Evaluation of GWAS chip-genotyping of fetal DNA extracted from umbilical cord tissue and WGA-DNA extracted from filter paper bloodspots

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The InterPregGen consortium is searching for maternal and fetal pre-eclampsia susceptibility genes by genome-wide association screening (GWAS). As part of this work we have extracted DNA from umbilical cord tissue and filter paper (Guthrie) blood spots and examined the suitability of these samples for chip-genotyping of fetal DNA.

Cord-DNA was extracted from 50-100mg of umbilical cord. DNA extracted from Guthrie spots was whole genome amplified (WGA) using the Illustra GenomiPhi-V2 kit (GE Healthcare). The subsequent DNA quality was assessed both by agarose gel electrophoresis and Sequenom genotyping. 36 cord-DNA samples and 18 WGA-DNA samples were genotyped using Illumina Human OmniExpress BeadChips. Genotyping quality was assessed by subject call rate (CR) and heterozygosity (HET), as well as by comparing fetal GWAS P-value distributions with distributions for case-control association (ASSOC) and Hardy-Weinberg Equilibrium (HWE) in 2000 pre-eclamptic mothers and 5000 controls already subjected to rigorous quality control.

Figure 1: Association results comparing GWAS p-value distributions

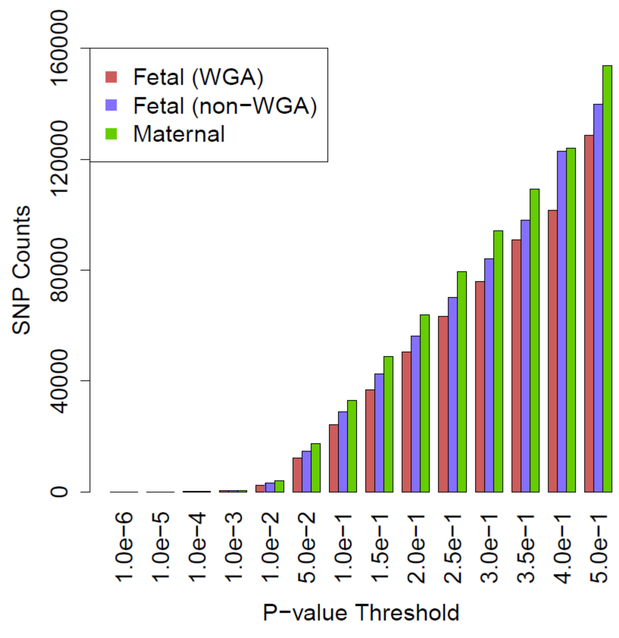


Figure 2: Call rate and heterozygosity for cord DNA and WGA-DNA samples.

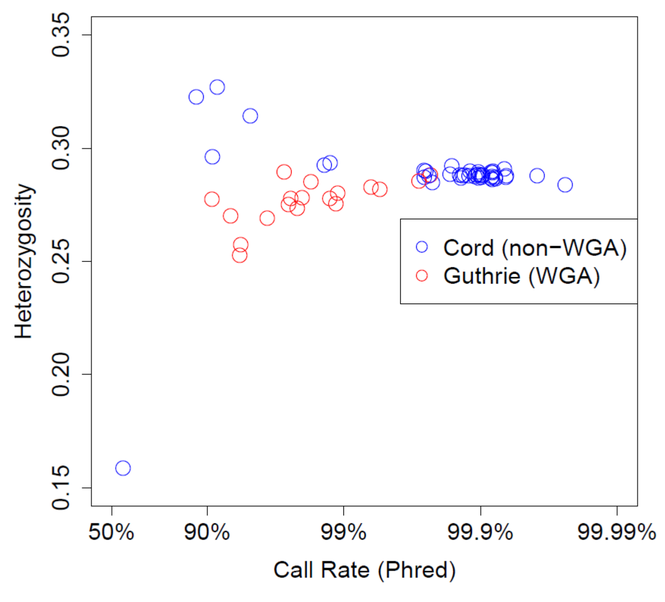
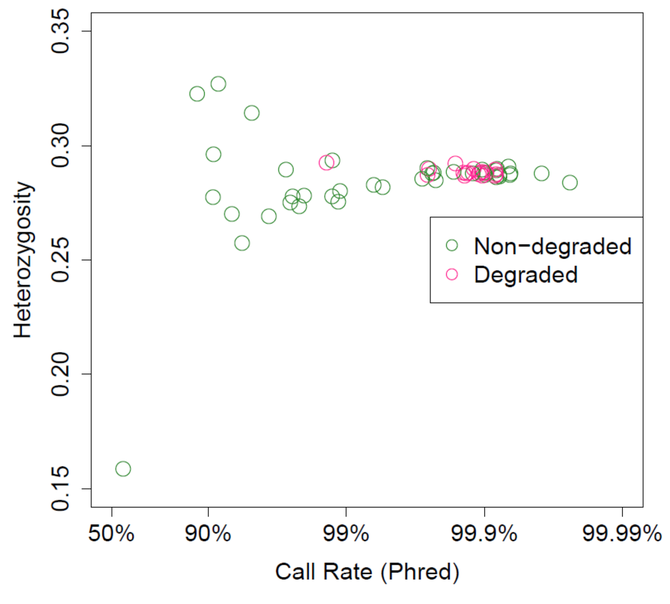


