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DNA Methylation of *DLG4* and *GJA-1* of Human Hippocampus and Prefrontal Cortex in Major Depression is Unchanged in Comparison to Healthy Individuals

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Abstract

Epigenetic alterations provide a potential mechanism to account for the numerous gene-environment interactions that have been reported in association with neuropsychiatric phenotypes. In context to major depression disorder (MDD), where postmortem and neuroimaging studies provide insights into dysfunctional brain regions, involvement of genetic heterogeneity also revealed the complexity of this disorder. Despite intensive research during the past several decades and information from genome wide studies, pathophysiology of depressive disorders remained elusive. To evaluate the impact of epigenetic pressure on this disease, we took advantage of DNA isolated from different sections of human brain (prefrontal cortex and hippocampus) from clinically well-defined depressed patients and healthy individuals and performed pyrosequencing for DNA methylation analysis. Herein, we focused on two genes *DLG4* (*PSD-95*) and *GJA-1* (*Connexin43*) known to be associated with neuropsychiatric behavior. Comparing MDD with controls we found no differences of DNA methylation. Our results clearly demonstrate that DNA methylation levels on these particular genes are not associated with depression related phenotype.

Keywords: Depression; DNA methylation; *DLG4 (PSD-95; Connexin43 (GJA-1)***3**

Introduction

Epigenetic regulations have been implicated in several human diseases, including neuropsychiatric disorders [1-3]. Studies from animal models have shown that early life stress can leave persistent epigenetic marks in the genome, which alter gene expression and can later influence neural and behavioural function through adulthood [4-6]. Furthermore, administration of epigenetic inhibitor has also been shown to produce an antidepressant effect in an animal model system [7]. Involvement of epigenetics in human depression, which is a psychological condition that presents with wide-ranging symptoms along with neuronal structural changes in brain has been widely discussed [8-12]. In this particular study, we selected two independent genes (*DLG4* and *GJA-1*) which are known to be associated with neuropsychiatric behaviours. We determined their epigenetic status by pyro sequencing method by using tissues samples isolated from different sections of brain regions (PFC: Prefrontal cortex, HIP: Hippocampus) from individuals with MDD vs. healthy controls. First, we selected *DLG4* (*discs large homolog 4)* gene, which encodes a protein named *PSD-95* (post-synaptic density protein 95) also known as *SAP-90* (*synapse-associated protein 90*) and has been previously implicated in studies related to depression [13-15]. Interestingly, levels of NMDA receptors and *PSD-95*, were found to be reduced in the post-mortem samples of prefrontal cortex Brodmann's area 10 (BA10) in depressed patients as compared to psychiatrically healthy controls [16]. However, increased immunoreactivity levels of *NR2A* and *PSD-95* were reported in amygdala samples of depressed individuals [17]. Therefore, for this particular gene on chromosome 17, we selected six *CpG* sites embedded between *CpG* Island 41 and neighbouring *ACADVL* gene. To evaluate DNA methylation, we designed pyro sequencing analysis for two independent genomic locations within *DLG4* locus: *CpG* 1-3 (Chromosome 17: 7215813-7215862 bp) and *CpG* 4-6 (Chromosome 17:7216811-7216860 bp) respectively (Figure 1A). In the absence of a well-defined 4 promotor region, our analysis on selected *CpG* sites clearly demonstrates that although methylation levels for *CpG* 1-3 are higher (10% to 15% methylation range) than *CpG* 4-6 (5% to 10% methylation range), no observable differences exists among patients and healthy controls (Figure 1B). Furthermore, we extended our analysis on *a* second gene named *GJA-1* (Gap junction alpha-1 protein) which encodes protein known as *Connexin43 (Cx43)* and has been previously shown as down regulated in expression specifically in major depressive disorder [18-21]. Reduced levels of *Cx43* and *Cx30* mRNA in the dorsal lateral prefrontal cortex (DLPFC) of subjects diagnosed with psychiatric disorder and committed suicide are described previously [22]. Similarly, low levels of *Cx43* mRNA were detected in the locus coeruleus (LC) in depression subjects [23]. At this chromosome 6 locus, we determined methylation status at seven *CpG* sites of *GJA-1* genomic region as previously described by Jinn et al. [24]. Here again, our pyro sequencing analysis did not detect any significant difference among patients and healthy individuals. The DNA methylation levels were found to be very low (1% to 3%) for these selective sites (Figure 1C). Moreover, it has been argued that women have stronger tendency towards depression than men [25], but in our analysis no difference was observed among male and female subgroups, indicating that gender has no impact on DNA methylation levels for these particular genes in depression phenotype. In conclusion, *DLG4* and *GJA-1* did

