The Effect of Single Nucleotide Polymorphisms from Genome Wide Association Studies in Multiple Sclerosis on Gene Expression

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Abstract

Background: Multiple sclerosis (MS) is a complex neurological disorder. Its aetiology involves both environmental and genetic factors. Recent genome-wide association studies have identified a number of single nucleotide polymorphisms (SNPs) associated with susceptibility to MS. We investigated whether these genetic variations were associated with alteration in gene expression.

Methods/Principal Findings: We used a database of mRNA expression and genetic variation derived from immortalised peripheral lymphocytes to investigate polymorphisms associated with MS for correlation with gene expression. Several SNPs were found to be associated with changes in expression: in particular two with HLA-DQA1, HLA-DQA2, HLA-DQB1, HLA-DRB1, HLA-DRB4 and HLA-DRB5, one with ZFP57, one with CD58, two with IL7 and FAM164A, and one with FAM119B, TSFM and KUB3. We found minimal cross-over with a recent whole genome expression study in MS patients.

Discussion: We have shown that many susceptibility loci in MS are associated with changes in expression using an unbiased expression database. Several of these findings suggest novel gene candidates underlying the effects of MS-associated genetic variation.

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Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system characterised by demyelination and axonal loss.[1] Studies conducted in mono- and dizygotic twin pairs and siblings have shown that genetics plays a role in MS susceptibility.[2] Linkage was effective in identifying the locus exerting the single strongest genetic effect in MS, namely, the human leukocyte antigen (HLA) class II region.[3] The risk associated with this region has since been shown to be determined by epistatic interactions between different HLA alleles.[4] and is thought to be responsible for approximately 50% of the genetic risk of MS.[5] Beyond this powerful determinant of MS genetic susceptibility, research has taken considerably longer to bear useful fruit. Finally, after the genotyping of hundreds of thousands of single nucleotide polymorphisms (SNPs) in many thousands of MS patients and controls, we are beginning to establish a network of loci outside of the HLA region involved in determining MS susceptibility.[6,7,8,9,10,11,12,13,14,15,16,17] It is worth considering that even the most strongly associated of these with MS is still a significantly weaker determinant of MS susceptibility than HLA alleles. For some of these loci, functional studies have been undertaken.[11,18,19] However, such studies are rarely carried out in an unbiased manner since these generally correlate genetic variations with the expression of a candidate gene. A recent study of mRNA levels in MS patients and healthy controls showed a great multitude of differentially expressed genes; however it is uncertain to what extent this reflects the aetiology of disease as opposed to the disease process or adaptive biological pathways.[20] A recent investigation has performed whole genome expression analysis in lymphoblastoid cell lines (LCLs) from healthy volunteers who were also genotyped for a large number of SNPs.[21] We used the data from this study to examine the effects of current susceptibility loci in MS on gene expression.

Methods

Gene expression analysis

This was carried out as described in Dixon et al.[21] Briefly, peripheral lymphocytes were transformed using Epstein-Barr virus before being cultured, pelleted and frozen for storage. cDNA
templates were created using the One-Cycle cDNA Synthesis Kit (Affymetrix). In vitro transcription of cDNA was performed using the IVT Labeling Kit (Affymetrix) and, after hybridisation on U133 Plus 2.0 GeneChips (Affymetrix), this was scanned using a high-resolution scanner (Affymetrix). Whole-genome genotyping was carried out according to manufacturers’ instructions using the Sentrix Human-1 Genotyping BeadChip and the HumanHap300 Genotyping BeadChip. The analysis of expression was carried out on the publically available database of mRNA by SNP Browser 1.0 as described.[21]

mRNA by SNP analysis
We investigated the mRNAs significantly altered in expression by the SNPs reported in the literature to be at or close to genome-wide significance.[6,7,8,9,10,11,12,13,14,15,16,17] If the susceptibility SNP was not available on the database, we used the SNP with the strongest linkage disequilibrium (LD) with the susceptibility SNP as provided by SNP Browser 1.0 based on r². For SNPs where no proxy was provided, we investigated all genotyped SNPs within 500 kb for LD with r²≥0.4 for a suitable proxy SNP. We also assessed the degree of LD with potentially interesting SNPs within 500 kb of the original susceptibility SNP. Finally we assessed the SNPs associated with expression of putative candidate genes to ensure that we did not miss any important associations with expression.

