Genome Medicine

REVIEW

Prenatal diagnosis of fetal aneuploidies: post-genomic developments

Sinuhe Hahn[1]*, Laird G Jackson[2] and Bernhard G Zimmermann[3]

Abstract

Prenatal diagnosis of fetal aneuploidies and chromosomal anomalies is likely to undergo a profound change in the near future. On the one hand this is mediated by new technical developments, such as chromosomal microarrays, which allow a much more precise delineation of minute sub-microscopic chromosomal aberrancies than the classical G-band karyotype. This will be of particular interest when investigating pregnancies at risk of unexplained development delay, intellectual disability or certain forms of autism. On the other hand, great strides have been made in the non-invasive determination of fetal genetic traits, largely through the analysis of cell-free fetal nucleic acids. It is hoped that, with the assistance of cutting-edge tools such as digital PCR or next generation sequencing, the long elusive goal of non-invasive prenatal diagnosis for fetal aneuploidies can finally be attained.

Analysis of fetal material gained by invasive procedures

The current gold standard for prenatal diagnosis for fetal aneuploidy is a full karyotype obtained from the culture of amniocytes or chorionic villus cells, which are obtained by invasive procedures such as amniocentesis or chorionic villus sampling (CVS) [1-3]. It is unclear, however, how long this practice will remain standard operating procedure because the classical karyotype yields a limited amount of information by today's standards, and because the lengthy culture period of typically 10 to 14 days is no longer acceptable in our high-speed society [1,2].

The most frequent severe chromosomal anomaly in live births is trisomy 21 (Down syndrome), and trisomies 13 and 18 are associated with intra-uterine lethality. Strategies have evolved to detect the most common anomalies rapidly following an invasive procedure. These include direct preparations of uncultured chorionic villus cells, multi-color fluorescent *in situ* hybridization (FISH) [4,5], quantitative fluorescent PCR (qf-PCR) [6,7], real-time quantitative PCR [8], PCR coupled with mass spectrometry [9], multiplex ligation-dependent probe amplification, and most recently digital PCR [10,11]. Usually the FISH- or PCR-based tests offer information concerning the ploidy of chromosomes 13, 18, 21, X and Y, as these analyses should in theory cover about two-thirds of the chromosomal anomalies that are most commonly found at the time of amniocentesis and about 85% of those found at the time of birth [12]. Both qf-PCR and rapid FISH methods, such as Fast-FISH, enable informative results to be obtained in a matter of hours [4,6], so the expectant couple can be informed if the fetus is affected by Down syndrome or not within a very short time-frame, instead of having to wait for almost 2 weeks. The introduction of such services has been so successful that it has been suggested that they replace conventional karyotyping completely, as a cost-saving measure [12]. These rapid tests, however, provide only a limited amount of information, and large-scale studies conducted in the UK have shown that their sole use may lead to the failure to detect 30 to 45% of the fetal chromosomal anomalies occurring in the study population [13]. For this reason, conventional G-banded karyotyping is still routinely performed on fetal material obtained by invasive means.

New technologies such as microarray comparative genomic hybridization, also termed chromosomal microarray (CMA), enable a more precise assessment of chromosomal structure and have thus been proposed to be useful for prenatal diagnosis [1]. However, as it would be too costly to perform CMA and conventional G-banded karyotyping in parallel on the same sample, the question has been raised as to whether the former should replace the latter [1,14,15]. In a large-scale meta-analysis of 33 studies involving over 21,000 patients performed by the International Standard Cytogenomic Assay Consortium, it was determined that CMA yielded a 15 to 20% higher diagnostic yield than G-banded karyotyping for the

*Correspondence: shahn@uhbs.ch
[1]Department of Biomedicine, University Women's Hospital, University Clinics Basel, Hebelstrasse 20, CH-4031, Switzerland
Full list of author information is available at the end of the article

detection of disorders involving submicroscopic deletions or duplications [14]. Such alterations have been shown to be involved in disorders such as unexplained development delay/intellectual disability, autism spectrum disorders and multiple congenital anomalies.