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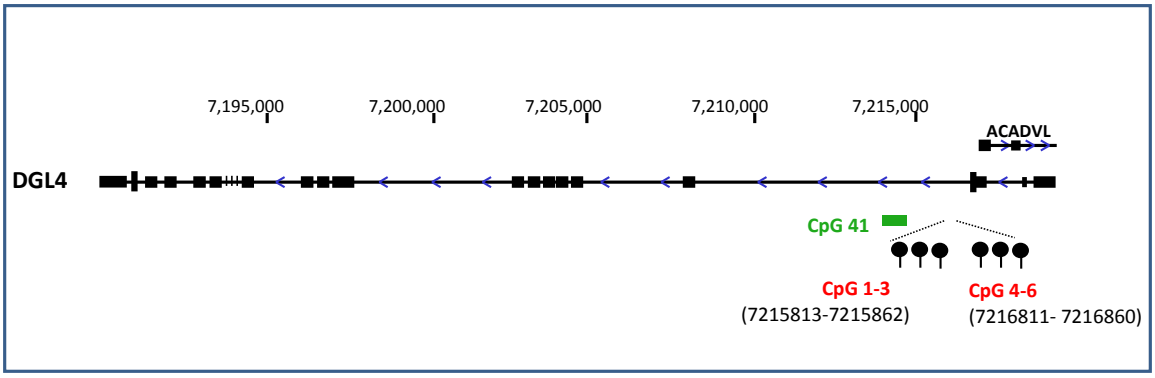
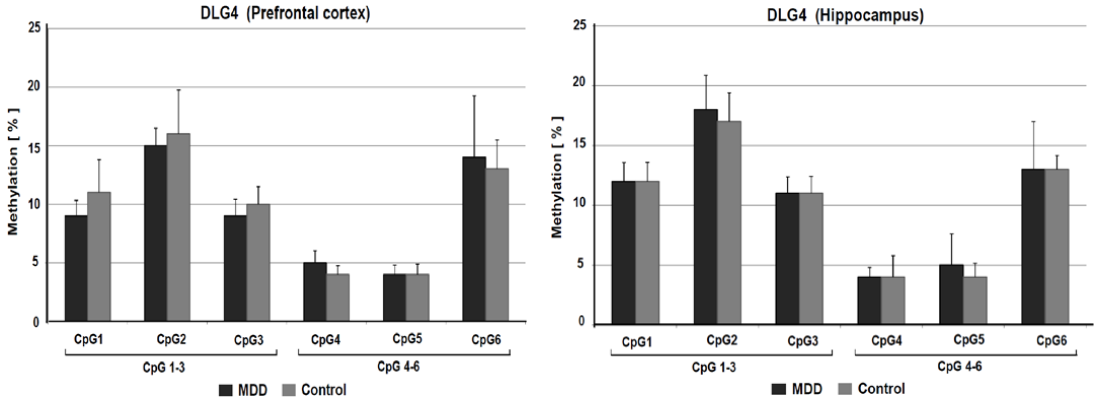
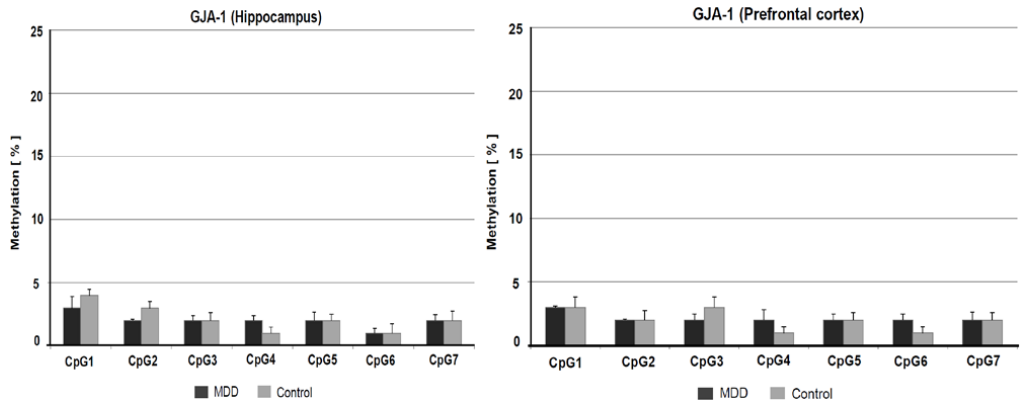


