Author's response to reviews

Title: Estrogen Receptor-Beta Gene Polymorphism in women with Breast Cancer at the Imam Khomeini Hospital Complex, Iran

Authors:

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Version: 7 Date: 25 June 2010

Author's response to reviews: see over
Dear Dr. Rebecca Simmons,

Thank you for consideration of our manuscript. MS: 1416919219343530
Estrogen Receptor-Beta Gene Polymorphism in women with Breast Cancer at the Imam Khomeini Hospital Complex, Iran by: Sakineh Abbasi and Cyrus Azimi, for publication in your journal.
We have reviewed the above manuscript according to your reviewer’s comments.

Referee 1: 
http://www.biomedcentral.com/imedia/9710483903999213_comment.pdf

Reviewer's report
Title: Estrogen Receptor-Beta Gene Polymorphism in women with Breast Cancer at the Imam Khomeini Hospital Complex, Iran
Version: 2 Date: 28 May 2010
Reviewer: Ivana Beatrice da Cruz
Reviewer's report:
The manuscript reviewed
Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
'I declare that I have no competing interests'

Referee 2: 
http://www.biomedcentral.com/imedia/7221136573913342_comment.pdf

Reviewer’s report
Title: Estrogen Receptor-Beta Gene Polymorphism in women with Breast Cancer at the Imam Khomeini Hospital Complex, Iran
Version: 2 Date: 11 May 2010
Reviewer: Piero Tosi
Reviewer’s report:
Major compulsory revisions have been made
Minor essential revisions have been made
Discretionary revisions such as clarifications have been made or answered
Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests
Abstract:
..found only on exon 7 The SNP was found...
..of allle 1..
…polymorphisms in exon..

Introduction
..30% (9), suggesting..
..incidence (10-16).
ER-α has been studied extensively, but supporting evidence for involvement of ER-β needs further work (26-33).

Methods:
median age 47.49 ± 11.43 years. Is it median age or mean ± SD?
Race is repeated twice
..breast cancer tissue.. A
..v. 0.4.0) soft ware (Table 1).
...(16capillaries)

Results:
…C convesion to G (C 1176 G) was..
...(Table 5). Moreover, the allelic frequency of 392C was… (is it 392G?)

Discussion
a) …important players in the mechanism…
…cancer (Table3). SNPs…
…suggest the associate of ER-β polymorphisms (is it association?)…
…of 10.5%) suggesting that GC genotype (is it CG?)…
…again, the frequency of GC genotype (is it CG?)…
…than those without LN metastasis in comparison…
…indicating that GC genotype (CG?)…
…population (Table4)…
..disease (Table5). In terms..
…This indicates that codon 329 polymorphism.. (392?)

b) Clarify these two sentences that seem to be contradictory.
…it cannot be considered as a breast cancer risk factor.

…We have identified codon 392 CTC/CTG polymorphism to be associated with an increased risk of breast cancer. Moreover, CG heterozygosiy increases in familial breast cancer and LN metastasis…

References
Ref 28:.. Endocrinol Metab. 1998, 83, 4524-4527.
Some references have no space and others have a space between volume and pages.
Ref 31-33 and 40 are in a different format
KupierKupier? In ref 40.
Abstract:

All requested Suggestions have been done in Abstract as follow:
ER-alpha and ER-beta genes have been proven to play a significant role in breast cancer. Epidemiologic studies have revealed that age–incidence patterns of breast cancer in Middle East differ from those in the Western countries. Two selected coding regions in the ER-β gene (exons 3 and 7) were scanned in Iranian women with breast cancer (150) and in healthy individuals (147). PCR single-strand conformation polymorphism was performed. A site of silent single nucleotide polymorphism was found only on exon 7. The SNP was found only in breast cancer patients (5.7%) ($\chi^2=17.122$, $P=0.01$). Codon 392 (C1176G) of allele 1 was found to have direct association with the occurrence of lymph node metastasis. Our data suggest that ER-β polymorphism in exon 7 codon 392 (C1176G) is correlated with various aspects of breast cancer and lymph node metastasis in our group of patients.

Keywords: breast cancer, estrogen receptor beta, ER-beta, polymorphism, LN metastases, SSCP-

Introduction

In Introduction corrections have been done as follow:

Only a small fraction (<5%) of women diagnosed with breast cancer have a clear hereditary predisposition (6-8), and of these, about one half have predisposing mutations in BRCA1, BRCA2, PTEN, TP53, or other known cancer predisposing genes. However, twin studies indicate that the heritability of breast cancer is about 30% (9), suggesting that genes other than the well-mapped regions act as modifiers of breast cancer risk. Although it is likely that low penetrance as well as high penetrance genes may be involved in the etiology, it remains unclear which genomic regions and which biochemical functions or signal transduction pathways account for the additional heritability of breast cancer incidence or progression.

