Genetic Variant rs755622 Regulates Expression of the Multiple Sclerosis Severity Modifier D-Dopachrome Tautomerase in a Sex-Specific Way

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Multiple sclerosis (MS) is a sex-specific autoimmune disease involving central nervous system. Previous studies determined that macrophage migration inhibitory factor (MIF) and its homologue D-dopachrome tautomerase (DDT) sex-specifically affect MS progression. Moreover, other studies reported that rs755622 polymorphism in promoter region of MIF gene is associated with risk of MS and affects the promoter activity to regulate MIF expression in a sex-specific way. Given that MIF and DDT share a part of promoter sequence, we surmise that rs755622 can also regulate DDT expression in a sex-specific way. However, this has not yet been studied. Here, we used five large-scale expression quantitative trait loci (eQTLs) and two RNA-seq datasets from brain and blood to assess the potential influence of rs755622 variant on expression of DDT in different genders by the linear regression and differential expression analysis. The results show that the minor allele frequency of rs755622 and expression of DDT are significantly increased in males for MS subjects and this minor allele variant can significantly upregulate DDT expression for males but not females, which suggests that the regulation of DDT expression level by rs755622 can affect MS progression in males. These findings further support and expand conclusions of previous studies and may help to better understand the mechanisms of MS.

1. Introduction

Multiple sclerosis is one of the most common immune-mediated diseases involving central nervous system and presents the sex-specific rates of morbidity [1–3]. According to the report provided by Atlas of MS in 2013, the number of individuals affected by MS is about 2.3 million [4]. Previous Genome-wide association studies (GWAS) uncovered that about 200 variants in the human genome are associated with MS and they mainly affect immunological process related genes and lie in their regulatory regions [5, 6].

An immunoregulatory cytokine, macrophage migration inhibitory factor (MIF), plays an important role in the modulation of macrophages and microglia immune response and is associated with autoimmune and inflammation-related diseases including MS [7–11]. D-dopachrome tautomerase (DDT, also called MIF-2) is highly homologous to MIF and has a similar structure and functions as it [12–14]. Moreover, the promoter regions of MIF and DDT gene share sequences which contain some transcription factor binding sites [13, 14]. A recent study found that DDT is a sex-specific disease modifier for MS, and its high expression can promote MS progression in males but not females [15].

The single nucleotide polymorphism (SNP) rs755622 (G > C) lies in the promoter region of MIF, and previous studies showed that it is significantly associated with some immune-mediated diseases including MS [16, 17]. And then, this recent study further indicated a significant sex difference in the association between rs755622 polymorphism and MS [15]. Moreover, previous studies determined that rs755622 polymorphism affects the activity of MIF promoter and regulates MIF gene expression [18–20]. Given the relationship between
MIF and DDT, we surmise that rs755622 polymorphism can also affect the expression of DDT. However, it is still unclear whether and how rs755622 polymorphism regulates DDT expression and the resulting impact on MS in different genders.

Evidence showed that regulating gene expression is an important class of the biological functions of the genetic variants [21–30], and the expression quantitative trait loci (eQTL) analysis is an effective method to discover the correlations between genetic variants and quantitative changes in gene expression [21, 22, 24, 25, 27, 31–33]. Therefore, in this study, we first selected five large-scale expression quantitative trait loci (eQTLs) datasets to assess the potential influence of rs755622 variant on expression of DDT in normal brain tissues and blood by a linear regression analysis. And then, we further investigated how the rs755622 polymorphism affects DDT expression in brain and blood of the MS patients using two RNA-seq datasets. Finally, we performed the differential expression analysis of DDT between genders and explored whether there is a sex-specific regulation of DDT expression level by the rs755622 polymorphism.

2. Methods

2.1. eQTL Analysis Using Five Large-Scale Datasets. To validate the effect of rs755622 polymorphism on DDT expression level, we selected five large-scale eQTL datasets which primarily consist of the European ancestry individuals without MS diagnosis [34–38]. In particular, 10 brain regions (cerebellar cortex, frontal cortex, hippocampus, medulla, occipital cortex, putamen, substantia nigra, temporal cortex, thalamus, and intralobular white matter) of 134 individuals are included in Brainac [34]; 13 brain regions (amygdala, anterior cingulate cortex, caudate, cerebellar hemisphere, cerebellum, cortex, frontal cortex, hippocampus, hypothalamus, nucleus accumbens, putamen, spinal cord, and substantia nigra) of 1,497 individuals are included in GTEx [35]; the blood of 369, 2,765, 2,116, and 5,257 individuals is included in GTEx [35], CAGE [36], BIOS QTL Browser [37], and FHS_eQTL [38], respectively. Then, we selected the rs755622 genotype and DDT expression data from Brainac and conducted the eQTL analysis to assess the influence of rs755622 variant on expression of DDT by the R package ‘Matrix eQTL’, which is based on a linear regression model with the parameters, gender and age, as the covariates [39]. Finally, we analyzed the eQTL results for rs755622 (including beta and P values) from the other four datasets.

