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# Innovations in Down Syndrome Screening

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## 1. Introduction

Down syndrome (DS) is the most common chromosomal abnormality, with an incidence of approximately 1 per 500 to 800 live births (Egan et al., 2004). DS is associated with an impairment of cognitive ability and physical growth, and a particular set of facial characteristics. Moreover, about 50% of all people with DS suffer from a congenital heart defect and DS patients are more prone to develop serious illnesses such as Alzheimer's disease, leukaemia and epilepsy. These factors all contribute to a shorter life expectancy.

For decades, people developed methods to prenatally diagnose DS. In this chapter an overview is given of non-invasive screening methods for DS. The research described in this chapter was performed at the Dutch National Institute for Public Health and the Environment (RIVM). The RIVM acts as the reference laboratory for DS screening in the Netherlands and processes over 10,000 first-trimester combined tests per year. The RIVM therefore possesses an extensive collection of sera of pregnant women carrying a foetus with DS and other congenital abnormalities. For our scientific studies, serum samples from this large database were used. The aim of our research was to identify new biochemical screening markers using proteomics techniques to improve the performance of the current DS screening.

## 2. Down syndrome screening

### 2.1 A historical perspective of Down syndrome

An accurate phenotypic description of Down syndrome (DS) was published by John Langdon Down in 1866 (Down, 1866). Following descriptions of Esquirol and Séguin (Esquirol, 1838, Séguin, 1846), who wrote about phenotypic differences between mentally retarded humans, Down was the first to make the distinction between the phenotype which is now called DS and other disorders. He made this distinction based on an ethnic classification in which he discerned four types; the Ethiopian type, the Malay type, the American type and the Mongolian type (Down, 1866). Down noticed that the 'mongolism' occurred in more than 10% of all mentally retarded children and that it was always congenital.

At the end of the 19<sup>th</sup> century, the principle of inheritance was explained by the discovery of chromosomes in living organisms. In 1909, Morgan and colleagues began to study the

chromosomes of *Drosophila* (fruit flies), which were very suitable for genetic studies because they bred quickly and only have four chromosomes. During their experiments it was, among others, discovered that occasionally *Drosophila* possessed three sex chromosomes instead of two showing a pattern of XXY or XYY, an abnormality which they called 'trisomy' (Morgan et al., 1925, Morgan et al., 1915). Since this trisomy occurred when two copies of a chromosome failed to disjoin properly, it was described as non-disjunction.

Somewhat later, in the 1930s, two researchers independently linked non-disjunction to DS. Waardenburg stated that, due to the extended clinical features of humans with DS, the syndrome might very well be caused by something as complicated as a chromosomal disorder (Waardenburg, 1932). Bleyer proposed that DS occurs with fertilization or has already occurred before, during the period of maturation of the ovum or spermatozoon (Bleyer, 1934). Therefore, he thought that a chromosomal abnormality such as non-disjunction was most likely to cause DS.

Finally, in 1959, a few years after it had been established that human tissues normally contain 46 chromosomes, Lejeune and Jacobs independently discovered the presence of an extra chromosome in children with DS (Jacobs et al., 1959, Lejeune et al., 1959). Lejeune suggested, principally based on the *Drosophila* research, that the presence of an extra chromosome could well be explained in terms of non-disjunction. As individual chromosomes were identified, it appeared that the extra chromosome in DS was always the 21<sup>st</sup> chromosome. Therefore, DS was since then referred to as trisomy 21.

## 2.2 Prenatal screening for Down syndrome

The discovery of a trisomy of chromosome 21 as the underlying cause for DS and the possibility to perform a chromosome analysis on amniotic fluid allowed for the prenatal diagnosis of DS (Valenti et al., 1968).

In 1966 the first chromosome analysis of amniotic fluid was performed (Steele and Breg, 1966). This development allowed for the prenatal detection of DS, which was first achieved in 1968 (Valenti et al., 1968). The relationship between the risk of having a child with DS and advanced maternal age had been known for a long time (Penrose, 1933, Shuttleworth, 1909). A statistical estimation of this relationship is shown in figure 1. Because of an increased risk of DS, in many countries women above a certain age (usually above 35-38 years) were offered prenatal diagnosis by means of amniocentesis.

In 1972 it was discovered that very high levels of alpha fetoprotein (AFP) were present in the amniotic fluid of women carrying a child with a neural tube defect (NTD) (Brock and Sutcliffe, 1972). Two years later the association between high AFP levels and NTD was also seen in second trimester maternal serum samples (Brock et al., 1974, Wald et al., 1974), allowing for a non-invasive screening method for NTD (Wald et al., 1977). Again a few years later, in 1984, it was found that, in contrast to the elevated AFP levels in NTD pregnancies, decreased maternal serum levels of AFP in the second trimester of pregnancy could be linked to DS (Cuckle et al., 1984, Merkatz et al., 1984). This meant that prenatal screening for NTD could be extended with the screening for DS. This way, women of advanced maternal age could now be offered a screening test before opting for an invasive amniocentesis that bears a certain risk of miscarriage (Eddleman et al., 2006, Odibo et al., 2008).

The discovery of AFP as a second trimester screening marker for DS triggered researchers to look for other potential screening markers to even further improve the prenatal detection by screening. In 1987, two new screening markers were presented. Maternal serum levels of

human chorion gonadotropin (hCG) were shown to be, on average, higher in DS pregnancies (Bogart et al., 1987) while levels of unconjugated estriol ( $uE_3$ ) were mostly decreased in DS (Canick et al., 1988). A year later, Wald and colleagues reported on a new method of screening using the three biochemical markers (AFP, hCG and  $uE_3$ ) together with maternal age as parameters in a single test (Wald et al., 1988). This test became known as the 'triple test'. With the triple test 60% of all DS cases could be prenatally detected at a 5% false positive rate (FPR) (Wald et al., 1988), which was a significant improvement compared to the detection of the previous screening method based on maternal age and AFP only (Cuckle et al., 1984). The triple test became increasingly popular as a screening test for DS and started to be carried out routinely in several countries. The most optimal cut-off risk for the screening was calculated to be 1 in 250 (Baumgarten, 1985). During the early 1990s, the triple test was adjusted by the replacement of hCG with the free beta subunit of hCG ( $f\beta$ -hCG) (Macri et al., 1990, Ryall et al., 1992). Moreover, in 1996, inhibin-A was found to contribute to the current triple test (Wald et al., 1996) and with the addition of inhibin-A the 'quadruple test' was conceived.

