Author's response to reviews

Title: Implication of Xenobiotic Metabolizing Enzyme gene (CYP2E1, CYP2C19, CYP2D6, mEH and NAT2) Polymorphisms in Breast Carcinoma.

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Version: 2 Date: 2 January 2008

Author's response to reviews: see over
January 2, 2008

Dr Maria Kowalczuk
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Manuscript ID: 1231888224155443

Manuscript title: Implication of Xenobiotic Metabolizing Enzyme gene (CYP2E1, CYP2C19, CYP2D6, mEH and NAT2) Polymorphisms in Breast Carcinoma.

Dear Dr Maria Kowalczuk,

Please find enclosed the revised manuscript above-referenced. The reviewers’ comments were very useful and allowed the improvement of the manuscript. We have made revisions based on their comments. A copy of the revised manuscript with all changes marked in blue is included.

We have performed all the suggested corrections and we give responses to all the referees’ comments point by point. Responses to referees’ comments are noted in the attached pages.

We thank you for the opportunity to improve our paper and hope that our revised manuscript meets the standards of publication in your journal.

Yours sincerely,

Dr Elham HASSEN
Responses to the referees’ comments

Reviewer 1: Valentina Paracchini

Major Compulsory Revisions:

1. **Abstract section** (page 2; results paragraph; line 5):

   According to the reviewers’ comments, all associations found with OVS and DFS were added in the abstract section and more details were also added to describe the association between mEH and NAT2 genes and OVS and DFS in axillary lymph node negative or positive patients.

2. **Subjects paragraph** (page 5; Materials and methods section):

   For this study we collected controls from the same patients’ area and at the same period (between 1994 and 2002). We tried as much as possible to meet the age matching between patients and controls. The patients had a mean age of 52 ± 24 years and the controls a mean age of 41 ± 14 years. Controls were healthy Tunisians having no evidence of any personal or family history of cancer or any other illnesses. To avoid any bias due to consanguinity, both study population groups (patients and controls) included only unrelated subjects.

3. **Polymorphism analysis** (page 6; line 5):

   As proposed by the reviewer we provided more details about laboratory controls we used.

4. **Results section** (page 10; XMEs gene polymorphisms and susceptibility to breast carcinoma paragraph; line 8):

   In the result section we added a sentence that states that in our study population heterozygous CYP2D6 genotype seems to be protective.
5. **Discussion section (page 13; line 13):**

In the discussion section the hypothesis which explains the fact that individuals with at least one copy of CYP2D6 mutant allele (means heterozygous or mutant homozygous genotypes) was more clarified.

6. **Table 1:**

We corrected the title of Table 1 that became Table 2 in the revised version of the manuscript.

7. **Table 2:**

Concerning the missing genotypes we added the following sentence in the beginning of the **Results section** (page 10; XMEs gene polymorphisms and susceptibility to breast carcinoma paragraph; line 1): “The number of polymorphism-genotyped individuals was dependent upon DNA availability.” And the exact sample sizes were added in the table that sums up genotype frequencies (Table 4 in the revised manuscript).

8. **Table 2 and 3:**

According to the reviewer’s comment we added the 95% CI for all the ORs calculated to Table 4 and Table 5 of the revised manuscript.

**Minor Essential Revisions:**

Both the Minor and Discretionary revisions were corrected according to the reviewer’s suggestions.

1. **Subjects paragraph** (page 5; line 1) the following sentence: “…subjects selected form the SAME population… “ was replaced by: “… living in Sousse on the middle coast of Tunisia, …”.
2. **Subjects paragraph** (page 5; line 10): “control subjects’ mean age of 41 + 14 years…” was corrected and replaced by “control subjects having a mean age of 41 ± 14 years…”

3. **Statistical analysis paragraph** (page 8; line 1): “Hardy-Weinberg” was typed in capital letters.

4. **Results section** (page 10; XMEs gene polymorphisms and susceptibility to breast carcinoma paragraph; line 2): The sentence “All genotype distributions did not diverge significantly from H-W equilibrium” was corrected as follows: “All genotype distributions did not diverge significantly from Hardy-Weinberg equilibrium for both patient and control groups separately”.

5. **Results section** (page 10; XMEs gene polymorphisms and susceptibility to breast carcinoma paragraph; line 5): We added the word “when” before the sentence “the patients were stratified according to their menopausal status…”.

