Increased methylation of the oxytocin receptor gene in motor functional neurological disorder: a preliminary study

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Increased Methylation of the Oxytocin Receptor Gene in Motor Functional Neurological Disorder: a preliminary study.

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Background

Functional Neurological Disorder (FND) represents a paradigmatic neuropsychiatric disorder; patients present neurological physical symptoms but associated psychosocial stressors play a role as predisposing and precipitating factors\(^1\). Recent neuroscience research has shed light on how the physical symptoms arise in terms of aberrant brain mechanisms\(^2\) but little is known in terms of why certain individuals develop the disorder. Childhood abuse and life adversities have been linked to FND but cannot be viewed solely/exclusively as causal\(^3\), as this association is partially non-specific. A multifactorial causal model has to be considered and a gene-environment interaction is plausible, as it could theoretically integrate life stressors as precipitating factors in a subset of susceptible individuals. There is to date no evidence for genetic risks of FND with only one study reporting familial cases suggesting disease modeling in families rather than genetic transmission\(^4\). Twin studies in other functional symptoms (chronic fatigue, irritable bowel syndrome, etc.)\(^5\) have found potential, even if weak, genetic influences but no studies looked specifically at neurological functional symptoms. In order to explore potential gene-environments interaction, epigenetics offers promising new routes. In particular, epigenetics in stress research and related psychopathologies has generated novel findings that remain to be replicated in large-scale datasets\(^6\). Methylation of the promoter of the oxytocin receptor gene (OXTR) has been implicated in stress and emotional regulation in health and disease\(^7,8\) as well as in social cognition\(^9\). The oxytocinergic system has been found to suppress HPA axis basal and post stress activity\(^10\). As evidence of hyperarousal of the HPA-axis has been found in FND\(^11,12\) we set out to explore epigenetic changes in the promoter of the OXTR gene in a cohort of FND patients.
Methods

Participants Fifteen patients with motor Functional Neurological Disorder (FND) according to DSM-5 criteria (F44.4) were recruited from the Neurology Department of Geneva University Hospital. Sixteen healthy controls (HC) matched for age and gender were recruited through announcements. All participants signed a written informed consent (Swiss ethical approval CER 14-008) and participated in a study assessing stress biomarkers (salivary cortisol and alpha-amylase) and mood (BDI depression score and STAI trait anxiety score). Duration of the disorder is reported in months from symptom onset to study day. Severity of the symptom was measured by the neurologist with the Clinical Global Impression (0=no symptoms, 1= minimal, 2= moderate, 3= severe) and by patients report on a visual analog scale from 0-10.

Blood sampling and epigenetic testing

Genomic DNA was extracted from blood samples using the Illustra Nuclen Genomic DNA Extraction Kit (Bioscience Amersham, GE Healthcare, UK). 1μg of genomic DNA was bisulfite-modified by using the EpiTect Bisulfite Kit (Qiagen, Hilden, Germany) and 2μl of the post-bisulfite-treated DNA were used for subsequent PCR amplification.

Polymerase chain reaction (PCR) Amplification and Pyrosequencing

The reactions were performed with a PCR reaction mixture containing oligonucleotides at 0.5 mM with the following sequences: OXTR_F: 5’-TTGCGTTTTGGATTTAGATAATT-3’ / OXTR_R: 5’-BIO-TTAGGATAAGGAGTGCGAGGTATTTTATT-3’. The biotinylated PCR products were purified using streptavidin-sepharose beads (Amersham) and sequenced using the PSQ 96 Gold reagent kit (Qiagen, Hilden, Germany) with the following primer: OXTR_S: 5’-AGAAGTTATAGAAGTTATTTTATAATT-3’. This amplified a region on the coding strand of the OXTR gene containing two methylation sites, -944 and -934 (hg19, chr3:8,810,729-
8,810,845). The sequencing was performed using the Q96 system with Pyromark Q96 analysis software in CpG (Qiagen) and the sequence to analyze OXTR wasYGGAATATTYTGGAGTTTTT.

