Editorial

Cell-free fetal DNA: the new tool in fetal medicine

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Introduction

Since the discovery of cell-free fetal DNA (cffDNA) in maternal plasma in 1997[1] there has been rapid progress in harnessing this as a source of fetal genetic material for prenatal diagnosis. The majority of cell-free DNA (cfDNA) is maternal in origin[2], with the fetal proportion emanating from the placenta[3], detectable in the maternal circulation from around 5 weeks' gestation[2] and constituting around only 10% of cfDNA in early pregnancy[4]. However, as cffDNA is cleared rapidly from the maternal circulation after delivery, it offers great potential as a source of fetal genetic material for prenatal diagnosis[5]. Initially, in view of the high background of maternal cfDNA, technological restrictions only enabled the detection or exclusion of alleles that were not present in the mother but were present in the fetus because they were paternally inherited or arose *de novo* at conception. Thus, early indications were for fetal sex determination using Y-chromosome alleles[6], fetal Rhesus D (RhD) genotyping in RhD-negative mothers[7,8] or for the diagnosis of certain genetic conditions, such as achondroplasia[9], in which the majority of cases arise as a result of a new mutation. Technological advances associated with the development of next-generation sequencing (NGS) have enabled accurate counting of DNA sequences that are associated with specific chromosomes[10,11] present in maternal blood, which has allowed very rapid development of non-invasive prenatal testing (NIPT) for aneuploidy[12]. Furthermore, quantification of cffDNA may also be useful in the early identification of pregnancies at risk of other adverse outcomes, such as pre-eclampsia and fetal growth restriction (FGR)[13,14].

These developments are delivering the biggest change seen in antenatal care over the last few decades, as the need for invasive diagnostic testing reduces dramatically. It is also likely that they will impact on the need for some therapeutic interventions, such as *in-utero* fetal transfusion, as well as offering a new diagnostic tool in fetal medicine for diagnosis of a dysmorphic fetus and earlier diagnoses in pregnancies at prior risk of a genetic disorder. Here, we review the potential of cffDNA, highlighting its use in fetal medicine, and discuss how it is impacting on the practice of fetal medicine.

X-linked disorders and disorders of genital ambiguity

The earliest clinical use of cffDNA was for the determination of fetal sex[1]. This relies on the detection of sequences, SRY or DYS14, in the maternal plasma that derive from the Y-chromosome. The technique has already become incorporated into standard care in several European countries, including the UK, for management of pregnancies at risk of severe X-linked genetic disorders[15,16], such as Duchenne muscular dystrophy. It has the potential to reduce the incidence of invasive testing for such conditions by up to 50% by allowing targeted testing in male-bearing pregnancies[16]. In pregnancies at risk of congenital adrenal hyperplasia, determining fetal sex can enable early cessation of steroid treatment in male-bearing pregnancies or, as occurs in several centers, steroid administration could be delayed until fetal sex is determined through early cffDNA testing and only offered in pregnancies in which the fetus is known to be female[17]. However, close co-ordination with fetal-medicine services is required as testing is only reliable after 7 weeks' gestation, with false-positive results possible in twin pregnancies or in those with early fetal demise of a cotwin[16].

Ambiguity of the genitalia is a rare finding on ultrasound, and even with the advent and improvement of three-dimensional (3D) imaging techniques, differentiation between clitoromegaly in the female fetus and hypospadias in the male remains difficult (Figure 1). In cases in which genital ambiguity is isolated and cffDNA testing indicates that the fetus is male, the most likely diagnosis is hypospadias, although some rare endocrine disorders cannot be excluded completely without sequencing of the androgen receptor gene (Table 1). If, however, cffDNA testing indicates that the fetus is female genetically, referral to a team specialized in disorders of sexual development is advised as abnormalities in SRY can cause disorders of sexual differentiation and multiple markers should be assessed for determination of fetal sex in these cases (Table 1)[15]. Another relatively common association with hypospadias is FGR; therefore, maternal uterine artery Doppler examinations should be performed and, if Down syndrome screening is performed, maternal serum biomarker results should be reviewed for low levels of pregnancy-associated plasma protein A (PAPP-A) and high levels of human chorionic gonadotropin (hCG) or α-fetoprotein levels (Table 1).

