ATXN1: Expanding the spectrum of polyglutamine repeats in ALS.

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ENCALS meeting 2017 Ljubljana
Polyglutamine & PolyQ diseases

PolyQ disease gene

DNA

C A T C A G C A G C A G G T A A T C

Amino acids

His Gln Gln Gln Val Ile

normal protein
Polyglutamine & PolyQ diseases

PolyQ disease gene

DNA

Amino acids

CAG repeat expansion

normal protein

PolyQ protein
Polyglutamine & PolyQ diseases

PolyQ disease gene

DNA

Amino acids

CAG repeat expansion

normal protein

RNA toxicity (?)

PolyQ protein

Aberrant interactions

Aggregate formation

Dysregulation of transcription factors

Neurodegeneration
Spinocerebellar ataxias (SCA) & Polyglutamine expansions

Many caused by CAG-expansion
  - SCA1 (ATXN1), SCA2 (ATXN2), SCA3 (ATXN3), SCA6, SCA7, SCA17

Clinical symptoms:
  - Adult onset
  - Slow progressive (15-30 yrs)
  - Cerebellar symptoms
  - Non-cerebellar symptoms: spasticity, hyperreflexia, weakness, cramps, fasciculations, atrophy, cognitive impairment, Parkinsonism, autonomic dysfunction
ALS & Polyglutamine expansions

Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS

Andrew C. Elden¹*, Hyung-Jun Kim²*, Michael P. Hart¹*, Alice S. Chen-Plotkin³,⁴*, Brian S. Johnson¹,

Yeast screen for TDP-43 toxicity: Pbp1 (orthologue of Ataxin-2)
Ataxin-2 in ALS

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Total</th>
<th>≤26 repeats</th>
<th>27–33 repeats</th>
<th>Percentage of 27–33 repeats</th>
<th>P value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS</td>
<td>915</td>
<td>872</td>
<td>43</td>
<td>4.7%</td>
<td>$3.6 \times 10^{-5}$</td>
<td>2.80</td>
</tr>
<tr>
<td>Neurologically normal</td>
<td>980</td>
<td>966</td>
<td>14</td>
<td>1.4%</td>
<td></td>
<td>(1.54–5.12)</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval

ATXN2

<table>
<thead>
<tr>
<th>PolyQ repeat length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal 15</td>
</tr>
<tr>
<td>ALS 27</td>
</tr>
<tr>
<td>SCA2 34</td>
</tr>
<tr>
<td>Normal 64</td>
</tr>
</tbody>
</table>
What about other polyQ genes in ALS?

<table>
<thead>
<tr>
<th>PolyQ gene</th>
<th>Patients with ALS tested</th>
<th>Controls tested</th>
<th>PolyQ repeat lengths(^a)</th>
<th>Differences in polyQ length in ALS vs control?</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA1 (ataxin 1)</td>
<td>526</td>
<td>526</td>
<td>21-37 (27, 28)</td>
<td>No</td>
</tr>
<tr>
<td>SCA2 (ataxin 2)(^b)</td>
<td>915</td>
<td>980</td>
<td>4.7% Q27-Q33 ALS; 1.4% Q27-Q33 control</td>
<td>Yes; (p = 3.6 \times 10^{-5})</td>
</tr>
<tr>
<td>SCA3 (ataxin 3)</td>
<td>488</td>
<td>623</td>
<td>13-33 (13, 21)</td>
<td>No</td>
</tr>
<tr>
<td>SCA6 (CACNA1A)</td>
<td>146</td>
<td>167</td>
<td>3-17 (10, 12)</td>
<td>No</td>
</tr>
<tr>
<td>SCA7 (ataxin 7)</td>
<td>156</td>
<td>170</td>
<td>1-12 (5)</td>
<td>No</td>
</tr>
<tr>
<td>SCA17 (TBP)</td>
<td>116</td>
<td>85</td>
<td>25-41 (35, 36)</td>
<td>No</td>
</tr>
<tr>
<td>DRPLA (atrophin 1)</td>
<td>120</td>
<td>106</td>
<td>19-36 (27)</td>
<td>No</td>
</tr>
<tr>
<td>Huntington disease</td>
<td>121</td>
<td>115</td>
<td>7-31 (18)</td>
<td>No</td>
</tr>
<tr>
<td>(huntingtin)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AR (androgen receptor)(^c)</td>
<td>146</td>
<td>100</td>
<td>Male: 22-26 (23 ALS; 23, 25 control); female: 21-26 (22, 23 ALS; 25 control)</td>
<td>No</td>
</tr>
</tbody>
</table>
ALS case in Dutch SCA1 family (ATXN1 polyQ)
ALS case in Italian SCA1 family

Rossella Spataro & Vincenzo La Bella; J Neurol (2014) 261:1442–1443
# ATXN1 & ALS in Italian ALS cohort:

