Decades after being hit by poliovirus (PV), 20-70% polio survivors develop the “post-polio syndrome” (PPS), a progressive condition characterized by chronic fatigue, pain, new muscular weakness, cold intolerance. The etiology and pathogenesis of PPS are undefined. The literature suggests that PV genome fragments may persist for decades in the central nervous system of affected patients.

Over the last two years, we developed extremely sensitive molecular tests for detecting polioviruses (and other enteroviruses). To this end, we tested huge numbers of different primer pairs directed to conserved genome regions of PVs. Reference strains and clinical samples were used. Most recent tools allow direct differentiation of PV types (i.e., PV-1, PV-2, PV-3).

Using the above assays together with tissue culture methods and immunofluorescence, low-level PV infectivity and genome fragments have been detected in 43/47 patients aged 50 to 76 years. PVs could not be detected in 49 negative controls (CSF from 11 adult patients with non-infectious, non-autoimmune, non-neoplastic neurologic disorders; blood from 26 healthy blood donors; blood from 12 patients’ family members).

In a few patients undergoing surgical procedures, PV genome fragments could be detected in primary cultures of skeletal muscle, peripheral nerve, and duodenal mucosa cells.

In clinical samples, PV genome fragments were present at extremely low levels. Thus, whole genome sequencing has been impossible so far.

Partial sequencing of the 5'UTR, VP1, and 3D genome regions indicated that amplicons obtained from most patients were compatible with reference sequences of PV-1. A few patients, however, appeared to carry PV-2 or PV-3 sequences.
Extensive mutations/deletions were detected in the 5'UTR and VP1 regions. Immunofluorescence with PV-specific mAbs showed that capsid proteins were produced at low levels in primary cultures of muscle and peripheral nerve cells as well as in cell lines that had been exposed to biological samples of PPS patients for 1-3 weeks.

These data indicate that PV genome fragments can indeed persist for several decades in polio survivors. The data, however, do not provide a pathogenetic link between virus persistence and PPS development. The highly sensitive tools now available can contribute to detect and characterize PV strains in PPS patients, with the aim of clarifying PPS pathogenesis and proposing preventive and therapeutic measures.

Prosecution of these studies includes investigation of additional patients and their family members together with testing of antivirals against reference and PPS-derived PV strains.

Our diagnostic methods will be made available to interested laboratories.

**A manuscript is being prepared for publication. Preliminary results have been presented at national and international meetings:**


Toniolo A, Baj A. Role of enteroviruses in neural and endocrine pathology. Department of Microbiology, National University of Singapore, Singapore, July 30, 2008.


Toniolo A, Baj A, Maccari G, Molteni F, Monaco S. Poliovirus genome fragments in patients with the post-polio syndrome. 8th Asia Pacific Congress of Medical Virology, Hong Kong 25-28 Feb 2009 (p. 60)

Toniolo A, Baj A, Maccari G, Monaco S. Persistence of poliovirus type 1 genome in patients with the Post-Polio Syndrome. 9th International Symposium on Neurovirology, Miami Beach, FL 2-6 June 2009 (p.98).
Results are summarized in the following slides:

**Poliovirus detection: methods**

**PPS patients (n = 47)**

<table>
<thead>
<tr>
<th>Male/Female</th>
<th>Age (years, M ± SD)</th>
<th>Years from APP (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36.2%</td>
<td>57.4 ± 7.3</td>
<td>53 ± 7.0</td>
</tr>
</tbody>
</table>

**Controls (n = 49)**

Blood donors (n=26); neurologic patients with non-infectious, autoimmune, or neoplastic disease (n=11); family members of PPS patients (n=12)

<table>
<thead>
<tr>
<th>Male/Female</th>
<th>Age (years, M ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>67.3%</td>
<td>39.7 ± 13.4</td>
</tr>
</tbody>
</table>
Poliovirus genome fragments in PPS patients (n = 47)
PATIENT LL:
detection of poliovirus genome fragment in duodenal cells (DC)
acute infection, 1933; virus detection, 2009

