**Author's response to reviews**

**Title:** CD58 polymorphisms associated with the risk of neuromyelitis optica in a Korean population

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**Author's response to reviews:** see over
Dear Editor,

Thank you very much for giving us the opportunity to submit a revised version of our manuscript. We are grateful to the reviewers for their constructive comments and have revised the text in the areas suggested (changes in the manuscript are highlighted in blue font). Our responses are given below.

We hope that our responses and the associated changes to the manuscript adequately address the reviewer’s comments, and that the manuscript will now be acceptable for publication.

With my best regards,

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Reviewer 1’s report

There are only a few grammatical errors to correct throughout the manuscript.

Thank you for your comment. In order to correct grammatical errors, a natural English speaker has edited the manuscript thoroughly. Corrected errors are marked in the manuscript.

Reviewer 2's report

The authors performed a case-control study aimed at investigating whether six polymorphisms of the CD58 gene are associated with Neuromyelitis Optica (NMO). They genotyped 99 patients and 237 controls from Korea, perform haplotype analyses, and found significant differences in allele and genotype distribution between the two groups, suggesting an association with the disease. Furthermore, the authors deduced the functional background of the association between rs2300747 and rs1016140 polymorphisms with NMO. CD58 gene was investigated in Multiple Sclerosis as a susceptibility gene, but this study presents first investigation in NMO and there is a rationale for studying it. Therefore, this paper adds an useful contribution to the NMO association literature.

However, there are some areas in which the manuscript may be improved (minor essential revisions):

ABSTRACT:

In Abstract some language corrections could be made (page 2, line 11,14,16.)

Page 2, line 5: insert „multiple sclerosis (MS)” instead of „MS“;

Page 2, line 4: „analysis“ instead of „analyses“

Thank you for the suggestions. We have changed the abstract section accordingly, and also fixed other grammatical errors as follows:

“In a previous genome-wide association study, cluster of differentiation 58 (CD58) region was found to be susceptible for the risk of multiple sclerosis (MS) in Caucasian, and the association between CD58 variants and MS was replicated in Americans.”

“Logistic regression analysis was conducted to find a possible association between CD58 polymorphisms and NMO.”

“The analysis results showed that 6 variations~”

“Based on previous studies, we suspect that the A allele of rs2300747 may decrease CD58 RNA expression, thus increasing NMO risk. Also, we deduced that the G allele of rs1016140 caused an increase of T cell activity, which in turn eased the access of AQP4 antibody into CNS and ultimately leading to NMO development.”

INTRODUCTION:
Introduction is correctly written and concise.

Page 3, line 5: In MS symptoms could be added also disturbance of vision such as double vision.

Thank you for the suggestion. We have changed the sentence as follows:

“In MS, demyelination causes symptoms such as a loss of sensitivity, hypoesthesia, parenthesis, disturbance of vision such as double vision, and muscle weakness.”

METHODS:

NMO is, like MS, inflammatory demyelinating disease which is more frequent in women than in man, therefore, for further studies, it would be desirable to well-matched control group by gender (Table 1. Characteristics of study subjects). Therefore, in this paper, the authors adjusted gender using logistic model.

Hardy-Weinberg equilibrium could be mentioned in chapter Statistics.

Thank you for the suggestion. We have added following sentence describing Hardy-Weinberg equilibrium in the Methods section:

“P-values for Hardy-Weinberg equilibrium (HWE) were also calculated using the HaploView software.”

Page 5, line 3: insert longitudinally extensive transverse myelitis

Page 6, line 1: “healthy controls” instead of “normal controls subjects”

Page 6, line 3: In Supplementary Table 1. instead of “Primer” and instead of “Probe” should be written “Primer/probe”

Thank you for pointing out the mistakes. We have corrected them as follows:

In order to study biologically homogenous population, all the patients showed both optic neuritis and longitudinally extensive transverse myelitis following the revised diagnostic criteria for NMO

Then, the selected SNPs were genotyped in 99 NMO cases and 237 healthy controls using TaqMan assay on the ABI prism 7900HT sequence detection system (Applied Biosystems, USA)

Supplementary Table 1. Primer/probe information of CD58 SNPs

<table>
<thead>
<tr>
<th>Loci</th>
<th>Primer/probe sequence or Assay-by-design ID*</th>
<th>Methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17426456</td>
<td>Forward GAACTTAGGGCTGCTTGTGG</td>
<td>TaqMan assay by design</td>
</tr>
</tbody>
</table>
Reverse CGTCTCTGATCGGCAACC
VIC GTCCTCAGCGTG
FAM GTCCTCGGCGTG

rs2300747 C__15755405_10 TaqMan assay
rs1335532 C__8700717_10
rs12044852 C__31433800_10
rs1016140 C__26629015_10
rs12025416 C__31433762_10

*TaqMan assay IDs from Applied Biosystems, Foster City, CA, USA.

