The C9ORF72 repeat expansion in ALS
The C9ORF72 repeat expansion in ALS

It’s complicated…

…sometimes frustrating…

more questions than answers!

Steve Gleason
Overview
Adult onset neurodegeneration

aapp. 20’000 individuals in USA

Degeneration of upper and lower motor neurons

Paralysis and ultimately death

~ 90% sporadic, 10% familial cases

Various disease causing genes identified (SOD1, TDP-43, FUS, C9ORF72)

Vast majority of cases: cause unknown
Discovery of the Repeat

Recent efforts have revealed new genes involved in ALS.

2011: 2 publications linking a repeat expansion in C9ORF72 to ALS and FTD

How did they discover the repeat?
Discovery of the Repeat

Association of risk locus in the region of C9ORF72

Shatunov et al., Lancet Neurol 2010
Discovery of the Repeat

Next generation sequencing methods failed to amplify the repeat containing region...

Renton et al, Neuron 2011
Affected individuals seemed all homozygous via PCR based detection methods...

Or is amplification inhibited?

Back to the ancient methods...

DeJesus-Hernandez, Neuron 2011
Discovery of the Repeat

Back to the ancient methods...

Southern blot revealed repeat expansion
Discovery of the Repeat

Confirmation with repeat primed PCR methods...

Not able to determine repeat size
Why is C9ORF72 important in ALS?

- C9ORF72 mutations are the most frequent cause of familial ALS and the most frequent known cause of sporadic ALS.
Why is C9ORF72 important in ALS?

Where does it come from?
Origin and distribution of C9ORF72

High prevalence of the mutation in northern Europe

Distribution of this mutation worldwide varies extensively
Risk allele is common in healthy population in Europe

Associated with higher instability and bigger repeat length in healthy individuals

Most, but not all patients with C9ORF72 expansions carry this risk haplotype

Even without repeat expansion, allele still associates with disease

Is the repeat or the instability of the region inherited?
Existence of single founder allele spread by the Vikings?

- Theory currently debated – high prevalence in certain other populations difficult to be explained by this migration (S Europe + E Asia)

- Larger study including 82 SNPs indicates that this founder haplotype is likely older – found in European, African and distant Asian populations (Smith et al, Eur J Hum Genet 2013)
Overview
Pathogenic C9ORF72 repeat size

- Normal repeat size = variable – more than 90% of Europeans 2-10 repeats
- Small: Larger than 30 repeats can be found in healthy individuals, although rare
- Intermediate: 20 – several hundred can be pathogenic or not
- Big: Repeat size in patients usually several hundreds or thousands of repeats

Rohrer et al., Lancet Neurol 2015
Disease characteristics of C9ORF72

Clinical spectrum broad, even within families

$[G_4C_2]_n$ Expansion of C9ORF72

- Amyotrophic Lateral Sclerosis
  - Parkinsonism
  - Olivopontocerebellar Degeneration
  - Huntington’s Disease Phenocopies
  - Corticobasal Syndrome

- Frontotemporal Lobar Dementia
  - Alzheimer’s Disease

Motor Phenotypes

Non-Motor Phenotypes

Cooper-Knock et al, Acta Neuropathol 2014
Disease characteristics of C9ORF72

Mixed phenotype might be more common than usually thought in general

- Up to 50% of sporadic ALS cases could show cognitive impairments

Figure. Comparison of cognitive performance in normal controls vs four amyotrophic lateral sclerosis groups. BFRT = Benton Facial Recognition Test; VSAT = Verbal Series Attention Test; AMNART = American National Reading Test; LMI = Logical Memory subtest from the Wechsler Memory Scale-Revised (immediate recall); LMII = Logical Memory subtest from the Wechsler Memory Scale-Revised (delayed recall); VRI = Visual Reproduction subtest from the Wechsler Memory Scale-Revised (immediate recall); VRII = Visual Reproduction subtest from the Wechsler Memory Scale-Revised (delayed recall).

Ringholz et. al, Neurology 2005
Disease characteristics of C9ORF72

ALS: Amyotrophic Lateral Sclerosis

- Most common adult onset motor neuron disorder
- 1-2/100’000 individuals, onset 50-60 years
- Average survival ~ 3 years

FTD: Frontotemporal Dementia

- Second most common form of pre-senile dementia after Alzheimer’s disease
- 10-30/100’000 individuals, onset 45-65 years
- Average survival ~ 7 years

Short disease length = increased life time risk

Diverse clinical phenotype implicates presence of disease modifiers
Disease characteristics of C9ORF72

Variation in time and type of onset

- Penetrance = age dependent, but nearly 100% at the age of 80
- Several studies show overrepresentation of bulbar onset for C9ORF72-ALS
- Incidence of dementia or family history of dementia is higher in C9ORF72-ALS cases
- Potential evidence for younger age of disease onset in ALS caused by C9ORF72 repeat expansions
- Potential evidence for shorter survival of C9ORF72 ALS cases compared to non-C9ORF72 cases
- Gender might be important – males showed earlier onset in 1 study

