Letter to the Editor

Does DNA methylation in the promoter region of the \textit{ATXN3} gene modify age at onset in MJD (SCA3) patients?

\textit{To the Editor:}

Machado–Joseph disease (MJD/SCA3) is a neurodegenerative disease caused by an unstable CAG repeat expansion in \textit{ATXN3} gene, leading to an expanded polyglutamine tract in ataxin-3, the corresponding protein (1).

The distribution of age at onset (AO) is inversely correlated with CAG expansion size; however, repeat length is responsible only for 45–60\% of AO variation in MJD (2, 3), indicating other still unidentified factors (genetic, environmental or others).

The degree of neurodegeneration induced by the polyQ protein is correlated with protein storage levels (4). We therefore hypothesized that DNA methylation, specifically targeting the mutant allele and leading to transcriptional deregulation, might influence the levels of mutant ataxin-3 in affected cells and thus AO in MJD patients.

Our aim was to assess the methylation degree at six CpG dinucleotides at the \textit{ATXN3} promoter and explore their role as potential modifiers for AO in MJD.

One hundred and twenty-three Brazilian patients from 62 families with MJD were ascertained in Rio Grande do Sul, Brazil. The inclusion criterion was molecular confirmation in a symptomatic patient. AO was defined as the age at which the patient, or a close person, noticed the first symptoms (usually gait unbalance); 35 healthy individuals were also neurologically examined to be used as controls. This study was approved by the Hospital Ethics Committee.

DNA was isolated from leukocytes, as described (5). Evaluation of the (CAG)$_n$ tract was performed by fluorescently labeled polymerase chain reaction (PCR) and capillary electrophoresis. Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) analysis was carried out according to manufacturer’s instructions (MRC Holland, Amsterdam) (6). We designed four specific probes for the \textit{ATXN3} promoter, which were able to detect the methylation degree at six CpG dinucleotides, through restriction enzyme recognition. We have also designed three control probes, lacking a restriction enzyme recognition site, in genes located outside this region. Data analysis was performed by exporting peak areas to an Excel-based analysis program.

Methylation differences between patients and controls, for each probe, were evaluated using the Student’s unpaired \textit{t}-test. Generalized linear models (GLMs) by generalized estimating equations (GEEs) were used to test the effect of the methylation degree in AO variation. Controlled variables were the family and (CAG)$_n$ length (normal and expanded). Statistical analyses were performed using \textit{spss} for Windows v.16.

Length distribution of (CAG)$_n$ with AO is shown in Table 1. A significant inverse correlation was found between AO and repeat length ($r^2 = 0.57$, $p < 0.001$).

Probe 1 (containing one CpG) was predominantly methylated (average 94.8\%). Probe 2, also containing one CpG dinucleotide, had an average methylation degree of 40.1\%. Probes 3 and 4, both containing two CpGs, were predominantly non-methylated (averages of 6.7\% and 3.6\%) (Fig. 1).

No statistically significant differences were found when comparing methylation status between the whole patients’ cohort and controls ($p > 0.05$). A stepwise analysis of each probe was performed. Given the factor effect under study being the family, a trend toward a direct relation between methylation degree for probe 1 and AO was suggested ($p = 0.055$), when GLM analyzed methylation status against AO only (Fig. 1a). The regression coefficient relating probe 1 and AO was 24.0; i.e. each 10\% decrease in probe 1 methylation status was related to a 2.4-year reduction in AO. The effect of the methylation status was small; however, when compared to the effect of the expanded repeat: when (CAG)$_n$
Table 1. Distribution of age at onset and (CAG)$_n$ length in Machado–Joseph disease (MJD) patients

