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PERSISTING NONINFECTIOUS FRAGMENTS OF POLIOVIRUS IN PPS PATIENTS

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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:

Baj A, Maccari G, Monaco S, Toniolo A. Detection of persistent polioviruses in patients with the post-polio syndrome. Atti XV Meeting of the European study Group on the molecular biology of picornaviruses (EUROPIC). Sitges, Barcelona, Spain, May 26-30, 2008 (p. 91).

Toniolo A, Baj A. Chronic enterovirus infections: the post-polio syndrome and insulin-dependent diabetes. Proceedings First Annual World Summit on Antivirals, Kunming, China, July 20-22, 2008 (p. 146).

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

Maccari G, Baj A, Monaco S, Toniolo A. Infezioni croniche da enterovirus: persistenza del poliovirus tipo 1 in pazienti con sindrome Post-Polio. 36° Congresso Nazionale della Società Italiana di Microbiologia. Roma, 12-15 ottobre 2008.

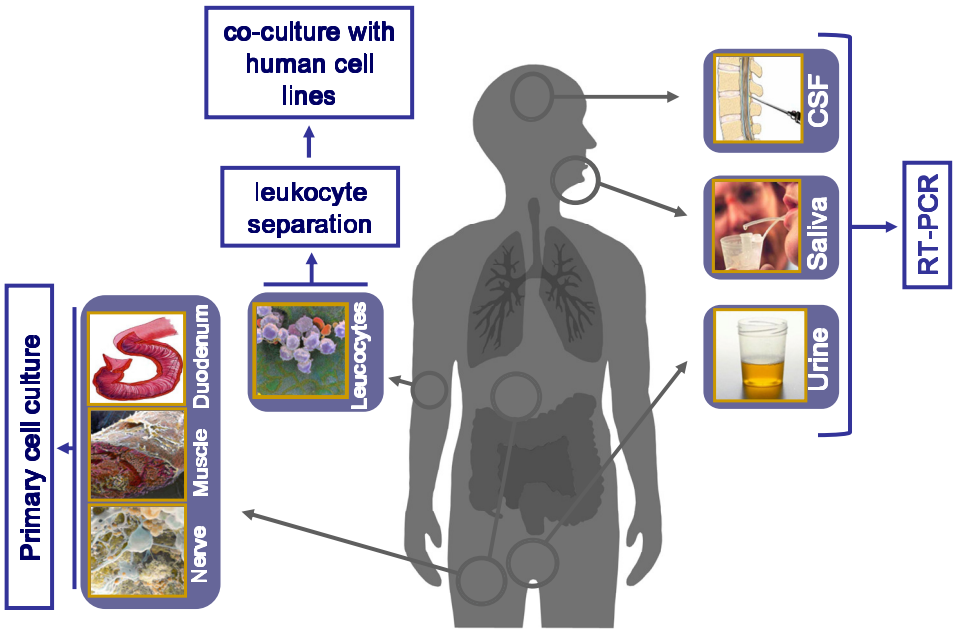
Maccari G, Baj A, Monaco S, Toniolo A. Infezioni croniche da enterovirus: persistenza del poliovirus tipo 1 in pazienti con Sindrome Post-Polio. Atti 36° Congresso della Società Italiana di Microbiologia, Roma 12-15 Ottobre 2008.

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)

Male/Female	Age (years, M ± SD)	Years from APP (mean ± SD)
36.2%	57.4 ± 7.3	53 ± 7.0

Controls (n = 49)

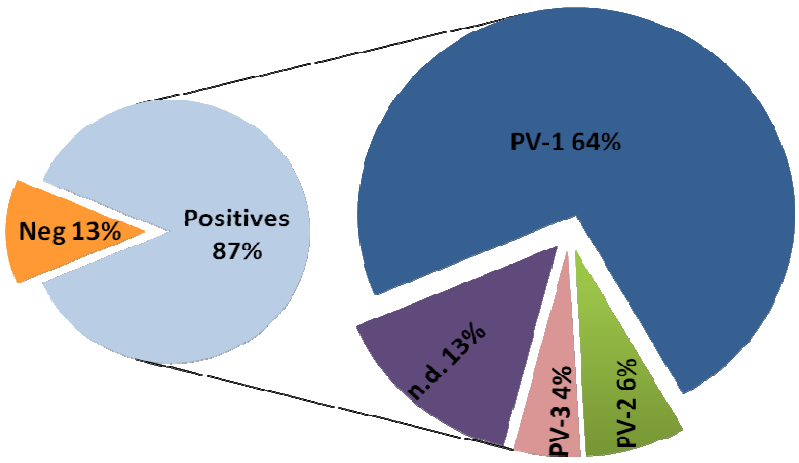
Blood donors (n=26); neurologic patients with non-infectious, autoimmune, or neoplastic disease (n=11); family members of PPS patients (n=12)

