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ORIGINAL RESEARCH PERIOPERATIVE MEDICINE

Correlation between human BDNF rs6265 gene polymorphism and type-2 diabetes and diabetic peripheral neuropathy

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ABSTRACT

Background & objective: Diabetes mellitus is quite common and globally prevalent condition affecting patients' quality of life. Patients who have type 2 diabetes mellitus (T2DM), are more likely to get diabetic peripheral neuropathy (DPN), which may affect feet, legs, hand, and arm. A BDNF gene rs6265 polymorphism in humans results in the substitution of valine with methionine at codon 66 (Val66Met). We aimed to find the possible link between human BDNF rs6265 gene polymorphism and T2DM with and without peripheral neuropathy and compare it with healthy subjects in Kirkuk City, Iraq.

Methodology: One hundred subjects were chosen to participate in this research, aged from 35 to 75 y and divided into three groups: 35 DPN, 35 T2DM patients, and 30 healthy controls were selected randomly for BDNF gene rs6265 SNP screening by using conventional PCR with specific sets of primers. Products of PCR for patients and control groups were run on the gel electrophoresis to detect the SNP rs6265 fragment. A nerve-conducting study was used to examine DPN.

Results: The observed results demonstrated the presence of two types of alleles identified as genotypic variants (GG and GA) among all participants in this investigation. By applying the Hardy-Weinberg Equilibrium principle (HWE) for both patient and control groups, the results proved that there was a statistically significant variance ($P < 0.05$) in the genotypic frequencies between each of the groups that had been studied. The wild homozygous GG genotype in type 2 diabetic without neuropathy, with DPN, and healthy control group were 51.4%, 40% and 80% respectively. The heterozygous GA genotype were 48.6%, 60% and 20%, respectively in the three groups.

Conclusion: The present study showed that the BDNF (Val66Met) rs6265 polymorphism is associated with type 2 diabetes mellitus and diabetic peripheral neuropathy in heterozygous allele G/A (Met/Met) and homozygous allele GG (Val/Val) genotype. Also, the current study found the absence of the mutant genotype AA, possibly due to the evidence that the distribution of the BDNF polymorphisms varies widely among different ethnic groups.

Abbreviations: BDNF - Brain-derived neurotrophic factor; DPN - Diabetic peripheral neuropathy

Keywords: Type2 diabetes mellitus; Diabetic peripheral neuropathy; Brain-Derived Neurotrophic Factor; BDNF rs6265 SNP; BDNF Val66Met polymorphism.

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1. INTRODUCTION

When the pancreas fails to produce sufficient insulin or cells have problems using it, a chronic illness known as diabetes mellitus develops. When diabetes fails to be controlled, it may lead to hyperglycemia, which, over time, damages many internal organ systems, including the nervous system and blood vessels.[1]

Diabetes related late consequences are classified as macro and microvascular problems; diabetic neuropathy is a typical microvascular consequence of diabetes.[2] Diabetic neuropathy primarily affects the distal lower extremities at first and is a symmetrical sensory neuropathy.[3] Diabetic peripheral neuropathy (DPN) begins from the tips of the toes and then progresses gradually upwards. It spreads to the upper limbs after it becomes well-established in the lower limbs, causing sensory loss, that typically manifests itself as "glove and stocking".[4] These sensations typically worsen at night and cause sleep disturbances. The patients do not usually display weakness; when symptoms appear, they are generally sensory.[5] Significant morbidity, including depression, ulceration, leg amputations, and risk of ankle or foot fractures, is associated with DPN.[6]

The main factor behind the start of the many metabolic processes that underlie DPN, appears to be chronic hyperglycemia. In the context of increased free fatty acid depletion and insulin resistance development, nerve ischemia results from a confluence of factors, including endothelial damage, microvascular dysfunctions, insulin resistance, toxic obesity, and direct axonal injury brought on by metabolic consequences of hyperglycemia.[7] Several neurotrophic substances, such as brain-derived neurotrophic factor (BDNF), activate the damaged neuron. The maturation, plasticity, and nervous system repair entirely depend on BDNF and it encourages the regeneration of neurons as well.[8] There are two types of receptors that BDNF may bind to. The apoptotic-responsible pan neurotrophic receptor (p75) is the initial one to be identified. It has a low affinity for BDNF but can attach to all members of the neurotrophic family. The second is the BDNF-binding tropomyosin receptor kinase B (TrkB).[9]