The use of cffDNA for sex determination can be an extremely useful aid to sonographic diagnoses of a number of genetic syndromes that present with multiple
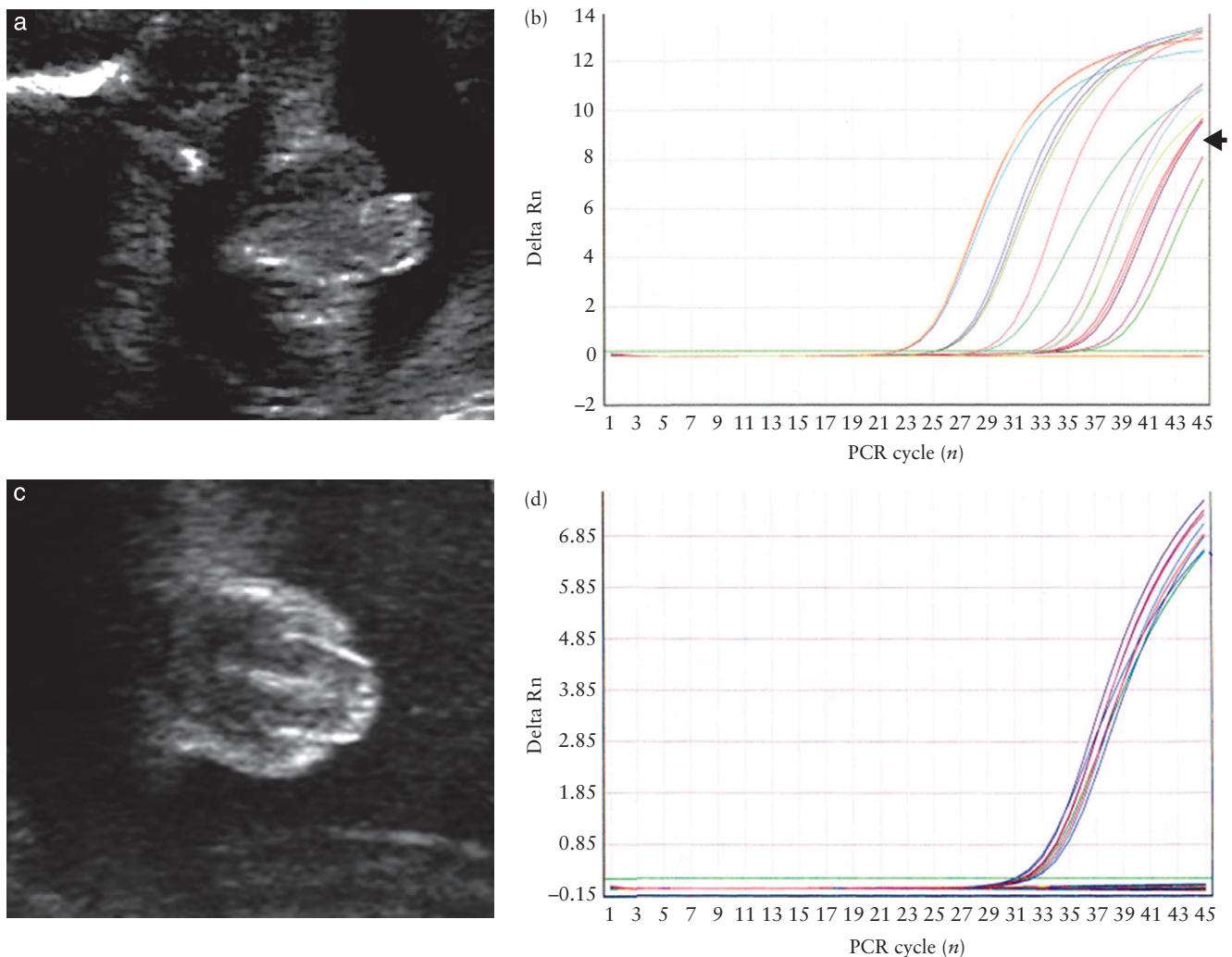
Figure 1 Genital ambiguity in a male fetus (a), as evidenced by amplification (arrow) of SRY sequences in cell-free fetal DNA (cffDNA) (b), and in a female fetus (c), in which there is amplification only of the control DNA sequences (d). PCR, polymerase chain reaction; Rn, normalized reporter.

abnormalities, usually genital ambiguity in which determination of genetic sex can be diagnostic when combined with the presence of other relevant sonographic findings (Table 2). For example, in cases of campomelic dysplasia, which presents with varying degrees of lower limb shortening and bowing, talipes and micrognathia, at least 50% of affected male fetuses have genital ambiguity or complete sex reversal. Therefore, if a fetus has ambiguous or female genitalia with these sonographic features and cffDNA testing indicates a genetic female, the diagnosis can be made (Table 2 and Figure 2). Non-invasive fetal sex determination using cffDNA can also be very useful in the presence of some urogenital anomalies, such as bladder cloacal exstrophy, as knowledge of the genetic sex can aid counseling with regard to long-term outcome[18].

