

Author's response to reviews

Title: Frequency of Common HFE Variants in the Saudi Population: A High Throughput Molecular Beacon-Based Study

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Author's response to reviews: see over

Dear BioMed Central Editorial Team,

Thank you for the positive feed back from both reviewers. We addressed all the concerns of both reviewers as outlined in a point by point basis below.

First reviewer's report: Finn Cilius C Nielsen

“My major reservation is that the technology previously has been described and that HFE genotyping in most laboratories already is automated and does not provide a major technical obstacle. Nevertheless this study may provide a useful alternative”.

We added at the end of the first paragraph in the discussion:

“Even though *HFE* genotyping in other laboratories is already automated and does not provide a major technical obstacle, molecular beacon-based real-time PCR genotyping may provide a cheaper and more efficient alternative, particularly for large studies.”.

Second reviewer's report: James Barton

Major Compulsory Revisions

1. *High throughput testing to identify the HFE polymorphisms in large-scale screening studies is not new. Other investigators have used various techniques to accomplish this. Examples of such projects are reported in these articles:
Adams et al. N Engl J Med. 2005 Apr 28;352(17):1769-78.
Asberg et al. Scand J Gastroenterol. 2001 Oct;36(10):1108-15.
Beutler et al. Ann Intern Med. 2000 Sep 5;133(5):329-37.
Olynyk et al. N Engl J Med 1999 Sep 2;341(10):718-24 (already cited in the present paper).*

The authors should compare and contrast the outcomes of the molecular-based beacon approach to detection of common HFE missense mutations to the methods used in some previous large-scale studies.

On page 4, line 6, we added:

“Several molecular methods have been used to screen for the common HH mutations, p.C282Y and p.H63D. The methods include allele-specific oligonucleotide hybridization (ASO) [12], restriction enzyme (RE) analysis [4], and the invader assay [13]. High throughput ASO methods require up to two days to complete, include multiple post PCR steps and often use radioactive reagents, all factors likely to discourage application of this format. RE also requires several post PCR steps including an overnight digestion and analysis of ethidium bromide stained gels, and can potentially lead to PCR contamination as a result of excess handling. The invader assay on the other hand, is an isothermal high throughput assay, but requires complicated design of both signal and invader probes which must work in tandem and involve multi-step intermolecular events. Genotypes

are assigned after determination of the net wild-type/variant signal ratios, which can potentially lead to genotyping miscalls. The use of these techniques have enabled several *HFE* population studies, however, these techniques remain limited to research laboratories as they are relatively expensive, time-consuming, and in some cases difficult to transform into a robust high throughput format.”

Also on page 5, line 11, we added:

“In addition, they eliminate the need for agarose gel analysis of PCR products because the entire assay from amplification to detection is performed in the same microwell, allow SNPs to be analyzed in less than 2 hours and genotyping calls can be reliably automated.”.

2. *“The authors should specify the source(s) of the 23 samples of known genotypes and the source(s) of the 540 anonymized newborn dry bloodspots, including race/ethnicity, in the first paragraph of the Methods. This should state whether any of the newborns from which the blood spots were taken are closely related”.*

On line 3, page 6, Method section, DNA samples subsection, we added:

“A total of 23 samples of known genotypes for the p.C282Y and p.H63D mutations were selected and anonymized prior to being used in assay development and validation. The samples originated from patients referred to our laboratory for *HFE* genotyping. Routinely, these samples are assayed using a restriction digestion based methodology. For population studies, we utilized a total of 540 random, anonymized newborn dried blood spots (DBS) as a source of template DNA. The neonatal DBS are routinely sent from multiple birth centers across Saudi Arabia to the National Laboratory for Newborn Screening in our institution. The likelihood of these samples originating from closely related subjects is virtually negligible but cannot be completely excluded.”.

3. *“A major contribution of the present work is the estimate of allele frequency of C282Y and H63D in Saudi people. However, this aspect of the work is not stated in the Title or Abstract. This should be corrected. The present allele frequency results should also be compared briefly with previously published estimates of C282Y and H63D allele frequencies in Saudis or closely related peoples in the Discussion”.*

The title has been changed to:

“Frequency of Common *HFE* Variants in the Saudi Population: A High Throughput Molecular Beacon-Based Study”

On page 2, line 20, the abstract was modified as follows:

“Additionally, these assays were used in a high throughput format to establish the allele frequency of C282Y and H63D in Saudis for the first time”.

4. *“The authors mention "cost per sample" and "more economical than either restriction enzyme analysis or direct sequencing" in the Results, last paragraph. These issues are important to planning large-scale screening programs. However, the authors provide no cost data that permit the reader to estimate the relative economy or expense of the present method. Thus, it seems important to provide some cost-related information. Such estimates must stipulate general assumptions made in formulating the costs analysis estimates, such as cost and availability of acquiring and storing test samples, hiring qualified personnel, possession of adequate laboratory equipment, reagents, etc. However, an extensive cost analysis is not requested”.*

On page 13, line 12, we added:

“This is based upon a requirement for 1,000 or more assays which introduces an economy of scale with respect to the synthesis of molecular beacons and assumes availability of real-time PCR instrumentation. Based upon these numbers, the reagent cost of each of the beacon assays described here, has been calculated at <US\$ 1.0, This is compared to a 3-4 fold more reagent cost when alternative methodology such as RE or sequencing is considered and reflects the single step beacon procedure as opposed to the multi-step nature of alternatives. Clearly the single step beacon procedure is similarly advantageous when viewed in terms of technician time”.

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5. *Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)*

In the first paragraph of the Background, the authors may wish to mention that many other mutations of HFE have been described that are associated with hemochromatosis phenotypes, although those other than C282Y, H63D, and S65C appear to be rare or "private" mutations.

On page 3, line 17, we added:

“Whilst many other mutations of *HFE* associated with the hemochromatosis phenotype have been described, they appear to be rare or “private” mutations [9].”.