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Common genetic variants in the plasminogen activation pathway are not associated with multiple sclerosis

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Abstract

Matrix metalloproteinase 9 (MMP9) is involved in multiple sclerosis (MS) aetiology. Previously, we identified differential gene expression of plasminogen activation cascade genes in MS patients. Based on our gene expression results, we wanted to identify whether polymorphisms in the genes associated with the plasminogen pathway could predict MS risk. We genotyped 1153 trio families, 727 MS cases and 604 healthy controls for 17 polymorphisms in MMP9, plasminogen activator urokinase (PLAU), PLAU receptor (PLAUR) and serpin peptidase inhibitor/clade 2/member B2 (SERPINB2) genes. No associations were found between the 17 polymorphisms and MS. Also, gene expression levels were analysed according to genotype: no associations were observed. In conclusion despite the consistent evidence for the role of MMP9 and the plasminogen activation cascade in MS, we found no associations between genotype nor gene expression. This suggested there are other potentially modifiable factors influencing gene expression in MS.

Keywords

Gene expression, genotype, matrix metalloproteinase 9, multiple sclerosis, plasminogen, plasminogen activation pathway, plasminogen activator urokinase, polymorphism, serpin peptidase inhibitor

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Introduction

Multiple sclerosis (MS) is the most common neurological disorder in young adults, characterised by inflammatory demyelination within the central nervous system (CNS). An aberrant immune response results in the formation of inflammatory lesions within the CNS, causing neuronal damage and axonal loss.

We previously identified a differential gene expression profile of MS, compared to healthy controls,[1] containing four genes from the plasminogen activation cascade that show increased expression in MS, as compared to healthy controls: matrix metalloproteinase 9 (*MMP9*); plasminogen activator urokinase (*PLAU*); plasminogen activator urokinase receptor (*PLAUR*) and serpin peptidase inhibitor, clade B (ovalbumin), member 2 (*SERPINB2*). The plasminogen activation cascade is involved in the activation of MMP9. Expression of MMP9 is known to be at high levels in MS lesions, in serum and in the cerebrospinal fluid (CSF) of relapsing–remitting MS patients (RRMS).[2,3] MMP9 is involved in the breakdown of the blood-brain barrier, thereby allowing migration of T cells into the CNS. It was shown that MMP9 is required for the migration of T cells into the CNS parenchyma in experimental autoimmune encephalomyelitis (EAE), a mouse model of CNS inflammation.[4] MMP9 can also degrade myelin basic protein (MBP), a key protein in the myelin sheath that surrounds axons.

Single nucleotide polymorphisms (SNPs) may affect the expression levels of mRNA and of proteins, or cause conformational changes of the translated protein; therefore, they may have a direct effect on function, resulting in disease. To determine if there is any association with MS disease risk, we examined 17 SNPs present in four genes involved in the plas-

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Table 1. Unphased output for SNP association testing, with empirical 5% quantile *p*-value threshold = 0.004777.

Gene	SNP	Minor Allele	Trio family		Case/Control		*p* value	Odds ratio (95% CI)
			Transmitted Frequency	Untransmitted Freq.	Case Freq.	Control Freq.		
MMP-9	rs13969	A	0.383	0.390	0.361	0.386	0.124	0.935 (0.857–1.019)
MMP-9	rs2274756	A	0.147	0.149	0.141	0.149	0.553	0.946 (0.855–1.087)
MMP-9	rs13925	A	0.148	0.151	0.139	0.146	0.398	0.950 (0.842–1.071)
MMP-9	rs17576	G	0.346	0.351	0.325	0.358	0.129	0.934 (0.855–1.020)
MMP-9	rs3787268	A	0.199	0.200	0.190	0.212	0.456	0.961 (0.864–1.068)
MMP-9	rs3918253	C	0.419	0.420	0.388	0.420	0.116	0.935 (0.859–0.017)
MMP-9	rs3918241	A	0.145	0.150	0.141	0.146	0.429	0.953 (0.845–1.074)
PLAU	rs2227564	T	0.261	0.249	0.256	0.261	0.501	1.033 (0.940–1.136)
PLAU	rs2227562	A	0.147	0.138	0.147	0.142	0.363	1.057 (0.939–1.189)
PLAU	rs4065	C	0.428	0.412	0.425	0.428	0.378	1.039 (0.954–1.131)
PLAU	rs2227551	G	0.285	0.275	0.284	0.291	0.552	1.028 (0.937–1.128)
PLAU	rs2227566	C	0.436	0.421	0.438	0.433	0.274	1.048 (0.963–1.139)
PLAUR	rs4760	G	0.141	0.162	0.165	0.170	0.434	0.955 (0.851–1.072)
PLAUR	rs344787	A	0.459	0.489	0.489	0.492	0.381	0.963 (0.887–1.047)
PLAUR	rs2302524	C	0.171	0.172	0.163	0.157	0.679	0.977 (0.873–1.092)
PLAUR	rs4251854	C	0.128	0.126	0.118	0.129	0.625	0.970 (0.856–1.098)
SERPINB2	rs6104	G	0.222	0.215	0.197	0.207	0.500	0.965 (0.870–1.071)