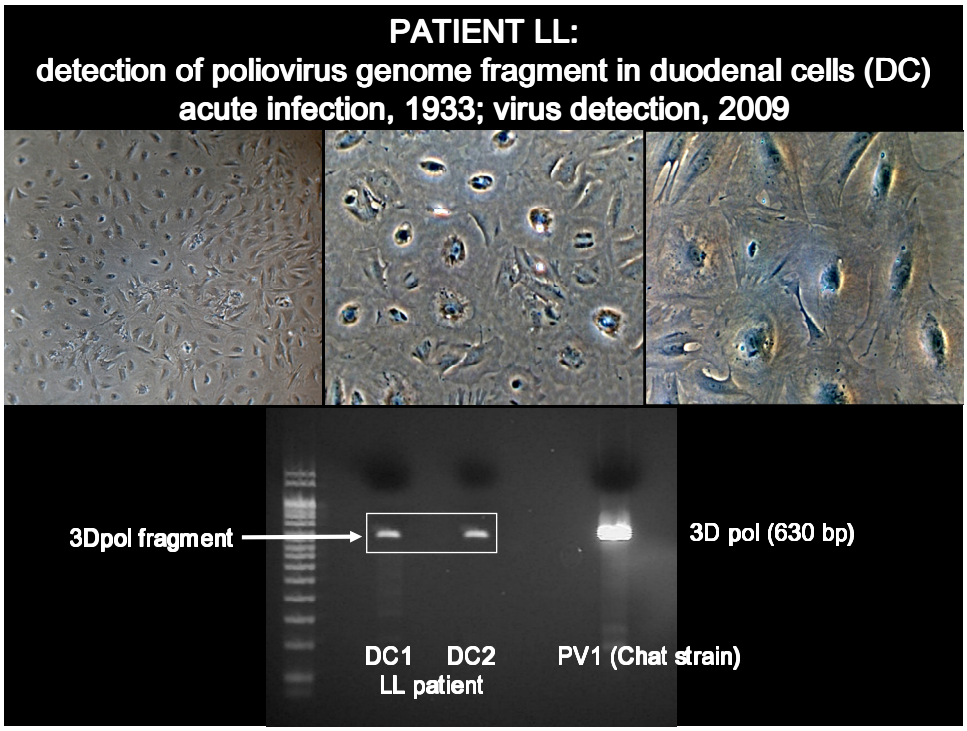
Male/Female	Age (years, M ± SD)
67.3%	39.7 ± 13.4

Poliovirus genome fragments in PPS patients (n = 47)

