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Increased burden of rare variants in GWAS associated genes in familial multiple sclerosis

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Multiple sclerosis (MS) is an immune-mediated neurodegenerative disease affecting the central nervous system with many known genetic risk factors. Although genome-wide association studies (GWAS) have identified common genetic variants with small effects associated with MS, the role of rare variants with large effects in MS aetiology remains underexplored. We hypothesized that rare variants in MS-associated genes from GWAS studies (GWAS-associated genes) are more likely to contribute to familial MS (FMS) risk than to sporadic MS (SMS). Therefore, we aimed to assess the burden of rare, predicted pathogenic (RPP) variants in GWAS-associated genes in FMS and SMS patients compared to controls. Rare genetic variants in 111 GWAS-associated genes were assessed in 87 FMS, 89 SMS and 3866 control cases. We demonstrate that RPP variants were significantly overrepresented in the FMS cohort whereas their frequency was not increased in the SMS cohort compared to controls (p-values 5.27×10^{-74} and 1.00, respectively). Six genes (*ALPK2*, *ANKRD55*, *INTS8*, *IQCB1*, *JADE2*, and *MALT1*) significantly contributed to the burden of RPP in the FMS group. We conclude that rare variants in genes identified by GWAS might contribute to the genetic predisposition of familial MS patients.

Keywords Whole exome sequencing (WES), Multiple sclerosis, Rare variants, Rare pathological changes, Candidate genes, Burden analysis

Multiple sclerosis (MS) is a chronic, immune-mediated disease affecting the central nervous system (CNS)[1]. It is characterized by inflammation and the formation of focal areas of demyelination in the CNS known as plaques or lesions[2]. While the aetiology of MS is unclear, both environmental and genetic factors are known to contribute to MS risk[3]. Research into MS genetics has yielded significant results, and so far genome-wide association studies (GWAS) have identified over 200 common genetic variants associated with MS[4]. However, common variants detected by GWAS only explain up to approximately 50% of expected MS heritability[4]. Furthermore, while GWAS can successfully identify disease-associated common variants[5] rare variants are not typically evaluated[6].

The rare variant hypothesis suggests that a significant portion of susceptibility to relatively common chronic diseases is due to the cumulative effects of low-frequency variants of moderate effect, as opposed to a large number of common variants with a small effect size[7]. Under this model, a sizeable portion of complex disease genetic variance could be due to rare variants with an allele frequency (AF) < 1%[5]. These variants represent a significant portion of human genetic variation[8] and are predicted to have a stronger effect on various phenotypes than common variants, due to functional variants being subjected to purifying selection pressure[9]. Estimates based on heritability modelling suggested that rare and low-frequency variants in gene-coding sequences might contribute up to 5% of MS heritability[10]. First-degree relatives of MS cases are at a 15–25 times higher relative risk for developing the disease than the background population[11]. Familial MS (FMS) cases represent about 12.6% of all MS patients and tend to experience earlier worsening of conditions and increased severity of long-term disability compared to sporadic MS (SMS) cases[12]. Additionally, the distribution of disease courses differs between FMS and SMS cases. FMS cases exhibit a higher risk of relapsing-remitting MS (RRMS) compared to sporadic cases, as well as an increased likelihood of progressing from RRMS to secondary progressive MS (SPMS)[13].

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In several common and complex CNS disorders, such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, rare genetic variants have been identified as the underlying cause of monogenic forms of these conditions[14–16]. While candidate monogenic causes for MS have been proposed, the existence of monogenic causes for MS remains unresolved[17,18].

This study aimed to investigate the presence of rare, potentially pathogenic (RPP) variants in genes associated with GWAS within cohorts of FMS and SMS compared to controls. We hypothesized that the burden of RPP variants would be higher in MS patients, particularly within the FMS group.

Methods

Study participants

The study included 4042 participants of Slavic ethnic backgrounds, which included Slovenian, Croatian and Serbian ancestry. The study participants were categorized into three groups: FMS patients, SMS patients, and control subjects. Patients were diagnosed with MS in accordance with McDonald diagnostic criteria[19]. The FMS cohort consisted of 87 individuals with MS, each having at least one first-degree relative diagnosed with the condition. The SMS cohort included 89 individuals with no first-degree relatives with MS. The control cohort consisted of 3866 individuals over the age of 40 of matching ethnic backgrounds to the FMS and SMS groups. The average age in the control group was 58 years and the average age of MS patients was 37 years. The female-to-male ratio was 1.6:1 in the MS cohorts and 1:1 in the control group.

