Influence of Genetic Variants on Underlying Neuropathology in Neurodegenerative Disorders

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Overview

- Clinical-pathological associations in PPA
- Genetic-pathological associations in PPA-related neuropathology
- Rare pathogenic variants in PPA-related neuropathology
- Common genetic variants in PPA-related neuropathology
Protein aggregation in neurodegenerative disease

- Intraneuronal/glial inclusions composed of abnormally processed proteins
  - Correlation with neuronal loss and clinical symptoms
  - Diagnostic “gold standard”
- Pathogenic mutations in these proteins cause disease
  - Altered function
  - Increased fibrillization
- Toxicity in animal/cell models
  - Transmission studies

FTDP-17 (P301L MAPT) Cingulate Gyrus

Image courtesy of PENN CNDR

From Iba et al. J Neurosci 2013
Disease-modifying therapy: Targeting protein aggregation

What is PPA? - Ask a Neurologist

Primary Progressive aphasia
- **Subjective** language difficulty
- **Objective** cognitive findings (testing)
  - Language > memory, spatial impairment
- **Non-independent** activities of daily living
- History or evidence of progressive decline
- Exclusion of other disorders (mimics)
  - MRI brain
  - Blood, CSF, ancillary tests
What is PPA- Ask a neuropathologist?

- **Frontotemporal Lobar Degeneration**
  - **Gross** atrophy of the brain in frontal, temporal lobes.
  - **Microscopic** findings of protein aggregates:
    - *Tau* protein (cell “skeleton”)
    - *TDP-43* protein (cell “genetic regulator”)

- **Alzheimer’s disease**
  - **Gross** atrophy of the brain in frontal, temporal lobes.
  - **Microscopic** findings of protein aggregates:
    - *Tau* protein (cell “skeleton”) + *Amyloid* protein plaques (cell membrane protein)
PPA diagnosis 2016: “Tear down the wall”

CLINICAL

CLINICOPATHOLOGICAL

BIOMARKERS

FTLD- TAU  FTLD- TDP  AD
Importance of accurate diagnosis of PPA pathology

- Protein aggregation and spread through brain is key mechanism of disease.
- Developing therapies aimed at slowing or halting disease progression target these specific proteins
  - Tau
  - TDP-43
  - AD
Improving Diagnostics in PPA

- Can these neuropathological subtypes of PPA accurately be differentiated during life?

Primary Progressive Aphasia

- AD
- TDP
- TAU

Appropriate Clinical Trial Selection
Overview

- Clinical-pathological associations in PPA
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- Rare pathogenic variants in PPA-related neuropathology
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Clinicopathological complexity of young onset dementia

Clinical Syndromes

- bvFTD
- svPPA
- naPPA
- ALS
- PSP
- CBS

Cognitive Disorders

- FTLD-tau
- FTLD-TDP
- AD
- SOD-1
- FUS/Other

Motor Disorders

Legend

- MAPT
- GRN
- C9orf72
- TARDBP
- VCP
- FUS
- SOD-1

From Irwin et al, Acta Neuropathologica 2015
Frontotemporal Lobar Degeneration Neuropathology

FTLD-TAU

FTLD-TDP

From Irwin et al. Acta Neuropathologica 2015
Classes of Proteinopathies

- Tau
- TDP-43
- AD
- ALS
- PSP
- FTLD-TDP
- FTLD-Tau
- CBD
- PiD
- FTDP-17
**Function:** MT stability

**Genetics:** Chromosome 17-\(MAPT\) = FTDP17

6 isoforms (3-4 MTBDs)

Adapted from: Brunden, et al. *Nat Rev.* 2009
**Clinical:** AD Amnestic symptoms

- Later progress to include language, visuospatial and executive dysfunction

**Pathology:** Medial temporal lobe, limbic and neocortical Grey Matter.

**Genetics:** APP and APP processing enzymes (i.e. PSN-1,2)

Images courtesy of PENN CNDR

From: Irwin et al, *Brain* 2012
AD: Atypical presentations

- Posterior Cortical Atrophy
- Corticobasal Syndrome
- Logopenic Progressive Aphasia
- Frontal Variant AD
Tauopathies: Pick’s disease

