Methylation at a transcription factor binding site on the 5-HT1A receptor gene correlates with negative symptom treatment response in first episode schizophrenia

Citation for published version (APA):

Published in:
International Journal of Neuropsychopharmacology

Citing this paper
Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights
Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy
If you believe that this document breaches copyright please refer to the University of Manchester’s Takedown Procedures [http://man.ac.uk/04Y6Bo] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.
Methylation at a transcription factor-binding site on the 5-HT1A receptor gene correlates with negative symptom treatment response in first episode schizophrenia

Hao Tang1,2, Caroline F. Dalton1, Umarat Srisawat1, Zhi Jun Zhang2 and Gavin P. Reynolds1

1 Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK
2 Department of Neurology, ZhongDa Hospital and Institute of Neuropsychiatry, Southeast University, Nanjing, People’s Republic of China

Abstract

Individual variability and inadequate response of negative symptoms are major limitations of antipsychotic treatment in schizophrenia. A functional polymorphism, rs6295, in the 5-HT1A-receptor gene (HTR1A) contributes to this variability in negative symptom response. The DNA sequence containing rs6295 is rich in cytosine methylation (CpG) sites; CpG methylation is an epigenetic factor that, like rs6295, can modify transcriptional control. To investigate whether DNA methylation influences response to antipsychotic treatment, we determined methylation at CpG sites close to rs6295 in DNA from 82 Chinese subjects with a first psychotic episode. Methylation of one CpG site within a recognition sequence for HES transcriptional repressors was found to correlate with changes in total PANSS score ($p=0.006$) and negative factor sub-score ($p<0.001$) following 10 wk initial antipsychotic treatment, as well as with baseline negative factor score ($p=0.019$); the effect on symptom change remained after correction for this baseline score. An effect of rs6295 on negative symptom response was not seen in this sample, which may not have provided sufficient power for the pharmacogenetic association. These preliminary results indicate that epigenetic modification of transcriptional regulation by specific cytosine methylation may modulate HTR1A expression, resulting in effects on emotional dysfunction and negative symptom response to antipsychotic treatment.

Received 16 August 2013; Reviewed 11 September 2013; Revised 8 October 2013; Accepted 30 October 2013; First published online 16 December 2013

Key words: Antipsychotic drugs, DNA methylation, HTR1A, negative symptoms, schizophrenia.

Introduction

Response to antipsychotic drug treatment shows substantial inter-individual variability, as well as a generally poorer improvement of negative than positive symptoms. In pharmacogenetic studies addressing this differential response, a replicated finding is the association of negative symptom response with the −1019C/G polymorphism (rs6295) in the 5-HT1A-receptor gene (HTR1A) (Reynolds et al., 2006; Wang et al., 2008; Mossner et al., 2009). This polymorphism, first found to be associated with depression and suicide (Lemonde et al., 2003), is also associated with response to antidepressant drugs (Lemonde et al., 2004; Yevtushenko et al., 2010). It is found to influence gene expression; the risk allele (G) disrupts the repressor activity of the DEAF1, HES1 and HES5 transcription factors, resulting in overexpression of the presynaptic 5-HT1A-receptor (Lemonde et al., 2003; Jacobsen et al., 2008).

Methylation of DNA sequences is another established influence on transcription factor binding (Sharma et al., 2010). Cytosine residues at CpG dinucleotide sequences are sites of DNA methylation, one of several epigenetic mechanisms for the modulation of DNA function. It is notable that rs6295 is found within an island of 13 CpG sites in the HTR1A promoter sequence; these adjacent methylation sites could conceivably contribute, along with rs6295, to modifying the binding of transcription factors such as DEAF1. We hypothesised that the percentage methylation of one or more CpG sites close to the rs6295 polymorphism in the promoter sequence of HTR1A may also influence symptom response to treatment with antipsychotic drugs.