BDNF rs6265is one of the most prevalent and significant polymorphisms located in exon XI within the prodomain area of the BDNF that causes valine to be substituted for methionine at codon 66 (G196A or Val66Met).[10] Mechanistically, the non-conservative replacement of a single nucleotide modifies the gene expression, localization, and signal transduction of BDNF, leading to phenotypic changes.[11] This BDNF rs6265 polymorphism at the 5' end of the pro-BDNF sequence does not affect the generation and signaling of mBDNF in neuronal cells. As an alternative, SNP rs6265 has been associated with other neurological and neuropsychiatric disorders, such as an increased risk of memory loss and cognitive impairment, an imbalance in the secretion and distribution of BDNF, and more. SNP rs6265 and BDNF protein levels were previously investigated in type II diabetes.[12]

We aimed to study the possible link between human BDNF rs6265 gene polymorphism and type II diabetes with and without peripheral neuropathy patients and compare it with healthy subjects in Kirkuk City, Iraq.

2. METHODOLOGY

After the scientific committee approved the study's protocol, all patients of type-2 diabetic mellitus and DPN were included in this study collected from the Azadi-Teaching Hospital Specialized Diabetes, Endocrine, Metabolism Center, and the Department of Neuro-medicine in Kirkuk, Iraq.

This study was conducted from October 2022 to July 2023. The 100 participants, aged 35 to 78 y, were a combination of men and women, and the research was designed using a case-control design. The subjects were divided into 30 healthy controls, 35 T2DM patients, and 35 patients clinically diagnosed with DPN. Patients with a history of depression or dementia or any other related peripheral nervous system pathology (such as carpal tunnel syndrome, asymmetrical neuropathies, or chronic inflammatory demyelinating polyneuropathy) and type 1 diabetes were excluded from the study.

All participants provided written consent for their health records to be used in this study. A quick but uniform clinical examination was performed following the questionnaire. All patient's with DPN, identified by an expert, will have a neurological exam through the Neuropathy Disability Score (NDS). The patient's medical history and current symptoms were reviewed as part of the clinical investigation. Signs of damage to the peripheral nervous system were identified. These included a burning sensation, pinching, "tingling sensations," severe pain like "electric shock," foot paralysis, instability while walking, and muscle cramps. Nerve Conduction Study (NCS) in the arms and legs, was done to ensure the presence of DPN.[13]

All participants had about 3 mL of fresh blood added to an EDTA tube to do the molecular study. The primers were designed by the researcher using the NCBI website. The primer designer was detected to amplify 360 base pairs (product size) of the human BDNF gene at RS 6265. The sequences of the Forward primer were GCCCAAGGCAGGTTCAAGAG and the reverse primer GCCGGACTCATGGACATG. DNA Extraction Kit was used for sample preparation.