<table>
<thead>
<tr>
<th>Alleles</th>
<th>sALS</th>
<th>Controls</th>
<th>p Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATXN-1, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥32 repeats</td>
<td>57 (7.07)</td>
<td>13 (2.4)</td>
<td>0.0001(^b)</td>
<td>2.396 (1.26-4.56)</td>
</tr>
<tr>
<td>&lt;32 repeats</td>
<td>749 (92.9)</td>
<td>531 (97.6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# ATXN2 vs ATXN1

## Similarities:
- Both have variability in CAG repeat size
- Both polyglutamine repeats can be interrupted
- Both are TDP-43 RNA targets
- Neuronal cell loss in overlapping CNS regions

## Differences:

<table>
<thead>
<tr>
<th></th>
<th>ATXN2</th>
<th>ATXN1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range</td>
<td>15-32</td>
<td>27-36</td>
</tr>
<tr>
<td>Interruption</td>
<td>CAA (Glutamine)</td>
<td>CAT (Histidine)</td>
</tr>
<tr>
<td>TDP-43 toxicity</td>
<td>Yes</td>
<td>Unknown</td>
</tr>
<tr>
<td>Inclusion bodies</td>
<td>TDP-43</td>
<td>Intranuclear</td>
</tr>
</tbody>
</table>

**SAME SAME**

**BUT DIFFERENT**
Aim(s) of the study

To investigate whether there is an increase in the number of CAG/CAT repeats in ATXN1 in ALS compared to non-ALS controls
<table>
<thead>
<tr>
<th>Cohort</th>
<th>ALS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Netherlands</td>
<td>1434</td>
<td>1425</td>
</tr>
<tr>
<td>France</td>
<td>496</td>
<td>425</td>
</tr>
<tr>
<td>Belgium</td>
<td>537</td>
<td>212</td>
</tr>
<tr>
<td>Ireland</td>
<td>205</td>
<td>354</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2672</strong></td>
<td><strong>2416</strong></td>
</tr>
</tbody>
</table>

**ALS Clinical data (N = 2531)**

- Male (%): 1494 (59.0)
- Age at onset in years (SD): 61.9 (11.5)
- Survival after onset in months (SD): 42.9 (38.8)
- Bulbar onset (%): 774 (30.6)
- C9ORF72 expansion (%): 211 (8.3)
Methods

Step 1: Sanger sequencing – 850 samples
• Advantage: The actual length of the repeat
• Disadvantage: Artifacts and Low Call Rates
• Outcome: 29 & 30 Most common alleles

Step 2: Repeat PCR – All samples
• Automated calling
ROC-analysis: Best 32 or 33 (sens=0.75, spec=0.28)
ROC-analysis + Distribution: Intermediate = 33 - 40

ATXN1 - CAG/CAT Repeat Size
## ATXN1 & ALS

<table>
<thead>
<tr>
<th>ATXN1 Repeat Size Genotype (&gt;32 CAG/CAT)</th>
<th>normal</th>
<th>expanded (%)</th>
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<tr>
<td>ALS</td>
<td>2344</td>
<td>328 (12.3%)</td>
</tr>
<tr>
<td>Controls</td>
<td>2172</td>
<td>244 (10.1%)</td>
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<tr>
<td></td>
<td>normal</td>
</tr>
<tr>
<td>ALS</td>
<td></td>
</tr>
<tr>
<td>C9ORF72 -</td>
<td>1915</td>
</tr>
<tr>
<td>C9ORF72 +</td>
<td>195</td>
</tr>
</tbody>
</table>
Association analysis

**OR = 1.28**

95% CI : 1.07-1.53

*p = 0.0036* (one-sided)

*Fixed & Random effects model*

Heterogeneity n.s.
ATXN1 & Survival after onset

Corrected for Sex, Site of onset & C9ORF72 ($p = 0.14$)
ATXN1 & Age at onset

![Graph showing onset probabilities over time for normal and expanded conditions.](image)
(Preliminary) Conclusions - Genetics

Significant association with an increased length of CAG(/CAT) trinucleotide repeats in $ATXN1$ and ALS
- Cut-off 33
- Dominant model

No enrichment for intermediate $ATXN1$ expansions in C9ORF72 expanded ALS individuals

No significant difference in survival

No difference in age at onset
Discussion

ATXN1 vs ATXN2

- Allele frequency intermediate expansion
  - ALS: 1-2% in ATXN2 vs 6% in ATXN1
  - Controls: 0.4% in ATXN2 vs 5% in ATXN1

- RR: ATXN2 3.06 (pooled, Sproviero et al.) vs ATXN1 1.22 (p=0.01)

- Genetic differences (eg. CAT interruptions)
- Biological differences
Discussion – The next step

Steven Boeynaems – KU Leuven
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Stéphanie Millecamp

Steven Boeynaems
Matthieu Moisse
Philip Van Damme

Russell McLaughlin
Orla Hardiman
Now this is not the end
It’s not even the beginning of the end
But it is, perhaps

THE END OF THE BEGINNING.