

FRAMMENTI GENOMICI DEI POLIOVIRUS NEI PAZIENTI CON SINDROME POST-POLIO E AUMENTATA PRODUZIONE DI CHEMOCHINE NELLE COLTURE CELLULARI ESPOSTE AI LEUCOCITI DEL SANGUE PERIFERICO DEI PAZIENTI

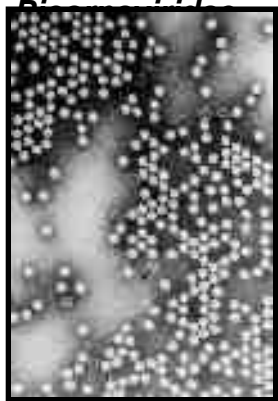
TONIOLO A1, MACCARI G1, STEFANOLI M1,

Facciamo per modificare lo stile del sottotitolo dello schema
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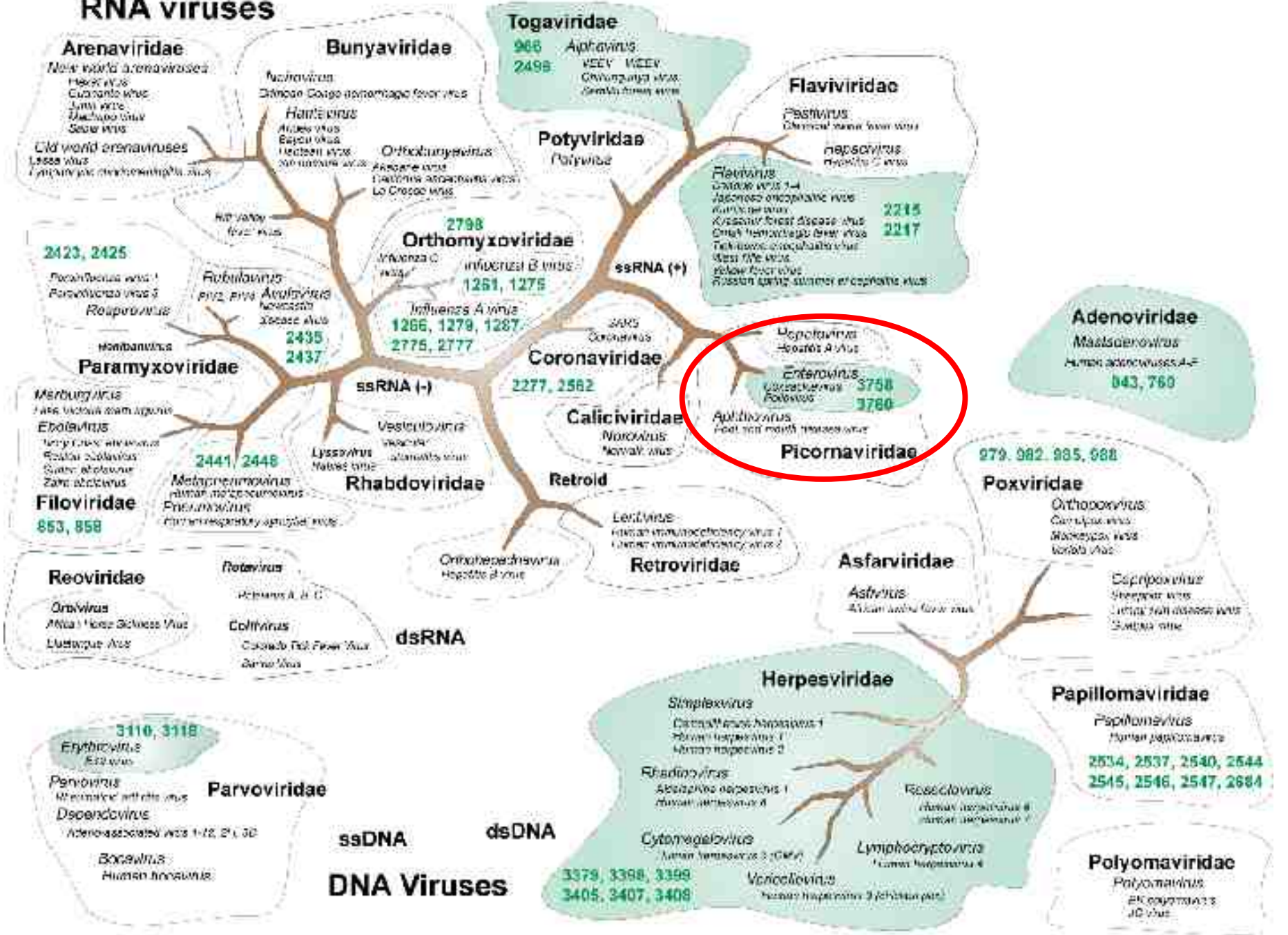


Picornaviridae classification: enteroviruses and polioviruses



Family	Genus	Species	Serotypes
Picornaviridae	Enterovirus (>100 serotypes)	Human enterovirus A (17)	CVA-2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16; Enterovirus-71, 76, 89, 90, 91, 92
		Human enterovirus B (58)	CBV-1, 2, 3, 4, 5 (incl. SVDV), 6; CVA9; Echovirus-1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 24, 25, 26, 27, 29, 30, 31, 32, 33; Enterovirus-69, 73, 74, 75, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 93, 97, 98, 100, 101, 106, 107
		Human enterovirus C (19)	CVA-1, 11, 13, 17, 19, 20, 21, 22, 24; Enterovirus- 95, 98, 99, 102, 104, 105, 109 Poliovirus-1, 2, 3
		Human enterovirus D (3)	Enterovirus-68, 70, 94
	Parechovirus	Human parechovirus (14) Ljungan virus (4)	HPeV-1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14
Kobuvirus	Human Aichi virus (1) Bovine Kobuvirus	AiV	
Cardiovirus	Encephalomyocarditis virus (1) Theilovirus (12)	animal EMC; Theiler's murine encephalomyelitis virus; Rat Theravirus; Human Vilyuisk encephalomyelitis virus; Human Saffold virus (9)	

RNA viruses





Where do we go from here?

The term Post-Polio Syndrome (PPS) was introduced by Halstead in 1985 to cover medical, orthopaedic and psychological problems related to the new disabilities occurring many years after the onset of acute paralytic polio.

15 or more years from the onset, 40-70% polio survivors develop PPS

New neuromuscular symptoms appear (Halstead, 1985; Dalakas, 1991):

- musculoskeletal complaints
- slowly progressive muscular weakness
- wasting
- chronic pain
- cold intolerance

Symptoms concern the affected and sometimes the spared bodily regions.

Elevated serum CPK values may be seen.



GIVEN THE LARGE NUMBERS OF POLIO SURVIVORS, THE HIGH FREQUENCY OF NEW WEAKNESS MAKES PPS THE MOST PREVALENT MOTOR NEURON DISEASE TODAY

The Post- Polio Syndrome: clinical manifestations

Clinical manifestation	U.S. patients (%)	European patients (%)
Generalized fatigue	83-89	7-62
Joint pain	71-73	33-57
Muscle pain	71-86	34-58
Weakness in muscles		
previously affected	63-91	54-85
previously unaffected	42-66	
Total new	87-95	32-79
Atrophy	28-59	30-80
Cold intolerance	29-56	42-62
Dyspnea	39-42	4-43
Bulbar muscle involvement	27-36	17
Sleep disturbances (restless leg syndrome)	NA	9-48
Impaired attention/Word finding difficulty	NA	39

