



Universiteit
Leiden
The Netherlands

Noninvasive prenatal detection of genetic defects

Oever, Jessica Maria Elisabeth van den

Citation

Oever, J. M. E. van den. (2016, February 3). *Noninvasive prenatal detection of genetic defects*. Retrieved from <https://hdl.handle.net/1887/37582>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/37582>

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/37582> holds various files of this Leiden University dissertation.

Author: Oever, Jessica Maria Elisabeth van den

Title: Noninvasive prenatal detection of genetic defects

Issue Date: 2016-02-03

The background of the page is a complex, abstract pattern of overlapping, semi-transparent gray shapes. These shapes resemble organic, cell-like structures or a network of interconnected nodes. The shapes vary in size and opacity, creating a sense of depth and movement. The overall effect is a textured, monochromatic design that frames the central text.

References

References

- Abrahams VM, Kim YM, Straszewski SL, Romero R, Mor G. 2004. Macrophages and apoptotic cell clearance during pregnancy. *Am J Reprod Immunol* 51:275-282.
- Agathangelou A, Cooper WN, Latif F. 2005. Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. *Cancer Res* 65:3497-3508.
- Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. 2014. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*.
- Alberry M, Maddocks D, Jones M, Abdel HM, Abdel-Fattah S, Avent N, Soothill PW. 2007. Free fetal DNA in maternal plasma in anembryonic pregnancies: confirmation that the origin is the trophoblast. *Prenat Diagn* 27:415-418.
- Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, Lamy T, Le Prise PY, Beauplet A, Bories D, Semana G, Quelvenec E. 2002. Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. *Blood* 99:4618-4625.
- Almomani R, van der Stoep N, Bakker E, den Dunnen JT, Breuning MH, Ginjaar IB. 2009. Rapid and cost effective detection of small mutations in the DMD gene by high resolution melting curve analysis. *Neuromuscul Disord* 19:383-390.
- Anunziato AT. 2008. DNA Packaging: Nucleosomes and Chromatin. *Nature Education* 1:26.
- Arnemann J, Epplen JT, Cooke HJ, Saueremann U, Engel W, Schmidtke J. 1987. A human Y-chromosomal DNA sequence expressed in testicular tissue. *Nucleic Acids Res* 15:8713-8724.
- Ashoor G, Syngelaki A, Poon LC, Rezende JC, Nicolaides KH. 2013. Fetal fraction in maternal plasma cell-free DNA at 11-13 weeks' gestation: relation to maternal and fetal characteristics. *Ultrasound Obstet Gynecol* 41:26-32.
- Bachmann N, Bergmann C. 2012. Epigenetics and imprinting. *Arch Pediatr* 19:1145-1147.
- Bayindir B, Dehaspe L, Brison N, Brady P, Ardui S, Kammoun M, van d, V, Lichtenbelt K, Van Den Bogaert K, Van HJ, Peeters H, Van EH, de RT, Legius E, Devriendt K, Vermeesch JR. 2015. Noninvasive prenatal testing using a novel analysis pipeline to screen for all autosomal fetal aneuploidies improves pregnancy management. *Eur J Hum Genet* 23:1286-1293.
- Beaudet AL. 2011. Progress toward noninvasive prenatal diagnosis. *Clin Chem* 57:802-804.
- Bellido ML, Radpour R, Lapaire O, De B, I, Hosli I, Bitzer J, Hmadcha A, Zhong XY, Holzgreve W. 2010. MALDI-TOF mass array analysis of RASSF1A and SERPINB5 methylation patterns in human placenta and plasma. *Biol Reprod* 82:745-750.
- Benirschke K. 1994. Anatomical relationship between fetus and mother. *Ann N Y Acad Sci* 731:9-20.
- Benirschke K, Willes L. 2010. Deportation of trophoblastic emboli to maternal lung: A source of cell-free DNA in maternal blood? *Chimerism* 1:15-18.
- Benn P, Cuckle H, Pergament E. 2012. Non-invasive prenatal diagnosis for Down syndrome: the paradigm will shift, but slowly. *Ultrasound Obstet Gynecol* 39:127-130.
- Bianchi DW. 1995. Prenatal diagnosis by analysis of fetal cells in maternal blood. *J Pediatr* 127:847-856.
- Bianchi DW. 2004. Circulating fetal DNA: its origin and diagnostic potential-a review. *Placenta* 25 Suppl A:S93-S101.

- Bianchi DW, Parker RL, Wentworth J, Madankumar R, Saffer C, Das AF, Craig JA, Chudova DI, Devers PL, Jones KW, Oliver K, Rava RP, Sehnert AJ. 2014. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med* 370:799-808.
- Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP. 2012. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. *Obstet Gynecol* 119:890-901.
- Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. 1996. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. *Proc Natl Acad Sci U S A* 93:705-708.
- Bianchi DW, Zickwolf GK, Yih MC, Flint AF, Geifman OH, Erikson MS, Williams JM. 1993. Erythroid-specific antibodies enhance detection of fetal nucleated erythrocytes in maternal blood. *Prenat Diagn* 13:293-300.
- Bidard FC, Madic J, Mariani P, Piperno-Neumann S, Rampanou A, Servois V, Cassoux N, Desjardins L, Milder M, Vaucher I, Pierga JY, Lebofsky R, Stern MH, Lantz O. 2014. Detection rate and prognostic value of circulating tumor cells and circulating tumor DNA in metastatic uveal melanoma. *Int J Cancer* 134:1207-1213.
- Bischoff FZ, Lewis DE, Simpson JL. 2005. Cell-free fetal DNA in maternal blood: kinetics, source and structure. *Hum Reprod Update* 11:59-67.
- Boon EM, Schlecht HB, Martin P, Daniels G, Vossen RH, den Dunnen JT, Bakker B, Elles R. 2007. Y chromosome detection by Real Time PCR and pyrophosphorolysis-activated polymerisation using free fetal DNA isolated from maternal plasma. *Prenat Diagn* 27:932-937.
- Brar H, Wang E, Struble C, Musci TJ, Norton ME. 2013. The fetal fraction of cell-free DNA in maternal plasma is not affected by a priori risk of fetal trisomy. *J Matern Fetal Neonatal Med* 26:143-145.
- Buermans HP, den Dunnen JT. 2014. Next generation sequencing technology: Advances and applications. *Biochim Biophys Acta* 1842:1932-1941.
- Bustamante-Aragones A, Rodriguez de AM, Perlado S, Trujillo-Tiebas MJ, Arranz JP, Diaz-Recasens J, Troyano-Luque J, Ramos C. 2012. Non-invasive prenatal diagnosis of single-gene disorders from maternal blood. *Gene* 504:144-149.
- Bustamante-Aragones A, Trujillo-Tiebas MJ, Gallego-Merlo J, Rodriguez de AM, Gonzalez-Gonzalez C, Cantalapiedra D, Ayuso C, Ramos C. 2008. Prenatal diagnosis of Huntington disease in maternal plasma: direct and indirect study. *Eur J Neurol* 15:1338-1344.
- Buysse K, Beulen L, Gomes I, Gilissen C, Keesmaat C, Janssen IM, Derks-Willems JJ, de LJ, Feenstra I, Bekker MN, van Vugt JM, Geurts van KA, Vissers LE, Faas BH. 2013. Reliable noninvasive prenatal testing by massively parallel sequencing of circulating cell-free DNA from maternal plasma processed up to 24h after venipuncture. *Clin Biochem* 46:1783-1786.
- Canick JA, Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE. 2013. The impact of maternal plasma DNA fetal fraction on next generation sequencing tests for common fetal aneuploidies. *Prenat Diagn* 33:667-674.
- Chan KC, Ding C, Gerovassili A, Yeung SW, Chiu RW, Leung TN, Lau TK, Chim SS, Chung GT, Nicolaides KH, Lo YM. 2006. Hypermethylated RASSF1A in maternal plasma: A universal fetal DNA marker that improves the reliability of noninvasive prenatal diagnosis. *Clin Chem* 52:2211-2218.
- Chan KC, Zhang J, Hui AB, Wong N, Lau TK, Leung TN, Lo KW, Huang DW, Lo YM. 2004. Size distributions of maternal and fetal DNA in maternal plasma. *Clin Chem* 50:88-92.
- Chang F, Li MM. 2013. Clinical application of amplicon-based next-generation sequencing in cancer. *Cancer Genet* 206:413-419.

- Chen EZ, Chiu RW, Sun H, Akolekar R, Chan KC, Leung TY, Jiang P, Zheng YW, Lun FM, Chan LY, Jin Y, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. 2011. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. *PLoS One* 6:e21791.
- Chiang DY, Getz G, Jaffe DB, O'Kelly MJ, Zhao X, Carter SL, Russ C, Nusbaum C, Meyerson M, Lander ES. 2009. High-resolution mapping of copy-number alterations with massively parallel sequencing. *Nat Methods* 6:99-103.
- Chim SS, Jin S, Lee TY, Lun FM, Lee WS, Chan LY, Jin Y, Yang N, Tong YK, Leung TY, Lau TK, Ding C, Chiu RW, Lo YM. 2008. Systematic search for placental DNA-methylation markers on chromosome 21: toward a maternal plasma-based epigenetic test for fetal trisomy 21. *Clin Chem* 54:500-511.
- Chim SS, Tong YK, Chiu RW, Lau TK, Leung TN, Chan LY, Oudejans CB, Ding C, Lo YM. 2005. Detection of the placental epigenetic signature of the maspin gene in maternal plasma. *Proc Natl Acad Sci U S A* 102:14753-14758.
- Chinnapapagari SK, Holzgreve W, Lapaire O, Zimmermann B, Hahn S. 2005. Treatment of maternal blood samples with formaldehyde does not alter the proportion of circulatory fetal nucleic acids (DNA and mRNA) in maternal plasma. *Clin Chem* 51:652-655.
- Chitty LS, Khalil A, Barrett AN, Pajkrt E, Griffin DR, Cole TJ. 2013. Safe, accurate, prenatal diagnosis of thanatophoric dysplasia using ultrasound and free fetal DNA. *Prenat Diagn* 33:416-423.
- Chiu RW, Akolekar R, Zheng YW, Leung TY, Sun H, Chan KC, Lun FM, Go AT, Lau ET, To WW, Leung WC, Tang RY, Au-Yeung SK, Lam H, Kung YY, Zhang X, van Vugt JM, Minekawa R, Tang MH, Wang J, Oudejans CB, Lau TK, Nicolaides KH, Lo YM. 2011a. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *BMJ* 342:c7401.
- Chiu RW, Cantor CR, Lo YM. 2009. Non-invasive prenatal diagnosis by single molecule counting technologies. *Trends Genet* 25:324-331.
- 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. 2008. Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma. *Proc Natl Acad Sci U S A* 105:20458-20463.
- Chiu RW, Chim SS, Wong IH, Wong CS, Lee WS, To KF, Tong JH, Yuen RK, Shum AS, Chan JK, Chan LY, Yuen JW, Tong YK, Weier JF, Ferlatte C, Leung TN, Lau TK, Lo KW, Lo YM. 2007. Hypermethylation of RASSF1A in human and rhesus placentas. *Am J Pathol* 170:941-950.
- Chiu RW, Lo YM. 2011b. Non-invasive prenatal diagnosis by fetal nucleic acid analysis in maternal plasma: the coming of age. *Semin Fetal Neonatal Med* 16:88-93.
- Chiu RW, Sun H, Akolekar R, Clouser C, Lee C, McKernan K, Zhou D, Nicolaides KH, Lo YM. 2010. Maternal plasma DNA analysis with massively parallel sequencing by ligation for noninvasive prenatal diagnosis of trisomy 21. *Clin Chem* 56:459-463.
- Chou LS, Meadows C, Wittwer CT, Lyon E. 2005. Unlabeled oligonucleotide probes modified with locked nucleic acids for improved mismatch discrimination in genotyping by melting analysis. *Biotechniques* 39:644, 646, 648.
- Chu T, Bunce K, Hogge WA, Peters DG. 2010. A novel approach toward the challenge of accurately quantifying fetal DNA in maternal plasma. *Prenat Diagn* 30:1226-1229.
- Chung GT, Chiu RW, Chan KC, Lau TK, Leung TN, Lo YM. 2005. Lack of dramatic enrichment of fetal DNA in maternal plasma by formaldehyde treatment. *Clin Chem* 51:655-658.
- Daley R, Hill M, Chitty LS. 2014. Non-invasive prenatal diagnosis: progress and potential. *Arch Dis Child Fetal Neonatal Ed* 99:F426-F430.