MMP9: Matrix metalloproteinase 9; PLAU: plasminogen activator urokinase; PLAUR: PLAU receptor; SERPINB2: serpin peptidase inhibitor, clade B, member 2; SNP: single nucleotide polymorphism.

minogen activation cascade pathway, seven genes involved in *MMP9*, five in *PLAU*, four in *PLAUR* and one in *SERPINB2*.

Methods

We used 1153 trio families, 727 MS cases and 604 healthy controls from the UK and Australia for this study, as was previously described.[5] All MS patients fulfilled the diagnostic McDonald criteria for MS affection status.[6] Informed written consent was obtained from all individuals. Study controls recruited in Australia self-reported no MS nor any family history of MS. The study was approved by the ethics committees of the University of Cambridge and Hunter New England Health Service.

Common variants present within *MMP9, PLAU, PLAUR* and *SERPINB2* were selected for analysis (Table 1) based on a minor allele frequency of > 0.05 and the likelihood of tagging large regions of linkage disequilibrium (LD) present within the genes (based on the HAPMAP project data), and the possibility to impact the gene due to location within the gene.

We performed genotyping using an Applied Biosystems (Foster City, CA) platform, utilising TaqMan SNP genotyping assays, an ABI 7900 HT real-time detection system and SDS 2.1 software, as per the manufacturer's instructions. We repeated the genotyping of 277 and 94 samples from the UK and Australian cohorts, respectively, as a quality control measure to ensure genotype accuracy.

We removed individuals from the analysis if they failed genotyping for > 50% of SNPs. In the trio family dataset, all three individuals were removed from the analysis if any individual within the trio was not successfully genotyped for > 50% of SNPs. Also, we tested trio family data for Mendelian errors, using PedCheck. Statistical analysis was performed using 'Unphased' version 3.1.4., testing for allelic association with 1000 permutations, to control for multiple testing correction. We investigated the association in males and females separately (with trio families separated according to the sex of the affected child).

The level of gene expression, as measured previously by whole-genome gene expression analysis,[1] was tested for correlation with genotype in a subset of samples included in both the gene expression and genotyping studies. These were: 17 MS patients without disease-modifying therapy, 11 MS patient on interferon-beta therapy and 37 healthy controls. Statistics were performed in GraphPad Prism, using the Mann Whitney or Kruskal Wallis test for association, depending on the number of groups (genotypes).

Results and discussion

We examined > 4500 samples, to test for a MS disease association of 17 common variations, across four genes: *MMP9, PLAU, PLAUR* and *SERPINB2*. A total of 1124 trio families and 556 MS cases/473 healthy controls (UK); plus 147 MS cases/122 healthy controls (Australia), passed the quality control measures and thus, were included in this analysis.

We found no association between allele frequency and disease risk for any SNP investigated (Table 1). We identified no associations based on the sex of the affected individual (data not shown). Furthermore, none of the genes demonstrated a correlation between expression level and their respective SNP genotypes (data not shown); however, this is based on only a small sample population and therefore the statistical power of this test to detect an association was low. Although there is no association identified with clinical course in the largest genome wide association study (GWAS) to date,[7] it would be of interest to investigate these SNPs in a population composed of only RRMS cases, due to the investigation of gene expression in RRMS cases only. Unfortunately, the clinical characteristics of the individuals were not available; therefore, this could not be investigated.

High levels of MMP9 in serum, CSF and lesions were previously associated with MS, and we previously identified increased *MMP9* mRNA expression in MS; however, here we demonstrated that this increased expression of *MMP9* and plasminogen activation pathway genes is not a consequence of the variants tested in this study.

None of the variants investigated in this study are reported to be associated with MS in GWAS performed to date.[8] Of the 17 polymorphisms, only rs2227564 in PLAU was previously reported to be associated with inflammatory bowel disease, in any GWAS.[8,9]

In conclusion, the SNPs investigated in this study showed no association with MS disease risk and did not alter the expression levels of their respective genes. It would be of interest to examine other regulators of MMP9, such as tissue inhibitor of metaloproteinases (TIMPs) for genetic association with disease and MMP9 levels; however, as this gene is not found to be differentially expressed in a previous study[1], it was not investigated in this study. Further studies will be required to investigate if, for example, epigenetic changes like methylation, miRNA or histone modification are responsible for the altered expression.

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Conflict of interest statement

The author declares that there is no conflict of interest.

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