NUMBERS OF POLIO SURVIVORS

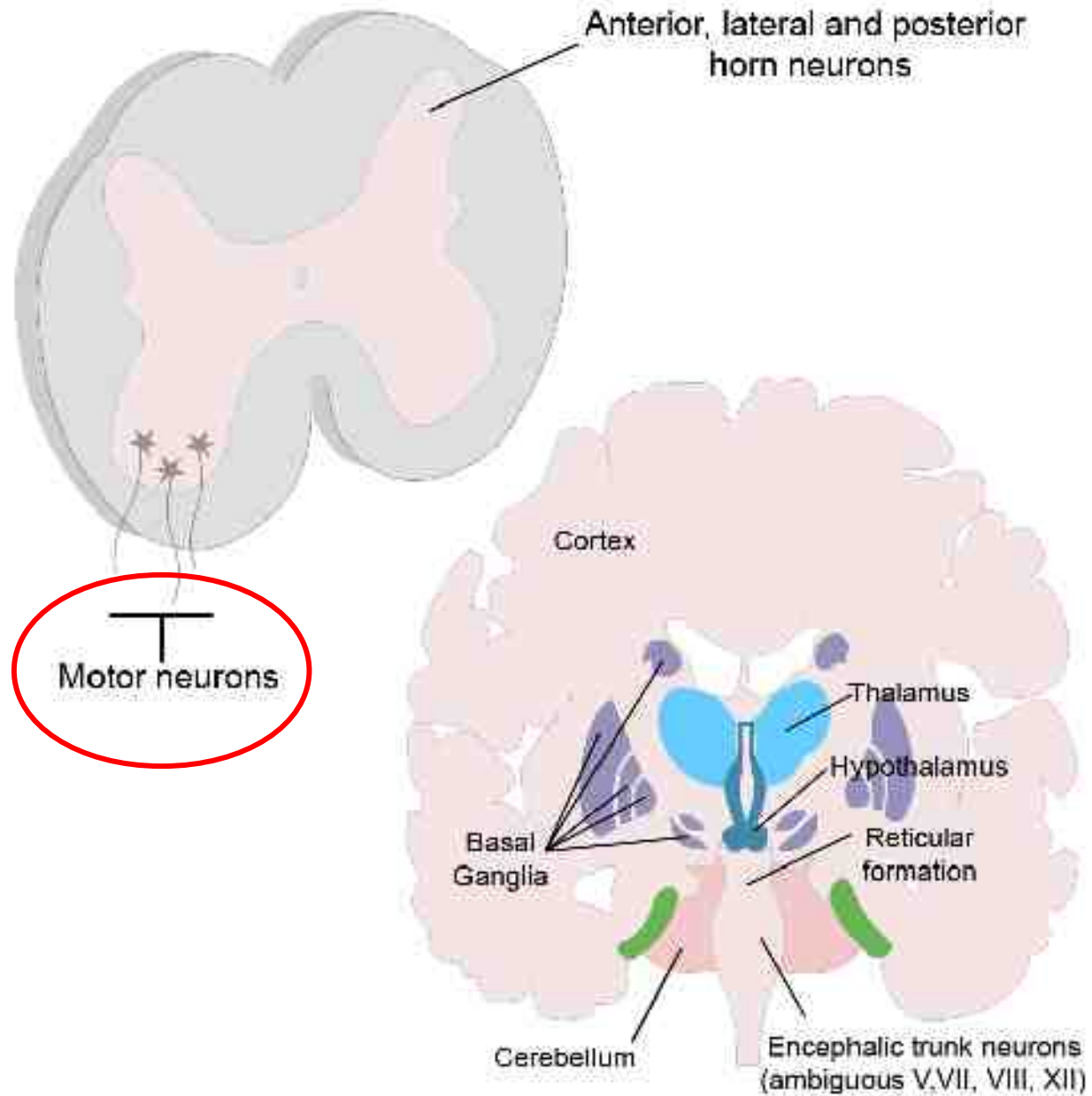
Worldwide: 10-20 million

USA: 0.5 million

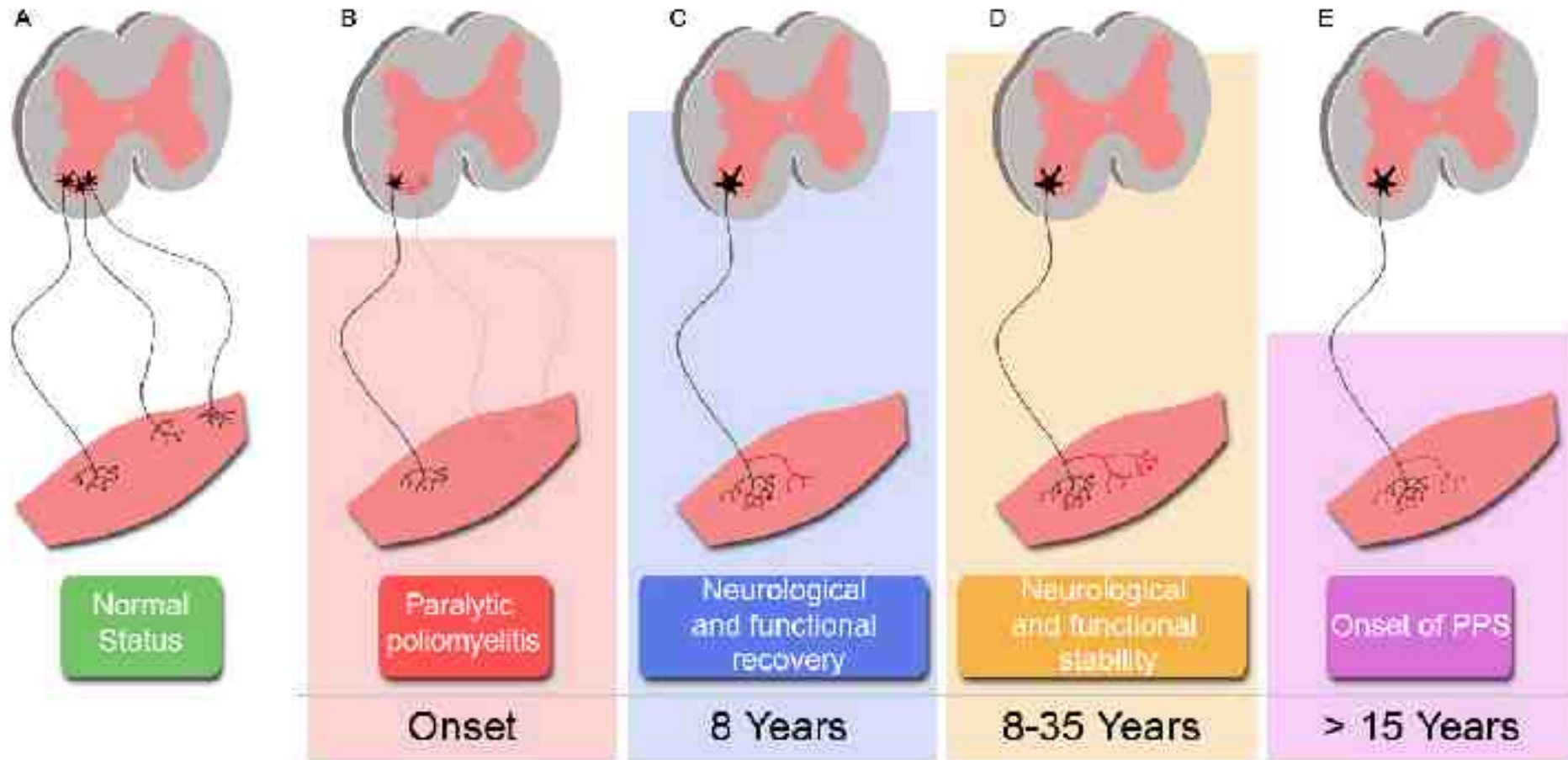
Europe: >0.5 million

Asia: >5.0 million

Poliomyelitis: CNS areas infected by PVs



Late effects of polio: progression from poliomyelitis to PPS



The Post-Polio Syndrome: pathogenesis

CO-FACTORS

- Aging, loss of motor neurons
- Overuse, muscle degeneration
- Disuse, muscle atrophy

PERSISTENT VIRAL REPLICATION:

- Virus-induced cell damage
- Chronic inflammatory response
- Immune-mediated injury
- Decreased expression of neurotrophic factors

DISTAL DEGENERATION HYPOTHESIS:

- Distal degeneration of axonal sprouts in enlarged motor units *and/or*
- Loss of entire motor units

Methods

PPS patients (n = 65)

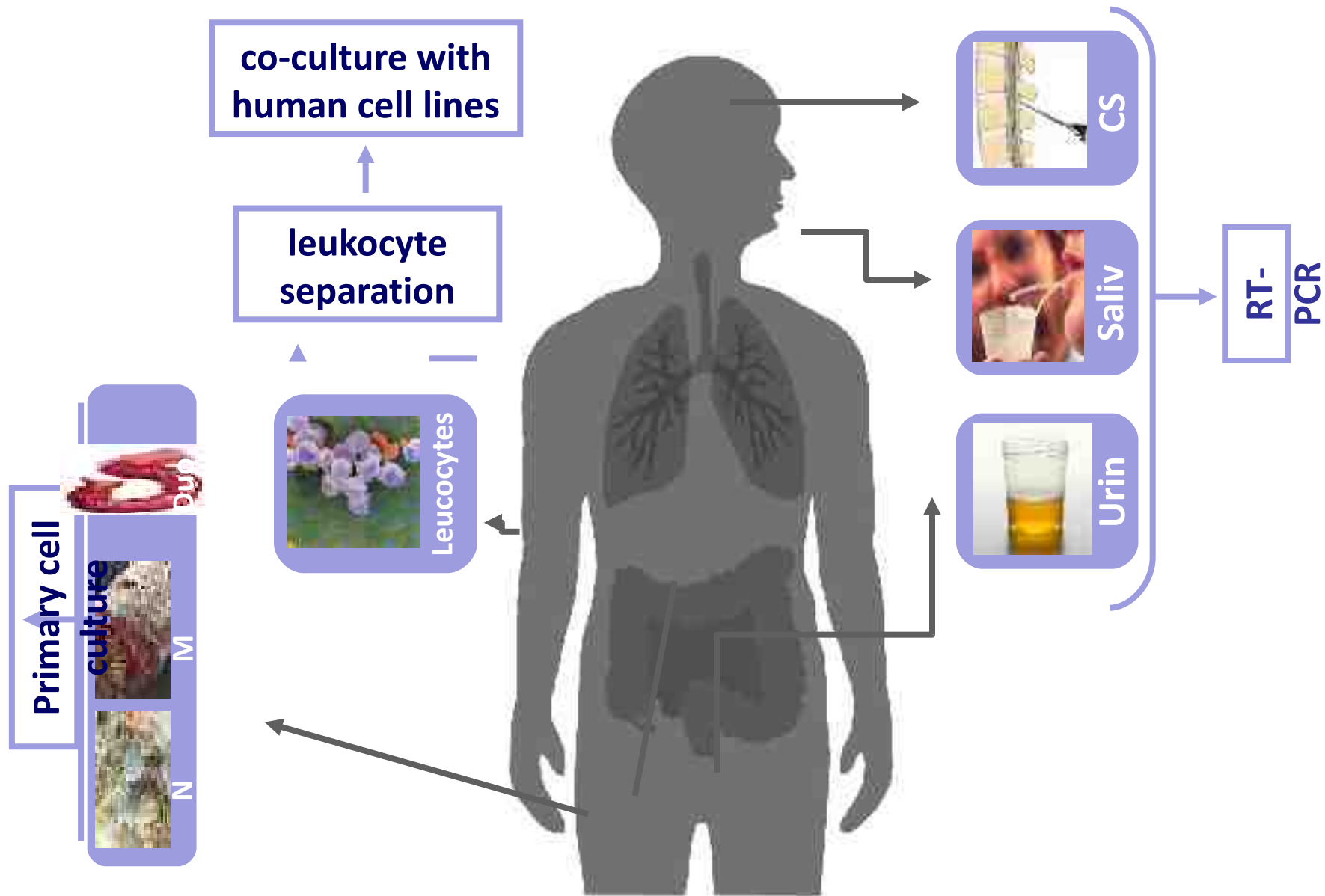
Male/Female	Age (years, M \pm SD)	Years from APP (mean \pm SD)
36.2%	57.4 \pm 7.3	53 \pm 7.0

