Discovery of Novel Functional SNPs in the IFNGR2 Region Associated with Multiple Sclerosis Pathogenesis Starting from GWAS Data

Endri Visha[1] Alaa Habeeb[1] , Nicola Pomella[1] , Ferdinando Clarelli[2] , Lucia Corrado[1], Elisa Gorla[1] , Fjorilda Caushi[1] , Beatrice Piola[1] , Diego Cotella[1] , Laura Follia[1] , Gianluca Baldanzi[1] , Martina Tosi[1] , Domizia Vecchio[3] , Massimo Filippi[4] , Maurizio Leone[5] , Filippo Martinelli Boneschi[6] , Federica Esposito[7] , Nadia Barizzone[1] , Sandra D'Alfonso[8] .

1 Università Del Piemonte Orientale, Novara, Italy, 2 IRCCS San Raffaele Scientific Institute, Laboratory of Human Genetics of Neurological Disorders, Institute of Experimental Neurology, Division of Neuroscience, Milano, Italy, 3 University Hospital "Maggiore Della Carità", Department Of Neurology, Novara, Italy, 4 San Raffaele Scientific Institute, Neurology and Neurorehabilitation Unit, Neurophysiology Service, Neuroimaging Research Unit, Division of Neuroscience, Vita-Salute San Raffaele University, Milano, Italy, 5 Scientific Direction, Fondazione IRCCS Casa Sollievo della Sofferenza, San Giovanni Rotondo, Foggia, Italy, 6 ASST Santi Paolo Carlo, Department Of Health Sciences, Neurology Unit And Multiple Sclerosis Center, Milan, Italy, 7 IRCCS Ospedale San Raffaele, Laboratory of Human Genetics of Neurological Disorders, Institute of Experimental Neurology, Division of Neuroscience, Neurology and Neurorehabilitation Unit, Milano, Italy, 8 Università del Piemonte Orientale, NOVARA, Italy. Contact: endri.visha@uniupo.it

Abstract

Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system driven by complex genetic and environmental factors. GWAS have identified over 200 risk loci, mostly in noncoding regions, suggesting regulatory roles. However, linkage disequilibrium (LD) complicates the identification of causal variants, requiring integrating computational and experimental approaches. Our aim is to identify causal variants that regulate drug-targetable genes in MS by combining fine-mapping with MPRA validation. From 36 MS-associated loci near known drug targets, five high-LD regions were selected for functional analysis to uncover regulatory mechanisms and support drug repurposing. We analyzed GWAS data from an Italian cohort (4,259 MS patients, 1,644 controls), imputed with HRC r1.1. Using DGIdb, we filtered variants near drug-target genes, identifying 36 regions linked to 238 druggable genes.

Fine-mapping (PAINTOR, CAVIAR BF) and annotations (GWAVA, CADD, FINSURF, RegulomeDB) prioritized candidates, with drug-gene links validated via Open Targets.

We tested 83 high-LD SNPs ($r^2 > 0.77$) from five loci (CD40, TEC-TXK, IFNGR2, PRDX5, CHRNA9) using MPRA. Reference, alternative, and scrambled constructs were transfected into SH-SY5Y, HEK293T, and Jurkat cells; cDNA was sequenced and analyzed with MPRAlm. Significant SNPs were further assessed with MotifBreakR and MEME. Selected variants were validated by luciferase assays in Jurkat cells.The IFNGR2 locus showed consistent regulatory activity across all cell lines and in both enhancer and promoter contexts. Two variants (rs28653198, rs17880053) showed strong allele-specific expression, with significant promoter ($p = 7.08\times10^{-5}$, 1.64×10^{-4}) and enhancer effects ($p = 1.78\times10^{-3}$, 3.4×10^{-3}), respectively. TF analysis identified TGIF2 and PROX1 binding, highlighting IFNGR2 as a therapeutic target with INTERFERON GAMMA-1B as a repurposing candidate. CD40, PRDX5, and TEC also showed regulatory SNPs, but IFNGR2 stood out.

We identified causal variants in MS loci, with IFNGR2 variants showing the most consistent regulatory effects. These findings will undergo further CRISPR-Cas9 and FREP validation.

This is a promising result for MS therapeutic repurposing.