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SYMPOSIUM: FUTURES IN REPRODUCTION
REVIEW

Non-invasive prenatal testing using massively parallel sequencing of maternal plasma DNA: from molecular karyotyping to fetal whole-genome sequencing

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Dennis Lo is the Director of the Li Ka Shing Institute of Health Sciences and the Chairman of the Department of Chemical Pathology of The Chinese University of Hong Kong. In 1997, Dennis Lo and his co-workers reported the presence of cell-free fetal DNA in the plasma of pregnant women. Since then, he and his co-workers have elucidated the fundamental biological characteristics regarding circulating fetal DNA as well as its clinical applications in non-invasive prenatal testing. In recognition of his work, Dennis Lo has won numerous awards, including a 2005 State Natural Science Award from the State Council of China and the Ernesto Illy Trieste Science Prize from the Academy of Sciences for the Developing World. He was elected to the Royal Society in 2011 and as a Foreign Associate of the US National Academy of Sciences in 2013.

Abstract The discovery of cell-free fetal DNA in maternal plasma in 1997 has stimulated a rapid development of non-invasive prenatal testing. The recent advent of massively parallel sequencing has allowed the analysis of circulating cell-free fetal DNA to be performed with unprecedented sensitivity and precision. Fetal trisomies 21, 18 and 13 are now robustly detectable in maternal plasma and such analyses have been available clinically since 2011. Fetal genome-wide molecular karyotyping and whole-genome sequencing have now been demonstrated in a number of proof-of-concept studies. Genome-wide and targeted sequencing of maternal plasma has been shown to allow the non-invasive prenatal testing of β-thalassaemia and can potentially be generalized to other monogenic diseases. It is thus expected that plasma DNA-based non-invasive prenatal testing will play an increasingly important role in future obstetric care. It is thus timely and important that the ethical, social and legal issues of non-invasive prenatal testing be discussed actively by all parties involved in prenatal care.

KEYWORDS: cell-free fetal DNA, circulating nucleic acids, Down syndrome screening, fetal DNA in maternal plasma, next-generation DNA sequencing, non-invasive prenatal testing

Introduction

The discovery of cell-free fetal DNA in maternal plasma in 1997 has opened up new possibilities for non-invasive prenatal testing (NIPT) (Lo et al., 1997). Circulating fetal DNA exists in maternal plasma at a mean fractional concentration of approximately 15% (Chiu et al., 2011). Fetal DNA is cleared rapidly from maternal plasma following delivery

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and thus there is no risk of fetal DNA persistence from one pregnancy into the next one (Lo et al., 1999). These characteristics have made circulating fetal DNA an attractive platform for developing NIPT. Indeed, early work had focused on the detection of fetal sex, for the prenatal investigation of sex-linked genetic diseases (Costa et al., 2002) and congenital adrenal hyperplasia (Rijnders et al., 2001) and for the detection of paternally inherited sequences which are absent in the mother's genome: for example, for fetal *RHD* genotyping (Faas et al., 1998; Finning et al., 2008; Lo et al., 1998) and for detecting paternally inherited gene mutations (Chiu et al., 2002).

With the advent of massively parallel sequencing (MPS), the sensitivity and precision with which one can analyse circulating fetal DNA for NIPT have been greatly enhanced. This review attempts to summarise a number of recent developments in this area.

Detection of fetal chromosomal aneuploidies

If a pregnant woman is carrying a fetus with trisomy 21, then the fetus would release an increased amount of chromosome 21-derived DNA sequences into maternal plasma, when compared with the other chromosomes. Thus, provided that one could measure the relative contributions of DNA from different chromosomes in maternal plasma precisely, then one should be able to detect the presence of a trisomic fetus non-invasively. In 2007, Lo et al. (2007) first reported single-molecule counting as one approach through which such precise measurement could be achieved. For such an approach, the higher the number of molecules that are counted, the higher is the analytical precision. Furthermore, the precision that one would need to achieve to detect fetal trisomy is related to the fractional fetal DNA concentration. Hence, the lower is the fractional fetal DNA concentration in a particular maternal plasma sample, the higher is the number of molecules that one would need to count to achieve the detection of the trisomic fetus. This concept was first demonstrated using digital PCR (Lo et al., 2007), a process whereby PCR is carried out on extremely diluted samples containing either one or no target molecules and many amplification reactions are carried out simultaneously. Then, the number of positive reactions is counted, which should provide a measure of the number of target molecules in the original sample.