- **Clinical:** bvFTD- social comportment disorder, executive difficulties. Visuospatial and memory relatively spared. Also naPPA, CBS

- **Pathology:** Ventromedial and dorsolateral frontal, anterior temporal. *Knife-edge atrophy.*
Tauopathies: Corticobasal Degeneration

**Clinical:** CBS Asymmetric akinetic-rigid syndrome. Apraxia, cortical sensory loss, myoclonus. Also bv-FTD, naPPA.

**Pathology:** brainstem, basal ganglia, peri-sylvian cortex. **White matter glial inclusions**

From: Irwin et al, *Brain* 2012
**Clinical:** PSP akinetic-rigid syndrome, supranuclear gaze palsy, axial rigidity, gait imbalance. Also CBS, bvFTD, naPPA.

**Pathology:** Midbrain, pons, dentate nucleus (cerebellum) Variable cortical involvement.

From: Irwin et al, Brain 2012.
Classes of Proteinopathies

- Tau
- AD
- PSP
- CBD
- PiD
- FTDP-17

[Boxed]
- TDP-43
- ALS
- FTLD-TDP
**Function:** RNA binding protein

**Genetics:**
- Chromosome 1 *TARDBP* (TDP-43)
  - ALS >> FTLD
- Chromosome 17 *PGRN*
  - FTLD-TDP (Type A)
- Chromosome 9 *C9orf72*
  - FTLD-TDP/ALS (Type B)
TDP-43 proteinopathies: ALS-FTD

- **Clinical:** upper and lower motor neuron +/- bvFTD/naPPA

- **Pathology:**
  - TDP-43 inclusions
    - *Skeins*
    - *Lewy-like*
    - *Glial inclusion*
  - Variable non-motor involvement

**Clinical:**
- bvFTD, svPPA
- naPPA or CBS (*GRN*)
- +/- ALS (*C9ORF72*)

**Pathology:**
- Neuronal Cytoplasmic/Nuclear Inclusions, Dystrophic neurites
- Glial inclusions
- Subtypes= Genetics

Images courtesy of PENN CNDR

Overview

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- Rare pathogenic variants in PPA-related neuropathology

- Common genetic variants in PPA-related neuropathology
Genetic Variance and Frontotemporal Dementia

- Rare alleles causing Mendelian disease
- Low-frequency variants with intermediate effect
- Common variants implicated in common disease by GWA
- Few examples of high-effect common variants influencing common disease

Effect size vs. Allele frequency graph:
- High
- Intermediate
- Modest
- Low

Very rare, Rare, Low frequency, Common

Genetic variants of FTLD-TDP

Primary Progressive Aphasia

TDP

C9orf72

GRN

Sporadic
30-50% of ALS has cognitive impairment during course of disease\textsuperscript{1-3}
- \textasciitilde{}10-15% meet criteria for FTD clinical syndromes (bvFTD, naPPA)

ALS and FTD share common pathological substrate of TDP-43 neuronal/glial inclusions.
- Extent of non-motor TDP-43 pathology correlates with cognitive impairments.

Shared molecular etiologies in familial and sporadic cases

2. Lomen-Hoerth et al. Neurol. 2005
3. Robinson et al. JNNP. 2005
~50% of FTLD and ~90% of ALS is TDP-43 proteinopathy

30-45% FTD, 10% ALS is familial

C9orf72 = 25% of familial FTD, 40% of familial ALS
• ~5-10% sporadic cases

C9orf72 = Common in ALS-FTD

Advantages for study

- Uniform underlying neuropathology for homogenous patient cohorts
- **Ability to study pre-clinical/early disease**
- Insights from biology associated with pathogenic mutations may identify novel therapeutic targets
- *Could clinical trial outcomes be influenced by hereditary cases of ALS-FTD?*

