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Beyond screening for chromosomal abnormalities: Advances in non-invasive diagnosis of single gene disorders and fetal exome sequencing

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A B S T R A C T

Emerging genomic technologies, largely based around next generation sequencing (NGS), are offering new promise for safer prenatal genetic diagnosis. These innovative approaches will improve screening for fetal aneuploidy, allow definitive non-invasive prenatal diagnosis (NIPD) of single gene disorders at an early gestational stage without the need for invasive testing, and improve our ability to detect monogenic disorders as the aetiology of fetal abnormalities. This presents clinicians and scientists with novel challenges as well as opportunities. In addition, the transformation of prenatal genetic testing arising from the introduction of whole genome, exome and targeted NGS produces unprecedented volumes of data requiring complex analysis and interpretation. Now translating these technologies to the clinic has become the goal of clinical genomics, transforming modern healthcare and personalized medicine. The achievement of this goal requires the most progressive technological tools for rapid high-throughput data generation at an affordable cost. Furthermore, as larger proportions of patients with genetic disease are identified we must be ready to offer appropriate genetic counselling to families and potential parents. In addition, the identification of novel treatment targets will continue to be explored, which is likely to introduce ethical considerations, particularly if genome editing techniques are included in these targeted treatments and transferred into mainstream personalized healthcare. Here we review the impact of NGS technology to analyse cell-free DNA (cfDNA) in maternal plasma to deliver NIPD for monogenic disorders and allow more comprehensive investigation of the abnormal fetus through the use of exome sequencing.

1. Introduction

The presence of fetal cell-free DNA (cfDNA) in maternal blood has enabled the development of highly sensitive screening tests for fetal aneuploidy and diagnostic tests for fetal RHD type and monogenic disorders [1]. As cfDNA is a mix of maternal and fetal cfDNA and the fetal component emanates from the placenta [2], it may reflect cell lines that are confined to the placenta ('confined placental mosaicism') or that arise in the mother. Thus, when testing for aneuploidy, confirmation of an abnormal result by analysis of fetal genetic material obtained by invasive testing is required [3]. However, when testing for monogenic disorders, either because of a family history or because of suspicious ultrasound findings, analysis is targeted and is therefore diagnostic [4], circumventing the documented small risks associated with invasive testing. Until recently, subsequent to a huge commercial drive, non-invasive methods for prenatal testing have focused on screening for aneuploidy [5]. The use of this technology for the diagnosis of monogenic disorders has attracted less interest, largely being confined to the research arena as it represents a much smaller market opportunity and the majority of cases require development on a bespoke, patient or disease-specific basis using methods and workflows that are labour intensive and not readily scalable. Here, we review the progress that has been made to date, the different technologies and their associated challenges, and we show how we have established a clinical non-invasive prenatal diagnosis (NIPD) service in an accredited public sector laboratory.

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2. Non-invasive prenatal diagnosis

Prior to NIPD, prenatal diagnosis of genetic disease involved direct analysis of the fetal genetic material obtained using chorionic villus sampling or amniocentesis, procedures that may be unpopular due to the small associated miscarriage risks [6]. NIPD is therefore an attractive option eliminating these risks and evidence suggests that there will be a high uptake, particularly by parents who have declined, or would decline, invasive testing [7,8]. Cell-free DNA in maternal plasma is predominantly maternal in origin, with the fetal proportion, or fraction, varying both between and within pregnancies, often increasing as pregnancy progresses and influenced by a variety of factors including maternal smoking, maternal weight, and pre-eclampsia [9]. Thus, initial goals in NIPD focused on the identification of variants inherited from the father [10], such as determination of fetal sex or *RHD* status, and the detection of de-novo mutations. The advent of nest generation sequencing (NGS), and notably desk-top sequencers, has allowed expansion of NIPD to include the development of larger gene panels [4,7] and more complicated genetic analysis for diagnosis of recessive, X-linked and dominant conditions inherited from the mother [11,12].