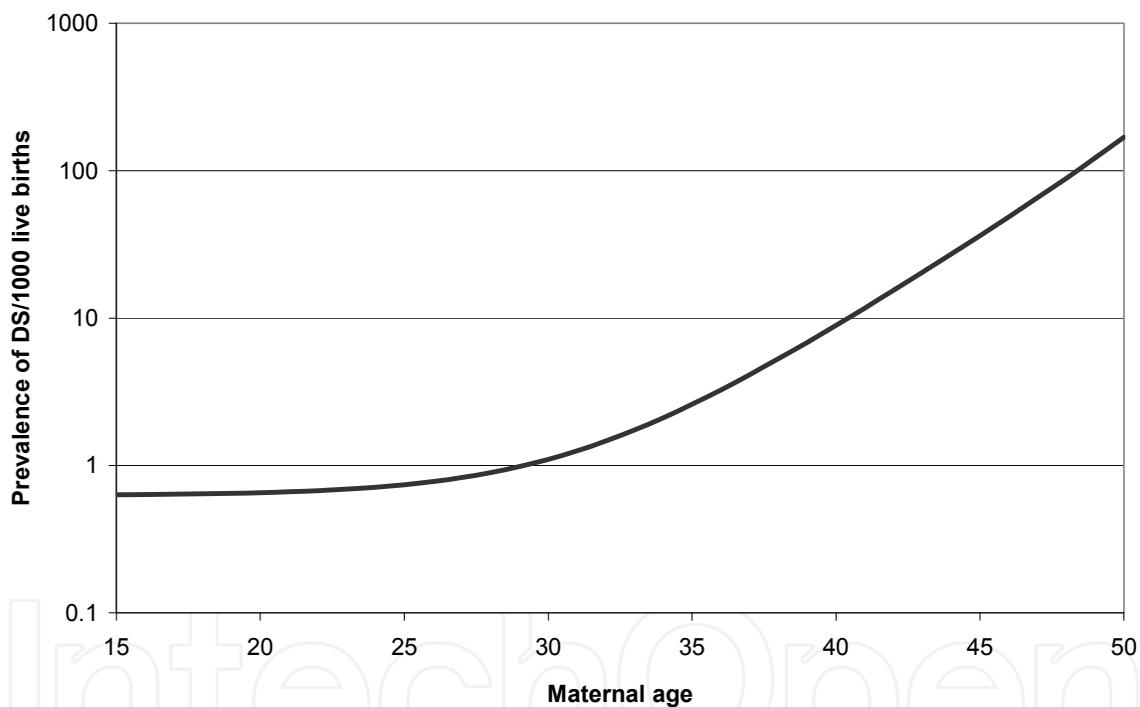


Fig. 1. The relationship between maternal age and the risk of having a child with Down syndrome based on data from Cuckle *et al.* (Cuckle et al., 1987).

In the meantime the focus of prenatal screening for DS shifted more towards the first trimester of pregnancy. This development was in part due to the applicability of chorionic villus sampling, a technique that allows for karyotyping already in the first trimester. Thus, it became possible to detect DS earlier in pregnancy, what subsequently allowed for earlier termination of pregnancy. On the other hand, first trimester screening would not include screening for NTD. However, advanced ultrasound techniques were developed promising high detection rates for NTD in the second trimester.

Except for  $f\beta$ -hCG (Spencer et al., 1992), the parameters in the current triple test did not perform well in the distinction between DS and euploid pregnancies in the first trimester.

So, to come up with a proper test, new first trimester screening markers were necessary. In 1991, it was found that maternal serum pregnancy associated plasma protein-A (PAPP-A) was reduced about 50% in DS pregnancies (Brambati et al., 1993). Besides PAPP-A, more potential markers were studied (e.g. SP1 (Kornman et al., 1998) and CA125 (Van Lith et al., 1993)), but none of those turned out to be worth adding to the screening test. The search for DS screening markers was not limited to biochemical markers; an enlarged nuchal translucency (NT) on a first trimester ultrasound scan also turned out to be predictive for DS (Nicolaidis et al., 1992, Pandya et al., 1994). Combining these three screening markers ( $\beta$ -hCG, PAPP-A and NT) with maternal age, using a risk calculation method similar to that of the triple test, originated the 'first trimester combined test' (Wald and Hackshaw, 1997). Over the years, numerous studies have been published showing that with the first trimester combined test approximately 85-90% of all DS cases could be detected at a 5% FPR (Jaques et al., 2007, Nicolaidis et al., 2005, Spencer and Nicolaidis, 2003, Valinen et al., 2007, Wojdemann et al., 2005).

Under strict guidelines issued by the Dutch Centre for Population Research the first-trimester screening policy for DS was fully implemented in the Netherlands as of January 1, 2007. Since then, all pregnant women are offered such prenatal screening for DS, but the uptake of the test is only 23% (Schielen et al., 2008), which is rather low as compared to other countries. The detection rate (DR) of DS screening in the Netherlands is currently 76% (Wortelboer et al., 2009a).

### **3. Proteomics techniques to identify new screening markers for Down syndrome**

The development of methods for DS screening has so far mainly been based on coincidences. The screening really is a spin-off of the neural tube defect (NTD) screening, and the most effective markers were discovered by fishing expeditions, not by thorough analysis of the causal relationship of genes on chromosome 21 and foetal or placental proteins that are likely to cause an excess or shortage in maternal serum as a result.

A proteome is the entire complement of proteins including the modifications made to a particular set of proteins, produced by an organism or system. Proteomics is the field of research that aims at examination of the proteome in a certain tissue, cell type or body fluid at a certain time point. A plethora of emerging methodological tools allows for the study of proteins, e.g. their quantity, cellular location and post-translational modifications. Understanding the proteome, the structure and function of each protein, and the complexities of protein-interactions during a DS pregnancy may help in the search for additional biomarkers for current first-trimester DS screening.

Our proteomics research consists of three phases (figure 2): i) the discovery of new biomarkers for first-trimester DS screening, ii) the feasibility and validation of proteomics techniques to analyze multiple markers simultaneously, iii) the implementation of a cost-effective assay for large-scale screening programmes.

The presence of an extra chromosome in DS not only leads to anomalies of the foetus, but also of the placenta. In human trophoblast cells, the excess of oxygen radicals produced during oxygen metabolism are eliminated by natural antioxidants and superoxide dismutase (SOD). The gene responsible for this reaction is Zn-SOD and is encoded by chromosome 21. SOD expression and protein levels and activity are significantly higher (about 50%) in trophoblast cells from DS placentas (Pidoux et al., 2004). Over-expression of

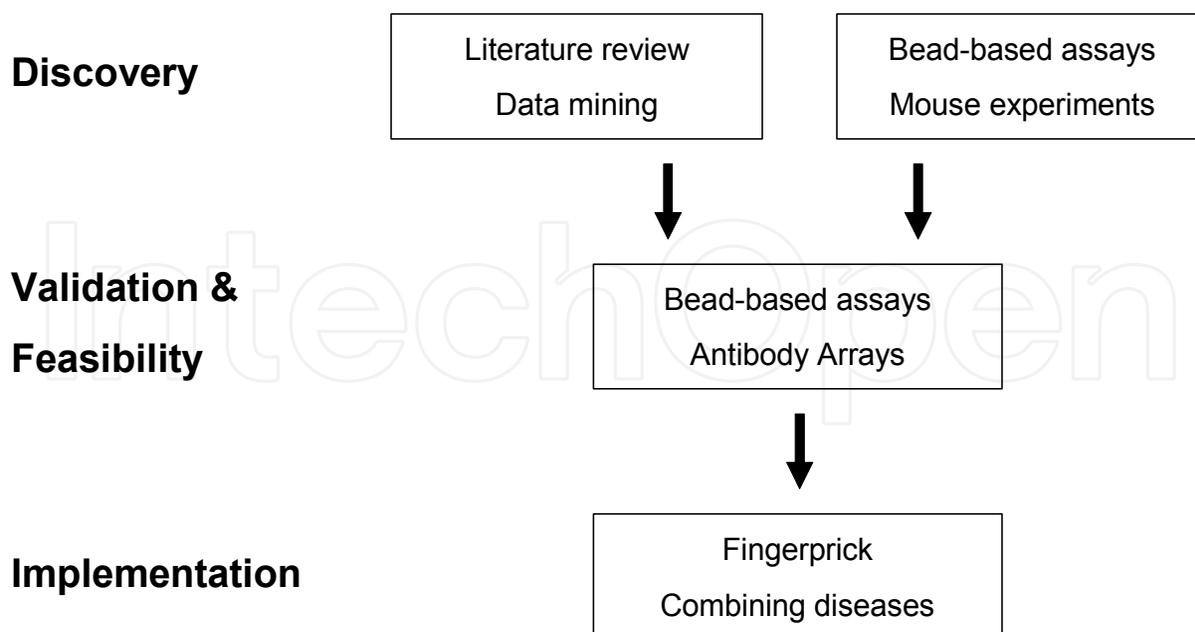


Fig. 2. Three phases of proteomics research for DS screening described in this chapter.

