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Comparison of next generation sequencing-based and methylated DNA immunoprecipitation-based approaches for fetal aneuploidy non-invasive prenatal testing

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Conflict-of-interest: Elisavet A Papageorgiou is currently employed by and owns shares of NIPD Genetics. Philippos C Patsalis also owns shares of NIPD Genetics. Elisavet A Papageorgiou and Philippos C Patsalis have filed a PCT patent application for the MeDIP real time qPCR based NIPD approach (PCT Patent Application No.PCT/1B2011/O00217). Voula Velissariou and Georgia Christopoulou declare that they have no conflict of interest.

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Abstract

Over the past few years, many researchers have attempted to develop non-invasive prenatal testing methods in order to investigate the genetic status of the fetus. The aim is to avoid invasive procedures such as chorionic villus and amniotic fluid sampling, which result in a significant risk for pregnancy loss. The discovery of cell free fetal DNA circulating in the maternal blood has great potential for the development of non-invasive prenatal testing (NIPT) methodologies. Such strategies have been successfully applied for the determination of the fetal rhesus status and inherited monogenic disease but the field of fetal aneuploidy investigation seems to be more challenging. The main reason for this is that the maternal cell free DNA in the mother's plasma is far more abundant, and because it is identical to half of the corresponding fetal DNA. Approaches developed are mainly based on next generation sequencing (NGS) technologies and epigenetic genetic modifications, such as fetal-maternal DNA differential methylation. At present, genetic services for non-invasive fetal aneuploidy detection are offered using NGS-based approaches but, for reasons that are presented herein, they still serve as screening tests which are not readily accessed by the majority of couples. Here we discuss the limitations of both strategies for NIPT and the future potential of the methods developed.

Key words: Next generation sequencing; Differential methylation; Epigenetics; Fetal aneuploidy; Methylation dependent immunoprecipitation; Non-invasive prenatal testing

Core tip: Non-invasive prenatal screening and diagnosis of fetal aneuploidies has been a challenging field

for many researchers. Different methodologies have been developed, mainly based on next-generation sequencing and epigenetic modifications. At present, non-invasive prenatal testing services are offered using next generation sequencing-based technologies which have great potential, but currently they present with certain limitations. Epigenetic approaches may overcome some of these limitations and seem to have promising potential for wider applications.

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INTRODUCTION

Invasive procedures such as chorionic villus sampling (CVS) and amniocentesis are a prerequisite for the prenatal diagnosis of fetal chromosomal abnormalities, either by conventional and/or molecular fetal karyotyping, or other molecular cytogenetic methods. Although these approaches yield accurate results, the rate of pregnancy loss attributed to CVS or amniocentesis is estimated to be 1.0% to 2.0%[1]. This considerable procedure-related risk of pregnancy loss has motivated researchers to try to develop non-invasive approaches in order to provide safer healthcare service.

Since the discovery that fetal cells circulate in the maternal blood during pregnancy[2], numerous researchers worldwide have put great effort towards exploring the possibility of non-invasive prenatal investigation of the fetal genetic constitution. Initially, the focus of investigation was on circulating fetal nucleated cells, where detection of fetal gender and aneuploidies was made possible, mainly by applying FISH subsequent to cell sorting[3-5]. Even though preliminary results were promising, the development of a commercially available application has failed to date, mainly due to certain inherent limitations of the method. Firstly, the rarity of fetal cells in the maternal circulation made it very difficult to isolate a satisfactory number for investigation[4-6]. Secondly, the poor quality of the isolated fetal cells made the application of FISH on the nuclei problematic, resulting in less reliable results. Most importantly, the observation that fetal cells may remain in the maternal circulation for several years after their release, presents a serious problem for non-invasive prenatal investigation of subsequent pregnancies[7,8]. Nevertheless, researchers have not given up this approach entirely and attempts are still being made to overcome limitations[9-13].

