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Norepinephrine transporter methylation profile and association with symptoms of ADHD and in vivo NET expression measured by PET

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BACKGROUND

Attention Deficit Hyperactivity Disorder (ADHD) is one of the most frequent neurodevelopmental disorders [1]. The exact molecular mechanism of ADHD is complex and studies indicate it to be a polygenetic disorder. Recently, the possible modulation by epigenetic processes has received increased attention [2]. We therefore aimed to examine possible differences in the DNA methylation levels of cytosine-phosphate-guanine (CpG) sites in the norepinephrine transporter (NET) promoter between patients with ADHD and healthy controls. Furthermore, we tested for possible associations between behavioural symptoms of ADHD with methylation levels. Lastly, we tested whether differences detected are observed in vivo expression of the NET.

METHODS

23 adult ADHD patients (age±SD: 30.9±10.6, 16 males) and 23 healthy controls (age±SD: 32.2±10.9, 16 males) participated in this study. Participants were subject to *(S,S)*-[^{18}F]FMeNER-D$_2$ PET scan with an advance full-ring scanner (General Electric Medical Systems, Milwaukee, WI, USA) in 3D acquisition mode. DNA was extracted and converted into bisulfite using the EZ-96 DNA methylation kit [3]. The quantification of NET binding potential (BP_{ND}) values were computed using the caudate as the reference region. Means across regions (region 1, 2, 3) of the NET promoter were averaged and selected from previous publication showing these regions to be transcriptionally important [4]. Statistical analysis was carried out using SPSS.

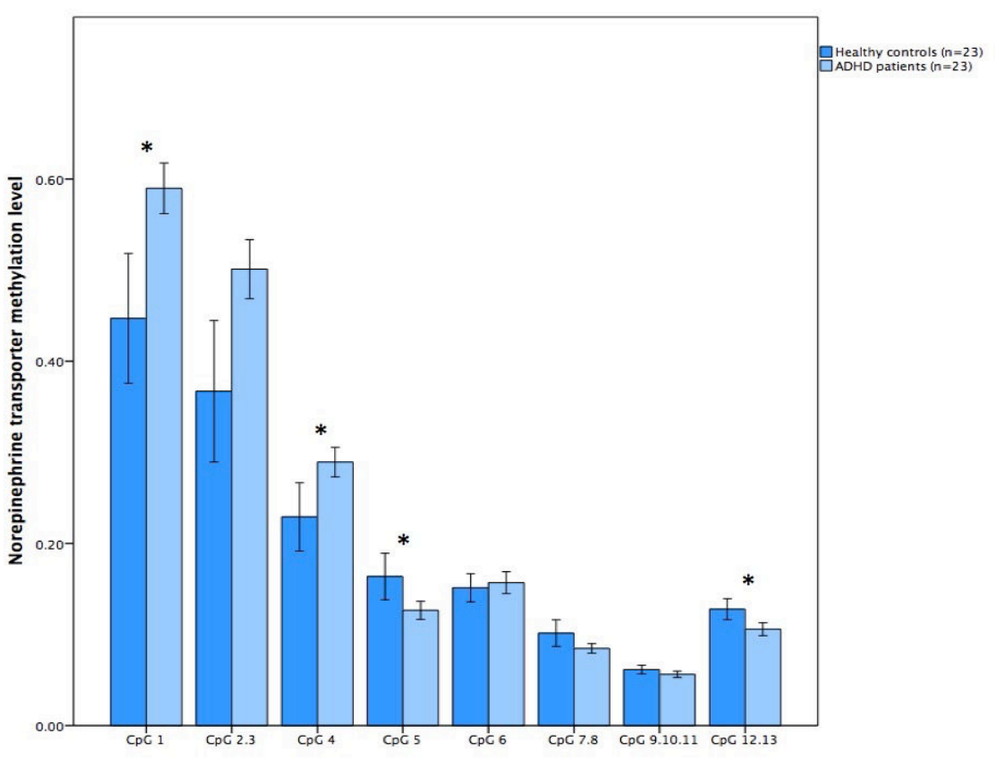

Figure 1. Norepinephrine transporter methylation levels are depicted on the Y-axis for several CpG sites across the promoter region 1 on the x-axis. Light blue colour illustrates patients with ADHD while darker blue represents healthy controls. Significant difference between groups after multiple correction testing ($p<0.05$) is indicated by an asterisk.

RESULTS

Across region 1, patients with ADHD had higher methylation (0.27) in comparison to healthy controls (0.22)($p<0.01$). No difference was detected between averaged means in region 2 and 3. Differences in individual CpG sites in region 1 were also detected between groups. Negative correlation was detected between a single CpG site with BP_{ND} in the thalamus ($r=-0.60$), raphe nuclei (-0.61) and locus coeruleus ($r=-0.51$) in patients only. Negative association was found between CpG sites 1 and 2.3 with hyperactivity-impulsivity behavioural symptom scores.

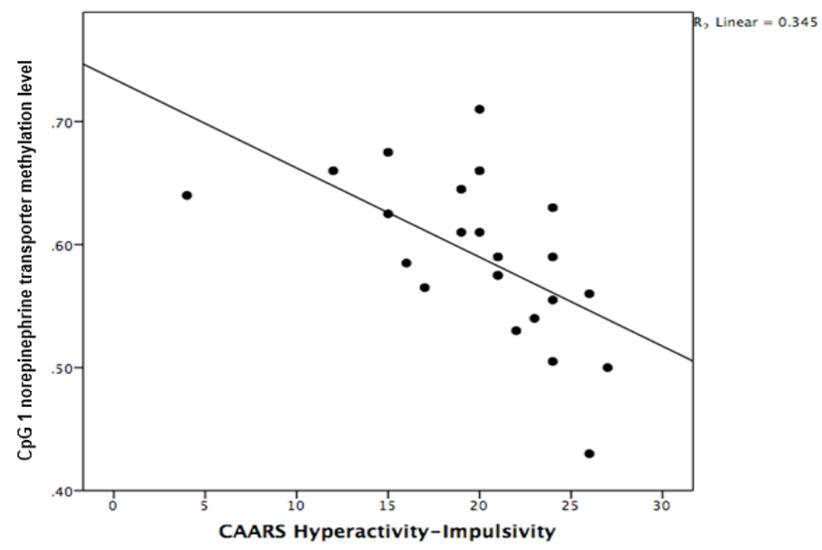

Figure 2. Negative correlation between Conners' Adult ADHD Rating Scale (CAARS) hyperactivity-impulsivity scale and NET methylation level in CpG site 1 ($r=-0.677$, $p=0.003$).

CONCLUSIONS

Our results point to an epigenetic dysregulation in ADHD. Increased methylation was detected in patients with ADHD towards the 5' end while this effect reversed towards the 3' end. Negative association between methylation levels and hyperactivity-impulsivity scores was detected. More specifically, lower methylation values were associated with increased symptom severity, reflecting higher transporter expression and thus lower extracellular norepinephrine. This is of great importance as common medication for ADHD significantly improve symptoms by increasing norepinephrine levels. We also found higher methylation to be correlated with lower NET expression in patients only, supporting the evidence of molecular effect of DNA on expression [5]. This is potentially due to a compensatory mechanism or other factors involved in transcription. Further studies with larger sample sizes are warranted.

DISCLOSURE

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