

Determination of fetal chromosome aberrations from fetal DNA in maternal blood: has the challenge finally been met?

Sinuhe Hahn^{1,*}, Olav Lapaire², Sevgi Tercanli², Varaprasad Kolla¹ and Irene Hösli²

The analysis of cell-free fetal nucleic acids in maternal blood for prenatal diagnosis has been transformed by several recent profound technology developments. The most noteworthy of these are 'digital PCR' and 'next-generation sequencing' (NGS), which might finally deliver the long-sought goal of noninvasive detection of fetal aneuploidy. Recent data, however, indicate that NGS might even be able to offer a much more detailed appraisal of the fetal genome, including paternal and maternal inheritance of point mutations for mendelian disorders such as β -thalassaemia. Although these developments are very exciting, in their current form they are still too complex and costly, and will need to be simplified considerably for their optimal translation to the clinic. In this regard, targeted NGS does appear to be a step in the right direction, although this should be seen in the context of ongoing progress with the isolation of fetal cells and with proteomic screening markers.

In the past few years it has become clear that the demographic shift towards increasing maternal age for pregnancies in developed nations, with its associated risks of fetal chromosomal anomalies, will necessitate a change in current strategies for prenatal screening and detection of fetal aneuploidies (Refs 1, 2, 3, 4). This is illustrated by a recent analysis of the English and Welsh National

Down Syndrome (DS) Cytogenetic Register over the period 1989–2008 (Ref. 5), which reported a 71% increase in the number of diagnosed cases with DS, with no comparable increase in birth rate. The increase in the number of cases with DS was largely attributed to the concomitant increase in maternal age, as more than 20% of pregnancies now occur in mothers older than 35 years (Ref. 5).

¹Laboratory for Prenatal Medicine, Department of Biomedicine / Department of Obstetrics and Gynecology, University Hospital Basel, Switzerland.

²Department of Obstetrics and Gynecology, University Hospital Basel, Switzerland.

*Corresponding author: Sinuhe Hahn, Laboratory for Prenatal Medicine, Department of Biomedicine/Department of Obstetrics and Gynecology, University Hospital Basel, Hebelstrasse 20, CH-4031 Basel, Switzerland. E-mail: shahn@uhbs.ch

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This development is likely to add additional costs to already strained healthcare budgets, because positive cases from initial noninvasive screening based on ultrasounds and maternal serum biochemical analysis need to be verified by invasive analysis such as amniocentesis or chorionic villous sampling, which are labour-intensive examinations requiring highly skilled personnel (Ref. 6). To break this spiralling cost, it would be advantageous for any future noninvasive method to be so accurate that it could function as a 'stand-alone' test and not require further invasive verification. Naturally, it will also need to be considerably cheaper than current invasive practices. An alternative scenario might be to use such a noninvasive test to reduce the cost and risk associated with the invasive analysis of the high number of false-positive cases resulting from current screening practice (Refs 7, 8). In either case, strenuous efforts will need to be undertaken in order to ensure that cost-effective noninvasive prenatal diagnosis of DS cases finally becomes a reality (Ref. 8).

Has research in the field of noninvasive prenatal diagnosis become 'mature'?

Since the discovery of fetal cell-free DNA (cf-DNA) in maternal plasma or serum in 1997 (Ref. 9), more than 1000 papers have been published on this topic. A cursory review of publications listed in public depositories such as PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) suggests that the most prolific period was probably the latter half of the previous decade. In this period, several large-scale studies were initiated to test the efficacy of this new-found tool for the noninvasive determination of fetal genetic traits such as the fetal RhD (Rhesus D) gene or gender (Refs 10, 11, 12, 13). These data indicated that this approach was indeed sufficiently robust that widespread clinical application could be safely implemented, and as a result concerted efforts were undertaken by multicentre consortia, such as the European Union (EU)-funded SAFE Network, to standardise these assays (Refs 11, 14). These data also indicated that the analysis of cf-fetal DNA could form the secure and sound basis for subsequent developments, such as the noninvasive detection of fetal aneuploidies (Ref. 15).

In recent years this prolific output in publications seems to have largely tapered off,

although the number of reviews dealing with the field has increased tremendously. Hence the scenario appears very similar to economic models of the 'product life cycle', where a particular item has progressed through development, introduction and growth, and is settling into a pattern of maturity and subsequent decline (Ref. 16). Although this simplistic view might lead to the impression that research in this field has waned, this is far from the truth, for this punctuated modicum in published reports indicates that the field has finally entered a phase where quality, using a quantum leap in technological development, rather than quantity is the prevailing trend. This facet will become very evident in this review.

The question of fetal cell-free DNA fragment size and concentration

It is necessary to reiterate that the major problem still hampering the use of fetal cf-DNA for the noninvasive detection of fetal genetic loci that are not distinct from maternal loci is that fetal sequences constitute only 5–10% of the total cf-DNA in maternal plasma, and even less in maternal serum (Refs 17, 18, 19).

