Could a Fungal Infection Cause Some Cases of ALS?

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Outline

- Origin of this idea
- Evidence for fungi in people with ALS
- Possible interpretations of the evidence
- Suggested next steps
Origin of This Idea

- ALSUntangled
  - (www.alsuntangled.org)
- By early 2019, >500 requests to review “Anti-fungals” for ALS
- Review published, has nearly 2,000 downloads to date
  - (Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration, 2019; 20: 625–629)

**ALSUntangled** reviews alternative and off label treatments (AOTs), with the goal of helping people with ALS make more informed decisions about them.
Origin of This Idea

• In 2006, Dr. William Reid filed a patent for treating ALS and other neurodegenerative disease with antifungals

• He hypothesized that people with ALS (PALS) were immunodeficient, colonized with fungi, succumbed to fungal toxins
  • Reid W. Immunosuppression & mycotoxins causing amyotrophic lateral sclerosis. The winnower. 2017. Available at: [http://www.webcitation.org/76MCrRWq0](http://www.webcitation.org/76MCrRWq0)
Evidence-Clinical?

- Dr. Reid found some PALS with low IgG levels, lymphopenia, metabolic acidosis, abnormal urine porphyrins, abnormal urine organic acids, abnormal levels of the mycotoxin Trichothecene, all of which he felt supported his hypothesis
  - ALSUntangled review noted most PALS have normal IgG levels and lymphocyte counts
Dr. Reid treated 5-10 PALS with antifungals, in some cases along with PLEX or IVIG, and reported improved motor function. ALSUntangled review noted these improvements were generally small, transient, which can happen spontaneously in PALS. ALSUntangled was unable to independently verify the ALS diagnoses or the improvements in these patients (no sufficient records sent to us).
Evidence-Neuropathology

• A Spanish group published 3 papers claiming neuropathological evidence of fungi in the brains of PALS
• CSF from 5 PALS, 3 healthy controls
• Brain tissue from 6 PALS, 4 healthy controls

• Polyclonal antibodies detected various fungal antigens in CSF from PALS, not healthy controls

• PCR analysis detected fungal DNA in CSF and brain tissue from PALS, not healthy controls
• Immunohistochemistry detected intracellular fungal antigens in frontal cortex of PALS, not healthy controls
Evidence-Neuropathology 2

- Brain tissue from: 6 PALS, 11 patients with AD, 6 patients with PD, 5 healthy controls

- Immunohistochemical analyses of corpora amylacea (CA, glycoproteinaceous inclusions that accumulate in the brain during the course of normal aging and to a greater extent in some neurodegenerative diseases)
**Evidence-Neuropathology 2**

- Polyclonal antibodies detected several different fungi in CA of patients with ALS, AD and PD but not controls
Evidence - Neuropathology 3

- Brain tissue from 11 PALS, 4 healthy controls

- Immunohistochemistry again showed intracellular fungi in PALS (not controls)

- 3d reconstructions suggested fungi in or on the nucleus of cells from the motor cortex, brainstem, spinal cord
• DNA extracted, nested PCR technique used to amplify specific fungal regions for subsequent DNA sequencing. The genomic regions chosen were the intergenic sequences located between the ribosomal RNA genes—many specific fungal species identified.

<table>
<thead>
<tr>
<th>REGION ITS1</th>
<th>REGION ITS2</th>
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<tbody>
<tr>
<td><strong>Species</strong></td>
<td><strong>Patients</strong></td>
</tr>
<tr>
<td>Aspergillus sp</td>
<td>ALS9-MC</td>
</tr>
<tr>
<td>Candida famata</td>
<td>ALS2-SC1, ALS1-SC1</td>
</tr>
<tr>
<td>Cladosporium sp</td>
<td>ALS6-SC1, ALS2-MC</td>
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<tr>
<td>Cryptococcus curvatus</td>
<td>ALS7-SC2</td>
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<tr>
<td>Cryptosporidium sp</td>
<td>ALS8-SC1</td>
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<tr>
<td>Davidella tassona</td>
<td>ALS1-MD, ALS4-SC1, ALS5-SC1</td>
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<tr>
<td>Malassezia globosa</td>
<td>ALS3-SC1, ALS7-MD</td>
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<tr>
<td>Malassezia restricta</td>
<td>ALS3-MC, ALS4-MD</td>
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<tr>
<td>Penicillium sp</td>
<td>ALS5-MD, ALS1-MC</td>
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<tr>
<td>Rhodotorula mucilaginosa</td>
<td>ALS4-MC</td>
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<tr>
<td>Trichoderma sp</td>
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<tr>
<td>Uncultured basidiomycota</td>
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<td>Uncultured fungus</td>
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<td>Uncultured malassezia</td>
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<tr>
<td>Uncultured Sporidobolales</td>
<td>ALS6-MD</td>
</tr>
</tbody>
</table>

MC: Motor cortex; MD: Medulla; SC: Spinal cordal
Critiques of the Neuropathology Studies

• Small numbers of patients and controls
• Are the same participants being studied in all 3 papers?
• No clinical details on PALS (were they known to have fungal infections in life?)
• Scant details on brain processing methods (contaminants?)
• Polyclonal antibodies (may not be specific for fungi)
• Not all patients’ data are included in different analyses
• Not yet independently replicated
• How can this explain the anatomic specificity of ALS (and other degenerative diseases)?
Possible Interpretations

- Artifacts/contaminants?
- Part of the “CNS Microbiome”? 
- Coincidental infection?
- Part of the pathophysiology of ALS (and other degenerative diseases)?
  - Longshot, but even in a subset might have huge implications for treatment
Suggested Next Steps

• I would like to see the VABB try to replicate and extend the neuropathological findings I described today
  • Well-characterized patients and controls
  • Well-described, sound protocols for brain acquisition and prep
  • Immunohistochemistry with monoclonal anti-fungal antibodies (if available)
  • PCR to look for fungal DNA