A high-throughput approach for multi-omic testing for prostate cancer research

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INTRODUCTION
The proliferation of genetic testing technologies and genome-scale studies has increased our understanding of the genetic basis of complex diseases. However, this information alone tells an incomplete story of the underlying biology. Integrative approaches that combine data from multiple sources, such as the genome, transcriptome and/or proteome, can provide a more comprehensive and multi-dimensional model of complex diseases. Similarly, the integration of multiple data types in disease screening can improve our understanding of disease in populations. In a series of groundbreaking multi-omic, population-based studies of prostate cancer, researchers at the Karolinska Institutet in Stockholm, Sweden identified sets of genetic and protein biomarkers that when evaluated together with other clinical research data performed significantly better in predicting cancer risk (1,2) than the most widely used single protein biomarker, the prostate-specific antigen (PSA). These studies are the basis of what is referred to as the Stockholm3 model which can potentially increase the detection of aggressive cancers by 20% while reducing the number of unnecessary biopsies by 50% when compared to PSA testing alone. Like many multi-omic approaches, these studies used different platforms for nucleic acid and protein analysis, which add a layer of complexity to data collection and interpretation. In the current study, we have simplified and streamlined these processes by measuring both genetic and protein markers on the same platform, the Applied Biosystems QuantStudio™ 12K Flex Real-time PCR System. As in the previous studies, our workflow uses TaqMan® Genotyping Assays in OpenArray® Plates; however, we replaced the ISAC intra-assay technology with TaqMan® Protein Assays in 384 well plates. Both TaqMan® chemistries are optimized to maximize sensitivity and specificity and require only a blood draw research sample. In addition, we automated the liquid handling steps for the protein assays which further simplifies the workflow. This approach enables prostate cancer researchers to perform both large-scale studies and routine testing of genetic and protein markers on a single system.

MATERIALS AND METHODS
149 SNPs from the Stockholm3 studies were used in the current study, including 15 assays redesigned. Genotyping was performed in OpenArray plates on the QuantStudio 12K Flex Real-time PCR system using standard methods (Thermo Fisher Scientific, USA). Initial analysis of real-time PCR data was performed with TaqMan Genotyping Data Analysis Software (Thermo Fisher Scientific). Further analysis was performed with GAPP Software (Rigenera AB, Sweden). ID DNA control samples (Coriol, USA) were used for validating genotyping assays. A set of five TaqMan Protein Assays for prostate cancer (Thermo Fisher Scientific) were used to measure protein concentrations in EDTA plasma samples. Test samples were provided by the Karolinska Institutet Biobank. CCMD Software (Thermo Fisher Scientific) was used to determine protein concentrations. Protein concentrations obtained with the ISAC platform were provided by OncorGenomics AB (Sweden) for comparison.

RESULTS

Figure 2. Genotyping Workflow for OpenArray™ Plates

Figure 3. Genotyping Panel Content and Performance

Figure 4. TaqMan Protein Assays Use Proximity Ligation Assay (PLA)™ Technology

Figure 5. Protein Assay Workflow for 384-well Plates

Figure 6. Real-time PCR Results For FPSSA Assay Calibration Curve and Plasma Sample Set

Figure 7. Stockholm3 Model Comparison with ISAC or TaqMan Protein Assay Data for 24 Samples

Table 1. Protein Assay Content and Assay Ranges

Table 2. Protein Assay Uniformity (Intra-assay) and Precision (Inter-assay)

CONCLUSIONS
In this study we present the development of an integrated, multi-omic workflow that enables genetic and protein biomarkers for prostate cancer to be measured on the sample analytical platform, the QuantStudio 12K Flex Real-time/PCR System. Proteins are measured with TaqMan Protein Assay technology which has several advantages over traditional ELISA methods including, homogeneous (no wash) assay, low sample requirement (2μL), and broad dynamic range (~4-5 logs). Fully automated liquid handling further simplifies the protein assay workflow and reduces hands-on-time. When applied to a set of 24 high value samples, our workflow performed well in the Stockholm3 model, demonstrating good concordance with previously published results. While this workflow has been used specifically for prostate cancer research, it can be easily adapted for research of other diseases and model systems.

REFERENCES

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