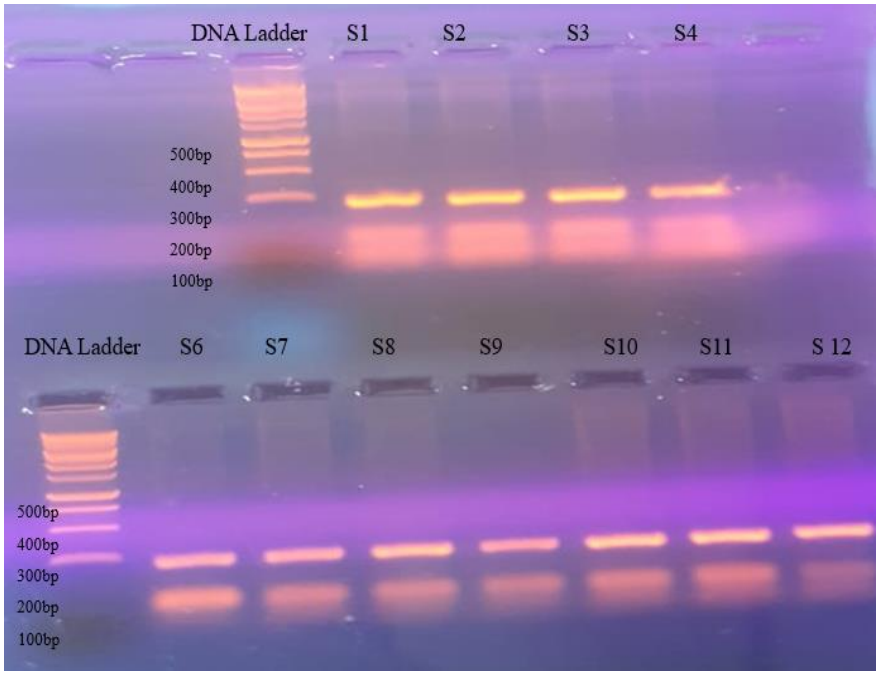

Figure 1: Gel electrophoresis of human BDNF gene fragment corresponding to rs6265 gene polymorphisms standard PCR with predetermined primers. Screening the PCR result with ethidium bromide was done. The length of the fragment was 360 base pairs, the product size. The sample (S1, S2, S3, S4) represent DPN patients, while (S5, S6, S7, S8) represent diabetics without neuropathy and (S9, S10, S11, S12) for control.

The PCR reaction mix from Promega /USA is in one conventional PCR kit containing all necessary compounds, such as MgCl2, dNTP, and DNA polymerase. The master mix was added to each PCR tube, and the PCR template was added to the corresponding tube; the PCR tube was centrifuged to pellet the compound into the bottom of the PCR tube and then added to the PCR thermal cycling machine. Following the PCR amplification process, the PCR product was run of agarose gel; to ensure the presence of amplification, agarose gel electrophoresis was performed.

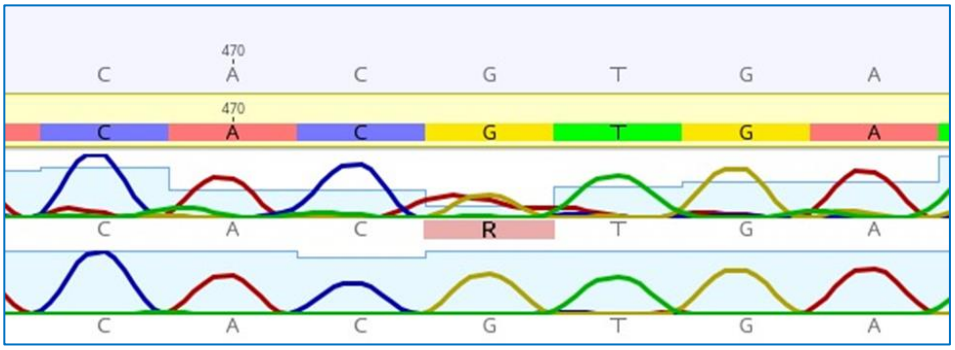

Figure 2. The frequency distribution of BDNF rs6265 genotypes GG and GA throughout the studied categories. GG is a homozygous wild genotype, and GA is a heterozygous genotype represented as R.

A purified band was prepared of a total liquid of 50 µL alongside sequences at a final concentration of 10 pmol/ mL. This was kept refrigerated and sent to KOREA. PCR products were sent for Sanger sequencing by Macrogen Corporation – Korea. The results received by email were analyzed and compared to standard reference NCBI gene bank using BIO EDITE sequencing software.

Data analysis

The SPSS (version 26) program was used for the statistical analysis. An ANOVA test was performed. The statistical test made use of an estimate by analyzing linear regression. We employed probability values of $P \leq 0.05$ and $P \geq 0.05$ as markers of statistical significance—and statistical non-significance, respectively. Genotype and allele frequencies of the *BDNF gene* among study groups were calculated using a Hardy-Weinberg equilibrium (HWE) equation and the chi-square ($\chi 2$) test.