With the advent of MPS (Schuster, 2008), an even more powerful method for counting millions or even billions of DNA molecules has become available. Compared with digital PCR, the use of MPS allows the DNA contained within a particular maternal plasma sample to be used much more efficiently. Thus, for digital PCR, the DNA molecules that can be counted are those containing the primer-binding sites. The remaining DNA molecules within the sample, which do not contain the primer-binding sites, will be 'wasted', even if they are derived from the chromosome of interest (e.g. chromosome 21). MPS, on the other hand, would allow virtually any DNA molecule contained within the plasma sample to be sequenced and to contribute towards the counting process (Chiu et al., 2009). Indeed, multiple studies have shown that such theoretical predictions are indeed correct and that MPS would allow fetal trisomy 21 to be detected robustly from maternal plasma (Chiu et al., 2008, 2011; Ehrich et al., 2011; Palomaki et al., 2011; Sehnert et al., 2011). It has been demonstrated that the precision of using MPS to measure the representation of chromosomes 13 and 18 is less than that for chromosome 21 (Chiu et al., 2008), possibly as a result of the so-called 'GC bias' (Guanine/Cytosine), as most MPS sequencers in common use require the use of an amplification step prior to performance of the actual sequencing. Nonetheless, through the use of appropriate bioinformatics algorithms that correct for such bias, it has been shown that trisomies 13 and 18 can also be detected with high sensitivity and specificity from maternal plasma (Bianchi et al., 2012; Chen et al., 2011; Dan et al., 2012; Lau et al., 2012). However, the current data suggest that, even with such corrections, the testing accuracies for trisomies 13 and 18 are still inferior to that for trisomy 21, thus indicating that either the GC bias has not been corrected completely by the current algorithms and/or that there are additional factors that have yet to be optimized. There are also recent data suggesting that the use of a single-molecule sequencer, which does not require a prior amplification step, might be beneficial for the detection of trisomy 18, although the application of such a platform for detecting trisomy 13 would require additional optimization (van den Oever et al., 2013).

The MPS process used in the above-mentioned publications is based on the random or shotgun sequencing of DNA molecules in maternal plasma (Chen et al., 2011; Chiu et al., 2008, 2011; Ehrich et al., 2011; Lau et al., 2011; Palomaki et al., 2011; Sehnert et al., 2011; Bianchi et al., 2012; van den Oever et al., 2013). As such random sequencing protocols would analyse sequences from across the whole-genome, including sequences which are of clinical interest (e.g. chromosome 21 sequences) as well as those that might not be clinically significant for a particular application, several groups have argued that it might be more cost effective to use targeted sequencing protocols. Such targeted sequencing protocols have been described and successfully used for detecting a number of chromosomal aneuploidies, focusing on targets on the chromosomes at risk of aneuploidies which are either non-polymorphic (Ashoor et al., 2012; Nicolaides et al., 2012; Norton et al., 2012; Sparks et al., 2012a,b) or those that are polymorphic (Liao et al., 2012; Zimmermann et al., 2012).

MPS-based methods for the NIPT of fetal chromosomal aneuploidies have been available clinically since 2011 in a number of countries, including those in North America, Asia and Europe. The American College of Obstetricians and Gynecologists has recently issued an opinion recommending the use of such testing for screening fetal aneuploidies in high-risk women (Committee Opinion, 2012). A similar opinion has also been published by the Society of Obstetricians and Gynaecologists of Canada (Langlois et al., 2013). It is anticipated that guidelines will be forthcoming in other countries.

Apart from abnormalities involving the entire chromosome, MPS-based analysis of maternal plasma DNA has also been shown to be useful for detecting Down syndrome caused by Robertsonian translocation (Lun et al., 2011), as well as microdeletions and microduplications (Jensen et al., 2012; Peters et al., 2011; Srinivasan et al., 2013; Yu et al., 2013). The latter two publications are particularly interesting because they show that a non-invasive molecular

karyotype of the fetus can be determined using maternal plasma, at a resolution that is comparable to conventional karyotyping: i.e. down to 3 Mb in the study by Yu et al. (2013) and down to 300 kb in the study by Srinivasan et al. (2013). Hence, subchromosomal gains in chromosomes 3q, 6q, 7q, 17q and 22q and subchromosomal losses in chromosomes 4q, 7q, 8p, 10q, 15q, 22q and Xp have been detected. Thus, with further reduction in the costs of sequencing, it is likely that non-invasive prenatal molecular karyotyping will become a practical modality for clinical application. However, large-scale validation would be required, to investigate if the diagnostic performance might vary for different chromosomal regions.

Application to multiple pregnancies

Most of the publications in NIPT using fetal DNA in maternal plasma have focused on singleton pregnancies. Recently, a number of workers have started exploring the application of NIPT to twin pregnancies. Thus, Canick et al. (2012) showed in 25 twin pregnancies that MPS-based analysis of maternal plasma was able to correctly determine whether these pregnancies involved euploid or trisomic fetuses.