Groundbreaking research performed in the middle of the past decade on the biophysical properties of cf-DNA indicated that fetal cf-DNA was more fragmented and had a shorter size than comparable maternal cf-DNA fragments (Refs 20, 21). Interestingly, this topic still continues to be the focus of intense research efforts and considerable debate (Refs 22, 23, 24). The fragmentation was exploited for the selective enrichment of fetal cf-DNA sequences (to up to 50% of total cf-DNA), thereby permitting the detection of otherwise masked fetal genetic loci such as short tandem repeats, single-nucleotide polymorphisms (SNPs) and paternally inherited point mutations, such as those involved in β -thalassaemia or achondroplasia (Refs 21, 25, 26, 27, 28). These experiments, however, used conventional agarose gel electrophoresis for the physical-size-based separation of fetal and maternal cf-DNA species – a cumbersome, labour-intensive, inefficient procedure that is prone to contamination. For the widespread applicability of this approach in the clinic, new approaches such as microfluidics will be required.

Another key development during this period was verification of the long-standing suspicion

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that fetal cf-DNA was of placental origin, and was not the result of the demise of trafficking fetal cells. This was confirmed by a number of different approaches, such as analysis of (1) placental mosaicism, where key fetal loci such as the Y chromosome were missing both in the placenta and in fetal cf-DNA, but not in the male fetal karyotype (Ref. 29), (2) molar pregnancies, where cf-DNA specific for the molar karyotype (46 XY) could be detected (Ref. 30), and (3) epigenetic markers specific for placental tissues, such as the hypomethylated maspin (*SERPINB5*) or the hypermethylated *RASSF1* genetic loci (Refs 31, 32, 33).

The latter markers could prove to be useful as gender-independent tools to verify the presence of fetal cf-DNA in ambiguous diagnostic cases, such as when determining fetal gender or RhD status (Ref. 10). It might also be possible to use these as tools to quantify fetal cf-DNA levels (Ref. 34). Reliable, accurate assessment of fetal cf-DNA levels, however, appears to require the use of high-copy sequences, such as *DYS14* on the Y chromosome (Ref. 18).

Indirect methods for the noninvasive detection of fetal aneuploidy using cell-free nucleic acids

Allelic transcript ratios

The first indication that cell-free fetal nucleic acids could be used for the noninvasive prenatal detection of DS, in 2007, came from an indirect approach in which chromosomal dosage was inferred from allelic gene transcript copy numbers (Ref. 35). The study focused on the gene *PLAC4* (placenta-specific 4), located on chromosome 21, which was specifically transcribed in the placenta but not in any maternal tissues. In this manner, the analysis of this gene product would be similar to that of the Y chromosome, in that it would not be hindered by maternal background. To assess the dosage of each allelic transcript, heterozygous SNP loci were used. These could then be quantitatively assessed using mass spectrometry. In the analysis of samples from 10 DS cases and 56 healthy controls, DS cases could be detected with a sensitivity of 90% and a specificity of 96.5%.

However, a serious caveat of this approach is that the SNP in *PLAC4* needs to be heterozygous in order to derive a conclusion concerning chromosomal dosage. Hence, a very large number of cases had to be excluded from

the study, because they failed to meet this important criterion. Furthermore, the approach makes a fundamental assumption concerning the underlying biology for it to work effectively – namely, that the interrogated alleles are transcribed at exactly the same rate. This might not be the case in many instances (Refs 36, 37), which would make subsequent analysis unreliable.

Although this RNA-based approach was subsequently explored by Sequenom, Inc., USA, it appears not to have been successful. Unfortunately, details of this study have not been divulged; it would have been interesting to determine the cause for its apparent failure, which perhaps could have been rectified in subsequent studies.

Epigenetic allelic ratios

A second indirect approach, which was explored around the same time, involved epigenetic differences of methylation to distinguish placental (fetal) genetic loci from maternal loci (Refs 31, 32, 38, 39, 40). The first report, on the maspin gene on chromosome 18 (Ref. 38), again used heterozygous SNP alleles to determine chromosomal dosage; in this study, however, no clear distinction could be discerned between cases with trisomy 18 and unaffected healthy controls, even when using 'pure' fetal and maternal genetic material, such as fetal material obtained by amniocentesis. Subsequently, however, the epigenetic approach has been explored successfully for cases with trisomy 21 and trisomy 18 (Edwards syndrome; ES) (Refs 39, 40). In these recent studies much more encouraging results were obtained, in that all five DS cases and eight out of nine cases with ES were correctly identified by the analysis of epigenetic markers in plasma DNA. The efficacy of these assays might be improved by the inclusion of further candidate epigenetic biomarkers (Refs 39, 40).

