Author’s response to reviews

Title: Association of BAK1 single nucleotide polymorphism with a risk for dengue hemorrhagic fever

Authors:

Tran Ngoc Dang (ngocdangytcc@gmail.com)
Izumi Naka (izumin-tky@umin.ac.jp)
Areerat Sa-Ngasang (areerat.sa@dmsc.mail.go.th)
Surapee Anantapreecha (surapee.a@hotmail.com)
Nuanjun Wichukchinda (wichukchinda@gmail.com)
Pathom Sawanpanyalert (pathoms@fda.moph.go.th)
Jintana Patarapotikul (jintana.pat@mahidol.ac.th)
Naoyuki Tsuchiya (tsuchiya-tky@umin.net)
Jun Ohashi (juno-tky@umin.ac.jp)

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Author’s response to reviews:

May 26, 2016
Dr. Tim Sands
BMC Medical Genetics

Dear Dr. Sands,

MGTC-D-15-00048:
Association of BAK1 single nucleotide polymorphism with a risk for dengue hemorrhagic fever

Tran Ngoc Dang; Izumi Naka; Areerat Sa-Ngasang; Surapee Anantapreecha; Nuanjun Wichukchinda; Pathom Sawanpanyalert; Jintana Patarapotikul; Naoyuki Tsuchiya; Jun Ohashi
Thank you for your decision letter on the above-mentioned manuscript. We acknowledge the time and effort that you and two reviewers have put into assessing our manuscript. We have revised a manuscript in accordance to comments raised by two reviewers, and the revised points are highlighted by yellow. Please find our point-by-point responses to them below.

Reviewer comments:

Reviewer #1:

There are several articles related to the association of SNPs with DHF have been published (e.g. Xavier-Carvalho C et al., 2013; Wang L et al., 2011). The possible confounding of other SNPs in the role of G allele of rs5745568 in BAK1 in the severity of dengue should be discussed before making the conclusion.

Our reply:

We thank this suggestion. We should have considered the possible confounding factors more carefully. Wang L et al. (2011) showed that the AG or GG genotype at a CD209 promoter SNP (2336 A/G; rs4804803) was significantly associated with susceptibility to DHF. Xavier-Carvalho et al. (2013) reported that GG genotype of CD209 promoter SNP was significantly associated with protection against severe dengue. As for the association of CD209 SNP with severe dengue, these two studies provided the contradictory results. Sakuntabhai et al. (2005) first reported that the G allele of CD209 promoter SNP (-336 A/G; rs4804803) was associated with strong protection against DF in three independent cohorts from Thailand.

To examine if a CD209 promoter SNP (-336 A/G; rs4804803) affects the association of BAK1 SNP with DHF, we have genotyped our dengue patients for rs4804803 in the revised manuscript. Single point analyses showed that rs4804803 was not associated with DHF in Chi-square test and logistic regression analysis (revised Table 2). A logistic regression analysis adjusted for genotype of rs4804803 as a possible confounding factor was performed for rs5745568. The results revealed that the BAK1 SNP was associated with DHF independent of rs4804803 [P = 0.0167 and adjusted OR (95% CI) = 1.29 (1.05-1.58)]. To mention this, in the revised manuscript, a new sentence “In addition, rs4804803 in CD209 was also genotyped to examine the possible interaction with rs5745568 in BAK1.” has been inserted into the subsection “Genotyping” of “METHODS”, and a new paragraph “Several studies have shown significant..."
association of a CD209 promoter SNP (rs4804803) with DF or DHF [17-19]. To examine if a CD209 promoter SNP affects the association of BAK1 SNP with DHF, we analyzed dengue patients for rs4804803. The rs4804803 SNP was not significantly associated with DHF in this study (Table 2). A logistic regression analysis adjusted for age, sex, hospital, immune status, virus serotype, and genotype of rs4804803 revealed that rs5745568 was significantly associated with DHF [P = 0.0167 and adjusted OR (95% CI) = 1.29 (1.05-1.58)]. Thus, we conclude that the association of rs5745568 with DHF is independent of rs4804803.” has been inserted into the second paragraph of “Results and Discussion”.

In addition, two sentences “In addition, rs4804803 in CD209, that has been reported to be associated with dengue infection, was also genotyped to examine if rs4804803 affects the association detected in this study” and “The result was not influenced by rs4804803 [P = 0.0167 and adjusted OR (95% CI) = 1.29 (1.05-1.58)]. No other SNPs including rs4804803 showed significant association” have been inserted into the revised ABSTRACT.

Accordingly, the following papers have been cited in the revised manuscript:


Reviewer #2:

1. Since the SNPs studied are previously associated with thrombocytopenia, are these SNPs associated with thrombocytopenia in dengue also???? The authors can compare cases with and without thrombocytopenia. Moreover, since the paper suggests the low-level constitutive production of platelets caused by the G allele of rs5745568 may increase the risk of bleeding in dengue infection, the authors should also compare whether it is true that G allele is associated with low platelet counts in dengue cases. They should compare the median platelet counts between patients with different genotypes of rs5745568. The platelet count data will be available for all the patient and lowest platelet count available for each patient during the disease time can be considered for such comparisons.

