Author's response to reviews

Title: Facile whole mitochondrial genome resequencing from nipple aspirate fluid using MitoChip v2.0

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Author's response to reviews:

Dear Editor,

Please find a revised version of the manuscript entitled ‘Facile whole mitochondrial genome resequencing from nipple aspirate fluid using MitoChip v2.0’ to be considered for publication in BMC Cancer. Point-by-point responses to the reviewer’s comments are provided below.

Yours truly,

Gabriel Dakubo

Responses to reviewer’s report

This study had 3 main aims.

First, to demonstrate that they have a high rate of NAF recovery from symptomatic women. The recovery rate is indeed good (23/28 women) however this in itself is not novel as a number of studies have reported on the rate of NAF recovery.

Second, they demonstrate convincingly that the entire mitochondrial genome can
be sequenced from NAF using the MCv2 array and importantly this data was cross validated by capillary electrophoresis methods.

Finally they have assessed the somatic mtDNA mutation rate in NAF compared to blood samples from the same patients as a potential tool for monitoring early somatic mutations associated with breast disease. The data obtained is clearly described but I think the case for this being a potential tool for monitoring breast disease has not yet been made (see comments below)

Minor Essential Revisions
1: The authors should discuss the most fundamental problem with studying NAF and that is that the ductal system has several (7-12) openings at the human nipple. When NAF is collected it is usually only from one or two ducts. Consequently it may well be that NAF is not collected from a duct where there is an early breast lesion. It would therefore seem that this approach is limited for the detection of early breast lesions. The authors should comment on this.

Response: This is indeed true since the FIRSTCYTE® Aspirator we used successfully collects NAF from 1-3 ducts. A comment on this is provided on page 5 under ‘materials and methods’.

NAF was recovered by a qualified practitioner, using a FIRSTCYTE® Aspirator (Cytyc Health Corporation) following the recommendations of the manufacturer, and stored in CytoLyt Solution until extracted. Using this device, NAF was expressed from 1-3 ducts and pooled for the study. This method of NAF collection is therefore not representative of the entire ductal system, and could miss ducts with lesions. For diagnostic purposes, a better method of NAF collection is needed. The total volume of NAF collected per patient ranged from 50-100 μL.

2: The authors should provide more details in the materials and methods about the NAF collection. What was the mean volume of NAF collected per breast? How many ducts produced NAF? If NAF was produced from more than one duct, was the sample pooled for that breast?

Response: See response 1 above and page 5 of manuscript.

3: I do not understand the argument made on p13 (para 2). The authors are discussing that 4/19 samples contained a single point mutation difference from the blood samples. They then state ‘This rate among women at risk is slightly elevated over the normal mtDNA mutation rate of 16% found in healthy women and men’ What does this latter study relate to. Is it a NAF study? What do they mean by normal mtDNA mutation rate? This needs to be clarified and better described.

Response: This is an inaccurate statement and has been removed.

4: Figure 1 needs a much more detailed legend. What are the black circles? The writing on the figure itself is too small to read. Why are there 2 red and 3 yellow circles (on average) for each patient? Why are only 18 patient samples shown?
Response: See the revised figure legend on page 17. We have revised this to include all their concerns.

Figure legend

Maximum likelihood tree showing the relationship between individual patients’ mtgenomes derived from both blood and NAF. There are two independent sequences for each blood sample (MCv2 and CE; red circles), and three independent sequences for each NAF sample (MCv2 sequences from GGI and NIST, and CE sequences; yellow circles). Results are also clustered according to haplogroups. Individual CMG1182 has several identical polymorphisms to haplogroup M and therefore clusters with this group even though she is haplogroup K. Individual CMG1071 was excluded from this analysis because the three NAF sequences had several nucleotide differences. Black circles represent outlier sequences.

5: The biggest question is whether the 4 mtDNA mutations found in the left breast NAF samples were also found in the right breast NAF samples which were not analyzed.

Response: We did not address this question in the present study. However, this is an important point, and we will consider it in our future studies.

6: There are a number of spelling mistakes in this manuscript. The authors need to proof read it thoroughly. e.g. p7, line 3 should be WERE not WHERE, p13 (para 2) line 2 WOMEN not WOMAN etc etc

Response: These have been corrected and the manuscript is proof-read by three people.