Reviewer’s report

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

Version: 0 Date: 12 Sep 2016

Reviewer: Manuel AS Silva Santos

Reviewer's report:

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? 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 an background review.

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.

III) The Figures have low graphical quality.

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 is it not needed. In the conclusion section please delete "at the same position".
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 (...)"

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 those 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.

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

V) Methods (PCR amplification). The authors should write "three primers 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)? Also, in Figure 1, what was the sample used?

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?

VII) Bioinformatic analysis. Please refer the name of database in line 18. Moreover, the additional nucleotide sequences used in the alignment were removed from NCBI and are obsolete. I would strongly advice the authors to withdraw these sequences from the analysis.

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.

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. One control is not enough in this type of study. Also, when referring to Figure 2 the authors should mention the specific panel. Regarding Figure 2, the authors have to mention that only one allele bears the mutation. Concerning Figure 3, the authors did not provide any statistical analysis of the data; therefore the differences found may not be relevant.

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.

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.

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

Are the methods appropriate and well described?  
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?  
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?  
If not, please explain in your comments to the authors.

No

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I am able to assess the statistics

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