Genetic susceptibility to multiple sclerosis in
the Shanghai Chinese is not linked to the
myelin basic protein gene microsatellite

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Abstract

Aim—To investigate the role of myelin
basic protein (MBP) gene polymorphisms
in determining susceptibility to multiple
sclerosis in a Shanghai Chinese popula-
tion.

Methods—Forty seven unrelated patients
with multiple sclerosis and 94 healthy con-
trol subjects were included in the study.
Genomic DNA was extracted from peri-
pheral blood lymphocytes and amplified
using the polymerase chain reaction to
characterise two adjacent tetranucleotide
repeats ([ATGG]_n and [TGGG]_n) located
5' to exon 1 of the MBP gene.

Results—Two polymorphic loci were iden-
tified: locus A, comprising both repeats,
and locus B, comprising the [ATGG]_n re-
peat only. Nine allelic variants were iden-
tified at locus A and six at locus B, ranging
from 212 to 244 and 122 to 146 base pairs,
respectively. The 244 base pair allele at
locus A has not been reported before. The
allele frequencies observed in the controls
differed from those seen in normal white
populations.

Conclusions—The present study dem-
onstrates a race specific pattern of allelic
distribution in the tetranucleotide re-
peat of the MBP gene. Further studies are
needed to fully elucidate the role of the
MBP gene in inherited susceptibility to
multiple sclerosis.

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Keywords: Multiple sclerosis, Shanghai Chinese, myelin
basic protein gene, polymorphism.

Multiple sclerosis is a chronic inflammatory
disease of the central nervous system, char-
terised by destruction of the myelin sheath
and gliosis. Both environmental and genetic
factors are implicated in disease susceptibility,
although the precise identity of these com-
ponents is unknown. Inherited predisposition
to multiple sclerosis has been mapped to the
HLA complex located on chromosome 6. In
north European Caucasian populations the
disease is strongly associated with alleles of
the DRB1*1501.DQA1*0102.DQB1*0602 haplo-
type. Segregation studies in multiplex families
with multiple sclerosis, however, suggest that
non-HLA genes may also play a significant role
in disease development.1

Multiple sclerosis is a rare disease in the
Chinese, with an approximate prevalence of
0.88 per 10^5 population.2 Previous in-
vestigations of disease susceptibility de-
terminants in this race have demonstrated no
consistent association between multiple scler-
osis and HLA-DR or -DQ alleles.23 The non-
HLA loci may play a more important role in
the aetiology of the disease in the Chinese.
This ethnic group may therefore be valuable
in the investigation of non-HLA encoded sus-
ceptibility.

The aetiology of multiple sclerosis is un-
known but it may involve an autoimmune re-
sponse with myelin basic protein (MBP) as a
putative target antigen. This concept is based
on similarities between multiple sclerosis and
experimental allergic encephalomyelitis, which
can be induced in rodents by immunisation
with MBP or transfer of MBP specific T cells.4
Polymorphisms of a tetranucleotide repeat re-
gion, located 5' to the MBP gene on chro-
mosome 18, have been implicated in sus-
cceptibility to multiple sclerosis in Canadian
and Finnish populations.5,6 The Finnish study6
supported genetic linkage with this locus in
familial disease. In both cases, however, a low
resolution technique was used to type this com-
plex polymorphism and it is possible that
different allelic variants were assigned the same
fragment size. An improved method has been
reported, permitting the analysis of two perfect
tetranucleotide repeat motifs at the 5' end of
the complex repeat.7 We have used this tech-
nique to investigate the role of MBP gene
polymorphisms in determining susceptibility
to multiple sclerosis in a Shanghai Chinese
population.

Methods

Forty seven unrelated patients with multiple
sclerosis and 94 healthy control subjects were
recruited from Shanghai and the neighbouring
provinces of Zhejiang and Jiangsu. All the
subjects were of Han nationality. Genomic DNA
was prepared from peripheral blood lympho-
cytes from all the subjects. The primers de-
scribed by Polymeropoulos et al7 were used to
amplify (using the polymerase chain reaction)
two adjacent perfect tetranucleotide repeats
([ATGG]_n and [TGGG]_n) located 5' to exon
1 of the MBP gene. The forward primer was
5'-labelled with 32P using T4 polynucleotide
kinase. The amplified products were separated
on a 6% denaturing acrylamide gel at 40 watts
for three hours, along with radiolabelled size
markers and a DNA sequencing ladder pre-
pared using the TA cloning kit (Invitrogen,
Abingdon, UK). The alleles were detected by autoradiography.

