Molecular and Cellular Endocrinology 429 (2016) 106–113

Contents lists available at ScienceDirect

Molecular and Cellular Endocrinology

journal homepage: www.elsevier.com/locate/mce

Brain derived neurotrophic factor in newly diagnosed diabetes and prediabetes

Wei Liu, Xueyao Han, Xianghai Zhou, Simin Zhang, Xiaoling Cai, Lihua Zhang, Yufeng Li, Meng Li, Siqian Gong, Linong Ji*

Department of Endocrinology, Peking University People's Hospital, Beijing, China

ARTICLE INFO

Article history:
Received 18 January 2016
Received in revised form
4 April 2016
Accepted 6 April 2016
Available online 7 April 2016

Keywords:
Brain derived neurotrophic factor
Glucose
Polymorphism
Methylation

ABSTRACT

Brain derived neurotrophic factor (BDNF) is thought to play an important role in glucose metabolism, but the exact mechanism has not been elucidated. The aim was to assess differences in serum BDNF levels across individuals with varying levels of glucose tolerance, and the association of serum BDNF levels with genetic variants and DNA methylation. Participants were selected from an ongoing population-based cohort study in rural China. In a randomly selected subsample of healthy participants (n = 33 males, n = 52 female), we assessed serum BDNF and in n = 50 of these, also DNA methylation. In a second subsample (all women; n = 28 with diabetes, n = 104 with prediabetes, and n = 105 age- and body mass index (BMI)-matched controls), we assessed serum BDNF and genetic variants. In a third subsample (all with diabetes; n = 7 normal BMI + low insulin level, n = 9 normal BMI + high insulin level, n = 9 obese + high insulin level), we assessed DNA methylation. Compared to age- and BMI-matched controls (24.71 (IQR, 20.44, 29.80) ng/ml), serum BDNF was higher in participants with prediabetes (27.38 (IQR, 20.64, 34.29) ng/ml), but lower in those with diabetes (23.40 (IQR, 18.12, 30.34) ng/ml) ($P < 0.05$). Two genetic variants near BDNF (rs4074134 and rs6265) were confirmed to be associated with BMI. BDNF CpG-6 methylation was positively associated with waist-to-hip ratio ($P < 0.05$). Furthermore, hypermethylation in this site was found in participants with diabetes and high fasting insulin levels compared to those with diabetes and low fasting insulin levels, regardless of BMI status ($P < 0.001$ and $P = 0.001$, respectively). Observed differences in serum BDNF levels, genetic variants, and DNA methylation patterns across different glucose metabolic state suggest that BDNF may be involved in the pathophysiological process of insulin resistance and type 2 diabetes.

1. Introduction

Brain derived neurotrophic factor (BDNF) is a neurotrophin encoded by the BDNF gene and is involved in regulating survival, growth, and maintenance of neurons. Most studies have focused on its role in mental disorders such as schizophrenia, Alzheimer's disease, and depression. However, recent evidence suggests that the brain plays an important role in the maintenance of glucose homeostasis (Schwartz et al., 2013), and BDNF has been shown to be involved in the central control of glucose metabolism, with functions downstream of the leptin-proopiomelanocortin (POMC) signaling pathway (Xu et al., 2003).

The clinical significance of BDNF levels in individuals with type 2 diabetes mellitus (T2DM) is unknown. Some studies have found that BDNF levels are lower in individuals with T2DM compared to non-diabetic individuals, both in plasma and serum, and in different populations (Krabbe et al., 2007; Fujinami et al., 2008). Other studies have found higher serum BDNF levels in individuals with T2DM compared to non-diabetic individuals (Suwa et al., 2006). Higher plasma and serum BDNF levels have been associated with cardiometabolic risk factors, including body mass index (BMI) (Golden et al., 2010; Nofuji et al., 2008), suggesting that differences in circulating BDNF levels in individuals with and without T2DM may be secondary to dysregulated energy balance.

Genome-wide association studies (GWAS) have provided evidence that the common BDNF gene polymorphism, Val66Met (rs6265: valine > methionine) is associated with obesity (Takeuchi et al., 2011; Wu et al., 2010). Our previous work has shown that the common BDNF variant, rs4074134, is associated with T2DM

* Corresponding author. No 11, Xi Zhi Men Nan Da Jie, Beijing, China.
E-mail address: jiln@bjmu.edu.cn (L. Ji).

http://dx.doi.org/10.1016/j.mce.2016.04.002
0303-7207/

independent of obesity in the Chinese Han population, and that this variant also has an effect on plasma glucose concentration, BMI, and insulin sensitivity (Han et al., 2013). However, it is still unclear whether these single nucleotide polymorphisms (SNPs) are associated with serum BDNF levels.

A cross-sectional study found that food responsiveness was higher and satiety responsiveness lower among obese versus non-obese female children, and analysis of the BDNF promoter revealed associations between methylation level and altered satiety responsiveness (Gardner et al., 2015). However, robust evidence is still lacking regarding changes in BDNF methylation in association with disruptions in glucose metabolism.

The objectives of the present study were to: (1) define the average level of serum BDNF in a healthy Chinese population and evaluate levels across different glucose metabolic states; (2) assess the association between common SNPs related to the BDNF gene and serum BDNF level; and (3) explore the relationship between BDNF methylation and markers of cardio-metabolic function.

2. Subjects and methods

2.1. Subjects

From March 2012 to May 2013, we conducted a population-based study of diabetes and metabolic syndrome in a rural region of China (Pinggu, located about 50 miles outside Beijing). Using two-stage stratified random cluster sampling, 5004 residents aged 25–75 years were randomly selected and 3345 took part in the study (response rate: 66.85%). Individuals with known major depressive disorder or other mental disorders were not eligible. Anthropometric assessment (height, weight, waist circumference, and blood pressure) and biological specimen collection were completed on all participants by trained study staff using standardized procedures. Participants not reporting a physician diagnosis of diabetes were given a 75-g oral glucose tolerant test (OGTT). The institutional review board at Peking University People's Hospital approved the protocol and written informed consent was obtained from all participants before initiating the study protocols.

