Neuroscience Research 91 (2015) 1–7

Contents lists available at ScienceDirect

Neuroscience Research

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

BDNF promoter I methylation correlates between post-mortem human peripheral and brain tissues

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ARTICLE INFO

Article history:
Received 19 June 2014
Received in revised form 25 August 2014
Accepted 6 October 2014
Available online 17 October 2014

Keywords:
Epigenetics
BDNF
Promoter
Methylation
Prefrontal cortex
Quadriceps

ABSTRACT

Several psychiatric disorders have been associated with CpG methylation changes in CG rich promoters of the brain-derived neurotrophic factor (*BDNF*) mainly by extracting DNA from peripheral blood cells. Whether changes in peripheral DNA methylation can be used as a proxy for brain-specific alterations remains an open question. In this study we aimed to compare DNA methylation levels in *BDNF* promoter regions in human blood cells, muscle and brain regions using bisulfite-pyrosequencing. We found a significant correlation between the levels of *BDNF* promoter I methylation measured in quadriceps and vPFC tissues extracted from the same individuals ($n = 98$, Pearson, $r = 0.48$, $p = 4.5 \times 10^{-7}$). In the hippocampus, *BDNF* promoter I and IV methylation levels were strongly correlated (Pearson, $n = 37$, $r = 0.74$, $p = 1.4 \times 10^{-7}$). We found evidence for sex-dependent effect on *BDNF* promoter methylation levels in the various tissues and blood samples. Taken together, these data indicate a strong intra-individual correlation between peripheral and brain tissue. They also suggest that sex determines methylation patterns in *BDNF* promoter region across different types of tissue, including muscle, brain, and blood.

1. Introduction

The brain-derived neurotropic factor (BDNF) is a family member of neurotrophins, which are important regulators of neuronal development, plasticity and survival (Lu and Figurov, 1997). Alterations in BDNF related pathways are thought to be involved in various neurological and psychiatric disorders (Autry and Monteggia, 2012). For example, genetic variation in the *BDNF* gene has been associated with bipolar disorder (Neves-Pereira et al., 2002; Lohoff et al., 2005; Sears et al., 2011), Rett syndrome (Zeev et al., 2009), Parkinson disease (Chen et al., 2007), suicidal behaviours (Perroud et al., 2008; Pregelj et al., 2011) and schizophrenia (Zakharyan and Boyajyan, 2014). At an epigenetic level, environmental factors have been shown to affect the methylation status of various *BDNF* promoters among which promoter IV was the most investigated. In rodents and humans, early-life adversity has been associated with changes in the methylation status of *BDNF* (Berton et al., 2006; Ivy et al., 2008; Roth et al., 2009; Perroud et al., 2013). In rodent models of early-life stress, increased methylation of *BDNF* promoter IV was found to be associated with decreased mRNA expression of *BDNF* in the prefrontal cortex (Ivy et al., 2008; Roth et al., 2009). In addition to early-life adversity, *BDNF* methylation patterns appear to be sex-dependent (Ikegame et al., 2013). A recent study comparing *BDNF* promoter I and IV methylation levels between 100 controls and 100 schizophrenic patients reported a gender-dependant DNA methylation status in peripheral blood (Ikegame et al., 2013). A major limitation to these human studies relates to the fact that *BDNF* methylation levels are generally measured in DNA extracted from blood cells (Ikegame et al., 2013; Perroud et al., 2013). Whether methylation levels in blood cells are relevant indicators of changes occurring in the brain remains an open question. To test this hypothesis, we aimed to compare DNA methylation patterns in *BDNF* promoter regions in blood cells extracted from a cohort of living bipolar patients

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http://dx.doi.org/10.1016/j.neures.2014.10.003
0168-0102/

and muscle and brain regions extracted from patients who died from different causes. We also aimed at determining whether sex-differences in *BDNF* methylation status could be observed across tissues. Subjects with psychiatric disorders were selected for this study as *BDNF* has been previously shown to be dysregulated in this population affecting brain functions. Moreover, such samples are influenced by environmental factors known to contribute to epigenetic dis-regulations and psychopathology.

