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# DNA methylation of *ANKK1* and response to aripiprazole in patients with acute schizophrenia: A preliminary study

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ABSTRACT

Epigenetic modification including DNA methylation may affect pathophysiology and the response to antipsychotic drugs in patients with schizophrenia. The objective of the present study was to investigate the effect of the DNA methylation of *ANKK1* (ankyrin repeat and kinase domain containing 1) on the response to aripiprazole and plasma levels of monoamine metabolites in antipsychotic-free acute schizophrenia patients. The subjects were 34 Japanese patients with schizophrenia who had been treated with aripiprazole for 6 weeks. Comprehensive DNA methylation of *ANKK1* was determined using a next-generation sequencer. DNA methylation levels at CpG site 387 of *ANKK1* were higher in responders to treatment with aripiprazole and correlated with the changes in Positive and Negative Syndrome Scale scores, although the associations did not remain significant after Bonferroni correction. In responders, methylation at all CpG sites was significantly correlated with plasma levels of homovanillic acid ($r = 0.587$, $p = 0.035$) and 3-methoxy-4hydroxyphenylglycol ($r = 0.684$, $p = 0.010$) at baseline. Despite our non-significant results after multiple correction, our preliminary findings suggest that methylation levels at CpG site 387 of *ANKK1* may be associated with treatment response to aripiprazole. Furthermore, methylation of *ANKK1* may affect dopaminergic neural transmission in the treatment of schizophrenia, and may influence treatment response. Caution is needed in interpreting these findings because of the small sample size, and further studies are needed to confirm and expand our preliminary results.

## 1. Introduction

Previous pharmacogenetic studies have focused on various genes to identify clinically meaningful predictors for treatment response to antipsychotics in schizophrenia. However, these previous studies have not yielded compelling results (Malhotra et al., 2012), and it is therefore necessary to investigate epigenetic factors such as DNA methylation, which may influence drug response (Reynolds et al., 2014). DNA methylation affects the modification of gene expression (Bird, 2007), which may explain the interaction between genetic and environmental factors in schizophrenia at the molecular level (Nishioka et al., 2012). In addition, epigenetic modification including DNA methylation may affect pathophysiology (Dempster et al., 2013) and the response to antipsychotic drugs (Tang et al., 2014) in patients with schizophrenia.

Taq1A (rs1800497), which is located 10 kb downstream of the dopamine $D_2$ receptor (*DRD2*), causes an amino substitution within the 11th ankyrin repeat of ankyrin repeat and kinase domain containing 1 (ANKK1), which may affect substrate-binding specificity (Neville et al., 2004). The Taq1A polymorphism affects DRD2density (Jönsson et al., 1999) and expression of dopamine-related molecules (Kunii et al., 2014) in the brain, and many studies have investigated the association between variants in the *DRD2* locus and the response to antipsychotics (Lencz et al., 2006; Yasui-Furukori et al., 2011; Miura et al., 2012, 2015). In a meta-analysis (Zhang et al., 2010), no association was found between the Taq1A (rs1800497) polymorphism and the response to antipsychotics ($N = 748$, pooled odds ratio = 1.31 for the comparison between A1 allele carriers and non-carriers). However, no studies have investigated the association between DNA methylation in *DRD2/ANKK1* and the response to antipsychotics in schizophrenia.

On the other hand, a previous study showed that the Taq1A polymorphism may affect plasma levels of homovanillic acid (HVA), a dopamine metabolite, during the treatment of acute schizophrenia (Miura et al., 2012), suggesting that Taq1A may play a role in the regulation of dopaminergic neural transmission. Although it is difficult to regard

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plasma monoamine metabolites as direct reflections of central activity, plasma levels of monoamine metabolites are thought to be a possible indicator of the response to antipsychotics (Nagaoka et al., 1997; Yoshimura et al., 2010; Miura et al., 2012). Since epigenetic regulation may be involved in drug response, the objective of the present study was to investigate the effect of the DNA methylation of *ANKK1* on the response to aripiprazole (APZ) and plasma levels of monoamine metabolites in antipsychotic-free acute schizophrenia patients.

## 2. Materials and methods

### 2.1. Subjects

The study subjects were 34 Japanese patients (22 men, 12 women; mean age ± standard deviation, 41.2 ± 12.6 years) who were diagnosed with schizophrenia or schizoaffective disorder based on the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. These subjects were the same as those who participated in our previous study (Miura et al., 2015). Detailed descriptions of the study design are provided in another previous study (Miura et al., 2012). In brief, patients included both drug-naive patients (n = 14) and drug-free recurrent patients (n = 20) who had taken no antipsychotic medication for at least 2 weeks prior to entry into the study. The duration of illness and age of onset were 11.1 ± 11.3, and 30.0 ± 11.4 years, respectively. At baseline, total, positive, and negative scores on the Positive and Negative Syndrome Scale (PANSS) were 105.9 ± 12.9, 28.1 ± 4.2, and 24.1 ± 5.6, respectively. All patients were treated with 18 mg/day APZ on day 1 for early improvement in positive symptoms of acute schizophrenia. From day 2 to day 42, physicians were allowed to assign doses of APZ based on clinical assessments. Responders were defined as patients with a Clinical Global Impression-Improvement score of 1 or 2, or a ≥30% decrease from baseline in the PANSS total score. Benzodiazepines and anticholinergics were permitted as co-medication.

