**Reviewer's report**

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

**Version:** 2  **Date:** 16 December 2007

**Reviewer:** Clare Isacke

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This study had 3 main aims.

First, they 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.

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?

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.
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?

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 analysed.

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

What next?: Accept after minor essential revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

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