SOD hampers normal trophoblast formation; DS trophoblast cells cannot fully compensate for the reduced oxidative stress resulting in placental abnormalities. DS placentas show signs of impaired differentiation, aggregation and fusion of their trophoblast cells. This could lead to undervascularisation, hypotrophy and cell apoptosis of the placenta already in the first trimester of pregnancy (Koster et al., 2010a). As a result of these pathological changes deregulation and/or differential expression occurs for proteins, e.g. cytokines and growth factors, involved in implantation and placental development (Bromage et al., 2000, Vesce et al., 2002). This may cause an increased or decreased placental expression of biological markers (hormones and proteins). PAPP-A and  $f\beta$ -hCG, currently used as DS screening markers in the first-trimester combined test, are such markers. PAPP-A is a protein which is thought to be an important regulator of IGF bioavailability and cell growth (Giudice et al., 2002) and  $f\beta$ -hCG is a subunit of total hCG, which is the most important hormone involved in early pregnancy and provides for the maintenance of the corpus luteum and of pregnancy (Stenman et al., 2006). Based on this knowledge the differential expression of other placenta derived proteins, given that it is traceable in maternal blood, could be used in the search for new screening markers.

In the discovery phase of our research, an extensive review of the literature was carried out to study normal placental development and function during early pregnancy (Koster et al., 2010a). Using this knowledge, candidate biomarkers were proposed which may be useful in screening for DS. Current screening markers for DS indeed mainly originate from the placenta, but can also be traced to the foetal liver, e.g. alpha-fetoprotein (AFP). It is therefore hypothesized that new screening markers may also originate from these tissues. However, a prerequisite of a good screening marker is that concentrations of a protein are detectable in maternal serum. The amount of information on genes and proteins in public databases is increasing rapidly, which allows for a bioinformatics approach that involves automated collection and combination of information from biological databases, known as data mining. A bioinformatics approach was developed to use data from the literature on genes and

protein expression and data-textmining tools. This way, a list of 49 potential DS screening markers was generated (Pennings et al., 2009). The list included three biomarkers that are already used for DS screening (AFP, f $\beta$ -hCG and PAPP-A) and several others, among which proteins that have been examined as potential biomarkers before. Furthermore, there was a large overlap between the proposed screening markers based on the literature review and the data mining (table 1).

Biomarker discovery research within our proteomics project also included the use of mouse-models for biomarker identification (Pennings et al., 2011). Breeding healthy female mice and male transgenic mice with DS (type Ts(16C-tel)1Cje; The Jackson Laboratory, Bar Harbor, ME, USA) produces healthy females pregnant with, on average, 50% DS embryos. Blood was drawn from the pregnant mice during the first trimester, for the identification of potential screening markers in maternal serum. Then, the pregnancy was terminated and the placenta and foetal organs were collected. Gene profiles were analyzed using a whole genome microarray approach to study the difference between DS and unaffected siblings. Genes that showed over- or underexpression in the placentas of DS fetuses were C2cd2, Dyrk1a, Ifnar2, Morc3, Sfrs15, Sod1, Tmprss2, Fgfbp3. Ongoing research focuses on the serum detectability of these gene products, and their potential as a biomarker for DS screening in human serum.

We continued our search for potential screening markers by examining the proteins that have been suggested as first-trimester screening markers for aneuploidies in international studies. One of those markers is placental protein 13 (PP13) which plays an important role in the implantation and modelling of foetal-maternal blood spaces between placenta and endometrium. PP13 is produced by the placenta, which is hampered in trisomic pregnancies, and was found to be decreased in DS pregnancies and, to greater extent, in trisomy 18 and 13 pregnancies (Akolekar et al., 2010, Koster et al., 2009b). Serum concentrations of a disintegrin and metalloprotease 12 (ADAM12) and placental growth factor (PlGF) are also decreased in DS pregnancies (Wortelboer et al., 2009b, Zaragoza et al., 2009). Total hCG (thCG), which is a screening marker for DS in the second trimester of pregnancy, is increased in maternal serum from first-trimester DS pregnancies (Hallahan et al., 2000). However, when these four markers were added to the current first-trimester combined test algorithm the DR increased by only 3% (table 2a) (Koster et al., 2010d).

These studies show that the predictive power of maternal serum markers is not constant during the first trimester. For three markers (PAPP-A, ADAM12 and PP13) the difference between DS and unaffected pregnancies is more distinct early in the first trimester (before 11 weeks), while for the remaining markers (f $\beta$ -hCG, thCG and PlGF) the difference is more pronounced later on (after 11 weeks) (Kuc et al., 2010). Based on this knowledge, it would be useful to draw two separate blood samples (a so-called two-sample combined test) to increase the DR of first-trimester screening to almost 90% at a 5% FPR, which is obviously a tremendous improvement compared to the DR of the current screening program (table 2b). On the other hand, adding new markers to the screening test and taking an extra blood sample bears extra costs and complicates the logistic process of first-trimester screening. A cost-effectiveness analysis is therefore necessary to evaluate the potential of such a two-sample first-trimester screening setting.

A more experimental proteomics approach was carried out by analyzing 90 different proteins from a pre-existing non-pregnancy-specific bead-based multiplexed immunoassay. By comparing the protein concentrations in a small cohort of DS and control sera, seven