Controls (n = 58)

Blood donors (n=26); neurologic patients with non-infectious, non-autoimmune, or neoplastic disease (n=11); family members of PPS patients (n=21)

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

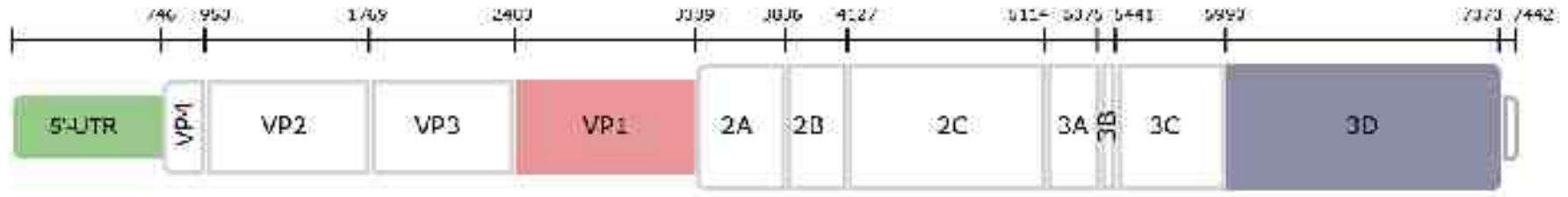
Detection of Polioviruses: methods



METHODS: detection of enteroviruses by immunofluorescence

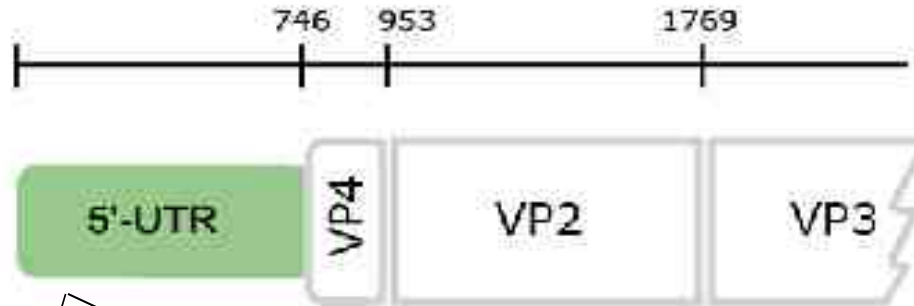
Antibody	Cell cultures infected with different enterovirus types															
	CBV 1	CB V2	CBV 3	CBV 4	CBV 5	CBV 6	CAV 2	CAV 4	CAV 9	echo 3	echo 7	echo 11	EV71	PV1	PV2	PV3
Pan- Enterovirus 9-D5 (Chemicon)	+++	++ +	+++	+++	+++	++	++	+++	++	+++	+++	++	+++	+++	+++	+++
CBV4-356.1	-	-	-	+++	-	-	-	-	-	-	-	-	-	-	-	-
Poliovirus Blend (Chemicon)	-	-	-	-	-	-	-	-	-	-	-	-	-	++	++	++

Methods: genomic regions for PV detection



Three genomic regions have been investigated:

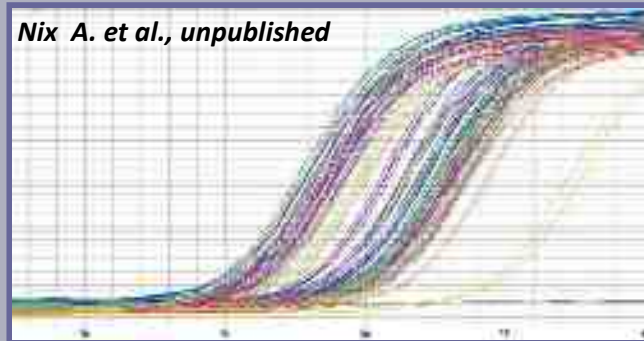
- **5'UTR (entry into ribosomes)**
- **VP1 (capsid protein)**
- **3Dpol (RNA polymerase)**



