Combination of genetic, viral and environmental factors in the susceptibility to pediatric Multiple Sclerosis (Pedigree Study Group): a gene-environment risk score

M. Tosi1, A. Zollo2, F. Caushi1, A. Corona2, A. Habeeb1, N. Pomella1, N. Barizzone1, S. Pilotto3, M. Simone4, A.Protti5, A. Berardinelli6, A. Gallo7, C. Canavese8, D. Vecchio9, L. Moiola10, M. Conti11, M. Borghi12, M. Viri13, P. Annovazzi14, L. Grimaldi15, R. Lanzillo16, S. Rasia17, S. Bova18, S. Sotgiu19, M. Fronza20, Agnese Suppiej21, Giacomo Lus22, M. Trojano23, M. Amato24, E. Cocco25, R. Bergamaschi6, M. Pugliatti3, A. Ghezzi1, F. Martinelli Boneschi2,26, S. D'Alfonso1 and the PEDIGREE Study Group

1 University Of Eastern Piedmont, Department Of Health Sciences, Novara, 2 Laboratory of Precision Medicine of Neurological Diseases, "Aldo Ravelli" Center for Neurotechnology and Experimental Brain Therapeutics, Department of Health Science, University of Milan, Milan, Italy, 3 Department of Neurosciences and Rehabilitation, University of Ferrara, Ferrara, Italy, 4 Child Neuropsychiatric Unit, Department of Precision and Regenerative Medicine and Jonic Area, University 'Aldo Moro' of Bari, Bari, Italy, 5 Department of Neurology - Grande Ospedale Metropolitano Niguarda – Milan, 6 IRCCS Fondazione Mondino - IRCCS Fondazione Mondino – Pavia, 7 Department of Advanced Medical and Surgical Sciences - University of Campania Luigi Vanvitelli – Naples, 8 Department of Pediatric Neurology - Children's Hospital Regina Margherita Città Della Salute E Della Scienza Di Torino – Turin, 9 Department Of Neurology - University Hospital Maggiore Della Carità – Novara, 10 San Raffaele Hospital - San Raffaele Hospital – Milan, 11 Asst Papa Giovanni XXIII - Asst Papa Giovanni XXIII – Bergamo, 12 Fondazione Cavalieri Ottolenghi - Fondazione Cavalieri Ottolenghi – Orbassano, 13 Child Neurology And Psychiatry Unit - University Hospital Maggiore Della Carità – Novara, 14 Neuroimmunology Unit-Multiple Sclerosis Center, Hospital of Gallarate, ASST della Valle Olona, Gallarate, Italy, 15 Department of Neurology - Fondazione Istituto G. Giglio – Cefalù, 16 Department Of Neuroscience, Reproductive Sciences And Odontostomatology – University Of Naples – Naples, 17 P.O. Montichiari, Multiple Sclerosis Regional Center, department of Neurology - Asst Spedali Civili Di Brescia – Montichiari, 18 Child Neurology Unit - Buzzi Children's Hospital – Milan, 19 Department of children neuropsychiatry - University Hospital of Sassari – Sassari, 20 Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy, 21 Pediatric Clinic, University of Ferrara, Ferrara, Italy, 22 Second Division of Neurology, Department of Clinical and Experimental Medicine, University of Campania Luigi Vanvitelli, Naples, Italy, 23 Department of Translational Biomedicine and Neurosciences – DiBraiN, University of Bari "Aldo Moro", Bari, Italy, 24 Department NEUROFARBA, Section of Neurosciences, University of Florence, Florence, Italy, 25 Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy, 26 Clinical Neurology Unit, Azienda Socio-Sanitaria Territoriale Santi Paolo e Carlo and Department of Health Sciences, University of Milan, Milan, Italy

Introduction

Multiple Sclerosis (MS) is a chronic, multifactorial, inflammatory disease that causes demyelination of oligodendrocytes in the Central Nervous System. MS etiology involves both genetic (201 loci + 32 HLA loci) and environmental factors, such as viral infections, low vitamin D level, obesity and smoking. We focused on pediatric MS (PedMS), which is a rare and more aggressive form of the disease with onset <18 years, that offers a unique opportunity to gain clinical and biological data, lifestyle and environmental information in proximity to the actual disease onset.

Aim of the study

This multi-omics project aims to analyze individually and in combination genetic, epigenetic, metagenomic, viral and environmental factors to identify markers associated with PedMS pathogenesis.

Materials and Methods

Pedigree is an Italian multicentric study group composed of MS patients with a clinical event between 12 and 18 years (PedMS) and pediatric Healthy Controls (HC) matched for sex and age to PedMS.

