Author’s response to reviews

Title: Monoallelic Characteristic-Bearing Heterozygous L1053X in BRCA2 Gene among Sudanese women with Breast Cancer

Authors:

Alsmawal Elimam (toteil1@hotmail.com)
Mohamed Aabdein (mogtaba8788@hotmail.com)
Mohamed Mohy Eldeen (elfatihnorth@gmail.com)
Hisham Altayb (hishamaltayb@yahoo.com)
Mohamed Taha (mohdil23@hotmail.com)
Mohammed Nimir (mohammednimir7@gmail.com)
Mohamed Dafaalla (mdafaallah200@gmail.com)
Musaab Alfaki (musabnoor946@live.com)
Mohamed Abdelrahim (mohd1991@gmail.com)
Abdelmohaymin Abdalla (shawrma214@gmail.com)
Musab Mohammed (musab.phyta@gmail.com)
Mona Ellaithi (Mona.Ellaithi@gmail.com)
Muzamil Abdel Hamid (mahdi@iend.org)
Mohamed Hassan (altwoh20002002@yahoo.com)

Version: 1 Date: 10 Mar 2017

Author’s response to reviews:

Reply to reviewer 1

In the current study, the authors determined a nonsense mutation in BRCA2 gene (Exon 11). This study is very weak for mutation screening related with BRCA1/BRCA2 genes. It provides a very little information about Sudan BC patients.
There are several major weaknesses in this manuscript:

1. **3385 T → G alteration is not novel. It has rs41293477 number. This mutation was previously reported.**

Authors checked the data base again, and we found the same protein alteration at the same position in a previous study. Accordingly, details from this study was introduced to the manuscript in discussion section.

2. **The number of patients were not enough. The authors mentioned different studies which determined BRCA2 gene mutation in different population. As they mentioned, all studies include a large number of patients. Thus, more patients should be added.**

3. **The authors screened only selected regions of exon 11 in BRCA2. The other regions in this exon and other exons of BRCA2 should be scanned due to the considerable effects of BRCA2 gene mutation.**

Technical facilities to establish the outcome/resulting truncation inactivation are not available and it is very difficult to handle such a technical assessment. Though all 45 patients’ DNA had been extracted, only 10 patient’s extracts were sequenced owing to financial constraints. According to literature, most of detected mutations in BRCA2 are found in exon 11. Also, due to these financial constraints only the product of one primer with the highest stability was subjected to further analysis in this study.

4. **The clinical parameters of patients should be added as table.**

The required table was added.

5. **In the discussion section, authors mentioned different studies. However, there is a lack between paragraphs. Thus, this section should be re-written.**

The discussion was rewritten.

6. **The English is below the required standard. Please read the paper carefully for English language style, grammar and spelling, and make appropriate corrections and changes.**

Sentences construction and grammar have been corrected. Please refer to the manuscript whole frame.

7. **Ethical approval number should be given.**

Ethical approval has been provided.
Reply to reviewer 2

Reviewer #2: Breast cancer cases with early onset are increasing in Sub-Saharan population and BRCA2 is one of the major causes of familial breast cancer. The majority of the mutations in this gene are frameshifting or nonsense. Based on this evidence, Elimam et al. searched exon 11 of BRCA2 for novel mutations in patients diagnosed with BC, using 3 primer sets that unveiled a new nonsense mutation at nucleotide 3385 in 4/9 patients. However, this mutation seems to occur only in one allele, as the WT allele is still present in the electropherogram. The authors did not describe this in the paper nor have they discussed this subject. Is the mutation in one allele deleterious? Or, is BRCA2 still able to perform its function with only one functional allele?

# Authors added Information on our finding regarding heterozygosity that conferred by one allelic genotype. We found some studies stated its association with Breast cancer predisposition as it results in distinctive phenotype with haploinsufficiency suggested to lead to cancer formation. All the above mention findings have been included in the introduction and discussion.

I have several concerns about this paper that I will describe below:

General comments:

I) First, of the 32 cited references, more than 20 were published before 2010. I would recommend a background review.

#updated literature were incorporated and new references were added. Please refer to the manuscript for the required added information.

II) The paper is sometimes hard to read due to poorly constructed sentences and nomenclature misuse. For example, when reporting a mutation, instead of writing "substitution of Thymine at position 3385 with guanine" the authors could simplify as T3385G mutation. Also, the authors should refer to different nucleotides with capital letter, according to IUPAC recommendations.

# Sentences construction and nomenclature have been corrected. Please refer to the manuscript whole frame.

III) The Figures have low graphical quality.

# Figure 2 graphic was re-adjusted. Please refer to the manuscript figures.
Detailed Comments:

I) Abstract. Rephrase "is the leading type of cancer" with "is the most common type of cancer. Please substitute "frame-shift" for "frameshift". In the Results section of the abstract, delete "at the same location" as it is not needed. In the conclusion section please delete "at the same position".