In a very recent report, it has been demonstrated that the enrichment of fetal cf-DNA fragments by methylated DNA immunoprecipitation can be used for the successful determination of chromosome 21 ploidy (Ref. 41). In this study, hypermethylated fetal cf-DNA fragments, identified in a previous study (Ref. 42), were enriched by immunoprecipitation and then examined by conventional real-time quantitative polymerase chain reaction (PCR). The fetal-

specific DNA methylation ratio for each sample was then calculated by comparing the sample CT (cycle threshold) value with the median CT value of a pool of normal control cases. In those cases where the fetus had a normal karyotype this ratio was determined to be of the order of 1, whereas in cases with trisomy 21 this ratio was larger than 1. To increase the accuracy of this system, eight methylated genetic regions on chromosome 21 were examined in parallel. In a blinded analysis, 14 cases with trisomy 21 could be correctly distinguished from 26 normal cases. The advantage of this system is that it does not require any specialised equipment, and can readily be performed by the instruments currently present in most routine diagnostic laboratories.

Digital PCR: first hint at direct noninvasive aneuploidy detection

Most of the studies examining fetal cf-DNA to date have used a form of real-time PCR and Y-chromosome-specific sequences (Refs 17, 43). These studies indicated that the amount of fetal cf-DNA increases during gestation, and disappears rapidly from the maternal circulation following delivery. Increases were also observed in several pregnancy-related disorders or conditions such as preeclampsia, preterm delivery and trisomy 21, suggesting that this phenomenon could serve as the basis for a new generation of screening tests.

During this period, studies had indicated that real-time PCR could be used for the rapid determination of chromosomal ploidy on pure fetal genetic material obtained by invasive means (Ref. 44). Because real-time PCR is not well suited for the detection of less than twofold differences in template copy numbers, special conditions had to be introduced to detect the 1.5-fold difference in template concentration occurring in trisomy 21. It was, however, very clear that this approach would not be suited for the noninvasive determination of DS, because of the overwhelming presence of maternal cf-DNA fragments.

Consequently, a different tack had to be taken, which was provided by 'digital PCR' (Ref. 45) and the advent of microfluidic devices (Ref. 46). Unlike real-time PCR, where an 'analogue' signal of the entire PCR reaction containing the entire input template is obtained, in 'digital PCR' the PCR reaction is split into thousands of

minute individual reactions, with each individual reaction containing at most a single template copy. A quantitative assessment of the concentration of input template in the sample examined is then made by counting the number of individual positive PCR reactions (Fig. 1).

This procedure has been demonstrated to permit a much more accurate quantification than more conventional approaches such as real-time PCR, but, more importantly, it permits the detection of very small changes in input DNA. Thus, 'digital PCR' could perhaps indicate whether a fetus was affected by DS, simply by counting the number of chromosome-21-specific target sequences in comparison to a similar locus on an unaffected chromosome (Refs 46, 47, 48, 49) (Fig. 1).

A strategy in which the amyloid gene locus on chromosome 21 and the *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) gene locus on chromosome 12 were co-examined by digital PCR was shown to be able to detect DS when examining pure fetal genetic material obtained by invasive means (Ref. 47) (Fig. 1). The report indicated that the method might also be suitable for the analysis of cf-DNA, in that 'DS cases' could be detected using artificial mixtures involving only 10% DS material, which is very similar to the concentration of fetal cf-DNA in maternal plasma. A crucial limitation of these observations, however, was that they would hold true only when 10 000 or more individual PCR reactions were monitored.

Next-generation sequencing and the possible advent of noninvasive DS detection

Although reports from two research groups indicated that digital PCR might offer success for the noninvasive detection of DS by the analysis of cf-DNA (Refs 47, 48, 49), these studies also made it clear that these analyses would be conducted at the limits of current digital PCR platforms, which at the time offered some 12 000 individual reaction events. Hence, if this strategy were to be pursued, then its successful transition would require an even greater level of 'individual event analysis' to permit the necessary degree of discrimination between normal and DS cases.

This was offered by the advent of 'next-generation sequencing (NGS)', also termed 'deep sequencing', whereby the entire genomic

