INTRODUCTION

The neurotrophin family contains growth factors that promote cell survival, differentiation, and death, and brain-derived neurotrophic factor (BDNF) is widely expressed in the adult mammalian brain [1]. It’s important for the long-term survival, differentiation, and expansion of neurons during development, as well as the preservation of neural systems in adults [2]. Neurotrophin theory proposes that failure of neurogenesis and neural plasticity causes many psychiatric disorders such as schizophrenia and major depression, and BDNF is regarded to be one of the essential components in this theory [3]. They’re made up of preforms that can be broken inside the cell to release mature, secreted ligands. Mature neurotrophins bind to the Trk family of receptor tyrosine kinases, promoting Trk-mediated differentiation or survival [4]. Proneurotrophins are not inactive precursors because they can be released and cleaved extracellularly and serve as high-affinity ligands for p75 NTR, which causes apoptosis in neurons and oligodendrocytes [5]. Each neurotrophin also activates the p75 neurotrophin receptor (p75NTR), which belongs to the tumor necrosis factor receptor superfamily. Neurotrophins activate Ras, phosphatidylinositol-3 (PI3)-kinase, phospholipase C-1, and signaling pathways mediated by these proteins, such as MAP kinases, through Trk receptors [6]. In obese, non-insulin-dependent diabetic mice with a simultaneous decrease in body weight, BDNF reduced nonfasting blood glucose levels without a significant reduction in food consumption per body weight. In the db/db mice model, which are identified by nonfunctional leptin receptors and provide a model for obesity and non-insulin-dependent T2DM, 3 weeks of intermittent BDNF injection dramatically lowered blood glucose concentrations and glycated hemoglobin (HbA1c) [7]. The nuclear factor-B (NF-B) and Jun kinase, as well as other signaling pathways, are activated when the p75NTR is activated [8]. The gene that also goes by the name BDNF codes for the BDNF protein. This gene is found on chromosome 11 in humans. Val66Met (rs6265) is a single nucleotide polymorphism in the gene that causes a difference in valine and methionine at codon 66 due to adenine and guanine alleles [9].

THE AIM

In this study, we looked into the possible link between the G196A polymorphism in the BDNF gene and DM in Iraqi patients.

MATERIALS AND METHODS

Our study included 100 participants in age from 30 to 65 years old (mean age47.5, SD 9.6) and were divided into two
groups: DM (50 patients) and control (50 participants). This study was conducted in Karbala City, the capital of Karbala Province, which is located in the heart of Iraq from December 2020 to August 2021.

GENOTYPING
All participants had four milliliters of venous blood drawn into EDTA anticoagulant polypropylene tubes. All participants gave written agreement to be included in the study and were told about it. The Karbala University of Medical Science Ethical Committee evaluated and approved our protocol. A simple salting-out process was used to extract genomic DNA from patients’ peripheral blood leukocytes [10]. A polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) approach was used to identify the BDNF Val66Met polymorphism (dbSNP ID rs6265) at position G196A, as previously reported. In brief, PCR was used to amplify the 272-bp region of the BDNF gene, which included the Val66Met polymorphism location. The restriction enzyme NlaIII was used to digest the PCR product overnight at 37 °C. Electrophoresis on an 8% polyacrylamide gel was used to identify the genotype, which was then stained with ethidium bromide and observed under ultraviolet light[11].

ANALYTICAL STATISTICS
Analysis of covariance (ANCOVA) was used to assess the relationship between the Val66Met polymorphism between control and DM genotypes/frequencies between patients. We considered probability values of 0.05 or less to be statistically significant in this investigation. SPSS v. 26 was used for statistical analysis.

RESULTS
Table (I) summarizes the baseline phenotypes and Val66Met genotypes of the participants in our study. The control group (normal HbA1C) and the patients were divided into two groups. Whenever the genotype frequencies of the Val66Met polymorphism were compared between these two groups, significant differences (P < 0.05) were found, table (II). Figure (1) was showed the Agarose Gel Electrophoresis of the DNA, figure (2) was showed that BDNF region Amplicons Bands, and figure (3) was showed Tex Shade of SNP sequences.

