Ultrasonography of the fetal nose, maxilla, mandible and forehead as markers for aneuploidy

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To my parents, my love and my children

Contents

1 General introduction **9**

1.1 Overview 10

1.2 A brief history of ultrasound in obstetrics and gynecology 11

1.3 Screening chromosomal abnormalities 13

1.4 Down and Edwards syndrome 14

1.5 Fetal dysmorphology 17

2 Three-dimensional ultrasound imaging and measurement of nasal bone length, prenasal thickness and frontomaxillary facial angle in normal second- and third-trimester fetuses **29**

Ultrasound Obstet Gynecol. 2012 Jun;39(6):636-41

3 Prenasal thickness-to-nasal bone length ratio: a strong and simple second- and third-trimester marker for trisomy 21 **43**

Ultrasound Obstet Gynecol. 2012 Feb;39(2):185-90

4 The facial profile of Down syndrome fetuses in the second and third trimester of pregnancy **55**

Ultrasound Obstet Gynecol. 2014 Nov 4.

5 Nasal bone length, prenasal thickness, prenasal thickness-to-nasal bone length ratio and prefrontal space ratio in second- and third-trimester fetuses with Down syndrome **67**

Ultrasound Obstet Gynecol. 2015 Feb;45(2):211-6.

6 Is 3D technique superior to 2D in Down syndrome screening? Evaluation of six second and third trimester fetal profile markers **79**

Prenat Diagn. 2015 Mar;35(3):207-13.

7 Trends in serial measurements of ultrasound markers in second and third trimester Down syndrome fetuses **91**

Fetal Diagn Ther. 2015 Jan 30.

8 Fetal facial profile markers in second and third trimester fetuses with Edwards syndrome **103**

Ultrasound Obstet Gynecol. 2014 Sep 5.

9 Summary, general discussion and future perspectives **117**

10 Summary in Dutch (Nederlandse samenvatting) **129**

Appendices

List of publications **137**

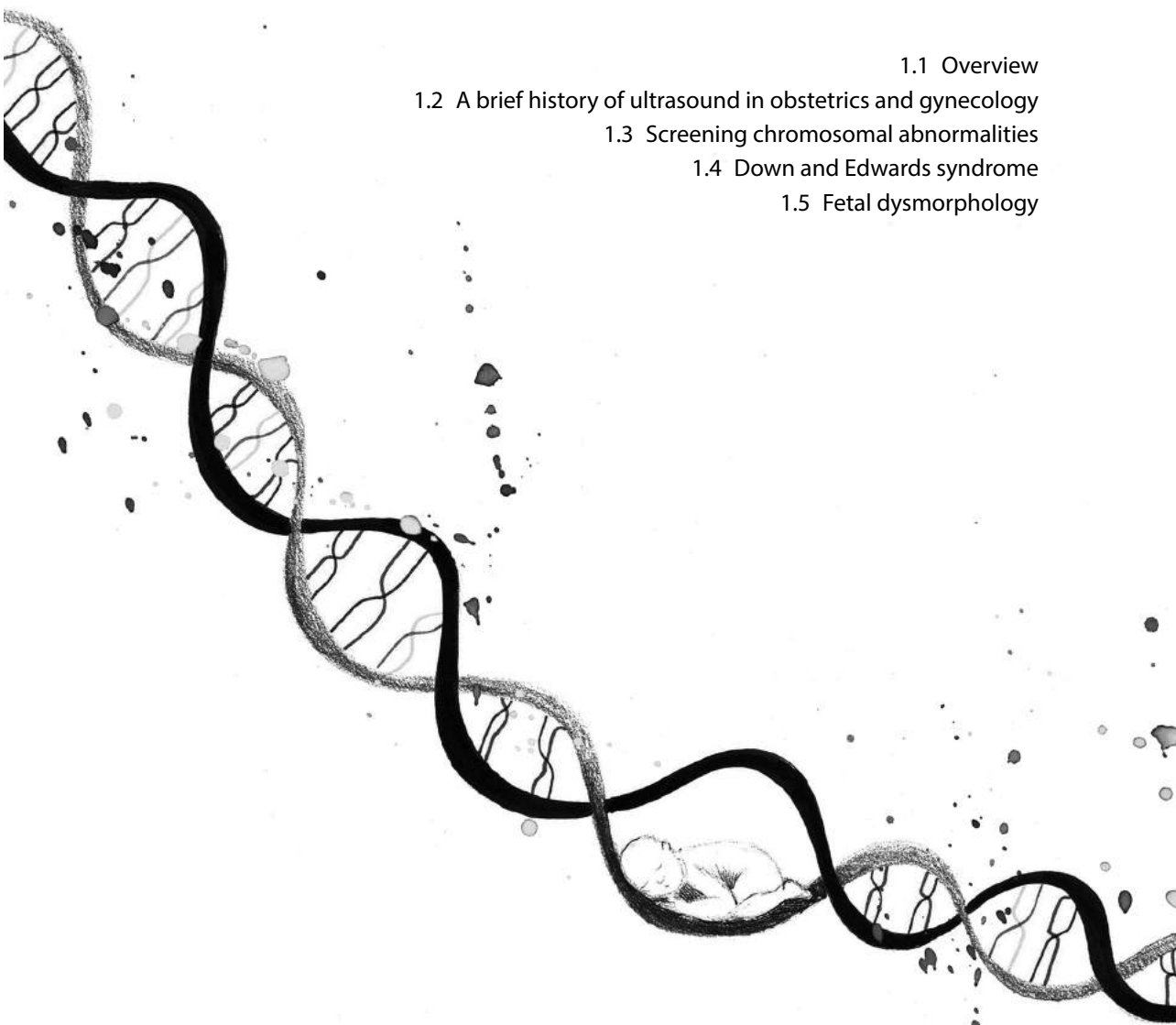
Dankwoord **139**

Curriculum Vitae **141**

CHAPTER 1

General introduction

1.1 Overview
1.2 A brief history of ultrasound in obstetrics and gynecology
1.3 Screening chromosomal abnormalities
1.4 Down and Edwards syndrome
1.5 Fetal dysmorphology



1.1 OVERVIEW

The face with its ability to express emotions is a very important element of human communication. If the eyes are the mirrors of the soul, the face can be regarded as the mirror of the mind. Furthermore, syndromes affecting the physical constitution of an individual are often characterized by typical facial features. In this thesis we link subtle facial features with fetal trisomies, the most common genetic disorders affecting the human fetus.

Since the introduction of ultrasound (US) technique in Obstetrics, one of the main goals of this discipline has been to diagnose congenital anomalies before birth. Initially, specific lethal and severe anomalies could be diagnosed prenatally. Examples are, for instance, anencephaly and spina bifida. In the 1980s, the ability to diagnose spina bifida was greatly improved[1] due to the introduction of the so called "cranial signs". These signs include the lemon and banana sign, which are typical "proxies" in the fetal head that might warrant the existence of an open defect in the spinal canal. As a result of these developments in prenatal ultrasound, the number of live births with this condition fell remarkably in many countries.

In the 1990s, attention shifted from structural anomalies to chromosomal anomalies, such as trisomy 21, 18 and 13, which are the three most common trisomies.

Whereas trisomy 18 (also known as Edwards syndrome) and 13 (Patau syndrome) are characterized by a variety of structural anomalies, trisomy 21 (Down syndrome) was more challenging to detect by US, due to the less frequent association with structural anomalies. The concept of "ultrasound markers for chromosomal anomalies" was introduced to remedy for this. Further improvement of US technique led to the possibility of examining the fetus in the first trimester, which moved screening for chromosomal anomalies to the first trimester. The technique of using a combination of US markers – the most important of which is the nuchal thickness (NT^2)- and of serum markers in an algorithm, achieved the best results.

However, search for effective second trimester US markers of aneuploidies has never ceased to exist as, for various reasons, first trimester screening is not performed in all pregnancies. The second trimester scan however, is offered more routinely. Recently, as a merit of the introduction of three-dimensional (3D) US, growing attention has gone out to the visualization of the fetal face. These increasing possibilities in imaging of the fetus, the growing knowledge of syndromes caused by chromosomal abnormalities and awareness of their corresponding phenotypes, has led to the birth of a new discipline defined as fetal dysmorphology.

The search for 2D ultrasound markers suggestive of fetal trisomies received further impulse when it became clear that analysis of the fetal profile could be of great value for this purpose.
Our research group has focused on combining the advantages of 3D ultrasound as a method for obtaining a perfect fetal profile view, with the exploration of new profile markers and assessment of their value when fetal trisomies are suspected in the prenatal phase.

In order to explore the profile markers, we have set out the following aims for this thesis:

- to study the natural history of several profile markers and their reproducibility in a cohort of euploid fetuses.
- to systematically investigate new and known fetal profile markers for aneuploidies in a large cohort of Down syndrome (DS) fetuses.
- to systematically investigate new and known fetal profile markers for aneuploidies in a cohort of Edwards syndrome (ES) fetuses.
- to study trends in facial markers serially in a group of DS fetuses.
- to determine the contribution of 3D US on top of 2D US, as a means to increase the performance of fetal profile markers.

In the remaining part of this chapter, we will shortly discuss the history of ultrasound in obstetrics and gynecology, set out what the current screening options are for fetal trisomies, briefly introduce the DS and ES, illustrate the discipline of fetal dysmorhpology with specific interest for facial markers.