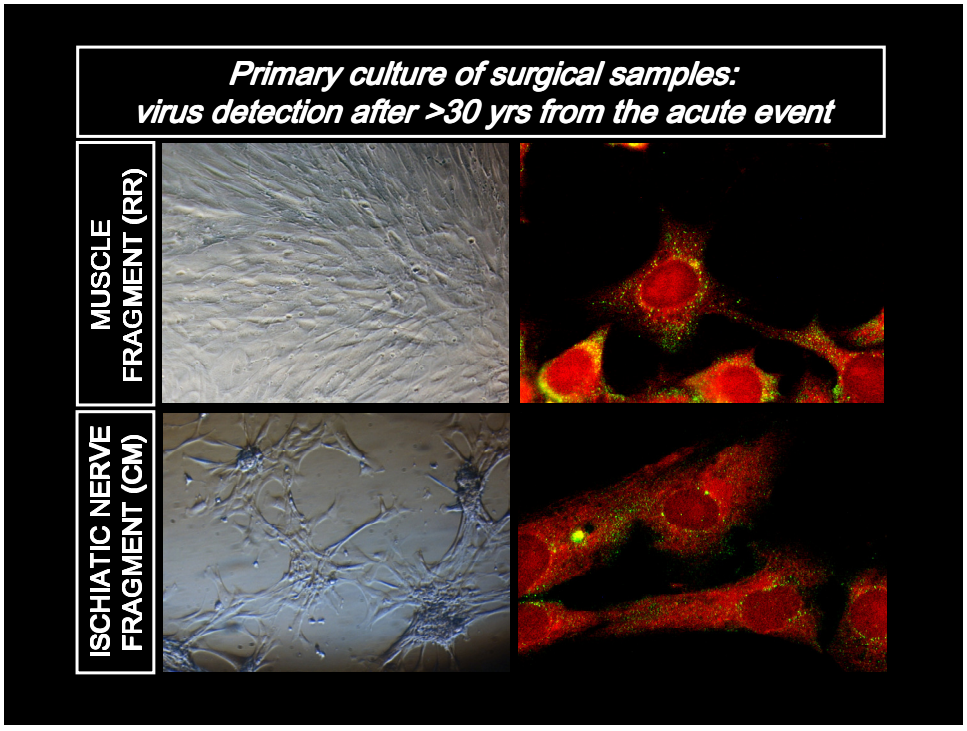
Patient	Gender	Age	Years from APP	Samples	Viral regions			ID
					5'UTR	VP1	3D Pol	
P01VN VR	F	53	48	CSF	-	+	+	PV-1
P02CR VR	M	59	51	CSF	+	+	+	PV-1
P03LL VR	F	74	73	CSF	+	+	+	PV-1
				Blood	+	+	+	
				Saliva	+	+	+	
				Duodenum	+	+	+	
P04CM VR	F	50	43	CSF	-	-	+	N.D.
P05BE VR	M	61	60	CSF	+	+	+	N.D.
P06PP VR	F	70	64	CSF	-	+	+	N.D.
P07FE VR	F	56	48	CSF	-	-	+	PV-1
P08CL VR	F	51	49	CSF	+	+	+	PV-1
P09BM VR	M	65	61	CSF	+	+	+	N.D.
P10LG VR	F	73	65	CSF	-	-	+	N.D.
P11DL VR	M	69	68	CSF	+	-	+	PV-1
P12PL VR	F	60	59	CSF	+	-	+	PV-2
P13BM VR	F	53	51	CSF	+	-	+	PV-2
P14MV VR	F	55	50	CSF	+	+	+	PV-1
P15AMG VR	F	58	49	CSF	-	+	+	PV-1
P16AA VR	F	59	52	CSF	+	+	+	PV-1
P17TR CO	F	57	55	CSF	+	+	+	PV-1
P18CS VA	F	54	50	CSF	-	-	+	N.D.
P19RR VA	F	58	57	CSF	-	+	+	PV-1
				Muscle	-	+	+	
				Nerve	-	+	+	
P20CF VA	F	52	49	CSF	+	+	+	PV-1
				Blood	+	+	+	
P21BL PI	F	57	55	Blood	+	+	+	PV-1
P22GS VA	F	60	57	Blood	+	+	+	PV-1
				Saliva	+	+	+	
				Urine	+	+	+	
P23SC VA	M	47	46	Blood	+	+	+	PV-1
				Saliva	+	+	+	
				Urine	+	+	+	
P25DLA VA	M	47	46	Blood	+	+	+	PV-1
P26CL ML	F	53	50	Blood	-	-	+	PV-1
P27PMG ML	F	62	58	Blood	-	-	+	PV-1
P28CAM VR	F	46	46	Blood	+	-	+	PV-1
P30AP VR	M	56	55	CSF	+	+	+	PV-1
P31CC VR	F	62	61	CSF	+	-	+	PV-1
				Blood	-	+	+	PV-1
P32GV VR	M	57	50	CSF	-	-	-	NEG
P33FV VR	M	49	48	CSF	+	+	+	PV-1
P34BN VR	F	71	69	CSF	-	-	-	NEG
P35CF VR	M	55	51	CSF	-	+	+	PV-1
P36RN VA	F	55	15	Blood	-	-	+	PV-1
P38RG VA	M	63	59	Blood	-	-	+	PV-1
P39RMA VA	F	49	47	Blood	+	+	+	PV-1
P40ZR VA	M	50	45	Blood	+	-	+	PV-1
P42BP VA	M	59	54	Blood	-	+	+	PV-1
P43BP VA	M	55	52	Blood	-	-	-	NEG
P44MA VA	M	57	54	Blood	+	+	+	PV-1
P51GG VA	M	51	50	Blood			+	PV-2
P53GO VA	F	52	51	Blood	-	-	-	NEG
P55CG VA	F	44	42	Blood	+	+	+	PV-3
P57BG VA	F	55	54	Blood	+		+	PV-3
P58CG VA	F	61	57	Blood	-	-	-	NEG
P59MG VA	M	47	46	Blood	-	-	-	NEG