**Table I. Participants’ baseline phenotypes and rs6265 (Val66Met) genotype distribution**

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual (n) DM group</td>
<td>50</td>
</tr>
<tr>
<td>Age mean (years)</td>
<td>47.5</td>
</tr>
<tr>
<td>HbA1C mean (kg/m2)</td>
<td>7.4</td>
</tr>
<tr>
<td>Individual (n) control group</td>
<td>50</td>
</tr>
<tr>
<td>Age mean (years)</td>
<td>40.9</td>
</tr>
<tr>
<td>HbA1C mean (kg/m2)</td>
<td>5.2</td>
</tr>
</tbody>
</table>
DISCUSSION
We investigated the putative link between the Val66Met (rs6265) polymorphism of the BDNF gene and DM in 40 patients in this study, and to our knowledge, this is the first study of its kind in Iraq. In this investigation, the Val66Met polymorphism was found to be strongly associated to DM, as well as DM with G/A (Met-Met) and GG (Val-Val) genotypes. DM is unquestionably exacerbated by metabolic and genetic variables, such as a lack of physical activity and the consumption of high-fat meals [12]. Differences in genetic makeup, on the other hand, may lead to variations in risk of DM in a group living in the same environment [13]. The BDNF gene encodes a neurotrophin family of growth factors that bind to the tyrosine kinase receptor tropomyosin-related kinase B (TrkB) and activate signaling [14]. It became pertinent to select and study such a gene that is common to DMT2 and Neurological disorder. BDNF was found to be one of such genes. Owing to this great significance of BDNF it was selected as target gene to be studied in terms of its SNPs [15]. A list of reported SNPs in BDNF were searched in dbSNP and one of them rs6265 at position 27,658,369 on chromosome 11 was selected for further studies. The aim was to check the frequency of this G to A Polymorphism in the test samples [16]. For this, the primer set was designed and amplification was performed on 100 diabetic samples. No control was taken as the purpose was to check the frequency of SNP in DMT2 samples only and not to compare with Non diabetic ones. The sequencing result when compared with Non diabetic ones. The sequencing result when compared with Non diabetic ones. No control was taken as the purpose was to check the frequency of SNP in DMT2 samples only and not to compare with Non diabetic ones. The sequencing result when analyzed by Bioinformatics tool clearly indicated that four diabetic samples (Sample number 2, 3, 4 and 6) were found to have A instead of G (rs6265) at position (chr 11: 27,658,369) that corresponds to val66met polymorphism.

CONCLUSIONS
The connection of the Val66Met polymorphism of the BDNF gene with DM as a DM trait was validated in the population of central Iraq in our study. Patient with the AG (Val-Met) genotype had a 40% of total DM patients than those and GG (Val-Val) genotypes.

RECOMMENDATION
Further study on a wider and large sample set is recommend-ed. As a future aspect of the report the work can be further extended on proteomic level wherein the corresponding change occurred due to the mutation in the protein can be further detected at structural and functional level.

REFERENCES

Table II. DM Patients genotype frequencies

<table>
<thead>
<tr>
<th>Genotype</th>
<th>DM occur</th>
<th>DM Not occur</th>
<th>OR</th>
<th>95% CI Lower</th>
<th>95% CI Upper</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/G</td>
<td>2 (8%)</td>
<td>23 (92%)</td>
<td>0.005</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/A</td>
<td>19 (95%)</td>
<td>1 (5%)</td>
<td>0.084</td>
<td>0.022</td>
<td>0.319</td>
<td></td>
</tr>
<tr>
<td>cohort DM = Occur</td>
<td>18.40</td>
<td>2.71</td>
<td>124.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cohort DM = Not occur</td>
<td>0.005</td>
<td>&lt; 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ORCID and contributionship:
Saly Naser Abbas: 0000-0002-4143-4142 A-F
Hajer Alaa Obeid: 0000-0001-9675-3450 A-F
Tahreer Shannan Alwan: 0000-0003-3522-3477 A-F
Saif M. Hassan: 0000-0003-4655-8045 A-F
Mahmood J. Jawad: 0000-0002-0949-3919 A-F
Mohammed J. Jawad: 0000-0002-6096-945X A-F
Najah R. Hadi: 0000-0001-9084-591X A-F

Conflict of interest:
The Authors declare no conflict of interest.

CORRESPONDING AUTHOR
Najah R. Hadi
University of Kufa
29CG+62H, Kufa, Iraq
e-mail: drnajahhadi@yahoo.com

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