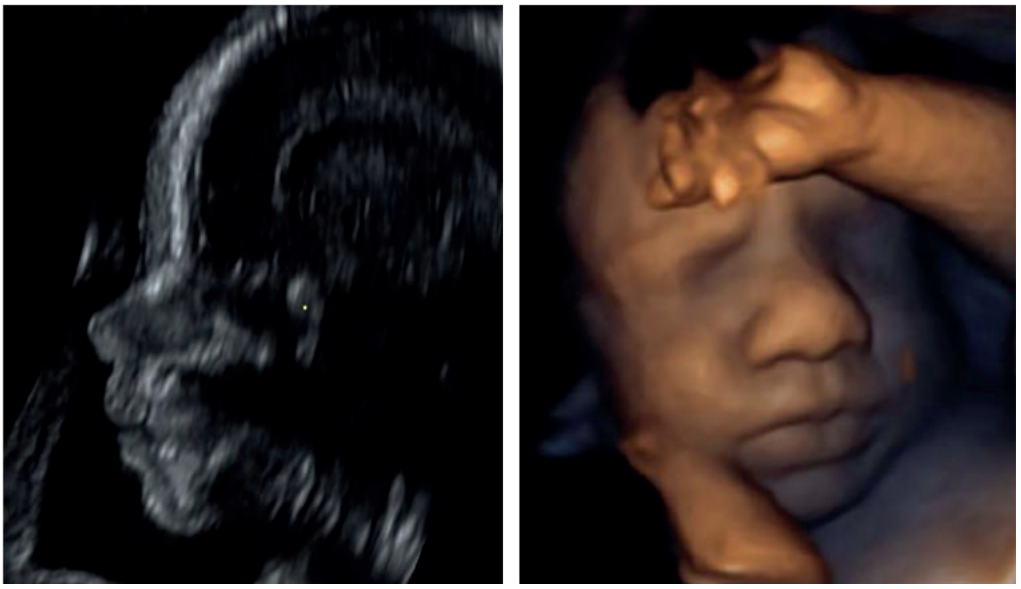
1.2 A BRIEF HISTORY OF ULTRASOUND IN OBSTETRICS AND GYNECOLOGY

In 1958, the first contact compound 2D ultrasound scanning machine (the Diasonograph), was introduced by Ian Donald. In the following decades, many different types of static scanning machines were developed. Using the Diasonograph, Ian Donald was the first to measure the fetal skull with ultrasonic A-mode cephalometry (by biparietal diameter) in 1961[3]. Subsequently, Stuart Campbell used cephalometry as a method of determining the exact gestation in the second trimester of pregnancy. Serial cephalometry was then further extended as a tool to identify and assess intra-uterine growth retardation[4].

Scanning techniques and equipment further developed over the years, and color and transvaginal ultrasound was developed in the late 1980s. Decades later harmonic imaging improved image resolution. The entire array of real time scanning with ultrasound modalities including high resolution images, color, and Doppler, facilities has been widely available since the beginning of this millennium.

Three-dimensional ultrasound

In 1974, Szilard was the first to describe the use of 3D US to investigate the fetus[5]. Halfway through the 1990s, articles concerning 3D reconstruction of the fetal face were published[6,7]. In these studies, the importance of visualization of the fetal face was stressed, with special regards to complex facial malformations, often found in syndromal abnormalities. The current academic consensus on the use of 3D US is that it contributes mostly to the evaluation of specific complex organs such as the brain, limbs, face and palate[8]. Rotten was the first to describe the use of 3D ultrasound in DS fetuses in 2002[9].



2D image (left) of the fetal profile of a euploid fetus in the second trimester. 3D reconstruction (right) of a euploid third trimester fetus.

Technique

3D US images are a reconstruction of multiple 2D images: the sonographic waves are being sent down but not reflected back immediately (as is the case in 2D imaging) but are sent from different angles. All these different 2D images together then construct a 3D volume by way of computer programming.

For evaluation of the profile, 3D volumes are acquired from fetuses facing the transducer, starting from as close as possible to the exact median profile view, during periods of quiescence and with an insonation angle of less than 45°. For the off-line measurement, the multiplanar images are magnified in order to obtain the maximal size possible of the area to be examined. When the fetal profile is to be examined, the planes are individually rotated to obtain symmetrical views of the orbits and nasal bone. To obtain an exact median view, the reference dot is then placed exactly at equal distance from the inner border of the orbits (which represents the midline) in the axial and coronal plane.

The measurement of fetal facial biometry by means of 3D volumes has many advantages compared to 2D images. (1) In a 3D volume, any desired plane can in fact be obtained by manipulating the volume with multiplanar mode[10]. (2) With respect to the relationship between parents and fetus, the expectation is that depicting the fetus by ultrasound would increase parental bonding[8,11,12]. In cases of visible malformations, such as for instance a facial cleft, actual visualization of the fetus may help the parents to understand the pathology and to prepare themselves for the birth of the baby. (3) Another major advantage of a 3D volume is the ability to analyze volumes off-line and in retrospect. A limitation of 3D imaging is that the resolution of the image in a calculated plane is usually lower than the resolution in the original starting plane for acquisition.

As 3D ultrasound is abstracted from 2D ultrasound, both techniques suffer from the same general limitations. These limitations concern the position of the fetus, the amount of amniotic fluid, the body mass-index of the mother and the experience of the sonographer.

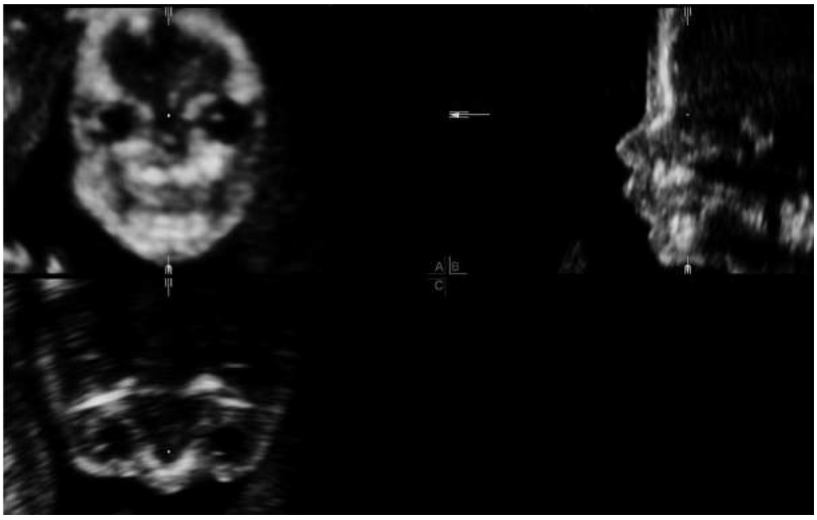


Figure of multiplanar view of a 3D volume in a euploid second trimester fetus.

1.3 SCREENING CHROMOSOMAL ANOMALIES

The most reliable diagnostic test for determining DS is karyotyping. This test can be performed by chorionic villous sampling, amniocentesis or cordocentesis, whereby fetal chromosomes are obtained and counted. More recently, molecular techniques and comparative genomic hybridization (CGH) arrays have substituted traditional karyotyping. However, tests aiming for obtaining fetal material have a disadvantage as they carry a risk of iatrogenic fetal loss (of about 0.1% – 2.8% within the first two weeks after the procedure)[13-16]. This is the reason why several non-invasive screening programs have been proposed. In the 1980s, several (second trimester) maternal serum markers combined with maternal age were used to calculate the risk of a DS pregnancy, reaching detection rates up to 60%[17]. At the beginning of the nineties, the NT was introduced as a first trimester marker[2]. Together with maternal serum markers and maternal age, this would later be installed as the combined test (CT) screening for DS, ES and Patau syndrome, which is still in use today.

Not all women undergo this early form of aneuploidy screening, with wide ranges of screening uptake reported across Europe; varying from 90% in Denmark and France[18,19] to 20 – 30% in parts of England and The Netherlands[20,21]. Factors that have been suggested to be of influence in decision making are maternal age, economic status, religion, rural demographic status, parity and type of referring health care professional[20-23].

Obviously, in large parts of the world first trimester serum screening is not available and ultrasonographic examination of the fetus takes place in later stages of pregnancy. In these settings, second trimester sonography is the first examination where aneuploidy can be suspected.

In most of Western Europe, the second trimester scan has proven to be a standard asset in prenatal care[24] with rates of uptake reported up to 99% in parts of Sweden and the UK[25,26]. The general aim of the scan is to evaluate the anatomical development of the fetus and to screen for major or minor anomalies. As several anatomical features like cardiac anatomy and intracranial structures are best visualized after eighteen weeks gestation, the scan is preferably performed between eighteen and twenty-two weeks gestation[27].

Introduction of screening for trisomies in the Netherlands was instituted many years ago. The issue of prenatal screening had to be examined by the Health Council, which, after a few years, produced two reports. The reports issued by the Health C. advised to offer to all women screening for DS and spina bifida by the CT and the 20-weeks scan, respectively. The introduction of screening needed a special concession of the Population Screening Act, the law regulating screening in the Netherlands.

In order to serve the principle of reaching out to "clients", the Ministry of Health chose to place screening extramurally, with the so-called 'first line'. Counseling concerning prenatal screening has also been delegated to the primary health care giver, which means the midwife in the majority of cases. When a woman decides to enrol for the CT, funding of the test used to be dependent on her age: women aged 36 years and older had free access to the test, all younger women paid a sum of 150 euros. From the beginning of 2015 however, everybody has to pay for the CT.

There are large regional variations between urban and rural areas concerning the uptake in first trimester screening. In a recent study, Bakker et al[21] made an inventory of the motivations for accepting or declining the CT in woman from the North-east and North-west of the Netherlands (which have an uptake of around 30%). A negative attitude towards termination of pregnancy (TOP) and an accepting attitude towards DS were reported to be the main reasons for declination of the CT. Another main reason reported for decline was unawareness of the pregnant women that a decision concerning the CT was being made. Opposed to the CT, the uptake of the 20-weeks scan (which is fully covered by insurance) is very high in the Netherlands, reaching more than 90%[28].

1.4 DOWN AND EDWARDS SYNDROME

The most common trisomy encountered in human fetuses and live born babies is that of the 21st chromosome, which is clinically classified as Down syndrome (DS)[29], followed by trisomy 18, the so-called Edwards syndrome (ES)[30].

Down syndrome

Down syndrome was first described by John Langdon Down in 1866[31]. Among other aspects, he described affected individuals to be characterized by a flat face and a small nose. Almost a century later, the French pediatrician and geneticist, Jérôme Lejeune[32], identified the origin of DS (which

was often referred to as 'mongolism') by establishing DS individuals having an extra copy of the 21st chromosome. This was a revolutionary discovery, not only because the genetic basis of DS was unraveled, but also because it was the first time that physical and mental disabilities were connected to a chromosomal anomaly.

The occurrence of and extra copy of the 21st chromosome is explained through the biological mechanism of gametogenesis. Gametogenesis[33] is a process in which cell division and differentiation create mature gametes. Oöcytogenesis is the female form of gametogenesis, as spermatogenesis is the male form. Oöcytogenesis, the formation of oöcytes, is initiated during fetal life and is completed in human females before or shortly after birth. At this time, oöcytes are called primary oöcytes, and their development halts in this stage at prophase I. Prophase I is the first phase of meiosis, in which final junction of chromosomes has not yet occurred. Oöcytes remain in this prophase I until menarche. From this time on, at each menstrual cycle a limited number of cells will develop into mature gametes. Spermatogenesis, in contrast to oöcytogenesis, is initiated at puberty. New sperm cells are created during the cycle of spermatogenesis, and this will be initiated throughout the male life. DS, which is caused by the presence of an extra copy of the 21st chromosome, is in the vast majority of cases the result of non-disjunction during meiosis[34].

