Olfactory Mucosa Brushing for the Intravital Diagnosis of Creutzfeldt-Jakob Disease

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The Peripheral Olfactory System

Expression of PrP$^C$ in Olfactory Mucosa

Olfactory bulb

Olfactory Mucosa

Glomeruli

Fila Olfactoria

Basal Cells

VGA

Fila Olfactoria

Basal Cells

PrP
Detection of Pathologic Prion Protein in the Olfactory Epithelium in Sporadic Creutzfeldt–Jakob Disease

Gianluigi Zanusso, M.D., Ph.D., Sergio Ferrari, M.D., Franco Cardone, Ph.D., Paolo Zampieri, M.D., Matteo Gelati, Ph.D., Michele Fiorini, Ph.D., Alessia Farinazzo, Ph.D., Marina Gardiman, M.D., Tiziana Cavallaro, M.D., Marina Bentivoglio, M.D., Pier Giorgio Righetti, Ph.D., Maurizio Pocchiari, M.D., Nicola Rizzuto, M.D., and Salvatore Monaco, M.D.
Olfactory epithelium is PrP<sup>CJD</sup>-positive in sporadic CJD

**Sporadic CJD**

**Alzheimer’s Disease**

**Olfactory Mucosa 10% homogenate**

<table>
<thead>
<tr>
<th>Lane</th>
<th>PK (20 µg/ml):</th>
<th>0'</th>
<th>20'</th>
<th>10'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mr</td>
<td>35</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>1</td>
<td>3F4</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
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**Olfactory Mucosa NaPTA treated (from 100mg)**

<table>
<thead>
<tr>
<th>Lane</th>
<th>Supernatant PK (20 µg/ml):</th>
<th>0'</th>
<th>20'</th>
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<tbody>
<tr>
<td></td>
<td>Mr</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>1</td>
<td>3F4</td>
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<tr>
<td>2</td>
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<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Lane</th>
<th>Pellet PK (20 µg/ml):</th>
<th>0'</th>
<th>20'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mr</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>3F4</td>
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<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Molecular types of PrP\textsuperscript{CJD} are conserved in the Olfactory Epithelium

**sCJD MM-1**

- **Respiratory Mucosa**
  - Sup. Pellet Pellet
  - PK: (20μg/ml) 20’
  - NaPTA treated

- **Olfactory Mucosa**
  - Sup. Pellet Pellet
  - PK: (20μg/ml) 20’
  - NaPTA treated

**sCJD VV-2**

- **Respiratory Mucosa**
  - Sup. Pellet Pellet
  - PK: (20μg/ml) 20’
  - NaPTA treated

- **Olfactory Mucosa**
  - Sup. Pellet Pellet
  - PK: (20μg/ml) 20’
  - NaPTA treated
Ultrastructural Deposition of PrP$_{CJD}$
Prion Detection in Olfactory Biopsy of Sporadic Creutzfeldt–Jakob Disease

Massimo Tabaton, MD,1 Salvatore Monaco, MD,2 Mario Paolo Cordone, MD,3 Monica Colucci, MD,1 Giorgio Giaccone, MD,4 Fabrizio Tagliavini, MD,4 and Gianluigi Zanusso, MD, PhD2

Annals of Neurology, 2004
Concerns about Olfactory Mucosa Biopsy

OM biopsy might be a potential procedure for in vivo diagnosis of CJD. However:

- **OM biopsy is an invasive procedure** and medical and/or surgical complications such as: bleeding, damage of lamina cribrosa, fistulas of the subarachnoid space etc. might occur.
- The low amount of OM sample (2mm$^2$) precluded PrP$_{CJD}$ detection by biochemical analysis.
- False negative cases might occur since OM is interspersed with PrP$_{CJD}$-negative respiratory mucosa or inconclusive result from ICH.

Why use OM brushing coupled with RT-QuIC?

- **OM brushing is a non-invasive procedure** without medical or surgical complications.
- Efficient sampling. PrP$_{CJD}$ is detected on neurons lining the nasal vault, therefore only the outer layers of OM need to be collected; the head surface of brush is 2 cm long.
- RT-QuIC assay did not show false positive and detects $>10^8$-fold dilutions of human sCJD brain homogenate and it has allowed identification of PrP$_{CJD}$ seeding activity in cerebrospinal fluid of sCJD affected patients with 80-90% sensitivity and 100% specificity (Atarashi R. et al. Nat Med 2011; McGuire L. et al. Ann Neurol 2012).
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The Nasal Brushing Procedure

(Done by e-learning group, University of Verona)
Olfactory Neurons obtained by OM Brushing

A single OM brushing procedure allows to collect $\sim 10^6$ cells. The $\sim 20\%$ are represented by OMP positive cells.
Aim of the study

- We investigated whether a non-invasive procedure such as nasal brushing coupled with RT-QuIC analysis might substantially improve sCJD diagnosis in living patients.

Patients and controls

- 31 CJD affected patients underwent to both CSF and OM brushing analyses by RT-QuIC.