Consequently, it seems that CMA would provide better value for money than the continued use of traditional G-banded karyotyping, and it was recommended by the International Standard Cytogenomic Assay Consortium that it should be considered as a 'first tier' option for prenatal diagnosis [14]. Unfortunately, in this regard no consensus has yet been attained, as is evident by the recent Committee Opinion no. 446 released by the American College of Obstetrics and Gynecology [16], which states that CMA is currently not a suitable replacement for classical cytogenetics in prenatal diagnosis. This is due to a perceived higher cost and apparent technical issues, such as a possible inability to detect balanced translocations or cases of triploidy by CMA. Given that several studies indicate, however, that array technologies may under certain conditions provide more detailed insight than classical G-banding with regard to chromosome rearrangements, it is possible that this issue will be resolved in future as CMA techniques become more technically proficient, robust and widespread [14].

Non-invasive prenatal diagnosis of fetal aneuploidies: direct versus indirect approaches

As invasive practices such as CVS or amniocentesis carry an inherent risk of fetal injury and loss, several alternative approaches that would allow a non-invasive assessment of the fetal genotype have been explored [2,17]. Initial attempts focused on the enrichment of fetal cells (erythroblasts or trophoblasts) from maternal blood and the retrieval of trophoblast cells by transcervical lavage [18]. Despite almost three decades of intensive efforts, none of these approaches has proven to be ready for clinical application. This may, however, change with the development of effictive enrichment devices using microfluidics or automated scanning microscopy [2].

Consequently, most attention has been focused on the potential use of cell-free placentally derived nucleic acids [19]. In this regard, two major strategies have emerged, relying on direct or indirect means of inferring whether a fetal chromosomal anomaly is present.

Indirect approaches: cell-free mRNA or epigenetic differences

Cell-free DNA is present in the serum and plasma of all normal individuals. It is assumed to arise from dying or damaged cells, and may be a consequence of normal cell turnover. Placentally derived cell-free DNA is derived from turnover of the placental trophoblast tissue. The use of placentally derived cell-free fetal DNA has been shown to be useful for the detection of fetal loci that are completely absent from the maternal genome, such as the Y chromosome or the fetal Rhesus D (*RHD*) gene in Rhesus D negative mothers [20,21]. The situation is, however, much more complex when studying fetal loci that are more similar to maternal ones because the few fetal cell-free DNA sequences present in maternal plasma are almost swamped by the preponderance of maternally derived ones. This renders the detection of fetal genetic loci that are not completely absent from the maternal genome difficult by current PCR-based approaches [22]. In order to overcome this problem, two avenues have been investigated: firstly, the use of placentally derived mRNA species not expressed by maternal tissues [23]; and secondly, epigenetic differences between placentally and maternally derived cell-free DNA sequences [24].

The hypothesis behind these approaches is that they should theoretically allow an absolute discrimination between fetal and maternal cell-free nucleic acid sequences, and thus should not be influenced by an overwhelming presence of maternal material. The analysis of the targeted fetal loci should then become as straightforward as that for the determination of fetal gender or Rhesus D status.

In order to determine chromosomal ploidy, the approaches rely on the quantitative assessment of heterozygous single nucleotide polymorphism (SNP) loci in the nucleic acid sequences being interrogated [23,25,26]. If the fetus is euploid, the SNP ratio should be 1:1, whereas if it were aneuploid, the SNP ratio would be 1:2 or 2:1.

In the mRNA approach, mRNA transcripts from genes located on chromosomes 21 (Placenta specific-4, *PLAC4*) and 18 (serpin peptidase inhibitor clade b2, *SERBINB2*) have been examined [23,27]. In the first report on the detection of trisomy 21 using *PLAC4* mRNA [23], 10 affected cases could be distinguished from 56 healthy cases with a sensitivity of 90% and a specificity of 96%. Unfortunately, almost 100 cases had to be excluded from analysis as they did not meet the necessary requirement for a heterozygous SNP locus in the *PLAC4* mRNA. In a recent follow-up study [26], it has been suggested that the accuracy of this assay could be improved by the use of digital PCR rather than mass spectrometry for the detection and quantification of the SNP alleles, as well as by quantitatively assessing cell-free *PLAC4* mRNA levels. This study [26] was performed on only four cases with trisomy 21, however, and although the sensitivity reached 100%, the specificity was only 89%.