Poliovirus genome fragments in PPS patients (n = 47)

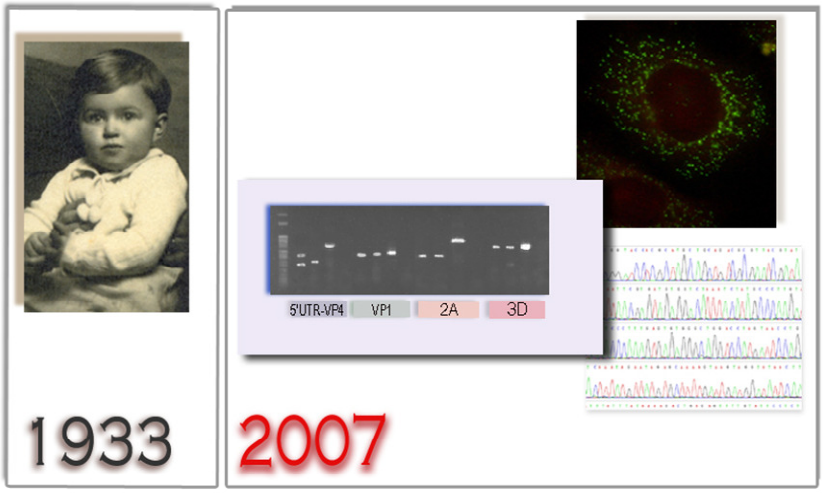




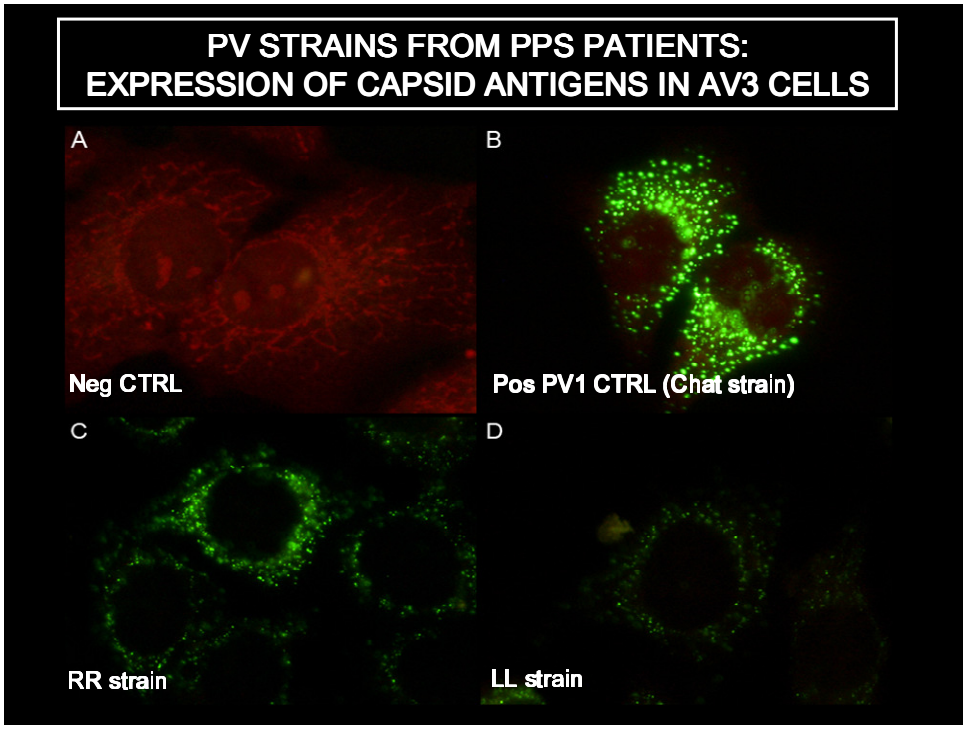
Controls n = 39		Gender
Neurology Patients non-infectious, non-autoimmune, non-neoplastic pathology (n=11)		7M, 4F
Blood donors (n=26)		19M, 7F
Relatives (n=12)		
P28CAM VR Female	P46OG VA -husband-	M
P31CC VR Female	P47GG VA -husband-	M
	P48GM VA -daughter-	F
	P49GM VA -son-	M
	P50GF VA -son-	M
P36RN VA Female	P37PRVA -husband-	M
P40ZR VA Male	P41ZE VA-daughter-	F
P44MA VA Male	P45VG VA -wife-	F
P51GG VA Male	P52GM VA -daughter-	F
P53GO VA Female	P54GD VA -brother-	M
P55CG VA Female	P56CG VA -brother-	M
P59MG VA Male	P60SY VA -wife-	F