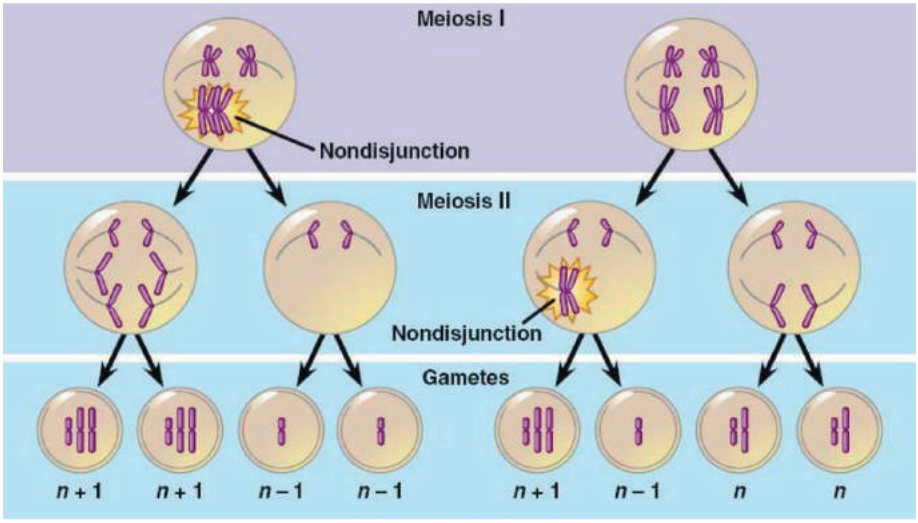


Figure of meiosis I and II and corresponding non-disjunctions.

The gamete with the additional chromosome is of maternal origin in an estimated 95%, opposed to paternal origin in 5%[35]. The only well documented risk factor for DS remains advanced maternal age[34]. This can be largely explained by the fact that oöcytes are developed many years before their actual maturation, in contrast to sperm cells which are newly developed throughout the male life.

There is no evident association between the incidence of DS and paternal age[34]. Several studies have suggested a male predominance of DS baby's[36] when paternal meiosis errors are concerned

(possibly as a result of the extra 21st chromosome to preferentially migrate with the Y chromosome)[34]. This could be a possible explanation of the 1:1.15 male predominance found in DS babies[36].

A limited increase in DS live births has been observed in the Netherlands during the last 18 years[29,37]: 10 out of 10,000 live born babies were diagnosed as having DS in 1996 versus 16 in 10,000 live births currently. Penrose[38] described the risk of DS to be related to maternal age in 1933, which is now considered common knowledge. In The Netherlands, the percentage of mothers over 35 years of age has increased from 5.7% in 1980 to 21.5% in 2010[39], while the number of terminations of DS pregnancies has also increased, but in a less pronounced way[37]. These trends are a possible explanation for the slight increase in the incidence of DS observed since 1996.

Down syndrome is characterized by both physical and intellectual disabilities (DS adults having an average IQ of 50[40] with large individual variations), as well as recognizable (facial) features. Most common birth defects are congenital heart disease (CHD), which affect over one-third of new-born DS babies[29,41,42]. The majority of CHD consists of atrioventricular septal defects, tetralogy of Fallot, aberrant right subclavian artery, ventricular septal defect, coarctation of the aorta and tricuspid dysplasia[41]. Other structural anomalies that affect DS babies are gastro-intestinal atresia, cleft lip and palate, megacolon and cataract[43].

Individuals with DS often have distinct physical features like a short neck, extra space between the first and second toe, excessive joint flexibility with poor muscle tone, short fingers and short stature. Specific facial features that are also common are a flat facial profile, enlarged and protruding tongue, epicanthic folds, up slanted palpebral fissures and a small nose with anteverted narices.

For the total population of 12 European countries (The Netherlands excluded) the EUROCAT group[44] mention a general prenatal detection of DS of 62% between 2005 and 2009, with very wide ranges between countries ranging from 9% in Eastern-Europe to over 80% in Western European countries[24]. In (the north of) The Netherlands, Cocchi et al[37] report a rate of 62% live births after a DS pregnancy, with a 38% percentage of TOP's, between 1993 and 2004 (opposed to 14% and 83%, respectively, in the general European population[24]). The neonatal mortality rate in DS (< 28 days after birth) is 1.65%, opposed to 0.36% for a control group of healthy neonates[29]. In a recent study[45], new-born DS babies who died in the post-neonatal period had significantly more heart-related causes of death. These findings were largely confirmed in other studies[46,47], who report the risk of death in the post-neonatal period to be nearly fivefold when CHD is present. CHD also continues to be one of the most significant predictors of mortality until age 20[46]. However, in the past 40 years, the life expectancy of DS individuals has increased drastically (to an estimated 60 years[48]), amongst other things due the safe and widespread availability of cardiac surgical treatments[49,50]. Finally, a trend has been observed that mothers of DS infants who died within the first day went to fewer prenatal visits, whilst the mortality of DS infants was not associated with mothers of certain race, marital status, education or residency[45]. This observation confirms our belief that it is important that DS pregnancies are identified prenatally to provide mothers and their babies with customized prenatal care.

Edwards syndrome

The Edwards syndrome is named after the British geneticist John Hilton Edwards, who first described the syndrome in 1960 and reported it to be associated with a trisomic disorder[51]. As is the case in DS, gametes containing the extra chromosome are of maternal origin in the vast majority (> 95%)[52].

The prevalence of live born babies with ES varies between countries, with reported prevalence's of 1.0 per 10,000 registered births between 2003 – 2007 in the UK[53], to 2.66 per 10,000 registered births between 2004 – 2006 in the US[54]. As the risk of fetal loss or stillbirth is high (72% at 12 weeks gestation and 65% at 18 weeks[55]) and TOP is carried out in a large percentage of affected pregnancies (83% – 86%[53,55]), the number of affected pregnancies is much higher (an estimated 6.5 in 10,000 registries[53]) than the amount of live births.

As for DS, maternal age is a risk factor for an ES pregnancy[56]. This is a probable explanation for the increase observed in ES pregnancies (2.0 in 10,000 pregnancies between 1985 – 1989 to 6.5 in 10,000 between 2003 – 2007[53]). However, the prevalence of live born ES babies has not increased, most likely due to advanced prenatal detection and subsequent TOP. Babies born with ES have a very poor prognosis: mean estimated survival rates range from two to four weeks[30,57], with 1-year survival rates ranging from 6% – 8.1%[30,53,57]. Female babies with ES are reported to have a better chance at survival both pre- and postnatally[30,53,57,58].

Frequently observed structural malformations before and after birth are heart defects (septal defects, patent ductus arteriosus, polyvalvular disease), kidney malformations, severe growth retardation, malformations of the central nervous system, orofacial clefts, micrognathia and deformities of the upper extremities (especially clenched hands)[57,58]. More subtle malformations are odd shaped skull, choroid plexus cysts, single umbilical artery, absent nasal bone and increased nuchal thickness[59-62].

Major causes of death are sudden death due to central apnea, cardiac failure and respiratory insufficiency due to hypoventilation, aspiration and upper airway obstruction[58].

1.5 FETAL DYSMORPHOLOGY

The continuous improvement of prenatal ultrasound (US) has resulted in the extension of the discipline of dysmorphology to the prenatal period. In this discipline, examination of the fetal profile is an integral part of routine ultrasound investigation in all trimesters. Until recently, one of the problems has been that many "clinical" observations were difficult to standardize. In addition, there was a lack of practical objective measurement tools capable to convert a clinical impression into a measurable marker.

Morphological abnormalities in fetuses with chromosomal abnormalities, especially in the facial area, can already be observed in the first trimester. Both a thickened nuchal translucency[2] and absent nasal bone are often encountered abnormalities[63]. Other distinct dysmorphologies such as micrognathia, clefts or a flat profile can also be observed at this stage. In the second trimester, the fetal forehead, nose, philtrum, lips, maxilla and mandible can be visualized with greater detail. Observation of the proportion and relationship between the various elements of the fetal profile

has become an essential part of the morphological fetal examination in order to exclude genetic syndromes characterised by a specific facial phenotype. Attempts to create standardized markers reflecting dysmorphic features encountered in clinical observations started over forty years ago. One of the first screening methods for DS was introduced by Buttery[64] in the late seventies. He proposed to use the cephalic index (occipitofrontal to biparietal diameter) as a marker for DS. However, this method of screening was discarded by other researchers in the mid-eighties[65-67]. At this time a thickened NT and a short femur length[67-70] were described in DS fetuses, and since the mid-1990's the second trimester scan has been described as a tool to detect DS related physical anomalies[71].

Additional second trimester markers for DS that are used today are a mild ventriculomegaly, hyperechoic bowel, aberrant right subclavian artery, echogenic focus, short humerus and several facial markers[27]. Some of these facial markers for DS assess the singular aspect of mid-facial hypoplasia or skin thickening. These markers include the nasal bone length (NBL), maxillary length, maxilla nasion mandibular-angle (MNM-angle), prenasal thickness (PT) and nuchal fold (NF). Markers that aim to incorporate both traits are prenasal thickness to nasal bone length ratio (PT-NBL ratio), prefrontal space ratio (PFSR) and the frontomaxillary facial angle (FMF-angle)[72-78].

Facial Markers for chromosomal anomalies

Facial markers are not an anomaly in itself. They represent the typical phenotype of specific syndromes, and can help identify affected fetuses. With the characteristic appearance that DS individuals have, many attempts have been made to use these features prenatally in routine second trimester ultrasound examinations. Based on the principle that DS fetuses are affected by mid-facial hypoplasia and thickening of the skin, many markers are situated in the fetal neck and profile[72, 73,79]. In ultrasound examination, this results in the finding of small or absent nasal bones, aberrant convexity of the fetal profile and thickened skin in the nuchal and prefrontal area[72,73,79]. Many different pathological mechanisms that cause these morphological irregularities have been proposed. Increased skin thickness has been associated with several mechanisms like changes of the extracellular matrix of the skin, abnormalities of lymphatic vessels and cardiac defects or disfunction[80-83]. Abnormalities in bone growth and development is thought to be a contributing factor to the abnormal facial anatomy observed in DS[72,84]. Several pathological reports have confirmed these conclusions by post-mortem examination and X-ray imaging[85,86].

