

Noninvasive prenatal screening and diagnosis

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Addendum: valorization

This addendum describes the **valorization** of the relevant findings from the research upon which this doctoral dissertation is based. This means that I translate these findings into available products for the society. The word “products” should be interpreted in a broad sense in this context, ranging from novel noninvasive prenatal diagnostic laboratory tests (**Part I**), to knowledge aiding the national debate and decision-making concerning noninvasive prenatal screening (**Part II**).

Valorization of Part I: Design of noninvasive prenatal diagnostic tests

Part I comprised the design of in-house noninvasive diagnostic tests for fetal sex determination as well as for single-gene mutation detection (**Chapters 2 and 3**).

A **noninvasive fetal sex determination assay** was developed and tested in 75 pregnant women from 9-34 weeks of gestation (**Chapter 2**). Noninvasive fetal sex determination in cell-free fetal DNA (cfDNA) in maternal blood is only available for a small group of pregnant women with a clinical indication. These are women carrying a fetus that presents with ambiguous genitalia during ultrasound examination, women carrying a fetus that is at risk of an X-linked disorder, or a fetus at risk of the autosomal recessive disorder congenital adrenal hyperplasia, where masculinization of the external genitalia of girls occurs.¹⁻³ The fetal sex was correctly determined in all 75 pregnant women without failure or false results. For clinical application, the assay is competitive opposed to current assays: it is a non-expensive, fast, single-tube assay that can determine the fetal sex within one or two working days, with an equal turnaround time for boys and girls. Up until now the blood from pregnant women with a clinical indication for prenatal diagnosis of fetal sex was sent to Sanquin, the national reference laboratory for noninvasive prenatal sex determination.⁴ In case of a female fetus the test in Sanquin can take more than a week, and sometimes fails to provide a certain result.

After publication of **Chapter 2**, our noninvasive fetal sex determination assay was further validated in 13 pregnant women from 10-12 weeks of gestation (Addendum Table 1).⁵ Comparison of the results of our new assay with the results of invasive prenatal testing revealed that the fetal sex was correctly determined in all samples. Before the assay can be offered to pregnant women in the Clinical Genetics department of the Maastricht UMC+, further validation is required, especially early in pregnancy (8-10 weeks of gestation). Currently, approval by the Medical Ethics Committee of the Academic Hospital Maastricht and Maastricht University is requested for this validation in 100 pregnant women. Plasma will be obtained in two

populations: (1) When a sample is sent to Sanquin for fetal sex determination, a second blood sample will be drawn for our new test. The results of the two sex determination assays will be compared. Annually, 5-10 samples are sent to Sanquin for X-linked disorders, and another 5-10 samples for ambiguous genitalia. (2) When blood is drawn for the first trimester fetal aneuploidy screening (combined test), an additional blood sample will be drawn. In these women, follow-up of the pregnancy should be guaranteed, e.g. by 20 weeks ultrasound or postnatal information, so that the fetal sex can be compared with the result of our assay.

Following implementation our new noninvasive fetal sex determination assay in the Clinical Genetics department of the Maastricht UMC+, pregnant women are provided a more robust and fast test, and the department can send an invoice to the health care insurers. In the future, after gaining some more experience and confidence conducting the test, our laboratory may even opt to establish itself as an additional national reference laboratory for noninvasive fetal sex determination.

Addendum Table 1 Further validation of the one-tube noninvasive prenatal sex determination assay

Sample		Noninvasive prenatal sex determination			Invasive testing
#	Gestational age (weeks + days)	<i>CSH</i> cfRNA	<i>CGB</i> cfRNA	<i>AMELY</i> cfDNA	
1	12+0	+	+	+	boy
2	12+1	+	+	+	boy
3	11+4	+	+	+	boy
4	12+4	+	+	+	boy
5	12+2	+	+	-	girl
6	11+2	+	+	+	boy
7	11+2	+	+	-	girl
8	11+2	+	+	-	girl
9	10+6	+	+	+	boy
10	12+3	+	+	+	boy
11	11+2	+	+	-	girl
12	12+3	+	+	-	girl
13	11+4	+	+	-	girl
Sensitivity	100%				
Specificity	100%				

The design of a newly developed in house **NIPD assay for single-gene disorders** was described in **Chapter 3**. Single molecule molecular inversion probes (smMIPs) were designed, together with the Human Genetics department of the Radboud UMC in Nijmegen, for targeted next-generation sequencing (NGS) for NIPD by haplotyping.⁶ We are the first to use smMIPs for NIPD of single-gene disorders. The designed MIPs were optimized for analysis of fetal cfDNA, which is fractionated and present in a low concentration. Adding single molecule tagging to the MIPs allows us to mark sequence reads derived from a common progenitor molecule (that is, the same genomic equivalent in source DNA). This results in counting of unique captured molecules, and tag-based consensus calling to minimize errors from PCR or sequencing. SmMIPs enable precise quantification of the variation in the cfDNA and are robust to relatively small amounts and poor quality of source DNA.

