50 Years of Progressive Supranuclear Palsy
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Genetics of PSP
(Scientific, diagnostic, and therapeutic relevance)

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Progressive Supranuclear Palsy

- Prevalence 4-6/100,000
- Most cases sporadic
- Complex etiology: Both genetic and environmental factors contribute to disease
- <1% of cases monogenic
Monogenic forms

- Both autosomal dominant and recessive modes of inheritance
- Linkage to chromosome 17
- Delineation of region harboring \textit{MAPT}
MAPT mutations in monogenic forms of PSP

Identification of 3 mutations in MAPT (R5L (AR) in exon1, ΔN296 (AR), and G303V (AD) in exon 10) in patients with characteristic phenotype
Monogenic segregation of PSP

AR
del N296

AD
G303V
Silent *MAPT* S305S mutation

- Pathogenic in several tauopathies (Frontotemporal dementia, PSP)
- AD mode of inheritance
- AGT > AGC transition in exon 10
- Increases splicing in of exon 10, results in overproduction of tau isoforms containing four repeats (4R)


MAPT plays major predisposing role in sporadic PSP

- Structure of MAPT gene
- Structure of tau protein
- Predisposing variant
H1 haplotype of \textit{MAPT} is the major genetic risk factor in PSP and contributes up to 68\% of the genetic risk

\textit{Melquist et al., 2007}
H1 haplotype more common in PSP than in Controls

<table>
<thead>
<tr>
<th>Subjects</th>
<th>N</th>
<th>H1</th>
<th>H2</th>
<th>H1/H1</th>
<th>H1/H2</th>
<th>H2/H2</th>
<th>OR</th>
<th>95% C.I.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>424</td>
<td>80.2</td>
<td>19.8</td>
<td>65.3</td>
<td>29.7</td>
<td>5.0</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSP</td>
<td>274</td>
<td>93.6</td>
<td>6.4</td>
<td>88.0</td>
<td>11.3</td>
<td>0.7</td>
<td>3.9</td>
<td>2.6-5.9</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

GWAS to detect additional gene loci predisposing to PSP since H1c haplotype confers less than 2/3 of the genetic risk
GWAS

Exploratory (discovery) cohort:
1,114 autopsy-confirmed PSP cases and 3,287 controls

531,451 SNPs analyzed

Validation cohort:
1,051 clinically-confirmed PSP cases and 3,560 controls

4,099 SNPs that yielded p <10^{-3} in exploratory study
**Quality Control (QC)**

1,163 autopsy-confirmed PSP

- n=6 matching outliers
- n=1 gender mismatch
- n=9 duplicate cases
- n=23 related
- n=10 <98% genotype completion rate

3,658 Controls

- n=2 gender mismatch
- n=1 <98% genotype completion rate
- n=368 matching outliers

1,114 autopsy-confirmed PSP

3,287 Controls
Table 2 Results from stage 1, stage 2 and the joint analysis among subjects of European ancestry

<table>
<thead>
<tr>
<th>Chr. band</th>
<th>SNP</th>
<th>SNP location</th>
<th>Gene or nearby gene</th>
<th>MAF(^b) case</th>
<th>MAF(^b) control</th>
<th>OR(^b) (95% CI)</th>
<th>(P_1)</th>
<th>MAF(^b) case</th>
<th>MAF(^b) control</th>
<th>OR (95% CI)</th>
<th>(P_2)</th>
<th>OR (95% CI)</th>
<th>Joint (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q25.3</td>
<td>rs1411478</td>
<td>179,229,155</td>
<td>STX6</td>
<td>0.50</td>
<td>0.42</td>
<td>0.73 (0.65–0.81)</td>
<td>1.8 × 10(^{-9})</td>
<td>0.46</td>
<td>0.43</td>
<td>0.85 (0.77–0.94)</td>
<td>1.5 × 10(^{-3})</td>
<td>0.79</td>
<td>(0.74–0.85)</td>
</tr>
<tr>
<td>2p11.2</td>
<td>rs7571971</td>
<td>88,676,716</td>
<td>EIF2AK3</td>
<td>0.31</td>
<td>0.26</td>
<td>0.75 (0.66–0.84)</td>
<td>7.4 × 10(^{-7})</td>
<td>0.31</td>
<td>0.25</td>
<td>0.75 (0.67–0.83)</td>
<td>8.7 × 10(^{-8})</td>
<td>0.75</td>
<td>(0.69–0.81)</td>
</tr>
<tr>
<td>3p22.1</td>
<td>rs1768208</td>
<td>39,498,257</td>
<td>MOBP</td>
<td>0.36</td>
<td>0.29</td>
<td>0.70 (0.63–0.79)</td>
<td>1.0 × 10(^{-9})</td>
<td>0.35</td>
<td>0.29</td>
<td>0.74 (0.67–0.82)</td>
<td>1.3 × 10(^{-8})</td>
<td>0.72</td>
<td>(0.67–0.78)</td>
</tr>
<tr>
<td>17q21.31</td>
<td>rs8070723</td>
<td>41,436,651</td>
<td>MAPT</td>
<td>0.05</td>
<td>0.23</td>
<td>5.50 (4.40–6.86)</td>
<td>2.1 × 10(^{-51})</td>
<td>0.06</td>
<td>0.23</td>
<td>4.74 (3.92–5.74)</td>
<td>4.8 × 10(^{-67})</td>
<td>5.46</td>
<td>(4.72–6.31)</td>
</tr>
<tr>
<td></td>
<td>rs242557</td>
<td>41,375,823</td>
<td>MAPT</td>
<td>0.53</td>
<td>0.35</td>
<td>0.48 (0.43–0.53)</td>
<td>2.2 × 10(^{-37})</td>
<td>0.50</td>
<td>0.36</td>
<td>0.54 (0.48–0.59)</td>
<td>5.0 × 10(^{-35})</td>
<td>0.51</td>
<td>(0.47–0.55)</td>
</tr>
<tr>
<td></td>
<td>rs242557/</td>
<td></td>
<td>MAPT</td>
<td>–</td>
<td>–</td>
<td>0.66 (0.58–0.74)</td>
<td>1.3 × 10(^{-11})</td>
<td>–</td>
<td>–</td>
<td>0.74 (0.67–0.83)</td>
<td>6.3 × 10(^{-8})</td>
<td>0.70</td>
<td>(0.65–0.76)</td>
</tr>
</tbody>
</table>
GWAS identified three additional risk genes in PSP and confirmed importance of *MAPT*:

- **STX6 (syntaxin 6)**: Vesicle membrane fusion, Golgi-endosomes \( (p=2.3\times10^{-10}) \)

- **EIF2AK3 (Eukaryotic translation initiation factor 2alpha kinase)**: Inhibits translation initiation upon accumulation of misfolded proteins in ER (ER-stress) \( (p=3.2\times10^{-13}) \)

- **MOBP (Myelin associated Oligodendrocytic Basic Protein)**: Abundant myelin constituent expressed exclusively in oligodendrocytes \( (p=1.0\times10^{-16}) \)
MAPT

controlling for H1/H2
Association results for the relationship between SNP genotypes and mRNA transcripts

MOBP

MAPT

MAPT controlling for H1/H2
EIF2AK3

Eukaryotic translation initiation factor 2-alpha kinase 3 (PERK)

UPR induces dimerization and transautophosphorylation of PERK ➔ phosphorylates translation-initiation factor 2 (eIF2α) ➔ inhibits global translation
**Unfolded protein response**

*EIF2AK3 ➔ PERK* eukaryotic translation initiation factor 2 alpha kinase

From Imaizumi, 2006
**STX6** ➔ Syntaxin 6

*Involved in intracellular vesicle fusion and trafficking*

Part of SNARE (SNAP (Soluble NSF Attachment Protein) REceptor)
Putative effect of gene variants discovered in GWAS

- **EIF2AK3**: altered gene expression?
- **STX6**: altered gene expression?
- **MAPT**: H1/H2 inversion polymorphism: altered expression plus preferential use of exon 3
- **MOBP**: evidence of small effect on gene expression
Epigenetic modification adds an additional layer of complexity to the pathogenesis of PSP
Frontal cortex PSP
Epigenetic analysis at DNA level, investigation of cytosine methylation
Bisulfite conversion of cytosine to uracil (thymine)
Infinium Assay for Methylation

Unmethylated Locus

- Captured gDNA
- Bisulfite Conversion
- Unmethylated Bead Type
- Unmethylated Locus
- Methylated Bead Type

Methylated Locus

- Captured gDNA
- Bisulfite Conversion
- Unmethylated Bead Type
- Methylated Bead Type
Differential methylation pattern in frontal lobe tissue of PSP patients

PSP
n=94
frontal lobe

vs.

control
n=88
frontal lobe

Infinium HumanMethylation450 BeadChip (Illumina)

485,577 potential methylation sites (CpG islands) in the promoter region of 99% of the RefSeq-genes
Differential methylation pattern in frontal lobe tissue of PSP patients

PSP
n=94
frontal lobe

vs.

control
n=88
frontal lobe

after QC:
data of 366,030 methylation sites

1,118 CpG-sites hypermethylated

530 CpG-sites hypomethylated

t-Test
Differential methylation pattern in frontal lobe tissue of PSP patients

1,118 CpG-sites hypermethylated

530 CpG-sites hypomethylated
Illumina-450k-Methylation Array
(456,838 genomic markers on autosomes)

94 PSP Patients vs. 72 Controls (age & gender matched)

- Number of markers hypermethylated
- Number of markers hypomethylated

<table>
<thead>
<tr>
<th>Methylation Difference</th>
<th>Number of Markers Hypomethylated</th>
<th>Number of Markers Hypermethylated</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>1,724</td>
<td>1,050</td>
</tr>
<tr>
<td>&gt;0.5%</td>
<td>1,586</td>
<td>879</td>
</tr>
<tr>
<td>&gt;1.0%</td>
<td>1,414</td>
<td>672</td>
</tr>
<tr>
<td>&gt;2.0%</td>
<td>893</td>
<td>422</td>
</tr>
<tr>
<td>&gt;5.0%</td>
<td>50</td>
<td>42</td>
</tr>
</tbody>
</table>

- p < 0.05
- p < 0.01
Conclusions:

Relevance of genetic approaches to analysis of PSP

Scientific: Unraveling of pathological mechanisms in PSP, improvement of understanding of tauopathies, explanation of aspects of neurodegeneration

Diagnostic: Presently limited application. Epigenetic studies might help identify PSP-specific biomarkers

Therapeutic: Discovery of predisposing genetic variants and of abnormally methylated genes supports development of causative therapies
Thank you!

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