In a study using *SERBINB2* mRNA for the detection of trisomy 18, three out of four samples with Edwards syndrome could be distinguished from healthy cases [27]. Unfortunately, because of the very low levels of *SERBINB2* mRNA in maternal plasma, the samples had to be pooled, thereby making a precise estimate of the usefulness of this approach difficult.

In the first study to explore whether epigenetic differences between placental and maternal tissues could be used for fetal aneuploidy detection [28,29], the use of the *MASPIN* gene on chromosome 18 was explored. This gene has been shown to be hypomethylated in placental tissues and hypermethylated in maternal blood [28]. However, as it was not possible for the authors [28] to reliably distinguish cases with trisomy 18 from healthy controls when using pure placentally derived fetal genetic material, it is unclear whether this approach will be suitable for the analysis of cell-free DNA, for which the quantities of fetal material are considerably lower [28,29].

More recently, the epigenetic approach has been tested in a more complex manner using a combination of fetus-specific genetic (*ZFY* on the Y chromosome) and epigenetic markers (holocarboxylase synthetase, *HLCS*, on chromosome 21) [30]. Instead of relying on the analysis of SNP ratios, this new test relies on a comparison of the relative dosage of the *HLCS* and *ZFY* loci by digital PCR (see below for a technical description). In their examination, Tong and colleagues [30] were able to discriminate 5 cases with Down syndrome from 24 normal euploid cases.

Although the latter results seem very promising, it is important to realize that several conditions need to be met for these methods targeting fetus-specific sequences to be functional. These are: that there is an absolute distinction between the maternal and fetal compartments; that the chromosomal loci being examined are transcribed at exactly the same rate, or are equally epigenetically altered; and for the *HLCS* and *ZFY* assay, that a reliable alternative to *ZFY* is obtained for gender-independent analysis. As such, considerable further improvement and multi-center large-scale studies will be necessary to reveal how valid these conditions are and whether these approaches are suitable for clinical applications.

Direct approaches: digital PCR and next generation sequencing

Several recent studies have, however, indicated that it may be possible to determine fetal ploidy through the direct analysis of cell-free DNA without having to resort to indirect means such as epigenetic markers or cell-free mRNA [31]. These findings are based on the development of new tools that enable a much more precise quantitative assessment of cell-free DNA sequences than was possible with techniques such as real-time PCR or PCR coupled with mass spectrometry.

In the first of these studies the technique of digital PCR [32] was used for the quantification of fetal DNA sequences [11,33]. Digital PCR differs from other quantitative approaches, such as real-time PCR, which use the exponential phase of the PCR reaction, in that digital PCR allows the reaction to proceed to its plateau and then simply uses a 'yes/no' method to monitor the presence or absence of input template [34]. Because this method relies on the monitoring of numerous single PCR reactions, it required the development of microfluidic devices with several thousand reaction chambers in order for it to become viable [11,32,33].

By these means two independent proof-of-principle studies [11,33] indicated that digital PCR could be used for reliable discrimination between aneuploid and euploid cases on pure fetal genetic material, and that this may be possible when only 10% of the input template was of aneuploid origin, provided that 4,000 individual events were monitored [11,32,33]. As the concentration of cell-free fetal DNA in maternal plasma is similar to 10%, this method may thus be useful for analyzing such samples [11,32,33].

The most spectacular evidence that the direct analysis of cell-free DNA in maternal plasma can be used to detect fetal aneuploidy is provided by studies using 'next generation' or 'shotgun' sequencing [35-37]. In this method, very short fragments from the entire genome are amplified and sequenced [37]. In this manner some 65,000 reads have been obtained for chromosome 21 and several million for the entire genome. However, instead of using these sequence data for genome analysis, the output data are examined in the same molecular counting manner as are digital PCR data. As the number of reads available is several orders of magnitude higher than what can currently be attained by digital PCR, the results would also be expected to be much more precise. This was indeed the case and, in both studies, all cases of aneuploidy could be reliably distinguished from euploid controls [35-37].