Comparative study of C9orf72 FTLD/ALS with ALS/FTLD-TDP

- Retrospective case-control study of a large C9orf72-positive (C9N) cohort compared to expansion negative (C9N) ALS/FTLD with known TDP-43 neuropathology.
The most common clinical presentation was ALS (48%) followed by bvFTD (26%)
  • 14% had ALS-bvFTD
PPA phenotype less common (8%)
  • naPPA> svPPA
Small subset of amnestic AD cases (3%)
  • Pathologically confirmed FTLD-TDP with co-morbid AD
Similar to C9N cohort
  • No CBS, lvPPA and less svPPA
C9orf72 expansion mutation in FTLD-ALS

- Comparative study of C9P ALS/FTD with autopsy/genetic confirmed C9N ALS/FTLD-TDP
- C9P vs C9N ALS
  - Earlier age of onset (~5 years)
  - Earlier age of death (~8 years)
  - Shorter disease duration (~1 year)
- C9P FTD had similar demographics to C9N FTD

<table>
<thead>
<tr>
<th></th>
<th>C9orf72 + (C9P)</th>
<th>C9orf72- (C9N)</th>
<th>P-value</th>
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<tbody>
<tr>
<td><strong>ALS</strong></td>
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<tr>
<td>Age Onset</td>
<td>55.1 (1.7) N=31</td>
<td>60.3 (1.9) N=36</td>
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<tr>
<td>Disease Duration</td>
<td>2.6 (0.3) N=24</td>
<td>3.8 (0.4) N=29</td>
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<td><strong>FTD</strong></td>
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<tr>
<td>Age Onset</td>
<td>56.4 (1.2) N=33</td>
<td>57.4 (1.2) N=43</td>
<td>&gt;0.1</td>
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<tr>
<td>Disease Duration</td>
<td>5.7 (0.8) N=19</td>
<td>5.8 (0.9) N=20</td>
<td>&gt;0.1</td>
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From Irwin et al. JNNP 2013
C9orf72 expansion mutation in FTLD-ALS

- C9P FTD had more rapid annualized decline in cognitive verbal fluency (~3 words/min) compared with C9N FTD.

<table>
<thead>
<tr>
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<th>C9P FTD</th>
<th>C9N FTD</th>
<th>P-value</th>
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<tbody>
<tr>
<td>MMSE Baseline</td>
<td>23.3 (1.4)</td>
<td>24.7 (0.9)</td>
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<td>N=26</td>
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<td>Annualized decline</td>
<td>4.8 (1.2)</td>
<td>2.7 (1.1)</td>
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<td>N=15</td>
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<td>Verbal fluency Baseline</td>
<td>6.2 (0.9)</td>
<td>6.4 (0.8)</td>
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<tr>
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<td>N=19</td>
<td>N=28</td>
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<tr>
<td>Annualized decline</td>
<td>4.5 (1.3)</td>
<td>1.4 (0.8)</td>
<td>0.02</td>
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<tr>
<td></td>
<td>N=10</td>
<td>N=16</td>
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</table>

From Irwin et al. JNPP 2013
**C9orf72 expansion mutation in FTLD-ALS**

- C9P FTD has more extensive cortical atrophy (green) compared with C9N FTD.

- Poor verbal fluency scores correlated to atrophy in frontoinsular, thalamic and inferior parietal regions (red).

From Irwin et al. JNPP 2013

n=5,000 permutations, p<0.01
C9orf72: Additional Neuropathologies

- Protein-degradation associated proteins
  - Ubiquillin-2 (UBQLN)\(^1\)
  - P62\(^2\)
- C9orf72 non-ATG translated di-peptide repeats (DPR)\(^3-4\)
  - P62 and UBQLN most likely markers of DPR
- Prominent in hippocampus and cerebellar molecular layer

The *C9orf72* genotype may confer:
- A shorter survival in ALS
- More rapid decline in cognition in FTD
- More severe neurodegeneration in frontoparietal, thalamic and cerebellar regions for FTD
- Additional protein inclusions not seen in sporadic TDP-43 proteinopathies

*C9orf72* genotyping may provide useful prognostic and diagnostic clinical information for ALS and FTD