In **Chapter 3** the first proof of concept was described. As a proof of concept, the smMIP NIPD assay was applied to cfDNA isolated from the plasma of a woman carrying a fetus affected with myotonic dystrophy type 1 (DM1), as diagnosed by invasive prenatal testing. DM1 is a single-gene disorder, caused by expansion of a CTG trinucleotide repeat.^{7,8} After sequencing the SNP markers from the pregnant woman, and from the father and a previous child (= reference) with DM1, informative SNPs for the mutated allele of the father and reference were selected. Subsequently, the cfDNA was sequenced to determine the fetal inheritance, calculated from the percentage of the informative SNPs in the cfDNA reads. In the proof of concept, it was demonstrated that the assay could detect the mutated *DMPK* allele, inherited from the father, in the fetal cfDNA.

Validation of the smMIPs NIPD assay is ongoing. Plasma samples are being collected in Maastricht and Nijmegen when a pregnant woman undergoes an invasive procedure for prenatal genetic diagnosis for one of the following indications: (1) a fetus at high risk of having inherited a dominant or recessive disorder of his/her affected parent(s), or (2) a fetus at risk of having a *de novo* disorder on the basis of ultrasound findings. Annually, 40-60 couples are referred for prenatal diagnosis of a single-gene disorder, both in Maastricht and Nijmegen. In anticipation of this study, smMIP assays have already been designed for three other single-gene disorders:

- autosomal dominant: spastic paraplegia 4 (SPG4, gene: *SPAST*)
- autosomal recessive: cystic fibrosis (CF, gene: *CFTR*)
- autosomal dominant, caused by a trinucleotide repeat expansion: Huntington's disease (HD, gene: *HTT*)

For SPG4 and CF, smMIPs were developed to cover all exons and to target intronic and gene surrounding common SNPs (combined direct and indirect mutation detection by SNP-based haplotyping). For HD, as for DM1, smMIPs were designed to target common repeat flanking SNPs (only SNP-based haplotyping).

Because of the unique smMIPs design, especially adapted to fetal cfDNA testing, the first steps for the development of an international competitive NIPD assay were taken. We are heading towards an in-house NIPD for paternally and maternally inherited disorders, even X-linked disorders and repeat expansion disorders. By combining the unique knowledge of the Radboud UMC regarding next-generation sequencing, as well as of the Maastricht UMC+ regarding direct and indirect detection of all types of genetic disorders in pre-implantation genetic diagnosis, translation of the newly developed assay into prenatal diagnostic tests for pregnant women is realistic.

Valorization of Part II: Noninvasive prenatal screening for fetal chromosome disorders

In **Part II** the diagnostic accuracy and clinical implementation of trisomy 21 screening by NIPT were examined (**Chapters 4 and 5**). Additionally, the opinion of pregnant women regarding the inclusion of fetal sex trisomies in the NIPT panel was explored, within the broader context of the expansion of the scope of screening (**Chapter 6**).

In 2007, in the Netherlands a national prenatal screening program was introduced aiming to provide all pregnant women the option of prenatal screening for trisomies 13, 18 and 21 with the first trimester combined test (FCT).⁹ The FCT combines a serum screening test with a nuchal translucency measurement in the first trimester of pregnancy. Traditionally, women with a risk of fetal trisomies 13, 18 or 21 of 1 in 200 or higher after the FCT were offered an invasive procedure to further investigate these fetal chromosome disorders.

In 2011, at the time I started the research upon which this doctoral dissertation is based, the first diagnostic accuracy studies were published of noninvasive prenatal testing (NIPT) for fetal trisomies 13, 18 and 21 in maternal plasma.¹⁰ In **Chapter 4** a systematic review was conducted and an overview was provided of all studies evaluating the diagnostic accuracy of NIPT for trisomy 21 between 1997 and the beginning of 2012.¹⁰ We concluded that the positive predictive value of NIPT (How likely is a pregnant woman with a positive cfDNA-based test result to actually carry a fetus with the trisomy?) declines if the *a priori* risk for a fetus with a trisomy declines.

NIPT requires a confirmation by a follow-up diagnostic test, for a definite diagnosis of fetal aneuploidy in the fetus. We predicted that NIPT was likely to replace the prenatal serum screening test that is currently combined with nuchal translucency measurement in the FCT. Due to the reduction in false-positive and false-negative results in comparison to the FCT, fewer trisomy cases would be missed at the first screening step and fewer invasive procedures would be needed, only to verify a positive NIPT result and to confirm non-inheritable or inheritable forms of Down syndrome, using the gold standard karyotyping. The results of our study were among others cited by the *Nederlandse Vereniging voor Obsetrie en Gynaecologie* (NVOG) in their opinion about NIPT in May 2013¹¹, and in the *Wet op het bevolkingsonderzoek niet-invasieve prenatale test bij verhoogd risico op trisomie* in December 2013, from the Health Council of the Netherlands Health Council, an advisory board of the Dutch Ministry of Health.¹²