Development of new highly specific screening markers using proteomics

Protein biomarkers have formed the basis for fetal aneuploidy screening tests for several decades, starting with the second trimester test that used maternal serum α-fetoprotein, human chorionic gonadotrophin (hCG) and estriol [38]. This test, which was routinely used to screen pregnancies at 15 to 20 weeks of gestation, has largely been replaced by the first trimester combined test, which is performed at around 11 to 13 weeks of pregnancy [39]. This test uses ultrasound for the detection of nuchal translucency (related to the size of a fold in the skin at the base of the neck, which is increased in cases of Down syndrome) in combination with serum protein markers such as free β-hCG and pregnancy-associated plasma protein-A (PAPP-A). In centers with skilled ultrasonographers, detection rates for pregnancies with a Down syndrome fetus of up to 80% can be attained. Unfortunately, both tests are hampered by high false positive rates of the order of 5 to 8%, thereby leading to a large

number of unnecessary invasive procedures being performed on healthy pregnancies. One way in which this problem has been proposed to be overcome is by the addition of more placenta-specific biomarkers, and indeed, slight improvements can be achieved by the addition of other markers, such as members of the inhibin/activin family [40].

Given that the placenta in Down syndrome has very characteristic defects in trophoblast differentiation, it may be possible that associated changes in protein expression are evident in the maternal plasma proteome [41]. For this reason several studies have used proteomic strategies to detect such potential biomarkers [38]. This approach is, however, not as simple as it would seem because of the incredible complexity of the plasma proteome, which contains peptides derived from every tissue of the body. Furthermore, the presence of very abundant proteins, such as serum albumin and immunoglobulin, effectively mask rare peptides, such as those of placental origin. An additional problem that hampered many previous studies is that the tools used to measure quantitative differences in plasma peptide levels between case and controls, such as two-dimensional differential in gel electrophoresis, were not adequately sensitive and reliable.

This has largely been overcome by the development of techniques such as the isobaric tag for relative and absolute quantification (iTRAQ) method [42]. In a recent pilot study [43], we have examined whether this approach will be suitable for the development of Down syndrome screening markers. In our study [43] we examined first-trimester plasma samples from mothers of fetuses with Down syndrome and matched healthy controls, which were labeled with quadruplex isobaric tags. Among the proteins found to be elevated in mothers of fetuses with Down syndrome, we were pleased to detect β-hCG, an important component of current screening strategies, suggesting that the iTRAQ method was working. Of particular interest was the detection of several molecules of the amyloid family associated with onset of senility in Alzheimer's and Down syndrome patients.

The true power of proteomic analyses, especially when coupled with high-throughput quantitative analyses such as selective reaction monitoring, comes from the use of very large panels (hundreds to thousands) of potential biomarkers [44]. Using such large panels it may be possible to minimize the effect of personal genomic differences.

Conclusions

Recent developments involving technologies such as digital PCR or shotgun sequencing may bring about the long-awaited dream of being able to detect fetal aneuploidies directly from a sample of maternal blood. The current problems hindering the immediate translation of this approach into the clinic are the cost of the instruments, the reagents and the experimental analysis, and the length of time taken to perform the subsequent bioinformatic analysis. This may, however, change as the next generation of machines becomes available, which will be priced at a fraction of the cost of current devices. Furthermore, by focusing on discrete targeted sequences (such as chromosomes 21, 18 and 13), it should be possible to perform smaller analytic runs and also cut down the time required for bioinformatic analysis enormously.

Although it is unlikely that proteomic approaches will become so effective as to render them diagnostic, it is possible that the quantitative analysis of large panels of potential biomarkers by mass spectrometry-based techniques such as selective reaction monitoring may increase current screening sensitivity and specificity to a very high level.