Epidemiologic evidence suggests that estrogen plays a crucial role in most breast cancers in women who have both early menarche and late menopause. Obesity is also associated with breast cancer risk; estrogen synthesis in adipose tissue is proposed to account for this increase in risk. There is data on the involvement of estrogen receptor in the molecular processes implicated in the rise of breast cancer incidence (10-16).

The last paragraph of the introduction has been changed as the reviewer indicates.

ER-α polymorphic variants have been associated with breast cancer risk (17-25) in Caucasians, as have certain clinical features including presence of a family history and lymph node (LN) metastasis. ER-α has been studied extensively, but supporting evidence for involvement of ER-β needs further work (26-33).
ER-α polymorphic variants have been associated with breast cancer risk (17-25) in Caucasians. Furthermore, ER-α has been studied extensively, but supporting evidence is required for proving the involvement of ER-β in breast cancer (26-33).

Methods:

The word of Median had changed to mean and other changes made in Methodology as indicated by the reviewer.

The breast cancer patients (n = 150; mean age 47.49 ± 11.43 years) were newly diagnosed and mostly living in Tehran. They were entered into the study if they had a confirmed pathological breast cancer diagnosis at the Imam Khomeini Hospital Complex…

Demographical and risk factor data were collected using a short structured questionnaire, including information on age, weight, height, race, religion, marital status, number of pregnancies and children, age at first child birth, average lactation term, family history of breast cancer (first-degree relatives), age at menarche, age at marriage, menopausal status, and age at menopause, ABO and Rhesus blood groups, race, age at onset, lymph node metastases, cancer stage at the time of testing and ER expression in breast cancer tissue. A protocol to collect and store blood samples for future genomic tests had been approved by the institutional review board. Peripheral blood was collected and genotyping analysis was performed.

**Screening for ER-β variants by single strand conformation polymorphism analysis**

In order to identify any change in the two selected exons (exons 3 and exon 7) of ER-β, PCR and SSCP was performed. Genomic DNA was extracted from blood leukocytes using the DNG™-Plus extraction kit (Cinnagen Inc, Tehran, Iran) in accordance with the manufacturer's instructions. Specific set of primers for ER-β gene exons 3 and 7 were designed by primer3 (v. 0.4.0) software (Table 1).

PCR was performed through 30 cycles with the following steps: denaturation 30 sec at 95°C, annealing 30 sec at 58°C and elongation 40 seconds at 72°C. The SSCP was carried out on 12% polyacrylamide gel (29:1 Acrylamide/ Bisacrylamide), with 200 V, for 20 h at 16°C. After electrophoresis, silver nitrate staining was used for visualizing the bands. To identify and confirm the exhibited irregular pattern in SSCP, PCR samples demonstrating varying band shift patterns as the result of first sequencing with forward primer, were re-purified on agarose gel using a DNA Extraction Kit (Fermentas # K0153, Germany) and directly sequenced by big dye Terminator V3.1 Cycle Sequencing kit protocol, (Applied Biosystem Kit, Microgen Co., USA), on a sequencer ABI 3130XL (16-capillaries).

The PCR product purification method was used in order to confirm sequencing by reverse primer. The PCR products were purified using QIAquick PCR purification Kit (50) (QIAGEN, USA).
Results:
The first and fourth paragraphs in Results has been changed as the reviewer indicates as follow:

..., a conversion of nucleotide C to G (C 1176 G) was observed. Both CTC and CTG code for Leucine amino acid (Table 5). Moreover, the allelic frequency of 392C was significantly higher in cancer patients with LN metastases (23.9%) than in those without LN metastases (2.4%) with a difference of 21.5%,...

Discussion:
A) GC changed to the correct form of CG and all other corrections were made as reviewer suggested:

... most important players in the mechanism of...
....breast cancer (Table 3). SNPs in different...
.....those without LN metastasis in comparison...

... population (Table 4).
... disease (Table 5). In....

And the paragraph before the lat in this section 329 corrected to 392.
...codon 392 polymorphism...

B) The following statements now has been changed in the first paragraph Conclusion section as follow:

We have identified codon 392 CTC/CTG polymorphism to be associated with an increased risk of breast cancer....

We have identified codon 392 CTC/CTG polymorphism to be associated with an increased risk of familial breast cancer with LN metastasis....

References:
The reference # 28 was corrected as the reviewer indicated. And a space between volume were made in all references as well.

Best Regards,
Dr. Sakineh Abbasi