Reviewer's report

Title: A Novel Multiplex Polymerase Chain Reaction Assay for Profiles Analysis of Peripheral Blood Gene Expression

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Reviewer: James Wingrove

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Summary

Using multiplex qRT-PCR, the authors have assessed the expression values of 15 candidate genes for association with the presence of coronary artery disease (CAD) and other CAD-associated clinical factors. Of the 15 genes evaluated, levels of IL-1beta, IL-6, IL-8 and MCP-1 increased significantly in patients with CAD, a positive correlation was observed between glucose levels and the expression levels of MCSF, HMOX-1, and TNF-alpha, and a positive correlation between L-Selectin and triglycerides. The authors also evaluated the performance of the multiplex qRT-PCR system used in the study, demonstrating intra-run CV values of 3.695 %-12.537 % and inter-run CV values of 4.405 %-13.405 %.

Major Revisions

Technical

- The authors validated each primer pair individually for appropriate size (Figure 1 and Table 1); it would be worthwhile to also report the efficiencies of the assays using a standard curve, both singularly as well as when multiplexed.

- On page 8 the authors state the reverse primer concentrations for 8 genes need to be either increased or decreased and show the results of these alterations in Figure 2. Figure 2 is of poor quality and uninterpretable (as is Figure 1); it would also be beneficial to summarize the results of the optimization in tabular form, quantifying the decrease or increase in peak height as a ratio to the positive control.

- As the samples are collected into tubes without RNA stabilization reagents, concern exists around the length of time prior to RNA purification; have the authors evaluated the effects of time on RNA stability?

Statistical

- The number of patients evaluated was rather low, it would be beneficial to see the results replicated in an independent set of patients to confirm the findings.

- As the patients in the study were evaluated by CT-angiography, it should be possible to define the amount of disease present in the cases; the disease severity here is unclear. Assuming there is a mixed amount of disease in this set
of patients (ranging from patients with low burden to multi-vessel disease) it would be interesting for the authors to look at the levels of gene expression versus amount of disease (including controls).

- Gene expression is known to be strongly associated with age, sex, and other clinical risk factors. The authors need to evaluate these in relationship to the changes that are being observed (e.g. multivariate analysis); the case and control sets were not completely balanced for age and sex.

- In the section describing precision results for gene expression, it is unclear what the range of CV’s given represent. Is this the range seen within the individual genes? Also, reporting the results as CV is not entirely correct, as gene expression is a quantitative measurement using an interval scale; using SD is more appropriate.

- Please provide the p values for the significant genes in Figure 5.

General

- Many typographical and grammatical errors are present in the manuscript, too numerous to list. Manuscript needs substantial editing.

- Figures, as presented, are of low quality, especially Figures 1 and 2. Please provide higher quality figures.

Discretionary Revisions

- A number of studies have been published examining peripheral blood gene expression in relationship to coronary disease; it is recommended that the authors look at these manuscripts to see what has already been done in this field (PubMed IDs 19750006, 21443790, 16433769)

Minor Revisions

- Please add label to Y axis in Figure 2, and label the peaks with corresponding gene names.

- Please use HUGO gene names for gene identification (e.g. SELL for L-Selectin, etc).

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

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
Employed by CardioDx, Inc.