Reviewed in Cooper-Knock et al, Acta Neuropathol 2013
Somatic heterogeneity of C9ORF72 repeat expansions

→ Tissue specific variation in repeat size

→ Expansions can increase throughout life span

→ Substantial problem for testing of patients due to CNS sample availability

Blitterswijk et al., Lancet Neurol 2013
Somatic heterogeneity of C9ORF72 repeat expansions

Repeat size in the CNS varies between regions

- Repeats are shorter and less variable in the cerebellum (mean ~ 1'667 vs 5'250 repeats in frontal cortex)

Blitterswijk et al., Lancet Neurol 2013
Cerebellum seems to have few alterations in repeat length in different mouse models of repeat expansion.

Similar observations found in mouse models of other repeat expansions.

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Repeat length</th>
<th>Brain</th>
<th>Cerebellum</th>
<th>Cerebral cortex</th>
<th>Heart</th>
<th>Kidney</th>
<th>Liver</th>
<th>Skeletal muscle</th>
<th>Spleen</th>
<th>Striatum</th>
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<tbody>
<tr>
<td>DM1 knock in</td>
<td>84</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>–</td>
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<tr>
<td>DM300</td>
<td>360</td>
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<td>++</td>
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<td>+++</td>
<td>–</td>
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<td>+++</td>
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<td>SCA1</td>
<td>154</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<td>94</td>
<td>+</td>
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<td>R6/1</td>
<td>116</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>+++</td>
</tr>
<tr>
<td>R6/2</td>
<td>144</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>+++</td>
</tr>
</tbody>
</table>

Blank, not tested; –, no instability detected; + to +++ marginal to extreme instability.
This list was updated from that in [108]. Only tissues with measurements in at least three different mouse models are shown.

Dion, Trends in Genetics, 2014

Cerebellum seems to have few alterations in repeat length in different mouse models of repeat expansion.
Origin somatic heterogeneity

How to cope with DNA damage

- Approximately 100'000 lesions in DNA PER cell PER day
- 3 major repair pathways were shown to participate in repeat instability
Origin somatic heterogeneity

How to cope with DNA damage

- A 4th one was recently suggested to be involved in repeat instability
  - DNA helicase
  - Replication fork progression after G4 resolution by FANCJ/DOG-1
  - G4 quadruplex formation in ssDNA

- G-quadruplexes were recently observed from C9ORF72 repeat expansion DNA
  - DSB
  - Theta-mediated end joining
  - deletion

Van Kregten, Experimental Cell Res, 2014
Origin somatic heterogeneity

- Repeat expansions per se have very high mutation rates
- Mutation rates depend on different DNA repair pathways and often lead to tissue specific disease phenotypes
- Different tissues preferably use different repair pathways
- Sensitivity to different types of damage depend on the mitotic state and metabolic rate and age
- Repeat instability could depend on frequency of repair initiation, variation in repair protein expression, replication rate, transcription and chromatin structure
- Recently described DNA structure formation requires special DNA helices for solvation

Disease characteristics of C9ORF72

Repeat length and disease

- Pure ALS:
  - no phenotypic aspect significantly correlated with length of expansion

Blitterswijk et al., Lancet Neurol 2013
Disease characteristics of C9ORF72

FTLD:

- Correlation: age of onset and repeat size in frontal cortex
- Correlation: reduced survival and repeats < 11.1kb in cerebellum

These studies might require the analysis of larger numbers of cases due to the noise introduced by additional modifiers (similar to Myotonic Dystrophy DM1 – 100+ patients needed)
Pathology of C9ORF72 repeat expansions

- TDP43+ inclusions
- Star-like p62+/TDP43-inclusions containing polypeptides

Dipeptide repeat protein inclusions are unique and highly characteristic for C9ORf72 cases

Rohrer et al, Lancet Neurol, 2015
Pathology of C9ORF72 repeat expansions

TDP43+ inclusions correlate with clinical phenotype and pattern of neurodegeneration

- C9ORF72 FTD patients with no signs of ALS: significantly less degeneration + TDP43 pathology in lower motor neurons compared to patients with mixed phenotype

  Stewart et al, Acta Neuropathol 2012

- C9ORF72 ALS patients: predominant degeneration and TDP43+ inclusions in upper, lower and brainstem and spinal cord motor neurons (extra-motor regions are only mildly affected)

  Davidson et al, Acta Neuropathol 2014
Pathology of C9ORF72 repeat expansions

RNA foci

- RNA foci in the nucleus were identified in FTD and ALS patients in the cortex and spinal cord in many studies.