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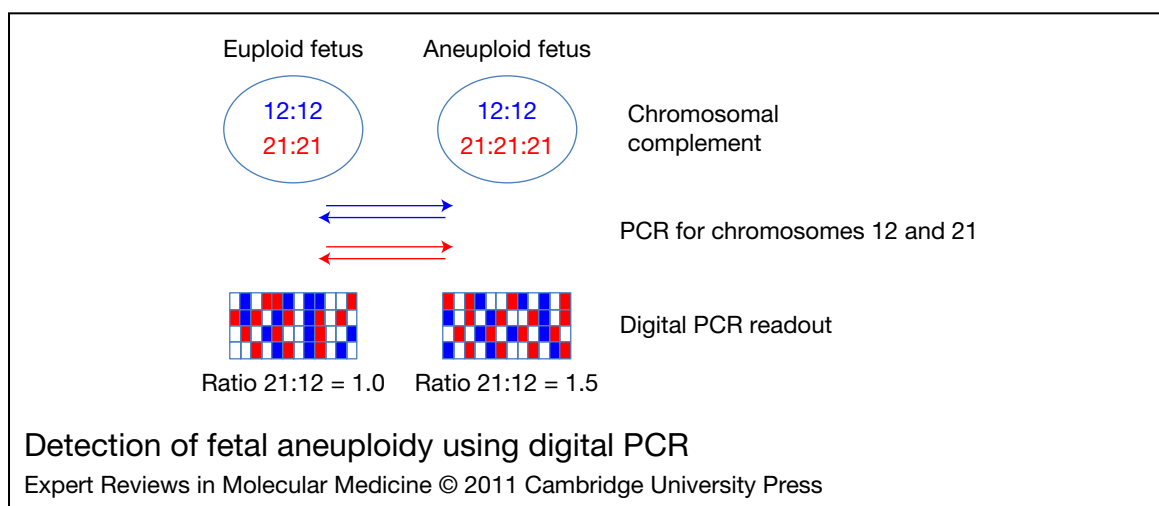


Figure 1. Detection of fetal aneuploidy using digital PCR. In this procedure, fluorescent PCR specific for sequences on chromosomes 12 and 21 is carried out in individual microreaction chambers. The amount of input template is titrated in such a fashion that each microreaction vessel contains >1 copy. After the plateau phase has been reached (approximately 40 cycles), the PCR reaction is terminated, and the number of positive reactions for each locus is counted. In euploid cases the ratio of blue (chromosome 12) to red (chromosome 21) signals should be 1, whereas in cases with Down syndrome the ratio of blue (chromosome 12) to red (chromosome 21) signals should be 1.5 (illustrated in more detail in Ref. 46). This method has to date not been successfully used for the detection of fetal aneuploidy using cell-free DNA in maternal plasma.

template is fragmented, sequenced in short reads and then reassembled through complex bioinformatic comparison with a genomic database (Ref. 50). In this manner, subtle mutations having key roles in tumour initiation and progression could be ascertained (Refs 51, 52).

Apart from offering unprecedented detail with regard to genomic alterations, the NGS approach also offered a unique opportunity to overcome the current limitation of digital PCR, by providing information concerning tens of thousands of sequence reads per chromosome; more than 60 000 reads might be recorded for chromosome 21, and many millions over the entire genome.

In the case of a DS fetus, there would be a small increase in the number of chromosome 21 reads, whereas there should not be any comparable alteration across the other chromosomes. As such, in a manner akin to digital PCR, by simply counting the number of chromosome 21 reads, and then comparing these to a much larger number of sequence reads over the entire genome, it should be possible to determine the ploidy of chromosome 21 (Fig. 2). This indeed was the case, and it was clearly demonstrated that even minute quantitative alterations in the