For twin pregnancies, one key consideration for performing NIPT is whether the twins are monozygotic or dizygotic. For a monozygotic twin pregnancy, as the two twins have essentially the same genome, one could perform NIPT as if it were for a singleton case. On the other hand, for a dizygotic twin pregnancy, the two twins are genetically distinct and would release different concentrations of fetal DNA into maternal plasma. The amount of sequencing that one would need to perform for maternal plasma to obtain a robust NIPT result is governed by the twin releasing the least amount of DNA into maternal plasma. Qu et al. (2013) have recently developed a technology that would allow one to address these issues. They reasoned that, for dizygotic twin pregnancies, the twins might share paternally inherited alleles that were absent in the maternal genome for parts of the genome and yet be discordant for such inheritance in other parts of the genome. For monozygotic pregnancies, on the other hand, the twins would share such paternally inherited alleles throughout the genome. Thus, if one uses such alleles to measure the fractional concentration of fetal DNA in maternal plasma, the levels would be constant across the genome for monozygotic twin pregnancies. In contrast, for dizygotic twin pregnancies, the observed fractional fetal DNA concentrations would vary across the genome. The data presented by Qu et al. (2013) demonstrated the feasibility of such a concept on four monozygotic twin pregnancies and four dizygotic twin pregnancies. For the monozygotic twin pregnancies, the fractional fetal DNA concentrations measured from each of the 14 targeted chromosomes were very close to each other (standard deviations 0.82 to 1.35). In contrast, for the dizygotic twin pregnancies, the fractional fetal DNA concentrations measured for these chromosomes had much larger variations (standard deviations 2.42 to 4.80). Furthermore, these authors showed that in dizygotic twin pregnancies, one could further determine the relative contribution of DNA by each fetus to maternal plasma. This information would be useful in determining if the depth of sequencing that one performs for NIPT would be sufficient for the fetus releasing the lower amount of DNA into maternal plasma. One could also potentially use this technology for monitoring the serial changes in the relative amounts of DNA released by each fetus in a twin pregnancy as gestation progresses or when pregnancy-associated complications (e.g. pre-eclampsia) arise.

Fetal whole-genome sequencing

In 2010, Lo et al. outlined an approach for obtaining a prenatal genome through the sequencing of DNA in maternal plasma (Lo et al., 2010). For the deduction of the fetal genome, they had used the genotype information from the father and maternal haplotype information deduced from pedigree analysis. For the maternal haplotype information, as an alternative to using pedigree analysis, other approaches, including the more recent ones based on MPS (Fan et al., 2011; Peters et al., 2012), can be used. This group has further shown that in situations where the father's DNA is not available, one could deduce the alleles that the fetus has inherited from its father through the deep sequencing of selected genomic regions through targeted sequencing (Liao et al., 2011).

The approach by Lo et al. has recently been confirmed by two other groups (Fan et al., 2012; Kitzman et al., 2012). The general approach described by Kitzman et al. is very similar to that by Lo et al. (2010) and involves maternal plasma DNA sequencing, paternal DNA genotyping and maternal DNA haplotyping. Compared with the data presented by Lo et al., Kitzman et al. (2012) performed maternal plasma DNA sequencing to a deeper extent (78-fold haploid genome coverage), as compared with 65-fold haploid genome coverage (Lo et al., 2010) and had a 10-fold higher coverage of single-nucleotide polymorphisms (SNP) across the genome. Kitzman et al. (2011) also directly determined the haplotype of the mother using large fragment cloning and sequencing. Due to the depth of sequencing that had been performed, they were also able to investigate if the approach would allow fetal de-novo mutations to be detected. They found that of the 44 de-novo mutations that the studied fetus actually possessed, 39 were detectable from the maternal plasma DNA sequencing results. The problem with this approach was that there were a total of 25 million candidate de-novo mutations and thus the false-positive rate of the method is impractically high at present.

