INNOVATION

Prenatal diagnosis: progress through plasma nucleic acids

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Abstract | Over the past 40 years, much effort has been spent on developing non-invasive prenatal diagnostic methods. Since 1997, the progress of this field has been accelerated by the unexpected finding of extracellular fetal nucleic acids in maternal plasma. These developments have been translated into many novel genetic, epigenetic and gene-expression markers, and are expected to have a fundamental impact on the future practice of prenatal diagnosis.

Prenatal diagnosis is an indispensable part of modern obstetrics care. Fetal genetic diseases that are targeted by most current programmes include chromosomal aneuploidies and several single-gene disorders. Although many non-invasive screening approaches are available, the definitive prenatal diagnosis of these disorders requires the analysis of fetal genetic material, which is obtained through invasive procedures such as amniocentesis and chorionic villus sampling. Because these procedures are associated with a small, but not immaterial, risk of fetal miscarriage, many researchers, over several decades, have searched for non-invasive alternatives to sample for fetal genetic material. One such approach is the isolation of fetal nucleated cells that have trespassed into the maternal circulation (BOX 1). However, such cells are extremely rare and require specialized isolation processes, prompting investigators to look for alternative approaches.

Since the 1940s, extracellular DNA has been reported to be present in the plasma of human subjects. In particular, it was demonstrated in the 1990s that a proportion of this extracellular plasma DNA in some cancer patients contains tumour-derived genetic signatures[1]. In 1997, Lo *et al.* reasoned by analogy that extracellular DNA of fetal origin might similarly be detectable in the plasma of pregnant women[2]. The first demonstration of the presence of cell-free fetal DNA in the plasma of pregnant women was the detection of Y-chromosomal DNA sequences in the plasma of women carrying male fetuses[2]. This discovery offered exciting new avenues for non-invasive prenatal diagnosis. Today, the clinical potential of this strategy has to some extent been realized, particularly with respect to the prenatal assessment of fetal rhesus D blood group status[3]. Here we discuss the current knowledge regarding the trafficking of fetal nucleic acids to the maternal circulation, and advances towards the elusive goal of non-invasive prenatal diagnosis.

Biology of circulating fetal DNA

After the surprising discovery of cell-free fetal DNA in maternal plasma, much had to be learnt about its nature. Circulating fetal DNA consists predominantly of short DNA fragments, of which 80% are less than 200 bp[4]. Furthermore, fetal DNA represents only a subfraction (on average, 3–6%) of the cell-free DNA that is present in maternal plasma[5]. The earliest gestational age at which circulating fetal DNA is detectable in maternal plasma is day 18 after embryo transfer by assisted reproduction[6]. Fetal DNA concentrations seem to increase as gestation progresses[5]; following delivery, fetal DNA is cleared extremely rapidly from maternal plasma, with a mean half-life of about 16 minutes[7]. It has been suggested that the renal system is one of the mechanisms of clearance of plasma DNA, as fetal DNA has been reported by some[8], but not all[9], investigators to be present in the urine of pregnant women. The consensus in the literature is that there is no long-term persistence of fetal DNA in maternal plasma[10,11], with the exception of an isolated report by Invernizzi *et al.*[12]. Their data might be explained by the persistence of some fetal nucleated cells for extended periods following delivery[13]. It has also been suggested that these cells could have contaminated a proportion of the plasma samples used in that study due to a suboptimal centrifugation step for the separation of cells from the plasma[11].

Many lines of evidence indicate that the placenta is the predominant source of fetal DNA in maternal plasma. This evidence includes the presence of placental genetic signatures in maternal plasma in cases with confined placental chromosomal abnormalities[14], the demonstration of placental epigenetic signatures in maternal plasma DNA[15], and the presence of circulating fetal DNA after the placenta has been formed but before the fetal circulatory system has been established[6]. Apoptosis and necrosis, which are normal cell turnover events in the placenta, have

Box 1 | **Fetal nucleated cells in maternal blood**

The search for fetal nucleated cells in maternal peripheral blood started in the late 1960s. Multiple fetal nucleated cell populations have been reported in maternal peripheral blood, including fetal nucleated red blood cells[54], lymphocytes[55] and trophoblasts[56]. Many methods have been attempted for their isolation and detection, including fluorescence-activated cell sorting[54,55], magnetic-activated cell sorting[57], microdissection[58] and size separation[56]. However, the extreme rarity of these cells, of the order of about one cell in each millilitre of maternal blood[59], is a major obstacle to their robust detection. In this regard, a landmark large-scale study, the National Institute of Child Health and Development Fetal Cell Isolation Study, reported a sensitivity for male fetal cell detection of 41.4% and a false-positive rate of 11.1% (REF. 57). This group of investigators concluded that technological advances are needed before clinical application of fetal cell analysis can be achieved.

been shown in a placental explant model to be responsible for fetal DNA release[16]. The association between fetal DNA release and placental cell death might also partly explain the elevation in circulating fetal DNA that has been reported in several pregnancy-associated disorders, such as pre-eclampsia[17,18].

