

Preterm Birth Genome Project (PGP) – validation of resources for preterm birth genome-wide studies

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Abstract

We determined a series of quality control (QC) analyses to assess the usability of DNA collected and processed from different countries utilizing different DNA extraction techniques prior to genome-wide association studies (GWAS). The quality of DNA collected utilizing four different DNA extraction techniques and the impact of shipping DNA at different temperatures on array performance were evaluated. Fifteen maternal-fetal pairs were used from four countries. DNA was extracted using four approaches: whole blood, blood spots with whole genome amplification (WGA), saliva and buccal swab. Samples were sent to a genotyping facility, either on dry ice or at room temperature and genotyped using

Affymetrix SNP array 6.0. QC measured included extraction techniques, effect of shipping temperatures, accuracy and Mendelian concordance. Significantly fewer (50%) single nucleotide polymorphisms (SNPs) passed QC metrics for buccal swab DNA ($P < 0.0001$) due to missing genotype data ($P < 0.0001$). Whole blood or saliva DNA had the highest call rates (99.2 0.4% and 99.3 0.2%, respectively) and Mendelian concordance. Shipment temperature had no effect. DNA from blood or saliva had the highest call rate accuracy, and buccal swabs had the lowest. DNA extracted from blood, saliva and blood spots were found suitable for GWAS in our study.

Keywords: DNA; genetic analysis; prematurity; samples; single nucleotide polymorphisms (SNPs).

Introduction

Preterm birth (PTB), delivery at <37 weeks' gestational age, is a complex disease resulting from multiple pathophysiologic pathways. This complexity is due to pathophysiologic, environmental and genetic heterogeneity [8]. Our lack of understanding of an individual's risk (genetic, environmental, gene × gene and gene-environment interactions) has led to our failure to impact the rate of PTB because, at best, only generic "one size fits all" treatments have been applied. One relatively simple approach to address this issue is to consider genetic factors and how they interact with the environment in the pathophysiology of PTB in the design of individualized treatments.

Over the last decade, our understanding of the genetics of complex disease has increased substantially due in large part to the robustness of genome-wide association studies (GWAS) [7]. In contrast, genetic studies into PTB aimed at identifying individualized risk have had some success as they have focused on candidate gene studies in determining the risk of PTB [1, 3–5, 11, 13–17]. To overcome the shortcomings of candidate gene studies, GWAS have been proposed as an alternate approach to avoid bias in gene selection. The Preterm Birth Genome Project (PGP) is a consortium initiated by the Preterm Birth International Collaborative (PREBIC), March of Dimes (USA) and the World Health Organization (Geneva) to study genetic predisposition in PTB using GWAS [2]. Candidate gene associations have been reported widely in PTB, and PREBIC has recently summarized these data in a systematic review by Dolan et al. This report listed several positive and negative associations as well as data that were reproduced in multiple studies [5].

Based on the minimal and optimal phenotype data sets outlined by Pennell et al., this consortium has accumulated

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DNA samples from PTB studies across the globe, currently totaling more than 5000 PTB cases and 5000 term controls [11]. Unlike other consortia, the PGP is utilizing a single genotyping center for GWAS using DNA samples collected from multiple countries, samples and extraction methods. Although this approach has a number of advantages, including uniformity in genotyping, it requires validation in quality control (QC) across recruitment sites and methods to ensure that sample processing is of adequate quality to generate reliable data. Therefore, the PGP performed a series of QC analyses to assess the usability of DNA collected and processed from four different countries utilizing different DNA extraction techniques, prior to GWAS. In this report, we describe the data from phase 1 (QC phase) of the five phases proposed by the PGP consortium (www.prebic.net). Further, we have evaluated the impact of shipping at different temperatures on down-stream array performance. These studies are intended to inform clinical investigators, key players in GWAS level studies of PTB, as to the best practices for sample processing at their respective centers.

Method

Fifteen maternal-fetal pairs were identified from four countries (Korea, Denmark, Mexico and Canada) that met the criteria for utilization for GWAS within the PGP consortium. The DNA was extracted in each country from different biological samples: 1) whole blood (Korea); 2) blood spots with whole genome amplification (Denmark); 3) saliva utilizing the salivate for DNA collection (Mexico); and 4) buccal swab (Canada). The 15 maternal samples (M1–M15) from each country were aliquoted twice, with one set of aliquots shipped to the centralized genotyping facility at University of Western Australia (Perth) on dry ice and the other sent at room temperature. The 15 fetal samples from each country were all sent on dry ice. All samples were shipped via expedited delivery and reached the genotyping facility within 3 days from the date of shipment.

