Author's response to reviews

Title: Lymphotoxin-alpha polymorphisms and presence of overall cancer in 1,536 consecutive autopsy cases

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BMC Cancer
Editor
Dear Dr. Scott Edmunds,

Thank you for your letter on the decision of our above manuscript. We are mostly pleased to know that our paper might be acceptable, if proper revisions are made.

We thoroughly reviewed the points raised by the reviewers' and made point-by-point answers, highlighting the changes that we made in the manuscript.

We now hope that the revised version suffices these requirements and will be found suitable for publication in BMC Cancer.

Sincerely yours,

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Referee : N.H.

1. In Table 1, the missing of smoking and alcohol is specified for "All subjects", but not for "Non-cancer" and "Cancer".

We corrected Table 1 as suggested by the reviewer.

2. In Table 1, the number and % of "Cancer, n (%)" should be placed at the column of "Cancer". Similarly, the number of % of "Cancer, sites, n" should be placed at the column of "Cancer".

We corrected Table 1 as suggested by the reviewer.

Discretionary revisions

1. The description on the similar genotype frequency may be informative for readers who judge the validity of the study.

We agreed to the reviewer's point and added the following.

“However, the genotype frequency of C804A was similar to that from other Japanese population studies [ref], and the effect of such potential bias did not cause the deviation from HWE.” (page 11, line 3 from the bottom)"

Referee 2: M.A.A.G·G.

1. The link between LTA·I± and cancer is not enough sustained in the introduction.

The literatures on the relation between LTA and cancer have been mainly reviewed in the discussion. However, we agree to the reviewer's point and added the followings in the introduction.

“The polymorphisms of LTA have been subjected to studies with regard to association with various cancers. The NcoI restriction fragment length polymorphism, A252G in the first intron, is in tight linkage disequilibrium
with C804A that results in the substitution of threonine with asparagine at codon 60 in exon3[ref]. The LTA 252G allele increases LTA at the mRNA and the protein level[ref]. The associations of various cancer risks and survivals with this LTA polymorphism have been reported, showing different influences depending on different types of cancers [ref]. However, the effect on the presence of whole cancer is not known.” (page 4, line 3~8)

2 Some considerations related to the sample size and the power of the current work should be provided and included in the statistical section.

The statistical power was calculated with the parameters of case/control=900/600, OR=1.5, and p<0.05, and this ensured the statistical power of 0.8. Accordingly, we added the following in the statistical section.

“This study had a statistical power of 0.8 to detect an OR of 1.5 with regard to the carriers of at least one polymorphic allele relative to the carriers of homozygous wild type.” (page 6, line 4 from the bottom)

3. Regarding genetic analysis, no data are provided about quality control.

Genetic analysis was done by melting temperature analysis, and randomly selected samples were subjected to direct sequencing to confirm the results. This was mentioned in the method section.

“The accuracy of the genotyping was confirmed by sequencing in randomly selected samples. There was a total concordance between the results of melting temperature analysis and direct sequencing.” (page 6, line 14)

4. The discussion is rather limited and misses out some potential explanations for their findings.

We added the following in the discussion.

“LTA gene is located within the class III region of the major histocompatibility complex in the chromosome 6p21.3[6], where many other genes including pro-inflammatory cytokine TNF reside. High degree of
linkage disequilibrium in this region is well known and thus the association observed in this study may reflect the effect of variants of other genes in this region.” (page 11, line 10~14)

5. There are several spelling and grammatical mistakes that need to be corrected.

We asked a native English speaking colleague to help us copyedit the paper.

Referee 3: H-Y. H.

1. The study was a cross-sectional study on autopsy samples. It was not designed to investigate the relationship between genetic polymorphisms and tumorigenesis (i.e., the risk of developing cancer).

We agreed the reviewer’s point and deleted the word “tumorigenesis” and changed to “presence” of cancer.

2. The study might have been subject to selection bias (i.e., those cases who had a particular genotype might have been associated with the decision on performing an autopsy) and survival bias (i.e., those who had a particular genotype might have been alive and could not be selected for autopsy). It is unclear what characteristics the non-cancer cases had. Did they have diseases that led to an autopsy? For these reasons, the conclusion drawn from the study findings could very possibly be biased.

The current study subjects are consecutive autopsy cases of a community-based general hospital of geriatrics. These subjects were collected regardless of the original disease. The rate of autopsy was about 40% of the whole death in the hospital. The causes of death among autopsy cases were malignant disease: 33%, coronary heart disease: 20%, and pneumonia: 13%. These proportions are similar to the death causes in a survey report from the Ministry of Health, Labor and Welfare (http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/tokusyu/gaikoku07/09d.html ), which shows: malignant disease: 30%, coronary heart disease: 16%, and pneumonia: 10%. Thus we believe, although not proven, that the study
samples are not significantly biased from that of the ordinary elderly population.

Accordingly, we added the followings in the methods and in discussion

“The causes of death of the subjects were malignant disease: 33%, coronary heart disease: 20%, and pneumonia: 13%, in this order. These proportions were similar to the death causes in a survey report from the Ministry of Health, Labor and Welfare (http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/tokusyu/gaikoku07/09d.html), which shows: malignant disease: 30%, coronary heart disease: 16%, and pneumonia: 10%. Thus, the cases of death among the study samples were not significantly biased from that of the ordinary elderly population.” (page 4, line 17~page 5 line 4).