For this ancillary study, we assessed serum BDNF, DNA methylation, and BDNF gene SNPs, using stored biological specimens from three subsamples (Fig. 1):

1) For serum BDNF, n = 33 males and n = 52 females meeting the following criteria were randomly selected from the larger cohort as the "healthy subsample": aged 25–50 years, no disease history, no medications in the past 2 weeks, not current or former smoker, normal weight (BMI ≥ 18.5 kg/m^2 and <25 kg/m^2), waist circumference <90 cm for males and <85 cm for females, systolic blood pressure (SBP) < 140 mmHg and diastolic blood pressure (DBP) < 90 mmHg, normal liver and kidney function (serum alanine aminotransferase (ALT) ≤50 U/L, serum aspartate aminotransferase (AST) ≤40 U/L, and serum creatinine <100 μmol/L), lipid levels within the normal range (total cholesterol (TC) < 6.2 mmol/L, triglycerides (TG) < 1.7 mmol/L, low density lipoprotein cholesterol (LDL-C) <4.1 mmol/L, and high density lipoprotein cholesterol (HDL-C) ≥0.9 mmol/L for males and ≥1.0 mmol/L for females), fasting plasma glucose <6.1 mmol/L, OGTT 2 h glucose >4.0 mmol/L and <7.8 mmol/L, hemoglobin A1c (HbA1c) < 6.0%, serum uric acid <428 μmol/L, urinary microalbuminuria and creatinine ratio <26 mg/g for males and <32 mg/g for females, white blood cell count >4 × 10^9/L, hemoglobin ≥120 g/L for males and ≥110 g/L for females. Because BDNF levels can be affected by many factors such as BMI, age, sex, menopause status, and smoking (Suwa et al., 2006), in order to better control these possible confounders, in all female participants who were non-smokers, non-menopausal, and without a history of hyperglycemia, pharmaceutical treatment, or behavior modification therapy, the World Health Organization (WHO) 1999 diagnostic criteria were applied to define diabetes (DM), prediabetes (PreDM) and normal glucose tolerance (NGT). A total of n = 28 DM and n = 104 PreDM participants were defined, and 105 age- and BMI-matched NGT participants were randomly selected as controls, and serum BDNF levels were assessed.
2) SNP analyses were conducted in the n = 28 DM, n = 104 PreDM, and n = 105 age- and BMI-matched controls.
3) A total of n = 50 randomly selected participants from the healthy subsample were selected for DNA methylation profiling of the BDNF gene. DNA methylation profiling was also performed for n = 7 participants with diabetes, a normal BMI, and low insulin level; n = 9 participants with diabetes, a normal BMI, and high insulin level; and n = 9 participants with diabetes, an obese BMI, and high insulin levels. Normal and obese BMI were defined by using BMI cut-point 25 kg/m^2 and 30 kg/m^2, and in-terquartile ranges of fasting insulin levels in the healthy cohort were used to define low and high insulin levels.

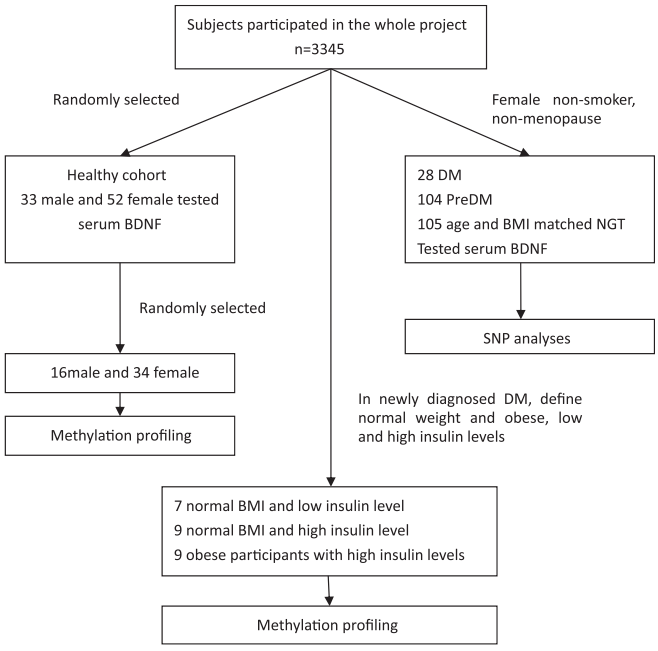

Fig. 1. Subject flowchart. In the whole population, we took out three parts for different analyses purposes. Serum BDNF level and methylation profiling were taken out in the healthy cohort; methylation profiling was taken out in newly diagnosed DM grouped by BMI and fast insulin level; Serum BDNF level and SNP analyses were performed in newly diagnosed DM, PreDM and age&BMI matched NGT. BMI, body mass index; NGT, normal glucose tolerance; PreDM, pre-diabetes; DM, diabetes; SNP, single nucleotide polymorphisms.

2.2. Laboratory examination

Blood samples were obtained by venipuncture in the morning after 8 h of fasting. Samples drawn into tubes containing EDTA were immediately placed at 4 °C and stored at −80 °C within 2 h, and the clotted blood was spun at 3500×g for 15 min at 4 °C within 1 h and isolated serum was stored at −80 °C. HbA1c was measured by automated high-performance liquid chromatography system (Primus Ultra^2, Trinity Biotech, Bray, Co Wicklow, Ireland). AST, ALT,