Author's response to reviews

Title: The C allele of the JAK2 rs4495487 is an additional candidate locus that contributes to myeloproliferative neoplasm predisposition in the Japanese population

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Author's response to reviews: see over
Dear Editor-in-Chief,

BMC Medical Genetics

I am sending herewith the revised manuscript entitled “The C allele of JAK2 rs4495487 is an additional candidate locus that contributes to myeloproliferative neoplasm predisposition in the Japanese population.” We found the reviewer’s comments to be most helpful, and we revised the manuscript accordingly. Here we provide point-by-point replies to the reviewer’s comments.

I hope that the revised manuscript is now suitable for publication.

Yours sincerely,

Junko Ohyashiki

We found the reviewer’s comments to be most helpful, and we changed the manuscript accordingly. First, we added several important references that were not included in the original version. Second, clinical data were reanalyzed using multivariate analysis, which proved to be very helpful in this study. Finally, in the Discussion section, we added some comments about how our findings differ from those of previous reports.

**Introduction (Background)**

Comment 1: The Authors have not cited the study by Olcaydu et al. “The role of the Jak2 GGCC haplotype and the TET2 gene in familial myeloproliferative neoplasms.” *Haematologica* 2011; 96; 367-74.

Reply: We added this important reference (Page 5, line 17~ Page 6, line 4, Reference no.12)

Comment 2: Moreover, recently, Amy V. Jones et al published on *Blood* 2010 115: 4517-4523 a study performed on patients enrolled in PT-1 trial.

Reply: We also added this reference (Page 6, line 4~5, Reference no.13).
Methods

Comment 1: Patients: It is not specified if the clinical data are recorded at diagnosis
Reply: In the current study, we studied 138 constitutive Japanese MPN patients. No familial MPN was included in the current study, because no patient experienced familial MPN. At diagnosis, however, the classification was reviewed according to the latest WHO classification, especially in patients with PV. Those PV patients who did not satisfy the latest WHO classification were excluded from the current study. We tried to clarify this point in the text (Page 6, line 13~Page 7, line 1, Methods section).

Comment 2: Patients: 9 PMF were included into the study. No data on degree of bone marrow fibrosis are provided. It would be interesting to know this variable to try to correlate this data with the presence of the Jak2 haplotype.
Reply: Unfortunately, we could not statistically analyze this question because of the small number of PMF patients in this single-institution study. We are trying to expand our research in a multi-centric study and collect more Japanese patient samples to clarify the genotype and clinical manifestations, including the degree of fibrosis. In the revised version, results of genotypic analysis are included in Table 3 (which was originally Table 2), as \textit{JAK2 V617F}-positive or -negative MPN. For instance, 33 \textit{JAK2 V617F}-positive PV, 57 \textit{JAK2 V617F}-positive ET, and 5 \textit{JAK2 V617F}-positive PMF are shown as \textit{JAK2 V617F}-positive MPN. Similarly, \textit{JAK2 V617F} allele burden of PMF is shown in Table 3 (Table 2 in the original manuscript)(Page 12, line 2~5).

To clarify this point, we changed what had been Supplementary Table 3 to in-text Table 2 in the revised manuscript. This table is rather complex, but the prevalence of the \textit{JAK2 46/1} haplotype in this Japanese population is clearly shown (Page 11, line 9~13).

Alternatively, the detailed genotypes of the 9 PMF patients are included in Supplementary Table 3 (Page 11, line 13~15).

Comment 3: Statistical Analysis
Reply: We performed a multivariate analysis to exclude possible false correlation especially between the molecular biological and clinical data. Indeed, several important points were clarified by the multivariate analysis (Page 9, line 14~16).
**Results**

Comment 1 (ET):

(1) The Authors found a significant major frequency of splenomegaly in Jak2 wild type ET without the 46/1 haplotype. This result does not agree with those from Vannucchi et al. *(Blood 2007; 110: 840)* and so it would be helpful to add some speculation in the discussion section.

Reply: We added the above-mentioned reference, and discuss this discrepancy in the Discussion section (Page 15, line 12~Page 16, line 2, Reference no. 16).

(2) About requirement of therapy, the Authors found that Jak2 V617F and haplotype positive patients required treatment more frequently than Jak2 V617F patients. This finding could be related to a different distribution of age, thrombotic complication and cardiovascular risk factor between the two subgroups. A multivariable analysis could solve this question.

Reply: We performed multivariate analysis and found there was no difference with regard to therapy requirement. As you suggested, this finding could be related to differences in distribution of age, thrombotic complication, and cardiovascular risk factor between the two subgroups. For this reason, we deleted text related to the therapy requirement from the revised manuscript.

(3) No difference in platelet count between the different group stratified on molecular biology. This data disagree with our personal data (Patriarca et al. Blood Transfusion 2011) and with Vannucchi et al. Blood 2007. A comment is required in “Discussion”.

Reply: We added these two references and described the discrepancy between our results and those of the previous studies (Page 15, line 14~Page 16, line 2, Reference nos. 16 and 17).

Comment 2 (PV):

(1) The Authors found a major rate of thrombotic complication in PV patients without the GCC genotype, but there is no stratification by cardiovascular risk. It’s possible that the patients without the GCC genotype affected by thrombosis has a worse cardiovascular risk profile which can explain the different complication rate. I think that
a multivariable analysis with correction by cardiovascular risk it would be helpful in this field.

Reply: For some reason, we made a serious mistake regarding the incidence of thrombosis in PV in the original manuscript. We corrected the incidence and performed a multivariate analysis, which indicated that there was no difference between the two groups. We redrafted Table 6 (originally Table 5) and changed the text accordingly (Page 13, line 15~16).

(2) A higher platelet count in PV patients without the genotype is reported. Has a correction by relative iron deficiency been made before analysis? As you know, it’s possible that during PV clone expansion a relative iron deficiency, causing a microcytic appearance of the RBC, could be present. This microcytic appearance could explain this result at least partially.

Reply: Although none of the PV patients exhibited microcytic anemia in the current study, we could not completely rule out the possibility of relative iron deficiency. We therefore commented on this point in the Discussion section (Page 16, line 17~Page 17, line 3).

Comment 3: No data are provided on the 9 PMF patients in “Results”.

Reply: Please see the revised versions of Tables 2, 3, and 4. Detailed genotypes of the 9 PMF patients are included in Supplementary Table 3.

Discussion

Comment: In the discussion the Authors cite the study from Colaizzo et al. regarding the frequency of Jak2 46/1 haplotype in splanchnic vein thrombosis. It would be correct, in my opinion, to cite both the work from Smalberg et al. entitled “The JAK2 46/1 haplotype in Budd-Chiari syndrome and portal vein thrombosis” published on Blood 2011; 117: 3968 and that from Kouroupi et al. “The JAK2 46/1 haplotype in splanchnic vein thrombosis” published on Blood 2011; 117: 5777. In this two works the conclusion are in agreement with your data and both the groups conclude that the haplotype is not a risk factor for splanchnic vein thrombosis.

Reply: These two papers are very informative, so we cited them (Reference nos. 19 and 20) and commented on them in the Discussion section (Page 16, line 9~16). Moreover,
using multivariate analysis we found that the JAK2 V617F-negative ET patients without GCC genotype were significantly free from thrombosis ($p=0.0446$) (Page 4, line 5–7, Abstract section, Page 12, line 16–Page 13, line 1, Result section). We discuss whether JAK2 germline variation is a risk factor for thrombosis (Page 16, line 16–17, Discussion section)