“There are some limitations in this study. First, this study is a hospital-based autopsy study and the information on life style factors that have a significant influence on cancer development is limited. Second, although the autopsy was done for relatively high proportions of the deaths (40%), and the cause of death among autopsy cases were similar to those of the national survey report, we cannot rule out the existence of selection bias. Such bias may arise from chance of admission, consent to autopsy, cause of death, and autopsy practice. Third, there may be a survival bias, since particular genotype may have been associated with other diseases and/or life span to affect the constitution of the subjects. However, the genotype frequency of C804A was similar to that from other Japanese population studies [ref], and the effect of such potential bias did not cause the deviation from HWE.” (page 11, line 15~bottom)

3. More detailed information about factors other than genotypes is needed.
   Why was aged dichotomized by 80? How were smoking and alcohol consumption measured? Inaccurate measurements on covariates often result in unchanged OR estimates after adjustment.

The information on the environmental factors is limited, since this is a hospital-based study. This was included in the limitation of the study
The age of 80 was used, since this was the median. (page 7, line 11)

The smoking and drinking status was retrospectively obtained from the medical records and was dichotomized. (page 5, line 6)

4. Overall, this analysis did not inform readers of useful information as to whether LTA genetic polymorphisms play a role in carcinogenesis cancer survivorship. The selection of cases/controls and the lack of sufficient information on the environmental factors undermine the validity of the study.

The aim of this study is to determine whether LTA genetic polymorphisms play a role in cancer-bearing status, but not in carcinogenesis cancer survivorship. We highlighted this point in the text (page 2, lane 2 from the bottom).

We also added the limitation of the study in the discussion (page 11, line 15~bottom)

Discretionary Revision:
1. The study is not based on a random sample, so "prevalence" is not the right word to describe the proportion of samples with a particular genotype.

We agree to the reviews point and changed “prevalence” to “presence”.

Referee 4: M.J.G.

1. The association observed for LTA SNPs in this investigation may reflect the effect of variants in TNF; thus a more comprehensive assessment of the entire TNF-LTA region would be desirable. In any case, the authors should address this point in the discussion as a possible limitation.

We agree the reviewer's point, and added the following in the discussion.
“The LTA gene is located within the class III region of the major histocompatibility complex in the chromosome 6p21.3[ref] where other pro-inflammatory cytokines such as TNF reside. High degree of linkage disequilibrium in this region is well known and thus the association observed in this study may reflect the effect of variants of other genes in this region.”

(page 11, line 10).

2. The authors should also discuss any potential bias that may arise as a result of the study design—specifically the use of autopsy patients. This is really a study of cancer-related death versus non-cancer-related death, and not cancer-prevalence; this needs to be highlighted in the manuscript.

The current study subjects are consecutive autopsy cases of a community-based general hospital of geriatrics. These subjects were collected regardless of the original disease. The rate of autopsy was about 40% of the whole death in the hospital. The causes of death among autopsy cases were malignant disease: 33%, coronary heart disease: 20%, and pneumonia: 13%. These proportions are similar to the death causes in a survey report from the Ministry of Health, Labor and Welfare (http://www.mhlw.go.jp/toukei/saikin/hw/jinkou/tokusyu/gaikoku07/09d.html), which shows: malignant disease: 30%, coronary heart disease: 16%, and pneumonia: 10%. Thus we believe, although not proven, that the study samples are not significantly biased from that of the ordinary elderly population. This was mentioned in the subject section (page 4, line 17~page 5, line 4).

The study limitation was also added in the discussion as follows.

There are some limitations in this study. First, this study is a hospital-based autopsy study and the information on life style factors that have a significant influence on cancer development is limited. Second, although the autopsy was done for relatively high proportions of the deaths (40%), and the cause of death among autopsy cases were similar to those of the national survey report, we cannot rule out the existence of selection bias. Such bias may arise from chance of admission, consent to autopsy, cause of
death, and autopsy practice. Third, there may be a survival bias, since particular genotype may have been associated with other diseases and/or life span to affect the constitution of the subjects. However, the genotype frequency of C804A was similar to that from other Japanese population studies [16, 17], and the effect of such potential bias did not cause the deviation from HWE. (page 11, line15~bottom).

Specific points

1. The patient recruitment criteria are not clearly described. What was the definition of elderly for this study? Were the 1,536 subjects all the deaths/autopsy patients that arose during the period 1995-2004? The eligibility criteria and definition of study population need to be more explicitly described.

All of the patients registered in the JG-SNP database were enrolled in the study. These are the death/autopsy patients that arose during the period of 1995 and 2004 at the Tokyo Metropolitan Geriatric Hospital. The autopsy rate was 40%, and was collected regardless of the original disease. This was described in page 4, line17~22.

The age of study samples were distributed from 46y to 104y, and the mean±SD was 80.2±8.9. The definition of elderly is usually over 60 years old. Since the one percentile of the subjects was 59 years old, we included all of the cases to ensure consecutiveness.

2. What proportion of samples were re-genotyped? (Last sentence, para 1, p.5) Were the re-genotyped specimens part of a quality assurance procedure?

This sentence means to be the follows.

“The accuracy of the genotyping was confirmed by sequencing in randomly selected samples. This showed total concordance between the results of melting curve analysis and direct sequencing.” (page 6, line 12)

3. The authors mention haplotype data but there is no description of haplotype estimation methods or how they reconstructed haplotypes from
the genotype data.

“The LTA haplotype frequencies were estimated using Haploview software. Chi-square value for comparing the distribution of LTA haplotypes in subjects with cancer and non-cancer were calculated. “ (page 6, bottom line)

4. Please insert P-trend values for the genotype data.

We inserted P-trend values in Table 3 and 4.

5. The manuscript requires significant editorial work to sharpen the language.

We had a native English speaking colleague to help us copyedit the paper.