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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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 (disc 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 a well-defined 4 promoter 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 not show any association with depression related phenotype. **

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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:

**Figure 1:** DNA methylation analysis in depression phenotype.
GTAGGGAAATATGTTGTTT-3’; DLG4 (CpG1-3) reverse biotinylated: 5’-ACCTAAACCTCCTAAACACTCT-3’; DLG4 (CpG1-3) pyrosequencing: 5’-AATATAAATTTTTAATTGGTG-3’; DLG4 (CpG4-6) forward: 5’-AGTTTTTTTGGGGAGGAAAG-3’; DLG4 (CpG4-6) reverse biotinylated: 5’-ACCCCTAAATA-ATCCCTTTATAC-3’; DLG4 (CpG4-6) pyrosequencing: 5’-TAG-TTTTTTTTAAATATGTTT-3’ and primers for GJA-1 are forward: 5’-GTTTTTTTTGAGGGAGGAAAG-3’; GJA-1 reverse biotinylated: 5’-CCCCAAC AAAAC ACTA AACCC-3’; GJA-1 pyrosequencing: 5’-GTTGGAAAAGTA AAAAAAATG-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).

References