

# Multidrug resistance is associated with different gene methylation in people with HIV: a matched cohort study with data from the PRESTIGIO Registry

T. Clemente<sup>1,2</sup>, G.M. Scotti<sup>3</sup>, E. Guidi<sup>3</sup>, A. Cervo<sup>4</sup>, S. Rusconi<sup>5,6</sup>, G.C. Marchetti<sup>7</sup>, S. Diotallevi<sup>2</sup>, S. Bagaglio<sup>2</sup>, R. Lolatto<sup>2</sup>, M. Tavio<sup>8</sup>, C. Torti<sup>9</sup>, B.M. Ceselia<sup>10</sup>, A. Castagna<sup>1,2</sup>, V. Spagnuolo<sup>2</sup>, on behalf of the PRESTIGIO Study Group

<sup>1</sup>Vita-Salute San Raffaele University, Milan, Italy; <sup>2</sup>Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy; <sup>3</sup>Center for Omics Sciences, IRCCS Ospedale San Raffaele, Milan, Italy; <sup>4</sup>Infectious Diseases Unit, Policlinico di Modena, Modena, Italy; <sup>5</sup>Infectious Diseases Unit, ASST Ovest Milanese, Legnano General Hospital, Legnano, Italy; <sup>6</sup>DIBIC, University of Milan, Milan, Italy; <sup>7</sup>Clinic of Infectious Diseases, Department of Health Sciences, San Paolo Hospital, ASST Santi Paolo e Carlo, University of Milan, Milan, Italy; <sup>8</sup>Infective Diseases, AOU Ospedali Riuniti, Ancona, Italy; <sup>9</sup>Unit of Infectious and Tropical Diseases, University Magna Graecia, Catanzaro, Italy; <sup>10</sup>Unit of Infectious Diseases, Garibaldi Hospital, Catania, Italy

## Introduction

- DNA methylation (DNAm) predicts disease progression, frailty, and mortality in people with HIV (PWH) [1].
- No data on this topic are available in PWH with 4-class drug resistance (4DR), characterized by a high disease and inflammation burden [2,3].
- Our aim was to investigate DNAm in PWH-4DR, as compared to PWH without drug resistance (no-DR).

## Study Design

- Cross-sectional, propensity-score-matched cohort study on PWH on antiretroviral treatment (ART), with viral load (VL) <200 copies/mL, classified as:
  - 4DR (defined as documented resistance to NRTIs, NNRTIs, PIs, and INSTIs) from the PRESTIGIO Registry (n=27);
  - no-DR (n=27).
- Groups matched by age ( $\pm 1.5$  years) and sex.

## Methods

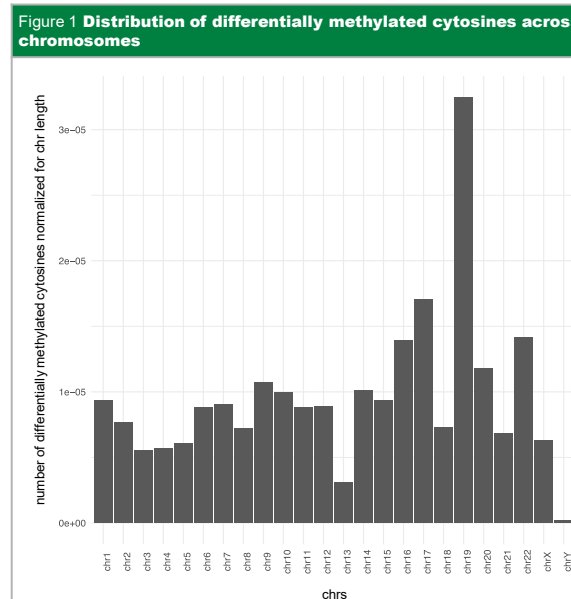
- Genome-wide methylation patterns were determined by reduced representation bisulfite sequencing from peripheral blood mononuclear cells.
- Differential DNAm evaluated on cytosines covered by  $\geq 10$  reads in  $\geq 10$  PWH for each group: bases with a minimum difference of 25% in medium methylation and a false discovery rate  $< 0.01$  were identified as differentially methylated.
- The number of significant cytosines per chromosomes (chrs) and genes were normalized according to chr and gene length, respectively.
- Enrichment analysis was performed for genes with  $\geq 10$  differentially methylated cytosines in absolute count and  $\geq 0.005$  after normalization for gene length.