Marker	Description	Function	Potential
ADAM12	ADAM metallopeptidase domain 12	* Involved in proteolysis, adhesion, fusion and intracellular signalling * Interacts with IGF binding proteins	biomarker
EGF	Epidermal growth factor ( $\beta$ -urogastrone)	* Promotes differentiation and prevents apoptosis in trophoblasts * Involved in trophoblast invasion and proliferation	examined
hCG	Chorionic gonadotropin	* Glycoprotein hormone that consists of a common $\alpha$ in use subunit and a unique $\beta$ subunit * Stimulates the ovaries to synthesize steroids to maintain pregnancy * Involved in trophoblast differentiation and cell aggregation	
hPL	Chorionic somatomammotropin hormone 1 (placental lactogen)	* Member of the somatotropin/prolactin family that is expressed mainly in the placenta * Plays an important role in growth control	biomarker
IGF-1	Insulin-like growth factor 1	* Regulates placental growth and transport, trophoblast invasion and placental angiogenesis	examined
IGF-2	Insulin-like growth factor 2	* Has large effects on cell proliferation and differentiation	examined
IGFBP-1	Insulin-like growth factor binding protein 1	* Bind both IGF I and II * Restrict trophoblast invasion	examined
IGFBP-2	Insulin-like growth factor binding protein 2	* Stimulate trophoblast cell migration and invasion	examined
IGFBP-3	Insulin-like growth factor binding protein 3	* Regulate IGF bioavailability and cell growth	examined
IGFBP-4	Insulin-like growth factor binding protein 4		unknown
IGFBP-5	Insulin-like growth factor binding protein 5		unknown
IGFBP-6	Insulin-like growth factor binding protein 6		unknown
IGFBP-7	Insulin-like growth factor binding protein 7		unknown
Leptin	Leptin	* Involved in angiogenesis, growth and immunomodulation * Regulation of foetal and uterine metabolism	examined
MMP-2	Matrix metallopeptidase 2 (gelatinase A)	* Involved in extracellular matrix degeneration in embryonic development, reproduction and tissue remodelling * Play a role in endometrial menstrual breakdown, regulation of vascularization and the inflammatory response	unknown
MMP-9	Matrix metallopeptidase 9 (gelatinase B)	* Regulate several growth factors and cytokines	unknown
PAPP-A	Pregnancy-associated plasma protein A	* Cleaves IGFBPs * Important regulator of IGF bioavailability and cell growth	in use
PGH	Placenta-specific growth hormone	* Member of the somatotropin/prolactin family that is expressed mainly in the placenta * Plays an important role in growth control. * Has a key role in the control of IGF-1 levels	biomarker
PLGF	Placental growth factor	* Mainly involved in angiogenesis * Has an autocrine function in regulating trophoblast function	biomarker
SOD-1	Superoxide dismutase 1	* Binds copper and zinc ions * Responsible for catalyzing free superoxide radicals	examined
TGF- $\beta$	Transforming growth factor $\beta$ 1	* Inhibits cytotrophoblast migration * Decreases trophoblast proliferation * Increases formation of placental giant cells * Regulates many other growth factors	unknown
TIMP-1	Tissue inhibitor of matrix metallopeptidase 1	* Natural inhibitors of MMPs * Promote cell proliferation * Anti-apoptotic function * Suppress endothelial proliferation	unknown
TIMP-2	Tissue inhibitor of matrix metallopeptidase 2	* Maintain tissue homeostasis	unknown
TIMP-3	Tissue inhibitor of matrix metallopeptidase 3	* Regulate platelet aggregation and recruitment	unknown
TIMP-4	Tissue inhibitor of matrix metallopeptidase 4	* Play a role in hormonal regulation and endometrial tissue remodelling	unknown
VEGF	Vascular endothelial growth factor	* Mediates vascular permeability * Induces angiogenesis, vasculogenesis and endothelial cell proliferation	examined

Table 1. Early biomarkers involved in placental development according to a literature study and data-mining. Potential for DS screening is indicated as follows: In use, currently widely used in DS screening; Biomarker, studies showed overall significant concentrations; Examined, examined as biomarker but not significant or inconclusive overall results.

A	One-sample test		DR at FPR		
	(8-13 wks)		5%	3%	1%
	<i>PAPP-A &amp; fβ-hCG</i>		77	71	59
	+ ADAM12		77	72	60
	+ thCG		77	71	60
	+ PP13		77	71	60
	+ PIGF		78	73	61
	<b>+ ADAM12, thCG, PP13 &amp; PIGF</b>		<b>80</b>	<b>74</b>	<b>63</b>
	+ ADAM12, thCG & PIGF		79	74	62
	+ ADAM12 & PIGF		79	73	62

B	Two-sample test		DR at FPR		
	1 <sup>st</sup> (8-10 wks)	2 <sup>nd</sup> (11-13 wks)	5%	3%	1%
	<i>PAPP-A</i>	<i>fβ-hCG</i>	83	79	68
	+ ADAM12		84	80	69
		+ thCG	85	80	70
	+ PP13		84	79	69
		+ PIGF	85	80	69
	<b>+ ADAM12 &amp; PP13</b>	<b>+ thCG &amp; PIGF</b>	<b>89</b>	<b>85</b>	<b>75</b>
	+ ADAM12	+ thCG & PLGF	88	84	74
	+ ADAM12	+ PIGF	85	81	70

Table 2. Modeled detection rates (DR) at given false positive rates (FPR) for NT at 11-13 weeks and several serum marker combinations in a one-sample (A) or two-sample test (B). Models containing all markers are displayed in bold.

potential screening markers were identified (Koster et al., 2009a): alpha fetoprotein (AFP), epidermal growth factor (EGF), extracellular rase binding protein (EN-RAGE), eotaxin, haptoglobin (HP), insulin (INS) and lipoprotein A (LPA). None of the identified proteins is linked to genes located on chromosome 21. However, some of the markers are known to be highly expressed in the placenta or foetal liver and were also proposed in the candidate biomarker lists from the previously described discovery studies. Unfortunately, none of the seven identified single markers showed significant differences between cases and controls. It might be that biomarkers with large distinctive power were not present on the immunoassay or, alternatively, that fold changes are inherently not high in maternal blood. Interestingly, the addition of the whole panel of seven biomarkers to the current screening test provided a significant improvement of the detection rate for DS.

Despite these promising results, it is obvious that test performance is always better when a screening test is applied to the same cases from which the markers are derived and therefore application of the proposed markers on a different cohort of cases is essential to establish the

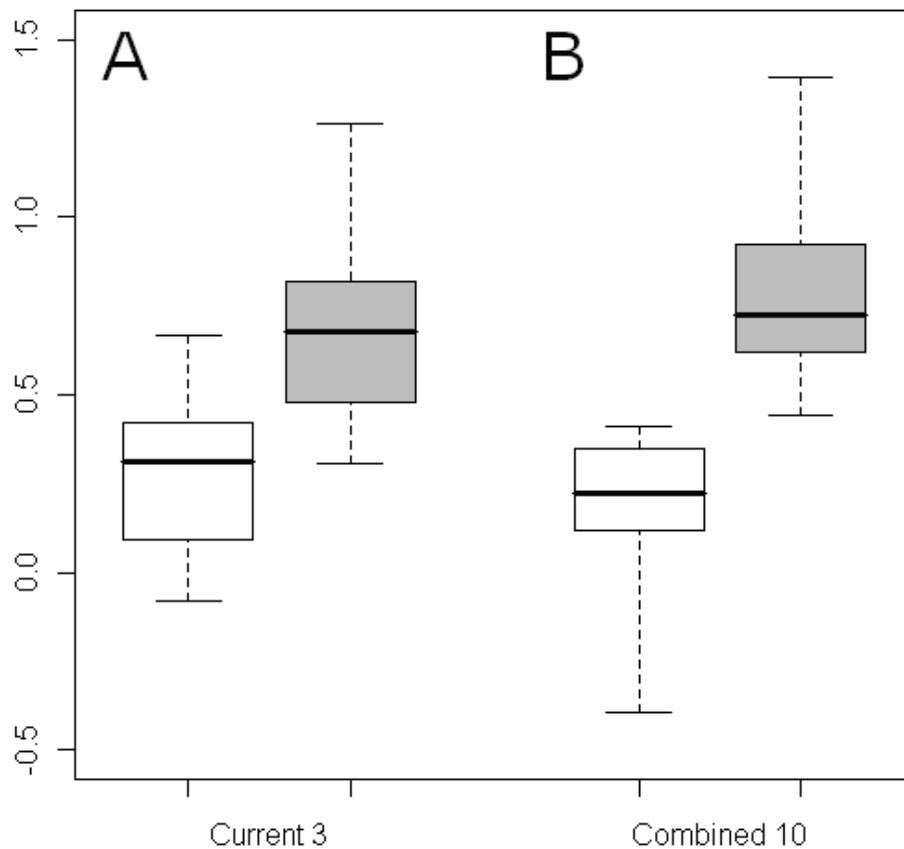


Fig. 2. Boxplots showing the distinction between Down syndrome cases (grey) and the controls (white) by plotting the median, quartiles and minimum/maximum values. (A) Difference between cases and controls when the three current screening markers (PAPP-A,  $\beta$ -hCG and NT) are used, (B) Difference between cases and controls when the current three screening markers are combined with the seven newly identified potential biomarkers (AFP, EGF, EN-RAGE, Eotaxin, HP, INS and LPA). Values along the vertical axis indicate prediction scores expressed as arbitrary units.

true diagnostic accuracy of the immunoassay. This was done in a subsequent validation study in which 34 DS cases and matching controls were included to confirm the predictive value of the seven markers found in the discovery study. EGF and EN-RAGE were confirmed to be potential screening markers for DS and improved the DR of the current first-trimester combined test with approximately 6% (table 3) (Koster et al., 2010b). This may seem rather disappointing considering the initial identification of seven potential markers. On the other hand, the finding that two markers again improved the DS screening performance in an independent study is highly encouraging. Clearly, large scale validation experiments need to be performed to provide sufficient evidence for potential markers before they can be implemented in a screening test.