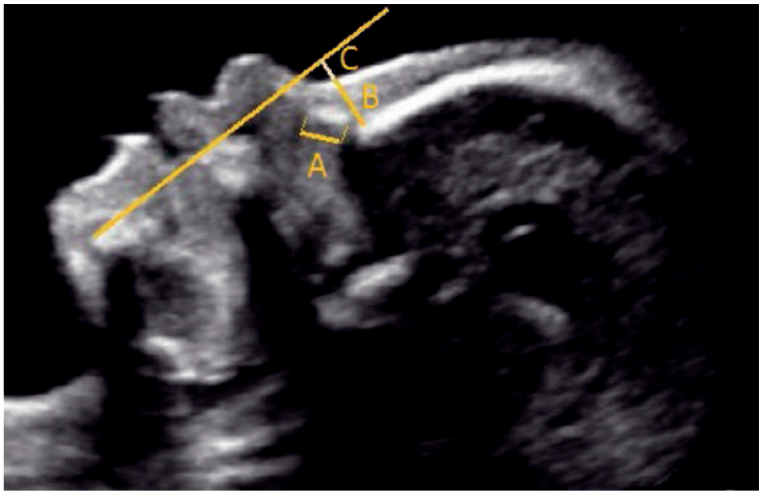
Nasal bone length

The most frequently studied facial marker in DS is undoubtedly the nasal bone. Studies have defined nasal bone hypoplasia variably: in a binary way as present or absent nasal bone[73,74], as continuous values[87,88], as percentiles[87], as multiple of the mean[89-91], in a ratio as biparietal diameter to nasal bone length ratio[73,74] and as the PT-NBL ratio[76,89].

Prenasal thickness, PFSR and the PT-NBL ratio

The PT is a measurement of the skin that lies anterior of the most distal part of the frontal bone. It is often thickened in DS, and the outcome has been studied as mean, delta, percentile, continues value[73], multiple of the mean and as the PT-NBL ratio[76,89]. Originally, PT is measured as the shortest

distance between the nasion (defined as the most anterior point in the junction between the frontal and nasal bones) and the leading skin edge. However, the PT is also part of other DS markers: the PFSR and the PT-NBL ratio. These two markers aim to combine mid-facial hypoplasia and prenasal thickening of the skin. The general consensus of all reports on the PT, PFSR and PT-NBL ratio is that PT measurements increase during gestation in both euploid and DS fetuses, while the PFSR and PT-NBL ratio remain constant throughout gestation.



Ultrasound image of a second trimester DS fetus. A, nasal bone length; B, prenasal thickness. The PFSR was calculated by dividing C by B. The PT-NBL ratio was calculated by dividing B by A.

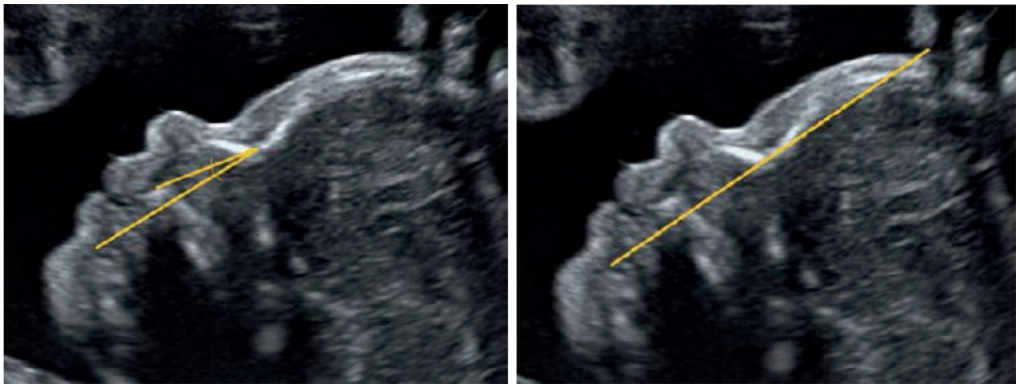
Angles in the fetal profile

In recent literature, many attempts have been made to construct markers that quantify the convexity of the fetal profile. Markers concerning the fetal forehead include the frontomaxillary facial angle (FMF angle)[92-94], nasofrontal angle[95] and frontonasal facial angle[96]. Even though DS individuals are known to have a flat profile, only the FMF angle is studied in DS fetuses. Angles that aim to describe the anatomical position of the fetal mandible, maxilla, or both, during the second half of pregnancy, are the sella-mandibular and sella-maxillary angle[97], the inferior facial angle[9] and the MNM angle[78]. All reports mention the angles to be independent of gestational age. To our knowledge only Rotten et al[9] describe the inferior facial angle (which quantifies the antero-posterior position of the mandible) in eight DS fetuses and found no apparent relation to DS. Our study of the MNM angle is the first study that describes the relation between mandible and maxilla in a large cohort of DS fetuses.

The fetal profile line

The final measurement in the fetal profile discussed in this thesis is the fetal profile line (FP line)[97], which assesses the position of the mandible in relation to the fetal forehead and the shape of the

forehead. The FP line passes through the midpoint of the anterior border of the mandible and the nasion and has been studied previously in euploid and pathological cases[97], but never in DS.



The MNM angle (left) and FP line (right) in a third trimester DS fetus.

An overview of all facial markers in DS mentioned above can be reviewed in table 1.

Table 1 | Overview of studies with detection rates of the PT, NBL, PT-NBL ratio and PFSR by analyzing both euploid and DS fetuses. If not mentioned differently, all studies use percentages as a cutoff value.

Marker	Study	Design	Dimension	Gestation	Euploid fetuses		DS fetuses		N.b.
					#	FP	#	DR	
NBL	Bunduki, 2003[87]	prospective	2D	16 – 24	1042	5,1%	22	59,1%	
	Maymon, 2005[89]	prospective	2D	14 – 27	500	5%	21	43%	
	Jung, 2007[98]	prospective	2D	16 – 28	2833	3,1%	9	33,3%	
	Cusick, 2007[99]	prospective	2D	16 – 21	371	3,5%	11	44,5%	
	Gianferrari, 2007[91]	retrospective	2D	15 – 25	2515	2,9%	21	85,7%	Uses 0.75 MoM af a cutoff
	Hung, 2008[100]	retrospective	2D	13 – 29	342	3,2%	14	64,3%	
	Odibo, 2008[90]	prospective	2D	16 – 22	4324	6%	49	47%	Uses 0.75 MoM af a cutoff
	Persico, 2012[101]	retrospective	3D	16 – 24	135	3,0%	41	75,8%	
PT	Persico, 2008[73]	retrospective	3D	16 – 24	135	11%	26	73,1%	Definition of PT: *
	Miguelez, 2010[102]	retrospective	2D and 3D	14 – 27	1385	5%	80	60%	Included fetuses of Persico in analysis. Definition of PT: *
	Chaveeva, 2013[103]	retrospective	2D	16 – 24	240	2.9%	45	73,3%	Definition of PT: **

Table 1 | Overview of studies with detection rates of the PT, NBL, PT-NBL ratio and PFSR by analyzing both euploid and DS fetuses. If not mentioned differently, all studies use percentages as a cutoff value (*Continued)*.

Marker	Study	Design	Dimension	Gestation	Euploid fetuses		DS fetuses		N.b.
					#	FP	#	DR	
PT-NBL ratio	Maymon, 2005[89]	prospective	2D	14 – 27	500	5%	21	63%	Definition of PT: *
	De Jong-Pleij, 2012[76]	retrospective	2D and 3D	15 – 33	219	2,3%	30	100%	Definition of PT: *
PFSR	Sonek, 2012[77]	retrospective	3D	15 – 25	90	5%	26	100%	Definition of PT: ***
	Yazdi, 2013[104]	retrospective	2D	15 – 40	279	5%	91	79.1%	Definition of PT: ***
	Chaveeva, 2013[103]	retrospective	2D	16 – 24	240	5%	45	100%	Definition of PT: **
FMF angle	Sonek, 2007[92]	retrospective	2D	14 – 24	100	9%	34	87.9%	Definition of FMF: ‡
	Molina, 2008[93]	retrospective	3D	16 – 25	150	3,3%	23	65.2%	Definition of FMF: ‡‡
	Odibo, 2009[94]	retrospective	2D	16 – 22	201	5,6%	21	14.3%	Uses > 30º above the mean as a cutoff. Definition of FMF: ‡‡
	Sooklim, 2010[105]	prospective	2D	17 – 19	386	3.9%	10	30,0%	Uses FMF values > 90º a cutoff. Definition of FMF: ‡‡

*As the shortest distance between lowest part of the frontal bone and the facial skin anteriorly.
**As the distance between skull and skin, tangential to Mandibula-maxillary line.
*** As a line parallel to maxilla.
‡ The first ray along the superior edge of the palate and the second ray from the upper anterior corner of the maxilla to the external surface of the frontal skin.
‡‡ The second ray from the upper anterior corner of the maxilla to the external surface of the frontal bone.
DS, Down syndrome; NBL; nasal bone length; PT, prenasal thickness; PT-NBL ratio, prenasal thickness to nasal bone length ratio; PFSR, prefrontal space ratio; FP, false positive rate; DR, detection rate; MoM, multiple of the mean.