### 2.2. Blood sampling and measurement of plasma monoamine metabolite levels

Blood samples were obtained at baseline and 6 weeks after treatment before breakfast. Plasma levels of HVA and 3-methoxy-4hydroxyphenylglycol (MHPG) were analyzed using high-performance liquid chromatography with electrochemical detection using methods previously described (Watanabe et al., 2015). The intra- and interassay coefficients of variation for plasma HVA in our laboratory were 4.1% and 6.9%, respectively. For plasma MHPG, the intra- and interassay coefficients of variation were 3.5% and 5.9%, respectively.

### 2.3. Determination of DNA methylation levels

Genomic DNA was extracted from white blood cells, and then sodium bisulfite modification was performed using the EZ Methylation Kit D500 (ZymoResearch, USA). The DNA methylation levels at all CpG sites ranging from −162 C to +260 C of the 5′ region of ANKK1 gene were determined using a next generation sequencing method. In brief, bisulfite polymerase chain reaction (PCR) was performed using F primer (5′-TATGTTTGGGATTTTTTTATTTTTG-3′) and R primer (5′-AAACTCAACCCACTCCTTC-3′) in a 50 μL solution containing 1 × PCR amplification buffer, 1 M betaine (Sigma Aldrich, USA), 0.2 mM dNTPs, 3.0 mM $MgCl_2$, 0.4 μM of each primer, 2.0 ng single-stranded DNA binding protein (Promega, USA), and 10 U of Platinum *Taq* DNA polymerase (Invitrogen, USA). The PCR conditions included an initial denaturation step for 3 min at 95 °C, 30 cycles of 10 s at 98 °C, 20 s at 60 °C and 20 s at 72 °C. PCR products were purified using the MinElute PCR Purification Kit (QIAGEN, Germany). The sequencing library was prepared using 1 ng PCR product with the Nextera XT DNA Library Preparation Kit (Illumina, USA) according to the manufacturer's instructions. Sequencing was performed using the MiSeq Reagent Nano Kit v2 (Illumina) according to the manufacturer's instructions. The mapping of sequence reads and the quantification of cytosine methylation levels were performed using Bismark v0.10.1 (Krueger and Andrews, 2011). The linearity of the assay at each CpG site was confirmed using test DNA samples comprising methylated and unmethylated genomic DNA (0, 20, 40, 60, 80, and 100%).

This study was carried out with accordance with the Declaration of Helsinki, and approved by the ethics committee of Fukushima Medical University. All patients provided their consent to participate after having been informed of the purpose of the study.

### 2.4. Statistical analysis

Student's *t*-test (unpaired) was used to compare responders and non-responders, while Student's *t*-test (paired) was used to compare measures from before and after APZ treatment. Pearson's correlation coefficients were calculated to examine relationships between methylation levels, plasma levels of HVA and MHPG, and PANSS scores. Significance was defined as a p-value $< 0.05$. We used Bonferroni correction for multiple comparisons, and significance level was set at $p < 0.0014$ (35 CpG sites, 0.05/35 = 0.0014).

## 3. Results

### 3.1. Comparison of responders and non-responders

All subjects completed the study, and doses of APZ ranged from 9 to 30 mg/day (9 mg/day: n = 2, 12 mg/day: n = 2, 18 mg/day: n = 6, 24 mg/day: n = 10, 25 mg/day: n = 1, 30 mg/day: n = 13, mean ± SD = 23.7 ± 6.6 mg/day) at endpoint. No significant correlation was found between the dosage of aripiprazole at endpoint and methylation levels at all CpG sites ($r = -0.204$, $p = 0.272$). Decreased PANSS total, positive, and negative scores were observed after 6 weeks of APZ treatment. Plasma levels of HVA ($p = 0.01$) and MHPG ($p = 0.002$) decreased in responders after 6 weeks of treatment with APZ, but no changes were seen in plasma HVA ($p = 0.39$) or MHPG ($p = 0.11$) in non-responders. Out of 34 subjects, three were excluded from statistical analysis because of deviated outliers for methylation levels. Fig. 1 shows methylation levels at each CpG site in responders (n = 13) and non-responders (n = 18). No difference was seen between responders and non-responders in methylation levels at overall CpG sites ($p = 0.592$). Methylation was higher in responders than in non-responders at CpG site 387 ($p = 0.017$, Fig. 1), although the association did not remain significant after Bonferroni correction.

### 3.2. Correlations between methylation levels of ANKK1 and PANSS scores or plasma levels of monoamine metabolites

Significant positive correlation was found between the age of the subjects and methylation levels at all CpG sites ($r = 0.622$, $p < 0.001$). Table 1 shows the significant correlations found between methylation levels at each CpG site and PANSS scores or plasma levels of monoamine metabolites adjusted for baseline data in the study subjects. Methylation levels at CpG site 387 were correlated with changes in PANSS total ($r = -0.393$, $p = 0.031$), positive ($r = -0.382$, $p = 0.037$), and negative ($r = -0.379$, $p = 0.039$) scores (Table 1), although the associations did not survive Bonferroni correction.

At some CpG sites, methylation levels were negatively correlated with changes in plasma levels of HVA (Table 1) after APZ treatment, although the associations did not remain significant after Bonferroni correction. Methylation at all CpG sites was significantly correlated with plasma levels of HVA ($r = 0.587$, $p = 0.035$) and MHPG ($r = 0.684$, $p = 0.010$) at baseline in responders, but not in non-responders (HVA: $r = 0.196$, $p = 0.435$; MHPG: $r = 0.035$, $p = 0.890$).

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