Iron traffic gene variants in MS

Donato Gemmati *[1] Giulia Zeri,[1] Elisa Orioli,[1] Francesca De Gaetano,[1] Salvi Fabrizio,[2] Ilaria Bartolomei,[2] Sandra D'Alfonso,[3] Rosanna Asselta,[4] Claudia Dall'Osso,[3] Patricia Momigliano-Richiardi,[3] Maurizio A. Leone,[5] Ajay V. Singh,[6] Erica Menegatti,[7] Paolo Zamboni.*

[1]Center Hemostasis & Thrombosis, Department of Biomedical Sciences & Advanced Therapies, University of Ferrara, Italy; [2]Department of Neurology, Bellaria Hospital, Bologna Italy; [3]Department of Medical Sciences and IRCAD, Eastern Piedmont University, Novara, Italy; [4]Department of Biology and Genetics, University of Milan, Italy; [5]Neurologic Clinic, Ospedale Maggiore della Carità and IRCAD, Novara, Italy; [6]Department of Physics, European School of Molecular Medicine (SEMM), University of Milan, Italy; [7]Vascular Diseases Center, University of Ferrara, Italy.

ABSTRACT

Anomalous cerebrospinal venous blood flow (CCSVI; Chronic Cerebrospinal Venous Insufficiency) and iron deposition in CNS have been recently proposed as a possible causative mechanism in multiple sclerosis (MS). Iron deposits encircle the veins in the CNS lesions documented by MRI and histochemics. Thus, iron involvement/unbalancing in MS etiopathogenesis is strongly suspected. We explore in MS patients the role of single nucleotide polymorphisms (SNPs) in the main iron homeostasis genes. By Pyrosequencing technique, we investigated in 400 MS cases (Relapse Remitting (RR), n=270; Secondary Progressive (SP), n=100; Primary Progressive (PP), n=30), and in 400 healthy controls, the following five SNPs: hemochromatosis (*HFE, C282Y, H63D*), ferroportin (*FPN1, -8CG*), transferrin (*TF, P570S*), and hepcidin (*HEPC -582 AG*). The homozygous *FPN1-8GG* condition increased the MS risk in the whole group of cases (OR=4.23, CI95%, 1.8-9.8; P<0.0001), and it was slightly higher among SP subgroup (OR=4.9, CI95%, 1.7-13.8; P=0.003). Conversely, the *HEPC -582GG* homozygous condition, was mainly associated among the progressive subgroups. In details: SP had (OR=1.94, CI95%, 1.1-4.1; P=0.02), and that value even doubled computing the PP cases alone (OR=4.8, CI95%, 1.9-12.3; P=0.001). It is noteworthy, that the number of -582GG homozygotes increased considering the most severe MS clinical phenotypes (i.e. RR, 5.5%; SP, 11.0%; PP, 23.3%; P-trend=0.01). No risk associations were instead found for the

remaining polymorphisms analyzed. The Expanded Disability Status Scale (EDSS) significantly increased as the number of the -8G-alleles increased in the FPN1 genotype. Accordingly, MS progression index (PI) was significantly higher among -8GG homozygotes when compared with the rest of genotypes and it was (PI, 1.36±2.18 vs 0.47±0.8; P=0.03) in the whole group of cases and (PI, 1.18±2.8 vs 0.43±0.77; P=0.006) in the RR subgroup. No significant risk associations were instead found computing EDSS scores for both the HFE gene variants, but the 63DD homozygotes had an increased PI of about 3.2-folds in the whole group (PI, 1.5±2.62 vs 0.47±0.86; P=0.0015) and the risk quite doubled among RR patients (PI, 0.82±1.45 vs 0.47±1.0; P=NS). As the HEPC -582AG variant is considered, EDSS significantly increased as the number of the -582G-alleles increased. Accordingly, comparing homozygotes -582GG vs the rest of genotypes the scores were (EDSS, 3.5±2.83 vs 2.57±2.29: P=0.025) in the whole group and no statistical significance was obtained among RR cases. On the contrary, PI did not show significant computing. Considering the PI values, TF P570S had a subtle trend among the whole group of cases when 570SS homozygotes were compared with the remaining genotypes (PI, 1.0±1.05 vs 0.48±0.92; P=0.08). Finally, in attempt to calculate MS risks associated to the coexistence of different genotypes, we compared those cases and controls carrying a combination of at least four polymorphic alleles in at least two different genes with subjects who were wild-type for all the five variants. Combined carriers were 11.37% in patients and 5.0% in controls. The complete wild-type condition was 11.5% in cases and 17% in controls. Appreciable risk-values were obtained (OR=2.93; CI95%, 0.87-15.9; P=0.10). In the same subgroups, EDSS score increased about 1.6-fold and PI was 3.0-fold higher (P=0.03 and P=0.02 respectively). A further important finding was the recognition of a *progression-switch* within the HEPC gene. The -582AG SNP yielded a different survival rate among RR-cases. In detail, by means a retrospective observational period of ten years, those RR-patients carrying the -582G-allele had a higher chance to progress in SP phenotype (log-rank; P=0.019).

We conclude suggesting that, selected genetic variants within peculiar iron homeostasis genes, acting together and in presence of local iron overload due to or exacerbated by CCSVI, might be responsible for a suboptimal iron handling. This might account for the significative variability observed in the clinical MS phenotypes, particularly affecting MS risk and progression. If confirmed in different and wider series of MS patients, these data could have strongly useful imaging and neurotherapeutic implications.