Clinical applications

Fetal DNA in maternal plasma can be used to obtain valuable information about the fetus or pregnancy, either for prenatal diagnosis or monitoring (TABLE 1). These applications can be divided into two groups: those that require the qualitative analysis of circulating fetal DNA, and those that require its quantitative assessment. These analyses typically start with the separation of plasma from the cellular components of maternal blood, followed by the extraction of DNA from the plasma fraction[19,20]. For both of these types of application, it is important to bear in mind that fetal DNA only represents a minor fraction of the DNA that is present in maternal plasma[5]. Therefore, the analytical method must detect this subpopulation of cell-free DNA with high sensitivity, and differentiate the target fetal DNA from the overwhelming background of maternal DNA. In practice, the easiest way to achieve this is by targeting chromosomes, genes or genetic polymorphisms and mutations that the fetus has inherited from the father, and that are either absent or different in the mother.

Qualitative assessments. Clinical information from such analysis can result in either the positive identification of, or the exclusion of, a genetic trait or disease (FIG. 1). At the chromosomal level, the most obvious applications are those that detect sequences from the Y chromosome of male fetuses in maternal plasma. These tests are close to 100% accurate, and are useful for the prenatal risk stratification of pregnancies that involve mothers who carry mutations for sex-linked diseases such as haemophilia A[21]. Fetal gender determination is also clinically useful for the prenatal management of congenital adrenal hyperplasia, as the virilization of the genitalia that is associated with this disease is only a problem for female fetuses[22]. At the genetic level, the most important procedure is, perhaps, the detection of the rhesus blood group antigen D (*RHD*) gene from a RHD-positive fetus in the plasma of RHD-negative mothers (15% of the Caucasian population)[23]. These mothers, when carrying a RHD-positive fetus, might have rhesus D incompatibility, a condition that requires further management. As this test is close to 100% accurate, it has now been introduced as a routine test in a number of centres[3].

The first polymorphisms or mutations to be detected from fetal DNA were those associated with multiple nucleotide differences between the fetal and maternal DNA, including microsatellite polymorphisms[24], trinucleotide repeat expansion (for example, within the gene that causes myotonic dystrophy)[25], and mutations caused by multiple base deletion (for example, the 4 bp deletion in codons 41 and 42 of the haemoglobin-β (*HBB*) gene, which causes β-thalassaemia)[26]. The technical challenge of distinguishing between fetal and maternal circulating DNA down to a single nucleotide has recently been overcome by the development of a single-allele base extension reaction protocol for mass spectrometry analysis[27]. This extends the plasma DNA approach to the non-invasive prenatal diagnosis of many single-gene disorders. For genetic diseases that are caused by more than one mutation, and in which the father and mother carry different mutations, this approach can allow direct detection of the unique paternally inherited mutations (FIG. 1). For situations in which the father and mother carry the same mutation, this method can be used to trace the inheritance of the mutant (or normal) chromosome by the fetus through the analysis of linked SNPs[27] (FIG. 1).

Table 1 | **Potential clinical applications of analysing fetal nucleic acids in maternal plasma**