Whole exome sequencing and variant annotation

DNA was isolated from whole blood using the FUJIFILM QuickGene-610 L system with a whole peripheral blood DNA isolation kit, following the manufacturer's instructions and best practices. WES was conducted on DNA extracted from study participants' whole blood samples using Illumina HiSeq-2000 with an average 30x coverage. Sequence reads were aligned with the hg38 reference genome using Burrows-Wheeler Aligner in line with genome analysis toolkit best practices[20]. Agilent-All-Exon 2/5/6 and Illumina Nextera-Exome exome capture kits were used for NGS library preparation. For quality control, variants were filtered by read depth ($DP \geq 10$), call quality ($GQ \geq 20$) and those that passed quality control by the Genome Analysis Toolkit (GATK) predictor[21]. Gene variants were annotated with the Ensembl Variant Effect Predictor (VEP), which was used to estimate their pathogenicity[22]. We also used the LOFTEE plugin for VEP in order to assess stop-gain, frameshift and splice-disrupting variants and classify them as loss-of-function variants with high or low confidence[23]. Variants were also annotated using the Combined Annotation Dependent Depletion (CADD) tool using the CADD plugin for VEP[24]. The gnomADe database was used as a source of gene AF data[25].

Gene panel and variant selection

The gene panel was selected based on previously published GWAS studies – specifically the work of the International Multiple Sclerosis Genetics Consortium (IMSGC)[4]. 233 loci were reported as having reached GWAS-level significance in the most extensive MS GWAS to date[4]. We selected MS-associated autosomal protein-coding genes with exonic, intronic, downstream, upstream or untranslated region (UTR) variants, as described in the supplementary data of the IMSGC publication[4]. A total of 111 genes were included: 99 genes outside of the extended major histocompatibility complex (MHC) region, and 12 MHC genes.

Variants containing synonymous genetic changes were excluded from the original data. Only rare ($AF < 0.01$) variants that were predicted as pathogenic were included. Variants were predicted as pathogenic if they were determined as frameshift, splice acceptor, splice donor, stop gained, stop lost or start lost by VEP, or were predicted to be missense by VEP and had deleterious predictions by 5 prediction algorithms (SIFT, Polyphen2 HDIV, Polyphen2 HVAR, LRT, MutationTaster) as well as having a CADD score ≥ 20. Variants without a documented AF in the gnomADe database were also included and classified as rare.

Burden analysis and rare variant reporting

A burden analysis of RPP variants was conducted with the generalised linear model (GLM) using the snpStats[26] and CMGgenomics[27] packages in the R statistical environment[28]. The analysis was conducted in three iterations: the combined MS cohort, the SMS cohort and the FMS cohort were individually compared to controls. Rare variants that fit the study criteria were aggregated by genes (Fig. 1, bottom track), and compared between the MS cohorts and controls using the GLM. The burden of RPP variants was calculated for the MS cohorts. Similarly, the RPP burden analysis was also conducted for all genes included in the present study's panel (Fig. 1, top track). The threshold for significance was set as $\alpha = 0.05$ after false discovery rate (FDR) correction. Candidate variants highlighted by the analysis were additionally manually reviewed for quality of reads using the IGV browser. The study workflow diagram is presented in Fig. 1.

Results

RPP variants were significantly overrepresented in both the total MS and FMS cohort compared to the control group, whereas the SMS cohort showed no significant overrepresentation of these variants (p-value: 0.00337, p-value: 5.27×10^{-74}, p-value: 1, respectively). The results show that patients with familial MS are substantially burdened with RPP variants in GWAS-associated genes.