- These are common hallmarks of many repeat expansion disorders.
Mechanisms of C9ORF72 mediated neurodegeneration

1. Reduced C9orf72 protein and function

2. RNA toxicity: sequestration of RNA-binding proteins

3. Translation into toxic DPR proteins

Repeat containing C9orf72 DNA

(GGGGCC)_n sense RNA

(GGCCCC)_n antisense RNA

Nucleus

Cytoplasm

Neuron

Rohrer, Lancet Neurol 2015
Mechanisms of C9ORF72 mediated neurodegeneration

1. Haploinsufficiency

- 3 mRNA variants described
- 2 potential protein isoforms
- Reduction of abundance of all 3 transcripts has been reported
- Evidence for v1 being more affected (repeat in promoter)
- Model organisms C. elegans and zebrafish show that C9ORF72 loss is pathogenic for motor neurons and causes motor deficits
Mechanisms of C9ORF72 mediated neurodegeneration

1. Haploinsufficiency

- C9ORF72 protein has strong homology with DENN-like proteins
- DENN protein family involved in membrane trafficking
- Members of this protein family have been linked to neurodegeneration

Levine, Bioinformatics 2013
Mechanisms of C9ORF72 mediated neurodegeneration

1. Haploinsufficiency

- Yeast homologue of C9ORF72 has been linked to sorting of endosome-localized proteins to cell surface (avoidance of lysosomes)

- Is endo-lysosomal pathway affected by reduced C9ORF72 protein levels?

- Disease modifying gene in FTD TMEM106B is involved in lysosome function and transport in dendrites

- p62+ inclusion pathology could point towards dysfunctional lysosomal degradation

- Mutations in another gene involved in lysosomal degradation of proteins (CHMP2B) causes FTD in a Danish family

Reviewed in Yokoyama et al, Am J Neurodegener Dis 2014
Mechanisms of C9ORF72 mediated neurodegeneration

2. RNA toxicity

[Diagram showing different C9orf72 variants and their effects on RNA toxicity]
Mechanisms of C9ORF72 mediated neurodegeneration

2. RNA toxicity

▌ Inclusions found in several disease affected cell types

Mechanisms of C9ORF72 mediated neurodegeneration

2. RNA toxicity

- Repeat RNA foci frequently found in affected brain regions in FTD
- Burden correlates with clinical disease phenotype
- Overexpression of repeat itself causes neurodegeneration in several animal models (zebrafish, drosophila)

Mizielinska, Science Reports 2014
Mechanisms of C9ORF72 mediated neurodegeneration

2. RNA toxicity

- Overexpression of 38x or 72x repeats sufficient to cause neurodegeneration in neuronal cell lines and zebrafish
  
  Le et al, Cell Reports 2013

- Likely via sequestration of RNA binding proteins involved in nuclear retention, pre-mRNA splicing or RNA trafficking (hnRNP H)
DNA hypermethylation which reduces repeat RNA expression is a disease modifier in FTD.

- Hypermethylation is associated with later age at death in FTD, longer disease duration.
- Hypermethylation is associated with shorter repeat length.

This correlation was not observed for ALS in the same study.

Russ, Acta Neuropathol 2014
Mechanisms of C9ORF72 mediated neurodegeneration

2. RNA toxicity

- Repeat RNA was shown to be toxic in other expansion disorders (DM1 and DM2) by sequestration of RNA binding proteins (muscle-blind-like proteins)
Mechanisms of C9ORF72 mediated neurodegeneration

3. Dipeptide repeat protein translation - RAN

Repeat Associated Non-ATG Translation Initiation: One DNA, Two Transcripts, Seven Reading Frames, Potentially Nine Toxic Entities!

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¹ Program of Genetics and Genome Biology, The Hospital for Sick Children, Toronto, Ontario, Canada, ² Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada
Mechanisms of C9ORF72 mediated neurodegeneration
Mechanisms of C9ORF72 mediated neurodegeneration