#### 4. Screening for Down syndrome using cell-free foetal DNA/RNA in maternal blood

Currently, not only knowledge to do in-depth evaluations based on proteomic techniques is available, but more and more research focuses on the genomic detection of DS in maternal

markers in the model	DR at a 5% FPR			
	discovery fit + discovery data	discovery fit + validation data	validation fit + discovery data	validation fit + validation data
current screening (PAPP-A, f $\beta$ -hCG, NT)	56.2	65.2	39.7	64.0
PAPP-A+ f $\beta$ -hCG	38.9	57.1	32.7	57.6
current+AFP	<b>58.9</b>	64.1	<b>40.6</b>	64.0
current+EGF	<b>62.6</b>	<b>67.1</b>	<b>51.7</b>	<b>68.0</b>
current+EN-RAGE	<b>58.7</b>	<b>68.4</b>	<b>47.7</b>	<b>68.1</b>
current+Eotaxin	<b>61.1</b>	55.8	<b>40.7</b>	63.8
current+Haptoglobin	<b>61.8</b>	62.3	36.1	<b>64.2</b>
current+Insulin	<b>59.7</b>	65.2	37.4	63.7
current+LPA	<b>61.0</b>	65.0	<b>51.6</b>	63.4
10 markers (current + 7 new)	<b>82.5</b>	42.4	<b>59.2</b>	<b>71.5</b>
current+EGF+EN-RAGE	<b>62.1</b>	<b>70.1</b>	<b>53.8</b>	<b>71.4</b>

Table 3. Modeled detection rates (DR) at a given 5% false positive rate (FPR) for several marker combinations. Models were fitted based on the data of the discovery study or based on the validation study and tested on both datasets. DRs displayed in bold indicate an improvement compared to the current screening model.

blood. We feel therefore that we should devote a paragraph on the potential value of this technique and its possible role within the screening process. One line of research concerning DS screening tries to put high-end sequencing quantification techniques for DNA and RNA into use to quantify foetal DNA or RNA, either in nuclei of foetal cells or free-floating in maternal serum (Lo, 2000). Non-invasive foetal genotyping became feasible when, foetal DNA was found to constitute approximately 10% of the DNA circulating in the mother's plasma (Lo et al., 1998). However, determination of the maternally inherited alleles was a considerable challenge because the foetal contribution to the plasma DNA can only result in subtle shifts to the total allelic balance, rather than providing a novel allele.

Currently, the most promising technique is so-called massive parallel genomic sequencing. This technique can identify and quantify many DNA fragments in a relatively short time span (Chiu et al., 2008, Fan et al., 2008). Recently, a large-scale study in a high risk cohort was conducted to investigate the performance of massive parallel genomic sequencing in terms of detection and false positive rates. In this study, all DS pregnancies could be identified at a 2.1% false positive rate (Chiu et al., 2011). The authors claim that if such a test would be carried out in all women who initially had a high risk pregnancy (based on first trimester screening), only very few women would need a referral for an invasive diagnostic procedure such as amniocentesis. However, it is unclear how the test would perform in a more representative low-risk pregnant population.

With this promising non-invasive technique, it seems possible to provide definite identification of DS. However, there are still limitations to the technique and some reputable experts in the field have expressed doubts concerning these developments (Community Corner Nature Medicine, 2011). This line of research has been going on for over ten years now; the major technical challenges of sequencing foetal DNA from maternal blood may have been largely solved, but now the practical issues raised by applying this technology need to be addressed. For a diagnostic test, a false positive rate of 2.1% is unacceptable, since it would lead to termination of pregnancy in an equal percentage unaffected pregnancies. For a screening test on the other hand, the technique is still too expensive and time-

consuming. And furthermore, if this technique keeps evolving it might even become possible to prenatally offer whole genome sequencing, which obviously raises a complex set of social and ethical issues.

So we feel that, although it is likely that, somewhere in the near future, massive parallel genomic sequencing will be used as an intermediate step in the prenatal detection of DS to decrease the number of invasive procedures such as amniocentesis, the technique is still a long way from implementation in a high-throughput screening setting.

## 5. Future perspectives and ongoing research

The research described in this chapter focuses on the directive search for new markers for DS using the ever expanding knowledge of the human genome and proteome and combines both laboratory techniques and digital evaluation of data (data mining). The current first trimester combined test is based on enzyme-linked immunosorbent assay (ELISA) methods, which is widely used for quantitative protein measurements. Recently, two-dimensional gel electrophoresis (2-D), tandem mass spectrometry (MS-MS) and bead-based multiplexed immunoassays have been used to identify several potential biomarkers in amniotic fluid and maternal blood (Busch et al., 2005, Kolialexi et al., 2008, Nagalla et al., 2007, Tsangaris et al., 2006), clearly demonstrating the potential of applying proteomics techniques in the quest for new biomarkers. Bead-based multiplexed immunoassays use color-coded tiny beads in up to 100 distinct sets. Coating each bead set with a specific reagent allows the capture and detection of many specific analytes, such as proteins, from a sample. Next, labeled beads are incubated with serum samples and, subsequently, with a detection antibody labeled with a reporter dye in a bead-based immunoassay. Flow cytometry equipment measures the internal dyes to identify each particle and the reporter dye captured during the assay (Krishhan et al., 2009). Another proteomics technique is the use of Antibody microarrays (Ab-arrays). Ab-arrays are a platform for protein expression profiling. Small amounts of capture antibodies for the selected targets are immobilized or spotted on a very small area on coated glass slides. The high density of the capture antibodies in the spots that is obtained enables high sensitivity (Ekins, 1989). These technologies allow analyzing many unique markers within a single sample, both rapidly and precisely, in a high-throughput setting. In the boost of new development the question arises whether these advanced detection techniques will be available at a reasonable cost, a prerequisite for screening tests. In principle however, Ab-array techniques are calculated to cost within the range of 20-50 Euros per screening.

In recent years, the focus of prenatal screening has expanded. Several studies have been performed to evaluate the potential of prenatal screening for foetal chromosomal abnormalities other than DS, in particular Edwards syndrome (trisomy 18) and Patau syndrome (trisomy 13). With the first-trimester combined test it is possible to detect these trisomies using the same algorithm as for DS screening (Spencer et al., 2000, Tul et al., 1999). In trisomy 18 and 13 pregnancies PAPP-A levels are decreased to a greater extent than in DS and the NT is often very large. However, as opposed to DS, serum concentrations of f $\beta$ -hCG are decreased in trisomy 18 and 13 pregnancies. Thus, with a slight adjustment of the DS risk calculation, it would be possible to provide separate risks specifically for trisomy 18 and 13. This would lead to the detection of many trisomy 18 and 13 cases with only 0.2% extra false positives (Koster et al., 2010c).