2. Material and methods

2.1. Subjects and clinical data

Detailed demographic information of patients used in this study is provided in supplementary material in Table 1S.

Ventral prefrontal cortex (vPFC) and quadriceps tissues of 98 individuals as well as hippocampus of another 37 subjects were collected post-mortem during medico-legal autopsy performed by forensic physicians.

Since we did not have access to the blood of deceased subjects from whom the vPFC and quadriceps tissues were collected, we used blood from one hundred and twenty-one subjects with a primary diagnosis of bipolar disorder (BD) who were recruited in our specialized mood disorder clinic. Subjects were diagnosed based on a clinical evaluation and assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al., 2012).

This study was approved by the research and ethics review board of the Department of Mental Health and Psychiatry of the University Hospitals of Geneva. Informed written consent was obtained either from patients or from their families when a patient was deceased.

2.2. Collection and dissection of post-mortem vPFC, hippocampus and muscle tissues

Human brain and muscle tissues were collected at autopsy from the Institute of Forensic Medicine, Geneva, Switzerland. The brain was removed from the cranium and was examined for neuropathological abnormalities. Dissection of the vPFC as well as the hippocampus was performed by forensic physicians and skilled technicians. Tissue blocks from the ventral prefrontal cortex (vPFC; Brodmann's area 11) were dissected in 0.5- to 1-cm coronal slices from the right hemisphere. Samples of muscle tissue were drawn from quadriceps. The samples were immediately stored at −80 °C. Brain samples were then carefully dissected on a glass surface with ice, in accordance with Brodmann's Atlas. Only grey matter was carefully isolated. Samples were then stored at −80 °C until DNA extraction.

2.3. DNA extraction and bisulfite conversion

Approximately 100 mg of vPFC, quadriceps or hippocampus tissues were homogenized using an Ultra-Turrax® T8 homogenizer (IKA, France). Genomic DNA was extracted from homogenized tissue using the Illustra Nucleon Genomic DNA Extraction kit (GE Healthcare, Dübendorf, Switzerland). The same kit was used to extract DNA from blood samples of living BD patients. Bisulfite conversion of unmethylated cytosines to uracil was performed on 2 μg of DNA using the EpiTect Bisulfite Kit (Qiagen, Germany). Bisulfite treated samples were resuspended in 20 μl EB Buffer and were stored at −20 °C.

2.4. PCR amplification and pyrosequencing

Methylation levels of 4 CpG sites were measured in *BDNF* promoter I in vPFC, quadriceps and blood samples using the pyrosequencing assay named "assay A". The original sequence is located in the coordinates chr11:27743930-27744048 on the hg19 human version on the negative strand and corresponds to: GGGCTGTTAACTCACATTTGGGAAGCCATAACCCATTAGAGCAAA[1]-(CG)CAGTCATAACTTCATTCAACTCAGC[2](CG)CT[3](CG)AGAGCT[4]-(CG)GCTTACACAGGTTCCTGT GGGCAACTAGTGGCT . Bases underlined show the binding sites of the amplification primers. The CpG sites tested are numbered and appear in parenthesis. The amplification primers to measure methylation levels in the four CpG sites within *BDNF* promoter I were designed using MethPrimer software (Li and Dahiya, 2002). Forward primer: 5′-GGG**T**TGTTAA**TTTAT**ATTTGGGAAG**T**-3′ (bold: C converted to T) and 5′-biotinylated reverse primer (5′-A**A**CCACTA**A**TT**A**CCCACA**AA**-AACC-3′ (bold: G converted to A) were used for PCR amplification of the bisulfite converted DNA leading to a 119 bp product. The product was sequenced with the forward primer. The sequence to analyze was: **T**ATAA**TTT**ATTAGAG**T**AAA[1](YG)**T**AGT-**T**ATAA**TTT**ATT**T**AA**TTT**AG**T**[2](YG)**T**T[3](YG)AGAG**TT**[4](YG)G**T**TTA**T**-A**T**AGGTT**TT**TGTGGG**T**AA**T**TAGTGG**T**T where the four "YG" (CG or TG) correspond to the four CpG sites from 1 to 4 respectively. Bold T corresponds to cytosine bisulfite-converted to thymine.

