20. Spaapen RM, Groen RW, van den Oudenalder K, et al. Eradication of medullary multiple myeloma by CD4+ cytotoxic human T lymphocytes directed at a single minor histocompatibility antigen. Clin Cancer Res. 2010; 16: 5481-5488.
21. Spaapen R, van den Oudenalder K, Ivanov R, et al. Rebuilding human leukocyte antigen class II-restricted minor histocompatibility antigen specificity in recall antigen-specific T cells by adoptive T cell receptor transfer: implications for adoptive immunotherapy. Clin Cancer Res. 2007; 13: 4009-4015.
22. Mutis T, van Rijn RS, Simonetti ER, et al. Human regulatory T cells control xenogeneic graft-versus-host disease induced by autologous T cells in RAG2-/-gammac-/- immunodeficient mice. Clin Cancer Res. 2006; 12: 5520-5525.
23. van de Donk NW, Kamps S, Mutis T, Lokhorst HM. Monoclonal antibody-based therapy as a new treatment strategy in multiple myeloma. Leukemia. 2011.
24. de Weers M, Tai YT, van der Veer MS, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. J Immunol. 2011; 186: 1840-1848.
25. van der Veer MS, de Weers M, van Kessel B, et al. Towards effective immunotherapy of myeloma: enhanced elimination of myeloma cells by combination of lenalidomide with the human CD38 monoclonal antibody daratumumab. Haematologica. 2011; 96: 284-290.

Ned Tijdschr Klin Chem Labgeneesk 2012; 37: 41-43

Placental DNA as a source for clinical diagnostics and a cause of maternal diseases

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The placenta is essential for embryonic growth and development, informative for genetic disorders of the fetus and a cause of maternal diseases. Here we describe the latest accomplishments in the use of placental DNA in maternal plasma as a source for non-invasive prenatal diagnostics of fetal genetic diseases, i.e. trisomy 21 and placental DNA as the cause of prevalent maternal diseases, i.e. pre-eclampsia.

The placenta as source for clinical diagnostics: from invasive to non-invasive, from indirect to direct, from focal to genome-wide and from protein to DNA

The genetic material (DNA sequence) retained in placental cells is identical to the genetic blueprint of the fetus and as such used routinely for the diagnosis of aneuploidy and other chromosomal anomalies of the fetus. However, to obtain placental cells for this clinical purpose invasive procedures like chorionic villus sampling are needed (1). These procedures carry a risk of miscarriage due to the procedure. Non-invasive methods with the same sensitivity and specificity were considered urgently wanted, both by patients and doctors. The landmark discovery in 1997 that placental DNA is present in maternal plasma from early pregnancy onwards in quantities that permit reliable and amenable molecular analysis reactivated the search for the holy grail: non-invasive prenatal diagnosis (NIPD) of common fetal genetic diseases (trisomy 21, 13 and 18) using placental i.e. fetal DNA in maternal plasma (2, 3). Pregnant plasma contains packages of released cellular particles (exosomes) of the placenta, nicely packed and thereby protected that contain all the genetic material (DNA and RNA) of the child, are present in large quantities in the plasma and can be analyzed by modern DNA methods for their contents. The clinical breakthrough came two years ago with the introduction of the second generation DNA sequencers and the vision, intelligence and fund raising power of Dennis Lo. It had been proven by Dennis Lo and Rossa Chiu that this test permitted diagnostics of Down's syndrome (trisomy 21). We, our colleague Kypros Nicolaides in London and Dennis Lo in Hong Kong had sufficient sample numbers to perform the ultimate test in 2010 performed in Hong Kong. In a study of about 800 pregnant women containing 90 proven cases of trisomy 21 the presence or absence of Down's syndrome was diagnosed with near perfect accuracy (2).