3. RESULTS

3.1. Molecular analysis for Single Nucleotide Polymorphisms in BDNF genome

BDNF rs6265 (Val66Met) was investigated in association with T2DM and DPN using conventional PCR with specific primers. PCR products for patients and control groups were run on the gel electrophoresis to detect the amplified SNP rs6265 fragment. The product size was measured at 360bp BDNF *gene* promoter region. Figure 1 shows the DNA ladder, and the amplified PCR DNA samples were subjected to gel electrophoresis. BDNF bands are seen between

Table 1: Chi-square analysis of genotypes and alleles distribution among study groups.

Genotypes of BDNF *rs6265 gene* polymorphism	Diabetics without neuropathy group (n = 35)	DPN group (n = 35)	Control group (n = 30)
GG (homozygous wild)	18 (51.4%)	14 (40%)	24 (80%)
GA (heterozygous)	17 (48.6%)	21 (60%)	6 (20%)
AA (homozygous mutant)	0 (0%)	0 (0%)	0 (0%)

Data presented as n (%)

300-400bp of the DNA markers, mainly at 360bp, indicating PCR successful amplification.

3.2. Genotypes and alleles frequency of human BDNF rs6265 gene polymorphism among study groups

The automated sequencers for the BDNF rs6265 gene of each sample produced a four-color graph presenting the result of the sequencing run. Additionally, each graph was accompanied by a text file of the sequenced nucleotides. The sequenced samples were further analyzed using the BioEdit program to detect the presence or absence of BDNF rs6265 gene polymorphism. Two genotypes (GG and GA) corresponding to two (G and A) alleles were determined, as shown in Figure 2.

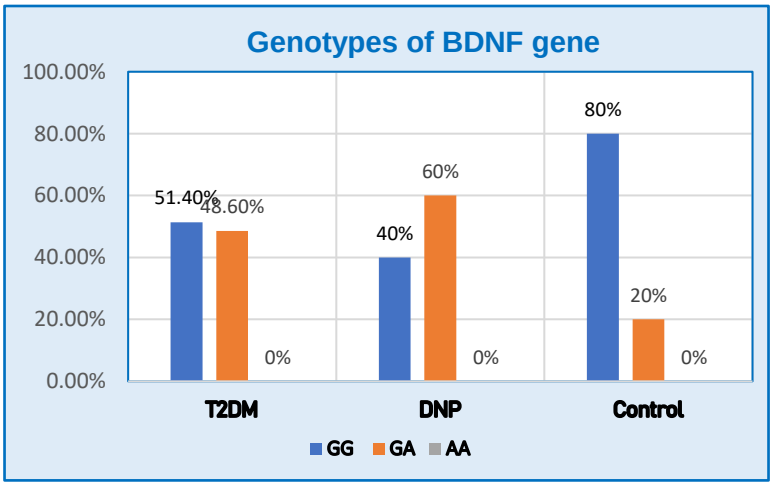

Figure 3: Genotypes and alleles frequency of human BDNF rs6265 gene polymorphism among the DPN, T2DM, and control groups. GC represents the heterozygous genotype; GG means homozygous, and AA.

The observed results demonstrated the presence of two types of alleles identified as genotypic variants (GG and GA) among all participants in this investigation; by applying the Hardy-Weinberg Equilibrium principle (HWE) for both patient and control groups, Following the genetic study, the results proved that there was a statistically significant variance ($P < 0.05$) in the genotypic frequencies between each of the groups that had been studied, the wild homozygous GG genotype in, type 2 diabetic without neuropathy, diabetic peripheral neuropathy, and healthy control group, (51.4%), (40%), (80%), and heterozygous GA genotype (48.6%), (60%), (20%), respectively as presented in Table 1. The higher distribution of allele G in the control group, 54 (90%), against the higher distribution of A allele in DPN 21 (30%) revealed significant differences (Figure 2).

