells [6] and was found to be associated with altered susceptibility to memory and cognitive impairment in a variety of neurological disorders [7,8].

Loss of nerve growth factor-mediated neuronal survival has been previously proposed as a candidate mechanism underlying CIPN [9,10]. Our previous report showed that dysregulated blood protein levels of BDNF in patients developing bortezomib-induced peripheral neuropathy [11]; thus, suggesting the need for investigating a possible link between genetic alterations in BDNF and vulnerability to CIPN.

## PATIENTS AND METHODS

### STUDY POPULATION

Inclusion criteria were patients diagnosed with breast cancer between the ages of 18 and 70 years who were candidates for treatment with paclitaxel-based protocol. These patients signed informed consent documentation and were recruited to the study in the outpatient oncology unit at the Galilee Medical Center, Nahariya, Israel.

**NEUROLOGICAL EXAMINATION**

Symptoms of polyneuropathy were assessed and graded at diagnosis, after each treatment cycle, and at the end of treatment protocol, using the reduced version of Total Neuropathy Score (TNSr) [12]. Patients with TNSr scores  $\geq 2$  at diagnosis were determined as patients with pre-existing peripheral neuropathy.

**BDNF VAL66MET GENOTYPING**

DNA purification from the patient’s peripheral blood was conducted by QIAamp DNA Mini kit (cat# 51106, Qiagen, Netherlands). The BDNF gene DNA region containing the rs6265 polymorphism (Val66Met) was amplified by polymerase chain reaction (PCR) using the primer sequences P1 (forward) CCTACAGTTCACCAGGTGAGAAGAGTG, and P2 (reverse) TCATGGACATGTTTGCAGCATCTAGGTA. The 401 bp PCR-product containing the SNP of interest was sequenced by Sanger sequencing method and the allele specific G→A substitution was determined by the ABI sequence analyzer.

**DATA ANALYSIS**

Genotype differences between patients with and without pre-existing peripheral neuropathy were conducted by non-parametric chi-square analysis. To avoid inter-patient variation in PIPN development, we analyzed the maximal clinical score that each patient achieved during the follow-up. Cross-sectional ANOVA analysis was performed to compare the baseline and maximal TNSr scores of the patients between the genotype groups. All statistical analyses were performed using JMP® (SAS Institute Inc., Cary, NC, USA) statistical software.

**RESULTS**

**PATIENT DEMOGRAPHICS AND BDNF GENOTYPE FREQUENCY**

Thirty-five patients, 34 women and 1 man, were recruited. Ten patients (28.57%) were diagnosed with pre-existing peripheral neuropathy (TNSr score  $\geq 2$ ), three with diabetes-related neuropathy and seven of unknown origin. The remaining 25 patients (71.43%) were diagnosed with TNSr scores  $\leq 1$  before initiation of paclitaxel treatment and identified without pre-existing peripheral neuropathy. With regard to BDNF genotype, we found Val/Val in 20 patients (57.14%), Val/Met in 15 patients (42.86%), and Met/Met in none of the patients (0%). Patients with the genotype Val/Met were defined as Met-BDNF, and those with Val/Val were classified as Val-BDNF. We found higher incidence of the Met-BDNF allele in the group of patients with pre-existing peripheral neuropathy compared to the group of patients defined without pre-existing peripheral neuropathy (7/10 [70%] vs. 8/25 [32%]) in pre-existing and non-pre-existing peripheral neuropathy, respectively ( $P < 0.05$  chi-square). All three patients with the diabetes-related pre-existing neuropathy were genotyped Met-BDNF [Table 1].