- 1678 HC + 743 MS patients with onset < 18 years
- Genotypes for HLA-DRB1*1501, HLA-A*02 and 200 non-HLA adultMS SNPs; wPRS

Genetic Analysis (GWAS)

Microbiome Analysis (16S)

- 109 PedMS and 64 HC
- I level analyses: SmartSeq-MicrobAT software. II level and comparative analyses: MicrobiomeAnalyst

Viral Status

- 72 PedMS and 35 HC
- Vit-D level and infection by EBV, CMV, VZV and HSV 1-2
- Significative association correlated with HLA-DRB1*1501 status

Questionnaire

- 114 PedMS and 66 HC
- Evaluation of parental smoking and normal or reduced (< 4 months) breastfeeding

Methylation Analysis (EPIC array)

- 122 PedMS and 53 HCs
- Differentially methylated probes (DMP) by RnBeads automated cut-off Differentially methylated regions (DMR): DMRcate; CpG ≥7 , FDR < 0.05 and |meandiff| > 0.02

Individual omics analysis

Multi omics analysis

MOFA

- Multi Omics Factor Analysis (MOFA)
- Latent factor explaining the variance across each data modality
- Inspection of factor values, of feature weights; gene set enrichment analysis; plot of molecular signatures

Gene-Environment (G-E) risk score

- **Environmental score (1-4)**: maternal or paternal smoking (1=y), no or reduced breastfeeding (1=no), EBV and HSV 1-2 positivity (1=y)
- **Genetic score (1-3)**: HLA-DRB1*15:01 (1=y), HLA-A02 (1=no), weighted PRS (1 > 95 perc)
- **G-E risk score**: environmental score + genetic score

Results

Individual omics analysis

- 282 suggestive SNPs
- 7978 DMPs
- 5 genera

Individual statistically significative results (p < 0.05)

HLA-DRB1*15:01

OR= 4.9
Association confirmed

HLA-A02

OR= 0.4
Association confirmed

EBV

- 4 PedMS tested negative for EBV
- **OR= 3.5**
- Association between EBV infection and risk of MS confirmed

PARENTAL SMOKING

OR= 2.03
Association confirmed

NO OR REDUCED BREASTFEEDING

OR= 2.04
Association confirmed

HSV 1-2

- **OR= 5.7**
- HSV 1-2 infection is more common in PedMS than in HC

HLA-DRB1*1501 stratification

DR15pos	EBV Positive	EBV Negative	OR	95% IC
PedMS (n=13)	13	0	6,5	0.27-151.13
HC (n=2)	2	0		

Possible additive effect of EBV positivity and DRB1*15:01+ (OR= 6.5) as reported in adult MS

DR15neg	HSV Positive	HSV Negative	Pval	OR	95% IC
PedMS (n=39)	20	19	0.002	6.8	1.62-41.48
HC (n=25)	3	20			

For HSV1-2 infection, higher OR in the DR15:01-group (OR= 6.8) as reported in literature

Multi omics analysis

ENVIRONMENTAL SCORE

- 30% PedMS and 27% HC have environmental and viral data
- 82% PedMS and 73% HC were exposed to at least two risk factors, especially smoking and EBV infection
- **Environmental** score significantly **higher in PedMS** than HC (p=0.0004, mean risk score: 2.6+0.77 vs 1.63+0.89)

GENETIC SCORE

- 81% PedMS vs 66% HC have HLA-DRB1*15:01 genotype; 76% PedMS and 68% HC have HLA-A02 genotype
- 74% PedMS and 60% HC have wPRS data
- **Genetic** score significantly **higher in PedMS** than HC (p=0.001, 1.04+0.68 vs 0.65+0.56)

GENE-ENVIRONMENT RISK SCORE

- 21% PedMS and 19% HC have environmental and genetic data
- **G-E risk score** significantly **higher in PedMS** than HC (p=4.731e-05, mean G-E risk score: 3.75+ 0.77 vs 2.21+0.86)
- Parental smoking, EBV infection and absence of HLA-A02 were the most prevalent risk factors

Conclusions

- In a new Italian PedMS cohort, several omics have been individually analyzed, obtaining statistically significative results that confirmed literature findings
- PedMS are significantly positive for HSV 1-2 infection (p= 0.002), specifically in DR15:01- subjects. EBV seropositivity increases the risk of developing MS (OR= 3.5), especially in DR15:01+ subjects (OR= 6.5)
- Genetic, viral and environmental factors have been combined to obtain a preliminary G-E risk score: PedMS are more likely to carry multiple risk factors compared to HC
- Our results confirm combined genetic and environmental contributions in disease susceptibility in PedMS
- To increase the power of our study, we will enlarge PedMS cohort ("Pedigree 3" study). We will perform multiomics data integration by applying different tools (i.e.: MOFA)

Grants: FISM – Fondazione Italiana Sclerosi Multipla (grants 2022/R-Multi/013, 2024/Special-Multi/002)

Authors declare no conflict of interests