The principle is as follows. During early pregnancy (week 9-13) blood is withdrawn from the mother by a standard procedure. The blood is processed with removal of the cells by means of centrifugation. In the remaining fraction (plasma) the total amount of DNA is subsequently isolated. This DNA originates from the particles (exosomes) and contains maternal (90%) and fetal (10%) DNA. The ends of the DNA are polished, glued to pieces of synthetic DNA that subsequently, by a combination of enzymes and DNA fragments complementary to the glued DNA, are recognized and transcribed leading to the simultaneous million fold amplification of the original DNA. All these DNA fragments are then immobilized on a car-

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rier and during a couple of days in a so called Massively Parallel Sequencer (MPS) copied, during which process the content is read. The end result is a large encyclopedia containing the genetic information of the starting material written in 4 letters, A, C, G and T. With common wisdom, a powerful computer and a couple of computer whizzkids all pages are ordered, compared with reference books, filtered for spelling mistakes after which the reading can start. This reading is nothing else then checking if, of the pages that contain the information of for example chromosome 21, additional pages are present. By this, with near perfect accuracy the presence or absence of a child with Down's syndrome can be diagnosed non-invasively (NIPD) with a simple blood test. No Down's syndrome case was missed, in 3 out of 1000 cases we incorrectly predicted the presence of one. We already know that this test is suitable and accurate for the diagnosis of the other two common trisomies, trisomy 13 and 18 (3). Together, these genetic anomalies account for the majority of genetic diseases in the unborn child. The implications are enormous, the test can (and has to) replace the current first trimester combination test. From a predictive one, the combination test only provides a probability calculation, the test has become a diagnostic one. The test is non-invasive (blood test), can be applied to all women in first trimester, yields direct information, is independent of the sex of the child or race of the parents, does not recognize signals from previous pregnancies, can be automated and will become rapidly cost-effective comparable to the first trimester combination test by the rapid progression in the development of DNA sequencers. Alert readers will have noticed that Dennis Lo in his articles refers to 'Massively Parallel Sequencing' (MPS) as 'Maternal Plasma Sequencing' (MPS) when applied on pregnant plasma. He has all rights to do so, his article is the first in his kind that fulfilled the high expectations of MPS in large scale validation studies.

The principle is applicable to all other situations where strange or changed DNA is released into the circulation. Consider tumor cells or fragments thereof that are shed from tumor cells and circulate in bodily fluids. Regarding trisomies we were faced with a relative easy task. We looked for the additional presence of a complete chromosome. In cancer patients, these are tiny changes. However, it is only a matter of time that this will become possible as well: finding the smaller needles in the haystack. This also holds for other genetic anomalies of the child. Smaller anomalies are not yet detectable but it will only be a matter of years to overcome this hurdle as well.

Much unnecessary agony will be prevented. Reduction of the number of invasive tests (chorionic villous sampling and amnion punction) performed unnecessarily. No uncertainty and fear of the pregnant female and difficult discussions with the doctor about the interpretation of the test as with the first trimester combination test. But will this lead to an increase in costs for health care? Not at all, on the contrary. More reliable? Yes. Safer? Indeed. Less cases of death? Surely. Will one or more tests become replaced? You bet. It should be noted that in the Netherlands prenatal screening is organized uniquely with an organization of 8 regional centers affiliated with the Academic Centers and regulated by law.

The fetal origin of maternal genetic disease: from multifactorial to multigenic, from late to early and from mother to child

During the development of the early placenta this fetal tissue directly contacts the tissue of the maternal uterus in the beginning of pregnancy. In the first weeks of pregnancy, these cells *walk* towards the blood vessels of the mother. These blood vessels, called spiral arteries, supply the growing embryo with nutrients throughout pregnancy. These placental cells, -they are cells originating from the child but located outside of the child, hence called extra-embryonic- induce by means of a bizarre and intriguing process with similarities of tumor growth, transplantation reactions, coagulation processes and hormone production essential changes in the maternal blood vessels. The inside layer is partially destroyed, hence widened, the vessels change from narrow to broad and become partially disconnected from maternal control. 'The child takes over control from the mother for the sake of its own blood supply'. The purpose is clear and essential: a connection is being established between the circulation of the child and the mother through the placenta.