## Results

- Overall, 54 individuals evaluated (Table 1).

Characteristics	Overall (n=54)	PWH-4DR (n=27)	PWH-no-DR (n=27)	P
Age (years)	53.4 (51.0-58.3)	53.6 (51.0-58.2)	53.2 (50.6-58.3)	0.993
Male sex	46 (85.2%)	23 (85.2%)	23 (85.2%)	1.000
Years of HIV	21.4 (13.6-25.1)	23.2 (20.9-25.8)	15.2 (8.33-23.7)	0.003
ART duration (years)	18.9 (10.4-21.1)	21.2 (18.8-22.6)	11.2 (5.8-18.9)	<0.001
Viral load				
<50 cps/mL	52 (96.3%)	26 (96.3%)	26 (96.3%)	1.000
50-199 cps/mL	2 (3.7%)	1 (3.7%)	1 (3.7%)	
CD4+ T-cell count (cells/mm <sup>3</sup> )	744 (604-927)	718 (556-886)	763 (620-970)	0.124
CD8+ T-cell count (cells/mm <sup>3</sup> )	987 (740-1208)	1120 (872-1367)	858 (636-1086)	0.020
CD4+ nadir (cells/mm <sup>3</sup> )	258 (115-350)	162 (37-242)	320 (262-392)	0.001
Number of ongoing antiretrovirals				
2	28 (51.9%)	1 (3.7%)	27 (100%)	<0.001
3	7 (13.0%)	7 (25.9%)	0 (0.0%)	
4	8 (14.8%)	8 (29.6%)	0 (0.0%)	
$\geq 5$	11 (20.4%)	11 (40.7%)	0 (0.0%)	
Positive HBsAg	1 (1.9%)	1 (3.7%)	0 (0%)	1.000
Positive HCV serostatus	9 (16.7%)	3 (11.1%)	6 (22.2%)	0.463
Active smoking habit	25 (46.3%)	13 (48.1%)	12 (44.4%)	0.868

- Comparing PWH-4DR with PWH-no-DR, there were 27060 differentially methylated cytosines distributed throughout all chrs, with a notable enrichment in chr 19 (Figure 1).



- The top 10 hypermethylated and the top 10 hypomethylated genes in PWH-4DR reported in Table 2.

Rank	Gene	Function
<b>Hypermethylated genes</b>		
1	ENSG00000268729	long non-coding RNA
2	ENSG00000224174	long non-coding RNA
3	DPY19L2P5	pseudogene
4	C19orf53	coding for a protein which could have a role in malignancies
5	ENSG00000233230	long non-coding RNA
6	RNA5SP378	pseudogene
7	IDH3B	coding for a subunit of isocitrate dehydrogenase, known to be downregulated in striatal cells of murine models of HIV
8	ENSG00000285853	long non-coding RNA
9	ENSG00000289832	long non-coding RNA
10	MGAT4B	coding for a glycosyltransferase
<b>Hypomethylated genes</b>		
1	ENSG00000243659	pseudogene
2	MIR4745	microRNA
3	ENSG00000272277	long non-coding RNA
4	ENSG00000226545	pseudogene
5	BHLHE22	coding for a transcription factor involved in neural and retinal development, known to be upregulated in macrophages with integrated HIV proviruses
6	B3GALT6	coding for a galactosyltransferase involved in synthesis of glycosaminoglycans (ligand of VISTA, a novel monocytic checkpoint regulator involved in immune tolerance and increased in HIV infection)
7	ENSG00000287765	long non-coding RNA
8	MIR412	microRNA
9	ENSG00000287296	long non-coding RNA
10	ENSG00000289888	long non-coding RNA

- Enrichment analysis of differentially methylated genes highlighted two pathways: 'estrogen response early' and 'serine/threonine kinases' (Figure 2)
  - 'Serine/threonine kinases' pathway was confirmed also in the analysis with hypomethylated genes only, suggesting a possible upregulation of the genes in the pathway in PWH-4DR.

## Conclusion

- Even when HIV replication is controlled by ART, multidrug resistance is associated with a different DNAm, a proxy for different gene expression.
- DNAm might play a role in the high inflammation and disease burden observed in the fragile population with 4DR.