Cell-free fetal DNA and management of complications arising from blood-group antigens

The second clinical application of cffDNA testing was for the determination of fetal RhD status in pregnant RhD-negative mothers[19]. As with fetal sex determination, this is possible because an RhD-negative mother does not produce any copies of the RhD gene (*RHD*), and thus the *RHD* identified in maternal blood originates from the fetus who has inherited the gene from the father. For the past decade, fetal *RHD* genotyping in RhD-negative women with significant titers of anti-RhD immunoglobulin has been possible using labor-intensive polymerase chain reaction (PCR)-based methods[19,20]. This approach to management of these high-risk pregnancies has avoided the need for invasive testing that was required previously. In addition to avoiding the associated risks of miscarriage, NIPT circumvents the need for assessment of paternal phenotype, which may not be known or available. If the fetus is RhD positive, increased surveillance in a tertiary center to monitor for the development of fetal anemia or hydrops is required, whereas if the fetus is predicted to be RhD negative, there is no risk of hemolytic disease of the newborn (HDN) and standard antenatal care is appropriate[21].

Table 1 Clinical management of fetuses with isolated genital ambiguity based on the cell-free fetal DNA (cffDNA) result

cffDNA result	Differential diagnosis	Other aids for management
Male	Isolated hypospadias	Look for markers of FGR:
	FGR	review PAPP-A, hCG and MSAFP results if available
	Inadequate production of testosterone because of Leydig cell hypoplasia or rare abnormalities of steroid metabolic pathway	Review maternal Doppler Consider referral to DSD team for sequencing of androgen receptor gene
	Partial androgen insensitivity syndrome	
	5α-reductase deficiency	
	True hermaphrodite	
Female	Congenital adrenal hyperplasia	Refer to DSD team for further investigations
	21-hydroxylase deficiency	Amniotic steroid levels
	11-hydroxylase deficiency	Maternal serum androgen levels
	3β-hydroxysteroid dehydrogenase deficiency	Maternal urinary estrogen levels
	True hermaphrodite	Maternal ovarian scan for multicystic change
	Maternally derived androgens (e.g. luteoma of pregnancy)	
	Placental aromatase deficiency	

DSD, disorders of sexual development; FGR, fetal growth restriction; hCG, human chorionic gonadotropin; MSAFP, maternal serum alpha-fetoprotein; PAPP-A, pregnancy-associated plasma protein A.

Introduction of high-throughput technologies for mass fetal *RHD* genotyping[22] has provided potential for routine fetal genotyping and targeted administration of anti-D immunoglobulin, a human blood product. Currently, many countries offer routine antenatal prophylaxis to all RhD-negative mothers. This has significantly decreased the incidence of Rhesus sensitization, and cases resulting in fetal anemia are now rare. However, this policy results in the unnecessary administration of anti-D immunoglobulin to around 38% of RhD-negative women who are carrying a RhD-negative fetus[23]. Routine fetal *RHD* genotyping and targeted anti-D prophylaxis have been introduced recently into routine obstetric care at 26–28 weeks' gestation for RhD-negative women in The Netherlands and Denmark[24,25]. However, a UK study has shown that high-throughput *RHD* genotyping is highly accurate from 11 weeks' gestation[26]. Introduction at this earlier stage in pregnancy would result in further avoidance of administration of anti-D immunoglobulin for sensitizing events that occur in early pregnancy. Subsequently, there have been calls for implementation of high-throughput *RHD* genotyping into routine antenatal care in the UK[27]. Current routine immunoprophylaxis programs do not achieve complete uptake as some women decline anti-D immunoglobulin treatment. Routine fetal *RHD* genotyping is likely to be very acceptable to women[28] and may improve immunoprophylaxis uptake, thereby targeting women at highest risk, causing a further decline in rates of alloimmunization and, subsequently, the need for *in-utero* transfusion.

Although anti-RhD is the most common cause of HDN, other antibodies, in particular anti-c and anti-K (and less commonly anti-C and anti-E), are responsible for an increasing proportion of cases. Unlike anti-D, there is no prophylaxis, so the use of cffDNA as routine screening is unlikely; however, it remains an important investigation in sensitized pregnant women. The accuracy of fetal genotyping in these cases approaches 100%, thereby obviating the need for invasive testing in these pregnancies[21,29,30].

Fetal or neonatal alloimmune thrombocytopenia (FNAIT) is caused by production of maternal alloantibodies directed against paternally inherited antigens present on fetal platelets[21]. Complications include intracranial hemorrhage, occurring in up to 20% of cases, which may cause severe long-term consequences to the child. Until recently, in cases with a heterozygous father, invasive testing was required to determine whether the fetus was affected by FNAIT, which can occur only if they are positive for human platelet antigen-1a (HPA-1a). Analysis of cffDNA in maternal blood can detect the HPA-1a gene[31,32], which again avoids the need for invasive testing in women with a heterozygous partner.