- de Die-Smulders CE, de Wert GM, Liebaers I, Tibben A, Evers-Kiebooms G. 2013. Reproductive options for prospective parents in families with Huntington's disease: clinical, psychological and ethical reflections. *Hum Reprod Update* 19:304-315.
- de Haas M, Finning K, Massey E, Roberts DJ. 2014. Anti-D prophylaxis: past, present and future. *Transfus Med* 24:1-7.
- Della RF, Mastrovito P, Campanile C, Conti A, Papageorgiou EA, Hulten MA, Patsalis PC, Carter NP, D'Esposito M. 2010. Differential DNA methylation as a tool for noninvasive prenatal diagnosis (NIPD) of X chromosome aneuploidies. *J Mol Diagn* 12:797-807.
- Deng YH, Yin AH, He Q, Chen JC, He YS, Wang HQ, Li M, Chen HY. 2011. Non-invasive prenatal diagnosis of trisomy 21 by reverse transcriptase multiplex ligation-dependent probe amplification. *Clin Chem Lab Med* 49:641-646.
- Devaney SA, Palomaki GE, Scott JA, Bianchi DW. 2011. Noninvasive fetal sex determination using cell-free fetal DNA: a systematic review and meta-analysis. *JAMA* 306:627-636.
- Dhallan R, Au WC, Mattagajasingh S, Emche S, Bayliss P, Damewood M, Cronin M, Chou V, Mohr M. 2004. Methods to increase the percentage of free fetal DNA recovered from the maternal circulation. *JAMA* 291:1114-1119.
- Dhallan R, Guo X, Emche S, Damewood M, Bayliss P, Cronin M, Barry J, Betz J, Franz K, Gold K, Vallecillo B, Varney J. 2007. A non-invasive test for prenatal diagnosis based on fetal DNA present in maternal blood: a preliminary study. *Lancet* 369:474-481.
- Dohm JC, Lottaz C, Borodina T, Himmelbauer H. 2008. Substantial biases in ultra-short read data sets from high-throughput DNA sequencing. *Nucleic Acids Res* 36:e105.
- Dokras A, Gardner LM, Kirschmann DA, Sefror EA, Hendrix MJ. 2002. The tumour suppressor gene maspin is differentially regulated in cytotrophoblasts during human placental development. *Placenta* 23:274-280.
- Donninger H, Vos MD, Clark GJ. 2007. The RASSF1A tumor suppressor. *J Cell Sci* 120:3163-3172.
- Driscoll DA, Gross SJ. 2009. Screening for fetal aneuploidy and neural tube defects. *Genet Med* 11:818-821.
- Du Y, Zhang J, Wang H, Yan X, Yang Y, Yang L, Luo X, Chen Y, Duan T, Ma D. 2011. Hypomethylated DSCR4 is a placenta-derived epigenetic marker for trisomy 21. *Prenat Diagn* 31:207-214.
- Dunham A, Matthews LH, Burton J, Ashurst JL, Howe KL, Ashcroft KJ, Beare DM, Burford DC, Hunt SE, Griffiths-Jones S, Jones MC, Keenan SJ, Oliver K, Scott CE, Ainscough R, Almeida JP, Ambrose KD, Andrews DT, Ashwell RI, Babbage AK, Bagguley CL, Bailey J, Bannerjee R, Barlow KF, Bates K, Beasley H, Bird CP, Bray-Allen S, Brown AJ, Brown JY, Burrill W, Carder C, Carter NP, Chapman JC, Clamp ME, Clark SY, Clarke G, Clee CM, Clegg SC, Copley V, Collins JE, Corby N, Coville GJ, Deloukas P, Dhami P, Dunham I, Dunn M, Earthrowl ME, Ellington AG, Faulkner L, Frankish AG, Frankland J, French L, Garner P, Garnett J, Gilbert JG, Gilson CJ, Ghori J, Grafham DV, Gribble SM, Griffiths C, Hall RE, Hammond S, Harley JL, Hart EA, Heath PD, Howden PJ, Huckle EJ, Hunt PJ, Hunt AR, Johnson C, Johnson D, Kay M, Kimberley AM, King A, Laird GK, Langford CJ, Lawlor S, Leon gamornlert DA, Lloyd DM, Lloyd C, Loveland JE, Lovell J, Martin S, Mashreghi-Mohammadi M, McLaren SJ, McMurray A, Milne S, Moore MJ, Nickerson T, Palmer SA, Pearce AV, Peck AI, Pelan S, Phillimore B, Porter KM, Rice CM, Searle S, Sehra HK, Shownkeen R, Skuce CD, Smith M, Steward CA, Sycamore N, Tester J, Thomas DW, Tracey A, Tromans A, Tubby B, Wall M, Wallis JM, West AP, Whitehead SL, Willey DL, Wilming L, Wray PW, Wright MW, Young L, Coulson A, Durbin R, Hubbard T, Sulston JE, Beck S, Bentley DR, Rogers J, Ross MT. 2004. The DNA sequence and analysis of human chromosome 13. *Nature* 428:522-528.
- Ehrich M, Decui 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. 2011. Noninvasive detection of fetal trisomy 21 by sequencing of DNA in maternal blood: a study in a clinical setting. *Am J Obstet Gynecol*.

- Faas BH, Beuling EA, Christiaens GC, von dem Borne AE, van der Schoot CE. 1998. Detection of fetal RHD-specific sequences in maternal plasma. *Lancet* 352:1196.
- Faas BH, de Ligt J., Janssen I, Eggink AJ, Wijnberger LD, van Vugt JM, Vissers L, Geurts van KA. 2012. Non-invasive prenatal diagnosis of fetal aneuploidies using massively parallel sequencing-by-ligation and evidence that cell-free fetal DNA in the maternal plasma originates from cytotrophoblastic cells. *Expert Opin Biol Ther* 12 Suppl 1:S19-S26.
- Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. 2008. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci U S A* 105:16266-16271.
- Fan HC, Blumenfeld YJ, Chitkara U, Hudgins L, Quake SR. 2010. Analysis of the size distributions of fetal and maternal cell-free DNA by paired-end sequencing. *Clin Chem* 56:1279-1286.
- Fan HC, Blumenfeld YJ, El-Sayed YY, Chueh J, Quake SR. 2009. Microfluidic digital PCR enables rapid prenatal diagnosis of fetal aneuploidy. *Am J Obstet Gynecol* 200:543-547.
- Fan HC, Ho LI, Chi CS, Chen SJ, Peng GS, Chan TM, Lin SZ, Harn HJ. 2014. Polyglutamine (PolyQ) diseases: genetics to treatments. *Cell Transplant* 23:441-458.
- Fan HC, Quake SR. 2007. Detection of aneuploidy with digital polymerase chain reaction. *Anal Chem* 79:7576-7579.
- Forest MG, Morel Y, David M. 1998. Prenatal treatment of congenital adrenal hyperplasia. *Trends Endocrinol Metab* 9:284-289.
- Futch T, Spinosa J, Bhatt S, de FE, Rava RP, Sehnert AJ. 2013. Initial clinical laboratory experience in noninvasive prenatal testing for fetal aneuploidy from maternal plasma DNA samples. *Prenat Diagn* 33:569-574.
- Gezondheidsraad. Prenatale screening: Downsyndroom, neuralebuisdefecten, routine-echoscopie.
- Ghanta S, Mitchell ME, Ames M, Hidestrand M, Simpson P, Goetsch M, Thilly WG, Struble CA, Tomita-Mitchell A. 2010. Non-invasive prenatal detection of trisomy 21 using tandem single nucleotide polymorphisms. *PLoS One* 5:e13184.
- Gil MM, Akolekar R, Quezada MS, Bregant B, Nicolaides KH. 2014. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: meta-analysis. *Fetal Diagn Ther* 35:156-173.
- Go AT, van Vugt JM, Oudejans CB. 2010. Non-invasive aneuploidy detection using free fetal DNA and RNA in maternal plasma: recent progress and future possibilities. *Hum Reprod Update*.
- Gonzalez-Gonzalez MC, Garcia-Hoyos M, Trujillo MJ, Rodriguez de AM, Lorda-Sanchez I, Diaz-Recasens J, Gallardo E, Ayuso C, Ramos C. 2002. Prenatal detection of a cystic fibrosis mutation in fetal DNA from maternal plasma. *Prenat Diagn* 22:946-948.
- Gonzalez-Gonzalez MC, Garcia-Hoyos M, Trujillo-Tiebas MJ, Bustamante AA, Rodriguez de AM, Diego AD, Diaz-Recasens J, Ayuso C, Ramos C. 2008. Improvement in strategies for the non-invasive prenatal diagnosis of Huntington disease. *J Assist Reprod Genet* 25:477-481.
- Gonzalez-Gonzalez MC, Trujillo MJ, Rodriguez de AM, Garcia-Hoyos M, Lorda-Sanchez I, Diaz-Recasens J, Ayuso C, Ramos C. 2003a. Huntington disease-affected fetus diagnosed from maternal plasma using QF-PCR. *Prenat Diagn* 23:232-234.
- Gonzalez-Gonzalez MC, Trujillo MJ, Rodriguez de AM, Ramos C. 2003b. Early Huntington disease prenatal diagnosis by maternal semiquantitative fluorescent-PCR. *Neurology* 60:1214-1215.
- Gonzalgo ML, Jones PA. 1997. Rapid quantitation of methylation differences at specific sites using methylation-sensitive single nucleotide primer extension (Ms-SNuPE). *Nucleic Acids Res* 25:2529-2531.