This spin-off of the current proteomics project may have opened a completely new field of research. Currently, a similar approach has been set up to identify potential screening markers for pregnancy complications such as pre-eclampsia (PE), intrauterine growth restriction and foetal death. PE is a serious complication of pregnancy that affects approximately 1-2% of all pregnant women and it is the leading cause of maternal and perinatal morbidity and mortality (Gaugler-Senden et al., 2006). Because of the serious health consequences of PE, risk assessment for PE is highly recommended. Early identification of women at risk might facilitate better antenatal surveillance, timely intervention and better outcomes.

Especially new proteomic techniques will need only minute amounts of test material; 10-20 micro-litres serum instead of 1-2 ml. Hypothetically, this downscaling opens the possibility to draw small amounts of blood and to replace the relatively laborious venous puncture with a finger prick. Our first studies comparing venous blood with capillary blood (derived from a finger prick), drawn at the same time from pregnant women, indeed show that capillary blood can be reliably used to determine the currently used biomarkers in serum. The laboratory will be able to analyze a combination of approximately ten markers. Based on these developments, it must surely be possible to detect, in the same samples, all parameters of prenatal screening (e.g. irregular blood types and infectious diseases, like HIV and hepatitis) and to identify high risks for foetal (e.g. chromosomal abnormalities) and maternal (e.g. pre-eclampsia) pregnancy complications.

In the next coming years, the outline of such a future prenatal screening is feasible, however; it will probably take some time before these methods can be tested in large cohorts that proof their efficacy as a screening tool, a bare necessity before actual implementation can take place.

## 6. Conclusion

It is anticipated that the introduction of a new screening method consists of a discovery phase, taking 1-3 years, a validation phase, taking 2-5 years, and an implementation phase, taking 5-7 years. This means that of all of the discoveries presented in this paper, which are clearly done in the realm of the discovery phase, very few will make it to becoming an element of an implemented screening test. While we cannot predict what the prenatal screening test for Down syndrome, other aneuploidies and foetal and maternal health will be in ten years time, we can state that it will not be the first trimester combined test. The past decades have learned sufficiently that the screening tests, while not being volatile, are liable to changes, and this will not stop in the next years. It is safe to say that the future test will use the complete array of proteomics, genomics and ultrasound markers, to provide a continuum of tests, with the sole purpose of improving in general the outcome of pregnancies, including the health perspective of the mother, worldwide.

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## 8. References

- Akolekar, R., Perez Penco, J. M., Skyfta, E., Rodriguez Calvo, J. & Nicolaides, K. H. 2010. Maternal serum placental protein 13 at eleven to thirteen weeks in chromosomally abnormal pregnancies. *Fetal Diagn Ther*, 27, 72-7.
- Baumgarten, A. 1985. AFP screening and Down syndrome. *Lancet*, 1, 751.
- Bleyer, A. 1934. Indications that mongoloid imbecility is a gametic mutation of degenerative type. *Amer J Dis Child*, 47, 342-348.
- Bogart, M. H., Pandian, M. R. & Jones, O. W. 1987. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. *Prenat Diagn*, 7, 623-30.
- Brambati, B., Macintosh, M. C., Teisner, B., Maguiness, S., Shrimanker, K., Lanzani, A., Bonacchi, I., Tului, L., Chard, T. & Grudzinskas, J. G. 1993. Low maternal serum levels of pregnancy associated plasma protein A (PAPP-A) in the first trimester in association with abnormal fetal karyotype. *Br J Obstet Gynaecol*, 100, 324-6.
- Brock, D. J., Bolton, A. E. & Scrimgeour, J. B. 1974. Prenatal diagnosis of spina bifida and anencephaly through maternal plasma-alpha-fetoprotein measurement. *Lancet*, 1, 767-9.
- Brock, D. J. & Sutcliffe, R. G. 1972. Alpha-fetoprotein in the antenatal diagnosis of anencephaly and spina bifida. *Lancet*, 2, 197-9.
- Bromage, S. J., Lang, A. K., Atkinson, I. & Searle, R. F. 2000. Abnormal TGFbeta levels in the amniotic fluid of Down syndrome pregnancies. *Am J Reprod Immunol*, 44, 205-10.
- Busch, A., Michel, S., Hoppe, C., Driesch, D., Claussen, U. & Von Eggeling, F. 2005. Proteome analysis of maternal serum samples for trisomy 21 pregnancies using ProteinChip arrays and bioinformatics. *J Histochem Cytochem*, 53, 341-3.
- Canick, J. A., Knight, G. J., Palomaki, G. E., Haddow, J. E., Cuckle, H. S. & Wald, N. J. 1988. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. *Br J Obstet Gynaecol*, 95, 330-3.
- Chiu, R. W., Akolekar, R., Zheng, Y. W., Leung, T. Y., Sun, H., Chan, K. C., Lun, F. M., Go, A. T., Lau, E. T., To, W. W., Leung, W. C., Tang, R. Y., Au-Yeung, S. K., Lam, H., Kung, Y. Y., Zhang, X., Van Vugt, J. M., Minekawa, R., Tang, M. H., Wang, J., Oudejans, C. B., Lau, T. K., Nicolaides, K. H. & Lo, Y. M. 2011. Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study. *Bmj*, 342, c7401.
- Chiu, R. W., Chan, K. C., Gao, Y., Lau, V. Y., Zheng, W., Leung, T. Y., Foo, C. H., Xie, B., Tsui, N. B., Lun, F. M., Zee, B. C., Lau, T. K., Cantor, C. R. & Lo, Y. M. 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, 20458-63.

- Community Corner: Opening the Pandora's box of prenatal genetic testing. *Nat Med*, 17, 250-1.
- Cuckle, H. S., Wald, N. J. & Lindenbaum, R. H. 1984. Maternal serum alpha-fetoprotein measurement: a screening test for Down syndrome. *Lancet*, 1, 926-9.
- Cuckle, H. S., Wald, N. J. & Thompson, S. G. 1987. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br J Obstet Gynaecol*, 94, 387-402.
- Down, J. L. 1866. Observations on an ethnic classification of idiots. *Lond Hosp Clin Lect Rep*, 3, 259-262.
- Eddleman, K. A., Malone, F. D., Sullivan, L., Dukes, K., Berkowitz, R. L., Kharbutli, Y., Porter, T. F., Luthy, D. A., Comstock, C. H., Saade, G. R., Klugman, S., Dugoff, L., Craigo, S. D., Timor-Tritsch, I. E., Carr, S. R., Wolfe, H. M. & D'alton, M. E. 2006. Pregnancy loss rates after midtrimester amniocentesis. *Obstet Gynecol*, 108, 1067-72.
- Egan, J. F., Benn, P. A., Zelop, C. M., Bolnick, A., Gianferrari, E. & Borgida, A. F. 2004. Down syndrome births in the United States from 1989 to 2001. *Am J Obstet Gynecol*, 191, 1044-8.
- Ekins, R. P. 1989. Multi-analyte immunoassay. *J Pharm Biomed Anal*, 7, 155-68.
- Esquirol, J. E. D. 1838. Des maladies mentales considérées sous les rapports médical, hygiénique et médico-légal. *Paris: JB Baillière*.
- Fan, H. C., Blumenfeld, Y. J., Chitkara, U., Hudgins, L. & Quake, S. R. 2008. Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood. *Proc Natl Acad Sci U S A*, 105, 16266-71.
- Gaugler-Senden, I. P., Huijssoon, A. G., Visser, W., Steegers, E. A. & De Groot, C. J. 2006. Maternal and perinatal outcome of preeclampsia with an onset before 24 weeks' gestation. Audit in a tertiary referral center. *Eur J Obstet Gynecol Reprod Biol*, 128, 216-21.
- Giudice, L. C., Conover, C. A., Bale, L., Faessen, G. H., Ilg, K., Sun, I., Imani, B., Suen, L. F., Irwin, J. C., Christiansen, M., Overgaard, M. T. & Oxvig, C. 2002. Identification and regulation of the IGFBP-4 protease and its physiological inhibitor in human trophoblasts and endometrial stroma: evidence for paracrine regulation of IGF-II bioavailability in the placental bed during human implantation. *J Clin Endocrinol Metab*, 87, 2359-66.
- Hallahan, T., Krantz, D., Orlandi, F., Rossi, C., Curcio, P., Macri, S., Larsen, J., Buchanan, P. & Macri, J. 2000. First trimester biochemical screening for Down syndrome: free beta hCG versus intact hCG. *Prenat Diagn*, 20, 785-9;discussion 790-1.
- Jacobs, P. A., Baikie, A. G., Court Brown, W. M. & Strong, J. A. 1959. The somatic chromosomes in Mongolism. *Lancet*, 1, 710.
- Jaques, A. M., Halliday, J. L., Francis, I., Bonacquisti, L., Forbes, R., Cronin, A. & Sheffield, L. J. 2007. Follow up and evaluation of the Victorian first-trimester combined screening programme for Down syndrome and trisomy 18. *BJOG*, 114, 812-8.
- Kolialexi, A., Tsangaris, G. T., Papantoniou, N., Anagnostopoulos, A. K., Vougas, K., Bagiokos, V., Antsaklis, A. & Mavrou, A. 2008. Application of proteomics for the identification of differentially expressed protein markers for Down syndrome in maternal plasma. *Prenat Diagn*, 28, 691-8.