Non-invasive prenatal testing for monogenic disorders

The use of cffDNA in the detection of monogenic disorders is considerably more challenging technically than fetal *RHD* genotyping or sex determination and is currently in clinical use only for the detection of alleles that have arisen *de novo* at conception, for example, achondroplasia[9], or that are inherited from the father[33]. In recessive or X-linked conditions for which the mother also carries the mutant allele, the high background of maternal mutation present in her plasma outweighs any fetal mutation and thus methods reliant on the detection of small differences in the ratio of mutant-to-wild-type alleles are required. Accurate estimation of this ratio is also dependent on the proportion of fetal DNA in the maternal plasma, the fetal fraction, and this can only be assessed consistently in male-bearing pregnancies as it requires measurement of an allele not present in the mother[34,35] or by use of the differential methylation in fetal and maternal DNA[36]; as yet, the reliability of these methods for use in routine clinical practice requires further development and evaluation. It is likely that this will become possible universally in the future, perhaps by exploiting the fact that fetal DNA is shorter than maternal cfDNA[37]. In recessive conditions, whereby the parents carry different allele mutations, exclusion or detection of

Table 2 Examples of genetic syndromes for which sex determination using cell-free fetal DNA (cffDNA) or targeted non-invasive prenatal testing for single-gene disorders may aid ultrasound diagnosis

Syndrome	Genitalia	Other sonographic findings	Differential diagnosis	cffDNA result	Other aids for diagnosis
Bardet–Biedel	Ambiguous/ hypospadias	Large echogenic kidneys, polydactyly	Trisomy 13	Male	Family history, consanguinity
Smith-Lemli–Opitz	Ambiguous	Polysyndactyly, cardiac and CNS anomalies, FGR, cleft lip, microcephaly	Other genetic syndromes, trisomy 13	Male	Family history, consanguinity, maternal urinary steroids
Malpeuch syndrome	Ambiguous	Cleft lip, FGR, renal anomalies	Trisomy 18	Male	Family history, consanguinity
Campomelic dysplasia	Ambiguous/ female	Short bowed lower limbs, talipes, cardiac anomalies, micrognathia	Osteogenesis imperfecta Type III/IV	Male	
Achondroplasia*	Normal	Rhizomelic shortening of long bones at >24 weeks, bowed femora, frontal bossing, relative macrocephaly, trident hands, polyhydramnios (small chest)	Down syndrome, Acromesomelic dysplasia, Hypochondroplasia	FGFR3 mutn	Normal limb length at <24 weeks' gestation
Thanatophoric dysplasia	Normal	Early-onset shortened long bones, bowed femora, short ribs, small chest, trident hands, frontal bossing, relative macrocephaly, clover-leaf skull, CNS anomalies, polyhydramnios	Short-ribbed polydactyly syndromes, Jeunes asphyxiating thoracic dystrophy, Spondyloepiphyseal dysplasia congenita	FGFR3 mutn	
Apert syndrome	Normal	Abnormal skull shape, mitten hands and feet	Other craniosynostosis syndromes	FGFR2 mutn	

*Shortened long bones may be the only presenting feature as this condition often presents in the third trimester when good visualization of other features can be difficult. CNS, central nervous system; FGFR2/3, fibroblast growth factor receptor 2/3 gene; FGR, fetal growth restriction; mutn, mutation.

the paternal allele can be used to refine the risk to the fetus, excluding risk if the paternal allele is not detected in maternal plasma and increasing risk if the paternal mutation is identified. In the latter situation, an invasive test is required to determine whether or not the fetus has inherited the maternal allele. This has recently been reported in conditions such as thalassemia[38] and cystic fibrosis[39].