Method

Chinese Han inpatients presenting with a first psychotic episode (45 male, 37 female; mean age 25.8±7.1 years) participated in our study. All patients met criteria for a diagnosis of schizophrenia according to the Diagnostic and Statistical Manual of the American Psychiatric Association 4th Edition (DSM-IV). Exclusion criteria
included prior history of medication with antipsychotics, antidepressants or mood stabilisers, co-morbid DSM-IV diagnosis of substance abuse or dependence or other physical illness. Study subjects were treated according to standard clinical practice; initial treatment was with chlorpromazine \((n=57)\), risperidone \((n=18)\), clozapine \((n=4)\) or fluphenazine \((n=3)\). Drug treatment was reviewed after approximately 6 wk and modified as needed; this resulted in a further 26 subjects receiving clozapine: 21 from the chlorpromazine group and 5 from the risperidone group. Anticholinergic and benzodiazepine co-medication were administered as required for the alleviation of extrapyramidal symptoms and need for sedation, respectively. The Positive and Negative Syndrome Scale (PANSS) was used for assessment and evaluation of psychopathology and therapeutic response to antipsychotic treatment. All patients were assessed on the day of admission by a psychiatrist trained in the use of PANSS and subsequently reassessed after 10 wk of antipsychotic treatment. PANSS items were divided into five symptom factors according to the consensus scheme of Wallwork et al. (2012). The five factors are defined by the following PANSS items: Positive Factor: P1, P3, P5, G9; Negative Factor: N1, N2, N3, N4, N6, G7; Disorganised/Concrete Factor: P2, N5, G11; Excited Factor: P4, P7, G8, G14; Depressed Factor: G2, G3, G6. The Nanjing Brain Hospital Ethical Committee approved the study, and all patients gave written informed consent.

Genomic DNA, extracted from blood samples taken on the day of admission prior to initiation of drug treatment using a standard chloroform-phenol method, was bisulphite-modified to convert unmethylated cytosine residues to uracil using the EpiTect Fast Bisulfite Kit (UK) with a calculated mean conversion of 99%. For analysis, a sequence containing 13 CpGs in the HTR1A promoter (GRCh37.p10, Chromosome 5 bases 63258525–63258684) including the rs6295 C/G polymorphism, (which enables/removes CpG12) was identified and amplified by PCR using primers, including a biotinylated reverse primer, as follows: 5'-AGTAAAGTTGGATTTAGATGATC-3' (forward) and 5'-[bnt]CTCTATTTCTCCTCTACATTA-3' (reverse) (Eurofins MWG Operon). PCR reaction was carried out with 20 ng bisulphite-converted DNA using the PyroMark PCR kit (UK) in a final volume of 25 μl containing 12.5 μl 1x PyroMark PCR Master Mix, 2.5 μl 1x CoralLoad Concentrate, 1 μl of each primer in a final concentration of 0.05 μM, 7 μl RNase-free water. Amplification conditions were as follows: 95°C for 15 min, 45 cycles of 94°C for 30 s, 56°C for 30 s and 72°C for 30 s, finally, 72°C for 10 min. Methylation status of the sequence within the CpG island around rs6295, containing sites CpG9-13 (Fig. 1a), was determined with a PyroMark Q24 pyrosequencer (Qiagen UK) using 15–20 μl PCR product and employing a sequencing primer, 5'-TTTAGTTGGAGTGTAAATG-3' (Eurofins MWG Operon). Pyrosequence setup and data reading were conducted by PyroMark Q24 2.0.6.20 software (UK). Samples underwent PCR and pyrosequencing in duplicate; any inconsistencies between samples were resolved following further repetition. Pyrosequencing also permitted determination of genotype for rs6295.

Data analysis was undertaken using SPSS version 16.0 (SPSS Inc., USA). Pearson’s correlation was used to determine individual relationships between clinical measurements and percentage methylation. As we initially investigated five CpG sites adjacent to, and including that of, the rs6295 polymorphism for their correlation with symptom response, statistical significance was set at \(p<0.01\) for a conservative Bonferroni correction; for subsequent, post-hoc analysis we applied \(p<0.05\). Stepwise regression analysis was used to determine the influence of clinical and demographic factors on symptom measures. Power analysis demonstrated that the sample size was adequate to identify a medium effect size \(r=0.3\) with 80% power at \(p<0.05\). Where shown, variance in data is expressed as standard deviation.