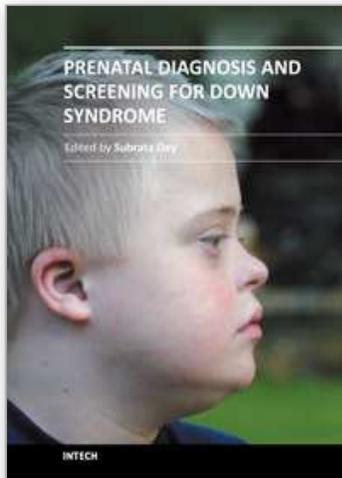
- Kornman, L. H., Morssink, L. P., Ten Hoor, K. A., De Wolf, B. T., Kloosterman, M. D., Beekhuis, J. R. & Mantingh, A. 1998. Schwangerschafts protein 1 (SP1) adds little to the age-related detection of fetal Down syndrome in the first trimester of pregnancy. *Prenat Diagn*, 18, 1086-90.
- Koster, M. P., Heetkamp, K. M., Pennings, J. L., De Vries, A., Visser, G. H. & Schielen, P. C. 2010a. Down syndrome screening: imagining the screening test of the future. *Expert Rev Mol Diagn*, 10, 445-57.
- Koster, M. P., Pennings, J. L., Imholz, S., Rodenburg, W., Visser, G. H., De Vries, A. & Schielen, P. C. 2009a. Bead-based multiplexed immunoassays to identify new biomarkers in maternal serum to improve first trimester Down syndrome screening. *Prenat Diagn*, 29, 857-62.
- Koster, M. P., Pennings, J. L., Imholz, S., Rodenburg, W., Visser, G. H., De Vries, A. & Schielen, P. C. 2010b. Proteomics and Down syndrome screening: a validation study. *Prenat Diagn*, 30, 1039-43.
- Koster, M. P., Stoutenbeek, P., Visser, G. H. & Schielen, P. C. 2010c. Trisomy 18 and 13 screening: consequences for the Dutch Down syndrome screening programme. *Prenat Diagn*, 30, 287-9.
- Koster, M. P., Wortelboer, E. J., Cuckle, H. S., Stoutenbeek, P., Visser, G. H. & Schielen, P. C. 2009b. Placental protein 13 as a first trimester screening marker for aneuploidy. *Prenat Diagn*, 29, 1237-41.
- Koster, M. P., Wortelboer, E. J., Stoutenbeek, P., Visser, G. H. & Schielen, P. C. 2010d. Modeling the Down syndrome screening performance using first trimester serum markers. *Ultrasound Obstet Gynecol*.
- Krishhan, V. V., Khan, I. H. & Luciw, P. A. 2009. Multiplexed microbead immunoassays by flow cytometry for molecular profiling: Basic concepts and proteomics applications. *Crit Rev Biotechnol*, 29, 29-43.
- Kuc, S., Koster, M. P., Visser, G. H. & Schielen, P. C. 2010. Performance of first-trimester serum screening for trisomy 21 before and from 11 + 0 weeks of gestational age in The Netherlands. *Prenat Diagn*, 30, 906-8.
- Lejeune, J., Gautier, M. & Turpin, R. 1959. Étude des chromosomes somatiques de neuf enfants mongoliens. *CR Acad Sci*, 248, 1721-1722.
- Lo, Y. M. 2000. Fetal DNA in maternal plasma. *Ann N Y Acad Sci*, 906, 141-7.
- Lo, Y. M., Tein, M. S., Lau, T. K., Haines, C. J., Leung, T. N., Poon, P. M., Wainscoat, J. S., Johnson, P. J., Chang, A. M. & Hjelm, N. M. 1998. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet*, 62, 768-75.
- Macri, J. N., Kasturi, R. V., Krantz, D. A., Cook, E. J., Moore, N. D., Young, J. A., Romero, K. & Larsen, J. W., Jr. 1990. Maternal serum Down syndrome screening: free beta-protein is a more effective marker than human chorionic gonadotropin. *Am J Obstet Gynecol*, 163, 1248-53.
- Merkatz, I. R., Nitowsky, H. M., Macri, J. N. & Johnson, W. E. 1984. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. *Am J Obstet Gynecol*, 148, 886-94.