Results

Two polymorphic loci were identified: locus A, comprised of both repeat motifs, and locus B, representing the [ATGG]_12 repeat only. Nine allelic variants were distinguished at locus A and six at locus B, ranging in size from 212 to 244 base pairs and 122 to 146 base pairs, respectively. The 244 base pair allele at locus A has not been reported previously. The distribution of these alleles among the Chinese patients with multiple sclerosis and control subjects is presented in tables 1 and 2. The allele frequencies observed in the control group differed significantly from those reported previously in normal white populations (p<0.001 for each locus based on an overall \( \chi^2 \) test). There was no significant association between multiple sclerosis and any of the alleles detected in our Chinese subjects.

Discussion

The present study demonstrates a race specific pattern of allelic distribution within the tetranucleotide repeat region of the MBP gene. None of the polymorphisms correlated with susceptibility to multiple sclerosis in the Chinese. This supports the findings of Graham et al., who demonstrated no association of multiple sclerosis with these alleles in a population of sporadic patients from Northern Ireland. Recent studies of multiplex families with multiple sclerosis have also concluded that there is no genetic linkage between the disease and the MBP alleles. Our data do not support the studies of the Finnish and Canadian populations, which implicated the MBP locus in genetic predisposition to multiple sclerosis. It is possible that the associations observed in these studies reflect population specific disease markers. Alternatively, the associations may be secondary to polymorphisms lying outside the two loci investigated in the present study. The method of Polymeropoulos et al. enabled us to investigate a 250 base pair portion of the total 994 base pair complex repeat. It is possible that polymorphisms exist within the remaining 740 base pair region and these may account for the associations observed between the MBP microsatellite and multiple sclerosis. Further characterisation of the polymorphisms present in the whole tetranucleotide repeat region is necessary to verify the disease associations observed in these populations and to fully elucidate the role of the MBP gene in inherited susceptibility to multiple sclerosis.

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Table 1 Allele frequencies for the polymorphic A locus of the MBP gene in Chinese patients with multiple sclerosis and control subjects

<table>
<thead>
<tr>
<th>Allele (bp)</th>
<th>Multiple sclerosis patients (N=92) n(%)</th>
<th>Control subjects (N=184) n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>212</td>
<td>11 (12.0)</td>
<td>17 (9.2)</td>
</tr>
<tr>
<td>216</td>
<td>7 (7.6)</td>
<td>5 (2.7)</td>
</tr>
<tr>
<td>220</td>
<td>21 (22.8)</td>
<td>30 (16.3)</td>
</tr>
<tr>
<td>224</td>
<td>4 (4.3)</td>
<td>15 (8.2)</td>
</tr>
<tr>
<td>228</td>
<td>8 (8.8)</td>
<td>21 (11.4)</td>
</tr>
<tr>
<td>232</td>
<td>25 (27.2)</td>
<td>47 (25.5)</td>
</tr>
<tr>
<td>236</td>
<td>10 (10.9)</td>
<td>34 (18.5)</td>
</tr>
<tr>
<td>240</td>
<td>9 (9.4)</td>
<td>15 (8.2)</td>
</tr>
<tr>
<td>244</td>
<td>1 ( 1.2)</td>
<td>0 ( 0)</td>
</tr>
</tbody>
</table>

Overall \( \chi^2 \) for locus A = 11.87, DF = 8, p>0.1; bp = base pairs.

Table 2 Allele frequencies for the polymorphic B locus of the MBP gene in Chinese patients with multiple sclerosis and control subjects

<table>
<thead>
<tr>
<th>Allele (bp)</th>
<th>Multiple sclerosis patients (n=94) n(%)</th>
<th>Control subjects (n=184) n(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>122</td>
<td>27 (28.7)</td>
<td>34 (18.5)</td>
</tr>
<tr>
<td>126</td>
<td>0 ( 0)</td>
<td>0 ( 0)</td>
</tr>
<tr>
<td>130</td>
<td>11 (11.7)</td>
<td>19 (10.3)</td>
</tr>
<tr>
<td>134</td>
<td>11 (11.7)</td>
<td>31 (16.8)</td>
</tr>
<tr>
<td>138</td>
<td>35 (37.2)</td>
<td>70 (36.0)</td>
</tr>
<tr>
<td>142</td>
<td>8 ( 8.5)</td>
<td>27 (14.7)</td>
</tr>
<tr>
<td>146</td>
<td>2 ( 2.1)</td>
<td>2 ( 1.1)</td>
</tr>
</tbody>
</table>

Overall \( \chi^2 \) for locus B = 6.74, DF = 6, p>0.1; bp = base pairs.