The discovery of cell free fetal DNA (cffDNA) in maternal plasma during pregnancy by Lo *et al*[14] in 1997, gave rise to a whole new opportunity in the field of non-invasive prenatal testing (NIPT). Its origin is proven to be either trophoblastic or from embryonic cells in the maternal blood which have undergone apoptosis[15]. It has also been demonstrated that cffDNA is cleared from maternal plasma within a few hours after delivery[16], making its study specific to the current pregnancy. Although cffDNA is detectable from the early stages of pregnancy[17] and increases during its progression[18,19], it is demonstrated to account only for 3.0% to 6.0%[14,20] of total free DNA in maternal plasma. A more recent study utilizing microfluidics, re-estimated the cffDNA fraction to a median of 9.7% in the first trimester[21,22]. The relatively small amount of fetal DNA in maternal plasma presents one of the most serious technical challenges for whichever technology is implemented for investigation. Furthermore, the fact that fetal DNA is 50% identical with that of the mother makes the attempts for fetal aneuploidy testing even more challenging.

CURRENTLY APPLIED METHODS

During recent years independent teams from all over the world have focused on developing methods for NIPT using cffDNA, mainly testing for fetal aneuploidy[23]. Despite applying different strategies including SNP and allelic ratio analyses, none have managed to produce a widely available test, mainly because they depend on informative genotypes or fetal gender[24-26]. On the other hand, next-generation sequencing (NGS) technologies have made great progress in the field, resulting in commercially available NIPT services. In recent years, the use of commercially available tests for NIPT for trisomy 13, 18, 21 and sex chromosome aneuploidies has been introduced into routine antenatal care. Massively parallel direct sequencing reads from a tested chromosome are compared to others with the aid of sophisticated bioinformatics software, resulting in a relative chromosome dose. NGS-based methods are polymorphism independent and have the ability to detect aneuploidies. In a recently published meta-analysis of clinical validation and implementation studies the pooled weighted detection rate for trisomy 21 is reported to be > 99% and the false positive rate to be < 0.01%[27]. Commercially available tests based on NGS technologies have been validated on large numbers of cases and have a very high sensitivity and specificity[28-34] as well (Table 1).

Another promising prospective in NIPT is provided by methylated DNA immunoprecipitation-based (MeDIP-based) approaches. The discovery of fetal-maternal differentially methylated regions (DMRs)[35] has facilitated the development of NIPT strategies by combining MeDIP with other downstream applications. Using the "epigenetic approach", a NIPT method based on MeDIP combined with quantitative polymerase chain reaction which proved to be of high precision in a proof of principle (100% sensitivity, 100% specificity)[36] and larger validation study[37] (100% sensitivity, 99.2%

Table 1 Validation and verification comparison of the most widely used commercially available non-invasive prenatal testing for trisomy 21

Company	Sequenome	Verinata (Illumina)	Ariosa	Natera
Test	"Materni T21 PLUS"	"Verify"	"Harmony"	"Panorama"
Sensitivity	99.6%-99% (209/212)	> 99% (90/90)	100% (81/81)	> 99% (25/25)
Specificity	99.8% (1468/1471)	99.8% (409/410)	99.97% (2887/2888)	> 99% (242/242)
False positive	0.2% (3/1471)	0.2% (1/410)	0.03% (1/2888)	0
No result rate	3.4%	5.8%	4.7%-5.7%	5.4%

specificity) was developed.

ADVANTAGES AND DISADVANTAGES

Approaches based on NGS are extremely powerful. Besides detecting whole chromosome aneuploidy, they have the potential to detect smaller chromosomal imbalances allowing for microdeletion/microduplication syndrome NIPT[38]. However, although NIPT is already commercially available for the detection of a certain number of microdeletion/microduplication syndromes, further validation studies are needed[39]. Taking into account the vast amount of data that NGS is capable of producing, it could be potentially be combined with other methodologies to generate non-invasive fetal whole genome sequencing[40]. As impressive as this may seem at present, it is quite possible that this will materialize in the near future.