- Grati FR. 2014. Chromosomal Mosaicism in Human Feto-Placental Development: Implications for Prenatal Diagnosis. *J Clin Med* 2014:809-837.
- Gupta PK. 2008. Single-molecule DNA sequencing technologies for future genomics research. *Trends Biotechnol* 26:602-611.
- Hagemann IS, Cottrell CE, Lockwood CM. 2013. Design of targeted, capture-based, next generation sequencing tests for precision cancer therapy. *Cancer Genet* 206:420-431.
- Hahn S, Lapaire O, Tercanli S, Kolla V, Hosli I. 2011. Determination of fetal chromosome aberrations from fetal DNA in maternal blood: has the challenge finally been met? *Expert Rev Mol Med* 13:e16.
- Hall AL, Drendel HM, Verbrugge JL, Reese AM, Schumacher KL, Griffith CB, Weaver DD, Abernathy MP, Litton CG, Vance GH. 2013. Positive cell-free fetal DNA testing for trisomy 13 reveals confined placental mosaicism. *Genet Med* 15:729-732.
- Harris TD, Buzby PR, Babcock H, Beer E, Bowers J, Braslavsky I, Causey M, Colonell J, Dimeo J, Efcavitch JW, Giladi E, Gill J, Healy J, Jarosz M, Lapen D, Moulton K, Quake SR, Steinmann K, Thayer E, Tyurina A, Ward R, Weiss H, Xie Z. 2008. Single-molecule DNA sequencing of a viral genome. *Science* 320:106-109.
- Hayden EC. 2014. Technology: The \$1,000 genome. *Nature* 507:294-295.
- Heazell AE, Crocker IP. 2008. Live and let die - regulation of villous trophoblast apoptosis in normal and abnormal pregnancies. *Placenta* 29:772-783.
- Hill M, Finning K, Martin P, Hogg J, Meaney C, Norbury G, Daniels G, Chitty L. 2010. Non-invasive prenatal determination of fetal sex: translating research into clinical practice. *Clin Genet*.
- Hillier LW, Marth GT, Quinlan AR, Dooling D, Fewell G, Barnett D, Fox P, Glasscock JI, Hickenbotham M, Huang W, Magrini VJ, Richt RJ, Sander SN, Stewart DA, Stromberg M, Tsung EF, Wylie T, Schedl T, Wilson RK, Mardis ER. 2008. Whole-genome sequencing and variant discovery in *C. elegans*. *Nat Methods* 5:183-188.
- Honorio S, Agathangelou A, Schuermann M, Pankow W, Viacava P, Maher ER, Latif F. 2003. Detection of RASSF1A aberrant promoter hypermethylation in sputum from chronic smokers and ductal carcinoma in situ from breast cancer patients. *Oncogene* 22:147-150.
- Hromadnikova I, Zejskova L, Kotlabova K, Jancuskova T, Doucha J, Dlouha K, Krofta L, Jirasek JE, Vlk R. 2010. Quantification of extracellular DNA using hypermethylated RASSF1A, SRY, and GLO sequences--evaluation of diagnostic possibilities for predicting placental insufficiency. *DNA Cell Biol* 29:295-301.
- Hudecova I, Sahota D, Heung MM, Jin Y, Lee WS, Leung TY, Lo YM, Chiu RW. 2014. Maternal plasma fetal DNA fractions in pregnancies with low and high risks for fetal chromosomal aneuploidies. *PLoS One* 9:e88484.
- Hui L, Bianchi DW. 2013. Recent advances in the prenatal interrogation of the human fetal genome. *Trends Genet* 29:84-91.
- Huppertz B, Kingdom JC. 2004. Apoptosis in the trophoblast--role of apoptosis in placental morphogenesis. *J Soc Gynecol Investig* 11:353-362.
- Illanes S, Denbow M, Kailasam C, Finning K, Soothill PW. 2007. Early detection of cell-free fetal DNA in maternal plasma. *Early Hum Dev* 83:563-566.
- Ioannides M, Papageorgiou EA, Keravnou A, Tsaliki E, Spyrou C, Hadjidaniel M, Sismani C, Koumbaris G, Patsalis PC. 2014. Inter-individual methylation variability in differentially methylated regions between maternal whole blood and first trimester CVS. *Mol Cytogenet* 7:73.

- Jeon YJ, Zhou Y, Li Y, Guo Q, Chen J, Quan S, Zhang A, Zheng H, Zhu X, Lin J, Xu H, Wu A, Park SG, Kim BC, Joo HJ, Chen H, Bhak J. 2014. The feasibility study of non-invasive fetal trisomy 18 and 21 detection with semiconductor sequencing platform. *PLoS One* 9:e110240.
- Jiang F, Ren J, Chen F, Zhou Y, Xie J, Dan S, Su Y, Xie J, Yin B, Su W, Zhang H, Wang W, Chai X, Lin L, Guo H, Li Q, Li P, Yuan Y, Pan X, Li Y, Liu L, Chen H, Xuan Z, Chen S, Zhang C, Zhang H, Tian Z, Zhang Z, Jiang H, Zhao L, Zheng W, Li S, Li Y, Wang J, Wang J, Zhang X. 2012a. Noninvasive Fetal Trisomy (NIFTY) test: an advanced noninvasive prenatal diagnosis methodology for fetal autosomal and sex chromosomal aneuploidies. *BMC Med Genomics* 5:57.
- Jiang P, Chan KC, Liao GJ, Zheng YW, Leung TY, Chiu RW, Lo YM, Sun H. 2012b. FetalQuant: deducing fractional fetal DNA concentration from massively parallel sequencing of DNA in maternal plasma. *Bioinformatics* 28:2883-2890.
- Jorgez CJ, Bischoff FZ. 2009. Improving enrichment of circulating fetal DNA for genetic testing: size fractionation followed by whole gene amplification. *Fetal Diagn Ther* 25:314-319.
- Juneau K, Bogard PE, Huang S, Mohseni M, Wang ET, Ryvkin P, Kingsley C, Struble CA, Oliphant A, Zahn JM. 2014. Microarray-based cell-free DNA analysis improves noninvasive prenatal testing. *Fetal Diagn Ther* 36:282-286.
- Kalousek DK, Vekemans M. 1996. Confined placental mosaicism. *J Med Genet* 33:529-533.
- Kersaudy-Kerhoas M, Amalou F, Che A, Kelly J, Liu Y, Desmulliez MP, Shu W. 2014. Validation of a fully integrated platform and disposable microfluidic chips enabling parallel purification of genome segments for assembly. *Biotechnol Bioeng* 111:1627-1637.
- Kersaudy-Kerhoas M, Sollier E. 2013. Micro-scale blood plasma separation: from acoustophoresis to egg-beaters. *Lab Chip* 13:3323-3346.
- Kim MJ, Kim SY, Park SY, Ahn HK, Chung JH, Ryu HM. 2013. Association of fetal-derived hypermethylated RASSF1A concentration in placenta-mediated pregnancy complications. *Placenta* 34:57-61.
- Kolialexi A, Tounta G, Apostolou P, Vrettou C, Papantoniou N, Kanavakis E, Antsaklis A, Mavrou A. 2012. Early non-invasive detection of fetal Y chromosome sequences in maternal plasma using multiplex PCR. *Eur J Obstet Gynecol Reprod Biol* 161:34-37.
- Landles C, Bates GP. 2004. Huntingtin and the molecular pathogenesis of Huntington's disease. Fourth in molecular medicine review series. *EMBO Rep* 5:958-963.
- Lapaire O, Holzgreve W, Oosterwijk JC, Brinkhaus R, Bianchi DW. 2007. Georg Schmorl on trophoblasts in the maternal circulation. *Placenta* 28:1-5.
- Lau TK, Chan MK, Salome Lo PS, Connie Chan HY, Kim Chan WS, Koo TY, Joyce Ng HY, Pooh RK. 2012a. Clinical utility of noninvasive fetal trisomy (NIFTY) test - early experience. *J Matern Fetal Neonatal Med*.
- Lau TK, Chen F, Pan X, Pooh RK, Jiang F, Li Y, Jiang H, Li X, Chen S, Zhang X. 2012b. Noninvasive prenatal diagnosis of common fetal chromosomal aneuploidies by maternal plasma DNA sequencing. *J Matern Fetal Neonatal Med*.
- Lau TK, Cheung SW, Lo PS, Pursley AN, Chan MK, Jiang F, Zhang H, Wang W, Jong LF, Yuen OK, Chan HY, Chan WS, Choy KW. 2014. Non-invasive prenatal testing for fetal chromosomal abnormalities by low-coverage whole-genome sequencing of maternal plasma DNA: review of 1982 consecutive cases in a single center. *Ultrasound Obstet Gynecol* 43:254-264.
- Laughlin TS, Moliterno AR, Stein BL, Rothberg PG. 2010. Detection of exon 12 Mutations in the JAK2 gene: enhanced analytical sensitivity using clamped PCR and nucleotide sequencing. *J Mol Diagn* 12:278-282.

- Lee dE, Kim SY, Lim JH, Park SY, Ryu HM. 2013. Non-invasive prenatal testing of trisomy 18 by an epigenetic marker in first trimester maternal plasma. *PLoS One* 8:e78136.
- Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. 1977. Free DNA in the serum of cancer patients and the effect of therapy. *Cancer Res* 37:646-650.
- Li LC, Dahiya R. 2002. MethPrimer: designing primers for methylation PCRs. *Bioinformatics* 18:1427-1431.
- Li Y, Page-Christiaens GC, Gille JJ, Holzgreve W, Hahn S. 2007. Non-invasive prenatal detection of achondroplasia in size-fractionated cell-free DNA by MALDI-TOF MS assay. *Prenat Diagn* 27:11-17.
- Li Y, Zimmermann B, Rusterholz C, Kang A, Holzgreve W, Hahn S. 2004. Size separation of circulatory DNA in maternal plasma permits ready detection of fetal DNA polymorphisms. *Clin Chem* 50:1002-1011.
- Liao C, Yin AH, Peng CF, Fu F, Yang JX, Li R, Chen YY, Luo DH, Zhang YL, Ou YM, Li J, Wu J, Mai MQ, Hou R, Wu F, Luo H, Li DZ, Liu HL, Zhang XZ, Zhang K. 2014. Noninvasive prenatal diagnosis of common aneuploidies by semiconductor sequencing. *Proc Natl Acad Sci U S A* 111:7415-7420.
- Lim JH, Kim MJ, Kim SY, Kim HO, Song MJ, Kim MH, Park SY, Yang JH, Ryu HM. 2011a. Non-invasive prenatal detection of achondroplasia using circulating fetal DNA in maternal plasma. *J Assist Reprod Genet* 28:167-172.
- Lim JH, Kim SY, Park SY, Lee SY, Kim MJ, Han YJ, Lee SW, Chung JH, Kim MY, Yang JH, Ryu HM. 2011b. Non-invasive epigenetic detection of fetal trisomy 21 in first trimester maternal plasma. *PLoS One* 6:e27709.
- Lim JH, Lee dE, Park SY, Kim dJ, Ahn HK, Han YJ, Kim MY, Ryu HM. 2014. Disease specific characteristics of fetal epigenetic markers for non-invasive prenatal testing of trisomy 21. *BMC Med Genomics* 7:1.
- Liu Q, Sommer SS. 2000. Pyrophosphorolysis-activated polymerization (PAP): application to allele-specific amplification. *Biotechniques* 29:1072-6, 1078, 1080.
- Liu Q, Sommer SS. 2004. PAP: detection of ultra rare mutations depends on P* oligonucleotides: "sleeping beauties" awakened by the kiss of pyrophosphorolysis. *Hum Mutat* 23:426-436.
- Lo KK, Boustred C, Chitty LS, Plagnol V. 2014. RAPIDR: an analysis package for non-invasive prenatal testing of aneuploidy. *Bioinformatics* 30:2965-2967.
- Lo YM, Chan KC, Sun H, Chen EZ, Jiang P, Lun FM, Zheng YW, Leung TY, Lau TK, Cantor CR, Chiu RW. 2010. Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. *Sci Transl Med* 2:61ra91.
- Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS. 1997. Presence of fetal DNA in maternal plasma and serum. *Lancet* 350:485-487.
- Lo YM, Lun FM, Chan KC, Tsui NB, Chong KC, Lau TK, Leung TY, Zee BC, Cantor CR, Chiu RW. 2007a. Digital PCR for the molecular detection of fetal chromosomal aneuploidy. *Proc Natl Acad Sci U S A* 104:13116-13121.
- Lo YM, Patel P, Sampietro M, Gillmer MD, Fleming KA, Wainscoat JS. 1990. Detection of single-copy fetal DNA sequence from maternal blood. *Lancet* 335:1463-1464.
- Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, Wainscoat JS, Johnson PJ, Chang AM, Hjelm NM. 1998. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet* 62:768-775.
- Lo YM, Tsui NB, Chiu RW, Lau TK, Leung TN, Heung MM, Gerovassili A, Jin Y, Nicolaidis KH, Cantor CR, Ding C. 2007b. Plasma placental RNA allelic ratio permits noninvasive prenatal chromosomal aneuploidy detection. *Nat Med* 13:218-223.

- Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. 1999. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 64:218-224.
- Luger K. 2003. Structure and dynamic behavior of nucleosomes. *Curr Opin Genet Dev* 13:127-135.
- Lui YY, Chik KW, Chiu RW, Ho CY, Lam CW, Lo YM. 2002. Predominant hematopoietic origin of cell-free DNA in plasma and serum after sex-mismatched bone marrow transplantation. *Clin Chem* 48:421-427.
- Lun FM, Chiu RW, Allen Chan KC, Yeung LT, Kin LT, Dennis Lo YM. 2008a. Microfluidics digital PCR reveals a higher than expected fraction of fetal DNA in maternal plasma. *Clin Chem* 54:1664-1672.
- Lun FM, Chiu RW, Leung TY, Leung TN, Lau TK, Lo YM. 2007. Epigenetic analysis of RASSF1A gene in cell-free DNA in amniotic fluid. *Clin Chem* 53:796-798.
- Lun FM, Chiu RW, Sun K, Leung TY, Jiang P, Chan KC, Sun H, Lo YM. 2013. Noninvasive prenatal methylomic analysis by genome-wide bisulfite sequencing of maternal plasma DNA. *Clin Chem* 59:1583-1594.
- Lun FM, Tsui NB, Chan KC, Leung TY, Lau TK, Charoenkwan P, Chow KC, Lo WY, Wanapirak C, Sanguanserm Sri T, Cantor CR, Chiu RW, Lo YM. 2008b. Noninvasive prenatal diagnosis of monogenic diseases by digital size selection and relative mutation dosage on DNA in maternal plasma. *Proc Natl Acad Sci U S A* 105:19920-19925.
- Maat W, Kilic E, Luyten GP, de KA, Jager MJ, Gruis NA, van der Velden PA. 2008. Pyrophosphorolysis detects B-RAF mutations in primary uveal melanoma. *Invest Ophthalmol Vis Sci* 49:23-27.
- Maat W, van der Velden PA, Out-Luiting C, Plug M, Dirks-Mulder A, Jager MJ, Gruis NA. 2007. Epigenetic inactivation of RASSF1a in uveal melanoma. *Invest Ophthalmol Vis Sci* 48:486-490.
- Macher HC, Martinez-Broca MA, Rubio-Calvo A, Leon-Garcia C, Conde-Sanchez M, Costa A, Navarro E, Guerrero JM. 2012. Non-invasive prenatal diagnosis of multiple endocrine neoplasia type 2A using COLD-PCR combined with HRM genotyping analysis from maternal serum. *PLoS One* 7:e51024.
- Madic J, Piperno-Neumann S, Servois V, Rampanou A, Milder M, Trouiller B, Gentien D, Saada S, Assayag F, Thuleau A, Nemati F, Decaudin D, Bidard FC, Desjardins L, Mariani P, Lantz O, Stern MH. 2012. Pyrophosphorolysis-activated polymerization detects circulating tumor DNA in metastatic uveal melanoma. *Clin Cancer Res* 18:3934-3941.
- Majchrzak-Celinska A, Paluszczak J, Kleszcz R, Magiera M, Barciszewska AM, Nowak S, Baer-Dubowska W. 2013. Detection of MGMT, RASSF1A, p15INK4B, and p14ARF promoter methylation in circulating tumor-derived DNA of central nervous system cancer patients. *J Appl Genet* 54:335-344.
- Mandel P, Métais P. 1948. Les acides nucléiques du plasma sanguin chez l'Homme. *C R Seances Soc Biol Fil* 142:241-243.
- Megarbane A, Ravel A, Mircher C, Sturtz F, Grattau Y, Rethore MO, Delabar JM, Mobley WC. 2009. The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. *Genet Med* 11:611-616.
- Mersy E, Smits LJ, van Winden LA, de Die-Smulders CE, Paulussen AD, Macville MV, Coumans AB, Frints SG. 2013. Noninvasive detection of fetal trisomy 21: systematic review and report of quality and outcomes of diagnostic accuracy studies performed between 1997 and 2012. *Hum Reprod Update* 19:318-329.
- Milbury CA, Li J, Makrigiorgos GM. 2009. PCR-based methods for the enrichment of minority alleles and mutations. *Clin Chem* 55:632-640.
- Milos PM. 2009. Emergence of single-molecule sequencing and potential for molecular diagnostic applications. *Expert Rev Mol Diagn* 9:659-666.

- Moise KJ, Jr. 2008. Management of rhesus alloimmunization in pregnancy. *Obstet Gynecol* 112:164-176.
- Mol BW, Opmeer BC, Veersema D, Mulders LGM. 2004. Gebruik van kansschattingen in diagnostische processen in de Verloskunde en Gynaecologie. *Maxima Medisch Centrum, Medisch Journaal* 33.
- Montgomery J, Wittwer CT, Palais R, Zhou L. 2007. Simultaneous mutation scanning and genotyping by high-resolution DNA melting analysis. *Nat Protoc* 2:59-66.
- Mouritzen P, Nielsen AT, Pfundheller HM, Choleva Y, Kongsbak L, Moller S. 2003. Single nucleotide polymorphism genotyping using locked nucleic acid (LNA). *Expert Rev Mol Diagn* 3:27-38.
- Mujezinovic F, Alfirevic Z. 2007. Procedure-related complications of amniocentesis and chorionic villous sampling: a systematic review. *Obstet Gynecol* 110:687-694.
- Muller SP, Bartels I, Stein W, Emons G, Gutensohn K, Legler TJ. 2011. Cell-free fetal DNA in specimen from pregnant women is stable up to 5 days. *Prenat Diagn*.
- Nicolaides K, Brizot ML, Patel F, Sniijders R. 1994. Comparison of chorionic villus sampling and amniocentesis for fetal karyotyping at 10-13 weeks' gestation. *Lancet* 344:435-439.
- Norton ME, Brar H, Weiss J, Karimi A, Laurent LC, Caughey AB, Rodriguez MH, Williams J, III, Mitchell ME, Adair CD, Lee H, Jacobsson B, Tomlinson MW, Oepkes D, Hollemon D, Sparks AB, 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* 207:137-138.
- Nygren AO, Dean J, Jensen TJ, Kruse S, Kwong W, van den Boom D, Ehrich M. 2010. Quantification of fetal DNA by use of methylation-based DNA discrimination. *Clin Chem* 56:1627-1635.
- Odeh M, Granin V, Kais M, Ophir E, Bornstein J. 2009. Sonographic fetal sex determination. *Obstet Gynecol Surv* 64:50-57.
- Oh JE, Lim HS, An CH, Jeong EG, Han JY, Lee SH, Yoo NJ. 2010. Detection of low-level KRAS mutations using PNA-mediated asymmetric PCR clamping and melting curve analysis with unlabeled probes. *J Mol Diagn* 12:418-424.
- Old RW, Crea F, Puszyk W, Hulten MA. 2007. Candidate epigenetic biomarkers for non-invasive prenatal diagnosis of Down syndrome. *Reprod Biomed Online* 15:227-235.
- Olins DE, Olins AL. 2003. Chromatin history: our view from the bridge. *Nat Rev Mol Cell Biol* 4:809-814.
- Oosterwijk JC, Mesker WE, Ouwkerk MC, Knepfle CF, van der Burg MJ, Wiesmeijer CC, Beverstock GC, Losekoot M, Bernini LF, Van Ommen GJ, van de Kamp JJ, Kanhai HH, Tanke HJ. 1996. Detection of fetal erythroblasts in maternal blood by one-step gradient enrichment and immunocytochemical recognition. *Early Hum Dev* 47 Suppl:S95-S97.
- Orozco AF, Jorgez CJ, Horne C, Marquez-Do DA, Chapman MR, Rodgers JR, Bischoff FZ, Lewis DE. 2008. Membrane protected apoptotic trophoblast microparticles contain nucleic acids: relevance to preeclampsia. *Am J Pathol* 173:1595-1608.
- Out AA, van Minderhout IJ, van der Stoep N, van Bommel LS, Kluijft I, Aalfs C, Voorendt M, Vossen RH, Nielsen M, Vasen HF, Morreau H, Devilee P, Tops CM, Hes FJ. 2015. High-resolution melting (HRM) re-analysis of a polyposis patients cohort reveals previously undetected heterozygous and mosaic APC gene mutations. *Fam Cancer*.
- Page-Christiaens GC, Bossers B, van der Schoot CE, de HM. 2006a. Use of bi-allelic insertion/deletion polymorphisms as a positive control for fetal genotyping in maternal blood: first clinical experience. *Ann N Y Acad Sci* 1075:123-129.

- Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW, Nelson SF, Canick JA. 2012. DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. *Genet Med* 14:296-305.
- Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Deciu C, Grody WW, Nelson SF, Canick JA. 2011. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* 13:913-920.
- Papageorgiou EA, Fiegler H, Rakyán V, Beck S, Hultén M, Lamnissou K, Carter NP, Patsalis PC. 2009. Sites of differential DNA methylation between placenta and peripheral blood: molecular markers for noninvasive prenatal diagnosis of aneuploidies. *Am J Pathol* 174:1609-1618.
- Papageorgiou EA, Karagrégoriou A, Tsaliki E, Velissariou V, Carter NP, Patsalis PC. 2011. Fetal-specific DNA methylation ratio permits noninvasive prenatal diagnosis of trisomy 21. *Nat Med* 17:510-513.
- Papantoniou N, Bagiokos V, Agiannitopoulos K, Kolialexi A, Destouni A, Tounta G, Kanavakis E, Antsaklis A, Mavrou A. 2013. RASSF1A in maternal plasma as a molecular marker of preeclampsia. *Prenat Diagn*:1-6.
- Patra SK, Szyf M. 2008. DNA methylation-mediated nucleosome dynamics and oncogenic Ras signaling: insights from FAS, FAS ligand and RASSF1A. *FEBS J* 275:5217-5235.
- Pearson HA. 1967. Life-span of the fetal red blood cell. *J Pediatr* 70:166-171.
- Phylipsen M, Yamsri S, Treffers EE, Jansen DT, Kanhai WA, Boon EM, Giordano PC, Fucharoen S, Bakker E, Harteveld CL. 2012. Non-invasive prenatal diagnosis of beta-thalassemia and sickle-cell disease using pyrophosphorolysis-activated polymerization and melting curve analysis. *Prenat Diagn* 32:578-587.
- Picchiassi E, Coata G, Centra M, Pennacchi L, Bini V, Di Renzo GC. 2010. Identification of universal mRNA markers for noninvasive prenatal screening of trisomies. *Prenat Diagn* 30:764-770.
- Ponomaryova AA, Rykova EY, Cherdyntseva NV, Skvortsova TE, Dobrodeev AY, Zav'yalov AA, Bryzgalov LO, Tuzikov SA, Vlassov VV, Laktionov PP. 2013. Potentialities of aberrantly methylated circulating DNA for diagnostics and post-treatment follow-up of lung cancer patients. *Lung Cancer* 81:397-403.
- Poon LL, Leung TN, Lau TK, Chow KC, Lo YM. 2002. Differential DNA methylation between fetus and mother as a strategy for detecting fetal DNA in maternal plasma. *Clin Chem* 48:35-41.
- Richter AM, Pfeifer GP, Dammann RH. 2009. The RASSF proteins in cancer; from epigenetic silencing to functional characterization. *Biochim Biophys Acta* 1796:114-128.
- Rijnders RJ, Christiaens GC, Bossers B, van der Schoot CE. 2002. [Congenital adrenal hyperplasia: clinical aspects and neonatal screening]. *Ned Tijdschr Geneesk* 146:1713-1714.
- Samango-Sprouse C, Banjevic M, Ryan A, Sigurjonsson S, Zimmermann B, Hill M, Hall MP, Westemeyer M, Saucier J, Demko Z, Rabinowitz M. 2013. SNP-based non-invasive prenatal testing detects sex chromosome aneuploidies with high accuracy. *Prenat Diagn* 33:643-649.
- Sato Y, Shinka T, Sakamoto K, Ewis AA, Nakahori Y. 2010. The male-determining gene SRY is a hybrid of DGCR8 and SOX3, and is regulated by the transcription factor CP2. *Mol Cell Biochem* 337:267-275.
- Scheffer PG, van der Schoot CE, Page-Christiaens GC, Bossers B, van EF, de HM. 2010. Reliability of fetal sex determination using maternal plasma. *Obstet Gynecol* 115:117-126.
- Scheffer PG, van der Schoot CE, Page-Christiaens GC, de HM. 2011. Noninvasive fetal blood group genotyping of rhesus D, c, E and of K in alloimmunised pregnant women: evaluation of a 7-year clinical experience. *BJOG* 118:1340-1348.