2.2. eQTL Analysis of MS Subjects Using the RNA-Seq Data. To further investigate the regulation of DDT expression by rs755622 polymorphism in MS patients, we first selected the RNA-seq data from 2 GEO datasets, GSE100297 (single-end reads produced by the Illumina HiSeq 3000) [40], and GSE66573 (paired-end reads produced by the Illumina HiSeq 2500) [41], which include the brain (optic chiasm) and blood samples of 5 and 6 MS patients, respectively. We next mapped these sequences to human reference genome (hg19) and calculated the transcript per million (TPM) values to measure DDT expression level in each of the MS patients using the Kallisto software, which is a quantification tool of transcript abundance based on RNA-seq data [42]. Then, these RNA-seq data were reused to detect the genotype of rs755622 polymorphism in each of the MS patients. This process includes the following: (1) the sequence reads are aligned to reference genome (hg19) using BWA software with the default parameter settings [43] and (2) SNPs are called on these aligned reads using SAMtools software with the default parameter settings (100 read depth) [44]. For the called genotype of rs755622 polymorphism, a Hardy–Weinberg Equilibrium (HWE) test based on a noncontinuity correction chi-squared method with the significance level $P < 0.05$ was performed using the R package ‘Genetics’ (https://cran.r-project.org/web/packages/genetics/index.html). Finally, we used the DDT expression and rs755622 polymorphism genotyping data of these MS patients to conduct the eQTL analysis by the R package ‘Matrix eQTL’ [39] as described in the previous step.

2.3. Differential Expression Analysis of DDT between Genders. According to a recent study, Benedek et al. observed that DDT expression level is significantly higher in brain (white matter) of male MS subjects compared with female MS subjects [15]. However, previous studies reported that the sex-specific expression has already existed in many human genes, including some MS-related genes, for the normal individuals [45, 46]. Therefore, we further explored whether the sex-specific expression of DDT is a general genetic model for healthy people or associated with MS. Brainac database provided the DDT expression data of 10 brain regions from 134 individuals free of known neurological diseases with the age and age and gender details [34]. We used these data to detect if there is significantly different expression of DDT between genders in the normal individuals by Student's t-test (the significance level was set at $P < 0.05$).

2.4. Effect of rs755622 Polymorphism on DDT Expression Level in Different Gender. To further explore whether the effect of rs755622 polymorphism on DDT expression level has a sex-specific pattern, we selected the rs755622 polymorphism genotype data of 99 male and 35 female subjects from Brainac database [34], respectively. The HWE test of rs755622 polymorphism in male and female groups was performed, respectively, using the R package ‘Genetics’ as described in the previous step. And then, we used Fisher’s exact test to compare the genotype frequencies of rs755622 minor allele variant (C) between genders by the R program (http://www.r-project.org/). Finally, in combination with the DDT expression data in the 10 brain regions, we performed the eQTL analysis to assess the influence of rs755622 variant on expression of DDT in males and females, respectively, by the R package ‘Matrix eQTL’ [39].

