NON-INVASIVE PRENATAL PATERNITY TESTING USING FETAL CELL FREE DNA FROM MATERNAL BLOOD

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Introduction: GSN presents here the first demonstration of non-invasive prenatal paternity testing using fetal cell-free DNA (cfDNA) found in maternal blood. The method involved measuring the genotypes present in the cfDNA found in maternal plasma at 317,000 genetic markers known as single nucleotide polymorphisms (SNPs). The SNP measurements were analyzed using an advanced informatics technology, Parental Support™, which was designed for single cell genotyping[1] in the context of *in vitro* fertilization (IVF) and non-invasive prenatal aneuploidy diagnosis.[2]

Background: Current methods of prenatal paternity testing are invasive, involving amniocentesis or chorion villus sampling. Non-invasive prenatal paternity testing from fetal cells isolated from maternal blood is complicated by the ability of nucleated fetal cells from previous pregnancies to persist in maternal blood for years.[3] In 1997, researchers showed that fetal cfDNA was present in maternal blood,[4] opening the prospect for the non-invasive detection of paternity. cfDNA is cleared from the mother's system within a few hours of birth, thus there is no chance that DNA from a previous pregnancy can interfere with the test results.[5] Until now, the low amount of fetal cfDNA in the maternal blood, combined with the fact that only a small percent of cfDNA is of fetal origin has prevented the reduction to practice of this concept.

Study Design: Twenty one samples of maternal blood from women with singleton pregnancies were collected at between six and 20 weeks gestational age, along with blood samples from the alleged father. Paternity was confirmed by either live birth follow up, products of conception sampling, or by control of fertilization by IVF. The cfDNA is isolated from the maternal blood was measured using the Illumina Infinium SNP microarray technology. Simultaneously, DNA from the mother and alleged father, found in the buffy coat fraction of the respective blood samples, were also measured using the SNP microarray technology to give a genetic fingerprint for the mother and the alleged father. The informatics algorithm known as Parental Support™ was then used to analyze the data by assessing whether the SNP identities found in the alleged father could account for the SNP profile observed in the cfDNA found in the maternal plasma containing fetal DNA. Specifically, Parental Support™ generated an individualized expected genotypic measurement distribution for a set hypothetical maternal plasma samples using the maternal genotype and the genotype of 800 unrelated potential fathers, and determined whether the measurements made on the actual maternal plasma sample can be excluded from that distribution (paternity inclusion) or not (paternity exclusion).

Results: Twenty out of 21 samples had the correct paternity confirmed, each with a p-value of $< 10^{-4}$. Each sample also had 800 incorrect fathers correctly excluded from paternity. One sample was a No Call due to low fetal fraction.

Conclusions: The current method was able to non-invasively determine the correct paternity for 20 women from maternal blood samples collected prenatally.

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