- Schmorl G. 1893. Untersuchungen über Puerperal-Eklampsie. Leipzig: Verlag FCW Vogel.
- Sehnert AJ, Rhees B, Comstock D, de FE, Heilek G, Burke J, Rava RP. 2011. Optimal Detection of Fetal Chromosomal Abnormalities by Massively Parallel DNA Sequencing of Cell-Free Fetal DNA from Maternal Blood. *Clin Chem*.
- Semaka A, Collins JA, Hayden MR. 2010. Unstable familial transmissions of Huntington disease alleles with 27-35 CAG repeats (intermediate alleles). *Am J Med Genet B Neuropsychiatr Genet* 153B:314-320.
- Shi J, Liu Q, Sommer SS. 2007. Detection of ultrarare somatic mutation in the human TP53 gene by bidirectional pyrophosphorolysis-activated polymerization allele-specific amplification. *Hum Mutat* 28:131-136.
- Sikora A, Zimmermann BG, Rusterholz C, Birri D, Kolla V, Lapaire O, Hoesli I, Kiefer V, Jackson L, Hahn S. 2010. Detection of increased amounts of cell-free fetal DNA with short PCR amplicons. *Clin Chem* 56:136-138.
- Simoni G, Fraccaro M. 1992. Does confined placental mosaicism affect the fetus? *Hum Reprod* 7:139-140.
- Sirichotiyakul S, Charoenkwan P, Sanguanserm Sri T. 2012. Prenatal diagnosis of homozygous alpha-thalassemia-1 by cell-free fetal DNA in maternal plasma. *Prenat Diagn* 32:45-49.
- Song N, Zhong X, Li Q. 2014. Real-time bidirectional pyrophosphorolysis-activated polymerization for quantitative detection of somatic mutations. *PLoS One* 9:e96420.
- Sparks AB, Struble CA, Wang ET, 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.
- Sparks AB, Wang ET, Struble CA, 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.
- Straszewski-Chavez SL, Abrahams VM, Mor G. 2005. The role of apoptosis in the regulation of trophoblast survival and differentiation during pregnancy. *Endocr Rev* 26:877-897.
- Straver R, Sistermans EA, Reinders MJ. 2014. Introducing WISECONDOR for noninvasive prenatal diagnostics. *Expert Rev Mol Diagn* 14:513-515.
- Tabor A, Alfirevic Z. 2010. Update on procedure-related risks for prenatal diagnosis techniques. *Fetal Diagn Ther* 27:1-7.
- Tang NL, Leung TN, Zhang J, Lau TK, Lo YM. 1999. Detection of fetal-derived paternally inherited X-chromosome polymorphisms in maternal plasma. *Clin Chem* 45:2033-2035.
- The Huntington's Disease Collaborative Research Group. 1993. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* 72:971-983.
- Tjoa ML, Cindrova-Davies T, Spasic-Boskovic O, Bianchi DW, Burton GJ. 2006. Trophoblastic oxidative stress and the release of cell-free fetoplacental DNA. *Am J Pathol* 169:400-404.
- Tong YK, Chiu RW, Akolekar R, Leung TY, Lau TK, Nicolaidis KH, Lo YM. 2010a. Epigenetic-genetic chromosome dosage approach for fetal trisomy 21 detection using an autosomal genetic reference marker. *PLoS One* 5:e15244.
- Tong YK, Chiu RW, Leung TY, Ding C, Lau TK, Leung TN, Lo YM. 2007. Detection of restriction enzyme-digested target DNA by PCR amplification using a stem-loop primer: application to the detection of hypomethylated fetal DNA in maternal plasma. *Clin Chem* 53:1906-1914.

- Tong YK, Ding C, Chiu RW, Gerovassili A, Chim SS, Leung TY, Leung TN, Lau TK, Nicolaides KH, Lo YM. 2006. Noninvasive prenatal detection of fetal trisomy 18 by epigenetic allelic ratio analysis in maternal plasma: Theoretical and empirical considerations. *Clin Chem* 52:2194-2202.
- Tong YK, Jin S, Chiu RW, Ding C, Chan KC, Leung TY, Yu L, Lau TK, Lo YM. 2010b. Noninvasive prenatal detection of trisomy 21 by an epigenetic-genetic chromosome-dosage approach. *Clin Chem* 56:90-98.
- Tsui DW, Chan KC, Chim SS, Chan LW, Leung TY, Lau TK, Lo YM, Chiu RW. 2007. Quantitative aberrations of hypermethylated RASSF1A gene sequences in maternal plasma in pre-eclampsia. *Prenat Diagn* 27:1212-1218.
- Tsui DW, Lam YM, Lee WS, Leung TY, Lau TK, Lau ET, Tang MH, Akolekar R, Nicolaides KH, Chiu RW, Lo YM, Chim SS. 2010. Systematic identification of placental epigenetic signatures for the noninvasive prenatal detection of Edwards syndrome. *PLoS One* 5:e15069.
- Tsui NB, Jiang P, Chow KC, Su X, Leung TY, Sun H, Chan KC, Chiu RW, Lo YM. 2012. High resolution size analysis of fetal DNA in the urine of pregnant women by paired-end massively parallel sequencing. *PLoS One* 7:e48319.
- Tsui NB, Jiang P, Wong YF, Leung TY, Chan KC, Chiu RW, Sun H, Lo YM. 2014. Maternal plasma RNA sequencing for genome-wide transcriptomic profiling and identification of pregnancy-associated transcripts. *Clin Chem* 60:954-962.
- Tynan JA, Mahboubi P, Cagasan LL, van den Boom D, Ehrich M, Oeth P. 2011. Restriction enzyme-mediated enhanced detection of circulating cell-free fetal DNA in maternal plasma. *J Mol Diagn* 13:382-389.
- van den Oever JM, Balkassmi S, Segboer T, Verweij EJ, van der Velden PA, Oepkes D, Bakker E, Boon EM. 2013. Mrass f1a-pap, a novel methylation-based assay for the detection of cell-free fetal DNA in maternal plasma. *PLoS One* 8:e84051.
- van den Oever JM, Balkassmi S, Verweij EJ, van Iterson M, Adama van Scheltema PN, Oepkes D, van Lith JM, Hoffer MJ, den Dunnen JT, Bakker E, Boon EM. 2012. Single molecule sequencing of free DNA from maternal plasma for noninvasive trisomy 21 detection. *Clin Chem* 58:699-706.
- van der Schoot CE, Hahn S, Chitty LS. 2008. Non-invasive prenatal diagnosis and determination of fetal Rh status. *Semin Fetal Neonatal Med* 13:63-68.
- van der Stoep N, van Paridon CD, Janssens T, Krenkova P, Stambergova A, Macek M, Matthijs G, Bakker E. 2009. Diagnostic guidelines for high-resolution melting curve (HRM) analysis: an interlaboratory validation of BRCA1 mutation scanning using the 96-well LightScanner. *Hum Mutat* 30:899-909.
- Verweij, E. J. NIPT: non-invasive prenatal testing: towards implementation in the Netherlands. Thesis, 2014.
- Walknowska J, Conte FA, Grumbach MM. 1969. Practical and theoretical implications of fetal-maternal lymphocyte transfer. *Lancet* 1:1119-1122.
- Walsh PS, Fildes NJ, Reynolds R. 1996. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. *Nucleic Acids Res* 24:2807-2812.
- Wang E, Batey A, Struble C, Musci T, Song K, Oliphant A. 2013. Gestational age and maternal weight effects on fetal cell-free DNA in maternal plasma. *Prenat Diagn* 33:662-666.
- Wang Y, Wen Z, Shen J, Cheng W, Li J, Qin X, Ma D, Shi Y. 2014. Comparison of the performance of Ion Torrent chips in noninvasive prenatal trisomy detection. *J Hum Genet* 59:393-396.
- Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, Platt L, Mahoney M, Johnson A, Hogge WA, Wilson RD, Mohide P, Hershey D, Krantz D, Zachary J, Snijders R, Greene N, Sabbagha R, MacGregor S, Hill L, Gagnon A, Hallahan T, Jackson L. 2003. First-trimester screening for trisomies 21 and 18. *N Engl J Med* 349:1405-1413.
- Warner JP, Barron LH, Brock DJ. 1993. A new polymerase chain reaction (PCR) assay for the trinucleotide

- repeat that is unstable and expanded on Huntington's disease chromosomes. *Mol Cell Probes* 7:235-239.
- Warszawsky I, Mularo F. 2011. Locked nucleic acid probes for enhanced detection of FLT3 D835/I836, JAK2 V617F and NPM1 mutations. *J Clin Pathol* 64:905-910.
- White HE, Dent CL, Hall VJ, Crolla JA, Chitty LS. 2012. Evaluation of a novel assay for detection of the fetal marker RASSF1A: facilitating improved diagnostic reliability of noninvasive prenatal diagnosis. *PLoS One* 7:e45073.
- Whiting P, Rutjes AW, Reitsma JB, Bossuyt PM, Kleijnen J. 2003. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. *BMC Med Res Methodol* 3:25.
- Xiang Y, Zhang J, Li Q, Zhou X, Wang T, Xu M, Xia S, Xing Q, Wang L, He L, Zhao X. 2014. DNA methylome profiling of maternal peripheral blood and placentas reveal potential fetal DNA markers for non-invasive prenatal testing. *Mol Hum Reprod* 20:875-884.
- Yan TZ, Mo QH, Cai R, Chen X, Zhang CM, Liu YH, Chen YJ, Zhou WJ, Xiong F, Xu XM. 2011. Reliable detection of paternal SNPs within deletion breakpoints for non-invasive prenatal exclusion of homozygous alpha-thalassemia in maternal plasma. *PLoS One* 6:e24779.
- Yenilmez ED, Tuli A, Evruke IC. 2013. Noninvasive prenatal diagnosis experience in the Cukurova Region of Southern Turkey: detecting paternal mutations of sickle cell anemia and beta-thalassemia in cell-free fetal DNA using high-resolution melting analysis. *Prenat Diagn* 33:1054-1062.
- Yu SC, Chan KC, Zheng YW, Jiang P, Liao GJ, Sun H, Akolekar R, Leung TY, Go AT, van Vugt JM, Minekawa R, Oudejans CB, Nicolaides KH, Chiu RW, Lo YM. 2014. Size-based molecular diagnostics using plasma DNA for noninvasive prenatal testing. *Proc Natl Acad Sci U S A* 111:8583-8588.
- Yu SC, Lee SW, Jiang P, Leung TY, Chan KC, Chiu RW, Lo YM. 2013. High-resolution profiling of fetal DNA clearance from maternal plasma by massively parallel sequencing. *Clin Chem* 59:1228-1237.
- Yuan Y, Jiang F, Hua S, Du B, Hao Y, Ye L, Liu J, Feng K, Huang X, Yi X, Wang W, Yang L, Mu F, Liu C, Liang Y. 2013. Feasibility study of semiconductor sequencing for noninvasive prenatal detection of fetal aneuploidy. *Clin Chem* 59:846-849.
- Zejskova L, Jancuskova T, Kotlabova K, Doucha J, Hromadnikova I. 2010. Feasibility of fetal-derived hypermethylated RASSF1A sequence quantification in maternal plasma--next step toward reliable non-invasive prenatal diagnostics. *Exp Mol Pathol* 89:241-247.
- Zhang Q, Hu G, Yang Q, Dong R, Xie X, Ma D, Shen K, Kong B. 2013. A multiplex methylation-specific PCR assay for the detection of early-stage ovarian cancer using cell-free serum DNA. *Gynecol Oncol* 130:132-139.
- Zhang X, Wang Y, Fricke BL, Gu LQ. 2014. Programming nanopore ion flow for encoded multiplex microRNA detection. *CS Nano* 8:3444-3450.
- Zhao F, Wang J, Liu R, Yang J, Cui K, Wu Y, Guo J, Mu Y, Wang X. 2010. Quantification and application of the placental epigenetic signature of the RASSF1A gene in maternal plasma. *Prenat Diagn* 30:778-782.
- Zheng YW, Chan KC, Sun H, Jiang P, Su X, Chen EZ, Lun FM, Hung EC, Lee V, Wong J, Lai PB, Li CK, Chiu RW, Lo YM. 2012. Nonhematopoietically derived DNA is shorter than hematopoietically derived DNA in plasma: a transplantation model. *Clin Chem* 58:549-558.
- Zimmermann B, El-Sheikhah A, Nicolaides K, Holzgreve W, Hahn S. 2005. Optimized real-time quantitative PCR measurement of male fetal DNA in maternal plasma. *Clin Chem* 51:1598-1604.
- 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.