Results

SNP selection
We chose to look at a set of 38 SNPs which were the top loci to reach genome-wide significance selected from currently reported genome wide association studies (GWAS) of which 14 had been independently replicated in 2 studies. 17 of these were not present in the genome-wide association mRNA expression library and so when possible proxy SNPs in strong-to-moderate LD were used instead. The SNPs and proxy SNPs used are detailed in Table 1.

mRNA expression
13 of the MS susceptibility SNPs or proxy SNPs were associated with changes in mRNA expression (Table S1). Two SNPs in strong LD with multiple MS-associated SNPs in the HLA region were related to expression of various HLA alleles, including HLA-DQA1, HLA-DQB1, HLA-DRB1, HLA-DRB4 and HLA-DRB5. One SNP in the HLA class I region was associated with altered expression of ZFP57. Both SNPs in CD58 were associated with expression of CD58. A SNP in the IL7 region was associated with expression of mRNA encoding IL7 and FAM164A. Three SNPs in the region of METTL1-CYP27B1-CDK4 altered the expression of several genes: FAM119B, TSFM and KUB3. The common gene of altered expression for all three SNPs was TSFM.

Overlap with previous mRNA expression studies
We used the supplemental data supplied by Gandhi and colleagues to examine cross-over between the results obtained in that study and the genes we identified as being altered in expression by susceptibility SNPs.[20] Only three genes were in common between the two sets: HLA-DQB1, HLA-DRB1 and STAT3. HLA-DRB1 was upregulated in MS, relapsing-remitting MS (RRMS) and secondary progressive MS compared with healthy controls. HLA-DQB1 expression was reduced in MS and RRMS compared with healthy controls. STAT3 was reduced in primary progressive MS compared with healthy controls.

Table 1. SNPs and proxy SNPs analysed.

| SNP        | putative gene association | proxy SNP | r²  |
|------------|---------------------------|-----------|-----|
| rs1054283  | IL7                       |           |     |
| rs10876994 | METTL1, CYP27B1, CDK4     | rs10083154| 0.70067|
| rs1132200  | TME39A                    |           |     |
| rs11554159 | IFI30                     | rs874628  | 0.949|
| rs11808092 | EVIS-RPL5                 |           |     |
| rs11865121 | CLEC16A                   | rs2041670 | 1    |
| rs12122721 | KIF21B                    |           |     |
| rs12368653 | METTL1, CYP27B1, CDK4     |           |     |
| rs12708716 | CLEC16A                   | rs725613  | 1    |
| rs12722489 | IL2RA                     | rs12722561| 0.9349|
| rs1335532  | CDS8                      | rs6677309 | 1    |
| rs1569723  | CD40                      |           |     |
| rs17445836 | IRF8                      |           |     |
| rs17824933 | CD6                       | rs2237997 | 0.43718|
| rs1800693  | TNFRSF1A                  |           |     |
| rs2051322  | CD226                     |           |     |
| rs2104286  | IL2RA                     | rs12722561| 0.548|
| rs2300747  | CDS8                      | rs6677309 | 1    |
| rs2523393  | HLA class I               | rs2394160 | 0.96552|
| rs2587156  | IL7                       |           |     |
| rs3129860  | HLA class II              | rs9271366 | 0.95557|
| rs3129934  | C6orf10, BTN2L2, NOTCH4   | rs9267992 | 0.95604|
| rs3135388  | HLA class II              | rs9271366 | 0.95699|
| rs3536443  | TYK2                      | No proxy available | |
| rs4149584  | TNFRSF1A                  | No proxy available | |
| rs441349   | SOCS1                     | rs1646042 | 1    |
| rs6074022  | CD40                      | rs6074022 | 0.70337|
| rs6604026  | EVIS-RPL5                 |           |     |
| rs6860438  | C7                        |           |     |
| rs6897932  | IL7R                      |           |     |
| rs703842   | METTL1, CYP27B1, CDK4     |           |     |
| rs7404554  | CLEC16A                   |           |     |
| rs744166   | STAT3                     |           |     |
| rs763361   | CD226                     |           |     |
| rs8118449  | TYK2                      | No proxy available | |
| rs9271366  | HLA class II              | rs11554159| 0.95557|
| rs9523762  | GPC5                      |           |     |