3. Results

3.1. eQTL Analysis Using Five Large-Scale Datasets. Using the large-scale eQTL data from Brainac, GTEx, CAGE, BIOS QTL Browser, and FHS_eQTL, we validated the effect of rs755622 polymorphism on DDT expression level.
Table 1: Polymorphism rs755622 C allele upregulates DDT expression in brain and blood for subjects without MS diagnosis.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Effect allele</th>
<th>P value</th>
<th>Effect size (β)</th>
<th>Tissues</th>
<th>Sample size</th>
<th>Dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.47E-02*</td>
<td>0.1282</td>
<td>Brain intralobular white matter</td>
<td>134</td>
<td>Braineac</td>
</tr>
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<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>3.97E-02*</td>
<td>0.1013</td>
<td>Brain hippocampus</td>
<td>134</td>
<td>Braineac</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>3.16E-01</td>
<td>-0.0436</td>
<td>Brain medulla</td>
<td>134</td>
<td>Braineac</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>4.27E-01</td>
<td>-0.0432</td>
<td>Brain occipital cortex</td>
<td>134</td>
<td>Braineac</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>9.63E-01</td>
<td>-0.0027</td>
<td>Brain putamen</td>
<td>134</td>
<td>Braineac</td>
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<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>2.09E-01</td>
<td>-0.0630</td>
<td>Brain substantia nigra</td>
<td>134</td>
<td>Braineac</td>
</tr>
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<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>6.74E-01</td>
<td>0.0219</td>
<td>Brain anterior cingulate cortex</td>
<td>109</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>3.61E-01</td>
<td>0.1453</td>
<td>Brain amygdala</td>
<td>88</td>
<td>GTEx</td>
</tr>
<tr>
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<td>rs755622</td>
<td>C</td>
<td>8.55E-02</td>
<td>0.1732</td>
<td>Brain caudate</td>
<td>144</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.36E-01</td>
<td>0.1241</td>
<td>Brain cortex</td>
<td>136</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.61E-01</td>
<td>0.1553</td>
<td>Brain frontal cortex</td>
<td>118</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>7.21E-01</td>
<td>0.0383</td>
<td>Brain hypothalamus</td>
<td>111</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>2.84E-01</td>
<td>0.1136</td>
<td>Brain nucleus accumbens</td>
<td>130</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>7.33E-01</td>
<td>0.0417</td>
<td>Brain putamen</td>
<td>111</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.24E-01</td>
<td>0.2706</td>
<td>Brain spinal cord</td>
<td>83</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>7.01E-01</td>
<td>0.0553</td>
<td>Brain substantia nigra</td>
<td>80</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.21E-04*</td>
<td>0.5072</td>
<td>Brain cerebellum</td>
<td>154</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.21E-03*</td>
<td>0.3480</td>
<td>Brain cerebellar hemisphere</td>
<td>125</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>2.91E-02*</td>
<td>0.2388</td>
<td>Brain hypothalamus</td>
<td>108</td>
<td>GTEx</td>
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<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>4.62E-01*</td>
<td>0.2260</td>
<td>Blood</td>
<td>369</td>
<td>GTEx</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>3.70E-01*</td>
<td>0.4214</td>
<td>Blood</td>
<td>2,765</td>
<td>CAGE</td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>4.31E-28 &gt; 0 (z=10.989)</td>
<td>Blood</td>
<td>2,116</td>
<td>BIOS QTL Browser</td>
<td></td>
</tr>
<tr>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>5.21E-16*</td>
<td>0.0427</td>
<td>Blood</td>
<td>5,257</td>
<td>FHS-eQTL</td>
</tr>
</tbody>
</table>

The rs755622 position (hg19), 22_24223692_G>C (G>C); the threshold of significant association is 0.05; β > 0 and β < 0 means that this effect allele upregulates and downregulates gene expression, respectively; β = z × SE (standard errors); the asterisk (∗) means a significant association.

Interestingly, we found a significant association between rs755622 and DDT expression in brain (intralobular white matter, hippocampus, cerebellum, cerebellar hemisphere, and hypothalamus) and blood when the significance level was set at P < 0.05. In addition, we further found that the minor allele variant (C) of rs755622 can significantly upregulate DDT expression (β > 0) in all of these tissues. More detailed information is described in Table 1.

3.2. eQTL Analysis of MS Subjects Using the RNA-Seq Data. According to the Ensembl database, there are 7 transcripts for the gene DDT (ENST00000398344.8, ENST00000350608.7, ENST00000404092.5, ENST00000430101.2, ENST0000043754.7, ENST00000428792.1, and ENST000004449472.2) [47]. We downloaded the sequences of these transcripts as reference and calculated their TPM values in the MS patients from 2 GEO datasets using the RNA-seq data and Kallisto software. We found that the expression levels of transcripts ENST000004350608.7 and ENST000004449472.2 are generally higher comparing with the other transcripts. These results are described in Supplementary Table S1 and Table S2.

After the genotype calling of rs755622 polymorphism using these RNA-seq data, we found that there are 2 and 1 minor allele variants (C) of rs755622 in 2 MS patients from GSE100297 and GSE66573, respectively, and in other individuals no variant was detected at this polymorphism (Supplementary Table S3). And then, by setting the significance level at P < 0.05, we further observed that the genotype distribution of rs755622 polymorphism in the 2 groups of MS patients did not deviate from HWE (Supplementary Table S4).

Next, we performed an eQTL analysis using expression level data of the 7 transcripts for gene DDT in combination with genotyping data of rs755622 polymorphism. The results show that the minor allele variant (C) of rs755622 can significantly upregulate the expression of transcript ENST000004449472 in blood and transcript ENST00000428792.1 both in brain (optic chiasm) and blood (P < 0.05 and β > 0), which is in accordance with the eQTL results of the individuals without MS as described in the previous step. More detailed information is summarized in Table 2.

3.3. Differential Expression Analysis of DDT between Genders. To explore the association between sex-specific expression of DDT and MS, we compared the DDT expression levels


Table 2: Polymorphism rs755622 C allele upregulates DDT expression in brain and blood for MS patients.