163-183 → ← 602-583

Qualitative PCR

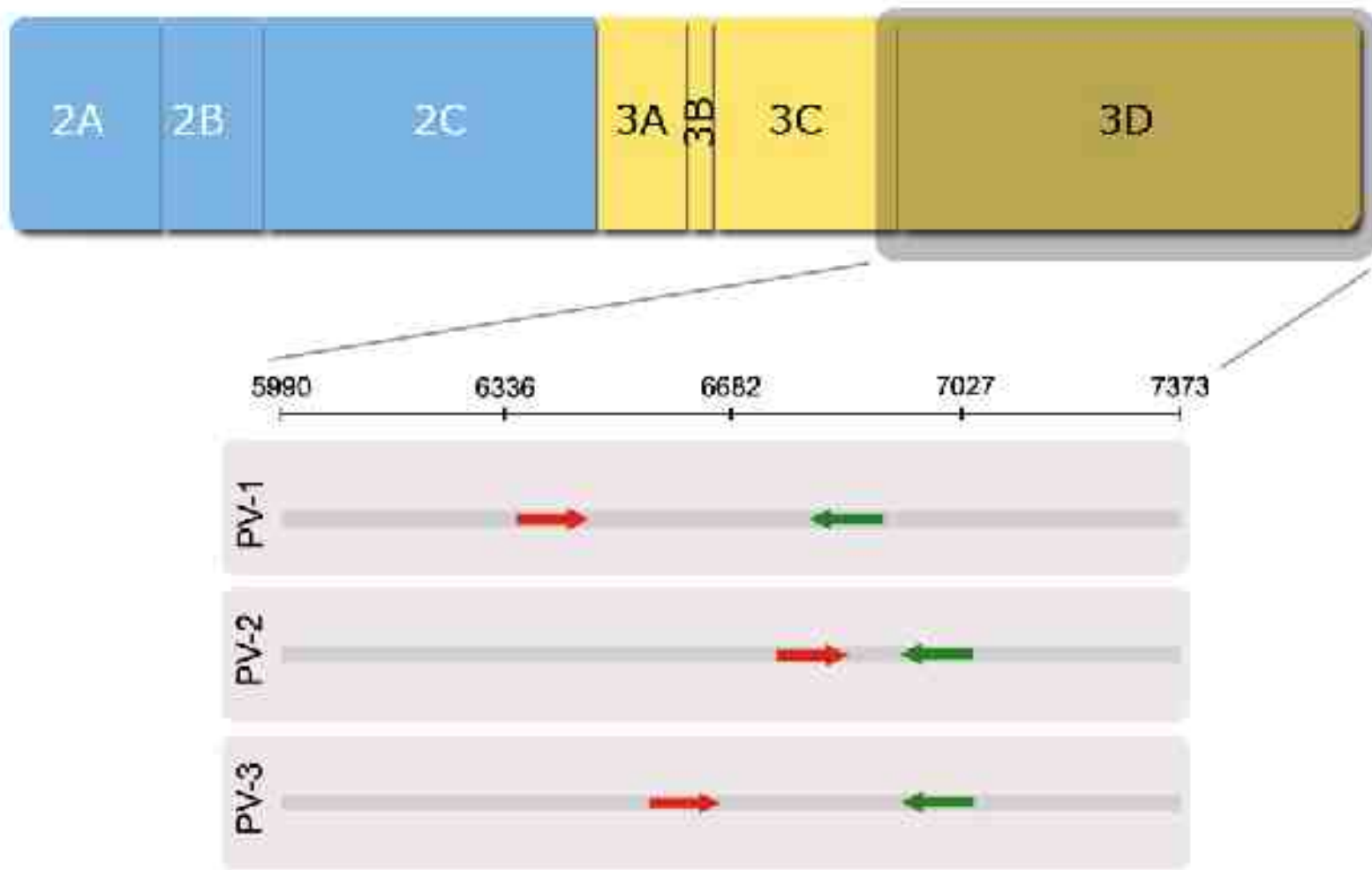
453-470 → ← 537-559 ← 595-577



Real time quantitative PCR

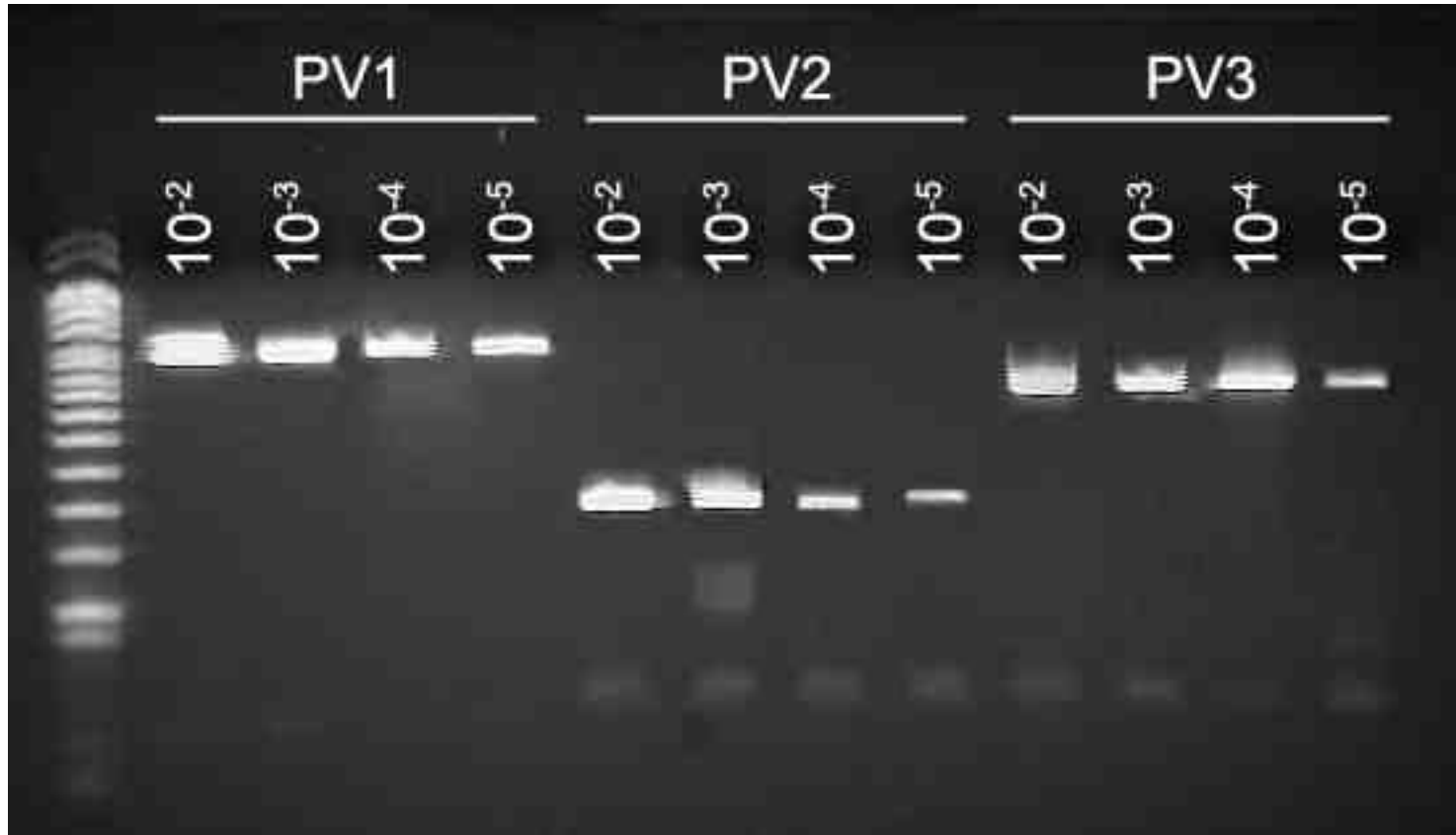
Nix A. et al., unpublished

Methods: PVs (3Dpol)

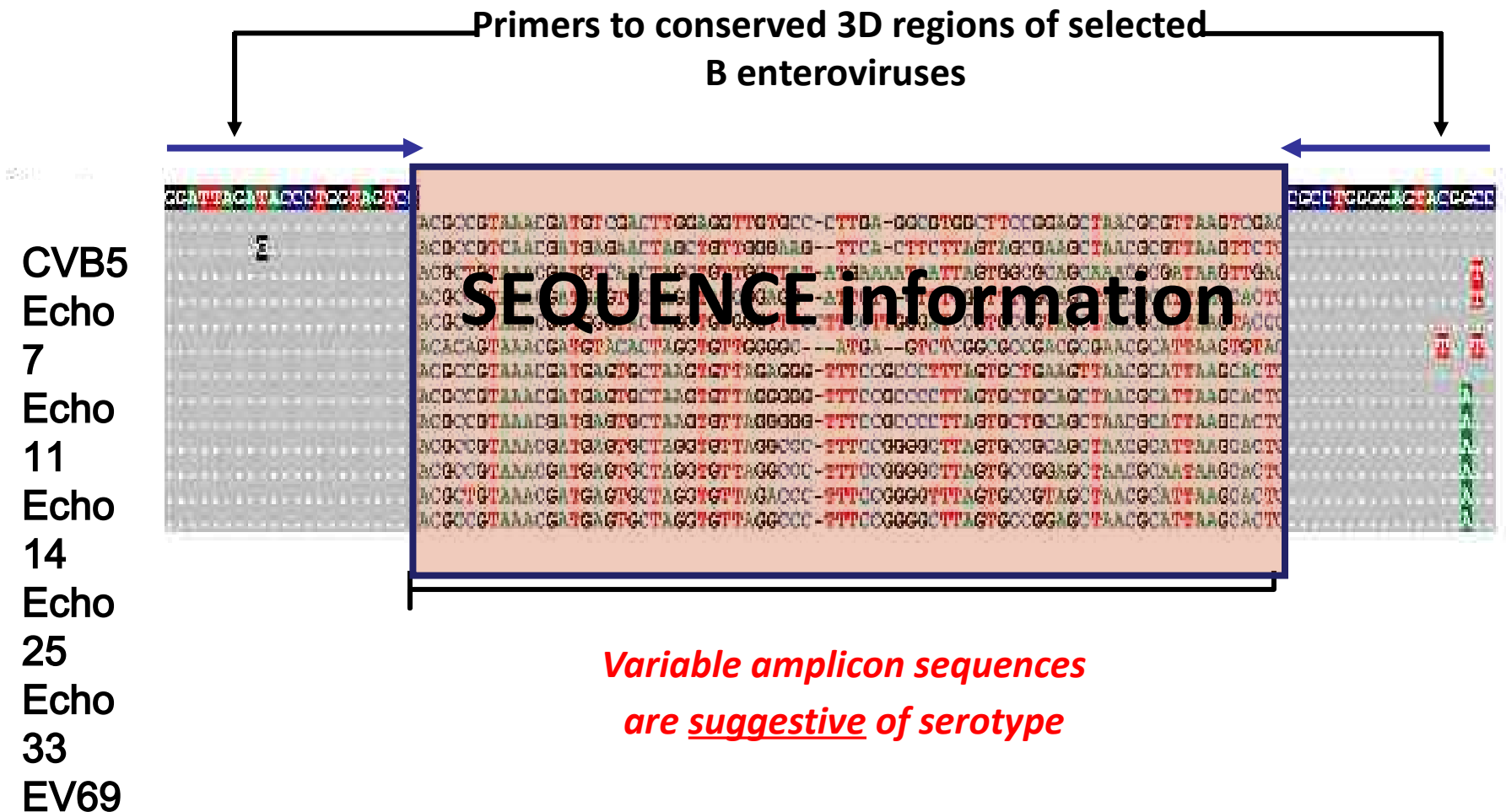


Methods: RT-PCR for Polioviruses (3Dpol)

3Dpol primers: PV positive controls



Enterovirus ID: primers to conserved regions of the 3D gene



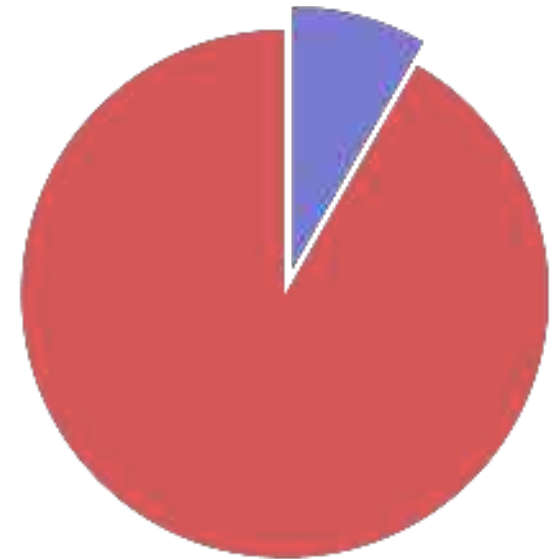
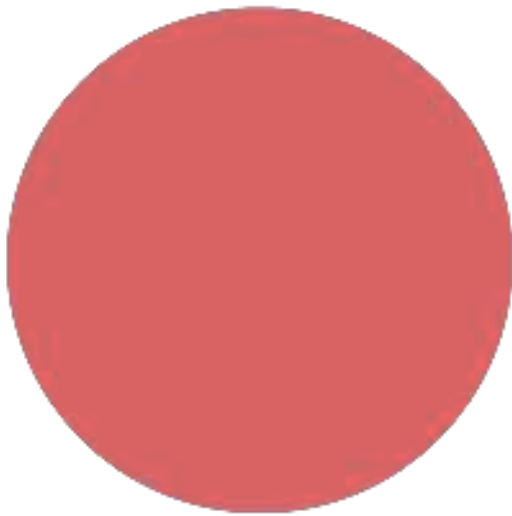
*Late effects of polio:
can poliovirus be detected
in PPS patients?*