**Results**

Initial mean PANSS score on admission was 99.6 ± 14.4; this reduced to 49.6 ± 9.6 after 10 wk initial treatment with antipsychotic drugs. Mean methylation was determined at methylation sites CpG9, CpG10, CpG11 and CpG13 as 13.8, 18.1, 20.5 and 13.9%, respectively; at CpG12 (rs6295 C-allele carriers) it was 21.0%. Results for CpG9 and CpG13 each contained one extreme outlier \((z>5)\) and were not normally distributed; removal of the two outlier data points normalised the distributions.
HTR1A methylation and antipsychotic response

Table 1. Methylation at CpG sites and their correlation with symptom changes after 10 wk’ treatment with antipsychotic drugs

<table>
<thead>
<tr>
<th>Percentage methylation (mean±s.d.)</th>
<th>CpG9 (n=81)</th>
<th>CpG10 (n=82)</th>
<th>CpG11 (n=82)</th>
<th>CpG12 at C allele (n=76)</th>
<th>CpG13 (n=81)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearman’s r and p values</td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Change in total PANSS</td>
<td>0.15</td>
<td>0.17</td>
<td>0.11</td>
<td>0.31</td>
<td>0.04</td>
</tr>
<tr>
<td>Changes in symptom factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Excited factor</td>
<td>0.25</td>
<td>0.024</td>
<td>0.15</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Negative factor</td>
<td>0.09</td>
<td>0.41</td>
<td>0.11</td>
<td>0.35</td>
<td>0.08</td>
</tr>
<tr>
<td>Disorganised/concrete factor</td>
<td>0.01</td>
<td>0.92</td>
<td>–0.06</td>
<td>0.61</td>
<td>−0.08</td>
</tr>
<tr>
<td>Positive factor</td>
<td>0.11</td>
<td>0.34</td>
<td>0.05</td>
<td>0.67</td>
<td>−0.02</td>
</tr>
<tr>
<td>Depressed factor</td>
<td>0.15</td>
<td>0.17</td>
<td>0.17</td>
<td>0.13</td>
<td>0.09</td>
</tr>
</tbody>
</table>

(Shapiro-Wilk test; p=0.264, 0.234 respectively). Only methylation at CpG13 showed a significant correlation with clinical measures (Table 1). CpG13 methylation was significantly correlated with change in total PANSS ($r=0.300$, $n=81$, $p=0.006$). This reflected a highly significant positive correlation with change in the negative factor ($r=0.410$, $p<0.001$) (Fig. 1b). Stepwise linear regression indicated a significant effect of negative factor score before treatment on change in negative symptoms, but no significant effect of age, sex or whether patients received clozapine. A significant correlation with CpG13 methylation was also observed with this negative factor baseline score ($r=0.261$, $p=0.019$) but not with the baseline score of any other factor. After controlling for baseline negative symptom score the correlation between CpG13 methylation and change in negative symptoms remained highly significant ($r=0.353$, $p=0.001$). CpG13 methylation had a small effect on the change in the depressed factor ($r=0.206$, $p=0.065$); correlations with changes in other factors were also not significant ($p>0.1$).

The subjects were found to have the following rs6295 genotypes: 46 CC, 30 CG and 6 GG. Genotype had no significant influence on methylation of the invariant CpG sites, nor was genotype significantly related to PANSS measures of symptom response to treatment, including negative symptom response. Thus CC genotype and G allele carriers had mean values for change in negative score of 7.93±3.93 and 7.31±4.22, respectively. We investigated the relationship between CpG13 methylation and change in negative symptoms separately within two rs6295 genotype subgroups of the sample. The correlation remained in both the CC genotype ($r=0.422$, $n=46$, $p=0.004$) and the G carrier ($r=0.421$, $n=35$, $p=0.012$) subjects (Fig. 1b).

Discussion

In a hypothesis-driven search for effects of DNA methylation in the promoter region of a gene (HTR1A) known to be associated with symptom response to antipsychotic medication, we have identified a correlation of the change in negative symptoms following initial antipsychotic drug treatment with methylation, determined prior to onset of treatment, at a specific CpG site adjacent to the functional polymorphism rs6295. Thus epigenetic variation, as well as a genetic polymorphism, in a specific DNA sequence in the HTR1A promoter region can influence negative symptoms in schizophrenia and their subsequent response to antipsychotic drug treatment.