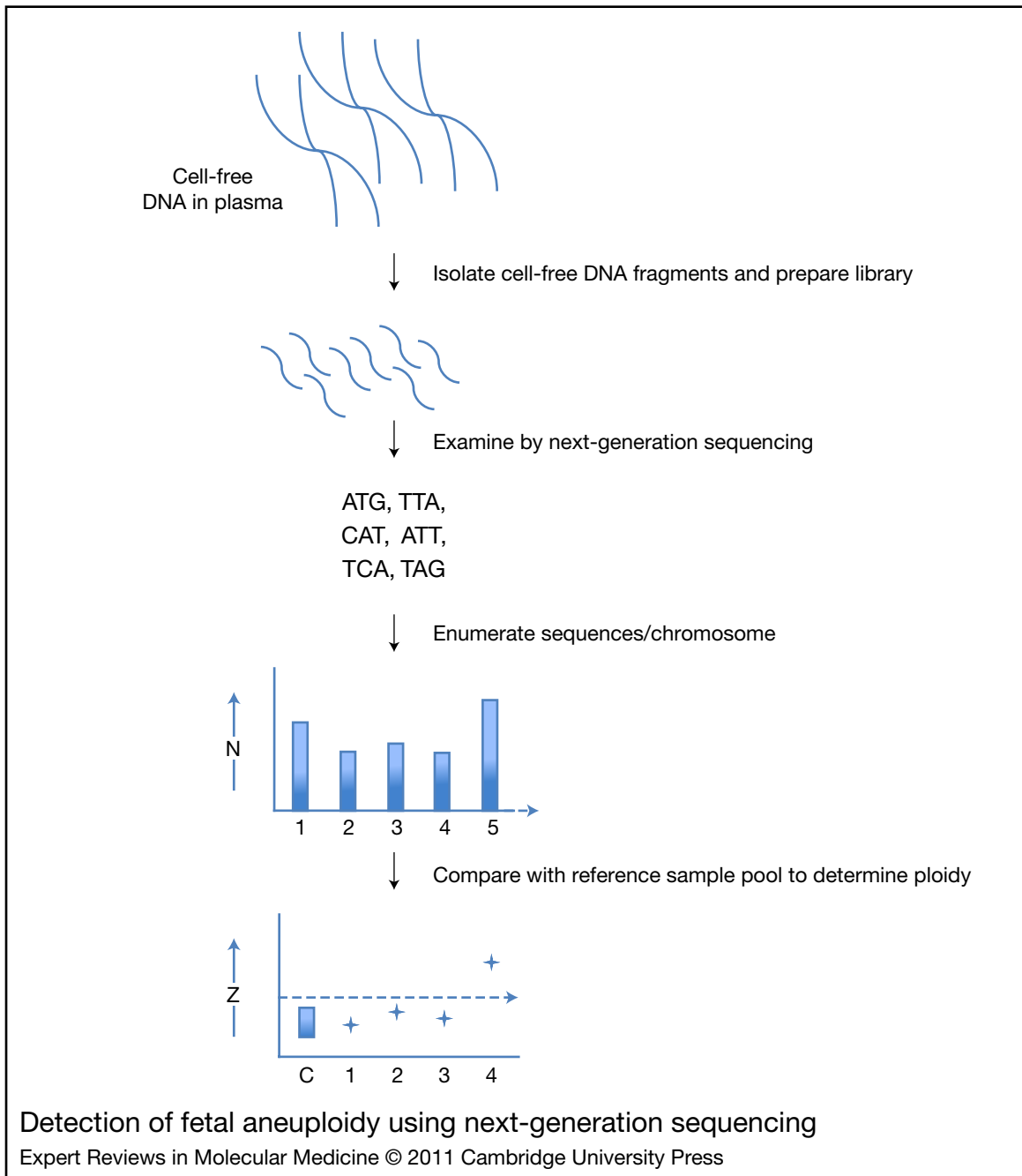
number of chromosome 21 reads could lead to an unparalleled discrimination between pregnancies bearing a fetus with trisomy 21 or those with a normal karyotype (Refs 53, 55). Furthermore, because this analysis was not restricted to chromosome 21, it was also possible to detect two cases with ES (trisomy 18) and one case with Patau syndrome (trisomy 13), thereby indicating the possible widespread applicability of this technology (Ref. 55).

The downside of this approach is the prohibitively high cost per sample and the length of time taken for sample preparation, sequencing and subsequent bioinformatic analysis, which occupies the better part of several days per sample (Ref. 56).

Is NGS ready for clinical application?

Nevertheless, two very recent publications have suggested that the NGS approach might be ready to make the transition from the research laboratory to clinical routine (Refs 7, 8, 34). In the first of these studies, 753 pregnant women at risk of having a fetus affected by DS, and who were therefore about to undergo an invasive prenatal diagnostic procedure, were recruited

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Figure 2. Detection of fetal aneuploidy using next-generation sequencing. In this procedure the cell-free DNA fragments in maternal plasma are isolated, and a library with special sequence tags is then made. These tags permit subsequent multiplex analysis. The library is examined by next-generation sequencing, which determines the sequence of each and every fragment. By bioinformatic analysis these sequences are ascribed to chromosomal locations. Following this, the number of sequence reads for each chromosome is counted. For chromosome 21 this is typically of the order of several thousand reads, which can then be compared with several million reads spread across the genome. If the fetus is affected by Down syndrome, then slightly more reads will be recorded for chromosome 21 compared with those from a euploid fetus. By comparing these data with a bank of reference samples, and by the use of predetermined cut-off values (Z score), the ploidy of the sample being examined can be determined (described in more detail in Refs 53, 54).

(Ref. 7); of these, full karyotyping determined that 86 had a fetus affected by DS. To reduce the complexity and cost of the NGS analysis, a multiplex approach was tested, whereby eight samples were examined in parallel (8-plex). This was compared with a more costly duplex analysis on 314 samples (two samples examined simultaneously). The 8-plex analysis yielded a sensitivity of 79.1% and a specificity of 98.9%, whereas the duplex assay yielded 100% sensitivity and 97.9% specificity (Ref. 7). To make the assay more efficient, a cohort of normal samples was used as a standard, permitting a threshold cut-off value (termed *Z*) to be determined: values above this were deemed to show DS cases (Fig. 2).