Clinical trials for ALS/FTD-TDP should stratify patients based on genotype
Overview

♦ Genetic-pathological associations in primary progressive aphasia

♦ Rare pathogenic variants in neurodegenerative disease

♦ Common genetic variants in neurodegenerative disease
Genetic Variants of FTLD-Tau

Primary Progressive Aphasia

TAU

MAPT

Sporadic

Genetic modifiers?
Genetic Variants of FTLD-Tau

♦ Genome-wide association study of Progressive Supranuclear Palsy (FTLD-Tau) found several disease associated single-nucleotide polymorphisms (SNPs).\(^1\)

♦ Our group found two of these SNPs associate with all FTLD-Tau.\(^2\)
  • rs8070723 in MAPT (tau) gene
  • rs1768208 in MOBP (myelin oligodendrocytic basic protein) gene

♦ *Do these SNPs have diagnostic/prognostic value in bvFTD?*
  • Genotyped 80 sporadic bvFTD cases
    – *Subset of autopsy confirmed FTLD-Tau =20 and FTLD-TDP=12*

Genetic Variants of FTLD-Tau

- **MOBP** risk variants (CT/TT) shortened total cohort and autopsy Tau (~7 years) compared to **MOBP** patients.

![Graphs showing disease duration by MOBP genotype](attachment:image)

- **Irwin et al., Neurology, 2014.**
MAPT protective allele + (GG/GA) patients had an earlier age at onset (~5 years) than MAPT (AA) genotype patients (p<0.01).

There was no difference in disease duration between MAPT genotype groups.

The association of MOBP genotype with earlier age at death and shorter disease duration appears to be specific for this variant.
Genetic Variants of FTLD-Tau

- **MOBP**
  - Second most abundant myelin-associated protein
  - Knockout of MOBP gene in mice is not lethal
  - MOBP may be important to stabilize myelin during times of cellular stress
  - MOBP gene is complex and involves alternative splicing to form different isoforms
  - rs1768208 is located in an intronic region between exons 1 and 2

- Does MOBP SNP influence transcription/function of MOBP protein?
- Does MOBP SNP mark another variant with biological effect or regulate a different distant gene?

From: Montague et al, *Dev Neurosci* 2006
Genetic Variants of FTLD-Tau

- Neuroimaging analysis in subset of patients (n=37) to examine for biological association of \textit{MOBP} genotype.

- \textit{MOBP} risk allele + (TC/TT) patients have increased white matter degeneration (RD) in midbrain and long association tracts (green).
**MOBP risk SNP in FTLD-Tau**

- **MOBP risk allele** was associated with shorter disease duration in bvFTD
  - Specific for FTLD-Tau sub-group
  - Effect not present for MAPT SNP

- **MOBP risk allele** was associated with more severe WM degeneration
  - Areas included subcortical WM implicated in tauopathies
  - Majority of neuroimaging cohort did not have autopsy information
  - 6/7 PSP/CBD (4R Tau) patients in autopsy cohort were MOBP RA+

- Is **MOBP a marker of 4R tauopathy** in bvFTD or is there a biological effect potentiating the neurodegenerative process?
  - Future studies will examine MOBP genotype and WM integrity using histology

- **MOBP genotype may be a useful diagnostic and prognostic marker** in bvFTD-Tau
Emerging disease modifying therapies for PPA are targeting Tau, amyloid and TDP-43 proteins
  • Ante mortem diagnosis is critical for these efforts
Hereditary forms of PPA are clinically and pathologically different compared with sporadic disease and could effect clinical trials.
Common genetic polymorphisms may have biological effects that contribute to clinical heterogeneity in PPA and may have diagnostic/prognostic clinical utility
Studies presented were supported by grants from the National Institute on Aging (P30 AG010124-20, T32-AG000255) P01 AG017586, R01 NS44266, R01 AG15116, P01 AG32953, and P01 NS53488), National Institute for Neurological Disorders & Stroke (K23NS088341) from the National Institutes of Health and grants from the Wyncote Foundation and PENN Institute for Translational Medicine and Therapeutics (ITMAT).

Patients and their families