A) Genomic structure of *DLG4 (PSD-95)* showing six CpG sites (CpG1-3 and CpG4-6) selected to compare patients with major depressive disorder (MDD) and healthy controls.



B) Pyrosequencing results of *DLG4* gene [Prefrontal cortex (Left panel) and *Hippocampus* (right panel)] have been demonstrated.



C) DNA methylation levels measured at seven CpG sites of *GJA-1* locus has been shown for prefrontal cortex and hippocampus regions of the brain.

Figure 1: DNA methylation analysis in depression phenotype.

not showed any alteration of DNA methylation in brain tissues isolated from depression patients.

Subjects and Methods

Genomic DNA from brain tissues of six individuals diagnosed with MDD (4 females, 2 males) were used, aged 76.3 ± 19.5 years (PFC) and 76.8 ± 19.6 years (HIP), and the mean post-mortem intervals were 5.6 ± 1.0 h (PFC) and 6.25 ± 1.6 h (HIP). Control tissue specimens had a mean tissue pH of 6.8 ± 0.3 and mean postmortem interval of 6.1 ± 0.7 h and were obtained from six healthy donors (4 females, 2 males) aged 78.8 ± 14.2 years. DNA was isolated using standard protocols. Detail description of patient samples has been given in Kaut et al., 2015 [26]. Bisulfite conversion for methylation analysis was performed using the EZ DNA Methylation-Gold Kit (Zymo Research, Hiss Diagnostics, Freiburg, Germany) according to the manufacturer's instructions. Bisulfite-treated DNA samples were used for PCR with the *PSD-95* and *GJA-1* gene specific primers using HotStartTaq (Qiagen). Detail of primer sequences are following : *DLG4* (*CpG1-3) forward:* 5′-TG-

GTAGGGAATATGTGTGTTT-3′ ; *DLG4 (CpG1-3) reverse biotinylated:* 5′-ACCTAAACTCTCCTTAAAAACTCT-3′; *DLG4 (CpG1-3) pyrosequencing:* 5′-AATATAATTTTTTTTTTAGTTTGTG-3′; *DLG4 (CpG4-6) forward:* 5′-AGTTTTTTTTTGGGGAGGAAAGAG-3′; *DLG4 (CpG4-6) reverse biotinylated:* 5′-ACCCCCTAAAATA-ATCCCTTTATAC-3′; *DLG4 (CpG4-6) pyrosequencing:* 5′-TTAG-GTTTTTTTTTAAATAAGTTTT-3′ and primers for *GJA-1 are forward:* 5′-GTGGAAGTATTTGTATTAGTGAATG-3′; *GJA-1 reverse biotinylated:* 5′CCCAAAACCRAACTAATTC-3′; *GJA-1 pyrosequencing:* 5′- TGTTGAAAAGTAAATAAAAATG-3′ respectively. Pyrosequencing was carried out on a PyroMark Q24 instrument (Qiagen) according to the manufacturer's instruction and methylation analysis was performed by taking mean of all analysed *CpG* sites. In all statistical calculation the significance was set at $p<0.05$. All subjects gave written consent and the study was approved by the local ethical committee (51/100).

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Conflicts of Interests

The authors declare no conflict of interests.

Ethical Approval

The Ethics Committee of the Medical Faculty of the University of Bonn approved the study (No. 51/00, 6th July 2000).

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