## References

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## Contact Information

Tommaso Clemente, MD  
Vita-Salute San Raffaele University, Milan, Italy  
Infectious Diseases, IRCCS San Raffaele Scientific Institute, Milan, Italy  
Tel: +39 0226437907; mail: [clemente.tommaso@hsr.it](mailto:clemente.tommaso@hsr.it)

## PRESTIGIO Study Group

STEERING COMMITTEE: Antonella Castagna (Coordinatore operativo), Vincenzo Spagnuolo (Coordinatore operativo), Daniele Armenia, Stefano Bonora, Leonardo Calza, Anna Maria Cattelan, Giovanni Cenderello, Adriana Cervo, Laura Comi, Antonio Di Biagio, Emanuele Focà, Roberta Gagliardini, Andrea Giacomelli, Filippo Lagi, Giulia Marchetti, Stefano Rusconi, Francesco Saladini, Maria Santoro, Maurizio Zazzi. VIROLOGY TEAM AND BIOLOGICAL BANK: Andrea Galli, Daniele Armenia, Francesco Saladini, Maria Santoro, Maurizio Zazzi. STUDY COORDINATORS: Elisabetta Carini, Sabrina Bagaglio, Girolamo Piromalli. STATISTICAL AND MONITORING TEAM: Riccardo Lolatto, Sara Diotallevi. ENROLLING CENTERS: ANCONA: Marcello Tavio, Alessandra Mataloni Paggi; BARI: Annalisa Saracino, Flavia Balena; BERGAMO: Laura Comi, Daniela Valentini, Claudia Suardi; BOLOGNA: Pierluigi Viale, Leonardo Calza, Federica Malerba, Silvia Cretella, Riccardo Riccardi; BRESCIA: Francesco Castelli, Emanuele Focà, Davide Minisci, Francesca Pennati; BUSTO ARSIZIO: Barbara Menzaghi, Maddalena Farinazzo; CATANIA: Bruno Cacopardo, Maurizio Spagnuolo, Elisabetta Carini, Sabrina Bagaglio, Riccardo Lolatto, Raddusa, Carmen Giarratana; CATANZARO: Paolo Fusco, Vincenzo Olivadese; CREMONA: Angelo Pan, Chiara Fornabaio, Paola Brambilla; FIRENZE: Alessandro Bartoloni, Filippo Lagi, Paola Corsi, Seble Tekle Kiro, Filippo Ducci, Susanna Giachè, Cecilia Costa, Alessio Bellucci, Elisa Mirabelli; FOGGIA: Teresa Santantonio, Sergio Lo Caputo, Sergio Ferrara, Arianna Narducci; GENOVA: Emanuele Pontali, Marcello Feasi, Antonio Sarà, Matteo Bassetti, Antonio Di Biagio, Sabrina Bianchi, MILANO: Antonella Castagna, Vincenzo Spagnuolo, Elisabetta Carini, Sabrina Bagaglio, Riccardo Lolatto, Andrea Galli, Sara Diotallevi, Rebecka Papaioannu, Tommaso Clemente, Girolamo Piromalli, Spinello Antinori, Andrea Giacomelli, Tiziana Formenti, Fabiola Schiavo, Giulia Marchetti, Lidia Gazzola, Fabiana Trionfo Fines, Massimo Puoti, Cristina Molit, Federico D'Amico; MODENA: Cristina Mussini, Adriana Cervo; NAPOLI: Elio Marzillo, Amadeo Lanzardo; PADOVA: Anna Maria Cattelan, Maria Mazzitelli; PALERMO: Antonio Cascio, Marcello Trizzino; PARMA: Elisa Fronti, Diletta Laccabue, Federica Carli; PAVIA: Roberto Gulminetti, Layla Pagnucco, Mattia Demitri; PERUGIA: Daniela Francisci, Giuseppe De Socio, Elisabetta Schiaroli; REGGIO EMILIA: Elisa Garlassi, Romina Corsini; ROMA: Roberta Gagliardini, Marisa Fusto, Loredana Samati, Vincenzo Malagnino, Tiziana Mulas, Mirko Compagno Carlo Torti, Simona Di Giambenedetto, Silvia Lamonica, Pierluigi Francesco Salvo; SANREMO: Giovanni Cenderello, Rachele Pincino; SIENA: Mario Tumbarello, Massimiliano Fabbiani, Francesca Panza, Ilaria Rancan; TORINO: Giovanni Di Perri, Stefano Bonora, Nicol Ferrara, Andrea Calcagno, Silvia Fantino; VERONA: Stefano Nardi, Marta Fiscon. SUPPORTED BY: ViiV Healthcare, Gilead Sciences, MSD, Janssen-Cilag