Poliovirus genome fragments in control subjects (n=58)

Blood donors (0/26)

*Family members of
PPS patients (2/21)*

*Pathologic controls
(1/11)*

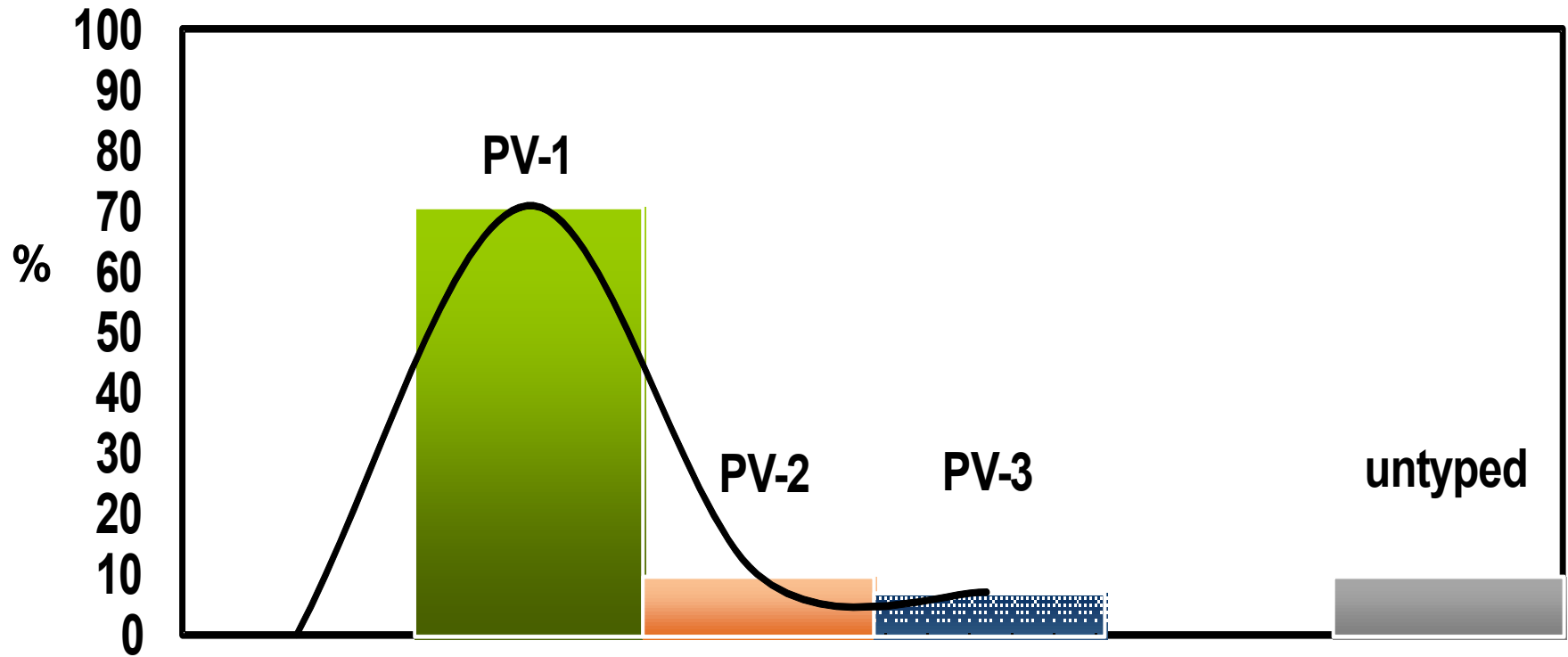


No. positive: 3/58 (5%)

Poliovirus genome fragments in PPS patients (n=65)



% Poliovirus types in PPS patients



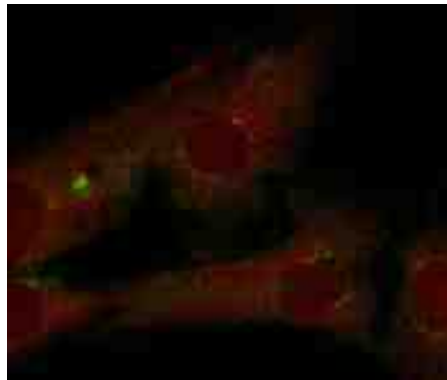
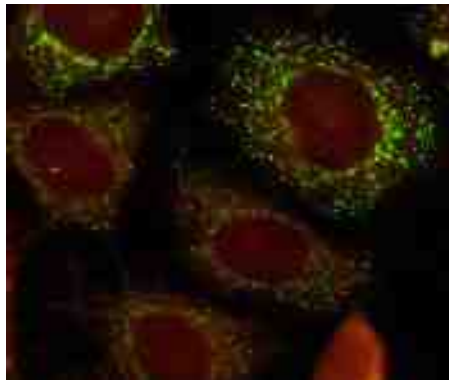
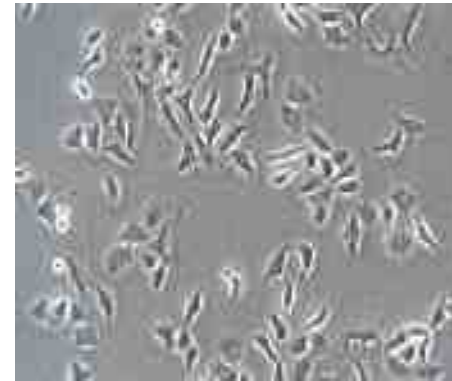
Post-Polio Syndrome: PV expression in primary cultures

*DUODENUM
(P03LL VR)*

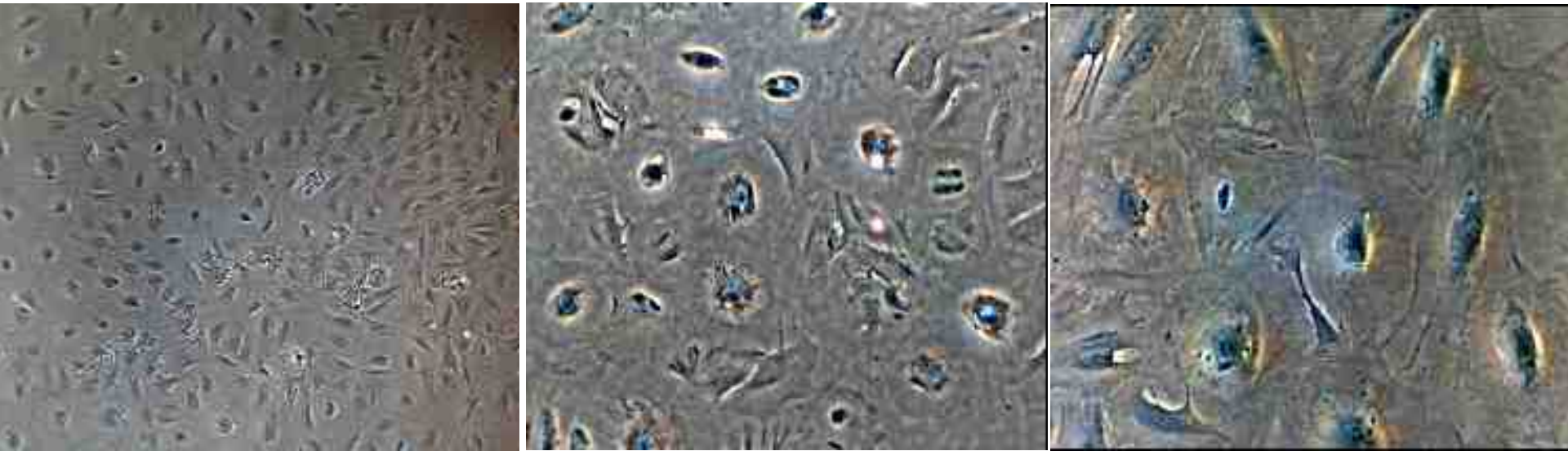
*ISCHIATIC NERVE
(P19RR VA)*

*MUSCLE
(P19RR VA)*

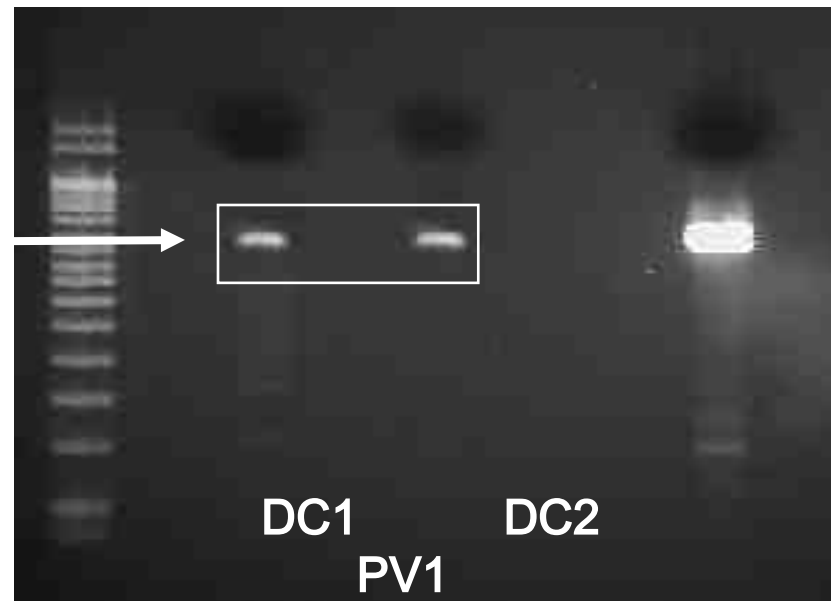
*HeLa plus PBL
(P39RMA VA)*



Post- Polio Syndrome: PV expression in primary duodenal cell cultures



Polio-1
3Dpol fragment



3D pol (630 bp)