The general approach by Fan et al. (2011) is also similar to that of Lo et al. (2010) and Kitzman et al. (2011). Thus, Fan et al. performed maternal plasma DNA sequencing and haplotyping of the mother. The main difference is that instead of genotyping the father, they deduced the fetal alleles inherited from its father through the identification of alleles that were not present in the mother's genome. This approach is similar to that previously described by Liao et al. (2011), who based their conclusion on SNP that were present on chromosome X and captured by the Illumina SureSelect Human X Chromosome Kit, while Fan et al. based their results on the SNP that were present in an Illumina Omni1Quad BeadChip array. At the current sequencing error rate of MPS, if such an approach were to be extrapolated on a whole-genome level, beyond regions known to be

polymorphic (e.g. in SNP databases), then it is likely that the false-positive rate would be very high. In other words, if the father's genotype information is not available for reference, then any sequencing errors would potentially be interpretable as a possible paternally inherited fetal allele. This is analogous to the high false-positive rate encountered by Kitzman et al. (2011) when they used essentially the same approach to look for fetal de-novo mutations. Hence, at the current accuracy of sequencing technologies, if one is interested in obtaining a fetal whole genome non-invasively, as opposed to just the part of the genome with known polymorphisms, then it would be the most straightforward, practical and robust to analyse the father's and the mother's DNA in conjunction with the maternal plasma DNA sequencing data than to deduce that paternal inheritance from the maternal plasma DNA data as suggested by Fan et al. (2012).

Detection of monogenic diseases

The application of NIPT to monogenic diseases involves the detection of a paternally inherited fetal mutation that is not present in the mother's genome (Amicucci et al., 2000; Chiu et al., 2002). For autosomal dominant disorders, such an approach is clinically valuable because the presence of the paternally inherited mutation in maternal plasma would signify a fetus suffering from the disease while its absence would suggest an unaffected fetus. For autosomal recessive disorders, the approach is most valuable for the scenario when one cannot detect that paternally inherited mutation in maternal plasma, which would suggest that the fetus cannot be homozygous for the disease-causing mutation. For this scenario, an invasive prenatal test can be avoided. On the other hand, if a paternally inherited mutation is detectable in maternal plasma, then the fetus could either be a heterozygous carrier or a homozygous sufferer of the disease. This scenario may not be too useful clinically as an invasive test would still be required.

In 2008, Lun et al. reported an approach called relative mutation dosage that could be used for the NIPT of virtually all monogenic diseases (Lun et al., 2008). This approach allows one to deduce a fetal genotype based on the measurement of subtle differences in concentrations of a mutant and a normal gene, as well as between two alleles of a SNP, using digital PCR. This approach has now been implemented successfully for the NIPT of haemophilia (Tsui et al., 2011) and sickle cell anaemia (Barrett et al., 2012). With the advent of MPS, one could perform mutation dosage and polymorphism dosage analyses through the sequencing of millions of DNA molecules from maternal plasma. Hence, the paternal inheritance of the fetus can be determined by finding paternal SNP alleles in maternal plasma that are absent from the maternal genome. These are alleles that the fetus has inherited from its father. On the other hand, the maternal inheritance of the fetus can be determined for genomic regions in which the mother is heterozygous and in which the maternal haplotype structure for those regions are known (e.g. by pedigree analysis). Then, the SNP alleles sequenced from maternal plasma are bioinformatically mapped to each of the two maternal haplotypes and their relative concentrations measured. This approach has been used for the NIPT of β-thalassaemia either through shotgun genome-wide sequencing (Lo et al., 2010) or targeted sequencing (Lam et al., 2012) of genomic regions of interest. This approach can potentially be applied to any genetic disease. The targeted sequencing variant of this approach is potentially a cost-effective approach to implement such a technology clinically. For example, it would be possible to develop capture probes that would target genomic regions implicated in genetic diseases common in a particular population.

There are advantages and disadvantages to the use of digital PCR versus MPS for the NIPT of monogenic diseases. The advantage of the digital PCR-based approach is that the platform is relatively simple and cheap. However, the number of molecules analysed is typically much lower than that analysed for MPS. As the robustness of NIPT is based on such molecular counting approaches, it is likely that a digital PCR-based approach would be less robust than one based on MPS.

Ethical, social and legal issues

The development of NIPT has raised numerous ethical, social and legal issues (Benn and Chapman, 2010; Greely, 2011). For example, the relative ease of performing NIPT compared with conventional invasive testing has raised the question as to whether such developments would encourage more women to undergo prenatal testing for screening purposes than would otherwise be the case (Franklin, 2013). With the development of technologies for non-invasive fetal molecular karyotyping (Srinivasan et al., 2013; Yu et al., 2013) and even fetal whole-genome sequencing (Fan et al., 2012; Kitzman et al., 2012; Lo et al., 2010), one has to consider the spectrum of genomic aberrations which would be clinically indicated, as opposed to being just technically feasible, for NIPT. In this regard, the current knowledge on the pathogenic implication, if any, of many genomic aberrations remains incomplete. Thus, the premature implementation of NIPT that would cover such aberrations would create difficulties in counselling and might generate unnecessary patient anxiety. There is also the concern that NIPT might be used for non-medical indications, e.g. sexing for social reasons. This is especially a concern when certain commercial vendors might provide such tests in a direct-to-consumer setting, which might allow patients to gain access to such tests without any or adequate counselling and having clinical professionals to monitor the performance of such tests (Bianchi, 2006). It is thus important that the discussion and research into the ethical, social and legal aspects of NIPT be encouraged alongside the rapid technological developments in the field.