Additionally, methylation levels of 13 CpG sites in the *BDNF* promoter I, using the pyrosequencing assay named "assay B", as well as 15 CpG sites in the *BDNF* promoter IV, using the pyrosequencing assay named "assay C", were measured in hippocampal tissue. In the pyrosequencing assay named "assay B" for measurements performed on 13 CpG sites in *BDNF* promoter I, the original sequence is located in the coordinate chr11:27743698–27744046 on the hg19 human genome version and corresponds to: GGGTAAAAAA-AGGAAACTTCTTAGAAAAGTT(CG)TGCCCCTCCCCCTCCCCCATC ATGACTAAGGGTC TCCAGC[1](CG)ATGAGGT[2](CG)TGAGTGATG-ATCAAATGGGGACTGGGGGGAGGGGGG[3](CG)AGTAAGTGACTTGT-CCTTGGGAACATCTGCATG[4](CG)T[5](CG)AAG[6](CG)[7](CG)AACCAGCC-CAACAACTTTCCCTTTTCCTCTTAGGTACTGATGACTAGG[8](CG)AGAG-GCACCAAGG[9](CG)AGCCACTAGTTGCCCACAGGAACCTGTGTAAG-C[10](CG)AGCTCT[11](CG)AG[12](CG)GCTGAGTTGAATGAAGTTATGAC-TG[13](CG)TTTGCTCTAATGGGTTATGGCTTCCCAAATGTGAGTTAACA-GC. Bases underlined show the binding sites of the amplification primers for human bisulfite converted DNA and resulted in a 349 bp amplicon. The bases located in the box represent the binding sites of the sequencing primer. The CpG sites tested are numbered and appear in parenthesis. Forward (F), reverse (R) and sequencing (S) primers used to measure methylation percentages in 13 CpG sites in *BDNF* promoter I were; F: 5′-GGGTAAAAAAAGGAAA**T**TT**T**TTA-GAAAAGT-3′ (bold T corresponds to cytosine converted to thymine), R: biotin-5′-**A**CT**A**TTAACTCACATTT**AAA**AAA**A**CCATA-3′ (bold A corresponds to guanine converted to adenine), S: 5′-**TTT**-T**TTTTT**ATT**A**TGAT**T**AAGGGT**T**-3′ (bold T corresponds to cytosine converted to thymine). The sequence to analyze was T**TT**AG**T**[1](YG)-ATGAGGT[2](YG)TGAGTGATGAT**T**AAATGGGGA**T**TGGGGGGAGGGG-GG[3](YG)AGTAAGTGA**T**TTGT**TT**TTGGGAA**T**AT**T**TG**T**ATG[4](YG)T[5]-(YG)AAG[6](YG)[7](YG)AA**TT**AG**TTT**AA**T**AA**T**TTT**TTT**TTTT**TT**TTTAGG-TA**T**TGATGA**T**TAGG[8](YG)AGAGG**T**A**TT**AAGG[9](YG)AG**TT**A**T**TAGTT-G**TTT**A**T**AGGAA**TT**TGTGTAAG**T**[10](YG)AG**T**T**T**[11](YG)AG[12](YG)G**T**T-GAGTTGAATGAAGTTATGA**T**TG[13](YG)TTTG**T** where the 13 "YG" representing either CG or TG correspond to the 13 CpG sites from 1 to 13 respectively. Bold T corresponds to cytosine bisulfite-converted to thymine.

Primers used for the pyrosequencing assay named "assay C" developed to measure methylation levels in 15 CpG sites in *BDNF* promoter IV as published previously (Keller et al., 2010) were F: 5′biotine-TTTG**T**TGGGG**T**TGGAAGTGAAAA**T**-3′ (bold T correspond to cytosine converted to thymine), R: 5′-CCCATCA**A**CAA-**A**AA**A**CTCCATTT**A**ATCTC-3′ (bold A correspond to guanine converted to adenine), S: 5′-ACA**AAA**AA**A**ATTTCAT**A**CTA**A**-3′ (bold