DC1

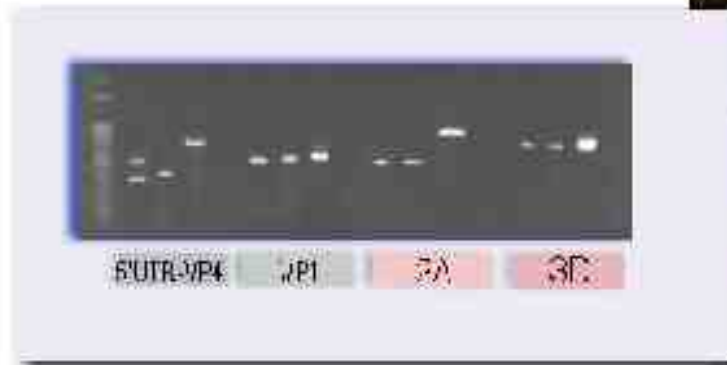
DC2

PV1

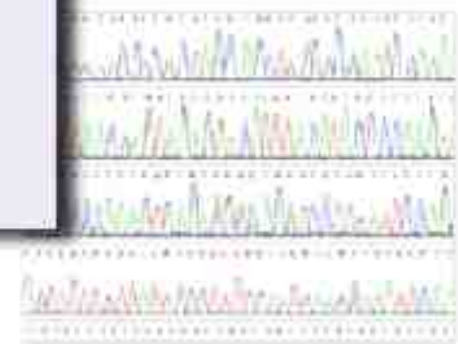
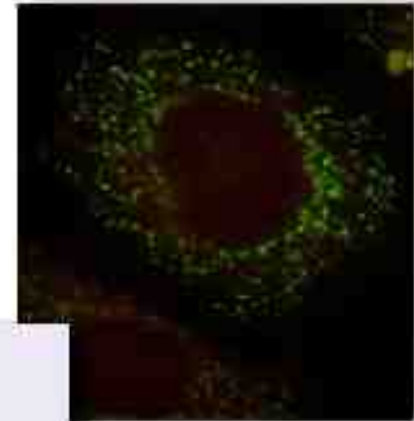
**PATIENT LL: acute infection (1933)
PV1 detection in leukocytes (2007)**



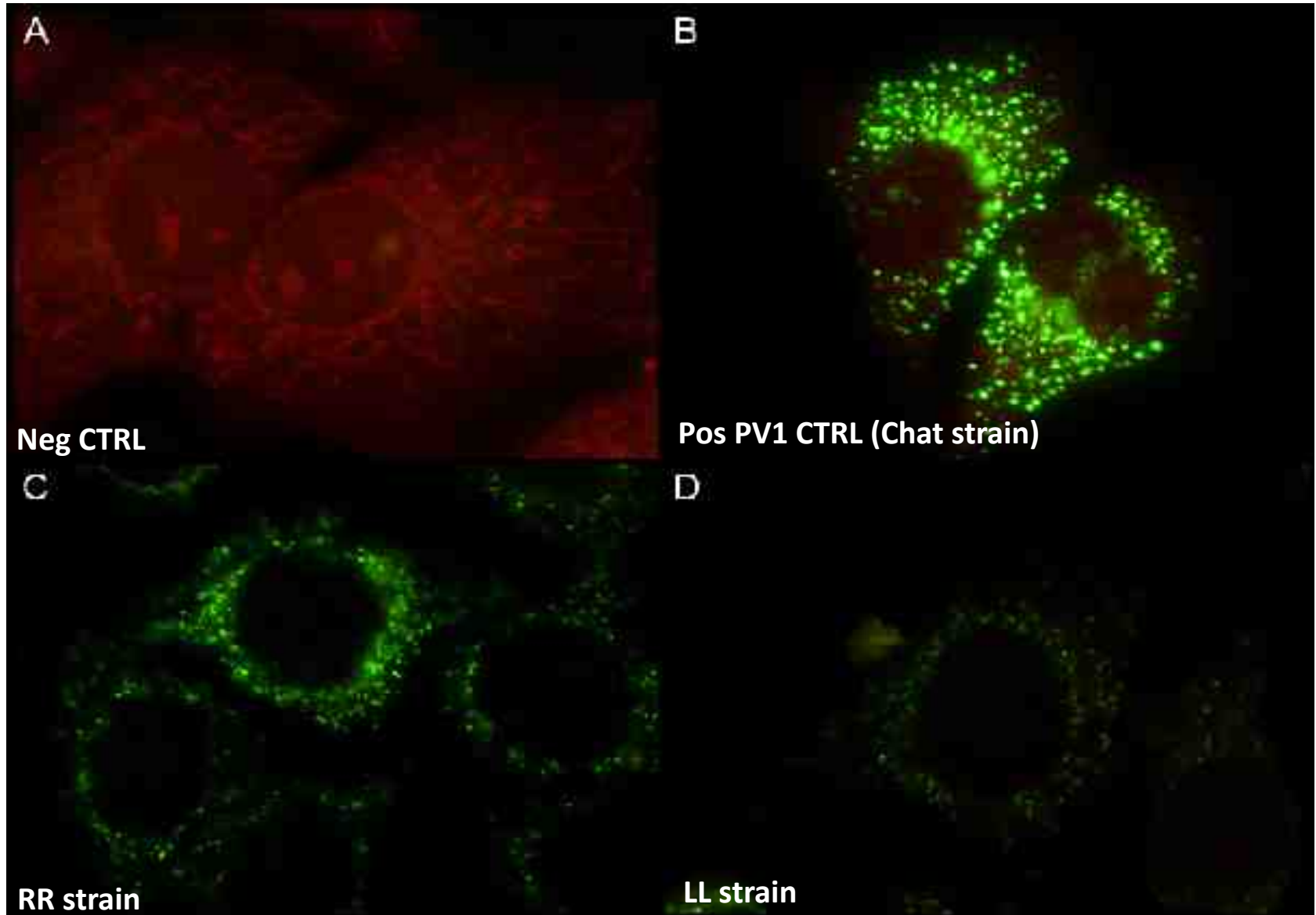
1933



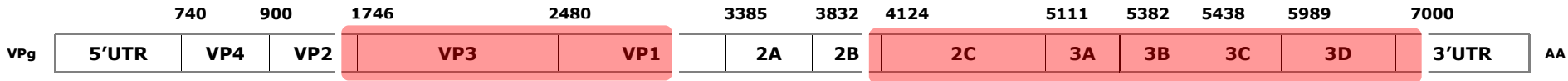
2007



EXPRESSION OF PV CAPSID ANTIGENS IN AV3 CELLS EXPOSED TO LEUKOCYTES



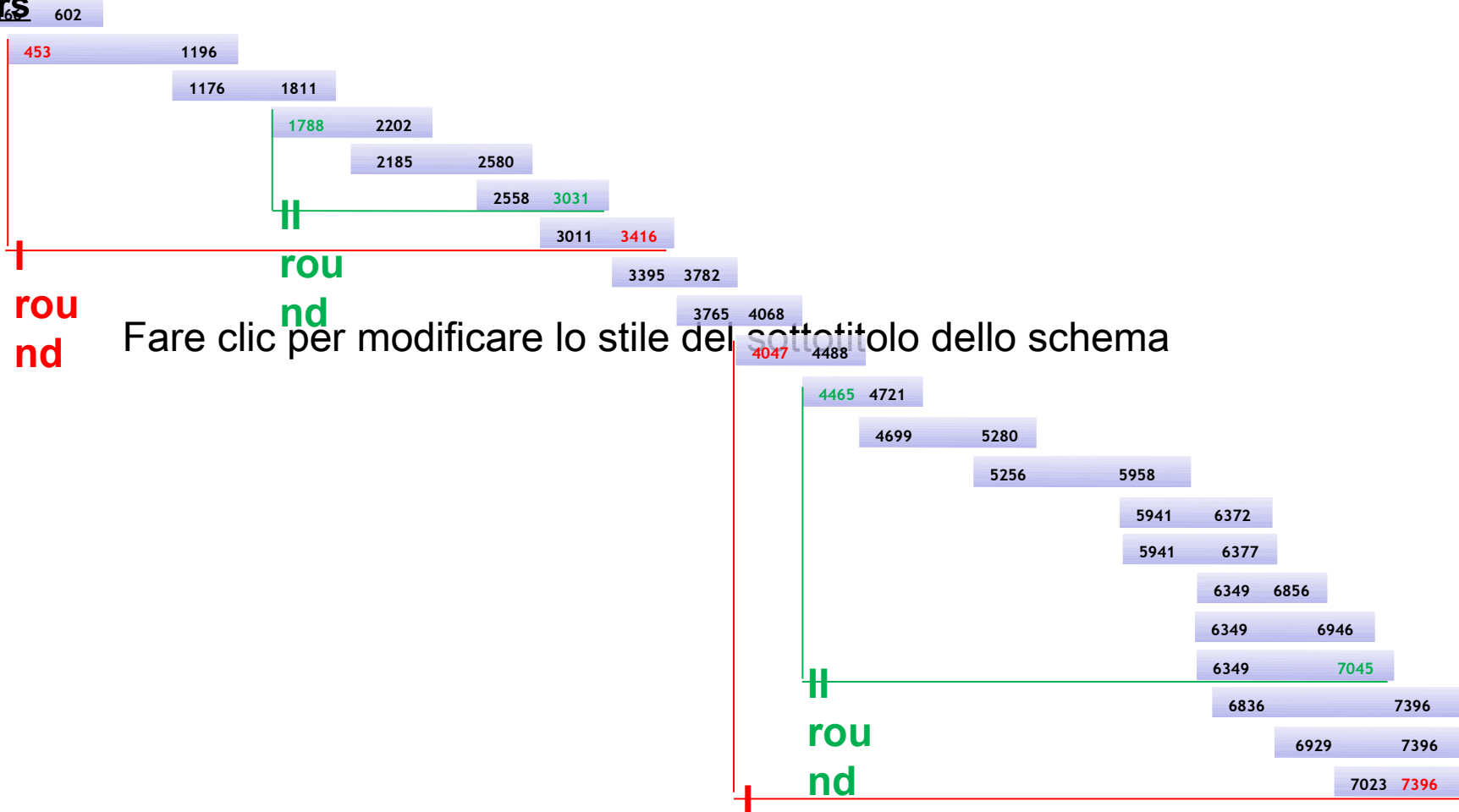
Full length amplification of persistent PV isolates from PPS patients