Zimmermann BG, Grill S, Holzgreve W, Zhong XY, Jackson LG, Hahn S. 2008. Digital PCR: a powerful new tool for non invasive prenatal diagnosis? *Prenat Diagn* 28:1087-1093.



Curriculum vitae

Curriculum vitae

Jessica Maria Elisabeth van den Oever werd geboren op 11 mei 1980 te Roosendaal, gemeente Roosendaal en Nispen. In 1997 behaalde zij haar HAVO diploma aan het Gertrudiscollege te Roosendaal, waarna zij in datzelfde jaar startte met de opleiding Biologie en Medisch Laboratorium Onderzoek aan de Hogeschool Brabant, Faculteit Techniek en Natuur te Etten-Leur (tegenwoordig onderdeel van Avans Hogeschool gevestigd te Breda).

Tijdens haar afstudeerstage op de afdeling Klinische Genetica van de Erasmus Universiteit Rotterdam onder supervisie van Dr. Rob Willemsen, deed zij onderzoek naar het fragiele X syndroom. Hiervoor bestudeerde zij het transport van FMRP in een PC12 neuronale cellijn.

Na het behalen van haar diploma, startte zij in 2001 met haar studie Biologie aan de Universiteit Leiden via een HBO-instroom programma. Ter afronding van deze studie werd stage gelopen bij de sectie Moleculaire Biologie van het instituut voor Moleculaire Plantkunde bij de Faculteit Biologie van Universiteit Leiden. Onder begeleiding van Prof. Herman Spaink en Prof. Michael Richardson werd gestart met een pilot studie met vertebraten waarbij er onderzoek gedaan werd naar het expressie patroon van Selenium Binding Protein in zebrafissen.

In 2005 begon zij als research analist op het project “Immunomodulatory properties of Mesenchymal Stem Cells” op de afdeling Immunohematologie en bloedtransfusie (IHB) van het Leids Universitair Medisch Centrum (LUMC) onder begeleiding van Prof. Wim Fibbe, Dr. Alma Nauta en Dr. Kirsten Canté-Barrett. Vanaf 2007 was zij tevens werkzaam als research analist op het project “Mechanismen van cytokine-geïnduceerde hematopoietische stam- en progenitorcel mobilisatie” onder begeleiding van Prof. Wim Fibbe en Dr. Melissa van Pel. Daarnaast heeft zij in die periode gewerkt als interim proefdiercoördinator voor de afdeling IHB.

Eind 2008 begon zij als research analist op het project “Gene expression profiling in a chronic restraint stress rat model” bij de afdeling Medische Farmacologie van het Leiden/Amsterdam Center for Drug Research (LACDR)/ LUMC onder begeleiding van Dr. Nicole Datson en Prof. Ron de Kloet.

In oktober 2010 werd gestart met het promotie onderzoek “Noninvasive prenatal detection of genetic defects” bij de afdeling Klinische Genetica op het Laboratorium voor Diagnostische Genoomanalyse (LDGA) van het LUMC te Leiden. Onder leiding van Dr. Elles Boon en Prof. Bert Bakker werden de experimenten verricht die staan beschreven in dit proefschrift. Van juli t/m september 2015 heeft zij onder supervisie van Prof. Joris Vermeesch gewerkt als interim project manager voor targeted NIPT bij het Centrum Menselijke Erfelijkheid van de Katholieke Universiteit Leuven in België.



**Publications
and
Presentations**

Publications

- Brison, N., Van Den Bogaert, K., **van den Oever, J.M.**, Dehaspe, L., Janssens, K., Blaumeiser, B., Peeters, H., Van Esch, H., de Ravel, T., Legius, E., Devriendt, K., Vermeesch, J.R., *Maternal incidental findings during non-invasive prenatal testing for fetal aneuploidies*, submitted.
- **Van den Oever, J.M.**, van Minderhout, I.J.H.M, Hartevelde, C.L., den Hollander, N.S., Bakker, E., van der Stoep, N., Boon, E.M.J., *A novel targeted approach for noninvasive detection of paternally inherited mutations in maternal plasma*, J Mol Diagn. 2015.
- **Van den Oever, J.M.**, Bijlsma, E.K., Feenstra, I., Muntjewerff, N., Mathijssen, I.B., Bakker, E., van Belzen, M.J., Boon, E.M.J., *Noninvasive prenatal diagnosis of Huntington disease: detection of the paternally inherited expanded CAG repeat in maternal plasma*, Prenat. Diagn. 2015.
- **Van den Oever, J.M.**, Balkassmi, S., Segboer, T., Verweij, E.J., van der Velden, P.A., Oepkes, D., Bakker, E., Boon, E.M., *Mrsf1a-pap, a novel methylation-based assay for the detection of cell-free fetal DNA in maternal plasma*, PloS One, 2013.
- Datson, N.A., **van den Oever, J.M.**, Korobko, O.B., Margarinos, A.M., de Kloet, E.R., McEwen, B.S., *Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus*, Endocrinology 2013.
- **Van den Oever, J.M.**, Balkassmi, S., Johansson, L.F., Adama van Scheltema, P.N., Suijkerbuijk, R.F., Hoffer, M.J., Sinke, R.J., Bakker, E., Sikkema-Raddatz, B., Boon, E.M., *Successful noninvasive trisomy 18 detection using single molecule sequencing*, Clin Chem 2013.
- Polman, J.A., Hunter, R.G., Speksnijder, N., **van den Oever, J.M.**, Korobko, O.B., McEwen, B.S., de Kloet, E.R., Datson, N.A., *Glucocorticoids modulate the mTOR pathway in the hippocampus: differential effects depending on stress history*, Endocrinology 2012.
- **Van den Oever, J.M.**, Balkassmi, S., Verweij, E.J., van Iterson, M., Adama van Scheltema, P.N., Oepkes, D., van Lith, J.M., Hoffer, M.J. den Dunnen J.T., Bakker, E., Boon, E.M., *Single molecule sequencing of free DNA from maternal plasma for noninvasive trisomy 21 detection*, Clin Chem 2012.
- Verweij, E.J., **van den Oever, J.M.**, de Boer, M.A., Boon, E.M., Oepkes, D., *Diagnostic accuracy of noninvasive detection of fetal trisomy 21 in maternal blood: a systematic review*, Fetal Diagn Ther 2012.
- De Jager, S.C., Canté-Barrett, K., Bot, I., Husberg, C., van Puijvelde, G.H., van Santbrink, P.J., Yndestad, A., **van den Oever, J.M.**, Kuiper, J., van Berkel, T.J., Lipp, M., Zwaginga, J.J., Fibber, W.E., Aukrust, P., Biessen, E.A., *Impaired effector memory T-cell regulation facilitates graft versus host disease in CCR7-deficient bone marrow transplant chimeras*, Transplantation, 2009.

Presentations

- Invited speaker: Prenatal Molecular Diagnostics; Trends, advances & prospects, Lisbon, April 2015.
- *Noninvasive prenatal diagnosis of Huntington disease in the Netherlands*: DHDRN, Amsterdam, mei 2014.
- Invited speaker: Werkgroep prenatale diagnostiek en therapie/ werkgroep foetale

echoscopie: *Gemetyleerd RASSF1A: een universele biomarker ter bevestiging van de aanwezigheid van foetaal DNA in maternaal bloed*; najaarssymposium, Utrecht, oktober 2013.

- *Single Molecule Sequencing of Free DNA from maternal plasma for noninvasive trisomy testing*; Medical Genetics Centre PhD student workshop, Luxemburg, mei 2013

- Invited speaker: *Noninvasive fetal aneuploidy detection using Next Generation Sequencing: towards application in diagnostics*; Integrated Data Analysis meeting organised by the dept. of Epidemiology in collaboration with SASC, LUMC, Leiden, november 2012.

- Invited speaker: *Noninvasive fetal aneuploidy detection using Helicos: Third generation vs Next Generation Sequencing*; NBIC Next Generation Sequencing meeting, Leuven, juni 2012.

- *Noninvasive fetal sexing and maternal discrimination tests: a validation study for application and implementation in diagnostics*; NVHG voorjaarssymposium, Veldhoven, maart 2011.

Grants/ Awards:

- VIVA400 Award 2015 nominee in the category “Knappe koppen”.
- Travel grant Stichting Simonsfonds (2014).

The background of the page is a complex, abstract pattern of overlapping, semi-transparent grey shapes. These shapes include circles of various sizes, teardrop-like forms, and irregular, organic blobs that resemble cells or molecular structures. The shapes are interconnected, creating a dense, interconnected network. The overall effect is a textured, organic, and somewhat futuristic aesthetic. The word "Dankwoord" is centered in the middle of the page, overlaid on this pattern.

Dankwoord

Eindelijk is het dan zover. Jaren van hard werken en studeren worden beloond met een mooi slot; een proefschrift. Ook al staat mijn naam op de voorkant, promoveren doe je niet alleen. Daarom wil ik via dit dankwoord iedereen bedanken die op welke wijze dan ook betrokken is geweest bij de totstandkoming hiervan. Er zijn ook een aantal mensen die ik graag persoonlijk zou willen bedanken:

Elles, wat ben ik ongelofelijk blij en trots dat ik je eerste OIO mocht zijn. Mede dankzij jouw tomeloze enthousiasme en passie zijn de jaren op het lab omgevlogen. Bedankt voor je hulp, advies en steun. De sushi diners om te vieren dat er weer een paper geaccepteerd was, waren een welkom excuus om de deur van het lab eens een keertje wat vroeger achter ons dicht te trekken. Jij hebt afgelopen jaar een nieuwe stap gemaakt in jouw carrière en helaas zien we je daardoor minder vaak. Maar gelukkig is er altijd een reden voor sushi te verzinnen.

Bert, als promotor heb je mij de kans gegeven om als promovendus aan de slag te gaan op het lab. Bedankt! Ik kon altijd bij je binnenlopen voor een vraag, advies of een praatje. Zeker tijdens een overleg verbaasde ik me toch elke keer dat je precies dat ene boek of proefschrift wist te pakken waar de benodigde informatie in stond. Bij deze kan er nog een exemplaar aan de verzameling worden toevoegd.

Nicole, bedankt voor onze gesprekken tijdens onze samenwerking. Ze hebben me doen inzien dat promoveren wel eens een goede vervolgstap voor mij zou zijn. En ik heb er zeker geen spijt van gehad. Joris, bedankt dat je het vertrouwen had in mij, en mij de kans hebt geboden om een paar maanden in Leuven te werken. Niet alleen heb ik een toptijd gehad bij het CME, het heeft me ook geholpen om goed na te kunnen denken over mijn toekomst na het behalen van mijn PhD.

De “borrelclub”, bedankt dat jullie me regelmatig van het lab haalden om wat iets leuks te gaan doen. Dat had ik echt even nodig!