The first commercial releases of NIPT were seen in Hong Kong in August 2011, and in the United States (US) in October 2011.¹³ Different companies offered commercial NIPT to high-risk pregnant women, such as the MaterniT21™ PLUS test from Sequenom (<http://www.sequenom.com>), the Praena-Test® from their European partner LifeCodexx (<http://www.lifecodexx.com>), the verifi® test from Verinata (<http://www.verinata.com>) and the Harmony™ prenatal test from Ariosa (<http://www.ariosadx.com>). During a 3 year-period after the first commercial release, the news about the option for NIPT had reached Dutch pregnant women through the internet, traditional media as well as family and friends. However, for them, it was not possible to undergo NIPT in The Netherlands. Following the publication of **Chapter 4**, we were approached for an interview for the newspaper NRC, in which we explained to pregnant women the principle of the NIPT, and when we expected the test to be available in the Netherlands.¹⁴

Since April 2014, a national implementation study has been organized in the Netherlands (the TRIDENT study).¹⁵ In this study, the genetic laboratories of the country collaborate to investigate the accuracy of NIPT and compare it to the published accuracy. Prenatal screening for trisomies 13, 18 and 21 by NIPT is offered as a part of the TRIDENT-1 study to women with an increased risk based on the FCT ($\geq 1:200$), or based on their personal history. Also in Maastricht UMC+, pregnant women can undergo NIPT as a part of the TRIDENT-1 study. Recently, the Health Council, an advisory board of the Dutch Ministry of Health, advised to offer all pregnant women the choice for NIPT as the first screening test.¹⁶ In September 2016, the Minister of

Health gave permission to start with the TRIDENT-2 study in April 2017. In the TRIDENT-2 study women can choose between three different scenarios: NIPT as the first screening test, the FCT as the first and only screening test, and pre-selection by the FCT prior to NIPT.¹⁵ At the moment, the Minister does not follow the suggestion of the Health Council that the NT measurement could be offered to pregnant women that only undergo NIPT. The university hospitals are allowed to offer these scenarios under the following conditions: adequate counseling about the advantages and disadvantages of these three scenarios, as well as counseling about incidental findings and the possibility to opt-out for incidental findings, scientific study of the impact of analysis filters (that preclude incidental findings) on the quality of NIPT, protecting the women's right not to know and avoiding routinization offer of NIPT.

In **Chapter 5** different hypothetical NIPT implementation strategies were compared for a national screening program. Decision trees were created to illustrate all plausible alternatives in a theoretical cohort of 100 000 pregnant women in five screening programs: classical screening by the first-trimester combined test (FCT), pre-selection of high-risk women prior to NIPT by the FCT, NIPT as the first screening test at 10 weeks and at 13 weeks, and the simultaneous conductance of NIPT and the FCT. We reflected upon the results of the quantitative analysis in the light of psychological and practical considerations, e.g. the chance that women are correctly reassured by a negative screening result, the chance of a correct positive screening result, the time until the result of the screening test, the time from the result of the screening test until the confirmation of a positive result by amniocentesis, the number of decision-making moments for women, etc. The results of **Chapter 5** were presented in the European Human Genetics Conference (31 May- 3 June 2014, Milan, Italy). The results may be used for to inform pregnant couples about the advantages and disadvantages of the three programs of TRIDENT-2.

In **Chapter 6**, we assessed pregnant women's opinions about NIPT for sex chromosome trisomies (SCTs) within the broader context of the expansion of disorders included in the NIPT panel. Individual semi-structured interviews were conducted with eight pregnant women. The results of our study revealed several topics to consider when offering NIPT for SCTs or expanding the NIPT panel. Our study identified several potential disadvantages of NIPT for SCTs: receiving a prenatal test result about a disorder with unclear consequences for the future child's life and for which they would take no further actions, a less worry-free pregnancy and childhood, and possible guilt about continuation of pregnancy. Two participants mentioned the option of

termination of pregnancy as a benefit of screening, while others firmly stated that they disagreed with offering pregnant women the possibility to terminate the pregnancy because of a fetal SCT. Furthermore, the limited positive predictive value was perceived as a negative aspect of NIPT for SCTs. In concordance with previous studies, not all participants agreed to undergo confirmatory invasive testing for these conditions during pregnancy, because of its additional miscarriage risk. All participants requested to be well-informed about every condition in the NIPT panel prior to testing and brought up that participation and all further steps in the screening process should be voluntary and based on adequate information. The overall opinion was that couples wanted to choose for themselves which disorders in the NIPT panel they wished to screen for, and which conditions they wanted to confirm by invasive testing. Hopefully, also this Chapter will have societal impact, contributing to the discussion about how NIPT should be offered to pregnant couples, and which conditions should be in the NIPT panel.

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