P03LLVR and P19RRVA: 1248bp fragment

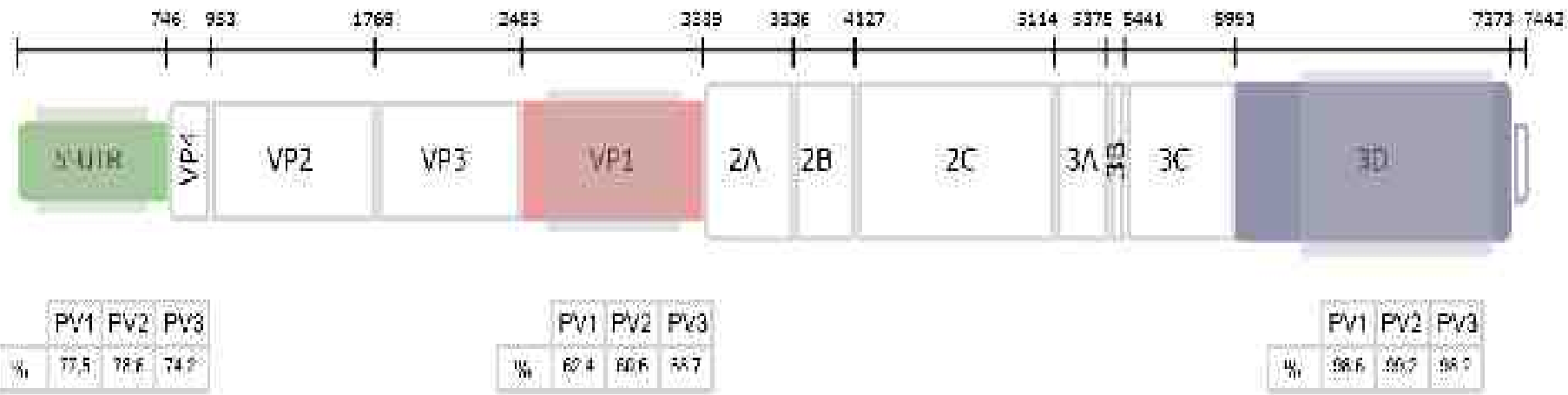
P03LLVR: 2580bp fragment

List of PV-specific primer pairs

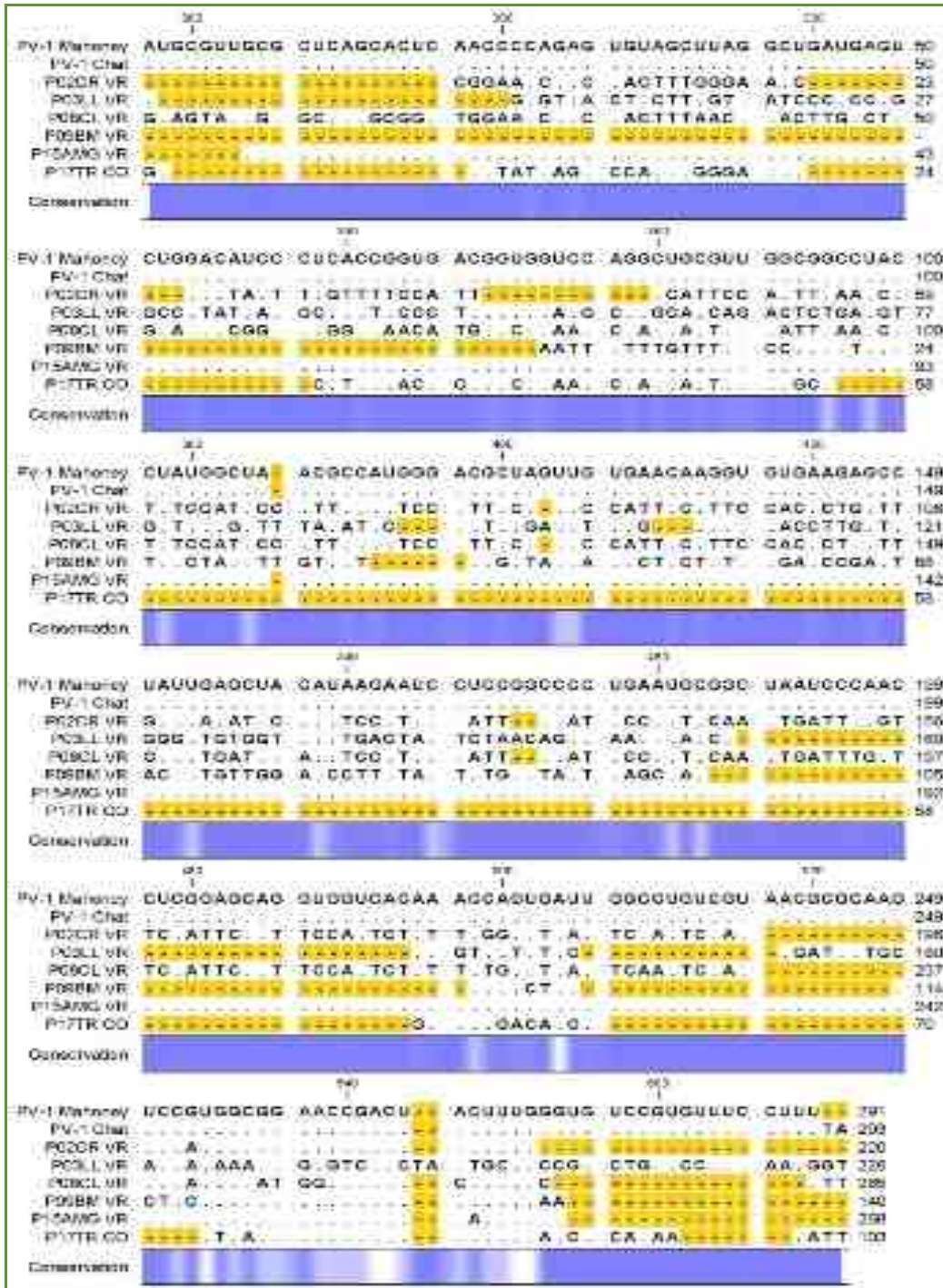


Fare clic per modificare lo stile del sottotitolo dello schema

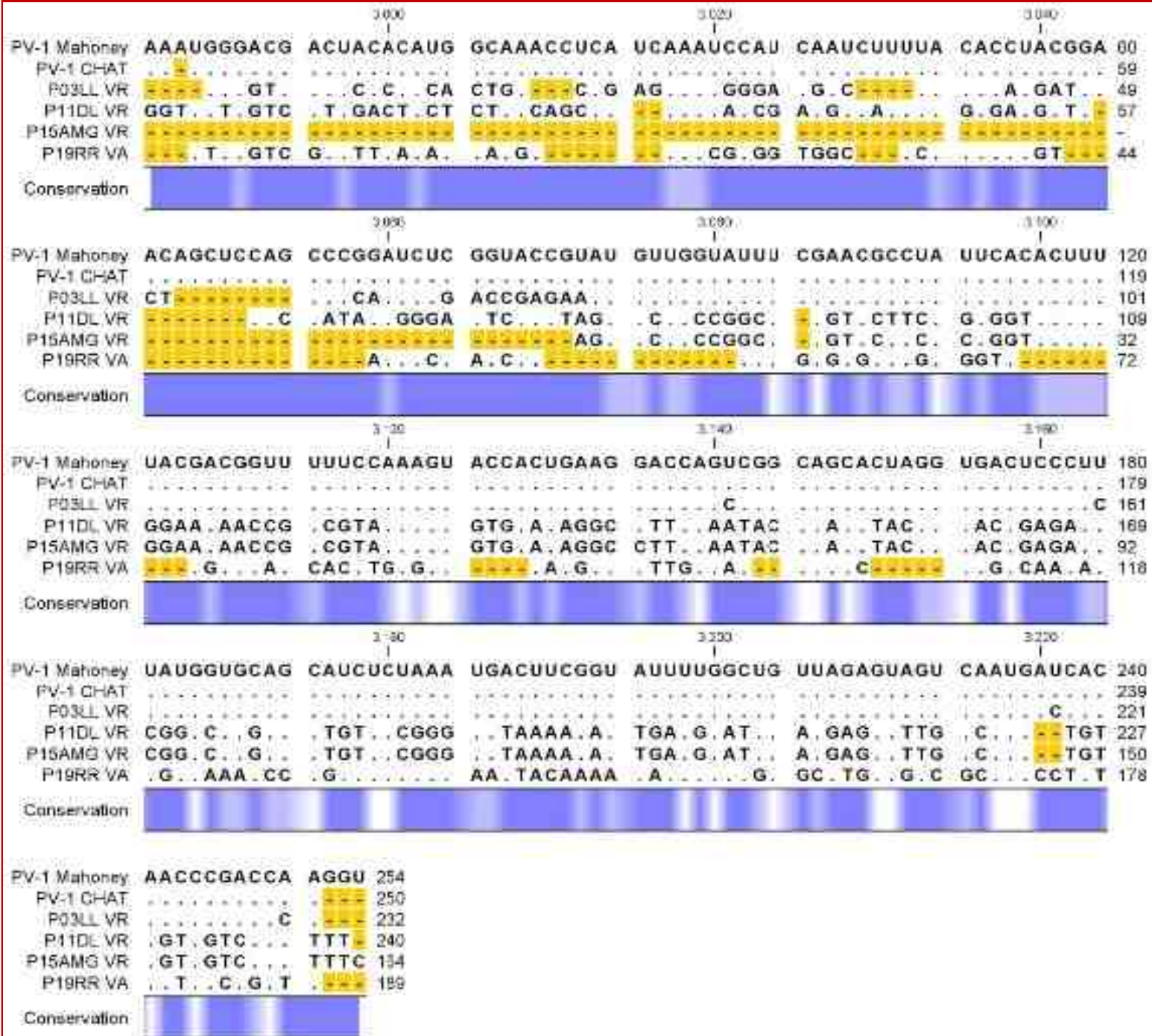
PV sequences obtained from different patients: % similarity with reference strains



5'UTR REGION



VP1 REGION



Institute of Human Virology, Baltimore

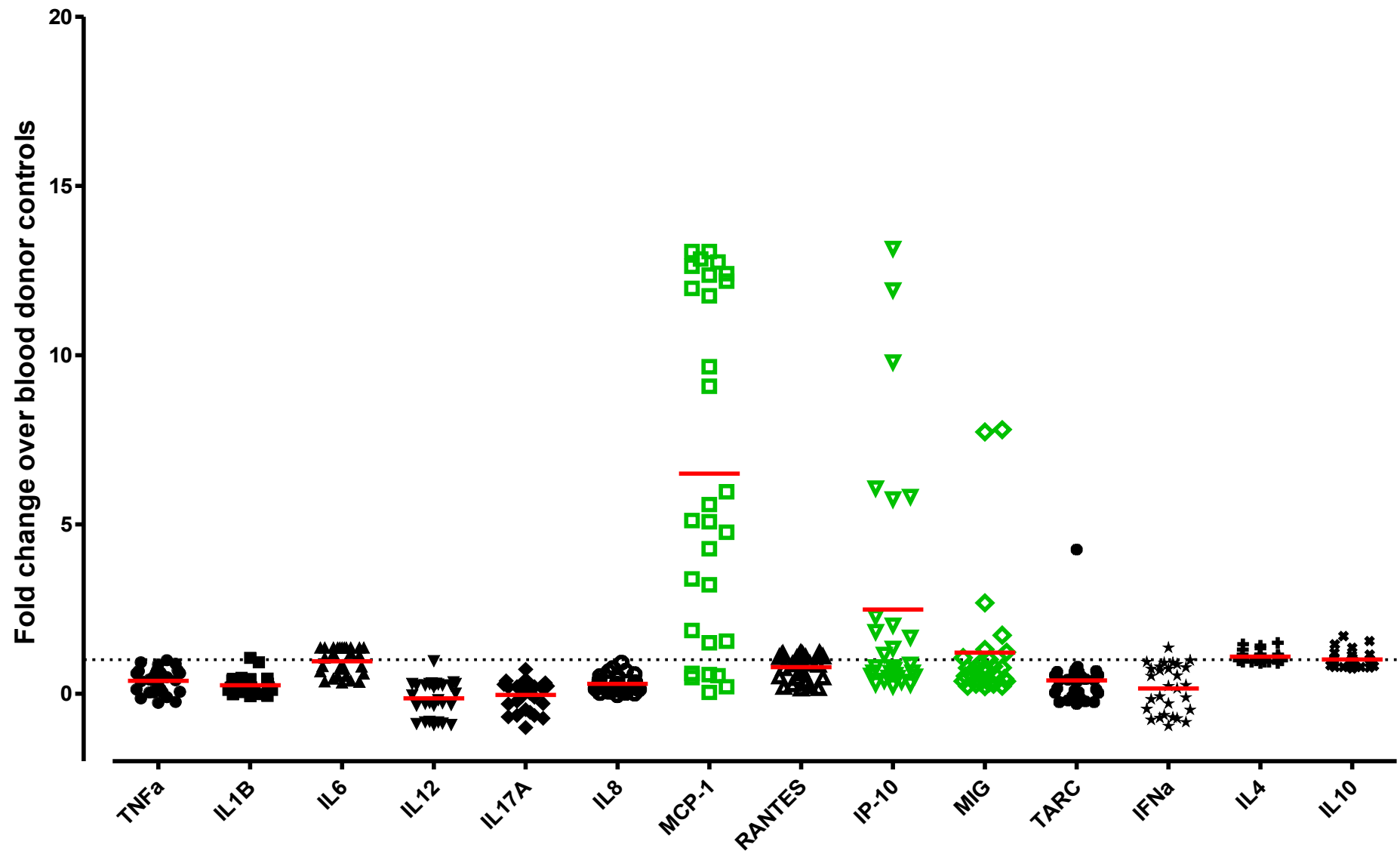


***DIFFERENT DELETIONS AND MUTATIONS ARE
DETECTED IN THE 5`UTR AND VP1 REGIONS;***

THE 3D POL REGION IS INSTEAD CONSERVED

Cytokine production by HeLa cells infected with PV isolates of PPS patients

[fold change : PPS patients (n=30) over blood donors (n=15)]



Chemokines upregulated by PV isolates

MCP-1: chemotactic factor for monocytes and basophils, but not neutrophils or eosinophils. It is implicated in the pathogenesis of **chronic diseases characterized by monocytic infiltrates**, like psoriasis, rheumatoid arthritis or atherosclerosis (receptor CCR2).

IP-10: (CXCL10) **Interferon gamma-induced protein 10 kDa (IP-10) or small-inducible cytokine B10** is secreted by several cell types in response to IFN- γ . CXCL10 has been attributed to several roles, such as **chemoattraction for monocytes/macrophages, T cells, NK cells, and dendritic cells**, promotion of T cell adhesion to endothelial cells, antitumor activity, inhibition of bone marrow colony formation and angiogenesis. Baseline pre-treatment plasma levels of CXCL10 are elevated in patients chronically infected with hepatitis C virus (HCV) who do not achieve a sustained viral response after completion of antiviral therapy. In patients co-infected with HIV, pre-treatment IP-10 levels below 150 pg/mL are predictive of a favorable response.

MIG: (CXCL9) **monokine induced by gamma interferon, a T-cell chemoattractant.**

