Brain Research: Imaging and Cognition in Duchenne Muscular Dystrophy

Nathalie Doorenweerd, PhD
n.doorenweerd@lumc.nl
Nathalie.Doorenweerd@Newcastle.ac.uk
Story outline

- Why we are interested in the brain in Duchenne muscular dystrophy
- Genetics and brain atlases

- Intermission / questions

- Neuroimaging
  - Brain structure / anatomy
  - Blood supply to the brain
  - Functional connectivity

- Future works

- Questions
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Duchenne muscular dystrophy

Increasing awareness of neuropsychological aspects
Guidebooks for parents, teachers & professionals
Duchenne muscular dystrophy

Reduced general intellectual ability
DMD - neuropsychological profile

- Information processing
- Verbal working memory
- Reading difficulties or even inability to read

- Autism spectrum disorder 3-15%
- Attention deficit hyperactivity disorder 11-32%
- Obsessive compulsive disorder 5-60%
- Anxiety 27%
- Epilepsy 6.3%
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**DMD gene and dystrophin protein in muscle**

Muscle biopsy
Red staining for Dystrophin

Healthy

Duchenne’s
DMD gene and multiple dystrophin proteins
DMD gene and multiple dystrophin proteins

DMD gene and multiple dystrophin proteins

Y chromosome
X chromosome

Proximal

Dp427 muscle and brain

Distal
Dystrophin gene

- Y chromosome
- X chromosome

DMD gene and multiple dystrophin proteins

Proximal → Distal

- Dp427: muscle and brain
- Dp260: retina
- Dp140: brain and kidney
- Dp116: peripheral nerve
- Dp71: ubiquitously
- Dp40
Unique position

- We actually know which gene is involved
- We know multiple proteins are generated from this gene

- We wish to know exactly where in the brain these proteins are expressed
- We wish to know when during development these proteins are important
- We wish to know the function of these proteins in the brain

Step 1. Determine where dystrophin is expressed in the healthy human brain across development
Adult human brain atlas

www.brain-map.org

19,991 genes mapped out in 6 healthy post-mortem brains (5 male, 1 female)
A total of 3702 samples from all different areas within the brain
DMD gene in the Allen adult human brain atlas
Three main dystrophin proteins of different sizes in the brain

- **Dp427**
- **Dp260**
- **Dp140**
- **Dp116**
- **Dp71**
- **Dp40**

**Proximal**

**Y chromosome**

**X chromosome**

**Distal**

**DMD gene**

- **retina**
- **peripheral nerve**

BrainSpan Atlas

www.brain-map.org

241,690 exons mapped out in 42 healthy post-mortem brains
Youngest: 8 weeks post-conception
Oldest: 40 years of age
Dystrophin protein RNA expression in the healthy human brain across development

![Graph showing dystrophin RNA levels across development stages: Fetus, Birth, Adult.](image-url)
Co-expression analysis

- We wish to know exactly where in the brain these proteins are expressed
- We wish to know when during development these proteins are important
- We wish to know the function of these proteins in the brain

Step 2. determine which of the 19,990 other genes in the atlas are expressed in the same place at the same time

Find out what is known about those genes’ function

Guilt by association → to generate hypotheses on the function of dystrophin in the brain
Functions of dystrophin in the brain

Different functions were found for each of the dystrophin sizes:

Dp427  Signal transmission / transmembrane transporter activity

Dp140  Developmental biology / neuron development and axon guidance

Dp71   Blood vessel development / extracellular structure organization
Likely part of the same mechanisms that are related to ASD, ADHD and OCD

Genes known from Autism, ADHD, OCD and dyslexia largely present in the same place at the same time in the brain

- Does dystrophin play a role in similar mechanistic pathways in the brain?
- Does this help explain some of the behaviors seen in DMD?
Dystrophin is expressed in the brain

Dystrophins in the brain are present during different life stages

Highly significant correlation with the genes known from Autism, ADHD, OCD and dyslexia

Helps us to better understand why these neurodevelopmental disorders have such a high incidence in DMD:
May play a role in the same mechanistic pathways
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## Study cohort

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>DMD</th>
</tr>
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<tbody>
<tr>
<td>No. of participants</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>Age, yr&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.1 (2.0)</td>
<td>12.3 (2.8)</td>
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<tr>
<td>Range, yr</td>
<td>8–16</td>
<td>8–18</td>
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<tr>
<td>Steroid treatment, No.</td>
<td>—</td>
<td>24</td>
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<tr>
<td>10 day on/10 day off cycle, No.</td>
<td>—</td>
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<tr>
<td>Brooke scale&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>2.1 (1.6)</td>
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<tr>
<td>Vignos scale&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>5.9 (3.1)</td>
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<tr>
<td>Wheelchair bound, No.</td>
<td>—</td>
<td>15</td>
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<tr>
<td>Age of loss of ambulation, yr&lt;sup&gt;a&lt;/sup&gt;</td>
<td>—</td>
<td>10.2 (2.1)</td>
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<tr>
<td>Regular education, No.</td>
<td>22</td>
<td>17</td>
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<tr>
<td>Reading composite score, No.</td>
<td>21</td>
<td>25</td>
</tr>
<tr>
<td>Information composite score, No.</td>
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<td>27</td>
</tr>
<tr>
<td>SDQ composite score, No.</td>
<td>22</td>
<td>28</td>
</tr>
</tbody>
</table>
Study design

30 min  60 min  45 min  30 min  30 min

Chemical shift (ppm)
T1-weighted MRI:
Gross brain anatomy – volume analyses
Structural scans - volume measurements

Control, 10 years old

DMD, 10 years old
Smaller total brain volume: less grey matter

- Total brain volume
  - CON
  - DMD
  - * indicates significant difference

- Grey matter volume
  - CON
  - DMD
  - ***) indicates highly significant difference

- White matter volume
Diffusion Tensor Imaging: visualizing white matter fiber bundles
DTI scans

Control, 10 years old

DMD, 10 years old
Group comparison of how streamlined the white matter connections are.
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Arterial spin labeling: measuring cerebral blood flow
How we measure blood flow to the brain non-invasively
Reduced cerebral blood flow in the grey matter

CON  DMD

Cerebral blood flow

mL/100g/min

80

60

40

20

0

CON  DMD

Decreased cerebral perfusion in Duchenne muscular dystrophy patients.

Back to the different dystrophins in the brain

Dystrophin RNA

Fetus  Birth  Adult
Subgroup results

Grey matter volume

Cerebral blood flow

Reading skills

Information processing

CON

DMD A

DMD B

Grey matter volume

Cerebral blood flow

Reading skills

Information processing

CON

DMD A

DMD B

Grey matter volume

Cerebral blood flow

Reading skills

Information processing

CON

DMD A

DMD B
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Where do we go from here?

Smaller grey matter volume and altered streamlining of the white matter fiber bundles

- Are these differences a result of different development of the brain, developmental delay, or compensation?

Cumulative loss of dystrophin proteins

- Do the boys who miss all dystrophin proteins show greater differences from controls?

Reduced blood flow in the brain

- Does this come from the head or the body?

Overall:
Still hard to tell ‘good’ versus ‘bad’ without more information
Acknowledgements

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J.G.M. Hendriksen, D.G. Schrans
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