Although it is unfortunate that the duplex analysis was determined to be superior to the less complex 8-plex analysis, it is not entirely surprising, in that for the 8-plex assay only approximately 300 000 total reads were recorded per sample. This translates to a small fraction of the billions of reads required to access the entire genome, and furthermore implies that perhaps less than 10 000 chromosome-21-specific reads were recorded. Because the NGS assay, like its digital PCR predecessor, relies simply on the counting of individual events, it would seem that here simply too few single events were being interrogated.

In the second, independent study, 449 samples were examined by a more detailed and robust pair-end 4-plex NGS procedure in which approximately 5 million reads per sample were obtained (Ref. 34). In this examination all 39 cases with DS were correctly identified, although one normal case was incorrectly classified as DS, thereby yielding 100% sensitivity and 99.7% specificity. An important quality control in this study is that the concentration of fetal cf-DNA was monitored, and samples with less than 3.9% were excluded (Ref. 34).

Even if the NGS assays are considered not to be 100% effective, and could not be used as a stand-alone diagnostic test for DS, it has been suggested that their use as a secondary-tier screen of high-risk cases could lead to a 98% reduction in the number of invasive procedures (Ref. 7). As such, this avenue certainly does appear to be an attractive route to pursue (Ref. 8). Accordingly, large-scale clinical studies are being launched in Europe and elsewhere.

Can NGS be simplified?

The above results have indicated that the use of complex cutting-edge genomic tools such as NGS can finally offer the long-sought dream of noninvasive detection of fetal aneuploidy (Refs 1, 56). However, this comes at a very high cost and unacceptably long analysis. A possible strategy to overcome these disadvantages is the targeting of only those chromosomes or regions of interest such as those on chromosome 21 for the detection of DS cases. Although several different strategies exist that permit some form of target enrichment prior to the subsequent sequencing step, they have to date been limited to examination of intact genomic DNA samples, and not fragmented DNA species such as those found in cf-DNA (Ref. 57). Furthermore, these procedures, such as on-array capture, require rather large concentrations of input template DNA (up to 7.5 µg per sample). Hence, an approach that permitted targeted sequencing of the small quantities of fragmented DNA in maternal plasma had to be sought.

Very recently, a solution hybrid selection method using ultralong oligonucleotides, commercially marketed by Agilent, USA, has been used for targeted enrichment (Ref. 57) (Fig. 3). A capture library specific for the X chromosome was hybridised with 500 ng of an amplified plasma DNA library, and the selected capture targets were pulled down using a combination of biotinylated oligonucleotide probes and streptavidin-coated magnetic beads. The enriched target DNA was then subjected to a further 12 rounds of PCR with specially tagged primers necessary for the subsequent sequence analysis. In the examination of 12 maternal plasma samples, this targeted capture procedure led to a mean enrichment of 213-fold. This enrichment was also reflected in the increased ability to detect fetal-specific loci on the X chromosome, which changed from 3.5% in the un-enriched samples to 95.9% in the samples subjected to a targeted enrichment step.

A concern with any enrichment approach is that it might lead to bias, by preferential enhancement of select sequences, including the preferential accumulation of maternal cf-DNA sequences over fetal ones. However, in this study the proportion of fetal to maternal sequences was similar in un-enriched loci (~16–30% from first to third trimester) and in enriched X-chromosome regions (~15–32%) (Ref. 57). These

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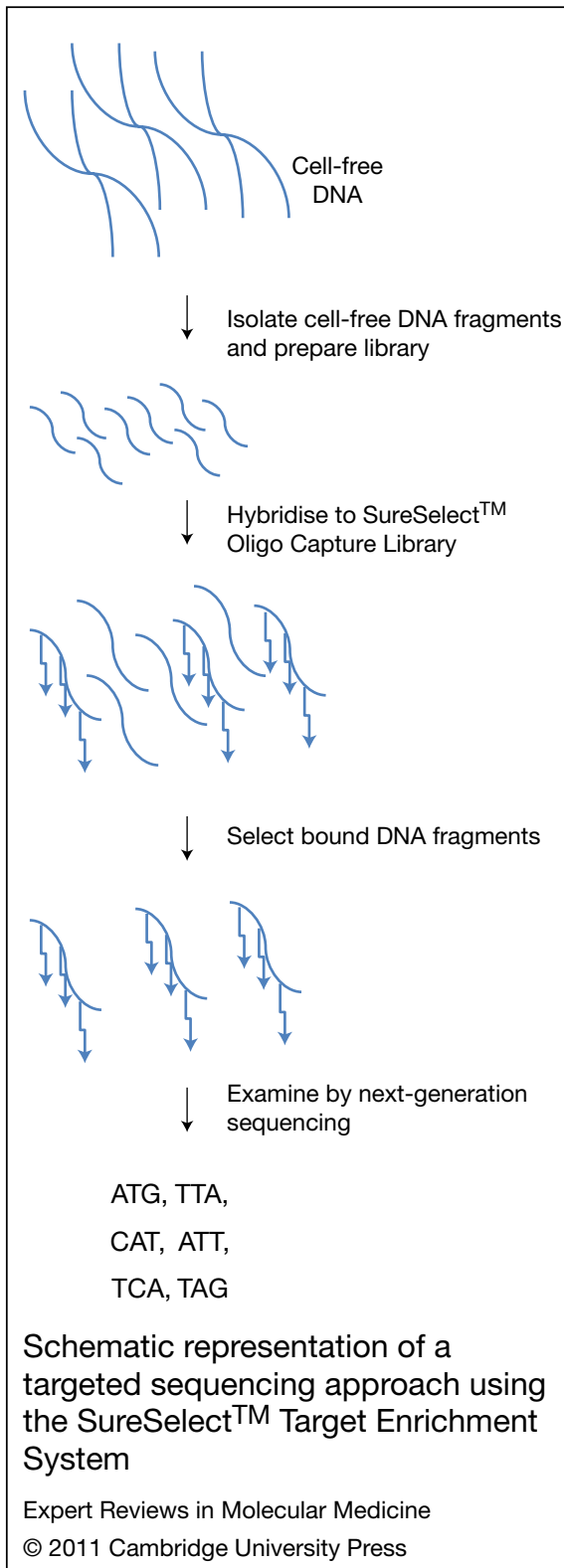