Definitive diagnosis of achondroplasia[9] and thanatophoric dysplasia[40] by NIPT has been available on a research basis since 2007 and was approved for use in routine clinical practice in the UK in 2012. The early tests were based on restriction-enzyme-digest-PCR methodology, whereby mutations had to be tested for individually. This is labor intensive, slow and costly, and the results can be difficult to interpret (Figure 3). The advent of NGS has allowed the development of gene panels for use in NIPT[33] that allow all possible disease-causing mutations to be tested for simultaneously and a digital result enables easier interpretation. This has greatly enhanced the utility of NIPT for conditions such as thanatophoric dysplasia, of which there are multiple possible mutations and all cases arise *de novo* as this is a lethal dominant condition. NIPT can be a very useful aid to clinical management of skeletal dysplasias. Thanatophoric dysplasia is detected increasingly in early pregnancy when the differential diagnosis includes the short-ribbed polydactyly syndromes and other autosomal recessively inherited conditions associated with a high recurrence risk[41] (Table 2), whilst thanatophoric dysplasia is a new dominant mutation with a low recurrence risk. If positive for thanatophoric dysplasia, NIPT can deliver a definitive diagnosis without the need for invasive testing and allows the option of a surgical termination, as a postmortem will not be required. Additionally, NIPT can allow the safe differentiation of thanatophoric dysplasia from achondroplasia, both of which arise from mutations in the fibroblast growth factor receptor 3 gene (*FGFR3*), but the former is lethal and the latter is the most common viable short-stature syndrome. Distinguishing between these two conditions can be challenging as they have many common features, such as frontal bossing, relative macrocephaly, short limbs, bowing of the femora, small chest and short fingers giving rise to the 'trident' hand appearance; these features are all more extreme in thanatophoric dysplasia. NIPT can also offer safer diagnostic testing in twin pregnancies with fetuses discordant for the abnormalities as it permits a safe definitive diagnosis whilst avoiding the risk of miscarriage of the normal fetus. In addition, it allows for conservative management of pregnancy in lethal conditions with no requirement for termination of pregnancy. In cases presenting at risk of achondroplasia late in pregnancy, NIPT allows for definitive diagnosis and accurate parental counseling without the risk of precipitating preterm labor. In all cases, it also allows for an early, safe, non-invasive test to exclude recurrence or inheritance of a paternal mutant allele in future pregnancies. This can be performed from
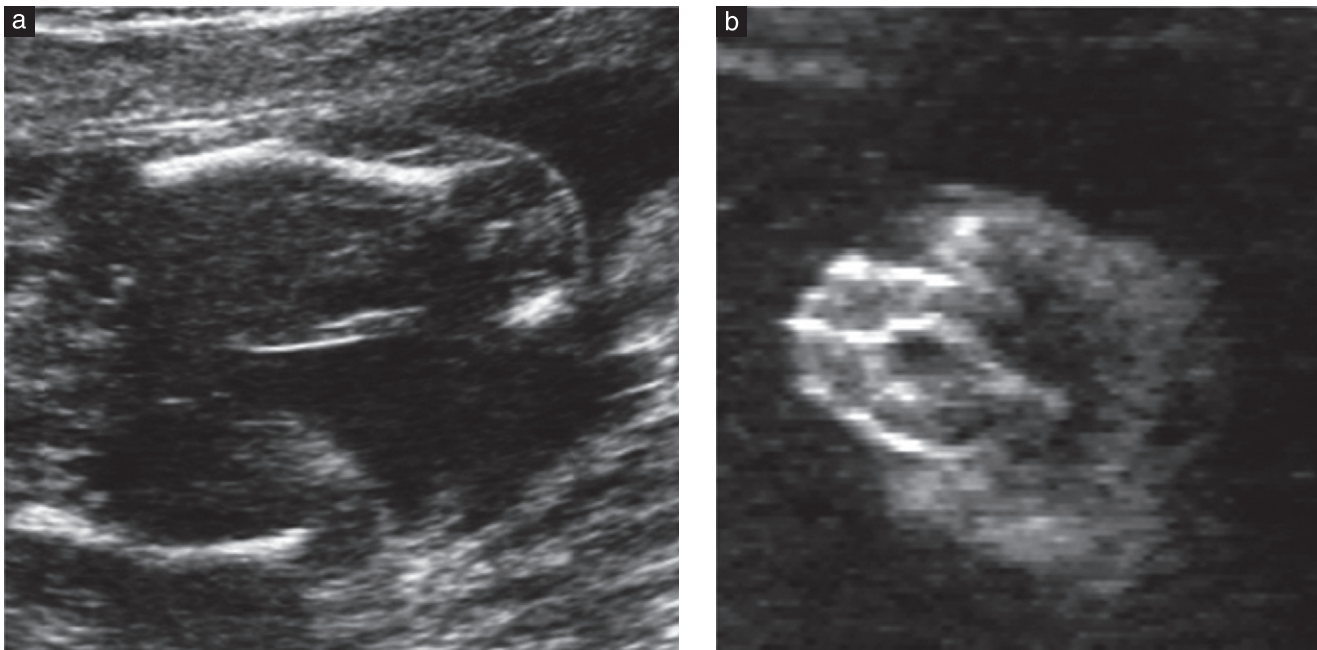
Figure 2 Features of campomelic dysplasia detectable on ultrasound include shortened 'bowed' limbs (a) and ambiguous genitalia (b).

9 weeks' gestation, earlier than invasive testing, which cannot be performed safely until 11 weeks[42,43], and much earlier than an ultrasound scan for women not wanting to put the pregnancy at risk by an invasive test.

The use of cffDNA for the diagnosis of monogenic disorders has great potential. In the UK it has been approved for use in clinical National Health Service (NHS) practice to screen for mutations in the *FGFR3* (achondroplasia and thanatophoric dysplasia) and *FGFR2* (Apert syndrome) genes and for paternal exclusion of common cystic fibrosis mutations. With the introduction of these safer tests we are seeing a dramatic decrease in the use of invasive testing for monogenic disorders (Table 3), with a further decline in the likely need for invasive testing as more non-invasive tests are developed and validated.