The results also showed considerable enrichment of RPP variants in genes. In the total MS cohort, five genes, with a total of 12 RPP variants, were considerably enriched compared to the control group: *ALPK2*, *INTS8*, *IQCB1*, *JADE2* and *MALT1*. In the FMS cohort, a substantial burden of the RPP variant in the *ANKRD55* gene was found in addition to those present in the total MS cohort. In the SMS cohort, RPP variants were considerably overrepresented in the *LIMK2* gene, with three RPP variants detected. Among the 16 RPP variants

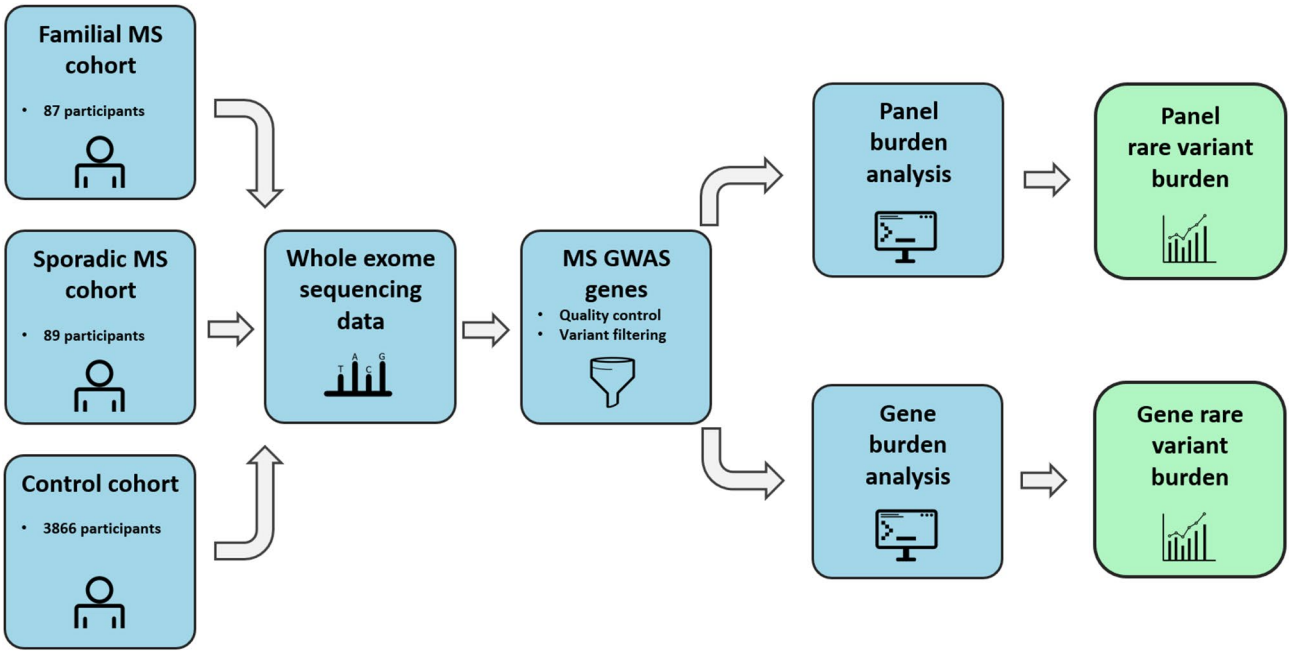

Fig. 1. Study workflow diagram. MS – multiple sclerosis. FMS – familial multiple sclerosis. SMS – sporadic multiple sclerosis. GWAS - genome-wide association study. The diagram represents a brief overview of the study workflow – the methods and results are explained in detail in their respective sections.

Gene symbol	Joint MS count Total: 176	FMS count Total: 87	SMS count Total: 89	Control count Total: 3866	Joint MS *p*-value	FMS *p*-value	SMS *p*-value
INTS8	2	2	0	3	0.000000257	4.84E-17	NS
JADE2	1	1	0	0	0.000003257	4.614E-12	NS
MALT1	1	1	0	3	0.0001133	7.213E-10	NS
IQCB1	4	3	1	6	7.456E-07	1.164E-09	NS
ALPK2	2	1	1	3	0.0005679	2.775E-08	NS
ANKRD55	1	1	0	1	NS	0.0002738	NS
LIMK2	1	0	1	5	NS	NS	0.02345

Table 1. Table of genes significantly burdened with rare, predicted pathogenic variants. The table includes the gene symbol, the counts of study participants with RPP variants in the control, joint MS, FMS and SMS cohorts, as well as the FDR-adjusted p-values for the joint MS, FMS and SMS. Values that did not reach statistical significance are labelled as NS - not significant.

in the enriched genes across all three cohort analyses combined, 10 did not have a recorded AF in the gnomADe database. The results are presented in Table 1. Detailed variant data is available in the Supplementary Materials – Table S1 contains panel burden analysis results by cohort, Table S2 contains a list of variants in burdened genes and Table S3 contains a list of case-only variants.