Approach	Analytical principles	Marker	Clinical applications	Refs
Fetal DNA analysis for diagnosis or exclusion of fetal genetic disorders	Qualitative detection of fetal-specific sequence (for example, Y chromosome, *RHD*, paternal-specific disease-associated alleles) in maternal plasma by real-time, fluorescent, nested or conventional PCR, and mass-spectrometry based methods, such as single-allele base extension reaction.	Fetal gender	Sex-linked disorders, congenital adrenal hyperplasia	21,22
		Fetal *RHD*	Early identification of pregnancies with rhesus D incompatibility, risk stratification of pregnancies requiring treatment with RHD immunoglobulin	3,23
		Fetal HLA haplotype	HLA-linked diseases; for example, congenital adrenal hyperplasia	60
		Paternally derived mutations	β-thalassaemia, haemoglobin E disease, cystic fibrosis, congenital adrenal hyperplasia, myotonic dystrophy, Huntington disease	25-27, 31,60
Fetal DNA analysis for assessment or monitoring of pregnancy-associated disorders	Quantification of fetal DNA concentration in maternal plasma by real-time quantitative or competitive PCR, and comparison with gestational age-matched normal pregnancies.	Fetal conditions	Chromosomal aneuploidy, intrauterine growth retardation, fetomaternal haemorrhage	29,61
		Other pregnancy complications	Pre-eclampsia, pre-term labour, ectopic pregnancy, invasive placentation, hyperemesis gravidarum, polyhydramnios	17,18,28, 62,63
Placental RNA analysis	Quantification of placentally expressed transcripts by real-time quantitative reverse-transcriptase PCR.	*CGB*	Gestational trophoblastic disease	49
		CRH	Pre-eclampsia	48
	Transcript quantification; RNA-SNP allelic ratio determination	Chromosome-21 encoded transcripts	Fetal trisomy 21	44,50
Epigenetic analysis	Real-time quantitative methylation-specific PCR; epigenetic allelic ratio determination	Hypomethylated *SERPINB5*	Pre-eclampsia, fetal trisomy 18	15

CGB, chorionic gonadotropin, beta polypeptide; *CRH*, corticotropin releasing hormone; HLA, human leukocyte antigen; *RHD*, rhesus blood group, D antigen.

Quantitative assessments. Abnormally high levels of circulating fetal DNA occur in several pregnancy-associated disorders, including pre-eclampsia[17,18], pre-term labour[28], and fetal chromosomal aneuploidies[29,30] (TABLE 1). Therefore, there have been suggestions that measuring the overall amount of circulating fetal DNA might be used as a general screening tool for pregnancy-associated disorders[18]. However, most groups have used loci on the Y chromosome as markers for circulating fetal DNA, limiting this approach to pregnancies that involve male fetuses. Therefore, as discussed below, one active area of research is the search for fetal DNA markers that can be used irrespective of the gender and genetic polymorphisms of the fetus. Another limitation is the significant overlap in circulating fetal DNA levels between normal and complicated pregnancies[17,29,30], which has reduced the sensitivity and specificity of this type of screening strategy. One possible solution is to search for fetal nucleic-acid markers that are specifically associated with a particular pathology; for example, fetal RNA (see below).

Recent advances

In this section we highlight a number of recent developments. It is expected that significant developments and discussion in these areas will take place over the next few years.

Fetal DNA enrichment. Many of the analytical and diagnostic limitations of fetal DNA in maternal plasma stem from the relative concentration of fetal DNA to the overwhelming background of maternal DNA, of the order of 1:20 or less[5]. The fractional concentration of fetal DNA could theoretically be increased by one of two approaches: through the selective enrichment of fetal DNA, or by suppressing the maternal DNA background. It has recently been demonstrated that the size of fragments of fetal DNA in maternal plasma is generally smaller than that of the background maternal DNA[4]. This size difference allows a degree of fetal DNA enrichment, by harvesting the DNA molecules of smaller molecular weight from the plasma following gel electrophoresis[31]. The main limitations of this approach are that the degree of enrichment that is achieved is only moderate, and the manipulations involved in the electrophoretic and subsequent DNA isolation steps are susceptible to exogenous contamination. The second concern could potentially be overcome by the development of devices which allow these steps to be carried out in an enclosed environment.