Cell-free DNA testing for aneuploidy

The use of cfDNA testing for aneuploidy is bringing the most radical change to the practice of fetal medicine. Early attempts at providing NIPT for Down syndrome relied on quantifying the amount of placenta-specific 4 (*PLAC4*) in the maternal plasma[44]. This gene originates from chromosome 21, is expressed only in the placenta and is therefore fetal in origin. By detection of two separate alleles in a 1:1 ratio, the fetus can be assumed to be euploid. If there is duplication of an allele (because of an extra copy of chromosome 21), the ratio will be 2:1 and the fetus can be assumed to be trisomic for chromosome 21. Although groundbreaking, this initial approach was flawed as it required the identification of genetic differences in the parents and was therefore only applicable in around 40% of pregnancies. The advent of NGS brought a new approach that could be applied universally. Rather than detection of a gene product derived from chromosome 21, all DNA circulating in maternal plasma is sequenced. This, by definition, includes both maternal and fetal DNA, and sequencing will analyze the entire fetal and maternal genome. There are three NGS-based approaches to NIPT for aneuploidy: whole-genome NGS; targeted NGS; and single nucleotide polymorphisms (SNPs). The whole-genome approach[10] requires sequencing of cfDNA from maternal plasma to generate millions of short sequence reads from the whole genome. These are then mapped to a reference human genome sequence to determine from which chromosome the fragment is derived; the number of fragments mapped uniquely to the chromosome of interest are then counted and compared with the number of counts obtained from other chromosomes. A variety of bioinformatics algorithms have been developed[10,45,46] to determine whether there is an increase or decrease in the expected number of counts around a set threshold which is suggestive of aneuploidy; for example, if the fetus has trisomy 21, more fragments from chromosome 21 will be present than expected in maternal plasma. Alternative NGS approaches involve the selective amplification of specific genomic loci on the chromosome of interest followed by sequencing. This approach may be more economical as the amount of sequencing required is reduced but has the limitation that only the preselected regions of interest can be studied and the development of these tests is potentially more labor intensive[12], although it does allow estimation of fetal fraction. Early studies validated this approach for the detection of trisomies 21 and 18 in high-risk pregnancies[47] but, more recently, the results of a large general-population study have shown similar high performance in pregnancies at low prior risk[48]. The third approach, a variation of the
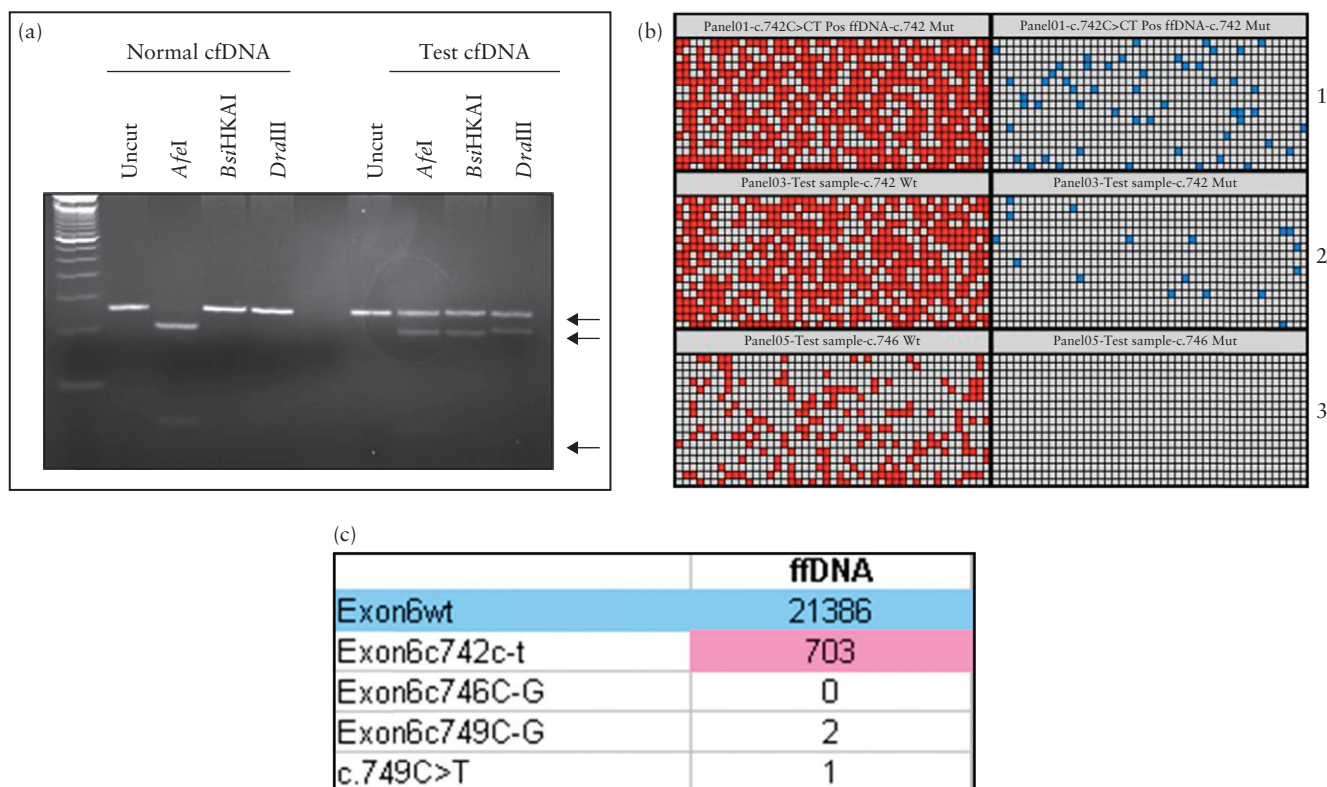
Figure 3 Detection of a mutation in the fibroblast growth factor receptor 3 (*FGFR3*) gene causing thanatophoric dysplasia, showing the increasing ease of interpretation between polymerase chain reaction (PCR)-based method (a), digital PCR (b) and digital readout obtained from sequencing (c). PCR-based method (a) relies on subjective interpretation; very faint bands for mutant alleles in affected cell-free (cf) DNA can be seen (bottom arrows). The wild-type (normal) allele is strongly present in all samples (upper arrow). This compares with digital PCR (b) for detection of the mutant allele c.742 C > T (blue dot) and wild-type alleles (red dot). Each row represents one sample. Wild-type signals are present in all samples but the mutant allele is only present in the positive control (panel 1) and test sample (panel 2). Panel 3 is the result obtained from a normal pregnancy and shows only wild-type alleles present. The digital readout obtained from sequencing (c) reveals a very high wild-type allele count (blue), as this represents both maternal and fetal alleles, and a lower mutant allele (pink) count, but is still very high compared with the counts for other disease-causing mutations, indicating that the fetus has thanatophoric dysplasia as a result of the c.742 C > T mutation.