The impressive developments of NGS technologies are accompanied however by certain drawbacks. One important limitation is the low level of fetal DNA which is available for testing. This is overcome in MeDIP-based technologies which are based on fetal DNA enrichment, which then increases sensitivity substantially. However, MeDIP by which cffDNA hypermethylated regions are selectively enriched is a stage wherein bias may be introduced, influencing the test results. Therefore, it is very important to carefully select DMRs, optimize this stage and evaluate the overall performance allowing for this. Another drawback of NGS-based approaches is that the equipment/technology required is still not available in all clinical settings, making the service feasible only in large centers, such as those in the United States and China. Furthermore, the requirements for significant infrastructure, complex laboratory procedures, highly trained personnel and challenging bioinformatics analyses make NGS-based technologies costly and complex. In contrast, the "epigenetic approach", uses equipment that is available in most genetic diagnostic laboratories offering established genetic services, it is considerably cheaper and simpler and therefore it may be applied potentially worldwide and offered to a broader population. However, current MeDIP-based approaches focus mainly on fetal trisomy 21 and at present have not yet demonstrated their ability to detect other fetal aneuploidies and submicroscopic aberrations that NGS-based technologies have proven to be capable of detecting. Moreover, large validation studies and future clinical application feedback data are awaited in order to re-evaluate the advantages and disadvantages of MeDIP-based NIPT tests.

THE FUTURE OF FETAL ANEUPLOIDY NIPT

Both NGS and MeDIP-based approaches yield risk classification results at present. This means that a probability is given for each condition investigated, and depending on whether the pregnancy is assessed as being high risk or not, the couples are counseled to proceed with confirmatory invasive diagnostic testing, usually fetal karyotyping after CVS or amniocentesis. False positive results lead to unneeded invasive procedures posing an undesirable risk of pregnancy loss, while false negative results may lead to the birth of an abnormal child. There is an argument that false negative NIPT results for trisomy 18 or 13 are unlikely to result in the birth of an abnormal child because both syndromes are most likely to present with serious ultrasound findings during pregnancy. Conversely, cases with trisomy 21 (Down syndrome) may not have any indications throughout the pregnancy and consequently, NIPT false negative trisomy 21 fetuses are more likely to be born[41]. Therefore, NIPT for trisomy 13, 18 and 21 should be considered as a screening test rather than a diagnostic test, which should be robust, rapid and cost efficient. We believe that MeDIP-based tests meet these requirements for the reasons already presented, and moreover have certain advantages compared to NGS-based methods and therefore show great potential for large scale public service access. At present, if treated as a replacement for current biochemical screening tests, the resulting risk could be combined with that derived from ultrasound markers such as nuchal translucency measurement and others. The combined NIPT-U/S risk for fetal aneuploidy may provide a safer screening strategy compared to that offered to most couples today[42]. The future aim is to eventually avoid invasive procedures and develop NIPT (testing) into NIPD (diagnosis).

For any NIPT used caution is needed when it comes to

genetic counseling, in order to avoid misunderstandings concerning diagnosis. There is an ongoing debate on ethical and policy issues related to NIPT and the European Society of Human Genetics/American Society of Human Genetics invite the scientific community to contribute to setting future guidelines for NIPT[43].

CONCLUSION

During recent years there have been enormous advances in the field of fetal aneuploidy NIPT. Relevant genetic services are offered by academic centers and commercial companies worldwide, but not all future parents have access to this service. Our team is working towards developing a commercially available MeDIP-based test, that will be relatively inexpensive and easy to apply and from which more people can benefit. Looking ahead, we predict that epigenetic based approaches in combination with genetic-based approaches and advanced technologies (digital PCR, NGS) will contribute to the development of NIPT for more subtle fetal genetic abnormalities[44], such as point mutations, microdeletion/microduplication syndromes, *etc*.