Figure 3. Schematic representation of a targeted sequencing approach using the SureSelect™ Target Enrichment System.

results are encouraging, because they could facilitate targeted enrichment of more crucial fetal loci, such as those on chromosome 21, thereby paving the way for a less complex noninvasive test for DS.

How much information is available from NGS?

The first series of experiments with NGS focused on select chromosomes and compared the number of reads obtained for these to those obtained for the entire genomic complement. Because close to 65 000 individual loci are counted on chromosome 21 alone, it was open to debate whether this approach would be sufficiently sensitive to detect more subtle chromosomal aberrations such as the Robertsonian translocation between chromosomes 21 and 15 (or 14), which has a role in 2–3% of cases with DS (Ref. 1). As the Down Syndrome Critical Region associated with this translocation is a lot smaller than the entire chromosome 21 commonly involved in DS, it was unclear whether the NGS approach would be able to detect this alteration (Ref. 1).

However, this view has been altered by a new landmark publication, in which the entire cf-DNA present in a maternal plasma sample was sequenced (Ref. 24). The data indicated that the entire fetal genome complement is present in cf-DNA in maternal plasma, and that this can be mined to show minute details such as mutations

Figure 3. Schematic representation of a targeted sequencing approach using the SureSelect™ Target Enrichment System.

In this procedure the cell-free DNA fragments are isolated and a library is generated as per the standard next-generation sequencing protocol. Prior to sequencing, however, this library is hybridised to the SureSelect™ Oligo Capture Library, which is manufactured in such a manner that it will recognise a specific chromosome, such as chromosome 21. These oligo sequences contain magnetic particles to permit their retrieval in a magnetic field. Hence, following hybridisation (65°C, 24 h), captured sequences are selected by magnetic selection, and unselected sequences are washed away. The bound fragments are then purified and prepared for sequencing and examined as described for Figure 2. This procedure was recently shown to permit a 213-fold enrichment of the targeted X chromosome (Ref. 57).

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involved in β -thalassaemia; surprisingly, the study correctly discerned that the fetus had inherited the paternal codon 41/42 mutation, involving a CTTT deletion, but not the maternal $-28A \rightarrow G$ mutation. Although this analysis was very complex, involving almost 4 billion reads and 900 000 SNPs, it is a striking indicator of what might lie ahead – namely, the ability to obtain a full fetal karyotype down to miniscule single-nucleotide detail from a single maternal blood sample (Ref. 24).

The question of intellectual property and how to optimise procedures

An important concern that needs to be addressed is how intellectual property and commercialisation will affect future research and applications (Ref. 58). For the best possible service to reach the patient, clarity is essential; otherwise, conflicting reports and views might prevent superior products from being developed. In the case of fetal cf-DNA, such an issue was raised by the commercialisation of a noninvasive test for fetal RhD determination in the EU, and subsequently in the USA (Ref. 58). Although it was clear to leading researchers in the field that the tests initially marketed might be flawed or were not state of the art, it was felt that it would be a weary process to convince the commercial parties involved to change their standard of practice and to adopt more modern effective approaches. This issue was further highlighted by the near-simultaneous reports concerning the application of digital PCR or NGS, leading to conflicting reports in the lay media as to who the main patent claimants were (Refs 1, 59). It is currently also unclear what business model or strategies should be used in translating this research from the bench to the clinic. Hence, it might be worthwhile to echo previous concerns that such uncertainty might hinder or restrict further research in the field (Ref. 60).

Are fetal cells in maternal blood still worth pursuing?