targeted approach, is based on the amplification of large numbers of polymorphic loci (SNPs) on the chromosome of interest[49]. Sensitivity and specificity for the detection of common aneuploidies are high for whole-genome, targeted and SNP approaches, irrespective of the sequencing platform or bioinformatic algorithms used[12].

Since the first reports describing the use of NGS for NIPT to detect trisomy 21[10,11], the pace of development has been extremely rapid and entirely commercially driven. NIPT is now available in the private sector in more than 50 countries, with detection rates in excess of 99% for trisomy 21 and slightly lower for trisomies 18 and 13, at around 96% and 92%, respectively[50,51]. Sensitivity for detection of sex-chromosome aneuploidy remains lower, at 88.6%, with a false-positive rate of 0.12% for monosomy X[50,51]. NIPT for the detection of aneuploidy has been shown to be a highly effective test in both high-risk[12] and low-risk[48] pregnancies.

Although more accurate than conventional combined screening (using nuchal translucency, PAPP-A and hCG) in the first trimester[48], NIPT should still be regarded as a highly sensitive screening test, rather than a diagnostic one[52–54], and any positive result should be confirmed by

Table 3 Shift from invasive to non-invasive prenatal testing (NIPT) for achondroplasia and thanatophoric dysplasia in the UK from 2009 to 2013, as tests became validated and approved for use in the North East Thames Regional National Health Service genetics laboratory

Year	Achondroplasia		Thanatophoric dysplasia	
	Invasive	NIPT	Invasive	NIPT
2009–2010	28	0	16	0
2010–2011	27	13	21	0
2011–2012	28	14	25	2
2012–2013	20	22	17	11
2013–	10	14	7	18

Data are given as *n*. Other conditions for which NIPT has been performed in high-risk families include Apert syndrome (*n* = 7), Crouzon syndrome (*n* = 2), Fraser's syndrome (*n* = 4), autosomal polycystic kidney disease, osteogenesis imperfecta (*n* = 2) and cystic fibrosis.

invasive testing[52–54], ideally by amniocentesis or, at minimum, karyotyping on cultured chorionic villi to avoid confined placental mosaicism. This is because of increasing, well-documented evidence of discordant results (mostly false positive, but false-negative results have also
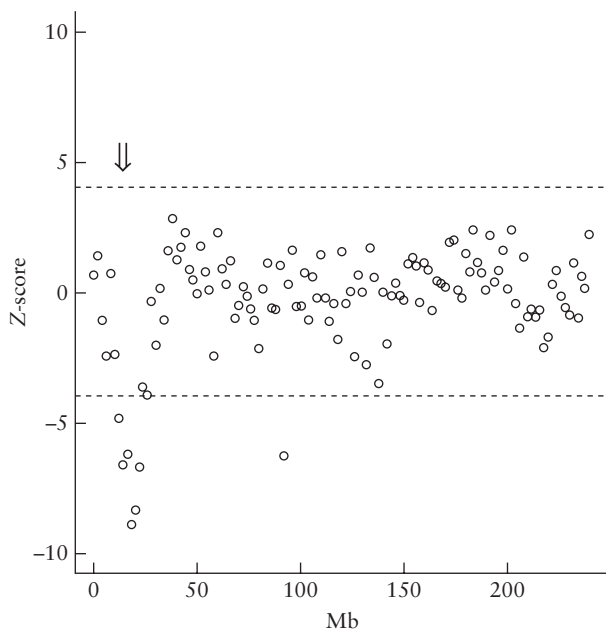
Figure 4 Detection of chromosomal rearrangements in cell-free fetal DNA (cffDNA) using standard aneuploidy sequencing. A small deletion (⇓) of chromosome 2, confirmed as 46,XY,del(2)(p23p25.1), is indicated when the expected number of reads falls outside a Z-score of ± 4 (- - - -).