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REFERENCES

1 **Mujezinovic F**, Alfirevic Z. Procedure-related complications of amniocentesis and chorionic villous sampling: a systematic review. *Obstet Gynecol* 2007; **110**: 687-694 [PMID: 17766619]
2 **Schröder J**. Transplacental passage of blood cells. *J Med Genet* 1975; **12**: 230-242 [PMID: 809586]
3 **Bianchi DW**, Simpson JL, Jackson LG, Elias S, Holzgreve W, Evans MI, Dukes KA, Sullivan LM, Klinger KW, Bischoff FZ, Hahn S, Johnson KL, Lewis D, Wapner RJ, de la Cruz F. Fetal gender and aneuploidy detection using fetal cells in maternal blood: analysis of NIFTY I data. National Institute of Child Health and Development Fetal Cell Isolation Study. *Prenat Diagn* 2002; **22**: 609-615 [PMID: 12124698]
4 **Jansen MW**, von Lindern M, Beug H, Brandenburg H, Wildschut HI, Wladimiroff JW, In ,t Veld PA. The use of in vitro expanded erythroid cells in a model system for the isolation of fetal cells from maternal blood. *Prenat Diagn* 1999; **19**: 323-329 [PMID: 10327136]
5 **Krabchi K**, Gros-Louis F, Yan J, Bronsard M, Massé J, Forest JC, Drouin R. Quantification of all fetal nucleated cells in maternal blood between the 18th and 22nd weeks of pregnancy using molecular cytogenetic techniques. *Clin Genet* 2001; **60**: 145-150 [PMID: 11553049]
6 **Bianchi DW**, Williams JM, Sullivan LM, Hanson FW, Klinger KW, Shuber AP. PCR quantitation of fetal cells in maternal blood in normal and aneuploid pregnancies. *Am J Hum Genet* 1997; **61**: 822-829 [PMID: 9382092]
7 **Bianchi DW**, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci USA* 1996; **93**: 705-708 [PMID: 8570620]
8 **Evans PC**, Lambert N, Maloney S, Furst DE, Moore JM, Nelson JL. Long-term fetal microchimerism in peripheral blood mononuclear cell subsets in healthy women and women with scleroderma. *Blood* 1999; **93**: 2033-2037 [PMID: 10068676]
9 **Hatt L**, Brinch M, Singh R, Møller K, Lauridsen RH, Schlütter JM, Uldbjerg N, Christensen B, Kølvraa S. A new marker set that identifies fetal cells in maternal circulation with high specificity. *Prenat Diagn* 2014; **34**: 1066-1072 [DOI: 10.1002/pd.4429]
10 **Emad A**, Drouin R. Evaluation of the impact of density gradient centrifugation on fetal cell loss during enrichment from maternal peripheral blood. *Prenat Diagn* 2014; **34**: 878-885 [DOI: 10.1002/pd.4387]
11 **Emad A**, Bouchard EF, Lamoureux J, Ouellet A, Dutta A, Klingbeil U, Drouin R. Validation of automatic scanning of microscope slides in recovering rare cellular events: application for detection of fetal cells in maternal blood. *Prenat Diagn* 2014; **34**: 538-546 [DOI: 10.1002/pd.4345]
12 **Hatt L**, Branch M, Singh R, Møller K, Lauridsen RH, Uldbjerg N, Huppertz B, Christensen B, Kølvraa S. Characterization of fetal cells from the maternal circulation by microarray gene expression analysis--could the extravillous trophoblasts be a target for future cell-based non-invasive prenatal diagnosis? *Fetal Diagn Ther* 2014; **35**: 218-227 [DOI: 10.1159/000356073]
13 **Hua R**, Barrett AN, Tan TZ, Huang Z, Mahyuddin AP, Ponnusamy S, Sandhu JS, Ho SS, Chan JK, Chong S, Quan S, Choolani M. Detection of aneuploidy from single fetal nucleated red blood cells using whole genome sequencing. *Prenat Diagn* 2014; Epub ahead of print [PMID: 25178640 DOI: 10.1002/pd.4491]
14 **Lo YM**, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. Presence of fetal DNA in maternal plasma and serum. *Lancet* 1997; **350**: 485-487 [PMID: 9274585]
15 **Lun FM**, Chiu RW, Sun K, Leung TY, Jiang P, Chan KC, Sun H, Lo YM. Noninvasive prenatal methylomic analysis by genomewide bisulfite sequencing of maternal plasma DNA. *Clin Chem* 2013; **59**: 1583-1594 [DOI: 10.1373/clinchem.2013.212274]
16 **Lo YM**, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 1999; **64**: 218-224 [PMID: 9915961]
17 **Illanes S**, Denbow M, Kailasam C, Finning K, Soothill PW. Early detection of cell-free fetal DNA in maternal plasma. *Early Hum Dev* 2007; **83**: 563-566 [PMID: 17234369]
18 **Lo YM**, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, Wainscoat JS, Johnson PJ, Chang AM, Hjelm NM. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet* 1998; **62**: 768-775 [PMID: 9529358]
19 **Smith SC**, Baker PN, Symonds EM. Placental apoptosis in normal human pregnancy. *Am J Obstet Gynecol* 1997; **177**: 57-65 [PMID: 9240583]
20 **Bischoff FZ**, Sinacori MK, Dang DD, Marquez-Do D, Horne C, Lewis DE, Simpson JL. Cell-free fetal DNA and intact fetal cells in maternal blood circulation: implications for first and second trimester non-invasive prenatal diagnosis. *Hum Reprod Update* 2002; **8**: 493-500 [PMID: 12498419]
21 **Lun FM**, Chiu RW, Chan KC, Leung TY, Lau TK, Lo YM. Microfluidics digital PCR reveals a higher than expected fraction of fetal DNA in maternal plasma. *Clin Chem* 2008; **54**: 1664-1672 [PMID: 18703764 DOI: 10.1373/clinchem.2008.111385]
22 **Ashoor G**, Syngelaki A, Poon LC, Rezende JC, Nicolaides KH. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. *Ultrasound Obstet Gynecol* 2013; **41**: 26-32 [PMID: 23108725 DOI: 10.1002/uog.12331]
23 **Papageorgiou EA**, Patsalis PC. Non-invasive prenatal diagnosis of aneuploidies: new technologies and clinical applications. *Genome Med* 2012; **4**: 46 [PMID: 22640877 DOI: 10.1186/gm345]
24 **Lo YM**, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, Gerovassili A, Jin Y, Nicolaides KH, Cantor CR, Ding C. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. *Nat Med* 2007; **13**: 218-223 [PMID: 17206148]
25 **Tong YK**, Jin S, Chiu RW, Ding C, Chan KC, Leung TY, Yu L, Lau TK, Lo YM. Noninvasive prenatal detection of trisomy 21 by