<table>
<thead>
<tr>
<th>CAG repeat length</th>
<th>66</th>
<th>67</th>
<th>68</th>
<th>69</th>
<th>70</th>
<th>71</th>
<th>72</th>
<th>73</th>
<th>74</th>
<th>75</th>
<th>76</th>
<th>77</th>
<th>78</th>
<th>79</th>
<th>80</th>
<th>81</th>
<th>82</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>4</td>
<td>13</td>
<td>14</td>
<td>13</td>
<td>20</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>123</td>
</tr>
<tr>
<td>Average Age at onset</td>
<td>53</td>
<td>42</td>
<td>49</td>
<td>45</td>
<td>40</td>
<td>38</td>
<td>39</td>
<td>37</td>
<td>32</td>
<td>30</td>
<td>25</td>
<td>23</td>
<td>19</td>
<td>15</td>
<td>18</td>
<td>16</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Minimum Age at onset</td>
<td>50</td>
<td>29</td>
<td>42</td>
<td>36</td>
<td>28</td>
<td>24</td>
<td>26</td>
<td>19</td>
<td>22</td>
<td>19</td>
<td>17</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>14</td>
<td>16</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Maximum Age at onset</td>
<td>57</td>
<td>55</td>
<td>56</td>
<td>55</td>
<td>53</td>
<td>56</td>
<td>49</td>
<td>50</td>
<td>50</td>
<td>40</td>
<td>36</td>
<td>36</td>
<td>26</td>
<td>20</td>
<td>24</td>
<td>16</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>SD CAG repeat length</td>
<td>4.9</td>
<td>9.8</td>
<td>5.1</td>
<td>8.0</td>
<td>9.1</td>
<td>7.7</td>
<td>7.4</td>
<td>7.5</td>
<td>7.7</td>
<td>8.0</td>
<td>9.1</td>
<td>8.2</td>
<td>5.6</td>
<td>5.3</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of affected patients</td>
<td>1.6</td>
<td>4.1</td>
<td>6.5</td>
<td>3.3</td>
<td>10.6</td>
<td>11.4</td>
<td>10.5</td>
<td>16.3</td>
<td>7.3</td>
<td>7.3</td>
<td>7.3</td>
<td>4.1</td>
<td>2.4</td>
<td>3.3</td>
<td>2.4</td>
<td>0.8</td>
<td>0.8</td>
<td>100.0</td>
</tr>
</tbody>
</table>

NA, not applicable.

Fig. 1. Scatter plot of methylation degree, age at onset (AO) (years) and (CAG)$_n$ length for (a) probe 1, (b) probe 2, (c) probe 3, and (d) probe 4. The direct relation between the higher probe 1 methylation degree and AO is indicated by an arrow. Note that methylation degree is in a different scale for each probe.

Length was also included in the model, the effect of probe 1 methylation was lost, at the expense of the effect of both normal and expanded CAG repeats ($p = 0.062$ and $p < 0.0001$). One reason might be that this CpG was methylated in the vast majority of patients, meaning that its effect might not be very prevalent, when the whole group was considered.

The ATXN3 promoter is divided into two large CpG islands (7). Probe 1 contains one CpG
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dinucleotide (-499) within the first island. The other five CpGs studied (-228, -205, -183, -103, and -93) are located in the second island. A previous analysis of the promoter sequence from −1089 to +1 showed several potential transcription regulation elements (7). The region between -768 and -495 contains an Alu repeat with 94% identity to the Alu–Sp subfamily consensus sequence (7). This region contains the CpG presently showing a trend toward association with AO. Alu sequences have been shown to have regulatory functions when located near promoters and are usually hypermethylated, thus the possible effect reported here may be common to other conditions (8, 9). Also, it has been shown previously that the region between -632 and -292, harboring a potential binding site for myelin transcription factor 1 (MYT1), had a positive effect on transcription (7). MYT1 has been found to be highly expressed in cells of the subventricular zone (10), a germinal area from which neurons, astrocytes, and oligodendrocytes arise, suggesting that this DNA-binding protein may regulate the differentiation of neuronal progenitors (7).

We conclude that an epigenetic control of the ATXN3 promoter cannot be excluded as a possible contributor for AO variation in MJD. Additional studies, with a larger sample or specially designed to investigate differential methylation patterns in MJD patients’ brains, are needed to confirm this putative epigenetic control. If ATXN3 hypermethylation is shown to be associated to a better prognosis, this effect would help the proof of concept for RNA silencing or other therapies aiming at reducing the expression of the expanded ataxin-3 in MJD.

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References


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