been reported)[55] between NIPT using cfDNA and conventional karyotyping following invasive testing[56]. cffDNA is derived placentally and discordant results can therefore be attributed to confined placental mosaicism[57,58] or to early demise of an aneuploid cotwin (vanishing twin)[55] as the placenta can continue to shed cffDNA after death of the fetus[3]. Other causes are derived maternally, including the detection of maternal chromosomal rearrangements[59–61] or mosaicism[62] as sequencing analyzes maternal as well as fetal cfDNA. Finally, maternal malignancy secondary to a tumor secreting abnormal cell lines has also been reported as the cause of a discordant NIPT result[63].

Failure of NIPT or inconclusive results occur in up to 5% of cases. These are usually caused by a low fetal fraction of cffDNA[50,51], most commonly because of either testing at an early gestation, as the quantity of cffDNA increases with placental mass and hence gestation[64,65], or high maternal body mass index[64,65], in which low fetal fraction has been attributed to high levels of maternal cfDNA derived from adipose tissue. In situations associated with a small placental volume, for example, trisomies 13 and 18[66,67], fetal fraction has been reported to be lower.

NIPT for aneuploidy is now available widely across the globe, albeit only in the private sector at present. The cost of testing remains high but it has already decreased significantly and there is considerable debate as to how it might be implemented into public-sector healthcare. It is likely that different approaches will be taken depending on local care pathways already in place and the local economy[68]. However, at the moment, most commentators advocate introduction of NIPT as a contingent test after traditional

Down syndrome screening[69] and there are at least two national studies ongoing in Europe evaluating different approaches[70,71]. Although NIPT for aneuploidy is not yet available outside the context of a research study in public health services, widespread availability in the private sector is having a significant effect on the practice of fetal medicine by decreasing the need for invasive diagnostic testing.

Currently, NIPT can only be used reliably for detection of the major trisomies and sex chromosome abnormalities and, as such, will fail to detect the majority of other chromosomal rearrangements that are the underlying pathology in a significant proportion of structurally abnormal fetuses. However, how long this remains the case is in question as there are already reports of NGS detecting other chromosomal rearrangements. Initial studies reported using very high depths of sequencing[72] but, more recently, rearrangements detectable from the karyotype have been detected using sequencing required for standard aneuploidy detection (Figure 4)[73–75]. The main drawback with this approach is that the false-positive rates and limits of detection are as yet unknown, thus limiting its value at present. Despite this, a number of companies have launched commercial tests for a limited range of microdeletion syndromes, including Di George (22q-), Wolf–Hirschhorn (4p-), Cri-du-Chat (5p-), Prader–Willi, Angelman and 1p36-. These tests have largely been developed using artificially produced samples, and reasonable validation data describing sensitivity, specificity and the positive predictive value in maternal plasma samples are yet to be published. Whilst this approach might increase the detection of pathogenic mutations, this targeted approach will only detect around 25% of pathogenic rearrangements as these occur across all chromosomes. There is also concern that using extended NIPT may increase the false-positive rate, potentially reversing the downward trend seen in invasive testing subsequent to the introduction of NIPT for aneuploidy. If this approach is to be used, it would seem sensible to confine its use to cases in which there is an increased incidence of pathogenic rearrangements, for example in euploid fetuses with multiple ultrasound anomalies[76–78].

Conclusions

The use of cffDNA for the diagnosis of fetal genetic and chromosomal conditions is having a profound effect on the practice of fetal medicine worldwide. The advent of NIPT for aneuploidy is reducing the need for invasive testing, the rate of which has declined dramatically in some countries[79], a fall that is met with approval from both women and health professionals alike as we move toward safer and earlier prenatal diagnosis[80,81]. The use of cffDNA to direct invasive testing or treatment in sex-linked diseases is also decreasing the need for invasive testing, whilst making prenatal diagnosis safer and more acceptable to high-risk families[82]; however, this may increase the economic burden on health services as

an increasing number of families elect to undergo NIPT for information[83,84]. These changes will inevitably impact on care pathways and fetal medicine in general, in respect to both training and service provision, as the indications for invasive tests decrease. The pace of change has been rapid and we must urgently address how we structure our services so that we can provide safe services for those who continue to need invasive testing and treatment.

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