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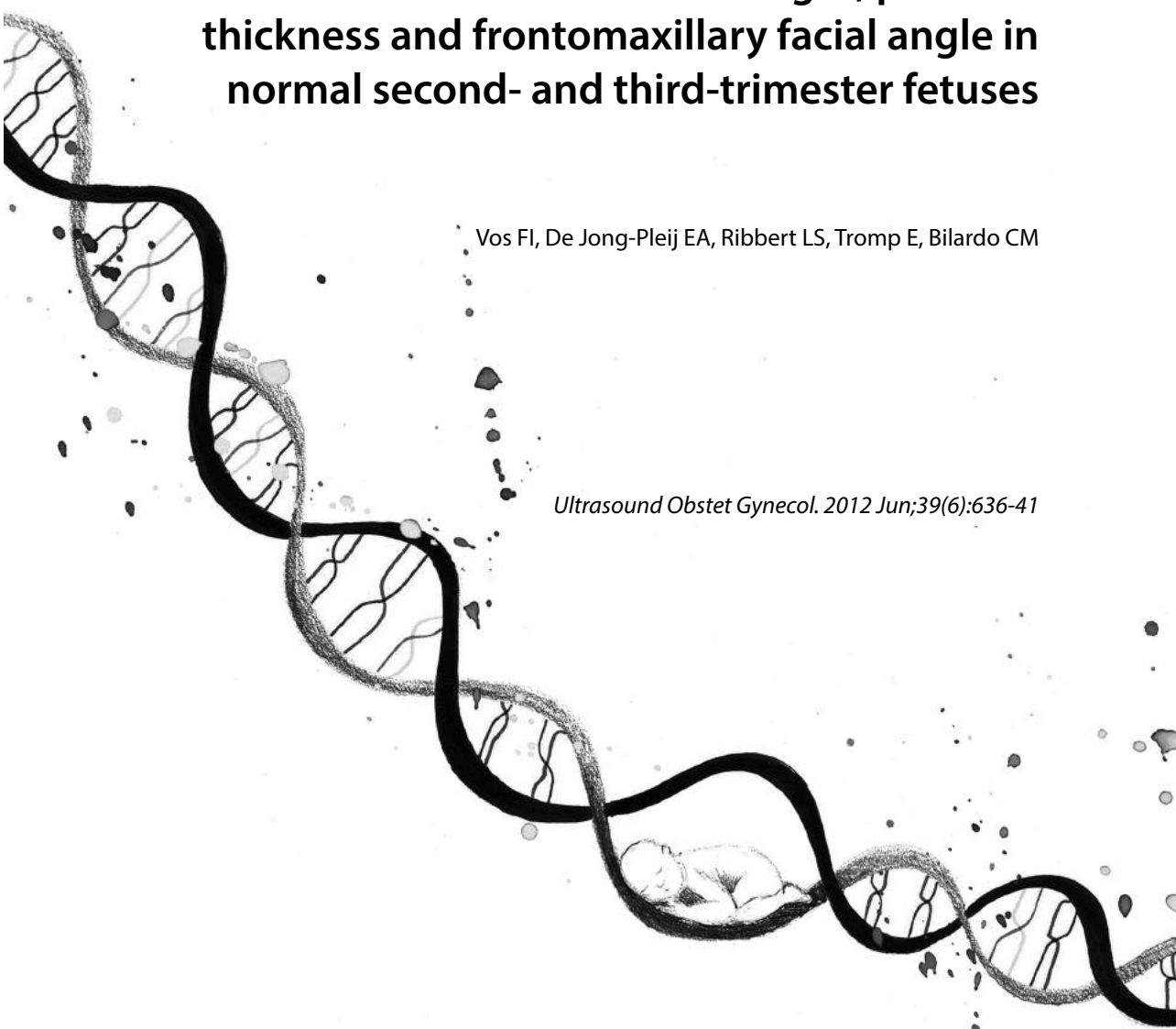
CHAPTER 2

Three-dimensional ultrasound imaging and measurement of nasal bone length, prenasal thickness and frontomaxillary facial angle in normal second- and third-trimester fetuses

Vos FI, De Jong-Pleij EA, Ribbert LS, Tromp E, Bilardo CM

Ultrasound Obstet Gynecol. 2012 Jun;39(6):636-41



ABSTRACT

Objectives

To assess the feasibility of nasal bone length (NBL), prenasal thickness (PT) and frontomaxillary facial (FMF) angle measurements performed on the same three-dimensional (3D) multiplanar-corrected profile view in euploid second- and third-trimester fetuses, to create reference ranges and to review published measurement techniques.

Methods

3D volumes of 219 euploid second- and third-trimester fetuses were retrospectively analyzed. The quality of images and measurability of the markers were assessed with 5-point and 3-point scoring systems, respectively. Measurements of NBL (with care to exclude the frontal bone), PT and FMF were obtained in the exact midsagittal plane. Reference ranges were constructed based on measurements from images with high-quality (4 or 5 points) and high measurability (2 or 3 points) scores and compared with those in the most relevant published literature.

Results

A high-quality score was assigned to 111 images. Among these, a high measurability score was significantly more often achieved for NBL (98.2%) and PT (97.3%) than for the FMF angle (26.1%) ($p < 0.001$). Both NBL ($NBL = -6.927 + (0.83 \times GA) - (0.01 \times GA^2)$) and PT ($PT = (0.212 \times GA) - 0.873$) (where GA = gestational age) showed growth with gestation, with less pronounced growth for NBL after 28 weeks. Our reference range for the NBL showed a systematically smaller length than those in other two-dimensional (2D) ultrasound based publications. The FMF angle measurements that we obtained did not show a significant change with GA.

Conclusions

NBL and PT, are easily measured using 3D ultrasound, whereas the FMF angle is more challenging. When it is measured in the exact midsagittal plane and care is taken to exclude the frontal bone, measurements of the NBL are systematically smaller than those in previous 2D ultrasound-based publications.

INTRODUCTION

Down syndrome (DS) is characterized by specific facial features such as a flat face and a small nose[1]. Continuous technical improvements in ultrasound techniques have enabled optimal visualization of these features which, in turn, have evolved into markers currently used as screening tools for the detection of DS. First-trimester nasal bone assessment, in combination with nuchal translucency measurements, was the first to be introduced[2], while second-trimester markers have also been proposed[3-5]. Nasal bone length (NBL), prenasal thickness (PT) and the frontomaxillary facial (FMF) angle are three second-trimester markers measurable in the misagittal profile view.

Improvements in three-dimensional (3D) ultrasound imaging have increased the accuracy of measurements by standardizing the examination plane through multiplanar correction of the acquired volume. The midsagittal plane obtained can differ considerably from the plane judged as midsagittal on two-dimensional (2D) ultrasound[6]. This has raised the question of whether the first published reference ranges, based on 2D images, are still valid and how they compare with the new ones obtained by 3D techniques. Reports on the role of 3D ultrasound in obtaining accurate NBL, PT and FMF angle measurements and individual reference ranges for these markers in the second trimester of pregnancy are available[7-10]. However, no study has thus far measured all three markers in the same fetus and extended the normal ranges to the third trimester. Although screening programs for trisomies are offered earlier in pregnancy, late diagnosis of chromosomal anomalies is not uncommon, especially in countries with a low uptake of screening programs. In addition, even when termination of pregnancy is no longer an option, the diagnosis of Down syndrome can be of value in establishing the optimal place of delivery and optimal perinatal management, and in preparing parents for the birth of a DS baby.

The aims of this study were to assess the feasibility of NBL, PT and FMF angle measurements performed on the same 3D-corrected profile view in normal second- and third-trimester fetuses and to create reference ranges for these markers. Furthermore, differences in definition or measurement techniques in the most relevant published literature on the individual markers were reviewed.

METHODS

The ultrasound unit of the Saint Antonius Hospital in Nieuwegein, The Netherlands, offers routine ultrasound investigation in the second and third trimesters of pregnancy. 3D images of the fetal face were collected cross-sectionally in 219 fetuses from a cohort of non-smoking, healthy, low-risk Caucasian women with a singleton pregnancy. Only non-anomalous fetuses from uncomplicated pregnancies were included. All images were obtained using a GE Voluson 730 Expert ultrasound system equipped with a RAB2-5L or RAB4-8L probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Volumes were acquired from fetuses facing the transducer, starting from as close as possible to the exact midsagittal profile view during periods of quiescence and with an insonation angle of less than 45°. An attempt was made to collect at least two such volumes per fetus. The volumes were stored on removable digital media for subsequent analysis on 4D View software version 7.0

(GE Medical Systems). These images were retrieved retrospectively for the purpose of this study and the markers measured offline using the multiplanar mode of the 4D View program. The study was approved by the local ethics committee and all women gave written consent.

Initially, the multiplanar images were magnified to obtain the maximum possible size of the fetal profile, and the reference dot was positioned in plane A (Figure 1a, upper left) just below the nasal bone. Planes B and C were then individually rotated to obtain symmetrical views of the orbits. When this multiplanar correction was carried out appropriately, the nasal bones and frontal processes of the maxilla automatically appeared in plane B as an 'inverted V-shape'. To obtain an exact midsagittal view in plane A, the reference dot was placed in planes B and C exactly at an equal distance from the inner border of the orbits at the level of the nasal bone. The adjusted planes, resulting in an exact midsagittal plane in A, are displayed in Figure 1a. NBL, PT and FMF angle were all measured in the enlarged image in plane A.

For each fetus, the volume with the best sagittal view was selected. Firstly, all images were corrected by multiplanar mode to the exact midsagittal view and scored from 1 – 5 in terms of quality for contrast and clarity (quality score), 1 being poor and 5 excellent. Specific points of interest were an optimal midsagittal view and clear contrast between the fetal profile and surrounding tissue or fluids. Only images with a quality score of 4 or 5 were used for further analysis. Subsequently, in the included images, each individual marker was scored from 1 – 3 in terms of visualization of landmarks (measurability score), 1 being poor and 3 excellent. Optimal contrast between bony and soft tissue at the location of the landmark was considered important. Only markers with a measurability score of 2 or 3 were used for further analysis. Each marker was measured three times and the average was taken as the final measurement.

The nasal bone was measured from the nasion to the distal end of the white ossification line (Figure 1b). The nasion was defined as the most anterior point at the junction between the frontal and nasal bones. As the frontal bone extends posteriorly of the nasal bone (Figure 1c), care must be taken to measure the nasal bone starting from the level of the nasion, without including the frontal bone in the measurement, as this would erroneously enlarge the measured NBL (Figure 1d). The PT was measured as the shortest distance between the nasion (same landmark as used for measuring the NBL) and the frontal skin (Figure 1b). In cases in which there was a gap between the nasal and the frontal bones (disjunction), for PT measurement the landmark nasion was set at the point of intersection of two lines drawn tangentially to the nasal bone and to the lower part of the frontal bone, whereas for NBL measurements only the white ossified part of the nasal bone was measured.

The FMF angle was measured according to the different techniques proposed in the literature by various researchers; Sonek et al[5] measured the FMF angle with the first ray drawn from the top edge of the palatal complex (Figure 1e) and the second line to either the frontal bone or the skin anteriorly of the frontal bone. In contrast, Molina et al.[7] made a distinction between two structures in the palatal complex: the vomer and the palate (Figure 1f). They placed the first ray along the palate and the second ray along the frontal bone. To determine which of these methodologies for FMF angle measurement was easier to perform and more reproducible, we measured the FMF angle in six different ways (Figures 1e and f).

To assess intraobserver variability, all markers were re-measured in the acquired volumes following a 1-week interval. Interobserver variability was assessed by a second examiner, who repeated the measurements as described above on all markers. Finally, results were compared with the most relevant literature. Data analysis was performed by Microsoft Excel for Windows 2000 (Microsoft Corp., Redmond, WA, USA) and SSPS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as mean (SD) or median (range). Bland-Altman analysis was used to describe intra- and interobserver variability. The best-fit polynomial line was used for constructing reference ranges. Differences between observed frequencies were compared by the chi-square test, and $p < 0.05$ was considered to be statistically significant.