Conclusions

The last 15 years have witnessed a rapid development of NIPT using cell-free fetal DNA in maternal plasma. With the development of MPS, selected fetal chromosomal aneuploidies have been robustly detected in maternal plasma and such tests have been clinically available since 2011. Proof-of-concept studies about fetal molecular karyotyping and even fetal whole-genome sequencing have recently been published. With the current momentum in the field, it is likely that such tests might impact the clinical practice

of prenatal diagnosis within a few years. It is thus timely and important that discussion on the ethical, social and legal implications of NIPT be carried out and that these catch up with the technological progress. When properly implemented, this technology will be expected to make prenatal testing safer and more informative in the future.

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References

Amicucci, P., Gennarelli, M., Novelli, G., Dallapiccola, B., 2000. Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma. Clin. Chem. 46, 301–302.

Ashoor, G., Syngelaki, A., Wagner, M., Birdir, C., Nicolaides, K.H., 2012. Chromosome-selective sequencing of maternal plasma cell-free DNA for first-trimester detection of trisomy 21 and trisomy 18. Am. J. Obstet. Gynecol. 206, 322 e321–325.

Barrett, A.N., Mcdonnell, T.C., Chan, K.C., Chitty, L.S., 2012. Digital PCR analysis of maternal plasma for noninvasive detection of sickle cell anemia. Clin. Chem. 58, 1026–1032.

Benn, P.A., Chapman, A.R., 2010. Ethical challenges in providing noninvasive prenatal diagnosis. Curr. Opin. Obstet. Gynecol. 22, 128–134.

Bianchi, D.W., 2006. At-home fetal DNA gender testing: caveat emptor. Obstet. Gynecol. 107, 216–218.

Bianchi, D.W., Platt, L.D., Goldberg, J.D., Abuhamad, A.Z., Sehnert, A.J., Rava, R.P. MatErnal BLood IS Source to Accurately diagnose fetal aneuploidy (MELISSA) Study Group, 2012. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet. Gynecol. 119, 890–901.

Canick, J.A., Kloza, E.M., Lambert-Messerlian, G.M., Haddow, J.E., Ehrich, M., van Den Boom, D., Bombard, A.T., Deciu, C., Palomaki, G.E., 2012. DNA sequencing of maternal plasma to identify Down syndrome and other trisomies in multiple gestations. Prenat. Diagn. 32, 730–734.

Chen, E.Z., Chiu, R.W., Sun, H., Akolekar, R., Chan, K.C., Leung, T.Y., Jiang, P., Zheng, Y.W., Lun, F.M., Chan, L.Y., Jin, Y., Go, A.T., Lau, E.T., To, W.W., Leung, W.C., Tang, R.Y., Au-Yeung, S.K., Lam, H., Kung, Y.Y., Zhang, X., van Vugt, J.M., Minekawa, R., Tang, M.H., Wang, J., Oudejans, C.B., Lau, T.K., Nicolaides, K.H., Lo, Y.M., 2011. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. PLoS ONE 6, e21791.

Chiu, R.W.K., Lau, T.K., Leung, T.N., Chow, K.C.K., Chui, D.H.K., Lo, Y.M.D., 2002. Prenatal exclusion of beta-thalassaemia major by examination of maternal plasma. Lancet 360, 998–1000.

Chiu, R.W.K., Chan, K.C.A., Gao, Y., Lau, V.Y.M., Zheng, W., Leung, T.Y., Foo, C.H.F., Xie, B., Tsui, N.B.Y., Lun, F.M.F., Zee, B.C.Y., Lau, T.K., Cantor, C.R., Lo, Y.M.D., 2008. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. Proc. Natl. Acad. Sci. USA 105, 20458–20463.

Chiu, R.W., Cantor, C.R., Lo, Y.M., 2009. Non-invasive prenatal diagnosis by single molecule counting technologies. Trends Genet. 25, 324–331.

Chiu, R.W., Akolekar, R., Zheng, Y.W., Leung, T.Y., Sun, H., Chan, K.C., Lun, F.M., Go, A.T., Lau, E.T., To, W.W., Leung, W.C., Tang, R.Y., Au-Yeung, S.K., Lam, H., Kung, Y.Y., Zhang, X., van Vugt, J.M., Minekawa, R., Tang, M.H., Wang, J., Oudejans, C.B., Lau, T.K., Nicolaides, K.H., Lo, Y.M., 2011. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. BMJ 342, c7401.