Despite the enormous strides that have been made with the determination of fetal aneuploidies through the use of cf-DNA, the latest reports have indicated that these will be very labour intensive, and require extremely high-tech devices and bioinformatic services. Hence it is

unclear how quickly these developments will be translatable into the clinic.

For this reason there has been a resurgent interest in the isolation of fetal cells from maternal blood. This has been fostered by the development of (1) sophisticated high-throughput automated scanning devices, which permit the rapid identification of putative target fetal cells among a large pool of maternal cells (Refs 61, 62), and (2) efficient microfluidic systems and electronic micromanipulation systems, which permit the effective retrieval of individual trafficking fetal cells (Refs 59, 63, 64).

Even though this process will most likely also be laborious, it has several advantages over cf-DNA analysis, in that fetal cells offer a pure source of fetal genomic material. Furthermore, widely standardised procedures commonly used in many diagnostic laboratories, such as FISH (fluorescence in situ hybridisation), can be applied for the very rapid detection of chromosomal anomalies (Ref. 65). In addition, use can now be made of a wide body of experience in the analysis of single cells, a routine practice in many in vitro fertilisation clinics offering preimplantation genetic diagnosis (PGD) (Ref. 66).

A possible further advantage is that the intellectual property situation in this area is not as complex as that of cf-DNA, in that many of the original patents are close to expiry, and no commercial entity holds a wide portfolio of key elements. As such, it will be of interest to monitor progress in this sphere of research, because it might yet have a few surprises in store.

The challenge of proteomics

The development of the first-trimester DS screening test using a combination of ultrasound measurement of nuchal translucency, serum analyte analysis and maternal age has led to a dramatic increase in the ability to detect pregnancies at-risk of bearing an aneuploid fetus (85% sensitivity) when compared with the previous generation of second-trimester screening tests (65% sensitivity), which relied solely on serum analyte analysis in combination with maternal age (Ref. 67). This facet is reflected in the report on the English and Welsh National Down Syndrome Cytogenetic Register analysis, which indicated that the vast majority of DS cases were detected by current screening practice (Ref. 5).

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The downside of this startling development is that the first-trimester screen is still hampered by a rather high false-positive rate of 5–8% (Refs 68, 69). This implies that a large number of healthy pregnancies are being subjected to unnecessary invasive procedures – a healthcare risk for mother and unborn child, as well as a considerable financial burden for healthcare services. Furthermore, almost 15% of cases remain undetected and, because these occur in the group of pregnant women not judged to be at a higher risk (<35 years old), could result in undesired live births.

Therefore, increasing the efficiency of this test has been proposed, perhaps by the addition of further biochemical analytes, such as members of the activin family (Refs 70, 71). The inclusion of further highly specific blood-based analytes might be possible as a result of the development of quantitative proteomic technologies as part of the Human Proteome Association, especially the affiliated plasma proteome project (Ref. 72). The placenta in DS cases exhibits structural alterations, which are most likely the result of alterations in protein expression, and so it is possible that these changes will be reflected in the maternal plasma proteome (Ref. 73). In this regard, the use of quantitative isobaric labelling has been shown to permit the detection of significant alterations in the levels of several placenta-derived peptides in DS cases when compared with controls (Ref. 74). Analogous observations have been made by other research groups using a variety of different proteomic approaches (Refs 75, 76, 77, 78). It now remains to explore how useful these potential biomarkers will be in increasing the detection rate of the current first-trimester screen.

Summary and conclusion

Tremendous strides have been made with the analysis of fetal cf-DNA in maternal blood, in that we now stand on the threshold of a new generation of noninvasive diagnostic tools, which might even permit a detailed analysis of the entire fetal genome or a full karyotype. However, it remains to be seen how rapidly these can be translated into the clinic, in a manner that is cost effective and accessible to all. In this context it will be interesting to see which system, namely NGS, digital PCR or enrichment of methylated fetal cf-DNA fragments, passes the final hurdle to enter

clinical service. Although the isolation and analysis of trafficking fetal cells might remain a peripheral test, it could be useful in those centres already offering FISH or PGD services. Should multiparameter mass spectroscopy and quantitative proteomics finally come of age, it might yield a set of biomarkers with unparalleled specificity, not only for DS screening but also for other pregnancy-related disorders such as preeclampsia.

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Further reading, resources and contacts

Publications

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Websites

A study funded by the UK National Institute for Health Research to assist with the transfer of noninvasive prenatal diagnostic techniques into the clinic is described at:
<http://www.rapid.nhs.uk/>

Features associated with this article

Figures

- Figure 1. Detection of fetal aneuploidy using digital PCR.
- Figure 2. Detection of fetal aneuploidy using next-generation sequencing.
- Figure 3. Schematic representation of a targeted sequencing approach using the SureSelect™ Target Enrichment System.

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