<table>
<thead>
<tr>
<th>Transcript ID</th>
<th>Gene</th>
<th>SNP</th>
<th>Effect allele</th>
<th>P value</th>
<th>Effect size (β)</th>
<th>Tissues</th>
<th>GEO dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENST00000428792.1</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>2.23E-308</td>
<td>6.72E+03</td>
<td>Brain optic chiasm</td>
<td>GSE66573</td>
</tr>
<tr>
<td>ENST00000428792.1</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>2.91E-15</td>
<td>3.60E+01</td>
<td>Blood</td>
<td>GSE66573</td>
</tr>
<tr>
<td>ENST000004449472</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.62E-02</td>
<td>1.23E+05</td>
<td>Blood</td>
<td>GSE66573</td>
</tr>
</tbody>
</table>

The rs755622 position (hg19), 22,24236392, G>C, (G>C); the threshold of significant association is 0.05; β > 0 and β < 0 means that this effect allele upregulates and downregulates gene expression, respectively.

4. Discussion

Both of MIF and DDT, which are highly homologous with each other, play an important role in the modulation of macrophages and microglia immune response [7, 8, 11–14]. Previous studies showed that both the MIF expression level and the variant of the rs755622 polymorphism in its promoter region are associated with MS progression [9, 10, 16, 17]. Moreover, other studies indicated that rs755622 polymorphism can regulate the expression of MIF gene by affecting the activity of its promoter [18–20]. Recently, Benedek et al. further found that DDT is a sex-specific disease modifier for MS [15]. Given the relationship between MIF and DDT, we surmise that rs755622 polymorphism may also sex-specifically affect the expression of DDT in MS.

We first assessed the influence of rs755622 variant on the expression level of DDT in the normal subjects using five large-scale eQTL datasets [34–38]. And then, we further used two RNA-seq datasets to genotype the rs755622 polymorphism and performed the eQTL analysis to assess this effect in MS patients. Interestingly, the results show that the minor allele variant (C) of rs755622 can significantly upregulate DDT expression level (P < 0.05 and β > 0) in brain tissues (intralobar white matter, hippocampus, cerebellum, cerebellar hemisphere, hypothalamus, and optic chiasm) and blood both for normal and MS subjects.

The differential expression analysis shows that there is no significant difference in the expression level of DDT between the healthy male and female subjects. In combination with the results of Benedek et al’s study [15], we further determined that the DDT is highly expressed in MS brain tissues and promotes MS progression for males but not females.

Finally, by the eQTL analysis on male and female subjects using Brainac data [34], respectively, we found that
the minor allele variant (C) of rs755622 can significantly upregulate DDT expression for males but not females. In addition, we also found that the distribution of the rs755622 polymorphism genotype is not significantly different between genders for healthy subjects, but the frequency of its minor allele variant (C) is significantly higher in male MS subjects comparing with females and closely associated with the high expression level of DDT in male MS subjects. These results indicate that the regulation of DDT expression level by the rs755622 polymorphism can affect MS progression in males, which further supports and expands the findings in previous studies [12–17] and may help to better understand the mechanisms of MS.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

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Supplementary Materials
Table S1: the expression levels of 7 transcripts for the gene DDT in MS patients from GSE100297. Table S2: the expression levels of 7 transcripts for the gene DDT in MS patients from GSE100297. Table S3: the genotype of rs755622 polymorphism in MS patients from 2 GEO dataset. Table S4: the results of HWE test for genotype distribution of rs755622 polymorphism in different groups. Table S5: the genotype distribution of rs755622 polymorphism between gender in MS and healthy subjects. (Supplementary Materials)

References

Table 3: Regulation of rs755622 polymorphism to DDT expression level in different gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Gene</th>
<th>SNP</th>
<th>Effect allele</th>
<th>P value</th>
<th>Effect size (β)</th>
<th>Tissues</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female+Male</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>2.80E-02</td>
<td>1.29E-01</td>
<td>Brain hippocampus</td>
<td>122</td>
</tr>
<tr>
<td>Female</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>1.05E-01</td>
<td>1.96E-01</td>
<td>Brain hippocampus</td>
<td>30</td>
</tr>
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<td>Male</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>4.41E-02</td>
<td>1.40E-01</td>
<td>Brain hippocampus</td>
<td>92</td>
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<td>Female+Male</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>2.26E-02</td>
<td>1.24E-01</td>
<td>Brain intralobular white matter</td>
<td>131</td>
</tr>
<tr>
<td>Female</td>
<td>DDT</td>
<td>rs755622</td>
<td>C</td>
<td>3.14E-01</td>
<td>1.28E-01</td>
<td>Brain intralobular white matter</td>
<td>34</td>
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<td>Male</td>
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<td>rs755622</td>
<td>C</td>
<td>2.03E-02</td>
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<td>Brain intralobular white matter</td>
<td>97</td>
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</table>

Significant associations (P < 0.05) marked in bold. The rs755622 position (hg19), 22:24236392_G, C, b37 (G > C); the threshold of significant association is 0.05; β > 0 and β < 0 means that this effect allele up-regulates and down-regulates gene expression, respectively.