The development of large panels of biomarkers, which take into account personal genomic differences, may increase the level of screening accuracy to such an extent that further testing, be it invasive or not, will be restricted to a well defined high risk group.

Abbreviations

CMA, chromosomal microarray; CVS, chorionic villus sampling; FISH, fluorescent *in situ* hybridization; qf-PCR, quantitative fluorescent PCR; hCG, human chorionic gonadotrophin; iTRAQ, isobaric tag for relative and absolute quantification; SNP, single nucleotide polymorphism.

Competing interests

BGZ is an employee of Fluidigm Corporation, USA. SH and LJ declare that they have no competing interests.

Authors' contributions

All authors contributed to the writing and editing of this manuscript.

Author details

[1]Department of Biomedicine, University Women's Hospital, University Clinics Basel, Hebelstrasse 20, CH-4031, Switzerland. [2]Division of Obstetrics and Gynecology, Drexel University School of Medicine, 245 N. 15th Street, Mail Stop 495, Philadelphia, PA 19102, USA. [3]Fluidigm Corporation, 7000 Shoreline Court, Suite 100, South San Francisco, CA 94080, USA.

Published: 5 August 2010

References

1. Fruhman G, van den Veyver IB: **Applications of array comparative genomic hybridization in obstetrics.** *Obstet Gynecol Clin North Am* 2010, **37:**71-85.
2. Hahn S, Jackson LG, Kolla V, Mahyuddin AP, Choolani M: **Noninvasive prenatal diagnosis of fetal aneuploidies and Mendelian disorders: new innovative strategies.** *Expert Rev Mol Diagn* 2009, **9:**613-621.
3. Philip J, Silver RK, Wilson, RD, Thom EA, Zachary JM, Mohide P, Mahoney MJ, Simpson JL, Platt LD, Pergament E: **Late first-trimester invasive prenatal diagnosis: results of an international randomized trial.** *Obstet Gynecol* 2004, **103:**1164-1173.
4. Choolani M, Ho SS, Razvi K, Ponnusamy S, Baig S, Fisk NM, Biswas A: **FastFISH: technique for ultrarapid fluorescence in situ hybridization on uncultured amniocytes yielding results within 2 h of amniocentesis.** *Mol Hum Reprod* 2007, **13:**355-359.
5. Weise A, Liehr T: **Rapid prenatal aneuploidy screening by fluorescence in situ hybridization (FISH).** *Methods Mol Biol* 2008, **444:**39-47.
6. Cirigliano V, Voglino G, Ordonez E, Marongiu A, Paz Canadas M, Ejarque M, Rueda L, Lloveras E, Fuster C, Adinolfi M: **Rapid prenatal diagnosis of**