Committee Opinion No. 545. American College of Obstetricians and Gynecologists, 2012. Noninvasive prenatal testing for fetal aneuploidy. Obstet. Gynecol. 120, 1532–1534.

Costa, J.M., Benachi, A., Gautier, E., 2002. New strategy for prenatal diagnosis of X-linked disorders. N. Engl. J. Med. 346, 1502.

Dan, S., Wang, W., Ren, J., Li, Y., Hu, H., Xu, Z., Lau, T.K., Xie, J., Zhao, W., Huang, H., Xie, J., Sun, L., Zhang, X., Wang, W., Liao, S., Qiang, R., Cao, J., Zhang, Q., Zhou, Y., Zhu, H., Zhong, M., Guo, Y., Lin, L., Gao, Z., Yao, H., Zhang, H., Zhao, L., Jiang, F., Chen, F., Jiang, H., Li, S., Li, Y., Wang, J., Wang, J., Duan, T., Su, Y., Zhang, X., 2012. Clinical application of massively parallel sequencing-based prenatal noninvasive fetal trisomy test for trisomies 21 and 18 in 11,105 pregnancies with mixed risk factors. Prenat. Diagn. 32, 1225–1232.

Ehrich, M., Deciu, C., Zwiefelhofer, T., Tynan, J.A., Cagasan, L., Tim, R., Lu, V., Mccullough, R., Mccarthy, E., Nygren, A.O., Dean, J., Tang, L., Hutchison, D., Lu, T., Wang, H., Angkachatchai, V., Oeth, P., Cantor, C.R., Bombard, A., van Den Boom, D., 2011. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. Am. J. Obstet. Gynecol. 204, 205 e201–211.

Faas, B.H., Beuling, E.A., Christiaens, G.C., Von Dem Borne, A.E., Van Der Schoot, C.E., 1998. Detection of fetal RHD-specific sequences in maternal plasma. Lancet 352, 1196.

Fan, H.C., Wang, J., Potanina, A., Quake, S.R., 2011. Whole-genome molecular haplotyping of single cells. Nat. Biotechnol. 29, 51–57.

Fan, H.C., Gu, W., Wang, J., Blumenfeld, Y.J., El-Sayed, Y.Y., Quake, S.R., 2012. Non-invasive prenatal measurement of the fetal genome. Nature 487, 320–324.

Finning, K., Martin, P., Summers, J., Massey, E., Poole, G., Daniels, G., 2008. Effect of high throughput RHD typing of fetal DNA in maternal plasma on use of anti-RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study. BMJ 336, 816–818.

Franklin, S., 2013. Conception Through a Looking Glass: the paradox of IVF. Reprod. Biomed. Online 27, 747–755.

Greely, H.T., 2011. Get ready for the flood of fetal gene screening. Nature 469, 289–291.

Jensen, T.J., Dzakula, Z., Deciu, C., Van Den Boom, D., Ehrich, M., 2012. Detection of microdeletion 22q11.2 in a fetus by next-generation sequencing of maternal plasma. Clin. Chem. 58, 1148–1151.

Kitzman, J.O., Mackenzie, A.P., Adey, A., Hiatt, J.B., Patwardhan, R.P., Sudmant, P.H., Ng, S.B., Alkan, C., Qiu, R., Eichler, E.E., Shendure, J., 2011. Haplotype-resolved genome sequencing of a Gujarati Indian individual. Nat. Biotechnol. 29, 59–63.

Kitzman, J.O., Snyder, M.W., Ventura, M., Lewis, A.P., Qiu, R., Simmons, L.E., Gammill, H.S., Rubens, C.E., Santillan, D.A., Murray, J.C., Tabor, H.K., Bamshad, M.J., Eichler, E.E., Shendure, J., 2012. Noninvasive whole-genome sequencing of a human fetus. Sci. Transl. Med. 4, 137ra176.

Lam, K.W., Jiang, P., Liao, G.J., Chan, K.C., Leung, T.Y., Chiu, R.W., Lo, Y.M., 2012. Noninvasive prenatal diagnosis of monogenic diseases by targeted massively parallel sequencing of maternal plasma: application to beta-thalassemia. Clin. Chem. 58, 1467–1475.