CXCL9 and CXCL10 elicit their chemotactic functions by interacting with the chemokine receptor CXCR2

SUMMARY AND CONCLUSIONS

SUMMARY

Detection of PV genomic fragments in 55/65 PPS patients (84.6%)

40/52: PV1

6/52: PV2

4/52: PV3

5/52: yet undefined PV

Thus, PVs (genomic fragments and partial infectivity) can persist for decades in polio survivors. However, PV fragments are not found in their family members

SINCE THE 3DPol REGION IS HIGHLY CONSERVED,
IT REPRESENTS A TARGET FOR:

Molecular detection

Antiviral drugs

CONCLUSIONS

Characterization of mutated PVs associated to PPS will lead to **molecular diagnosis of persisting PV infection**

It remains to be seen whether genomic PV fragments are of pathogenetic significance

Enhancement of **chemokine secretion** by persisting virus may contribute to inflammation

Viral diagnosis may pave the way to treating patients with **antiviral drugs and/or antibodies** in order to stop the progression of virus-induced cell damage

New antiviral drugs seem effective in vitro against polioviruses



*Post-Polio Health International
Saint Louis, Missouri, USA*

THE FIFTH AWARD (2009)

PHI award to a team from University of Insubria, Varese (Italy) led by Antonio Toniolo, MD. The study, *Persisting Noninfectious Fragments of Poliovirus in PPS Patients*, will set up methods for detecting polioviruses in PPS patients and sequencing their genome.



– JONAS SALK, M.D. –

“You NEVER have an idea of
what you MIGHT accomplish.
All you do is PURSUE A QUESTION.
And SEE where it leads.”

DEVELOPER, POLIO VACCINE

Thanks to the colleagues who introduced us to this area of research:
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**Our deepest appreciation goes to the many patients whose enthusiasm, patience,
and suggestions made these investigations possible.**