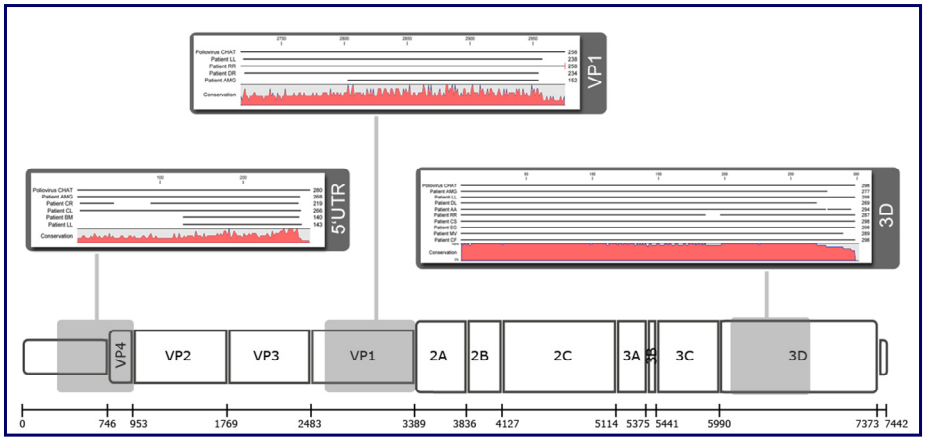
PATIENT LL: acute infection (1933)
virus detection in leukocytes (2007)



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



SEQUENCED PV REGIONS



PERSPECTIVES

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

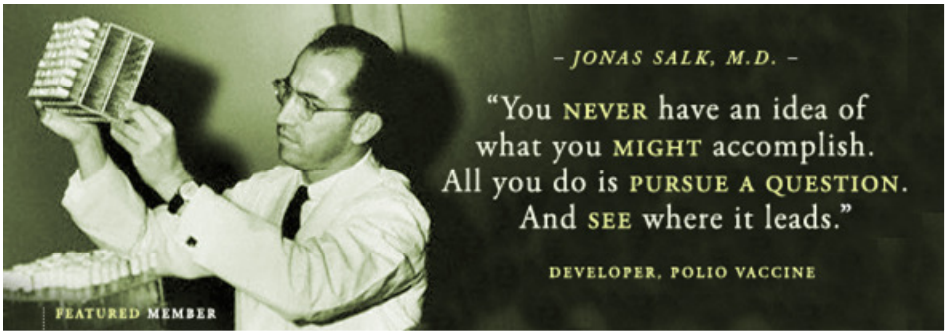
Failure to detect PV genomes in family members of PPS patients speaks against transmissibility of the mutated agents;

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.



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