Fifty arrays were performed on samples from each country: M1–M15 shipped on dry ice, M1–M15 shipped at room temperature, M1–M5 shipped on dry ice (replicates) and F1–F15 shipped on dry ice. All samples had OD260:280 ratios between 1.8 and 2.0. Genotyping was performed using the Affymetrix genome-wide human SNP array 6.0 following standard protocols.

Standard QC procedures were applied to data before analyses, including: minor allele frequency >0.01 , maximum missingness in genotype calls <0.05 and Hardy-Weinberg equilibrium $P>0.0001$. A comparison of QC metrics was performed using χ^2 -analyses. Analyses for comparing extraction techniques were performed utilizing all samples shipped on dry ice (15 maternal samples, five maternal samples replicates and 15 fetal samples). Analysis investigating the effect of shipping temperature from the four countries compared 15 maternal samples shipped on dry ice with 15 paired maternal samples shipped at room temperature. Accuracy of genotyping calls was assessed using two techniques: 1) replication of M1–M5 samples shipped on dry ice from each country and 2) Mendelian concordance on maternal-fetal pairs shipped on dry ice. Call rates are presented as mean \pm standard deviation (SD). Comparisons of call rates between countries were performed using Kruskal-Wallis non-parametric analysis of variance. P values <0.05 were considered significant. DNA samples were collected from respective institutions under specific IRB approved protocols.

Results

The biological source of the DNA affected the number of SNPs passing QC metrics (Figure 1), with significantly fewer SNPs passing QC metrics when DNA was isolated from buccal swabs ($P<0.0001$; Canadian samples 506,607/906,600) compared to DNA from blood (Korean 638,981/906,600; Danish 694,584/906,600) or saliva (Mexican 757,863/906,600). The difference in QC metrics was primarily driven by a significant increase in missing genotype data in chips utilizing DNA derived from buccal swabs ($P<0.0001$). Using DNA extracted from buccal swabs, only 11 of the 35 arrays could be processed to completion due to poor DNA quality and samples failing Affymetrix QC metrics.

The highest average call rates were obtained from arrays where DNA was extracted from whole blood or from Salivette™ containers (whole blood 99.21 ± 0.36 vs. Salivette™ $99.32\pm0.23\%$; $P=0.314$; Table 1). Significantly lower call rates were obtained from DNA obtained from blood spots with WGA (Danish, 98.89 ± 0.40 ; $P<0.0001$) or DNA obtained from buccal swabs (Canada, 96.14 ± 1.19 ; $P<0.001$). Taken together, these data suggest that DNA from buccal swabs was the worst performing of the four options evaluated in this study.

The temperature of DNA shipment did not alter the call rates between samples from any country (Table 2). These data were reassuring given the costs incurred with shipment of samples on dry ice.

Differences in call rate accuracy, as assessed by replication of genotyping, reveal higher rates of accuracy in arrays using DNA extracted from blood or saliva, whereas there was a significantly higher rate of inaccuracy in calls of buccal swab derived DNA ($P=0.004$, Table 3). Similarly, when assessed for Mendelian concordance, accuracy was significantly less with DNA extracted from buccal samples ($P=0.0009$, Table 4) compared to DNA extracted from blood or saliva.

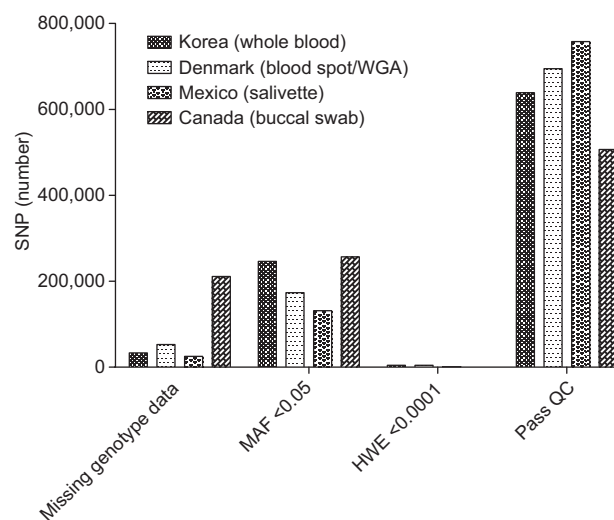


Figure 1 Comparison of QC metrics between DNA extraction techniques for GWAS.

Table 1 Comparison of extraction techniques.

Country of origin	DNA extraction technique	Arrays processed to completion*	SNPs passing QC metrics (%)	Call rate			Call rate% (Mean±SD)
				<98%	>98%	>99%	
Korea	Blood	33/35	638981 (71%)	0	33	34	99.21±0.36
Mexico	Salivette	34/35	757863 (84%)	0	41	17	99.32±0.23
Denmark	Blood spot + WGA‡	35/35	694584 (77%)	2	33	13	98.89±0.40
Canada	Buccal swab	11/35	506607 (56%)	11	0	0	96.14±1.19

*Objective to complete M1–M15 (dry ice), M1–M5 replicate (dry ice), F1–F15 (dry ice).