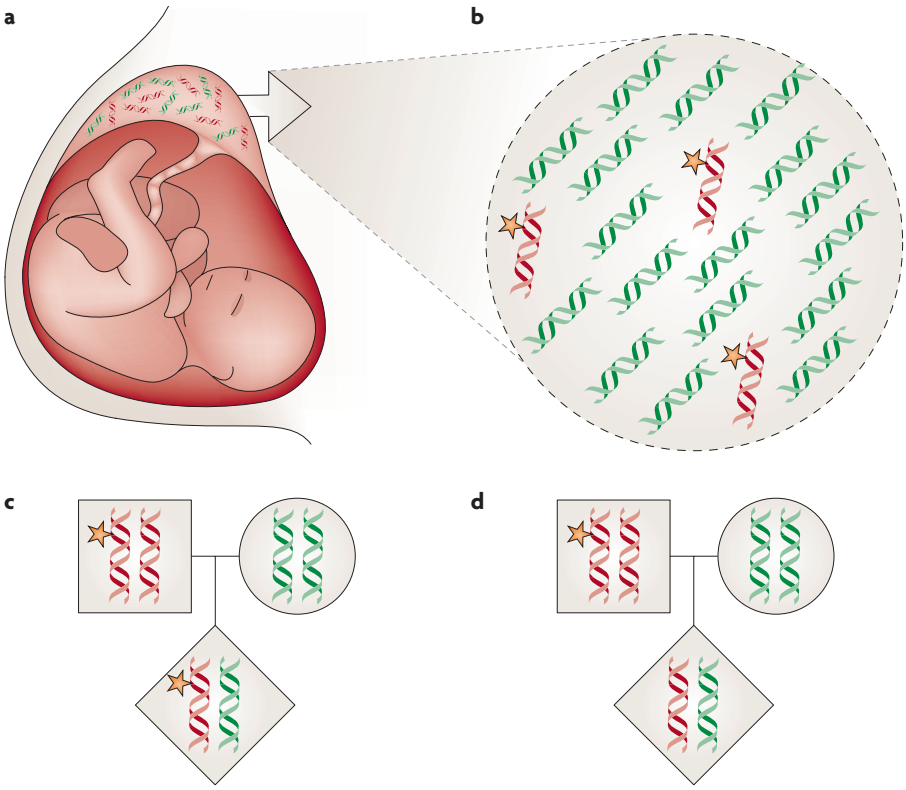

Figure 1 | **Principles of circulating fetal DNA analysis for prenatal diagnosis of single-gene disorders. a** | Release of fetal DNA into the maternal circulation. Before release, the numbers of paternally (red) and maternally inherited (green) DNA molecules are equal. The block arrow denotes the release of fetal DNA from the placenta into the maternal circulation. **b** | In maternal plasma, fetal DNA molecules circulate among a background of maternal DNA sequences. Because the fetal DNA allele that the fetus inherits from its mother (green) is genetically identical to the background maternal sequences (green), the allele that the fetus inherits from its father (red) is the most readily distinguishable fetal-specific sequence in maternal plasma. **c** | Non-invasive prenatal assessment of the fetal genotype could be made on the basis of the positive detection of a molecular feature (yellow star) that is unique to the paternally inherited fetal allele. Examples of unique molecular features include the Y chromosome, *RHD* blood antigen (when the mother is RHD-negative) and paternally inherited mutations or polymorphisms. **d** | Alternatively, non-invasive prenatal assessment could be made on the basis of excluding the inheritance of a molecular feature (yellow star) that is unique to the paternally inherited fetal allele. For example, non-invasive prenatal assessment of an autosomal recessive disease could be achieved by excluding the fetal inheritance of the paternal mutation if the mother and father each carry a different mutation. If the couple both carry an identical mutation, the disease could be excluded in the fetus through the detection of a polymorphism that is uniquely linked to the paternal wild-type allele.

To suppress the background maternal DNA, it is necessary to explore its possible sources. As this is relatively difficult to study directly in pregnant women due to the lack of markers for differentiating between DNA that originates from different tissues, Lui *et al.* studied individuals who had undergone successful sex-mismatched bone-marrow transplantation[32]: in these individuals, the haematopoietic and non-haematopoietic systems were of different gender. Interestingly, it was found that the 'gender' of the plasma DNA switched towards that of the donor, indicating that most of the plasma DNA (median ~60% in male-to-female bone marrow transplantation[32]) was of haematopoietic origin. The sources of the non-haematopoietic plasma DNA are not completely documented, but studies on patients following sex-mismatched heart, liver and kidney transplantations indicate that these organs contribute only a minor percentage of non-haematopoietic DNA in plasma[33]. If these findings can be extrapolated to pregnant women, then the haematopoietic cells should also be the main contributor to the background maternal DNA.

Haematopoietic DNA might be released *in vivo* before the blood is sampled and/or as a consequence of blood sampling, resulting

from the lysis of maternal nucleated blood cells during venesection or in transit in the blood-collection tubes. Dhallan *et al.* therefore reasoned that, if the maternal blood cells were stabilized through fixation with formaldehyde, the *in vitro* release of DNA from the maternal blood cells could be minimized[34]. However, several groups were unable to reproduce the data that Dhallan *et al.* presented to support this line of reasoning[35,36]. One possible explanation for their results could be the use of an imprecise method to determine fetal DNA concentration, which would yield fractional fetal DNA concentrations in some samples that are much higher than the actual values, therefore overestimating the effect, if any, of the formaldehyde treatment[35].