- Morgan, T. H., Bridges, C. B. & Sturtevant, A. H. 1925. The genetics of *Drosophila*. *The Hague: M Nijhoff*.
- Morgan, T. H., Sturtevant, A. H., Muller, H. J. & Bridges, C. B. 1915. The mechanism of Mendelian heredity. *London: Constable*, 149-50.
- Nagalla, S. R., Canick, J. A., Jacob, T., Schneider, K. A., Reddy, A. P., Thomas, A., Dasari, S., Lu, X., Lapidus, J. A., Lambert-Messerlian, G. M., Gravett, M. G., Roberts, C. T., Jr., Luthy, D., Malone, F. D. & D'Alton, M. E. 2007. Proteomic analysis of maternal serum in down syndrome: identification of novel protein biomarkers. *J Proteome Res*, 6, 1245-57.
- Nicolaides, K. H., Azar, G., Byrne, D., Mansur, C. & Marks, K. 1992. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ*, 304, 867-9.
- Nicolaides, K. H., Spencer, K., Avgidou, K., Faiola, S. & Falcon, O. 2005. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol*, 25, 221-6.
- Odibo, A. O., Gray, D. L., Dicke, J. M., Stamilio, D. M., Macones, G. A. & Crane, J. P. 2008. Revisiting the fetal loss rate after second-trimester genetic amniocentesis: a single center's 16-year experience. *Obstet Gynecol*, 111, 589-95.
- Pandya, P. P., Brizot, M. L., Kuhn, P., Snijders, R. J. & Nicolaides, K. H. 1994. First-trimester fetal nuchal translucency thickness and risk for trisomies. *Obstet Gynecol*, 84, 420-3.
- Pennings, J. L., Koster, M. P., Rodenburg, W., Schielen, P. C. & De Vries, A. 2009. Discovery of novel serum biomarkers for prenatal Down syndrome screening by integrative data mining. *PLoS One*, 4, e8010.
- Pennings, J.L., Rodenburg, W., Imholz, S., Koster, M.P., van Oostrom, C.T., Breit, T.M., Schielen, P.C. & de Vries, A. Gene expression profiling identifies new liver-derived potential biomarkers for Down syndrome screening. *Plos One* [in press].
- Penrose, L. S. 1933. The relative effects of paternal and maternal age in mongolism. *J Genet*, 27, 219.
- Pidoux, G., Guibourdenche, J., Frenco, J. L., Gerbaud, P., Conti, M., Luton, D., Muller, F. & Evain-Brion, D. 2004. Impact of trisomy 21 on human trophoblast behaviour and hormonal function. *Placenta*, 25 Suppl A, S79-84.
- Ryall, R. G., Staples, A. J., Robertson, E. F. & Pollard, A. C. 1992. Improved performance in a prenatal screening programme for Down's syndrome incorporating serum-free hCG subunit analyses. *Prenat Diagn*, 12, 251-61.
- Schielen, P. C., Koster, M. P., Elvers, L. H. & Loeber, J. G. 2008. First trimester combined test screening for Down's syndrome 2004-2006. *RIVM report 230024002/2008*.
- Séguin, E. 1846. Le traitement moral, l'hygiène et l'éducation des idiots et des autres enfants arrières. *Paris: JB Baillière*.
- Shuttleworth, G. E. 1909. Mongolian imbecility. *Brit Med J*, 2, 661-665.
- Spencer, K., Macri, J. N., Aitken, D. A. & Connor, J. M. 1992. Free beta-hCG as first-trimester marker for fetal trisomy. *Lancet*, 339, 1480.

- Spencer, K. & Nicolaides, K. H. 2003. Screening for trisomy 21 in twins using first trimester ultrasound and maternal serum biochemistry in a one-stop clinic: a review of three years experience. *BJOG*, 110, 276-80.
- Spencer, K., Ong, C., Skentou, H., Liao, A. W. & K, H. N. 2000. Screening for trisomy 13 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation. *Prenat Diagn*, 20, 411-6.
- Steele, M. W. & Breg, W. R., Jr. 1966. Chromosome analysis of human amniotic-fluid cells. *Lancet*, 1, 383-5.
- Stenman, U. H., Tiitinen, A., Alfthan, H. & Valmu, L. 2006. The classification, functions and clinical use of different isoforms of HCG. *Hum Reprod Update*, 12, 769-84.
- Tsangaris, G. T., Karamessinis, P., Kolialexi, A., Garbis, S. D., Antsaklis, A., Mavrou, A. & Fountoulakis, M. 2006. Proteomic analysis of amniotic fluid in pregnancies with Down syndrome. *Proteomics*, 6, 4410-9.
- Tul, N., Spencer, K., Noble, P., Chan, C. & Nicolaides, K. 1999. Screening for trisomy 18 by fetal nuchal translucency and maternal serum free beta-hCG and PAPP-A at 10-14 weeks of gestation. *Prenat Diagn*, 19, 1035-42.
- Valenti, C., Schutta, E. J. & Kehaty, T. 1968. Prenatal diagnosis of Down's syndrome. *Lancet*, 2, 220.
- Valinen, Y., Rapakko, K., Kokkonen, H., Laitinen, P., Tekay, A., Ahola, T. & Ryyanen, M. 2007. Clinical first-trimester routine screening for Down syndrome in singleton pregnancies in northern Finland. *Am J Obstet Gynecol*, 196, 278 e1-5.
- Van Lith, J. M., Mantingh, A. & De Bruijn, H. W. 1993. Maternal serum CA 125 levels in pregnancies with chromosomally-normal and -abnormal fetuses. Dutch Working Party on Prenatal Diagnosis. *Prenat Diagn*, 13, 1123-31.
- Vesce, F., Scapoli, C., Giovannini, G., Tralli, L., Gotti, G., Valerio, A. & Piffanelli, A. 2002. Cytokine imbalance in pregnancies with fetal chromosomal abnormalities. *Hum Reprod*, 17, 803-8.
- Waardenburg, P. J. 1932. Das menschliche Auge und seine Erbanlagen. *The Hague: M Nijhoff*.
- Wald, N. J., Brock, D. J. & Bonnar, J. 1974. Prenatal diagnosis of spina bifida and anencephaly by maternal serum-alpha-fetoprotein measurement. A controlled study. *Lancet*, 1, 765-7.
- Wald, N. J., Cuckle, H., Brock, J. H., Peto, R., Polani, P. E. & Woodford, F. P. 1977. Maternal serum-alpha-fetoprotein measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on alpha-fetoprotein in relation to neural-tube defects. *Lancet*, 1, 1323-32.
- Wald, N. J., Cuckle, H. S., Densem, J. W., Nanchahal, K., Royston, P., Chard, T., Haddow, J. E., Knight, G. J., Palomaki, G. E. & Canick, J. A. 1988. Maternal serum screening for Down's syndrome in early pregnancy. *BMJ*, 297, 883-7.
- Wald, N. J., Densem, J. W., George, L., Muttukrishna, S. & Knight, P. G. 1996. Prenatal screening for Down's syndrome using inhibin-A as a serum marker. *Prenat Diagn*, 16, 143-53.
- Wald, N. J. & Hackshaw, A. K. 1997. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. *Prenat Diagn*, 17, 821-9.

- Wojdemann, K. R., Shalmi, A. C., Christiansen, M., Larsen, S. O., Sundberg, K., Brocks, V., Bang, J., Norgaard-Pedersen, B. & Tabor, A. 2005. Improved first-trimester Down syndrome screening performance by lowering the false-positive rate: a prospective study of 9941 low-risk women. *Ultrasound Obstet Gynecol*, 25, 227-33.
- Wortelboer, E. J., Koster, M. P., Stoutenbeek, P., Elvers, L. H., Loeber, J. G., Visser, G. H. & Schielen, P. C. 2009a. First-trimester Down syndrome screening performance in the Dutch population; how to achieve further improvement? *Prenat Diagn*, 29, 588-92.
- Wortelboer, E. J., Linskens, I. H., Koster, M. P., Stoutenbeek, P., Cuckle, H., Blankenstein, M. A., Visser, G. H., Van Vugt, J. M. & Schielen, P. C. 2009b. ADAM12s as a first-trimester screening marker of trisomy. *Prenat Diagn*, 29, 866-9.
- Zaragoza, E., Akolekar, R., Poon, L. C., Pepes, S. & Nicolaides, K. H. 2009. Maternal serum placental growth factor at 11-13 weeks in chromosomally abnormal pregnancies. *Ultrasound Obstet Gynecol*, 33, 382-6.

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## **Prenatal Diagnosis and Screening for Down Syndrome**

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This book provides a concise yet comprehensive source of current information on Down syndrome. Research workers, scientists, medical graduates and paediatricians will find it an excellent source for reference and review. This book focuses on exciting areas of research on prenatal diagnosis - Down syndrome screening after assisted reproduction techniques, noninvasive techniques, genetic counselling and ethical issues. Whilst aimed primarily at research worker on Down syndrome, we hope that the appeal of this book will extend beyond the narrow confines of academic interest and be of interest to a wider audience, especially parents and relatives of Down syndrome patients.

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