RESULTS

The cross-sectional study group included 219 fetuses at 15–33 weeks' gestation (mean, 23 weeks). In 111 fetuses the mid-sagittal image obtained was given a quality score of 4 or 5. The quality scores of the images from all 219 fetuses and the measurability scores of the 111 high-quality images are presented in Table 1. The frequency distribution of the measurability scores of the 111 high-quality images was not equal for the three markers (chi-square $p < 0.001$). A measurability score ≥ 2 was obtained in 109 cases for the NBL (98.2%), in 108 cases for the PT (97.3%) and in 29 cases for the FMF angle (26.1%). A measurability score of ≥ 2 was obtained for both NBL and PT measured in the same midsagittal profile view in 106 cases (95.5%), for FMF angle and NBL in 26 cases (23.4%) and for FMF angle and PT in 28 cases (25.2%). The angle between the transducer and the nasal bone was less than 45° in all cases.

Table 1 | Quality score of 219 images and measurability score of facial markers in 111 images that had a quality score of 4 or 5. Data given as number of images. Quality was scored from 1 (poor) to 5 (excellent) for contrast and clarity. Measurability was scored from 1 (poor) to 3 (excellent) in terms of visualization of landmarks. FMF, frontomaxillary facial angle; NBL, nasal bone length; PT, prenasal thickness.

Score	Quality	Measurability		
	Number of images	Number of images		
		NBL	PT	FMF
1	7	2	3	82
2	47	105	102	28
3	54	4	6	1
4	108	–	–	–
5	3	–	–	–
Total	**219**	**111**	**111**	**111**

a
A
B
C
b
PT
NBL
c
d
A
B
e
B
A
1
2
C
f
B
A
3
4
5
6
C
D

Figure 1 | (**a**) Multiplanar ultrasound image showing the 'inverted V-form' of the nasal bones and frontal processes of the maxilla in plane B. In plane A the reference dot was placed just below the nasal bone and in both planes B and C exactly at equal distances from the inner borders of the orbits. (**b**) Ultrasound image showing prenasal thickness (PT) and nasal bone length (NBL) measurements. (**c**) Illustration of the fetal skull: the frontal bone continues posteriorly of the nasal bone. (**d**) Ultrasound image showing correct NBL measurement (A) and incorrect NBL measurement with inclusion of the frontal bone (B). (**e**) Ultrasound image of measurement of frontomaxillary facial angles between the frontal bone (A), skin (B) and palatal complex (C). In cases where only the palatal complex was visible (and no distinction was possible between vomer and palate) the first ray was drawn along the upper surface of the palatal complex. The second ray was directed to either the frontal bone (angle 1, complex–bone) or skin (angle 2, complex–skin) at the point of its greatest anterior excursion. In all cases the point of intersection was the upper corner of the anterior aspect of the maxilla. (**f**) Ultrasound image of measurement of frontomaxillary facial angles between the frontal bone (A), skin (B), vomer (C) and palate (D). In cases where the two structures, vomer and palate, could be identified, the first ray was drawn along the upper surface of the vomer or through the palate. The second ray was directed to either the frontal bone or skin at the point of its greatest anterior excursion. In all cases the point of intersection was the upper corner of the anterior aspect of the maxilla. 3, vomer–bone angle; 4, vomer–skin angle; 5, palate–bone angle; 6, palate–skin angle.

The intraobserver 95% limits of agreement were -1.03 to 0.86 mm, -0.61 to 0.76 mm and -8.18 to 5.29°, for NBL, PT and FMF angle, respectively. The respective interobserver 95% limits of agreement were -1.20 to 1.30 mm, -0.52 to 0.69 mm and -6.22 to 8.50° (Table 2).

Table 2 | Intra- and interobserver mean differences and 95% limits of agreement (LOA) with 95% CI's between paired measurements of facial markers. Diff., difference; FMF, frontomaxillary facial angle; NBL, nasal bone length; PT, prenasal thickness.

	Intraobserver		Interobserver	
Measurement	**Mean difference**	**LOA *(95%CI)***	**Mean difference**	**LOA *(95%CI)***
NBL	-0.08	-1.03 *(-0.87 – -1.19)* 0.86 *(0.71 – 1.02)*	0.05	-1.20 *(-0.99 – -1.40),* 1.30 *(1.09 – 1.50)*
PT	0.08	-0.61 *(-0.49 – -0.72)* 0.76 *(0.65 – 0.88)*	0.09	-0.52 *(-0.62 – -0.42),* 0.69 *(0.59 – 0.79)*
FMF angle	-1.45	-8.18*(-5.98 – -0.38)* 5.29 *(3.08 – 7.49)*	1.14	-6.22 *(-3.85 – -8.59),* 8.50 *(6.13 – 10.87)*

NBL increased significantly with gestational age (GA), from 3.3 mm at 15 weeks' gestation to 9.6 mm at 33 weeks (linear regression $p < 0.001$). NBL followed a second order polynomial relationship with GA: $NBL = -6.927 + (0.83 \times GA) - (0.01 \times GA^2)$, ($R^2 = 0.78$, $P < 0.001$) (Figure 2). Figure 2 shows the mean NBL derived from this study is compared with the mean published by Sonek et al[11].

PT increased significantly with GA from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks (linear regression $p < 0.001$). A linear relationship with GA was confirmed on polynomial regression: $PT = (0.212 \times GA) - 0.873$ ($R^2 = 0.74$, $p < 0.001$) (Figure 3). A comparison between the mean PT derived from this study and mean PT measured by Persico et al[9] is shown in Figure 3.

The palate and vomer were seen as a palatal complex in 21 out of 29 cases (72.4%), and as two separate structures in eight cases (27.6%). The likelihood of the two being observed as a palatal complex or as two separate structures seemed to be independent of GA. Median GA for visualization as a palatal complex was 19.5 (range, 15.4 – 28.2) weeks, and for separate structures it was 18.5 (range, 15.6 – 25.5) weeks. In view of the paucity of FMF angle data, the measurements of 'complex' angles (angles 1 and 2, Figure 1e) and 'vomer' angles (angles 3 and 4, Figure 1f) were pooled together; given the fact that in both measurements the first ray is placed at the same position, the angles 'complex-bone' and 'vomer-bone' are similar, as are 'complex-skin' and 'vomer-skin'. The difference between FMF angles measured to the skin or to the bone had a constant value of 10° (median 10.0°, range 6.1 – 14.6°) throughout gestation (Pearson's $r = -0.12$, $p = 0.54$), making it unnecessary to use these two different measurement techniques in this study. Consequently, further analysis of FMF angles was performed by analyzing two measurements only – complex/vomer–bone angle (i.e. complex-bone and vomer-bone pooled together) and palate-bone angle (Figure 4). The FMF angles did not change significantly with gestation, with a mean complex/vomer-bone value of 67.05° (range,

57.85 –77.78°; SD = 4.34) (p = 0.11). The median palate-bone angle was 85.08° (range, 80.8 – 94.9°; SD = 5.13) (p = 0.74).

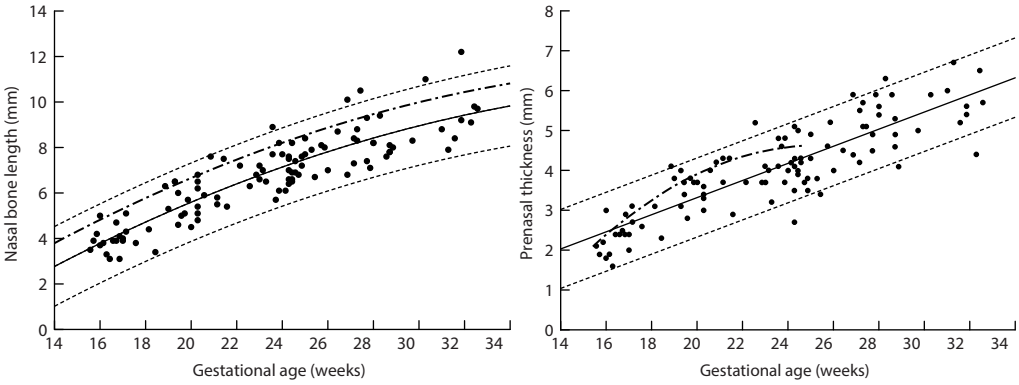


Figure 2 and 3 | Scatterplot of nasal bone length (NBL) and prenasal thickness (PT) with mean (—) and 5th and 95th percentiles (----) in 109 and 108 euploid fetuses, respectively. Also showing mean NBL from reference range of Sonek et al[11](— · — · —), mean PT from Persico et al[9] (– · – · –).

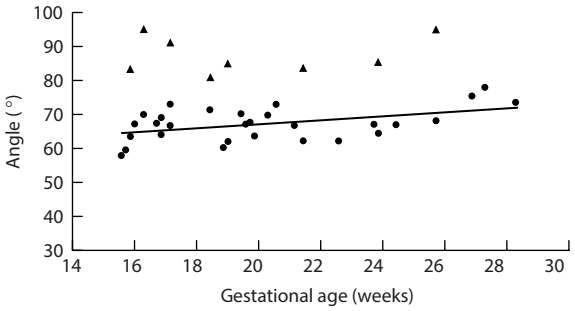


Figure 4 | Scatterplot of Palate–bone angle measurements (▲) in eight fetuses and complex/vomer–bone angle measurements (•) with corresponding mean trend in 29 fetuses (p = 0.11).

NBL and PT were highly correlated (p < 0.001). Owing to the paucity of FMF angle data, no analysis of correlation was performed between this and any other marker.

DISCUSSION

In this study we present novel reference ranges for NBL and PT measured on multiplanar view-corrected midsagittal plane using 3D volumes of normal second- and third-trimester fetuses. Both NBL and PT showed growth with gestation, with less pronounced growth for NBL after 28 weeks. Good visualization leading to high-quality measurements was achieved significantly more often for NBL and PT than for the FMF angle.

To the best of our knowledge this is the first study using 3D ultrasound to measure all three markers in the same fetus and extending the measurements into the third trimester.

Markers for Down syndrome are mainly studied early in pregnancy. However, uptake of first-trimester screening varies across countries as well as the rate of late bookers. It is therefore important to have effective Down syndrome markers available for later diagnosis in pregnancy.

The importance of measuring NBL, PT and FMF angle in the exact midsagittal view has recently been emphasized in the literature by a study showing that the use of 3D multiplanar mode improves the accuracy of profile measurements[6]. In addition, Persico et al[10] showed that the NBL is overestimated when measured in oblique midsagittal views and underestimated in parasagittal planes.