Discussion

In the present study we assessed the incidence of RPP variants in our MS cohorts versus controls. The findings highlighted that the FMS cohort was considerably burdened with RPP variants compared to controls; however, this variant overrepresentation was not observed in the SMS cohort. The results revealed that in the FMS cohort, RPP variants were substantially overrepresented in six genes - *ALPK2*, *ANKRD55*, *INTS8*, *IQCB1*, *JADE2* and *MALT1*. Conversely, only one gene was considerably burdened in the SMS cohort (*LIMK2*).

The results of the panel burden analysis revealed that RPP variants were significantly overrepresented in the FMS cohort (FDR-adjusted p-value: 5.27×10^{-74}), suggesting that the FMS cohort was likely the primary driver of the burden in the total MS cohort.

The burden analysis of RPP variants in GWAS-associated genes revealed that several genes were considerably encumbered in MS patients compared to controls. The involvement of genes such as *ALPK2*, *ANKRD55*, *INTS8*, *IQCB1*, *JADE* and *MALT1* in the pathogenesis MS is a subject of scientific interest due to their roles in immune regulation, inflammation, and neurological processes. While each gene may contribute independently to MS pathogenesis, there are also interconnected pathways and mechanisms involving several of these genes.

ALPK2 encodes a kinase, predicted to enable ATP binding activity[29]. While its biological function is unknown[30]ankyrin repeat domains are known to have important roles in mediating protein-protein interactions[15]. It has been suggested that *ALPK2* is involved in WNT/β-catenin signalling in zebrafish models[31]. The WNT/ β-catenin pathway has been shown to regulates the immune response in MS as well as playing a role in both myelinating and remyelinating processes[29]. Intronic single nucleotide polymorphisms (SNPs) in the ankyrin repeat domain-55 (ANKRD55) gene are linked to an increased chance of developing multiple sclerosis, rheumatoid arthritis, and other autoimmune diseases[30].

JADE2 is part of the HBO1 histone acetyltransferase complex[32]acting as a ubiquitin ligase[33] and is widely expressed in various tissues, with high levels observed in the brain, endocrine, reproductive and lymphoid tissues[34]. In murine and zebrafish models, it has been shown to affect neurogenesis, as it acts as a negative regulator of KDM1A (formerly LSD1)[33]. Interestingly, KDM1A promotes macrophage pro-inflammatory specialization by repressing catalase in response to the activation of TLR4 receptors[35]thus playing a role in inflammation – a key component of MS[36]. Similarly, *INTS8* - a subunit of the Integrator complex involved in RNA processing and transcriptional regulation, exhibits high expression in the brain and plays a crucial role in neuronal and brain development[37]. Biallelic alterations of *INTS1* and *INTS8*, both encoding subunits of the integrator complex, have been associated with neurodevelopmental disorder with cerebellar hypoplasia and spasticity (phenotype MIM number 618572)[37]. Additionally, *INTS8* has been shown to upregulate the TGF-β pathway[38]which is dysregulated in MS[39]. *IQCB1* (IQ Motif Containing B1) gene, encoding the nephrocystin protein, interacts with both the retinitis pigmentosa GTPase regulator protein and calmodulin and is involved in ciliary function (provided by RefSeq, January 2016)[29]. Additionally, *IQCB1* has been associated with immune infiltration as well as immune checkpoint mechanisms[40].