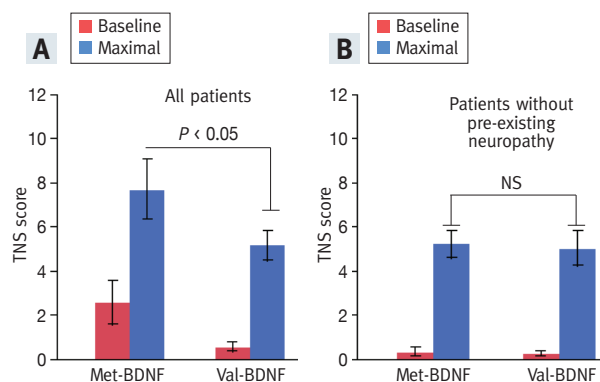
An analysis comparing the baseline TNSr scores between Met-BDNF and Val-BDNF patients showed higher mean TNSr scores in Met-BDNF patients compared to Val-BDNF patients (mean TNSr  $\pm$  SEM 0.70  $\pm$  0.23 vs. 2.60  $\pm$  0.99 in Val-BDNF and Met-BDNF, respectively,  $P < 0.01$  *t*-test). Analysis of the maximal TNSr score that the patients developed during the follow-up showed higher TNSr scores in Met-BDNF patients than in Val-BDNF patients (mean TNSr  $\pm$  SEM 4.80  $\pm$  0.62 vs. 7.73  $\pm$  1.34 in Val-BDNF and Met-BDNF, respectively,  $P < 0.04$  *t*-test). No difference in the maximal TNSr score between Met-BDNF patients and the Val-BDNF patients was shown after excluding the patients with pre-existing peripheral neuropathy from the analysis (mean TNSr  $\pm$  SEM 5.05  $\pm$  0.78 vs. 5.25  $\pm$  0.62 in Val-BDNF and Met-BDNF, respectively,  $P < 0.44$  *t*-test). [Figure 1].

**Table 1.** Higher maximal total neuropathy score in Met-BDNF patients resulted from their higher baseline score

| Parameter                              | Total             | Val/Val           | Val/Met          | P value |
|--|-------------------|-------------------|------------------|---------|
| N                                      | 35                | 20 (57.14%)       | 15 (42.86%)      |         |
| Age, mean $\pm$ SD, years              | 58.17 $\pm$ 10.55 | 56.65 $\pm$ 10.35 | 60.2 $\pm$ 10.84 | 0.332   |
| Paclitaxel mg/treatment, mean $\pm$ SD | 167.2 $\pm$ 59.57 | 176 $\pm$ 71.22   | 155.46 $\pm$ 38  | 0.32    |
| <b>TNM staging</b>                     |                   |                   |                  |         |
| T (1-4)                                | 24                | 15 (75%)          | 9 (60%)          |         |
| N (Positive)                           | 11                | 5 (25%)           | 6 (40%)          |         |
| M                                      | 0                 | 0                 | 0                | 0.32    |
| TNS $\leq 1$                           | 25 (71.43%)       | 17 (68%)          | 8 (32%)          |         |
| TNS $\geq 2$                           | 10 (28.57%)       | 3 (30%)           | 7 (70%)          | 0.03    |

BDNF = Brain-derived neurotrophic factor, SD = standard deviation

**Figure 1.** Baseline (red bars) and maximal (blue bars) TNSr scores in Met-BDNF and Val-BDNF patients. In all patients [A] and after exclusion of the patients with pre-existing neuropathy from the analysis [B]. Bars show the mean  $\pm$  SEM of the TNSr scores



BDNF = brain-derived neurotrophic factor, NS = not significant, TNSr = total neuropathy score

## DISCUSSION

Our observations show increased baseline TNSr scores and corresponding higher incidence of pre-existing peripheral neuropathy in Met-BDNF patients compared to Val-BDNF patients. Equivalent maximal TNSr scores between Met-BDNF and Val-BDNF patients without pre-existing peripheral neuropathy suggest that BDNF genotype has no direct effect on the peripheral neuropathy developed by paclitaxel treatment in our patients. However, at present we cannot exclude possible subclinical effects of the BDNF-SNP that we did not detect by the TNSr tool.

Pre-existing peripheral neuropathy may be the strongest clinical risk factor for CIPN development [2]. However, the mechanism underlying the development of pre-existing peripheral neuropathy is diverse and not completely understood. Diabetes is a distinctive cause of pre-existing neuropathy and was found in 30% of our patients with pre-existing neuropathy. Interestingly, in our cohort all the patients with the diabetes-related pre-existing peripheral neuropathy were genotyped Met-BDNF.

The involvement of the BDNF Val66met SNP in the development of peripheral neuropathy in diabetic patients has not yet been investigated. However, several studies support the involvement of BDNF in diabetes-related neuropathy. For example, altered BDNF protein level was shown in the muscles of patients with diabetes-related peripheral neuropathy [13].