Table 2 shows that the BDNF Val/Val (GG) genotype was found to have 18 (51.4%) patients with T2DM and

Table 2: BDNF genotype distribution (G>A nucleotide SNP rs6265) and allele frequency compared among the research groups

Genotypes	DM without PN	DNP Group	Control Group	X^2	P-value
Genotypes					
GG (%)	18 (51.4)	14 (40)	24 (80)	57.013	0.000**
GA (%)	17 (48.6)	21 (60)	6 (20)		
Alleles					
G (%)	53 (75.7)	49 (70)	54 (90)	200.923	0.000**
A (%)	17 (24.3)	21 (30)	6 (10)		

*X^2 =Chi-square; **P. = Highly significant; HWE: Hardy Weinberg Equilibrium; Data presented as n (%)*

14 (40%) DPN patients, while the Val/Met (GA) genotypes were detected in 17 (48.6%) T2DM and 21 (60%) DPN patients, respectively.

4. DISCUSSION

The study examined the connection between BDNF gene (rs6265) SNP with T2DM without PN and DPN and compared them to the control subjects. To our knowledge, this is the first study of this type in Iraq. The current study intended to investigate the relationship of BDNF rs6265 SNPs in humans with DPN, which could be used as DPN pathogenesis markers.

The observed results demonstrated the presence of two types of alleles identified as genotypic variants (GG and GA) among all participants in this investigation; by applying the Hardy-Weinberg Equilibrium principle (HWE) for both patient and control groups, a statistically significant variance ($P < 0.05$) was found in the genotypic frequencies between all groups that had been studied.

The rs6265 (Val66Met) polymorphism was discovered to be linked with T2DM and DPN by heterozygous allele G/A (Met-Met) and homozygous allele GG (Val-Val) genotype. The results are consistent with research by Azoulay et al. (2020) that showed a correlation between Val66Met polymorphism and the development of diabetic neuropathy.[14]

Research conducted by Zhou et al. (2013) in a cohort study of Chinese adults also conforms our findings that the BDNF (Val66Met) polymorphism is positively associated with type 2 diabetic patients.[15]

The findings of Krabbe et al. (2007), who examined a Caucasian population for differences in genotypic frequencies between diabetics and healthy controls, disagree with our research.[16] Similarly, Zhen et al. (2018) discovered no considerable difference in genotypic frequencies in those with diabetes and healthy groups in a Han Chinese community. The BDNF rs6265 (Val66Met) SNP may elevate the likelihood of diabetic complications without directly influencing the risk of diabetes.[17]

This study also found the presence of the wild genotype GG and the heterozygote genotype GA, while the absence of the genotype AA (0%) in sequencing results ; this may be due to the evidence that the distribution of the BDNF polymorphisms varies widely among different ethnic groups also population and factors related to the environment play a role in the genetic studies across the world and this result may be specific to the Iraqi population, this finding is in agreement with Abbas et al. (2022).[18] Therefore, ethnic and racial differences may account for the observed discrepancies in the BDNF rs6265 genotype distribution and allele frequencies across studies conducted on other populations. This finding agrees with that of Pivac et al. (2009), who found that variations in the distribution of this gene could be the product of environmental influences or natural selection acting on a particular allele.[19]

5. CONCLUSION

The present study showed that the BDNF (Val66Met) rs6265 polymorphism was associated with Type-2 diabetes mellitus and diabetic polyneuropathy in heterozygous allele G/A (Met/Met) and homozygous allele GG (Val/Val) genotype. Also, the current study found that the absence of the mutant genotype AA, is possibly due to the evidence that the distribution of the BDNF polymorphisms varies widely among different ethnic groups.

7. Data availability

The numerical data generated during this research is available with the authors.

8. Acknowledgement

We are particularly grateful to all the people who helped with our article.

9. Conflict of interest

The study utilized the departmental resources only, and no external or industry funding was involved.

10. Authors' Declaration

We certify that each one of the figures and tables included in this paper are original work. Moreover, the permission needed for re-publication has been added to the article, and any figures and photographs not ours have been included.

11. Authors' contribution

RH: writing the manuscript

EAW: conduction of the study work and manuscript editing

AMJ: conduction of the study work and manuscript editing

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