Fetal epigenetic markers. As fetal DNA is surrounded by a majority background of maternal DNA, it has generally been assumed that it would be technically challenging to detect a DNA sequence that the fetus has inherited from its mother. To challenge this assumption, Poon *et al.* studied an imprinted region between the insulin-like growth factor 2 (*IGF2*) and *H19* genes, which is methylated if inherited from the father and unmethylated if inherited from the mother[37]. Poon *et al.* reasoned that, in a pregnant woman who has inherited a copy of this imprinted region from her father (so it is methylated in the cells of the pregnant woman), and who has passed this copy on to her fetus (so it is unmethylated in the cells of the fetus), the methylation status of the fetal and maternal DNA in maternal plasma should be distinguishable from one another[37]. This has led to the first detection of maternally inherited fetal DNA sequences in maternal plasma, and has shown that epigenetic markers might allow one to overcome the limitations of genetic markers in non-invasive prenatal diagnosis.

Another limitation of fetal genetic markers for the detection of fetal DNA in maternal plasma is that no single marker is applicable to all pregnancies. For example, the use of Y-chromosomal markers is only applicable to women who are carrying male fetuses. By contrast, Chim *et al.* have recently demonstrated that *SERPINB5* (encoding maspin) is hypomethylated in the placenta and hypermethylated in maternal blood cells[15]. So, hypomethylated *SERPINB5* sequences can be used as a universal marker for fetal DNA in maternal plasma, irrespective of the gender and polymorphisms of the fetus[15]. This marker was used to detect the elevation of the concentrations of circulating fetal DNA in pre-eclampsia[15].

The main limitation of this marker is the need to carry out bisulfite treatment to convert the fetal-specific epigenetic signature into sequence differences that can then be detected by methods such as PCR. Bisulfite conversion is labour-intensive and inevitably destroys a large proportion of the treated DNA[38]. It would therefore be desirable to search for placental-specific (that is, fetal-specific) epigenetic signatures that are opposite to those exhibited by *SERPINB5*; that is, hypermethylated in the placenta, but hypomethylated in maternal blood cells. If found, such epigenetic markers could be detected with the help of methylation-sensitive restriction enzymes, which can be used to cut the hypomethylated maternal sequences, but will leave the hypermethylated fetal sequences intact for amplification and detection.

Plasma RNA markers. The presence of fetal RNA in maternal plasma was first demonstrated using transcripts from the Y chromosome[39]. This discovery was surprising in view of the known lability of RNA. It has subsequently been demonstrated that the unexpected stability of plasma RNA molecules might be due to their association with particulate matter[40], which might protect them against plasma RNase digestion[41]. The realization that the placenta is the main source of circulating fetal RNA[42] has greatly increased the number of molecular markers that can be developed for prenatal diagnosis and monitoring. The development of a microarray-based method for systematically identifying gene transcripts that are expressed in the placenta but absent in maternal blood cells has further increased the pace of plasma-RNA marker development[43]. To date, over ten placental mRNA species have been shown to be specific to pregnancy and detectable in maternal plasma[42–45].

Circulating placental mRNA is partially degraded, with a preponderance of 5′ fragments[46]. This indicates that assays that are designed towards the 5′ end of a transcript are likely to be more sensitive than those that are designed towards the 3′ end. Circulating placental mRNA is associated with particles[42], and it has been suggested that such particles might be related to syncytiotrophoblast microparticles, which are thought to be shed by the placenta, especially in pathological conditions such as pre-eclampsia[47]. The main advantages of the plasma RNA approach include its potential disease specificity and broad population coverage, irrespective of fetal gender and polymorphisms. In this regard, quantitative aberrations of selected placental mRNA species have been observed in various pregnancy-associated disorders, including corticotropin releasing hormone (*CRH*) mRNA in pre-eclampsia[48] and chorionic gonadotropin β-polypeptide (*CGB*) mRNA in gestational trophoblastic disease[49].

Glossary

Amniocentesis
A clinical procedure that involves the insertion of a needle through the womb to sample the amniotic fluid that bathes the fetus.

Chorionic villus sampling
A clinical procedure that is carried out to obtain a biopsy of the placenta during early pregnancy.

Confined placental chromosomal abnormalities
Chromosomal abnormalities that are restricted in distribution to the placenta, so are not found throughout all cells of the fetus.

Fetomaternal haemorrhage
Bleeding from a fetal source into the maternal circulation.