Figure 3: Call rate and heterozygosity for DNA assessed as degraded or non-degraded following agarose gel electrophoresis.



ASSOC and HWE distributions for cord- and WGA-DNAs with CR>95% did not show an excess of low fetal p-values and were similar to corresponding maternal-case distributions implying absence of frequent, widespread errors in genotypes of cord or WGA subjects (Figure 1). However, CR<95% occurred in 22% of WGA- versus 11% of cord-DNAs and lower CRs were associated with lower HET (Figure 2). Heterozygosity was significantly lower in WGA-DNA (0.277; SD 0.010) than cord-DNA (0.289; SD 0.007) (P<0.0001). DNA quality was assessed prior to any genotyping being carried out; this was done by visual assessment following agarose gel electrophoresis. There was no relationship between degradation assessed in this manner and the genotyping call rate (Figure 3). Cord-DNAs with CR <0.95 had passed initial QC checks; only 1 out of 18 degraded samples had CR <0.99.

Figure 4: Call rate and genotype concordance between 22 SNPs genotyped by Sequenom and on the GWAS chip.
(A) Cord DNA and WGA amplified DNA (B) DNA assessed as degraded or non-degraded following agarose gel electrophoresis.

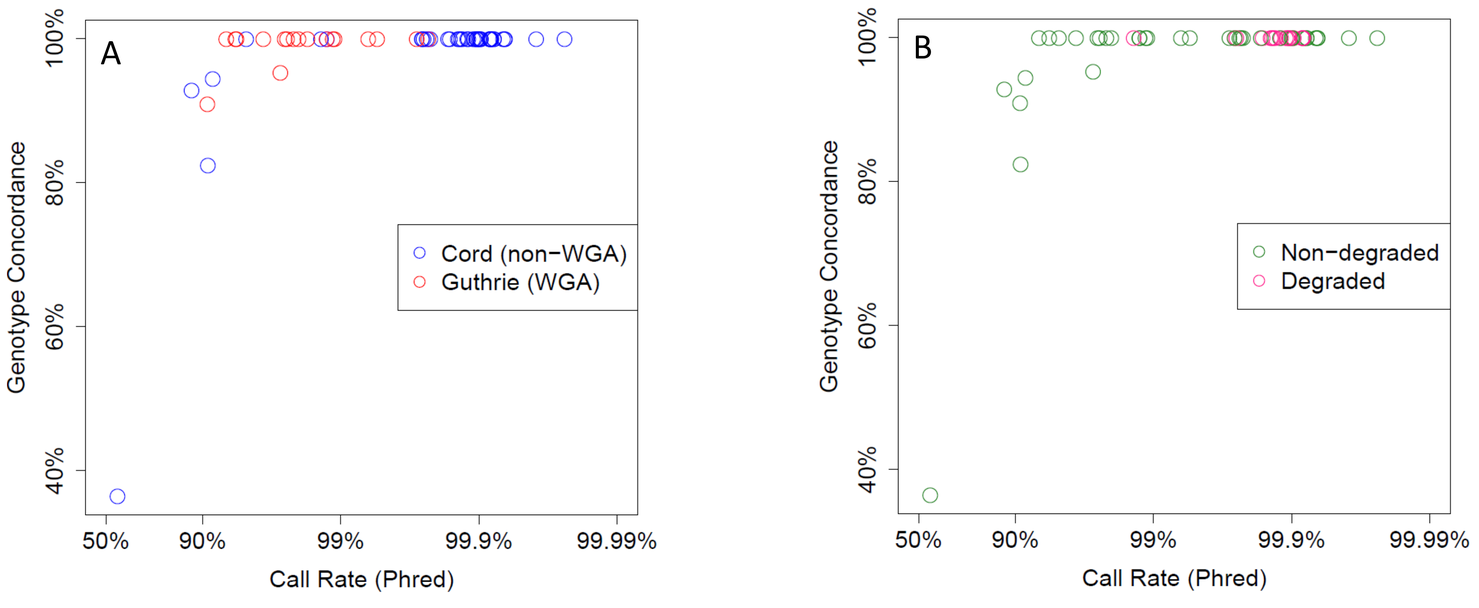
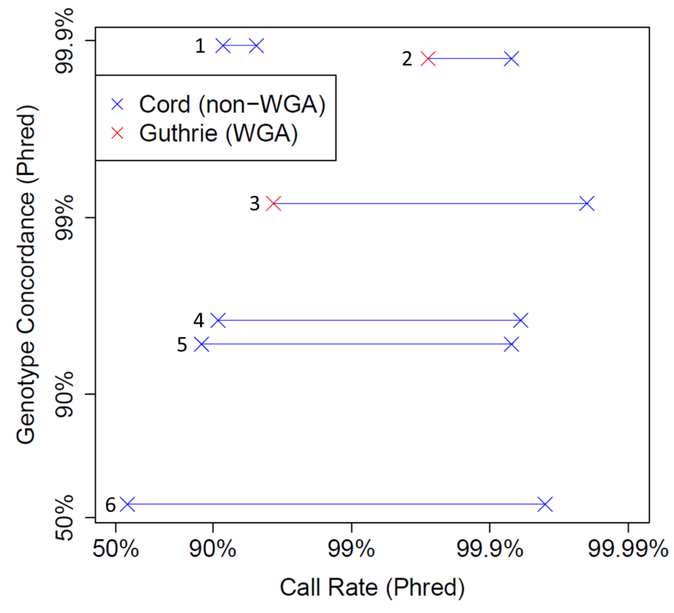


Figure 5: Call rate and genotype concordance between 6 replicate samples. Concordance rates are between all GWAS SNPs



As part of further QC checks carried out prior to genotyping using the GWAS chip, the samples were genotyped at 30 SNPs using Sequenom technology. Of these 30 SNPs, 22 are also present on the OmniExpress GWAS chip which allows assessment of genotype concordance between the two methods. This