an epigenetic-genetic chromosome-dosage approach. *Clin Chem* 2010; **56**: 90-98 [PMID: 19850629 DOI: 10.1373/clinchem.2009.134114]

26 **Tsui DW**, Lam YM, Lee WS, Leung TY, Lau TK, Lau ET, Tang MH, Akolekar R, Nicolaides KH, Chiu RW, Lo YM, Chim SS. Systematic identification of placental epigenetic signatures for the noninvasive prenatal detection of Edwards syndrome. *PLoS One* 2010; **5**: e15069 [PMID: 21152411 DOI: 10.1371/journal.pone.0015069]

27 **Gil MM**, Quezada MS, Revello R, Akolekar R, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for fetal aneuploidies: updated meta-analysis. *Ultrasound Obstet Gynecol* 2015; **45**: 249-266 [DOI: 10.1002/uog.14791]

28 **Bianchi DW**, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 2012; **119**: 890-901 [PMID: 22362253 DOI: 10.1097/AOG.0b013e31824fb482]

29 **Nicolaides KH**, Syngelaki A, Ashoor G, Birdir C, Touzet G. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. *Am J Obstet Gynecol* 2012; **207**: 374.e1-374.e6 [PMID: 23107079 DOI: 10.1016/j.ajog.2012.08.033]

30 **Nicolaides KH**, Syngelaki A, Gil M, Atanasova V, Markova D. Validation of targeted sequencing of single-nucleotide polymorphisms for non-invasive prenatal detection of aneuploidy of chromosomes 13, 18, 21, X, and Y. *Prenat Diagn* 2013; **33**: 575-579 [PMID: 23613152 DOI: 10.1002/pd.4103]

31 **Ehrich M**, Deciu C, Zwiefelhofer T, Tynan JA, Cagasan L, Tim R, Lu V, McCullough R, McCarthy E, Nygren AO, Dean J, Tang L, Hutchison D, Lu T, Wang H, Angkachatchai V, Oeth P, Cantor CR, Bombard A, van den Boom D. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol* 2011; **204**: 205.e1-205.11 [PMID: 21310373 DOI: 10.1016/j.ajog.2010.12.060]