Langlois, S., Brock, J.A., Genetics, C., Wilson, R.D., Audibert, F., Brock, J.A., Carroll, J., Cartier, L., Gagnon, A., Johnson, J.A., Langlois, S., Macdonald, W., Murphy-Kaulbeck, L., Okun, N., Pastuck, M., Senikas, V., 2013. Current status in non-invasive prenatal detection of Down syndrome, trisomy 18, and trisomy 13 using cell-free DNA in maternal plasma. J. Obstet. Gynaecol. Can. 35, 177–181.

Lau, T.K., Chen, F., Pan, X., Pooh, R.K., Jiang, F., Li, Y., Jiang, H., Li, X., Chen, S., Zhang, X., 2012. Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing. J. Matern. Fetal Neonatal Med. 25, 1370—1374.

Liao, G.J., Lun, F.M., Zheng, Y.W., Chan, K.C., Leung, T.Y., Lau, T.K., Chiu, R.W., Lo, Y.M., 2011. Targeted massively parallel sequencing of maternal plasma DNA permits efficient and unbiased detection of fetal alleles. Clin. Chem. 57, 92—101.

Liao, G.J., Chan, K.C., Jiang, P., Sun, H., Leung, T.Y., Chiu, R.W., Lo, Y.M.D., 2012. Noninvasive prenatal diagnosis of fetal trisomy 21 by allelic ratio analysis using targeted massively parallel sequencing of maternal plasma DNA. PLoS ONE 7, e38154.

Lo, Y.M.D., Corbetta, N., Chamberlain, P.F., Rai, V., Sargent, I.L., Redman, C.W., Wainscoat, J.S., 1997. Presence of fetal DNA in maternal plasma and serum. Lancet 350, 485—487.

Lo, Y.M.D., Hjelm, N.M., Fidler, C., Sargent, I.L., Murphy, M.F., Chamberlain, P.F., Poon, P.M., Redman, C.W., Wainscoat, J.S., 1998. Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N. Engl. J. Med. 339, 1734—1738.

Lo, Y.M.D., Zhang, J., Leung, T.N., Lau, T.K., Chang, A.M., Hjelm, N.M., 1999. Rapid clearance of fetal DNA from maternal plasma. Am. J. Hum. Genet. 64, 218—224.

Lo, Y.M.D., Lun, F.M.F., Chan, K.C.A., Tsui, N.B.Y., Chong, K.C., Lau, T.K., Leung, T.Y., Zee, B.C.Y., Cantor, C.R., Chiu, R.W.K., 2007. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. Proc. Natl. Acad. Sci. USA 104, 13116—13121.

Lo, Y.M.D., Chan, K.C., Sun, H., Chen, E.Z., Jiang, P., Lun, F.M., Zheng, Y.W., Leung, T.Y., Lau, T.K., Cantor, C.R., Chiu, R.W., 2010. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. Sci. Transl. Med. 2, 61ra91.

Lun, F.M., Tsui, N.B., Chan, K.C., Leung, T.Y., Lau, T.K., Charoenkwan, P., Chow, K.C., Lo, W.Y., Wanapirak, C., Sanguansermsri, T., Cantor, C.R., Chiu, R.W., Lo, Y.M., 2008. Noninvasive prenatal diagnosis of monogenic diseases by digital size selection and relative mutation dosage on DNA in maternal plasma. Proc. Natl. Acad. Sci. USA 105, 19920—19925.

Lun, F.M., Jin, Y.Y., Sun, H., Leung, T.Y., Lau, T.K., Chiu, R.W., Lo, Y.M.D., 2011. Noninvasive prenatal diagnosis of a case of Down syndrome due to robertsonian translocation by massively parallel sequencing of maternal plasma DNA. Clin. Chem. 57, 917—919.

Nicolaides, K.H., Syngelaki, A., Ashoor, G., Birdir, C., Touzet, G., 2012. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am. J. Obstet. Gynecol. 207, 374. e1—6.

Norton, M.E., Brar, H., Weiss, J., Karimi, A., Laurent, L.C., Caughey, A.B., Rodriguez, M.H., Williams 3rd, J., Mitchell, M.E., Adair, C.D., Lee, H., Jacobsson, B., Tomlinson, M.W., Oepkes, D., Hollemon, D., Sparks, A.B., Oliphant, A., Song, K., 2012. Non-Invasive Chromosomal Evaluation (NICE) study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am. J. Obstet. Gynecol. 207, 137. e1—8.

Palomaki, G.E., Kloza, E.M., Lambert-Messerlian, G.M., Haddow, J.E., Neveux, L.M., Ehrich, M., van Den Boom, D., Bombard, A.T., Deciu, C., Grody, W.W., Nelson, S.F., Canick, J.A., 2011. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet. Med. 13, 913—920.