enables assessment of the accuracy of GWAS chip genotypes in the fetal samples. As expected, samples with a lower call rate also have lower levels of genotype concordance, although this only seems to be a problem with CR of around 90% or lower (Figure 4A). There was no relationship between genotype concordance and the visual assessment of degradation, with samples initially assessed as degraded still giving high genotype concordance (Figure 4B).

6 samples which gave low call rates when first genotyped using the GWAS OmniExpress chip were subjected to repeat genotyping. This allowed us to assess genotype concordance between these two experiments across all the GWAS SNPs. As shown in Figure 5, the call rate in the repeat experiment was generally higher than the initial attempt, suggesting that factors other than DNA quality were affecting the genotyping. As expected, the concordance was lower when the initial attempt gave a very low call rate (e.g. sample 6). This analysis also showed good levels of genotype concordance between DNA whole genome amplified after extraction from Guthrie spots and DNA extracted from cord samples from the same individual (samples 2 and 3).

This work illustrates that umbilical cord and whole genome amplified DNA are suitable for GWAS when call rates are >95%. However, WGA-DNA yields more samples with call rates <95% and with lower heterozygosity; these samples appear to have marginally higher genotyping error than the non-WGA samples. Visual checks of cord-DNA quality are poor predictors of chip-genotyping success, suggesting that DNA degradation does not have a large impact on this method.

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