- OM brushings were also obtained from 31 normal controls and from 12 patients with neurodegenerative disorders (4 patients with clinical diagnosis of PD, 5 with AD; 1 patient with definite PSP and 1 with limbic encephaltis)
• OM brushings from sCJD patient positive down to 1:25,000 dilution
  • \( \rightarrow \) several \( \log_{10} \) of seeds in single brushings
Table 1. Demographic characteristics, clinical profiles, diagnostic parameters and RT-QuIC analyses of sCJD patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Gender</th>
<th>Codon 129</th>
<th>Clinical signs at onset</th>
<th>CSF 14-3-3/ Tau protein levels (pg/ml)</th>
<th>Typical MRI*</th>
<th>EEG (PSWCs)</th>
<th>Disease duration (months)</th>
<th>Diagnosis at the time of OM brushing</th>
<th>Definite diagnosis</th>
<th>Result</th>
<th>RT-QuIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>77/M</td>
<td>MM</td>
<td>Ataxia, visual hallucinations</td>
<td>1st positive/ 1297 2nd positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>3.85</td>
</tr>
<tr>
<td>2</td>
<td>50/F</td>
<td>MM</td>
<td>Ataxia, behavioural changes</td>
<td>Positive / &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>6</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>4.10</td>
</tr>
<tr>
<td>3</td>
<td>68/F</td>
<td>MV</td>
<td>Ataxia, dementia</td>
<td>Positive / &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>16</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>73/M</td>
<td>MM</td>
<td>Depression</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>8</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>3.35</td>
</tr>
<tr>
<td>5</td>
<td>64/F</td>
<td>VV</td>
<td>Ataxia</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>Probable sCJD</td>
<td>sCJD VV2</td>
<td>+</td>
<td>3.35</td>
</tr>
<tr>
<td>6</td>
<td>64/F</td>
<td>MV</td>
<td>Depression, ataxia</td>
<td>1st positive/ 892 2nd positive/ &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>17</td>
<td>Probable sCJD</td>
<td>sCJD MV2</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>66/M</td>
<td>MM</td>
<td>Visual hallucinations</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>2</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>Neg.</td>
</tr>
<tr>
<td>8</td>
<td>68/F</td>
<td>MM</td>
<td>Ataxia, visual hallucinations</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>4</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>77/M</td>
<td>MM</td>
<td>Ataxia</td>
<td>Positive/ ND</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>3.60</td>
</tr>
<tr>
<td>10</td>
<td>69/M</td>
<td>MM</td>
<td>Apraxia, epileptic seizures</td>
<td>Positive/ &gt;2400</td>
<td>No</td>
<td>No</td>
<td>2</td>
<td>Probable sCJD</td>
<td>sCJD MM1</td>
<td>+</td>
<td>Neg.</td>
</tr>
<tr>
<td>11</td>
<td>75/F</td>
<td>MM</td>
<td>Ataxia</td>
<td>1st negative/ ND 2nd positive/ ND</td>
<td>Yes</td>
<td>No</td>
<td>Still alive (2)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>Neg.</td>
</tr>
<tr>
<td>12</td>
<td>29/F</td>
<td>MM</td>
<td>Depression, choreic movements</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>Still alive (8)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>Neg.</td>
</tr>
<tr>
<td>13</td>
<td>65/M</td>
<td>MV</td>
<td>Depression, ataxia, extrapyramidal signs</td>
<td>1st positive/ 2297 2nd positive/ &gt;2400</td>
<td>Yes</td>
<td>No</td>
<td>Still alive (18)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>Neg.</td>
</tr>
<tr>
<td>14</td>
<td>61/M</td>
<td>MM</td>
<td>Ataxia, cortical blindness</td>
<td>Positive/ &gt;2400</td>
<td>Yes</td>
<td>Yes</td>
<td>Still alive (5)</td>
<td>Probable sCJD</td>
<td>Pending</td>
<td>+</td>
<td>3.60</td>
</tr>
</tbody>
</table>

Legend: *high signal abnormalities in Caudate nucleus and Putamen or at least two cortical regions (temporal-parietal-occipital) either in diffusion-weighted imaging (DWI) or fluid attenuated inversion recovery (FLAIR); M denotes methionine; V denotes valine; ND denotes not done; + denotes a positive RT-QuIC test result; Neg. denotes a negative result.
RT-QuIC comparisons of OM and CSF samples obtained from individual sCJD patients and from non-CJD controls:
First 14 patients (out of 31) and all negative controls
# Summary of the RT-QuIC results on OM and CSF samples

<table>
<thead>
<tr>
<th>Subjects</th>
<th>OM RT-QuIC Positive</th>
<th>CSF RT-QuIC Positive</th>
<th>CSF 14-3-3 Positive</th>
<th>CSF Tau &gt; 2400 pg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CJD#</td>
<td>30/31</td>
<td>23/30</td>
<td>28/31</td>
<td>23/25</td>
</tr>
<tr>
<td>sCJD MM</td>
<td>14/14</td>
<td>11/14</td>
<td>12/14</td>
<td>10/10</td>
</tr>
<tr>
<td>sCJD MV</td>
<td>8/9</td>
<td>6/8</td>
<td>8/9</td>
<td>6/8</td>
</tr>
<tr>
<td>sCJD VV</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
<td>2/2</td>
</tr>
<tr>
<td>CJD N.D.</td>
<td>4/4</td>
<td>3/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>gCJD E200K-129MM</td>
<td>2/2</td>
<td>1/2</td>
<td>2/2</td>
<td>1/1</td>
</tr>
<tr>
<td>OND</td>
<td>0/12</td>
<td>0/17</td>
<td>3/17</td>
<td>0/17</td>
</tr>
<tr>
<td>Normal controls**</td>
<td>0/31</td>
<td>0/26</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>
CONCLUSIONS

➢ The detection of high prion seeding activity in OM brushings from sCJD patients, so far with 97% sensitivity and 100% specificity, indicates that this procedure may provide a strong basis for definitive intravital sCJD diagnosis.

➢ The relatively high sCJD seeding activity in olfactory mucosa suggests that infectivity is likely present as well, posing biosafety implications.

➢ Other neurodegenerative protein misfolding diseases such as Alzheimer’s or Parkinson’s disease involve aggregation of their respective misfolded proteins in the olfactory anterior structures and olfactory epithelium.
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Contributors:

We also deeply acknowledge the many colleagues for their generous support of this project.