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Discussion
Our findings show that some, but by no means all, susceptibility SNPs in MS are associated with changes in gene expression. Some of these (CD58, had already been noted by previous investigators.[11] We were unable to find supporting evidence in this dataset for the previously reported allelic effect of the susceptibility SNP in IL7R on expression of the gene.[18] Similarly, SNPs in the IL2RA gene did not correlate with expression of IL2RA mRNA, despite previously finding altered levels of this in MS patients relative to controls.[19]

[1] Gandhi S, et al. (2008) Nature 456:919-923.
We also found several novel effects of susceptibility SNPs. Two SNPs in tight LD with susceptibility SNPs in the HLA region correlated with expression of several HLA class II mRNAs. However, measuring gene expression in the HLA is a complex task. There is haplotype specificity for some genes (HLA-DRB4 and HLA-DRB5) and thus we are not sure whether differential expression of HLA genes measured by microarray reflects different probe affinity for different HLA class II alleles and thus further work is needed to fully understand this association. Our identification that a SNP in the HLA class I region was associated with altered expression of ZFP237 is an interesting observation as this gene has been linked with DNA methylation changes across the genome resulting in transient neonatal diabetes. There is some epidemiological evidence that MS may be partly determined by epigenetic alterations and this would be an ideal candidate functionally linking MS to the epigenome. A SNP in IL7 recently confirmed as associated with MS was shown to correlate functionally linking MS to the epigenome. A SNP in IL7 and FAM164A. Naturally the most compelling candidate of these is IL7 due to its probable role in autoimmunity. However, the advantage of an unbiased screen is that it raises the possibility of candidate genes that would otherwise not be considered. This is especially so since the SNP is far more strongly associated with FAM164A expression than with IL7. FAM164A is a hypothetical protein encoded in the reverse direction to IL7 and its functional importance is largely unknown. The susceptibility region on chromosome 12 was previously linked with the expression of FAM119B. We feel that the relationship of all three major susceptibility SNPs with the expression of TSEM suggests this as a strong candidate. This is a plausible candidate in terms of function too as it is involved in the translation of mitochondrial proteins, providing a potential link with other susceptibility genes linked to mitochondrial function, such as KIF21B. Further functional work will be needed to better assess these candidates.

The limited cross-over between known and suspected susceptibility genes in the whole genome expression analysis of Gandhi and colleagues is likely due to a number of differences including the use of whole blood mRNA and individuals with established disease in the Gandhi study. It is possible that future whole genome analyses of expression conducted using RNA-seq in cell-sorted samples of patients with very early disease may reveal alterations in the level of susceptibility gene mRNA.

The advantage of an unbiased approach to linking the expression of genes with genetic variation associated with disease susceptibility is that there is no a priori hypothesis to blind investigators to the presence of other genes. There are several limitations to the approach we used. The mRNA screen was conducted in transformed LCLs and so it would not be informative about tissue-specific gene expression. Also, SNP coverage across the genome was not complete and so the functional effects of some SNPs for which no proxy was available will be concealed. Furthermore, despite using expression data from 400 LCLs, we may have been underpowered to detect relevant effects. However, our finding of several novel associations between MS SNPs and gene expression is worthy of further investigation and also raises the hypothesis that some disease associated SNPs may not exert their effects on MS susceptibility through simple effects on gene expression.

**Supporting Information**

**Table S1** Changes in mRNA expression associated with susceptibility SNPs.

**Author Contributions**

Conceived and designed the experiments: AEH SVR. Analyzed the data: AEH LH AJB CTW JMM SVR. Wrote the paper: AEH LH AJB CTW JMM SVR.
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