‡WGA, whole genome amplification.

Using DNA extracted from buccal swabs, only 11 of the 35 arrays were processed to completion due to poor DNA quality and samples failing Affymetrix QC metrics.

Discussion

DNA extracted from blood, Salivette™ and blood spots with whole genome amplification were found to be adequate and similar in outcomes in our pilot study. Our data suggest that DNA extracted from buccal samples were not suitable for GWAS analysis for the following reasons: 1) Only 11/35 samples passed the Affymetrix QC checks during sample preparation for GWAS and 2) the samples that qualified for GWAS had significantly lower call rates, reducing the number of informative analyses that could be performed.

Our data suggest that DNA obtained from blood samples provides the best combination of cost, call rate, accuracy and reproducibility. Although blood spots are cheaper to collect, easier to store and performed equally well on the arrays compared to DNA from whole blood, the process of whole genome amplification required for these samples is expensive and is technically challenging. Similarly, Salivette™ was effective at providing good quality DNA for GWAS analysis. Although this approach is more expensive than blood sample collection due to the cost of the sample containers, it is a non-invasive option and ideal for certain populations.

Our data indicate that DNA samples do not need to be shipped on dry ice. In our study, the shipping time was similar for dry ice and room temperature by design; therefore, we cannot address the effect of prolonged shipping of samples at room temperature in GWAS. Given the stability of DNA, we

do not anticipate that shipment time would be a major issue in most circumstances.

This study demonstrates that the source (whole blood, blood spots or saliva), extraction procedure and shipment temperature can have impact on the outcome of GWAS data in terms of accuracy and reproducibility. However, even the worst performing collection and processing method (buccal swabs) can yield a reasonably large amount of quality data if alternatives

Table 3 Assessment of accuracy using replication of genotyping for samples shipped on dry ice.

Country of origin	Pairs available for comparison*	Call rate% (Mean±SD)	Inconsistency replication% (Mean±SD)
Korea	5/5	99.21±0.36	0.26±0.22
Mexico	5/5	99.32±0.23	0.48±0.18
Denmark	5/5	98.89±0.40	0.47±0.55
Canada	4/5†	96.14±1.19	4.37±2.27‡

*M1–M5 samples were genotyped on two separate arrays for genotyping call comparisons.

†Using DNA extracted from buccal swabs, only four of the five pairs of arrays were processed to completion due to poor DNA quality and samples failing Affymetrix QC metrics.

‡P=0.004.

Table 4 Assessment of accuracy using Mendelian concordance.

Country of origin	Maternal-fetal pairs available for comparison*	DNA extraction technique	Mendelian concordance
Korea	15/15	Blood	99.95
Mexico	15/15	Salivette	99.96
Denmark	15/15	Blood spot+WGA	99.94
Canada	7/15	Buccal swab	99.30‡

*Paired comparison between M1–F1 to M15–F15 (all shipped dry ice).

Using DNA extracted from buccal swabs, only seven of the 15 pairs of arrays were processed to completion due to poor DNA quality and samples failing Affymetrix QC metrics.

‡P=0.0009.

Table 2 Comparison of shipping DNA on dry ice compared to room temperature.

Country of origin	Pairs available for comparison	Dry ice call rate% (Mean±SD)	Room temperature call rate% (Mean±SD)	Temperature comparison P-value
Korea	15/15	99.14±0.41	99.32±0.30	0.191
Mexico	15/15	99.32±0.22	99.35±0.16	0.945
Denmark	15/15	98.78±0.44	98.54±0.62	0.198
Canada	3/15†	96.91±0.43	96.45±0.43	0.400

†Using DNA extracted from buccal swabs, only three of the 15 pairs of arrays were processed to completion due to poor DNA quality and samples failing Affymetrix QC metrics.

are not feasible. Therefore, although providing evidence for differences among methods, our data provide support for study designs that pool existing resources and utilize common genotyping facilities for GWAS studies of pregnancy outcomes. In conclusion, our results inform clinical investigators as to best practices in studies of the genetics of preterm birth and related fields while guiding clinical researchers still justifying the use of less than optimally collected samples. Studies, such as ours can serve future research, especially in obstetrics, which is not yet substantially invested in genetic analyses. By using those methods that we demonstrated yield the best results, researchers can design approaches to sample collection that are the most cost effective in the long-term. However, our results also provide evidence that even sub-optimal methods can be used to minimize the need for new sample collection, thereby providing a cost effective strategy to perform GWAS studies on pregnancy outcomes utilizing existing resources.

Preterm birth is a complex disease [8, 6] and identification of risk factor(s) is of extreme importance for appropriate diagnosis and interventions.

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