Gestational trophoblastic disease
The tumorous growth of fetal tissues in a maternal host.

Hyperemesis gravidarum
Severe vomiting in pregnancy.

Mass spectrometry
An analytical technique that detects and identifies small molecules, for example, peptide fragments and oligonucleotides, on the basis of their molecular mass and charge.

Polyhydramnios
An excess of amniotic fluid.

Pre-eclampsia
A clinical condition that presents during the second half of pregnancy; hallmark manifestations are hypertension, oedema and proteinuria.

Rhesus D incompatibility
When a woman with rhesus D antigen (RHD)-negative blood group is pregnant with a RHD-positive fetus, immune destruction of the fetal red blood cells can occur if the maternal immune system has previously been sensitized by the RHD antigen.

Single-allele base extension reaction
An analytical protocol that involves an extension reaction with an oligonucleotide primer that targets the detection of a specific polymorphic allele or a mutation.

Syncytiotrophoblast microparticles
Subcellular particles derived from syncytiotrophoblasts, which are the outermost layer of cells that cover the floating villi of the placenta.

Chromosomal aneuploidy detection. The detection of fetal chromosomal aneuplodies using plasma nucleic acids represents a considerable challenge in the field. First, fetal nucleic acids only represent a minor fraction of the nucleic acids that are present in the plasma of a pregnant woman. Second, the dissociation of circulating nucleic acids from intact cells makes the elucidation of chromosome dosage difficult. One solution to the first problem is to focus on a subfraction of the nucleic acids in maternal plasma that is almost completely fetal-specific, thereby minimising the interference from the background maternal nucleic acids (FIG. 2). One such subfraction is mRNA that is expressed only in the placenta[42]. To provide the aneuploidy information, the placentally expressed mRNA species should be expressed by the chromosome that is involved in the aneuploidy; for example, chromosome 21 in Down syndrome[44]. Using this strategy, Lo *et al.* have recently demonstrated that transcripts from placenta specific 4 (*PLAC4*), a gene that lies on chromosome 21, are detectable in the plasma of pregnant women[50]. To address the problem of obtaining chromosome-dosage information, Lo *et al.* reasoned that, in a euploid fetus that is heterozygous for a SNP within the coding region of *PLAC4*, the ratio of the two alleles should be 1:1, whereas that for a fetus with trisomy 21 should be 1:2 or 2:1 (REF. 50). Using this approach, trisomy 21 was detected non-invasively with a sensitivity of 90% (9 cases were detected out of the 10 trisomy-21 cases that were tested) and a specificity of 96% (54 cases were excluded out of the 56 euploid cases that were tested) in informative cases[50]. These figures, which were achieved using only a single marker, are comparable to those that are currently possible only with multi-marker strategies. The false-positive and false-negative results of this 'RNA-SNP' approach are seen in cases with a relatively low absolute *PLAC4* mRNA concentration in maternal plasma. It is possible that the improvement in plasma processing and RNA extraction methods from maternal plasma will improve the total yield, and therefore the amount of *PLAC4* mRNA that is available for this kind of analysis, further reducing the chance of misdiagnosis.

The main limitation of the RNA-SNP approach is the need to find informative SNPs in the target RNA transcripts. For example, the first *PLAC4* SNP that was used for this approach has a heterozygosity rate of only ~0.45 (REF. 50). There are a number of other transcribed SNPs in *PLAC4*, and at least two of these seem to hold promise for RNA-SNP analysis (unpublished observations). Further increase in informativeness can potentially be achieved by exploring other placentally expressed genes, and their associated SNPs, on chromosome 21. A similar strategy might be applicable to the other trisomies that are important in prenatal diagnosis, trisomy 18 and trisomy 13.