3. Dipeptide repeat protein translation - RAN

- Initially discovered in SCA8, found in other repeat expansion disorders

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<tr>
<td>SCA8</td>
<td>CAG</td>
<td>polyGln</td>
<td>&gt;42 repeats</td>
<td>ATG-polyGln, cerebellum and brain stem (14)</td>
<td>(11)</td>
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<td>polyAa</td>
<td>&gt;73 repeats</td>
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<td></td>
<td>polySer</td>
<td>&gt;58 repeats</td>
<td>ND</td>
<td>(11)</td>
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<td></td>
<td>polyGln</td>
<td>ND</td>
<td>Myoblasts, skeletal muscle, peripheral blood leukocytes (11)</td>
<td>(11)</td>
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<tr>
<td>DM1</td>
<td>CAG</td>
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<tr>
<td>FXTAS</td>
<td>CGG</td>
<td>polyGly</td>
<td>&gt;30 repeats</td>
<td>Frontal cortex, cerebellum, hippocampus (24)</td>
<td>(24)</td>
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<td></td>
<td></td>
<td>polyAa</td>
<td>&gt;88 repeats</td>
<td>ND</td>
<td>(24)</td>
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<td></td>
<td></td>
<td>polyArg</td>
<td>UD</td>
<td>ND</td>
<td>(24)</td>
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<tr>
<td>ALS/FTD</td>
<td>GGGGCC</td>
<td>polyGlyPro</td>
<td>&gt;145 repeats</td>
<td>Cerebellum, hippocampus, iPSC-derived neurons, neocortex, medial and lateral geniculate nuclei, testes (25–27)</td>
<td>(25–27)</td>
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<tr>
<td></td>
<td></td>
<td>polyGlyAa</td>
<td>&gt;38 repeats</td>
<td>Cerebellum, hippocampus (26)</td>
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<tr>
<td></td>
<td></td>
<td>polyGlyArg</td>
<td>UD</td>
<td>Cerebellum, hippocampus (26)</td>
<td>(26)</td>
</tr>
</tbody>
</table>

ND, not examined and not determined; UD, examined but undetermined.

*Antisense transcript.
Mechanisms of C9ORF72 mediated neurodegeneration

3. Dipeptide repeat protein translation - RAN

Many studies on C9ORF72 found evidence for DRPs in C9ORF72 ALS and FTD
Mechanisms of C9ORF72 mediated neurodegeneration

3. Dipeptide repeat protein (DRP) translation - RAN

- Overexpression of Proline-Arginine DRP is toxic in vitro and in vivo

Wen, Neuron 2014
Mechanisms of C9ORF72 mediated neurodegeneration

3. Dipeptide repeat protein (DRP) translation - RAN

- RNA repeats that are not translated do not cause neurodegeneration
- Repeat RNA toxicity and DRP peptide production seem to be linked

Mizielinska, Science Reports 2014
But...dipeptide repeat protein pathology does not always seem to correlate with either phenotype or degeneration pattern.

More studies will be needed to decipher the correlation between disease course, clinical manifestation and DRPs.

Summary of potential toxic mechanisms

Cleary and Ranum, Hum Mol Genet 2013
Overview
Thank you!
Other repeat expansion disorders causing neurological disorders

<table>
<thead>
<tr>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myotonic Dystrophy type 1 (DM1)</td>
</tr>
<tr>
<td>Myotonic Dystrophy type 2 (DM2)</td>
</tr>
<tr>
<td>Spinocerebelar ataxia 1 (SCA1)</td>
</tr>
<tr>
<td>Spinocerebelar ataxia 2 (SCA2)</td>
</tr>
<tr>
<td>Spinocerebelar ataxia 3 (SCA3)</td>
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<td>Spinocerebelar ataxia 6 (SCA6)</td>
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<td>Spinocerebelar ataxia 12 (SCA12)</td>
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<td>Spinocerebelar ataxia 17 (SCA17)</td>
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<td>Spinocerebelar ataxia 31 (SCA31)</td>
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<tr>
<td>Spinocerebelar ataxia 36 (SCA36)</td>
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<tr>
<td>Fragile X mental retardation 1 (FMR1)</td>
</tr>
<tr>
<td>Fragile X-associated tremor ataxia syndrome (FXTAS)</td>
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<tr>
<td>Fragile X mental retardation 2 (FMR2)</td>
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<tr>
<td>Huntington’s disease (HD)</td>
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<tr>
<td>Huntington’s disease-like 2 (HDL2)</td>
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<td>Friedreich’s Ataxia (FRDA)</td>
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<td>Epilepsy progresive myelonol (EPMI)</td>
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<tr>
<td>Oculopharyngeal muscular dystrophy (OPMD)</td>
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<tr>
<td>Spinal and bulbar muscular atrophy (SBMA)</td>
</tr>
<tr>
<td>X-linked mental retardation</td>
</tr>
<tr>
<td>Dentatorubral-pallidoluysian atrophy (DRPLA)</td>
</tr>
<tr>
<td>ALS and/or FTD</td>
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</tbody>
</table>

For more information we refer to recent reviews [84,87–89,90,92,93-97], articles [85,86**,98–103], and GeneReviews (http://www.ncbi.nlm.nih.gov/sites/GeneTests/review).

Blitterswijk, Curr Opinion Neurol 2012
Other repeat expansion disorders

- At least 24 other neurological disorders
- Non-coding repeat expansions usually display RNA foci (DM2, FXTAS, HDL2, SCA36, SCA31, SCA8, SCA10)
- Commonality points towards similar RNA gain-of-function mechanism
- Intronic repeats usually long

Add table Marka van Blitterswijk, Curr Opin Neurol 2012 how do c9orf72 repeat expansions cause als and ftd