CpG13 is found within a recognition site for the repressor activity of transcription factors HES1 and HES5 (Fig. 1a); HES5 activity is also influenced by the nearby rs6295 allele (Jacobsen et al., 2008). The strong repressor activity of HES1 is thought to play an essential role in the developmental repression of HTR1A expression, while the inhibitory effect of HES6 on HES1, HES5 and DEAF1 repressor activity may regulate HTR1A expression (Jacobsen et al., 2008). Thus a methylation-induced variability in the balance between effects of these transcriptional regulators may modulate the developmental control of HTR1A transcription and its effects on affective circuitry (Richardson-Jones et al., 2011); leucocyte DNA methylation as determined here may well reflect epigenetic changes during, or even prior to, early development (Rosa et al., 2008). However, effects on expression of HTR1A in the adult may also be important; it is notable that a negative correlation between 5-HT1A-receptor binding potential in the amygdala and PANSS-derived negative and depression/anxiety symptom scores has been reported in drug-free schizophrenia (Yasuno et al., 2004). Certainly dysfunction of the amygdala and its connectivity with the prefrontal cortex are proposed to be involved in the emotional dysregulation underlying negative symptoms of schizophrenia (Aleman and Kahn, 2005).

By analogy with the association of rs6295 with antipsychotic drug response (Reynolds et al., 2006), we hypothesise that poor symptom response to drug treatment...
is associated with loss of inhibitory control of HTR1A expression, with a consequent increase in 5-HT1A auto receptors and reduction in serotonin neurotransmission. That we find poorer response to be correlated with diminished methylation at CpG13, suggests that methylation might enhance transcriptional repressor activity, perhaps by recruiting methyl-binding repressor proteins. However, the finding of a greater reduction in amygdala 5-HT1A receptors in patients with more severe negative symptoms (Yasuno et al., 2004), along with our observation of a positive correlation between CpG13 methylation and baseline negative factor score, would be consistent with an alternative view where greater methylation resulted in suppression of HTR1A expression. This could be brought about by disruption of the enhancer activity of DEAF1 on HTR1A expression that is found at post-synaptic sites (Czesak et al., 2006).

The 5-HT1A receptor is increasingly being recognised as a potential target for antipsychotic drug action, particularly with respect to negative and cognitive symptoms of schizophrenia (Newman-Tancredi and Kleven, 2011). Certainly the 5-HT system is implicated in negative symptoms and their response to drugs; selective serotonin reuptake inhibitors as an adjunct to antipsychotic treatment can improve negative symptoms in some subjects (Silver, 2004). Our findings add further evidence for 5-HT1A receptor involvement in negative symptoms and response to antipsychotic drug treatment. These receptors mediate the action of atypical antipsychotic drugs on dopamine release in the frontal cortex (Diaz-Mataix et al., 2005), one mechanism proposed to underlie drug effects on negative symptoms (e.g. Ichikawa et al., 2001). Thus genetically or epigenetically determined differences in 5-HT1A receptor density or its regulatory control may influence subsequent negative symptom response to antipsychotic drugs.

We recognise that the assessment of peripheral blood DNA, rather than DNA from brain tissue, is an inevitable limitation of our study. However, others have found psychiatric correlates of HTR1A methylation in blood DNA: methylation of the HTR1A proximal promoter region is reportedly increased in schizophrenia (Carrard et al., 2011). Notably, a site-specific hypomethylation of the 5-HT2A-receptor gene in schizophrenia and bipolar disorder is found in both brain- and saliva-derived DNA (Ghadirivasfi et al., 2011). We were unable to replicate in this sample the previously reported association of rs6295 with negative symptom response to treatment. This is not easily explained given that the original result was seen with a smaller sample (Reynolds et al., 2006), although the combination of sample size, lower G allele frequency and perhaps other factors associated with this Asian population may have contributed.

Nevertheless, the sample demonstrates a strong effect of methylation at the recognition site for transcription factors implicated in the effect of the rs6295 polymorphism on HTR1A gene expression (Jacobsen et al., 2008). This finding is replicated internally within the two genotype groups and represents a uniquely specific observation of an epigenetic factor relating to symptom response in the initial drug treatment of schizophrenia. As such the findings have an impact far beyond an understanding of the epigenetic influences on antipsychotic treatment response; as well as raising the question whether there might be an association of CpG13 methylation with schizophrenia, they immediately generate hypotheses relating to depression and antidepressant response, with which the adjacent HTR1A polymorphism has established associations (Lemonde et al., 2003; 2004).

Acknowledgments

No specific funding was received for this study. U. Srisawat is supported by a PhD scholarship from the Royal Thai Government, Thailand.

Statement of Interest

G.P. Reynolds has received honoraria for educational lectures, advisory panel membership and travel support from the following pharmaceutical companies: Lundbeck, Janssen-Cilag, Otsuka and Sunovion. No other authors have financial interests to disclose.

References