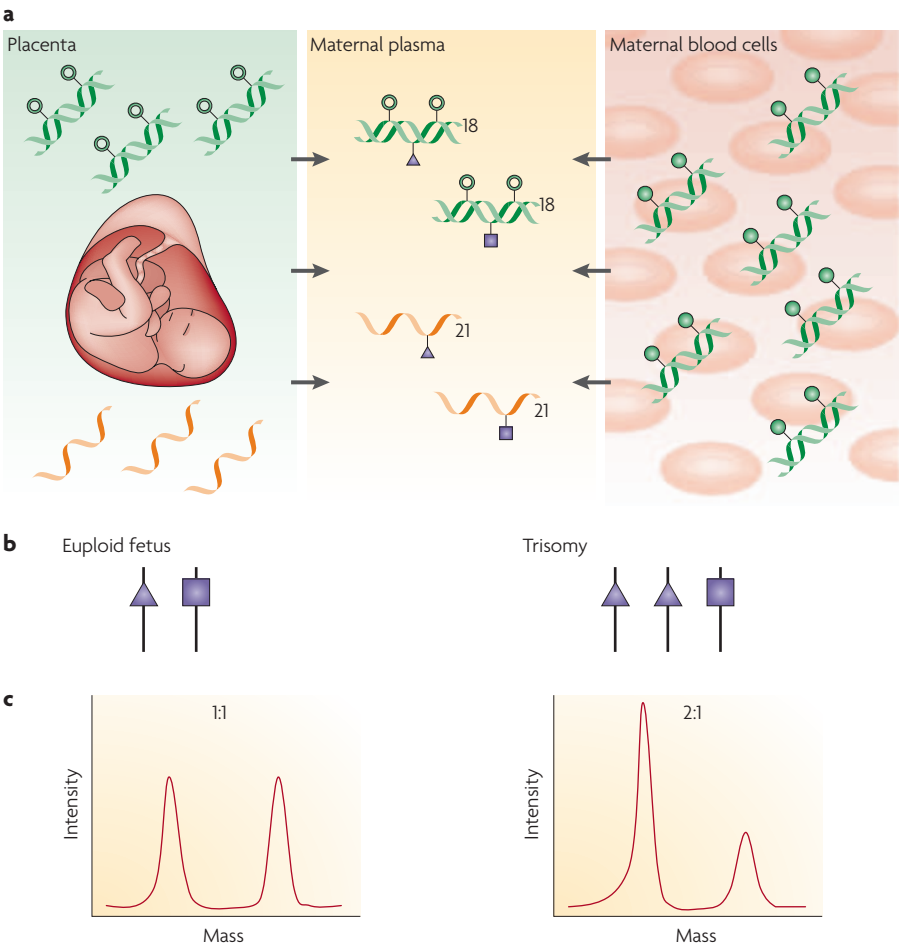

Figure 2 | **Principles of circulating fetal DNA and RNA analysis for the prenatal detection of trisomies. a** | In maternal plasma, most of the background maternal nucleic-acid sequences are derived from maternal blood cells; however, evidence suggests that the fetal-derived sequences originate from the placenta. Therefore, prenatal diagnosis of fetal trisomies could be achieved through the detection of placenta-specific DNA or RNA that is derived from the chromosome of interest. The placental specificity of the molecules could be ascertained by DNA-methylation differences between the placenta and maternal blood cells, as occurs in genes such as *SERPINB5*[15,51] (green double-stranded molecules), which is hypomethylated (open circles) in placental cells and hypermethylated (filled circles) in maternal cells. Alternatively, genes that are expressed by the placenta but not by maternal blood cells, such as *PLAC4* mRNA (orange single-stranded molecules), could be targeted[50]. *SERPINB5* and *PLAC4* are encoded by chromosomes 18 and 21, respectively. Grey squares and triangles denote base-pair differences, which are indicative of different parental origin. **b** | Chromosome-dosage information could be obtained by assessing the ratio of heterozygous alleles of polymorphic markers within the placenta-derived nucleic-acid molecules. For a biallelic polymorphism, we would expect an equal proportion of the two alleles in a heterozygous euploid fetus, whereas an additional dose of one of the alleles is expected for a trisomic fetus. **c** | Therefore, the allelic ratio of the placenta-specific sequence would be 1:1 for a euploid fetus but 2:1 for a trisomic fetus, which could be identified by methods such as mass spectrometry[50,51].

As an alternative to using placental mRNA to focus on the fetal-specific subfraction of circulating nucleic acids in maternal plasma, it is also theoretically possible to analyse circulating DNA that bears a fetal-specific epigenetic signature (FIG. 2). Analogous to the RNA-SNP strategy, the locus that bears the fetal-specific DNA-methylation signature should be on the

chromosome that is involved in the chromosomal aneuploidy. Chromosome-dosage information can then be obtained by SNP allelic ratio analysis. The theoretical basis of this 'epigenetic allelic ratio' approach is illustrated in FIG. 2 (REF. 51). *SERPINB5* (REF. 15), discussed above, is on chromosome 18, and non-invasive prenatal detection of trisomy 18 using the epigenetic allelic ratio approach with this gene has recently been achieved[51]. This strategy also has potential applications in the detection of other trisomies, provided that chromosome-specific and fetal-specific DNA-methylation signatures can be found. With the Human Epigenome Project (see online links), it is possible that such information will be forthcoming over the next few years.