32 **Savage M**. Merging into clinical practice: updates in non-invasive prenatal testing. Abstract 304 presented at the 31st National Society of Genetic Counselors Annual Education Conference; Boston, Mass, USA, 2012

33 **Chiu RW**, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, Foo CH, Xie B, Tsui NB, Lun FM, Zee BC, Lau TK, Cantor CR, Lo YM. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci USA* 2008; **105**: 20458-20463 [PMID: 19073917 DOI: 10.1073/pnas.0810641105]

34 **Verinata Health**. "Analytical Validation of the Verifi Prenatal Test: Enhanced Test Performance for Detecting Trisomies 21, 18 and 13 and the Option for Classification of Sex Chromosome Status." Redwood City, Calif, USA, 2012

35 **Papageorgiou EA**, Fiegler H, Rakyan V, Beck S, Hulten M, Lamnissou K, Carter NP, Patsalis PC. Sites of differential DNA methylation between placenta and peripheral blood: molecular markers for noninvasive prenatal diagnosis of aneuploidies. *Am J Pathol* 2009; **174**: 1609-1618 [PMID: 19349366 DOI: 10.2353/ajpath.2009.081038]

36 **Papageorgiou EA**, Karagrigoriou A, Tsaliki E, Velissariou V, Carter NP, Patsalis PC. Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. *Nat Med* 2011; **17**: 510-513 [PMID: 21378977 DOI: 10.1038/nm.2312]

37 **Tsaliki E**, Papageorgiou EA, Spyrou C, Koumbaris G, Kypri E, Kyriakou S, Sotiriou C, Touvana E, Keravnou A, Karagrigoriou A, Lamnissou K, Velissariou V, Patsalis PC. MeDIP real-time qPCR of maternal peripheral blood reliably identifies trisomy 21. *Prenat Diagn* 2012; **32**: 996-1001 [PMID: 22833530 DOI: 10.1002/pd.3947]

38 **Hahn S**, Lapaire O, Tercanli S, Kolla V, Hösli I. Determination of fetal chromosome aberrations from fetal DNA in maternal blood: has the challenge finally been met? *Expert Rev Mol Med* 2011; **13**: e16 [PMID: 21542948 DOI: 10.1017/S1462399411001852]

39 **Benn P**, Cuckle H. Theoretical performance of non-invasive prenatal testing for chromosome imbalances using counting of cell-free DNA fragments in maternal plasma. *Prenat Diagn* 2014; **34**: 778-783 [PMID: 24676912 DOI: 10.1002/pd.4366]

40 **Lo YM**. Non-invasive prenatal testing using massively parallel sequencing of maternal plasma DNA: from molecular karyotyping to fetal whole-genome sequencing. *Reprod Biomed Online* 2013; **27**: 593-598 [PMID: 24140310 DOI: 10.1016/j.rbmo.2013.08.008]

41 **Smith M**, Lewis KM, Holmes A, Visootsak J. A Case of False Negative NIPT for Down Syndrome-Lessons Learned. *Case Rep Genet* 2014; **2014**: 823504 [PMID: 24649382 DOI: 10.1155/2014/823504]

42 **Wright D**, Wright A, Nicolaides KH. A unified approach to risk assessment for fetal aneuploidies. *Ultrasound Obstet Gynecol* 2015; **45**: 48-54 [PMID: 25315809 DOI: 10.1002/uog.14694]

43 European Society of Human Genetics. Available from: URL: http://https://www.eshg.org/fileadmin/eshg/documents/consultations/NIPT-ChallengeOfresponsibleInnovationInPrenatalScreening-DraftOctober2014.docx

44 **Papageorgiou EA**, Koumbaris G, Kypri E, Hadjidaniel M, Patsalis PC. The Epigenome View: An Effort towards Non-Invasive Prenatal Diagnosis. *Genes* (Basel) 2014; **5**: 310-329 [PMID: 24722507 DOI: 10.3390/genes5020310]

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