De collega's van het LDGA voor al jullie hulp, het beantwoorden van mijn vragen, jullie geduld als ik weer eens met samples kwam waar iets apart mee moest gebeuren en alle leuke gesprekken tijdens de pauzes. Speciaal een dankje voor de collega's van sectie Prenataal die de meeste, zo niet alle samples uit dit proefschrift wel voorbij hebben zien komen. Zonder jullie hulp, harde werk en enthousiasme zou dit proefschrift niet zijn wat het nu is.

Dan mijn paranimfen. Sahila, eind 2010 zijn we samen aan deze uitdaging begonnen. Dankzij al jouw voorwerk had ik een vliegende start. Ook al zijn onze wegen (en werkzaamheden) daarna wat verder uit elkaar gaan lopen, het stond al heel lang vast dat jij een van mijn paranimfen zou worden. En gelukkig zei je “ja”! En Nienke, mijn vraagbaak en ervaringsdeskundige wat betreft het promoveren. Bedankt voor al je feedback, adviezen en antwoorden op al mijn vragen. Vergeleken met jouw promotie zijn we voor deze een stuk minder op pad geweest. En al heeft het even geduurd, nu dan eindelijk zelf ook een keer paranimf. Fijn dat ook jij naast me staat vandaag.

Lieve familie en schoonfamilie, “mijn boekje” is eindelijk klaar. Bedankt voor al jullie interesse in de afgelopen jaren!

Pa en ma, ik weet dat jullie trots op me zijn. Ook al is het weer een behoorlijke reis, fijn dat jullie er vandaag weer bij zijn in dit voor jullie reeds bekende gebouw.

En tot slot Danny, mijn grote steun en toeverlaat. Promoveren is niet altijd even makkelijk. Fijn dat je daar altijd begrip voor hebt gehad. Bedankt voor je onvoorwaardelijke steun, positiviteit en optimisme, zeker tijdens het afgelopen jaar.

The background of the page is a complex, abstract pattern of overlapping, semi-transparent gray shapes. These shapes resemble organic, cell-like structures or a network of interconnected nodes. The shapes vary in size and opacity, creating a sense of depth and movement. The overall effect is a textured, monochromatic design that frames the central text.

Appendices

Appendix 1: Confined placental mosaicism

Placental villi obtained with CVS can be analyzed by two distinct culturing methods. With a semi-direct method (short-term culture or STC) cells from the invading cytotrophoblast are analyzed. After culture (long-term culture or LTC) cells of the mesenchymal lineage are evaluated. Distinction between these two culturing methods and knowledge of the origin of the cells is very important for interpretation of the outcome of prenatal genetic testing on CVS material, since cells of different embryogenic progenitors are analyzed for chromosomal analysis of the fetus with these two distinct culturing methods (BIANCHI *et al.*, 1993). When culturing is successful and sufficient sample is provided, preferably a combination of both STC and LTC should be used to interpret prenatal findings.

In the majority of pregnancies the karyotype of the placental cells is similar to the karyotype of the fetus. However, in ~2% of the pregnancies studied by CVS a cytogenetic abnormality is found, most often a trisomy (KALOUSEK *et al.*, 1996). The existence of a discrepancy between the karyotype from chorionic tissue and embryonic/fetal tissue is caused by complex developmental events during early embryogenesis. When a trisomy is formed soon after fertilization before the trophoblast and the inner cell mass are differentiated, the discrepancy (or mosaic) can be generalized to both placenta and fetal tissues. When it is formed after the separation of the fetal and placental compartments, the abnormal tissue may be confined to either the placenta (**confined placental mosaicism** or **CPM**) or the fetus, but not necessarily to both tissues (SIMONI *et al.*, 1992). Therefore, in case of mosaicism it is very important to distinguish between a true fetal mosaicism (TFM) and CPM and confirmatory karyotyping on amniocytes is required to assess which type of mosaicism is present. Mosaicism can be classified according to the distribution of the abnormal cell line (Table 1) (GRATI, 2014).

Type	Nature	Trophoblast (direct)	Mesenchyme (culture)	Amniocytes
I	CPM	Abnormal	Normal	Normal
II	CPM	Normal	Abnormal	Normal
III	CPM	Abnormal	Abnormal	Normal
IV	TFM	Abnormal	Normal	Abnormal
V	TFM	Normal	Abnormal	Abnormal
VI	TFM	Abnormal	Abnormal	Abnormal
Evaluated in:		CVS(STC)/ NIPT	CVS (LTC)	Amniocentesis

Table 1: Different types of mosaic outcome: (CPM; confined placental mosaicism, TFM; true fetal mosaicism) found after chorionic villous and amniocytes karyotyping. Adapted from (GRATI, 2014).

Since cfDNA is derived from trophoblast cells, the presence of a possible CPM can also influence results of noninvasive prenatal testing (NIPT) for common fetal aneuploidies. Due to fetoplacental mosaicism potential false positive (CPM type I or III) and false negative (TFM type V) results may occur for mosaics in which the trophoblast is cytogenetically discrepant from the fetus. Even though a discrepancy between karyotypes occurs only in around 2% of the cases, in the majority of cases, the mosaicism is confined to the placenta (i.e. CPM type I, ~35%). CPM type III and TFM type V are found in around 10% and 6% of the cases respectively (GRATI, 2014). For NIPT, additional genetic analysis of either amniocytes or a combination of STC and LTC is therefore required to determine fetal karyotype in case of an aberrant result.

Appendix 2: Epigenetic allelic ratio, haplotype ratio analysis and relative mutation dosage.

Analysis of the epigenetic allelic ratio (EAR) is a method to assess copy number of a particular chromosome of interest for fetal aneuploidy detection by determining the ratio of an informative single nucleotide polymorphism (SNP) on fetal alleles. This SNP is present within a fetal specific amplified DNA molecule in a differentially methylated region (DMSR) on the chromosome of interest. Bisulfite converted DNA samples are amplified with methylation specific PCR (MSP). Subsequently, methylation differences are assessed with allele-specific primer extension. This primer extension utilizes internal primers which anneal to a PCR-generated template and terminate immediately 5' adjacent to the informative single base variation (GONZALGO *et al.*, 1997). Extension of these allele specific primers can distinguish between an allele with a nucleotide that is not affected by the conversion (allele A) and an allele with a polymorphism that is affected by the bisulfite conversion (allele B with an unmethylated cytosine (C) which is converted into a thymidine). The extension reactions are designed to generate products of distinct masses (and thus distinct peaks) when analyzing the alleles with Mass Spectrometry.

In euploid fetuses there are equal amounts of allele A and B. Therefore, the theoretical relative peak frequency of allele A and B is both 50% (or 0.5). The EAR can subsequently be calculated by dividing the relative peak frequency of A by the relative peak frequency of B (1). Hence, for euploid fetuses, the EAR is 1.

In case of a fetus with a trisomy, instead of two alleles, three alleles are present. Moreover, there is an overrepresentation of one of these alleles (e.g. either allele A (AAB) or allele B (ABB)). For AAB, there is twice the amount of A alleles contributing to the total amount of three alleles (e.g. twice as much A compared to B). Therefore, the contribution of A relative to the total amounts of alleles is 2 out of 3 (i.e. 67% or 0.67), while relative the B allele is present in only 1 of the 3 alleles (i.e. a relative peak frequency of 33% or 0.33 for B). Therefore, the EAR for AAB is 2 (i.e. 0.67/0.33). In case a fetal trisomy with only one A allele and two B alleles (i.e. ABB), the relative contribution for A is 0.33 and 0.67 for B, resulting in an EAR for ABB of 0.5 (i.e. 0.33/0.67). An EAR that has deviated from 1 (i.e. euploid fetus) is indicative for a fetal trisomy. Adapted from (TONG *et al.*, 2006).

$$(1) \quad EAR = \frac{\text{Relative peak frequency A}}{\text{Relative peak frequency B}}$$

Another method for fetal aneuploidy detection is the determination of the **haplotype ratio** (HR). In HR analysis only highly heterozygous tandem SNPs on the chromosomes of interest exhibiting three different alleles (haplotypes) are considered informative (i.e. two different maternal alleles and a third distinctive paternally inherited fetal allele) (**Fig. 1**). In contrast to EAR, for HR it is not required to have SNPs only present in DMR, since this method is not based on differences in methylation.

For HR, first Multiplexed Linear Amplification (MLA) is performed on DNA from maternal buccal swabs. This linear amplification product is used as a template in a "sequence specific" PCR and Cycling Temperature Capillary Electrophoresis (CTCE). When results of the maternal buccal swabs indicate that the maternal tandem SNP status is homozygous, the SNPs are not informative. When the maternal SNPs are heterozygous, maternal plasma can be processed similar to the maternal buccal DNA and analyzed subsequently. Fetal chromosome dosage can

be determined by calculating HR using the area under the curve of the three distinct peaks (p1, p2 and p3) in the electropherogram after CTCE (8). As a control, gDNA from maternal buccal swabs or maternal lymphocytes is analyzed and compared to the mixed profile of both mother and fetus in maternal plasma (Fig. 1). The maternal contribution to the fetal haplotype can be (quantitatively) compared to the paternally inherited haplotype (yellow peaks) to determine fetal aneuploidy status. In Fig. 1, each peak is schematically represented as a triangle and represents one haplotype; either only from the mother (e.g. no fetal contribution; white), only the fetus (e.g. the paternally inherited haplotype; only yellow) or a shared haplotype between mother and fetus (white and yellow). In a maternal plasma sample from a mother carrying a euploid fetus, the presence of three different alleles are informative (Figure 1 A and B). There is a unique non-shared maternal haplotype (white), a haplotype that is shared between mother and fetus (white and yellow) and a distinct unique paternally inherited haplotype (yellow). In a euploid fetus the paternally inherited peak p3 is equal to the relative difference between p1 and p2, resulting in $HR = 1$ (2). Both maternal and paternal contribution to the fetal genotype is equal.

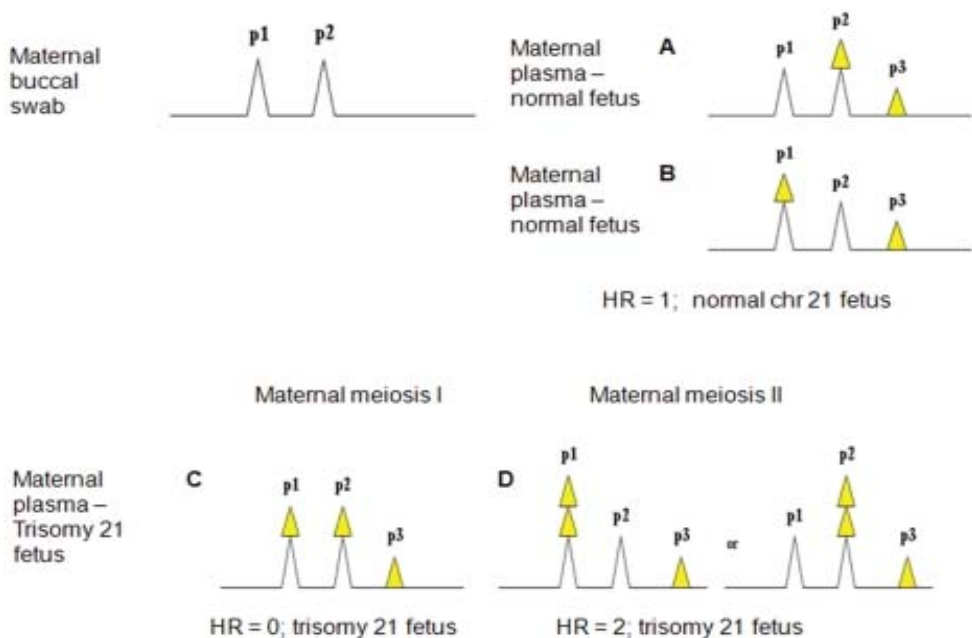


Figure 1: Theoretical CTCE electropherogram output from maternal buccal swab and maternal plasma using tandem SNP analysis. Adapted from (GHANTA et al., 2010).