Conclusions

In the nine years since the first discovery of cell-free fetal nucleic acids in maternal plasma[2], these molecules have provided many useful new markers for non-invasive prenatal diagnosis and monitoring. In particular, prenatal RHD genotyping from maternal plasma is now routinely carried out in a number of centres[3]. The recent demonstration that circulating fetal cell-free nucleic acids can be used for the direct detection of trisomy 21 in the fetus[50] will fuel further interest in this area, although much work is needed to replicate these data in a large-scale, multi-centre setting. Furthermore, the impact of this development on existing prenatal screening programmes and the current repertoire of tests, including ultrasound scanning and maternal serum biochemical screening[52], will need to be assessed and discussed. On the cost issue, judging from the current platforms of plasma nucleic-acid-based testing, this methodology should compare favourably with existing invasive methods of prenatal diagnosis, especially when the relatively expensive clinicians' time involvement is factored in.

The molecular characterization of plasma nucleic acids is still in its infancy. Currently, we have only basic information about the size distribution and concentrations of circulating fetal nucleic acids. Little or no information is available regarding possible complexing of these circulating nucleic acids with other biomolecules (for example, proteins and lipids) and the nature and biophysical properties of the particles that associate with circulating fetal RNA. Apart from the intrinsic biological interest of this information, such knowledge might also allow one to develop better methods for enriching fetal nucleic acids from maternal plasma. Such enrichment could simplify analytical procedures and further improve diagnostic accuracy.

Like many other rapidly developing areas of research, the social, ethical and regulatory discussions tend to lag behind the technological progress, although this will hopefully be rectified as we enter the second decade of the field. These issues were recently highlighted by several companies in their marketing of non-invasive molecular tests of fetal gender, which they claim are broadly based on the fetal nucleic acids in maternal blood[53].

Considering the recent progress in plasma nucleic acids, we could be entering the most exciting period of non-invasive prenatal molecular diagnosis so far. We expect that, over the next few years, we will witness the influence of these new developments on many fundamental aspects of the practice of prenatal diagnosis, making prenatal testing safer for the millions of pregnant women worldwide. We also await with anticipation the unravelling of the release and clearance mechanisms and functional implications of plasma nucleic acids.

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Competing interests statement
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OPINION

Recombination: an underappreciated factor in the evolution of plant genomes

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Abstract | Our knowledge of recombination rates and patterns in plants is far from being comprehensive. However, compelling evidence indicates a central role for recombination, through its influences on mutation and selection, in the evolution of plant genomes. Furthermore, recombination seems to be generally higher and more variable in plants than in animals, which could be one of the primary reasons for differences in genome lability between these two kingdoms. Much additional study of recombination in plants is needed to investigate these ideas further.

The nuclear genomes of plants are remarkably variable in terms of characteristics such as genome size, chromosome number, gene order and gene density. The grass family (Poaceae) serves as a fitting example. Since their origin ~77 million years ago[1], the grasses have diverged to range ~55-fold in diploid genome size and at least 10-fold in diploid chromosome number[2]. Some of this genome lability can be attributed to ancient polyploid events[3]. But paleopolyploidy by itself cannot explain the structural variation that exists among the genomes of extant plants. For one thing, paleopolyploid events might be too infrequent[4]. The most recent polyploidy event in the evolutionary lineage of *Arabidopsis thaliana* occurred anywhere from 25 million years to 100 million years ago[5,6], too long ago to contribute to differences in genome size and chromosomal number between *A. thaliana* and its closest relatives.

In addition to changes in ploidy, ongoing mechanisms of gene deletion, genome rearrangement and localized gene duplications that do not involve changes in ploidy undoubtedly contribute to genomic flux. In *A. thaliana*, for example, the proportion of genes that have been duplicated by localized events is comparable to that of genes that have been duplicated by polyploidy. These mechanisms might accelerate after polyploidy events occur, but also function in their absence[7].

In recent years, plant evolutionary genomicists have focused primarily on paleopolyploid events. However, one process that we believe has not received adequate attention is recombination, which generates mutations and influences the strength of natural selection. Here we highlight the increasing body of evidence that indicates that recombination has had an important role in plant genome evolution. We begin by briefly reviewing the mutational properties of recombination, its role in natural selection and genome-wide