2.1. Autosomal dominant de-novo and paternally inherited alleles

The first success of NIPD for monogenic disorder diagnosis was the exclusion or identification of paternally inherited variants and the identification of de-novo variants. Thus, in conditions that are caused by the inheritance of a paternal mutation in autosomal dominant disease, the presence of the paternal mutant allele would confirm a definitive diagnosis in the fetus. Conversely, its absence in recessive disease would confirm an unaffected carrier or normal fetus. Gonzales-Gonzales used polymerase chain reaction (PCR) and restriction analysis to identify a cystic fibrosis)-causing mutation in maternal plasma that was paternally inherited. However, the high background of maternal DNA contamination meant that they could only detect the paternal mutation and could not assess the likelihood that the fetus also carried the maternal mutation, fundamental in diagnosis of recessive disease where the fetal genotype might be compound heterozygous [13]. Li et al. had early success in using NIPD for beta-thalassemia in identifying point mutations that were inherited from the father. They reported high sensitivity (100%) and specificity (93.8%) by size-fractionating cfDNA in maternal plasma followed by a PCR approach that did not amplify the normal maternal allele. The paternal mutant allele was confirmed as either present or absent using allele-specific real-time PCR [14].

2.1.1. Polymerase chain reaction with restriction enzyme digest

Polymerase chain reaction with restriction enzyme digest (PCR-RED) was in use clinically on a research basis from around 2006 for NIPD for a small proportion of mutations in the *FGRF3* gene that cause achondroplasia or thanatophoric dysplasia [4,15]. PCR-RED, which uses restriction enzymes to cleave DNA at a specific site to include the sequence containing the mutation, gave a rapid and cheap method requiring analysis of agarose gel images (Fig. 1). However, as interpretation may vary between operators, it had an inconclusive rate of around 8%, and was not universally adaptable and applicable to all mutations [5]. In addition, PCR-RED can only detect one mutation at a time, and whereas this may be appropriate when offering NIPD in pregnancies at risk of a known mutation, this is not practical when offering prenatal diagnosis in pregnancies with ultrasound abnormalities that may be caused by a number of different mutations. For example, a fetus found to have short limbs and a small chest with features compatible with thanatophoric dysplasia could have any one of around 12 mutations, not all of which are amenable to developing a PCR-based assay [15]. Despite these limitations, PCR-RED was introduced into clinical practice in the UK National Health Service (NHS) in 2013 (Table 1).

2.1.2. Next generation sequencing

More recently, the use of NGS has afforded superior approaches to NIPD for paternally inherited and de-novo dominant disease through the use of panels designed to detect multiple mutations.

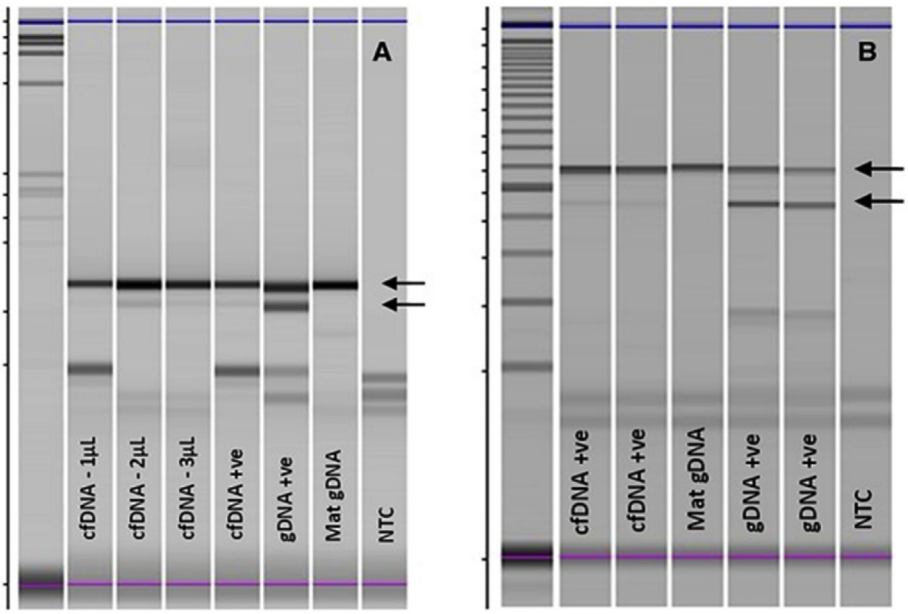

Fig. 1. Polymerase chain reaction–restriction enzyme digest data for (A) achondroplasia [fibroblast growth factor receptor 3 (*FGFR3*): c.1138G→A p.(Gly380Arg)/restriction enzyme BsrG1] and (B) thanatophoric dysplasia [*FGFR3*: c.742C→T p.(Arg248Cys)/restriction enzyme DraIII]. Upper arrows show normal allele is evident in all samples. Lower arrows show mutant alleles lighter in affected cell-free DNA (cfDNA+) and stronger mutation-positive control genomic DNA (gDNA + ve). Normal maternal genomic DNA (Mat gDNA) shows no band. Adapted from Chitty et al. [4].

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