Although the present study design was retrospective, volumes were rigorously selected in order to obtain optimal measurements. The stored volumes did not always allow optimal visualization of facial structures to enable high-quality measurements. This was dependent on the angle of insonation and fetal position. Although this may seem a limitation of the study, in our opinion it rather reflects a 'real-world' situation where, in a routine clinical setting, volumes are stored during the examination and markers could be measured retrospectively.

Measurement of the FMF angle was particularly challenging, being judged to be of high quality only in 26% of the cases, in contrast to 98% and 97% for NBL and PT, respectively. This suggests that measurement of the FMF angle is more difficult after the first trimester and probably requires a very specific insonation angle to avoid shadowing by the facial bony structures that hamper good visualization of the thin vomer.

After re-examining the nasal and frontal bones on multiplanar mode-corrected profile view using 3D volumes, we redefined our measurement technique. In the new technique care was taken not to add part of the frontal bone to the measurement of the NBL, as this would erroneously increase the measurement (Figures 1c and d). When in DS fetuses the nasal bone is hypoplastic, the nasal and frontal bones are not in contact, but are separated by a gap (nasal bone-frontal bone disjunction). In such cases we used the reconstructed landmark nasion as a starting point for the PT measurement, instead of the lowest part of the frontal bone. This landmark may be more difficult to reconstruct in case of absence of the nasal bone in the second and third trimesters of pregnancy. However, later in pregnancy the nasal bone is more commonly hypoplastic rather than absent. We preferred to measure PT from the (landmark) nasion, as this avoids combining bony tissue and skin tissue in the PT measurement. The advantage would be that only the skin is measured, which tends in our opinion to be more edemic in DS fetuses. However, comparative studies are needed to substantiate this assumption.

It is mandatory to adhere to standardized measurement techniques when using markers for the estimation of Down syndrome risk in order to prevent overestimation or underestimation of the calculated risk. Several measurement techniques for NBL have been described in the literature (Table 3)[3,8,10-12]. 2D ultrasound may lead to overestimation of the NBL if this is measured slightly obliquely and/or the measurement erroneously includes part of the frontal bone. This supposition is confirmed by the smaller NBL in our study and in that of Persico et al.[10]. Moreover, when our range is compared with the 2D reference range published by Sonek et al.[11], the NBL in our study is systematically smaller (by about 1–2 mm) while the means otherwise follow the same trend (Figure 2).

Both Maymon et al[4,13] and Persico et al[9] studied PT in normal fetuses. We chose to compare our results with those of the latter study, as it is recent and based entirely on 3D-corrected images examined offline. While our results show a linear trend of PT with GA, the reference range of Persico et al follows a second-order polynomial trend. Possible explanations for this discrepancy could be that our study has a wider gestational window (15 – 33 compared with 16 – 24 weeks) and that we used a different definition of PT in cases of disjunction. Nevertheless it seems unlikely that this different definition could play a major role in explaining the discrepancy between reference ranges, as disjunction was observed in only a very limited number of cases.

For FMF angle measurement we used six different techniques (Figure 1e and f) that have been described previously in the literature. The difference between the FMF angles using a ray towards the frontal bone or the frontal skin showed a non-significant change between 15 and 33 weeks' gestation, with a mean of 10°. We observed that (independently of GA) in our population the vomer and palate were more often seen as one complex than as two separate structures. For these reasons we decided to adopt the combination complex-bone/vomer-bone angle and the palate-bone angle. Of the three facial measurements we found the FMF angle to be the most difficult to visualize and measure.

FMF angle measurement in normal second-trimester fetuses has previously been performed by Sonek et al[5] and Odibo et al[14] using 2D ultrasound and by Molina et al[7] using 3D ultrasound. Consistent with the findings of Molina et al. and in contrast to those of Sonek et al and Odibo et al, our results show a constant FMF angle measured from the palate and a slight increase in the FMF angle measured from the vomer through gestation (Figure 4), although the latter was not statistically significant, possibly due to the small number of cases.

In conclusion, when measured on 3D volumes, NBL and PT are reproducible markers and easy to measure, whereas the FMF angle is more challenging. In this study we present novel reference ranges for NBL and PT. Both NBL and PT show growth with gestation, with less pronounced growth for the NBL after 28 weeks. Following measurement in the exact midsagittal plane and with care taken to exclude the frontal bone, our reference range for the NBL showed a systematically smaller length than in other publications.

Table 3 | Overview of definitions used for NBL, PT and the FMF angle.

Definition of NBL	Definition of FMF-angle	Definition of PT
Guis, 1995[3]: N = 376, 2D, 14-34 weeks. Synostosis has to be visible.	**Sonek, 2007[5]:** N = 100, 2D, 14-24 weeks FMF bone = 'the angle between the top edge of the upper palate and the bony forehead.' FMF skin` = 'the top edge of the upper palate and the skin over the forehead.' In the images it is seen that the vomer can be identified as the 'upper palate'.	**Maymon 2005[4]:** N = 500, 2D, 14-27 weeks Measured from the fronto-nasal angle to the outer part of the closest nasal skin edge.
Sonek, 2003[11]: N = 3537, 2D, 11-40 weeks Identified and measured at the level of the synostosis.	**Molina 2008[7]:** N = 150, 3D, 16-24 weeks FMF angle = 'angle between the palate and frontal bone.' Molina specifically states that, in contrast to Sonek, the FMF angle is measured from the palatine bone.	**Persico 2008[9]:** N = 135, 3D, 16-24 weeks The shortest distance between the anterior edge of the lowest part of the frontal bone (at the junction with the nasal bone when present) and the skin anteriorly.
Bergann, 2006[8]: N = 23, 3D, 18-28 weeks Measured from the base of the nose nearest to the frontal bone, to the farthest extent of ossification.	**Odibo, 2009[14]:** N = 201, 2D, 16-22 weeks Use same measuring technique as Sonek et al. Measuring the skin does not seem to make any difference.	**Vos, 2012:** N =108, 3D, 15-33 weeks Measured as the shortest distance between the nasion and the frontal skin. In the case of fronto-nasal disjunction, the landmark nasion is at the point of intersection between the lines tangential to the nasal bone and tangential to the lower part of the frontal bone.
Gianferrari, 2007[12]: N = 2515, 2D, 15-24 weeks measured from the base of the nose closest to the frontal bone to the most distal aspect of ossification.	**Vos, 2012:** N = 29, 3D, 25-33 weeks 1. Complex/Vomer-bone angle: angle between vomer or palatal complex and frontal bone. 2. Palate-bone angle: angle between palate and frontal bone.	
Persico, 2010[10]: N = 135, 3D, 16-24 weeks Measured in the exact median plane. Landmarks not specifically defined.		
Vos, 2012: N = 109, 3D, 15-33 weeks Measured from the nasion to the distal end of the white ossification line. Care taken not to include the frontal bone in the measurement.		

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CHAPTER 3

Prenasal thickness-to-nasal bone length ratio: a strong and simple second- and third-trimester marker for trisomy 21

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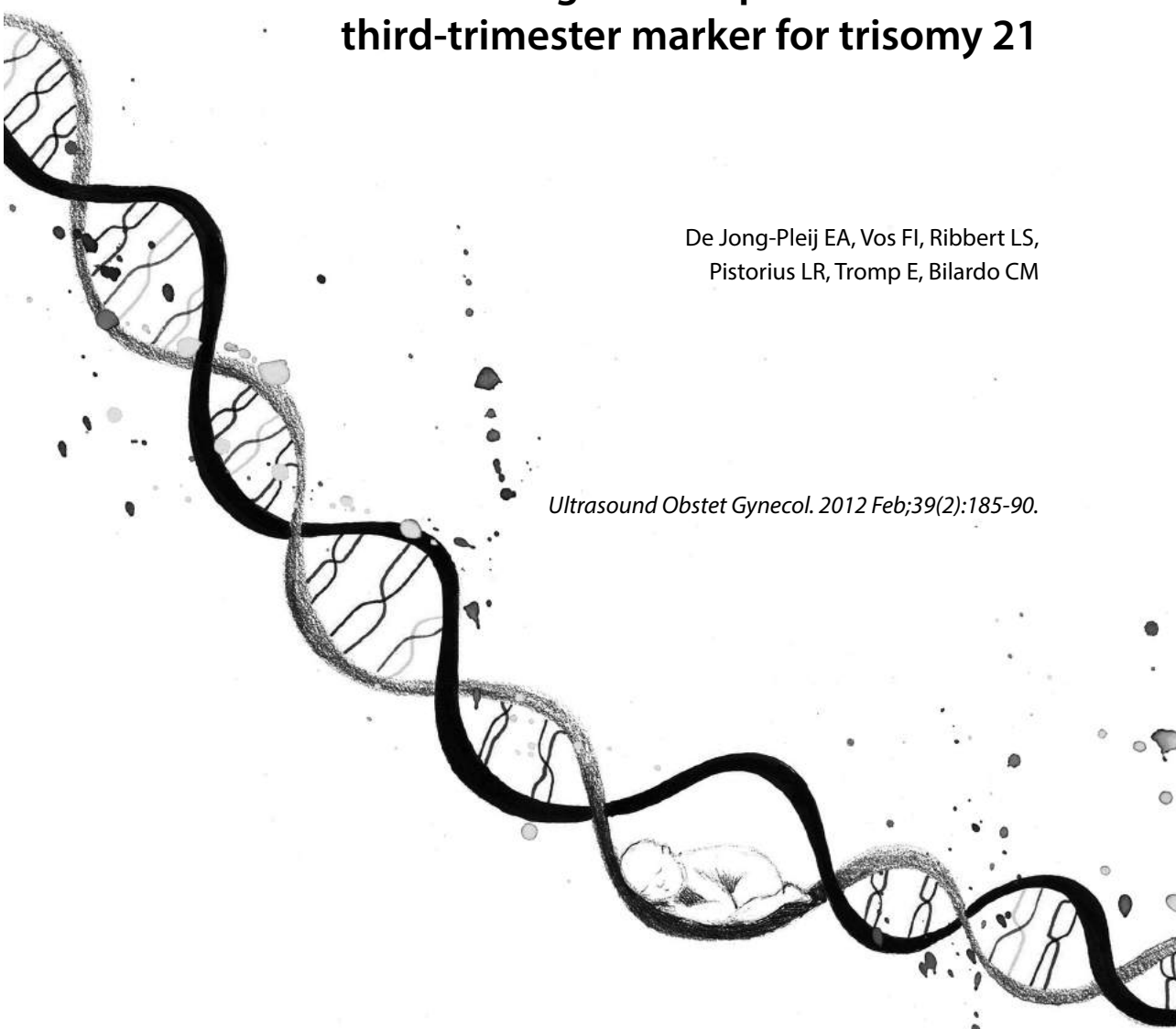

ABSTRACT

Objectives

To study the ratio of prenasal thickness (PT) to nasal bone length (NBL) in euploid and Down syndrome (DS) fetuses in the second and third trimesters of pregnancy.