This process takes place in all pregnancies but fails in 5-10% of all pregnancies. The placental cells stop walking and only partially fulfill their task. The effect, the necessary physiological changes in the vessels take place partially, the vessel remains narrow, the supply of oxygen and nutrients to the growing child becomes limited and the child at a certain stage alerts the mother: 'It is not so comfortable in your belly, mom!'

The placenta responds by the release of stress molecules. The mother reacts by increasing her blood pressure (hypertension), but becomes decompensated. The support to her child has limitations. Her vessels (endothelial activation) and kidneys (proteinuria) become damaged. It is only at that moment, much too late, weeks or months after the start of the problem, that the doctor tells the pregnant women: 'You have pre-eclampsia'. This is the paradox of pre-eclampsia. Late clinical symptoms of the mother, an early causative begin in the child, the placenta. 'The fetal origin of maternal disease.'

We consistently applied this point of view ('Explore the early stages of pregnancy, explore the placenta') in our quest for the cause of pre-eclampsia ('We won't deviate from our hypothesis until proven otherwise'). We were helped enormously by the unique collection of DNA samples collected between 1995-1997 with much effort by Guus Lachmeijer, currently working at the Department of Clinical Genetics. She tracked down a large set of families (150) with pre-eclampsia, with in each family two affected sisters or cousins, collected DNA from the patients and parents and scrutinized the clinical details. It is known that certain forms of pre-eclampsia in particular those that start in early pregnancy with growth retardation of the child runs in families. This means the cause is genetic.

We did one thing differently. In our genetic screen we not only compared the sharing of markers between the affected sisters, but also how they were transmitted to the children born from the affected pregnancies.
This is the way genetic linkage analysis is done. One compares the variations that we all have, investigate them on all chromosomes and subsequently pinpoint those variations that are identical between affected sisters in the same family and how these are transmitted.
We immediately noticed one thing. The father appeared unimportant (4, 5). In the chromosomal region where the gene had to be located only those genes inherited from the mother appeared transmitted to the affected child. This phenomenon is known as 'parent-of-origin effect' and in the development of the unborn child not uncommon, in fact essential, and is known as 'genomic imprinting'. However, we were faced with a new problem but found an escape.
A pregnancy exists known as molar pregnancy. In the Netherlands uncommon, but frequent in Asian countries like Japan and Indonesia. It originates because a (female) oocyte, that lacks, for unknown reasons, a nucleus becomes fertilized by a (paternal) sperm cell that duplicates. The effect: the genetic composition is completely paternal. A pregnancy develops but only consisting of a placenta without an embryo. We used this tissue for our search for the gene responsible for the cause of pre-eclampsia. If the gene was transmitted by the mother only, it would not be present in the molar placenta. This appeared to be the case. It became easy after that. Twelve candidate genes as little toddlers on the fence, eleven fell down, one remained. This was the *STOX1* gene (6).
The *STOX1* gene codes for a transcription factor. The protein it produces binds as an on- or off-switch to other genes and thereby controls whether these are activated or silenced. The finger that controls the light switch. The number of genes involved can be hundreds or thousands. More like the hand that controls the main switch. By a mistake in the readout of the STOX1 protein, as occurring in the Dutch females with pre-eclampsia we found that the binding of this protein to other genes becomes disturbed with their function changed.
Additional research by Marie van Dijk during her sabbatical at the Samuel Lunenfeld Institute in Toronto showed that one of the effects of the changed STOX1 protein is through a cell adhesion molecule on the walking placental cells. It prematurely stops walking. As a consequence, no changes in the necessary adaptations of the maternal blood vessels, the response of the mother with a final presentation of the clinical syndrome with high blood pressure and proteinuria (7-11). However, we are not there yet, we still lack a second factor. STOX1 is essential but not sufficient to cause pre-eclampsia.
The chromosomal area that contained the pre-eclampsia susceptibility gene contained a number of genetic marks that had been described for late onset Alzheimer's' disease as well. Remarkable, why should there be a connection with a disease so remote in symptoms, patients and affected organ? It then appeared the expression of the *STOX1* gene in brain tissue was very high. Coincidence? We decided to explore this in detail. The *STOX1* gene appeared to be overexpressed in the areas of the brain that are affected early in late-onset Alzheimer and to control a conserved pathway with an effect on a small protein essential in the etiology of Alzheimer, the amyloid protein (12-13).