Our reply:

We greatly thank this comment. We should have done as reviewer #2 recommends. However, unfortunately, we have only the presence/absence data of thrombocytopenia (platelet count of less than 100,000 per mm3) for most of patients (platelet count data are available for a part of patients), and our samples were unlikable anonymized (platelet count cannot be obtained from the clinical data anymore). Because we do not have the quantity data of platelet count, we cannot compare the median platelet counts between dengue patients with different genotypes of rs5745568. Because thrombocytopenia is one of characteristics required for the diagnosis of DHF in the WHO 1997 criteria, all the DHF patients in the present study have thrombocytopenia. Thus, we expect that SNPs associated with DHF also shows significant association with thrombocytopenia. Accordingly, we cannot examine the possible association of rs5745568 with thrombocytopenia in an appropriate manner.

We are sorry that we cannot make a proper reply to comment 1 of reviewer #2. So we would like to draw reader’s attention to the drawback of the present study. In the revised manuscript, the second paragraph of the original manuscript has been changed from “The G allele of rs5745568 is associated with low platelet count in healthy subjects [12]. Although the association of the G allele of rs5745568 with low platelet count remains to be clarified in patients with dengue in future studies, the low-level constitutive production of platelets caused by the G allele of rs5745568 may increase the risk of bleeding in dengue infection” to the third paragraph of the revised manuscript “Although the direct association of rs5745568-G with low platelet count in patients with dengue was not examined in this study, rs5745568-G has been reported to be associated with low platelet count in healthy subjects [12]. Together with our results, the low-level constitutive production of platelets caused by the G allele of rs5745568 may increase the risk of bleeding in dengue infection. To fully understand the biological significance of rs5745568
in the pathogenesis of dengue infection, the difference in the time course of the platelet count among dengue patients with different genotypes requires to be studied in future.”.

2. Secondary dengue infections have been shown to be associated with dengue disease severity. Whether the association reported is influenced by immune status.

Our reply:

We greatly appreciate this suggestion. In revised Table 1 of the revised manuscript, information on immune status has been presented. In the revised manuscript, all the logistic regression analyses have adjusted the immune status (i.e., primary or secondary), and the results have been shown in revised Table 2. The BAK1 SNP was significantly associated with DHF even after the immune status was adjusted [P = 0.016 and adjusted OR (95% CI) = 1.29 (1.05-1.58)].

3. Please provide 95% confidence intervals for odds ratio.

Our reply:

95% confidence intervals for odds ratio have been provided in text and Table 2 of the revised manuscript.

4. To control possible confounding factors, such as age, sex, hospital (i.e., geographic region), and virus serotype, the authors have performed logistic regression analysis with the number of risk alleles (i.e., 0, 1, and 2) being used as an independent variable. Since the number of alleles actually represent genotypes, the results should also be represented in terms of genotypic association. The association of G/G genotype with DHF is more evident than other genotypes. The association seems to be under a recessive model of association.

Our reply:

We greatly thank this comment. As reviewer #2 suggested, recessive model for rs5745568 showed more significant association with DHF than dominant model as shown in the below table. To mention this, a sentence “A further analysis based on the genotype revealed that the GG genotype of rs5745568 significantly increased a risk for DHG compared to GT and TT [P =
0.003 and crude OR (95% CI) = 1.50 (1.15-1.95)], while GG and GT did not significantly increase a risk compared to TT [P = 0.279 and crude OR (95% CI) = 1.26 (0.83-1.93)], indicating that a recessive model best explains the association of the G allele of rs5745568 with DHF.” has been inserted into the first paragraph of “Results and Discussion”.

Table. Genotypic models for the SNP rs5745568 in BKA1

<table>
<thead>
<tr>
<th>Model</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dominant Model</td>
<td>P = 0.279 and OR (95% CI) = 1.26 (0.83-1.93)</td>
<td></td>
</tr>
<tr>
<td>Recessive Model</td>
<td>P = 0.003 and OR (95% CI) = 1.50 (1.15–1.95)</td>
<td></td>
</tr>
</tbody>
</table>

In a logistic regression analysis, a recessive model, where TT or TG was coded as 0, and GG as 1, also showed the significant association with DHF [P = 0.003 and adjusted OR (95% CI) = 1.52 (1.54-3.30)].

Thanks to reviewers’ comments, we think that our manuscript has been greatly improved. To acknowledge their helpful comments, a sentence “The authors are grateful to Dr Chuan-Liang Kao and Dr Kalichamy Alagarasu for their valuable comments and suggestions that improved the manuscript.” has been inserted into the “Acknowledgements”.

If we might not have replied well enough, please let us know it. We would like to revise our manuscript again.

Addresses for correspondence to:

Dr. Jun Ohashi,
Graduate School of Science
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
e-mail: juno-tky@umin.ac.jp
Yours sincerely,

Jun Ohashi