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A Bidirectional Two-Sample Cis-Mendelian Randomization in the MHC Region of Immune Responses against Epstein-Barr Virus Nuclear Antigen 1 and Multiple Sclerosis

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INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system with a complex etiology involving both genetic and environmental factors [1]. Epstein–Barr virus (EBV), a ubiquitous herpesvirus infecting over 90% of the global population, has consistently been identified as one of the strongest environmental risk factors for MS. Observational studies have shown increased MS risk following infectious mononucleosis and elevated EBV antibody titers. Proposed mechanisms include molecular mimicry and impaired immune control of latent EBV infection [2]. A notable longitudinal study in U.S. military personnel demonstrated a >30-fold increase in MS risk following EBV seroconversion [3]. However, such findings remain susceptible to residual confounding, selection bias, and reverse causation, particularly considering emerging evidence suggesting that MS-related immune dysregulation may begin years before diagnosis.

OBJECTIVES

To address limitations in observational research and strengthen causal inference, we applied two-sample Mendelian Randomization (MR) to investigate whether humoral immune IgG responses to EBV antigen EBNA-1 is causally associated with MS risk specifically considering the MHC region, where the majority of genetic signals associated with EBNA-1 antibody levels are located [4]. To address the extensive linkage disequilibrium (LD) and pleiotropy in the MHC region, we applied a cis-MR framework centered on the constrained maximum likelihood method (cis-MR-cML), a methods allowing for correlated instrumental variables (IVs) and robust to IV assumption violations [5].

METHODS

We conducted a two-sample MR analysis using summary statistics from genome-wide association studies of antibody responses to EBV antigen EBNA-1 in 8,477 white British participants from the UK Biobank, and of MS risk in 115,803 individuals of European ancestry from the International MS Genetics Consortium. SNPs were selected using GCTA-COJO with UK Biobank cohort as LD reference panel[6,7]. To account for the extensive LD in the MHC region, it was treated as a single LD block. A stepwise regression approach was used, with p-value thresholds of 5×10-3 for SNPs associated to EBNA-1 levels (Ix, set of candidate IVs) and of 5×10^{-8} for SNPs associated to MS (Iy). Then, conditional effect of each candidate IV with both EBNA-1 and MS was estimated conditioning for all the other SNPs in Ix and in Iy in LD with it (considering an $R^2 > 0.005$). This approach aims to mitigate LD-driven horizontal pleiotropy. Instrument strength and directionality were then assessed using F-statistics and Steiger filtering to obtain the final set of IV to be used in the MR analysis. To obtain estimates for causal effect, we applied cis-MR-cML [5] and, as sensitivity analyses, additional MR methods (GSMR and LDA-Egger) [8,9] for robustness checks. We also conducted reverse MR analyses to evaluate whether MS genetic susceptibility may influence EBV antibody levels, reflecting potential bidirectional or disease-driven effects.

RESULTS

GCTA-COJO identified 19 SNPs, candidate IVs, jointly associated with EBNA-1 IgG levels. After conditioning the effect of this potential IVs for the other SNPs in Ix and Iy, we obtained a set of 6 IVs with F-statistic > 10 and passed the Steiger directionality test ($p<0.05$). Cis-MR-cML identified

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and removed one invalid IVs identified as outliers, leading to a final set of five IVs with global F-statistic = 24.6 and a significant correct causal directionality (p<0.001). The MR results indicated that a 1 SD increase in genetically predicted EBNA-1 IgG levels was associated with a two-fold increase in MS risk (OR=2.42 [95%CI: 1.87;3.14], p<0.001). GSMR provided further support for this significant causal association with similar effect estimate (OR ≈ 2). LDA-Egger did not detect significant directional pleiotropy (p>0.05), and the slope estimate further supported a significant risk association. Reverse MR analysis did not find a bidirectional relationship, as the estimated association between MS and EBNA-1 levels was not statistically significant (p>0.05).

CONCLUSIONS

Our findings support a causal role of increased humoral immune responses to EBV antigen EBNA-1 in increasing MS risk. Using a robust cis-MR framework and accounting for extensive MHC region LD, we showed that genetically predicted EBNA-1 IgG levels are significantly causally associated with MS unidirectionally. These results strengthen the hypothesis that impaired immune control of EBV may be a key mechanism in MS pathogenesis and highlight the potential of EBV-targeted prevention strategies.

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