References

1. Go ATJI, van Vugt JMG, Oudejans CBM. Non-invasive aneuploidy detection using free fetal DNA and RNA in maternal plasma: recent progress and future possibilities. Hum Reprod Update. 2011; 17: 372-382.
2. Chiu RWK, Akolekar R, Zheng YWL, et al. Noninvasive prenatal diagnosis of trisomy 21 by multiplexed maternal plasma DNA sequencing. Brit Med J. 2011; 342: c7401.
3. Chen EZ, Chiu RWK, Sun H, et al. 2011. Noninvasive prenatal diagnosis of fetal trisomy 18 and trisomy 13 by maternal plasma DNA sequencing. PloS One. 2011; 6: e21791.
4. Oudejans CBM, Mulders J, Lachmeijer AMA, et al. The parent-of-origin effect of 10q22 in preeclamptic females coincides with two regions clustered for genes with down-regulated expression in androgenetic placentas. Mol Hum Reprod. 2004; 10: 589-598.
5. van Dijk, M, Mulders J, Könst A, Janssens, B, van Roy, F, Blankenstein M, Oudejans C. Differential downregulation of alpha-T-catenin expression in placenta: trophoblast cell type-dependent imprinting of the CTNNA3 gene. Gene Expr Patterns 2004; 5: 61-65.
6. van Dijk M, Mulders J, Poutsma A, Könst AAM, Lachmeijer AMA, Dekker GA, Blankenstein MA, Oudejans CBM. Maternal segregation of the Dutch preeclampsia locus at 10q22 with a novel member of the winged helix gene family. Nat Genet. 2005; 37: 514-519.
7. Oudejans CBM, van Dijk M, Oosterkamp MJ, Lachmeijer AMA, Blankenstein MA. Genetics of preeclampsia: paradigm shifts. Hum Genet. 2007; 120: 607-612.
8. Oudejans CBM, van Dijk M. Placental gene expression and pre-eclampsia. Placenta. 2008; 9S: 78-82.
9. van Dijk M, van Bezu J, van Abel D, Dunk C, Blankenstein MA, Oudejans CBM, ye SJ. The STOX1 genotype associated with pre-eclampsia leads to a reduction of trophoblast invasion by αT-catenin upregulation. Hum Mol Genet. 2010; 19: 2658-2667.
10. van Dijk M, Drewlo S, Oudejans CBM. Differential methylation of STOX1 in human placenta. Epigenetics. 2010; 5: 736-742.
11. van Dijk M, Oudejans CBM. STOX1: key player in trophoblast dysfunction underlying early onset pre-eclampsia with growth retardation. J Pregn 2011, article ID 521826 doi 10.1155/2011/521826
12. van Dijk M, van Bezu J, Poutsma A, Veerhuis R, Rozemuller AJM, Scheper W, Blankenstein MA, Oudejans CBM. The pre-eclampsia susceptibility gene STOX1 controls a conserved pathway shared between placenta and brain with overexpression in late-onset Alzheimer disease. J Alzheimers Dis. 2010; 19: 673-679.
13. van Abel D, Hölzel D, Jain S, et al. SFRS7-mediated splicing of tau exon 10 is directly regulated by STOX1A in glial cells. PLoS One. 2011; 6: e21994.