common chromosome aneuploidies by QF-PCR, results of 9 years of clinical experience. *Prenat Diagn* 2009, **29:**40-49.
7. Mann K, Petek E, Pertl B: **Prenatal detection of chromosome aneuploidy by quantitative fluorescence PCR.** *Methods Mol Biol* 2008, **444:**71-94.
8. Zimmermann B, Holzgreve W, Wenzel F, Hahn S: **Novel real-time quantitative PCR test for trisomy 21.** *Clin Chem* 2002, **48:**362-363.
9. Huang DJ, Nelson MR, Zimmermann B, Dudarewicz L, Wenzel F, Spiegel R, Nagy B, Holzgreve W, Hahn S: **Reliable detection of trisomy 21 using MALDI-TOF mass spectrometry.** *Genet Med* 2006, **8:**728-734.
10. Fan HC, Blumenfeld YJ, El-Sayed YY, Chueh J, Quake SR: **Microfluidic digital PCR enables rapid prenatal diagnosis of fetal aneuploidy.** *Am J Obstet Gynecol* 2007, **543:**e1-e7.
11. Lo YM, Lun FM, Chan KC, Tsui NB, Chong KC, Lau TK, Leung TY, Zee BC, Cantor CR, Chiu RW: **Digital PCR for the molecular detection of fetal chromosomal aneuploidy.** *Proc Natl Acad Sci USA* 2007, **104:**13116-13121.
12. Evans MI, Henry GP, Miller WA, Bui TH, Snidjers RJ, Wapner RJ, Miny P, Johnson MP, Peakman D, Johnson A: **International, collaborative assessment of 146,000 prenatal karyotypes: expected limitations if only chromosome-specific probes and fluorescent in-situ hybridization are used.** *Hum Reprod* 1999, **14:**1213-1216.
13. Caine A, Maltby AE, Parkin CA, Waters JJ, Crolla JA: **Prenatal detection of Down's syndrome by rapid aneuploidy testing for chromosomes 13, 18, and 21 by FISH or PCR without a full karyotype: a cytogenetic risk assessment.** *Lancet* 2005, **366:**123-128.
14. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ: **Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies.** *Am J Hum Genet* 2010, **86:**749-764.
15. Regler DA, Friedman JM, Marra CA: Value for money? **Array genomic hybridization for diagnostic testing for genetic causes of intellectual disability.** *Am J Hum Genet* 2010, **86:**765-772.
16. ACOG Committee Opinion No. 446: **Array comparative genomic hybridization in prenatal diagnosis.** *Obstet Gynecol* 2009, **114:**1161-1163.
17. Maddocks DG, Alberry MS, Attilakos G, Madgett TE, Choi K, Soothill PW, and Avent ND: **The SAFE project: towards non-invasive prenatal diagnosis.** *Biochem Soc Trans* 2009, **37:**460-465.
18. Hahn S, Sant R, Holzgreve W: **Fetal cells in maternal blood: current and future perspectives.** *Mol Hum Reprod* 1998, **4:**515-521.
19. Lo YM: **Noninvasive prenatal detection of fetal chromosomal aneuploidies by maternal plasma nucleic acid analysis: a review of the current state of the art.** *BJOG* 2009, **116:**152-157.
20. Hahn S, Chitty LS: **Non-invasive prenatal diagnosis: implications for antenatal diagnosis and the management of high-risk pregnancies.** *Semin Fetal Neonatal Med* 2008, **13:**55-56.
21. van der Schoot CE, Hahn S, Chitty LS: **Non-invasive prenatal diagnosis and determination of fetal Rh status.** *Semin Fetal Neonatal Med* 2008, **13:**63-68.
22. Hahn S, Holzgreve W: **Prenatal diagnosis using fetal cells and cell-free fetal DNA in maternal blood: what is currently feasible?** *Clin Obstet Gynecol* 2002, **45:**649-656.
23. Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, Gerovassili A, Jin Y, Nicolaides KH, Cantor CR: **Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection.** *Nat Med* 2007, **13:**218-223.
24. Chim SS, Jin S, Lee TY, Lun FM, Lee WS, Chan LY, Jin Y, Yang N, Tong YK, Leung TY, Lo YM: **Systematic search for placental DNA-methylation markers on chromosome 21: toward a maternal plasma-based epigenetic test for fetal trisomy 21.** *Clin Chem* 2008, **54:**500-511.
25. Ding C, Chiu RW, Lau TK, Leung TN, Chan LC, Chan AY, Charoenkwan P, Ng IS, Law HY, Ma ES, Lo YM: **MS analysis of single-nucleotide differences in circulating nucleic acids: application to noninvasive prenatal diagnosis.** *Proc Natl Acad Sci USA* 2004, **101:**10762-10767.