6. **Discussion section** (page 13, line 3): We added the word “patients” after “postmenopausal”.

7. **Discussion section** (page 15, line 1):

   The association between NAT2 slow acetylator and breast cancer has not been seen in other studies means that our study is the first to find association between NAT2 slow acetylator and breast cancer. We reported discordant data with Alberg et al. (2004) that found the NAT2 rapid acetylator to be associated to increased breast cancer risk. To our knowledge, after carefully going through the literature, apart from Alberg et al. there were no previous studing provide any association with the NAT2 gene polymorphisms.

8. **Discussion section** (page 16; line 17): “mEH wild genotype” was changed into “mEH wild-type genotype”.


Discretionary Revisions:

1. **Results section** (page 10; NAT2 part; line 3): “but” was corrected with “while”

2. **Results section** (page 10; NAT2 part; line 4): “of” was corrected with “among the”

3. **Results section** (page 10; NAT2 part; line 5) “a risk factor to” was corrected with “a risk factor for”.

4. We included a table (Table 1 in the revised manuscript) that contains a detailed description of the clinical pathological characteristics of breast cancer patients containing information about axillary lymph node status.
Major Compulsory Revisions:

1. **Background section** (page 3; line 1): As suggested by the reviewer we included in this section a paragraph that sums up some epidemiological features (incidence and risk factors) about breast cancer in the world and in Tunisia.

2. **Materials and methods section:**

   **Polymorphism analysis paragraph** (page 6; line 8): The genotyping details including the PCR conditions, PCR enzymes, annealing temperatures and used controls were added.

   **Genomic DNA extraction paragraph** (page 5): We also added the exact protocol and the reference used for DNA extraction from peripheral blood leucocytes. In addition, enzyme restriction digestion conditions used for CYP2E1, CYP2C19, CYP2D6, mEH were added as well as a Table 3 summarising enzyme restriction digestion conditions for NAT2 genotyping polymorphisms.

3. **Results section:**

   According to the reviewer’s comment the genotyped sample size was clarified in the Results section (page 10; line 1) and added in the Table 4 of the revised manuscript. We added the 95% CI for all the ORs calculated in Table 4 and Table 5 of the revised manuscript.

   **Survival analysis and prognostic significance of XME gene polymorphisms paragraph** (page 11; line 8): When we explored whether the studied gene polymorphisms were associated with survival rate according to the different clinical pathological parameters (Age, nodal status, SBR tumor grade, clinical tumor size), the only significant associations were found with axillary lymph node
status. For that reason, we illustrated in our manuscript only the survival rate according to the nodal status.

4. Discussion section:

We agree with the reviewer comment concerning the study of lifestyle factors susceptibility and low penetrance candidate genes altogether and we also agree that lifestyle and environment risk factors markedly account for breast cancer occurrence, but it was not the emphasis of the current study. This study was rather aimed at establishing only the relationship between breast cancer and genetic polymorphism of some xenobiotic metabolizing genes without including lifestyle and environmental factors.

To improve the Discussion section the following recent references on epidemiological data on XMEs and cancer risk were added: Lemos et al., 2007; Kuo et al., 2007, page 13 line 9 and page 15 line 6, respectively.

Minor Essential Revisions:

Minor essential revisions were carried out.
Reviewer 3: Rui Medeiros

Minor Comments:

1. Abstract section (page 2; results paragraph; line 5):

   According to the reviewer’s suggestion we added to the Abstract section all the significant association we found regarding overall survival and disease free survival.

2. Materials and methods section:

   Given the number of the figures (three) and the tables (five) to be included in our manuscript, we decided to include only the figures of the survival analysis (OVS and DFS). However, we believe that the figures of the gel electrophoresis will be representative of our results. Thus, we gave for each studied polymorphism more details on the RFLP-PCR product size in the Table 3 and in the Materials and methods section.

3. Discussion section:

   As suggested we tried to add recent papers on epidemiological data on XMEs and cancer risk of other hormonal associated cancer in Mediterranean populations, but unfortunately we did not find any recent papers in this field (melting these three underlined requirements). However, two recent references were added: Lemos et al., 2007; Kuo et al., 2007 (page 13 line 9 and page 15 line 6, respectively).