# above sentences are rewritten as recommended. Please refer to the manuscript Abstract.

II) Background. As already mentioned, this section of the paper should include recent references. In line 9 I would recommend replacing "The human BRCA2 gene contains 27 exons. Exon 11 within the BRCA2 genes has the largest sequences base-pair (bp)" with "The human BRCA2 gene contains 27 exons, being exon 11 the largest one." In line-13 rephrase "Furthermore, in a genetic analysis performed on (…)"

# Recent references were included, and above sentences are rewritten as recommended.

III) Methods (Sampling). The authors did not provide the selection criteria of the patients used in this study. "Selected conveniently" is not a criterion. In this section the authors also mentioned that the control patients were suffering from other diseases. Which ones? May theses diseases have a potential impact in the results? Furthermore, what was considered "best bands" to proceed with the sequencing? The authors need to provide much more detail.

#criteria for patients’ selection and details about controls were added. We chose a control subject diagnosed with essential thrombocythemia. In addition, 4 healthy controls were added. Also, information about band selection according to quality was mentioned. Please refer to the methods (sampling) section in the manuscript.

IV) Methods (Ethical Approval). The authors must provide the reference of the ethical approval.

# Reference to ethical approval was stated in the materials and methods section.

V) Methods (PCR amplification). The authors should write "three primer sets" or "three primer pairs" because each primer is a oligonucleotide sequence rather than a pair of
oligonucleotides. Why is this study based on the product amplified by primer set B? The authors should explain why the other products were not used in the analysis. What is D.W? Distilled water? The authors provide the volumes of DNA and primers used in the reaction rather than their concentrations. The concentrations used must be presented. The 36 products were obtained with which primer set(s)?

# Statement was corrected as demanded, refer to the manuscript for further information. The study was based on region (exon 11) amplified primer B. Authors focused in depth sequence analysis of region B in this study. The results of analysis of primer A and C sets will be involved in subsequent studies.

# D.W refers to Distilled water. Requested DNA and primers concentrations were incorporated. The 36 products were amplified with the 3 primer sets.

Also, in Figure 1, what was the sample used?

# The patient samples – labeled (1 – 7) in both primers B and C and one sample patient number was labeled 1 for A primer. However, not all have been selected for sequencing.

VI) Methods (Sequencing of BRCA2 gene). This sub-section is rather confusing. When the authors say "run for both forward and reverse strands" do they mean strands of the genomic DNA?

# Sequencing was performed in two directions using forward and reverse primers targeting the amplified regions within the BRCA2 gene.

VII) Bioinformatic analysis. Please refer the name of database in line 18.

# Nucleotide database has been added - please refer to the Manuscript for the required added information.

Moreover, the additional nucleotide sequences used in the alignment were removed from NCBI and are obsolete. I would strongly advise the authors to withdraw these sequences from the analysis.

# the nucleotide sequences were removed and replaced by the following accession numbers (AY436640.1) and (X95161.1) as control measure from NCBI nucleotide database.
VIII) Results (Study population characteristics). This section does not provide enough data to characterize the population. For instance: 1) Stage of BC; 2) Histotype of BC; 3) Previous treatment(s); 4) Age interval of patients; 5) Previous mutations reported in these patients.

# Data was included in table 2 in the results section. Please refer to the figures and tables file for the requested added information.

IX) Results (Nonsense mutations). In this sub-section the authors must refer the motives by which one patient and one control were removed from the analysis.

# The motive was mentioned at the results Nonsense mutations subsection.

One control is not enough in this type of study.

#4 healthy subjects labeled and 1 diagnosed with thalassemia who are free of BC have been added as controls.

X) Results (Bioinformatic Analysis). The authors refer to an additional mutation found in two patients already bearing the new nonsense mutation but they did not discuss the data. Although this mutation occurs after the nonsense mutation and may not have additional impact I would like to see this issue mentioned in the paper.

#It was included in the discussion.

When referring to Figure 2 the authors should mention the specific panel.

We didn't get this, please, we need details about and thank you very much!

#The authors have to mention that only one allele bears the mutation:

We mentioned this in the manuscript.

Concerning Figure 3, the authors did not provide any statistical analysis of the data; therefore the differences found may not be relevant:

Table for these purposes have been added.
XI) Discussion. I feel that this section is a mere description of the literature. I would advise rewriting this part of the paper, acknowledging the fact that the nonsense mutation is present in one of the alleles only and the consequences that it may have.

# Discussion was rewritten and required information has been added.

In conclusion, the finding is relevant but is poorly presented and discussed. I would recommend at least 3 healthy individuals as controls.

#Healthy controls were added. Whole manuscript was adjusted and managed in order to present the data in an acceptable frame.

Replay to the Reviewer 3:

#Discussion was rewritten and required information has been added.

#Technical facilities to establish the outcome/resulting truncation inactivation are not available and it is very difficult to handle such a technical assessment of functional impact. #Though all 45 patients’ DNA had been extracted, only 10 patient’s extracts were sequenced owing to financial constraints. Also for the same reason, we could not test Family members of the variants carriers

#According to literature, most of detected mutations in BRCA2 are found in exon 11. Also, due to these financial constraints only the product of one primer with the highest stability was subjected to further analysis in this study

#Criteria for controls selection was added.

# Sentences construction and grammar have been corrected. Please refer to the manuscript whole frame.