Another study of Chinese diabetic patients reported higher frequency of the Met-BDNF allele in depressive patients [14]. Depression and chronic stress are reported to be comorbidities that could exacerbate symptoms of peripheral neuropathy [15]. However, the major difference in the frequency of the Met-BDNF allele between the Chinese and the Israeli populations necessitates verification of this data in the Israeli patients.

## CONCLUSIONS

Met-BDNF SNP was associated with pre-existing peripheral neuropathy in our cohort. Hence, BDNF gene polymorphism may be a pre-disposition for peripheral neuropathy developed by paclitaxel treatment. However, these data should be validated in a large-scale study, including more patients, other malignancies, and other neurotoxic agents.

## Acknowledgements

This study was supported by a grant from the Israel Cancer Research Fund (ICRF)

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## References

- Cundari S, Cavaletti G. Thalidomide chemotherapy-induced peripheral neuropathy: actual status and new perspectives with thalidomide analogues derivatives. *Mini Rev Med Chem* 2009; 9 (7): 760-8.
- Cavaletti G, Bogliun G, Marzorati L, et al. Early predictors of peripheral neurotoxicity in cisplatin and paclitaxel combination chemotherapy. *Ann Oncol* 2004; 15 (9): 1439-42.
- Rocamora N, Garcia-Ladona FJ, Palacios JM, Mengod G. Differential expression of brain-derived neurotrophic factor, neurotrophin-3, and low-affinity nerve growth factor receptor during the postnatal development of the rat cerebellar system. *Brain Res Mol Brain Res* 1993; 17 (1-2): 1-8.
- Lipsky RH, Marini AM. Brain-derived neurotrophic factor in neuronal survival and behavior-related plasticity. *Ann N Y Acad Sci* 2007; 1122: 130-43.
- Novikova L, Novikov L, Kellerth JO. Brain-derived neurotrophic factor reduces necrotic zone and supports neuronal survival after spinal cord hemisection in adult rats. *Neurosci Lett* 1996; 220 (3): 203-6.
- 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* 2003; 112 (2): 257-69.
- Borroni B, Archetti S, Costanzi C, et al. Role of BDNF Val66Met functional polymorphism in Alzheimer's disease-related depression. *Neurobiol aging* 2009; 30 (9): 1406-12.
- Bialecka M, Kurzawski M, Roszmann A, et al. BDNF G196A (Val66Met) polymorphism associated with cognitive impairment in Parkinson's disease. *Neurosci Lett* 2014; 561: 86-90.
- Richardson PG, Barlogie B, Berenson J, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. *N Engl J Med* 2003; 348 (26): 2609-17.
- Anand P. Neurotrophic factors and their receptors in human sensory neuropathies. *Prog Brain Res* 2004; 146: 477-92.
- Azoulay D, Lavie D, Horowitz N, et al. Bortezomib-induced peripheral neuropathy is related to altered levels of brain-derived neurotrophic factor in the peripheral blood of patients with multiple myeloma. *Br J Haematol* 2013 164 (3): 454-6.
- Smith EM, Beck SL, Cohen J. The total neuropathy score: a tool for measuring chemotherapy-induced peripheral neuropathy. *Oncol Nurs Forum* 2008; 35 (1): 96-102.
- Andreassen CS, Jakobsen J, Flyvbjerg A, Andersen H. Expression of neurotrophic factors in diabetic muscle--relation to neuropathy and muscle strength. *Brain* 2009; 132 (Pt 10): 2724-33.
- Zhou JX, Li HC, Bai XJ, Chang BC, Li CJ, Sun P, Chen LM. Functional Val66Met polymorphism of brain-derived neurotrophic factor in type 2 diabetes with depression in Han Chinese subjects. *Behav Brain Funct* 2013; 9: 34.
- Li MJ, Liu LY, Chen L, Cai J, Wan Y, Xing GG. Chronic stress exacerbates neuropathic pain via the integration of stress-affect-related information with nociceptive information in the central nucleus of the amygdala. *Pain* 2017; 158 (4): 717-39.

**“What lies behind us and what lies before us are tiny matters compared to what lies within us”**

Ralph Waldo Emerson, (1803–1882), American author, transcendental philosopher

**“The past is to be respected and acknowledged, but not to be worshipped. It is our future in which we will find our greatness”**

Pierre Trudeau, (1919–2000), 15th Prime Minister of Canada