Peters, B.A., Kermani, B.G., Sparks, A.B., Alferov, O., Hong, P., Alexeev, A., Jiang, Y., Dahl, F., Tang, Y.T., Haas, J., Robasky, K., Zaranek, A.W., Lee, J.H., Ball, M.P., Peterson, J.E., Perazich, H., Yeung, G., Liu, J., Chen, L., Kennemer, M.I., Pothuraju, K., Konvicka, K., Tsoupko-Sitnikov, M., Pant, K.P., Ebert, J.C., Nilsen, G.B., Baccash, J., Halpern, A.L., Church, G.M., Drmanac, R., 2012. Accurate whole-genome sequencing and haplotyping from 10 to 20 human cells. Nature 487, 190—195.

Peters, D., Chu, T., Yatsenko, S.A., Hendrix, N., Hogge, W.A., Surti, U., Bunce, K., Dunkel, M., Shaw, P., Rajkovic, A., 2011. Noninvasive prenatal diagnosis of a fetal microdeletion syndrome. N. Engl. J. Med. 365, 1847—1848.

Qu, J.Z., Leung, T.Y., Jiang, P., Liao, G.J., Cheng, Y.K., Sun, H., Chiu, R.W., Chan, K.C., Lo, Y.M.D., 2013. Noninvasive prenatal determination of twin zygosity by maternal plasma DNA analysis. Clin. Chem. 59, 427—435.

Rijnders, R.J., van Der Schoot, C.E., Bossers, B., de Vroede, M.A., Christiaens, G.C., 2001. Fetal sex determination from maternal plasma in pregnancies at risk for congenital adrenal hyperplasia. Obstet. Gynecol. 98, 374—378.

Schuster, S.C., 2008. Next-generation sequencing transforms today's biology. Nat. Methods 5, 16—18.

Sehnert, A.J., Rhees, B., Comstock, D., De Feo, E., Heilek, G., Burke, J., Rava, R.P., 2011. Optimal detection of fetal chromosomal abnormalities by massively parallel DNA sequencing of cell-free fetal DNA from maternal blood. Clin. Chem. 57, 1042—1049.

Sparks, A.B., Struble, C.A., Wang, E.T., Song, K., Oliphant, A., 2012a. Noninvasive prenatal detection and selective analysis of cell-free DNA obtained from maternal blood: evaluation for trisomy 21 and trisomy 18. Am. J. Obstet. Gynecol. 206, 319. e1—9.

Sparks, A.B., Wang, E.T., Struble, C.A., Barrett, W., Stokowski, R., Mcbride, C., Zahn, J., Lee, K., Shen, N., Doshi, J., Sun, M., Garrison, J., Sandler, J., Hollemon, D., Pattee, P., Tomita-Mitchell, A., Mitchell, M., Stuelpnagel, J., Song, K., Oliphant, A., 2012b. Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat. Diagn. 32, 3—9.

Srinivasan, A., Bianchi, D.W., Huang, H., Sehnert, A.J., Rava, R.P., 2013. Noninvasive detection of fetal subchromosome abnormalities via deep sequencing of maternal plasma. Am. J. Hum. Genet. 92, 167—176.

Tsui, N.B., Kadir, R.A., Chan, K.C., Chi, C., Mellars, G., Tuddenham, E.G., Leung, T.Y., Lau, T.K., Chiu, R.W., Lo, Y.M., 2011. Noninvasive prenatal diagnosis of hemophilia by microfluidics digital PCR analysis of maternal plasma DNA. Blood 117, 3684—3691.

van Den Oever, J.M., Balkassmi, S., Johansson, L.F., Adama Van Scheltema, P.N., Suijkerbuijk, R.F., Hoffer, M.J., Sinke, R.J., Egbert, B., Sikkema-Raddatz, B., Boon, E.M., 2013. Successful noninvasive trisomy 18 detection using single molecule sequencing. Clin. Chem. 59, 705—709.

Yu, S.C.Y., Jiang, P., Choy, K.W., Chan, K.C.A., Won, H.S., Leung, W.C., Lau, E.T., Tang, M.H.Y., Leung, T.Y., Lo, Y.M.D., Chiu, R.W.K., 2013. Noninvasive prenatal molecular karyotyping from maternal plasma. PLoS ONE 8, e60968.

Zimmermann, B., Hill, M., Gemelos, G., Demko, Z., Banjevic, M., Baner, J., Ryan, A., Sigurjonsson, S., Chopra, N., Dodd, M., Levy, B., Rabinowitz, M., 2012. Noninvasive prenatal aneuploidy testing of chromosomes 13, 18, 21, X, and Y, using targeted sequencing of polymorphic loci. Prenat. Diagn. 32, 1233—1241.

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