3D pol fragment

Primary culture of surgical samples:
virus detection after >30 yrs from the acute event

Controls n = 39

<table>
<thead>
<tr>
<th>Gender</th>
<th>Neurology Patients: non-infected, non-autoimmune, non-neoplastic pathology (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7M, 4F</td>
</tr>
</tbody>
</table>

| Blood donors (n=26) | 15M, 7F |

<table>
<thead>
<tr>
<th>Relatives (n=12)</th>
<th>Genotype</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2ICAM VR Female</td>
<td>P4GCO VA-husband-</td>
<td>M</td>
</tr>
<tr>
<td>P31DCD VR Female</td>
<td>P4GEO VA-husband-</td>
<td>M</td>
</tr>
<tr>
<td>P39CM VA Female</td>
<td>P4GMVA-daughter-</td>
<td>F</td>
</tr>
<tr>
<td>P45GM VA Female</td>
<td>P4GMVA-son-</td>
<td>M</td>
</tr>
<tr>
<td>P46GMVA Female</td>
<td>P50GA VA-daughter-</td>
<td>M</td>
</tr>
<tr>
<td>P41VA Female</td>
<td>P41ZE VA-daughter-</td>
<td>F</td>
</tr>
<tr>
<td>P4GMA VA Male</td>
<td>P4GVGA-wife-</td>
<td>F</td>
</tr>
<tr>
<td>P51GMA Male</td>
<td>P52GM VA-daughter-</td>
<td>F</td>
</tr>
<tr>
<td>P51GMAVA Female</td>
<td>P54GO VA-brother-</td>
<td>M</td>
</tr>
<tr>
<td>P53OSVA Female</td>
<td>P56CO VA-brother-</td>
<td>M</td>
</tr>
<tr>
<td>P54MDA Male</td>
<td>P56SY VA-son-</td>
<td>F</td>
</tr>
</tbody>
</table>

Blood donors (n=26):
- 15M, 7F

- 7M, 4F

- Relative (n=12)

- Control s n = 39

- Gender

- Relatives (n=12)

- Neurology Patients: non-infected, non-autoimmune, non-neoplastic pathology (n=11)

- Blood donors (n=26)

- Primary culture of surgical samples:
  - virus detection after >30 yrs from the acute event

- Controls n = 39
**The Post-Polio Syndrome: results**

**PATIENT LL: acute infection (1933)**

**virus detection in leukocytes (2007)**

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**PV STRAINS FROM PPS PATIENTS: EXPRESSION OF CAPSID ANTIGENS IN AV3 CELLS**

A: Neg CTRL  
B: Pos PV1 CTRL (Chatt strain)  
C: RR strain  
D: LL strain
**The Post- Polio Syndrome: results**

**SEQUENCED PV REGIONS**

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**PERSPECTIVES**

Effective methods for molecular diagnosis and differentiation of PV types are now ready and will be made available to interested laboratories;

Failure to detect PV genomes in family members of PPS patients speaks against transmissibility of the mutated agents;

Studies will be extended to more than 50 patients, 50 controls and as many family members as possible;

Complete sequencing of mutated PV genome fragments associated with PPS will help clarify PV persistence;

Viral diagnosis may pave the way to treating PPS patients in order to stop the progression of virus-associated cell damage or to prevent PPS development in polio survivors;

In vitro testing of new antivirals against reference and "mutated" PV strains is programmed.
Thanks to the colleagues who introduced us to this area of research:
Abner L. Notkins, Manos Dalakas, Takashi Onodera, Gianluigi Zanusso, Frans Nollet.

Thanks for support: Post Polio Health International, St. Louis, MO.

Our deepest appreciation goes to the many patients whose enthusiasm, patience, and suggestions made these investigations possible.