DO EBNA2 ALLELIC VARIANTS CONTRIBUTE TO THE EBV ROLE IN MULTIPLE SCLEROSIS?

Mechelli R[1,2], Ilari S[2], Buscarinu MC[3], Romano S[3], Chiara M[4], Bellucci G[3], Bigi R[3], De Robertis M[5], D'Erchia AM[5,6], Picardi E[5,6], Manzari C[5], Mansi L[5], Marnetto F[7], Valentino P[7], Bertolotto A[7], Capobianco M[7], Lorefice L[8], Cocco E[8], Castelli M[8], Marfia GA[9], Centonze D[9,10], Horner D[4], Pesole G[5,6], Ristori G[3], Salvetti M[3,10]

[1]San Raffaele Roma Open University, Via di Val Cannuta, 247, 00166, Rome, Italy
[2]IRCCS San Raffaele Roma, Via di Val Cannuta, 247, 00166, Rome, Italy
[3]Dipartimento di Neuroscienze, Salute Mentale e Organi di Senso (NESMOS) e Centro Neurologico Terapie Sperimentali (CENTERS), Sapienza Università di Roma, Azienda Ospedaliera Sant'Andrea, Via di Grottarossa, 1035, 00189, Rome, Italy
[4]Department of Biosciences, University of Milan, Via Celoria, 26, 20133 Milan, Italy.
[5]Dipartimento di Bioscienze, Biotecnologie e Biofarmaceutica, Università degli Studi di Bari «A. Moro», Via Edoardo Orabona, 70125 Bari, Italy
[6]Istituto di Biomembrane, Bioenergetica e Biotecnologie Molecolari, Consiglio Nazionale delle Ricerche, Via Giovanni Amendola 122/O - 70126 Bari, Italy
[7]Laboratorio di Neurobiologia Clinica-CRESM, Neuroscience Institute Cavalieri Ottolenghi, Regione Gonzole, 10, 10043 Orbassano (TO) Italy
[8]Dipartimento di Scienze Mediche e Sanita' Pubblica - Cittadella Universitaria di Monserrato -09042 Monserrato, Università di Cagliari, Italy
[9]Multiple Sclerosis Clinical and Research Unit, Dipartimento di Medicina dei Sistemi, Università di Roma "Tor Vergata", Italy
[10]IRCCS Istituto Neurologico Mediterraneo Neuromed, Via Ateniese, 18, 86077 Pozzilli (IS), Italy
rosella.mechelli@uniroma5.it

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system with unknown etiology. Many pieces of evidence support the involvement of both genetic and environmental factors in disease development [1; 2]. Recent findings strongly support the causal role of EBV infection in MS, although the mechanisms linking EBV infection and disease development are still unclear [3].
Our previous study and other recent findings showed that Epstein-Barr nuclear antigen 2 (EBNA2) occupy a substantial fraction of risk loci in MS and other autoimmune diseases [4-6], affecting the expression of the related genes [7]. Moreover, in an exploratory study, we showed the association between an EBNA2 allele and MS [8], hypothesizing that EBNA2 variants may affect the processes of early infection dysregulating the virus-host interactions in MS.
Our aim was to confirm the data on distribution of EBNA2 alleles in a larger and independent population of people with relapsing remitting MS (pwMS) and healthy donors (HD).
Genomic DNA was extracted from peripheral blood mononuclear cells or CD19+ cells using a commercial kit (QIAamp DNA mini kit, Qiagen). All the DNA samples were analyzed by a digital droplet PCR (ddPCR) approach using fluorescent primers/probes assays able to recognize the different EBV alleles/variants. Specifically, we used custom assays for the identification of 1.2, 1.3B, 1.3A and B95-8 EBNA2 alleles [4]. We analyzed 291 untreated pwMS and 154 age/sex matched HD. The mean age was 39,8±2,7 years for pwMS and 36,6±11,7 years for HDs; the female to male ratio was 1,9 in MS group and 2 in control group. Based on our previous work, we expected to find a percentage of undetectable samples in both groups, due to the low number of EBV-infected B cells in peripheral blood: we obtained 34% of undetectable samples in pwMS and 52% in HD (odds ratio [OR]= 0.48; 95% confidence interval [CI] = 0.3259 to 0.7151; p= 0.0004). We found the 1.2 allele in 24% of pwMS and in 9% of HD (odds ratio [OR]= 3.2; 95% confidence interval [CI] = 1.753 to 5.734; p= 0.0001) and the coexistence of two or more EBNA2 alleles with different combinations (1.2/1.3B; 1.3B/B95-8; 1.2/B95-8; 1.2/1.3/B95-8) in the 9% of pwMS and in the 2,6% of HD ([OR]= 3.7; 95% [CI] = 1.372 to 9.947; p= 0.01). No significant differences were observed in either 1.3B allele (21% in pwMS and 24% in HD), 1.3A (6% of both pwMS and HD) and B95-8 allele (5% in pwMS and 8% in HD). Our findings confirm the association between the 1.2 EBNA2 allele and the MS, reinforcing

the idea that EBV contributes to disease development. The higher level of co-infection and the lower number of undetectable samples observed in pwMS suggest a possible involvement of EBNA2 variants, with an increased permissiveness to infection and a reduced ability to keep it in check.

[1] OlssonT, BarcellosLF, AlfredssonL. 2017. Interactions Between Genetic, Lifestyle and Environmental Risk Factors for Multiple Sclerosis. Nat Rev Neurol 13:25–36.
[2] International Multiple Sclerosis Genetics Consortium (IMSGC). 2019. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. Science.365:eaav7188.
[3] Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y, Elledge SJ, Niebuhr DW, Scher AI, Munger KL, Ascherio A. 2022. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science.;375:296-301.
[4] Ricigliano VA, Handel AE, Sandve GK, Annibali V, Ristori G, Mechelli R, Cader MZ, Salvetti M. 2015. EBNA2 binds to genomic intervals associated with multiple sclerosis and overlaps with vitamin D receptor occupancy. PLoS One.;10(4):e0119605.
[5] Harley JB, Chen X, Pujato M, Miller D, Maddox A, Forney C, Magnusen AF, Lynch A, Chetal K, Yukawa M, Barski A, Salomonis N, Kaufman KM, Kottyan LC, Weirauch MT. 2018. Transcription factors operate across disease loci, with EBNA2 implicated in autoimmunity. Nat Genet. 50(5):699-707
[6] Hong T, Parameswaran S, Donmez OA, Miller D, Forney C, Lape M, Saint Just Ribeiro M, Liang J, Edsall LE, Magnusen AF, Miller W, Chepelev I, Harley JB, Zhao B, Kottyan LC, Weirauch MT. 2021. Epstein-Barr virus nuclear antigen 2 extensively rewires the human chromatin landscape at autoimmune risk loci. Genome Res.;31(12):2185–98
[7] Keane JT, Afrasiabi A, Schibeci SD, Swaminathan S, Parnell GP, Booth DR. 2021. The interaction of Epstein-Barr virus encoded transcription factor EBNA2 with multiple sclerosis risk loci is dependent on the risk genotype. EBioMedicine. 71:103572.
[8] Mechelli R, Manzari C, Policano C, Annese A, Picardi E, Umeton R, Fornasiero A, D'Erchia AM, Buscarinu MC, Agliardi C, Annibali V, Serafini B, Rosicarelli B, Romano S, Angelini DF, Ricigliano VA, Buttari F, Battistini L, Centonze D, Guerini FR, D'Alfonso S, Pesole G, Salvetti M, Ristori G. 2015. Epstein-Barr virus genetic variants are associated with multiple sclerosis Neurology. 84:1362-8.