In case of a plasma sample from a mother carrying a fetus with a trisomy, the CTCE electropherogram shows an uneven contribution between paternal (p3) and maternal haplotypes (p1 and/or p2) of the fetus. Fetal trisomy can be caused either by a familial form (e.g. Robertsonian translocation) or, in the majority of cases, by a meiotic nondisjunction event. A normale gamete (ovum or sperm) has one copy of each chromosome, containing 23 chromosomes in total (n). With nondisjunction, chromosomes fail to separate normally, resulting in a gain or loss of a chromosome in a gamete.

$$(2) \quad HR = \frac{(|p1 - p2|)}{p3}$$

Nondisjunction can occur both in mitosis and meiosis. Failure of sister chromatids to separate during mitosis may lead to mosaicism. Failure of a pair of homologue chromosomes to separate in meiosis I (i.e. primary nondisjunction) will result in both members of this homologues pair to be present into the same daughter cell (**Fig. 2 left**) resulting in a fertilized egg with an abnormal number of chromosomes (i.e. aneuploidy). Failure of the sister chromatids to separate during meiosis II (i.e. secondary nondisjunction) will result in both daughter chromosomes going into the same gamete, also resulting in an abnormal number of chromosome in the fertilized cell (**Fig. 2 right**).

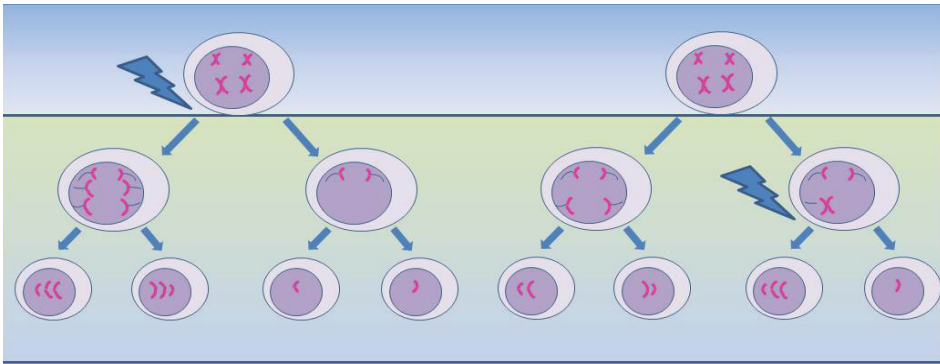


Figure 2: Nondisjunction occurring at meiosis I (left) and meiosis II (right). Nondisjunction in meiosis I will lead to two gametes with an extra chromosome (n+1) and two gametes with a missing chromosome (n-1). After fertilization this will result in a trisomy or monosomy respectively. Nondisjunction in meiosis II will lead to two normal gametes (n) and two abnormal gametes with either an extra chromosome (n+1) or missing one chromosome (n-1).

In case of fetal trisomy, the CTCE plots can also provide information about whether primary or secondary nondisjunction has occurred. When both maternal haplotypes (p1 and p2) are shared in the fetus, nondisjunction occurred during maternal meiosis I. The HR value for maternal meiosis I equals 0 (p1 = p2; therefore the relative difference between p1 and p2 = 0) (Figure 1 C). With secondary maternal nondisjunction, three alleles with different areas are reported whereas p1 or p2 is equal to twice the area of p3, resulting in HR = 2 (Figure 1 D). Adapted from (GHANTA *et al.*, 2010)

In addition to previous described methods used for fetal aneuploidy detection, also digital PCR can be used for fetal aneuploidy detection to determine the relative overrepresentation of a chromosome by calculating relative chromosome dosage (RCD). With RCD the total copy number of a chromosome is assessed in a sample to determine whether this chromosome is overrepresented when compared to a reference chromosome. However, the use of digital PCR is not only restricted to fetal aneuploidy detection in NIPT. Similar to RCD, the principle of digital relative mutation dosage (RMD) can also be applied to NIPD of monogenic diseases. With digital RMD it is no longer required to test only for paternally inherited mutations or fetal sequences that are different (e.g. methylated *RASSF1A*) or absent (e.g. *SRY*) in the mother. With RMD it is possible to compare and measure relative amounts of both the maternal mutant (M) and wild type alleles (N) in maternal plasma to determine the inherited dosage of the mutant allele by the fetus (**Fig. 3**). Therefore, it is no longer necessary to distin-

guish between fetal and maternal sequences. Digital RMD, performed by digital PCR, determines whether the M or N alleles are in balance in maternal plasma (**Fig. 3**). When a pregnant woman and her fetus are both heterozygous for a certain mutation, the amounts of the M allele and N allele are in balance ($M=N$). When the fetus is homozygous for the mutation, there will be an over-representation of the mutant allele ($M>N$). When the fetus is wild type, there will be an under-representation of the mutant allele ($M<N$) in the RMD. Adapted from (CHIU *et al.*, 2009; LUN *et al.*, 2008b).

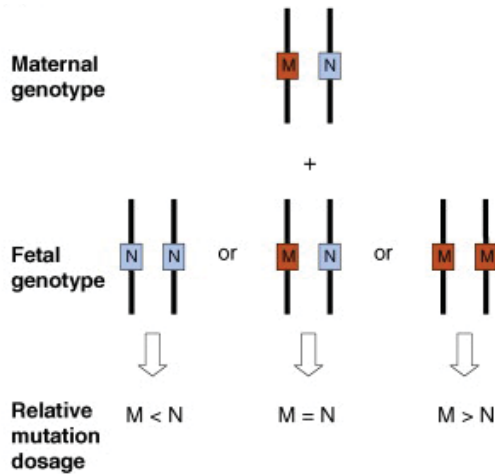


Figure 3: Relative mutation dosage of mutant (M) and normal (N) wild type alleles. Adapted from (CHIU *et al.*, 2009)

Appendix 3: Calculations for trisomy detection

Relative sequence tag density (RSTD):

Shotgun sequencing of numerous of short cfDNA sequences produced after massive parallel sequencing (MPS) are mapped to the chromosome of origin. For each chromosome, these short sequence fragments or reads are counted and summed. In addition, the median of these summed reads from all the autosomes was calculated. To correct for input, for each sample the sum of the reads per chromosome is normalized by dividing this value by the median of all autosomes; the sequence tag density (STD). Male plasma samples or maternal plasma samples from women carrying male euploid fetuses can be used as a reference. STD was also calculated for the controls or reference samples, by first calculating the average of summed tags per chromosome from all reference samples. Subsequently, the median is determined over the values of the autosomes. The average of summed tags per chromosome is normalized by dividing this number by the median value of the autosomes (3). Relative sequence tag density (RSTD) can be determined for each sample by calculating ratios between normalized value per chromosome from each maternal plasma sample and normalized value per chromosome for the controls (4) (Adapted from (FAN *et al.*, 2008).

By determining these ratios, the over- or underrepresentation of any chromosome in maternal plasma contributed by an aneuploidy fetus can be detected. This method does not

require the differentiation between maternal and fetal sequence tags. When a woman carries a healthy fetus, both mother and fetus have 2 copies of each autosome. The RSTD between the normalized value for each autosome as compared to the normalized value of the controls is therefore ~1.

$$(3) \quad \text{Normalized chr } N = \frac{\sum \text{tags chr } N}{\text{median autosomes}}$$

$$(4) \quad RSTD = \frac{\text{Normalized chr } N_{\text{sample}}}{\text{Normalized chr } N_{\text{controls}}}$$

A fetus with a trisomy (e.g. trisomy 21) has an additional copy of chromosome 21 as compared to mother. Theoretically, the RSTD of chromosome 21 between mother and this affected fetus would be 1.5 (e.g. the mother has 2 copies of chromosome 21 while the fetus has 3 copies). However, in maternal plasma, the fetal contribution to cfDNA in maternal plasma is only ~10% in the first trimester. Therefore, the RSTD for a plasma sample from a fetus with trisomy 21 is expected to be between 1 and 1.5 due to the relatively small contribution of the additional fetal chromosome 21 as compared to the maternal background (**Table 2**).

Sample	Fetal DNA content	↑ chr 21 (21)	↑ chr 21 (fold)
CVS	100 %	50 %	1.5
Maternal plasma	~10 %	5 %	1.05

Table 2: Theoretical example of fold increase of chromosome 21 in fetal trisomy 21 in gDNA from chorionic villus sampling (CVS) and maternal plasma.

Z-scores:

In fetal trisomy detection, the Z-score refers to the number of standard deviations that the percentage of reads from a particular chromosome in a test sample differs from the mean % of that particular chromosome in a reference data set. Such reference set contains plasma samples from pregnancies of women carrying euploid fetuses. The advantage of a reference set and reference values is that they have to be established only once for a certain run setting. It is therefore no longer required to run control samples together with unknown samples.

For Z-score calculations, first the % representation of unique sequences mapped to a chromosome is calculated by dividing the number of unique count for chromosome N (chr N) by the total counts from that sample (5). Subsequently, the difference between % chr N (x) and the mean % of chr N (μ) in the reference set is determined and divided by the standard deviation (SD; σ) of the % chr N in the reference set to determine the Z score for chromosome N (6). With this Z-score, disease status of the fetus is determined by looking at the overrepresentation of a certain chromosome. For example, a maternal plasma sample with a % chr 21 that is > 3 SD from the mean of the % chr 21 of the euploid reference set is considered to be a fetal trisomy 21 (Adapted from (CHIU *et al.*, 2008) and ISPD preconference NGS Course, 2012).

$$(5) \quad \% \text{ chr } N = \frac{\text{Unique count for chr } N}{\text{Total unique count}}$$

$$(6) \quad \text{chr } N \text{ Z-score for test sample} = \frac{\% \text{ chr } N_{\text{sample}} - \text{mean } \% \text{ chr } N_{\text{reference}}}{SD \% \text{ chr } N_{\text{reference}}}$$

Or
$$Z = \frac{x - \mu}{\sigma}$$

NCV:

Normalized chromosome value (NCV) calculations are based on correction for the intrarun and interrune sequencing variation in the chromosomal distribution of sequence reads. These variations may obscure the effects of fetal aneuploidy on the distribution of mapped sequence sites. For NCV calculations, a chromosome ratio is calculated (7), in which the count of mapped sites for the chromosome of interest is normalized to counts of another predetermined chromosome (or set of chromosomes) of the same sample (8); Modified from (SEHNERT *et al.*, 2011).

Sehnert *et al.* used a training set, consisting of pregnancies with unaffected fetuses. For each chromosomes of interest (e.g. chr 13, 18, and 21) they determined the denominator chromosome that minimized the variation of the chromosome ratios within and between the runs (**Table 3**). They also used the training set to determine parameters and boundaries for sample classification (i.e.. mean, SD and NCV classification). An NCV > 4.0 classifies the chromosome as affected (i.e. aneuploidy for that chromosome). An NCV < 2.5 classifies the chromosome as unaffected. Samples with an NCV between 2.5 and 4.0 were classified as “no call”. Similar to Z-score calculations, this method does not require additional control sample to be sequenced together with maternal plasma samples when all parameters and boundaries have been established. Adapted and modified from (SEHNERT *et al.*, 2011).

$$(7) \quad \text{ratio chr } N = \frac{\# \text{ reads}_{\text{numerator}}}{\# \text{ reads}_{\text{denominator}}}$$

$$(8) \quad \text{NCV chr } N = \frac{\text{ratio chr } N - \text{mean ratio}_{\text{trainingset}}}{SD \text{ ratio}_{\text{trainingset}}}$$

Chromosome of interest	Numerator (chromosome mapped sites)	Denominator (chromosome mapped sites)
21	21	9
18	18	8
13	13	Sum (2-6)

Table 3: Numerator and denominator combinations for noninvasive trisomy detection.

Note: Determination of appropriate control groups and calculation methods that have been addressed in Appendix 3 were used for fetal aneuploidy detection as described in chapters 3 and 4.