RESULTS:

Did the authors take into account the fact that the polymorphism rs17426456 is not in HWE for statistical analysis?

In the initial SNP selection process, we chose rs17426456 for the study because it causes a missense mutation S15G, despite its low MAFs. However, since the number of participants in our study was not large, a relatively small change in the minor homozygotes shifted HWE quite large. Although the SNP did not show any significance with NMO, we acknowledge that this could be a weakness in our study. Therefore, we included following sentences in the Discussion section:

“First, number of patients and controls enrolled in the study was relatively small, due to the rarity of the disease. This might have caused the low P-value of HWE for rs17426456.”

Figure legend 1, line 8: whole study subjects number is 336, not “(n=415)”

Thank you for pointing out the mistake. Furthermore, we have found that although we conducted the analysis with 98 NMO patients (not 99) and 237 normal controls (for a total of 335), throughout the manuscript we used 99 instead of 98. We are sorry about this mistake, and corrected them. There was no change in logistic analysis result, since the analysis was conducted with 335 samples to begin with.

Supplementary Table 2: The table could be better organized. It is correct to show genotype frequencies of CD58 gene in Korean population that including 237 healthy controls instead of patients and healthy cases together (N=336).

We have modified the Supplementary Table 2 as following to include genotype information of total, case, and control:
<table>
<thead>
<tr>
<th>Loci</th>
<th>Location</th>
<th>Allele change</th>
<th>Heterozygosity</th>
<th>Genotype</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs17426456</td>
<td>Exon1</td>
<td>A&gt;G</td>
<td>0.094</td>
<td>Total</td>
<td>305 25 4 0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>89 7 1 0.069</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>216 18 3 0.001</td>
</tr>
<tr>
<td>rs2300747</td>
<td>Intron1</td>
<td>G&gt;A</td>
<td>0.477</td>
<td>Total</td>
<td>126 155 54 0.583</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>27 47 24 0.693</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>99 108 30 0.948</td>
</tr>
<tr>
<td>rs1335532</td>
<td>Intron1</td>
<td>C&gt;T</td>
<td>0.484</td>
<td>Total</td>
<td>118 159 58 0.724</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>22 51 25 0.679</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>96 108 33 0.766</td>
</tr>
<tr>
<td>rs12044852</td>
<td>Intron1</td>
<td>A&gt;C</td>
<td>0.493</td>
<td>Total</td>
<td>107 159 67 0.571</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>21 46 29 0.732</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>86 113 38 0.931</td>
</tr>
<tr>
<td>rs1016140</td>
<td>Intron3</td>
<td>T&gt;G</td>
<td>0.491</td>
<td>Total</td>
<td>109 161 65 0.688</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>21 50 27 0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>88 111 38 0.762</td>
</tr>
<tr>
<td>rs12025416</td>
<td>3'-UTR</td>
<td>C&gt;T</td>
<td>0.448</td>
<td>Total</td>
<td>145 150 38 0.933</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Case</td>
<td>30 54 12 0.104</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>115 96 26 0.381</td>
</tr>
</tbody>
</table>

DISCUSSION

Discussion is rather relevant. However, in this chapter should be reported and discussed some limitation of the study such as a relatively small number of patients and differences in gender distribution between patients and controls.

Thank you for your input. We have added following sentences in the discussion section regarding the limitation of the study:

“Although our study reports a potential association between CD58 polymorphisms and NMO, some limitations are present which should be addressed in the future. First, number of patients and controls enrolled in the study was relatively small, due to the rarity of the disease. Second, there was a disparity in the gender ratio, as there were far more female subjects than male subjects in the study. However, it has been reported that NMO is approximately 3 to 5 times more common in women than men [31]. In addition, association analysis was adjusted for gender to accommodate for this disparity. Lastly, functional study would be required to examine the actual effect of CD58 SNPs.”