MALT1 gene (Mucosa-Associated Lymphoid Tissue Lymphoma Translocation Protein 1 gene) encodes a paracaspase and is important for lymphocyte activation[41]. It is also essential for NF-κB activation and proinflammatory gene expression[42]as well as functioning as a scaffold signalling protein[41]. Furthermore, in murine models, the administration of *MALT1* protease inhibitors reduced central nervous system demyelination, axonal damage and inflammatory cell infiltration and cytokine expression[41]. Dysregulated NF-κB signaling can lead to excessive inflammation and immune cell activation, which are hallmark features of MS pathology. Disruption of *MALT1* protease function in murine models or the presence of *MALT1* mutations in humans leads to diminished regulatory T cell (Treg) populations and the onset of progressive multiorgan inflammatory disorders[43]. Moreover, Skordos, et al. (2023) demonstrated, using *MALT1* protease-dead knock-in (Malt1-PD) mice, that *MALT1* regulates the homeostasis and function of both thymic and peripheral T cells in a T cell-intrinsic manner[44]. Finally, *MALT1* has also been associated with primary immunodeficiency-12 (phenotype MIM number 615468)[45]. Therefore, dysregulation of *MALT1* is implicated in excessive inflammation, immune cell activation, and the onset of inflammatory disorders, including those with multiorgan involvement.

A study conducted by Everest et al. (2022) found that SMS patients had a higher PRS than FMS patients[46]. While the difference in PRS scores did not remain significant after Bonferroni correction, it was speculated that this could be a result of higher rare variant loading in families with a history of MS[46]. The results of the present study indicate that RPP variants could be important contributors to MS development in FMS cases.

In conclusion, the genes *ALPK2*, *ANKRD55*, *INTS8*, *IQCB1*, *JADE* and *MALT1* represent a diverse array of molecular players implicated in the pathogenesis of multiple sclerosis (MS). Each gene contributes uniquely to various cellular processes, including immune regulation, inflammation and synaptic plasticity, all of which are relevant to MS pathology. *MALT1* stands out for its roles in immune regulation and inflammatory responses. Dysregulation of *MALT1* has been associated with excessive inflammation, immune cell activation, and an increased susceptibility to autoimmune disorders, including MS. Furthermore, *ANKRD55* and *ALPK2* while their precise functions remain elusive, are linked to pathways involved in protein-protein interactions, cellular signalling, and cytoskeletal regulation, all of which are implicated in neuronal function and synaptic plasticity, processes disrupted in MS.

While the precise mechanisms underlying the involvement of these genes in MS remain to be fully elucidated, their associations with immune dysregulation, neuroinflammation, and neuronal dysfunction position them as compelling candidates for exploring the intricate interplay of genetic and environmental factors in the development and progression of MS.

Conclusion

In this study we systematically analysed GWAS-associated genes in an FMS and SMS cohort compared to controls. We discovered an increased burden of RPP variants in MS-associated genes in FMS cases compared to SMS and controls. We identified six genes that were considerably enriched with RPP variants in the FMS group. The study provides further evidence supporting an association between rare, potentially pathogenic gene variants and MS pathology, while also identifying new compelling candidates for advancing our understanding of MS pathogenesis.

Study limitations

The FMS study cohort included only one patient per affected family, preventing us from performing a segregation analysis. The limited number of study participants could also represent a potential constraint. While the participant count was sufficient to detect the effect of rare variants with large effect size, rare variants with a smaller effect size may have gone undetected. Thus, a study on the burden of rare variants in MS with expanded cohort sizes may result in a higher detection yield. We acknowledge that common variants in linkage disequilibrium may contribute a part of the detected effect of rare variants. Furthermore, we did not conduct functional studies for the variants identified in our study.

Data availability

The datasets generated and/or analysed during the current study are available in the European Variation Archive repository, under accession number PRJEB83688.

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Author contributions
Manuscript preparation and drafting by A.T.; data analysis by A.T., P.J., A.M.; literature review by A.T., I.S.M.; study design by B.P.; patient recruitment by A.M., J.D., Š.M., I.N., N.D.Č., S.R., I.S.M., B.P. All authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Ethics statement
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee, the 1964 Helsinki Declaration and its later amendments or comparable ethical standards, the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine and the General Data Protection Regulation. Informed consent was obtained from all individual participants involved in the study. The study was approved by the Slovenian Committee for Medical Ethics (0120 -66/2024-2711-3).

Additional information
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