**Statistical Analysis**

Mean methylation rates were computed by summing the methylation of the 2 CpG sites tested. Group differences were assessed with two-sided Student T-test and effect sizes estimated with Cohen’s d coefficient. Correlations were computed with Spearman coefficient.

**Results**

Demographic and clinical data are presented in Table 1. Significant higher levels of methylation of the OXT receptor gene was found in patients compared to controls (68.1±4.3 versus 62.5±6.8, p=0.012, effect size 0.98). No correlation was found between OXTR mean methylation and depression or anxiety scores, nor with severity of functional symptoms and duration of the disease, nor with salivary stress biomarkers.

**Discussion**

A significant increase of methylation in the OXTR promoter was found in FND patients as compared to matched healthy controls. Despite the small sample, the effect size (0.98) suggests a reliable finding. Methylation in the OXTR promoter has been linked to certain psychopathologies related to social behavior and stress regulation. Increased methylation in the OXTR promoter is associated with repression of transcriptional activity in most tissues resulting in decreased levels of OXT receptor mRNA. In humans, such methylation assessed in blood cells has been linked to decreased circulating oxytocin. Thus, increased OXTR promoter methylation could reflect an overall downregulation of the oxytocinergic system that could contribute to FND symptomatology. Oxytocin has been shown to play a role in the central nervous control of neuroendocrine responses to stress by repressing HPA-axis reactivity and cortisol release. In our cohort, patients had significantly higher background
levels of salivary cortisol but no significant correlation was found between those and OXTR methylation levels, possibly due to the small sample size. Further studies should test whether such downregulation of the oxytocin pathway could explain the high background cortisol levels found in FND (both patients with non-epileptic and motor symptoms).

This is, to our knowledge, the first study reporting an epigenetic modification of OXTR gene in FND patients, suggesting that gene-environment interactions might play a role in the development and/or maintenance of this condition. Although preliminary, these findings are of particular interest for potential novel future treatment approaches, such as intranasal oxytocin administration in FND. It has to be stressed out, though, that OXTR methylation has also been found to be elevated in other neuropsychiatric disorders, such as obsessive compulsive disorder and anorexia questioning the specificity of our findings to FND. Further research is needed and larger cohorts of patients taking in account all multiple factors that contribute to FND.

Table 1: Demographical and Clinical Data
<table>
<thead>
<tr>
<th></th>
<th>FND patients (N = 16)</th>
<th>Healthy Controls (N = 15)</th>
<th>P value (T test*, Fisher test°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46 ± 15</td>
<td>39 ± 13</td>
<td>*ns</td>
</tr>
<tr>
<td>Gender(Female:Male)</td>
<td>14F:2M</td>
<td>12F:3M</td>
<td>*ns</td>
</tr>
<tr>
<td>BDI</td>
<td>8 ± 5.5</td>
<td>3.5 ± 4.6</td>
<td>*p &lt; 0.05</td>
</tr>
<tr>
<td>STAI-Trait</td>
<td>41.1 ± 12.1</td>
<td>37.9 ± 9.0</td>
<td>*ns</td>
</tr>
<tr>
<td>STAI-State</td>
<td>36.9 ± 10.1</td>
<td>33.7 ± 8.8</td>
<td>*ns</td>
</tr>
<tr>
<td>CGI</td>
<td>3 none, 2 minimal,</td>
<td>NA</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3 moderate, 8 severe</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>Symptoms duration (months)</td>
<td>73 ± 85 (range 4-300)</td>
<td>NA</td>
<td>10</td>
</tr>
<tr>
<td>Symptoms type</td>
<td>6 weakness, 5 tremor, 2 gait, 3 jerks</td>
<td>NA</td>
<td>11</td>
</tr>
<tr>
<td>OXTR methylation % (mean)</td>
<td>68.1 ± 4.3</td>
<td>62.5 ± 6.8</td>
<td>*p &lt; 0.05</td>
</tr>
</tbody>
</table>

BDI: Beck depression inventory score
STAI: Spielberger anxiety depression score
OXTR: oxytocin receptor
References