26. Tsui NB, Akolekar R, Chiu RW, Chow KC, Leung TY, Lau TK, Nicolaides KH, Lo YM: **Synergy of total PLAC4 RNA concentration and measurement of the RNA single-nucleotide polymorphism allelic ratio for the noninvasive prenatal detection of trisomy 21.** *Clin Chem* 2010, **56:**73-81.
27. Tsui NB, Wong BC, Leung TY, Lau TK, Chiu RW, Lo YM: **Non-invasive prenatal detection of fetal trisomy 18 by RNA-SNP allelic ratio analysis using maternal plasma SERPINB2 mRNA: a feasibility study.** *Prenat Diagn* 2009, **29:**1031-1037.
28. Chim SS, Tong, YK, Chiu RW, Lau TK, Leung TN, Chan LY, Oudejans CB, Ding C, Lo YM: **Detection of the placental epigenetic signature of the maspin gene in maternal plasma.** *Proc Natl Acad Sci USA* 2005, **102:**14753-14758.
29. Tong YK, Ding C, Chiu RW, Gerovassili A, Chim SS, Leung TY, Leung TN, Lau TK, Nicolaides KH, Lo YM: **Noninvasive prenatal detection of fetal trisomy 18 by epigenetic allelic ratio analysis in maternal plasma: Theoretical and empirical considerations.** *Clin Chem* 2006, **52:**2194-2202.
30. 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.
31. Chiu RW, Cantor CR, Lo YM: **Non-invasive prenatal diagnosis by single molecule counting technologies.** *Trends Genet* 2009, **25:**324-331.
32. Zimmermann BG, Grill S, Holzgreve W, Zhong XY, Jackson LG, Hahn S: **Digital PCR: a powerful new tool for noninvasive prenatal diagnosis?** *Prenat Diagn* 2008, **28:**1087-1093.
33. Fan HC, Quake SR: **Detection of aneuploidy with digital polymerase chain reaction.** *Anal Chem* 2007, **79:**7576-7579.
34. Vogelstein B, Kinzler KW: **Digital PCR.** *Proc Natl Acad Sci USA* 1999, **96:**9236-9241.
35. Chiu RW, Chan KC, Gao Y, Lau VY, Zheng W, Leung TY, Foo CH, Xie B, Tsui NB, Lun FM, 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.
36. Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR: **Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood.** *Proc Natl Acad Sci USA* 2008, **105:**16266-16271.
37. Lo YM, Chiu RW: **Next-generation sequencing of plasma/serum DNA: an emerging research and molecular diagnostic tool.** *Clin Chem* 2009, **55:**607-608.
38. Choolani M, Narasimhan K, Kolla V, Hahn S: **Proteomic technologies for prenatal diagnostics: advances and challenges ahead.** *Expert Rev Proteomics* 2009, **6:**87-101.
39. Avgidou K, Papageorghiou A, Bindra R, Spencer K, Nicolaides KH: **Prospective first-trimester screening for trisomy 21 in 30,564 pregnancies.** *Am J Obstet Gynecol* 2005, **192:**1761-1767.
40. Spencer K, Liao AW, Ong CY, Geerts L, Nicolaides KH: **Maternal serum levels of dimeric inhibin A in pregnancies affected by trisomy 21 in the first trimester.** *Prenat Diagn* 2001, **21:**441-444.
41. Malassine A, Frendo JL, Evain-Brion D: **Trisomy 21-affected placentas highlight prerequisite factors for human trophoblast fusion and differentiation.** *Int J Dev Biol* 2010, **54:**475-482.
42. Wiese S, Reidegeld KA, Meyer HE, Warscheid B: **Protein labeling by iTRAQ: a new tool for quantitative mass spectrometry in proteome research.** *Proteomics* 2007, **7:**340-350.
43. Kolla V, Jeno P, Moes S, Tercanli S, Lapaire O, Choolani M, Hahn S: **Quantitative proteomics analysis of maternal plasma in Down syndrome pregnancies using isobaric tagging reagent (iTRAQ).** *J Biomed Biotechnol* 2010, **2010:**952047.
44. Picotti P, Rinner O, Stallmach R, Dautel F, Farrah T, Domon B, Wenschuh H, Aebersold R: **High-throughput generation of selected reaction-monitoring assays for proteins and proteomes.** *Nat Methods* 2010, **7:**43-46.

doi:10.1186/gm171
Cite this article as: Hahn S, *et al*.: Prenatal diagnosis of fetal aneuploidies: post-genomic developments. *Genome Medicine* 2010, **2:**50.