Methods

The PT and NBL were measured retrospectively in 106 euploid fetuses (in three-dimensional (3D) volumes) and in 30 DS fetuses (10 on two-dimensional (2D) images and 20 in 3D volumes).

Results

In euploid fetuses the mean PT and NBL increased between 15 and 33 weeks' gestation from 2.3 to 6.1 mm ($r = 0.85$, $p < 0.001$) and from 3.3 to 9.6 mm ($r = 0.87$, $p < 0.001$), respectively. The PT-NBL ratio was stable throughout gestation, with a mean of 0.61 (95% CI, 0.59 – 0.63; $r = -0.04$, $p = 0.7$). The 5th and 95th percentiles were 0.48 and 0.80, respectively. In DS fetuses the mean PT and NBL increased between 14 and 34 weeks from 3.0 to 9.2 mm ($r = 0.86$, $p < 0.001$) and from 1.9 to 7.8 mm ($r = 0.85$, $p < 0.001$), respectively. The PT-NBL ratio was significantly higher than in euploid fetuses ($p < 0.001$), but also stable throughout gestation, with a mean of 1.50 (95% CI, 1.20 – 1.80; $r = -0.35$, $p = 0.07$). Twenty-three (77%) of the 30 DS fetuses had a PT above the 95th percentile and 20 (67%) had an NBL below the 5th percentile. All the DS fetuses had a PT-NBL ratio above the 95th percentile. When the 95th percentile of the PT-NBL ratio was used as a cut-off value the detection and false positive rates for DS were 100% (95% CI, 89 – 100%) and 5% (95% CI, 2 – 11%), respectively. The positive likelihood ratio was 21.2.

Conclusions

The PT-NBL ratio is stable in the second and third trimesters of pregnancy in both euploid and DS fetuses, but all DS fetuses in this series had a PT-NBL ratio above the 95th percentile. The ratio is therefore a strong marker for DS.

INTRODUCTION

The word 'syndrome' comes from the Greek 'syn' (together) and 'dramein' (to run) and means 'run together'. A syndrome is suspected when a combination of anomalies or dysmorphic features occur together in the same patient. The more characteristic features are recognized the higher the chance of a syndromal association. Prenatal identification of a syndrome is important, as it may change the management of pregnancy and perinatal care.

A variety of anomalies and dysmorphic traits are known to be associated with Down syndrome (DS)[1,2]. Major structural anomalies like heart defects account for only 27% of affected fetuses[3]. In contrast, more subtle deviations of the phenotype are present in the majority of affected individuals[3-5]. Currently there is overwhelming evidence that the observations reported by J.L.H. Down in 1866 such as a flat profile, a small nose and redundant skin are useful ultrasound markers[2].

Nasal bone length (NBL) was introduced in 1995 by Guis et al[6] as a possible marker for DS, while prenasal thickness (PT) was proposed in 2005 by Maymon et al[7]. Both markers are visualized in the same profile view and even share a landmark, the nasion. Because in DS NBL tends to be smaller while PT tends to be larger than in euploid fetuses, we speculated that their ratio may be a very sensitive and specific indicator for DS.

Recently we showed that three-dimensional (3D) ultrasound enhances the accuracy of facial measurements by enabling definition of the exact midline by multiplanar correction of the volumes[8].

In this study the PT-NBL ratio was evaluated in 3D volumes of second- and third-trimester euploid fetuses and subsequently compared with the PT-NBL ratio of DS fetuses.

METHODS

We retrospectively measured PT and NBL in two groups of patients. The first group comprised 219 fetuses with stored volumes collected cross-sectionally from non-smoking, healthy, low-risk Caucasian women with a singleton pregnancy. Only non-anomalous fetuses from uncomplicated pregnancies were included. Volumes were acquired from fetuses facing the transducer, starting as close as possible from the exact midsagittal profile view during periods of quiescence. An attempt was made to collect at least two such volumes per fetus. For each fetus, the volume with the best midsagittal view was selected. At first, all images were corrected by multiplanar mode to the exact midsagittal view and scored from 1 – 5 in terms of quality for contrast and clarity (quality score; 1 being bad and 5 excellent). Only images with an above-average quality (score 4 or 5) were included. Secondly, PT and NBL were scored from 1 – 3 in terms of visualization of landmarks (measurability score; 1 being bad and 3 excellent). Fetuses with score 1 for PT or NBL were excluded. The second group comprised DS fetuses confirmed by karyotyping. In prenatal databases of the Academical Medical Centre, Amsterdam, University Medical Centre, Utrecht and the Saint Antonius Hospital, Nieuwegein, 39 cases of second- and third-trimester DS fetuses were found, 19 on two-dimensional (2D) images and 20 on 3D volumes. Only images with satisfactory quality and with landmark visualization were included. Transabdominal ultrasonography had been carried out by experienced sonographers using a GE Voluson 730 Expert or E8 ultrasound system equipped with a RAB 2-5L

or RAB 4-8L abdominal transducer (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Images and volumes were stored and examined either off-line on 4D View software version 7.0 (GE Medical Systems) or on stored images in the GE ultrasound system. The nasal bone was measured from the nasion – defined as the most anterior point of the junction between the frontal and nasal bones – to the distal end of the white ossification line (Figure 1). Care was taken not to include the frontal bone in the measurement as the frontal bone extends posteriorly of the nasal bone[9]. PT was measured as the shortest distance between the nasion (same landmark as used for measuring the NBL) and the frontal skin (Figure 1). Calipers were placed on the outermost borders of the skin or bone, and the mean of three measurements was used for analysis. Multiples of the median (MoM) values were calculated using our own regression equation, but absolute values are reported except where indicated.

Data were analyzed using the statistical software SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for Windows 2000. Correlations were calculated by Pearson's correlation test after excluding outliers beyond three SD's from the mean. The statistical significance of the difference of the means of two groups was tested with the unpaired Student's t-test, and $p < 0.05$ was considered statistically significant.

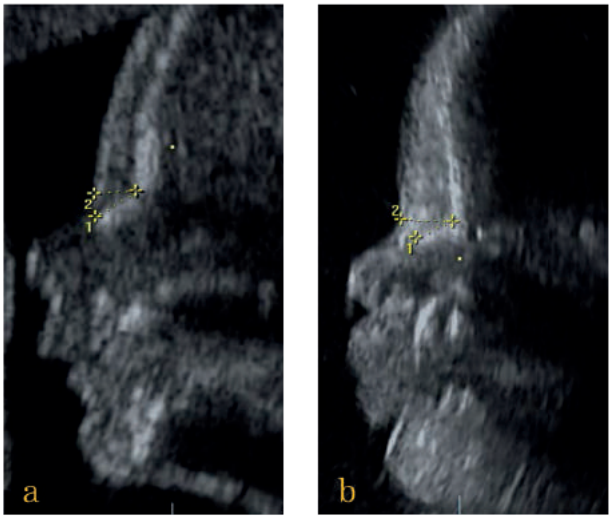

Figure 1 | Ultrasound images of a normal fetus (**a**) and a DS fetus (**b**) showing nasal bone length (caliper 1) and prenasal thickness (caliper 2) measurements.

RESULTS

One hundred and eleven of the 219 volumes had an above-average quality score. Five volumes were excluded because of a measurability score of 1 for PT or NBL. Median maternal age and median gestational age at measurement for the groups are given in Table 1. The median birth weight of the

babies was 3450 (range, 1590 – 4885) g, with 91% of the babies having a birth weight between the 5th and 95th percentiles.

The mean PT and NBL increased between 15 and 33 weeks' gestation from 2.3 to 6.1 mm ($r = 0.85$, $p < 0.001$) and from 3.3 to 9.6 mm ($r = 0.87$, $p < 0.001$), respectively (Table 1; Figures 2 and 3). There was a highly significant positive correlation between PT and NBL ($r = 0.83$, $p < 0.001$) and their MoM values ($r = 0.50$, $p < 0.001$) (Table 1). The PT-NBL ratio was stable throughout gestation, with a mean of 0.61 (95% CI, 0.59 – 0.63 (range, 0.36 – 0.85); SD 0.096; $r = -0.04$, $p = 0.7$) (Table 1). The 5th and 95th percentiles were 0.48 and 0.80, respectively (Figure 4).

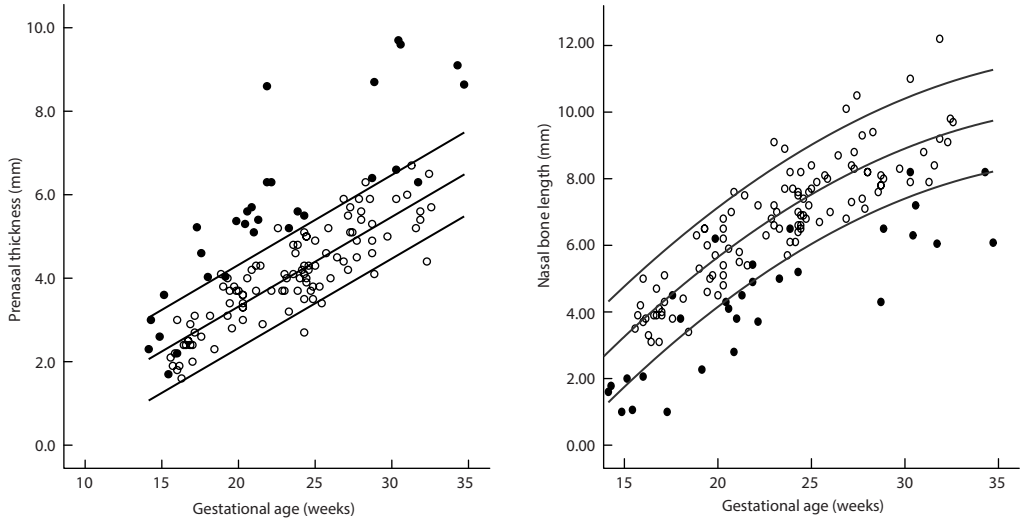

Figure 2 and 3 | Scatterplot of prenasal thickness (PT) and nasal bone length (NBL) against gestational age (GA) for 30 DS fetuses (•) plotted on reference curves (mean, 5th and 95th percentiles) derived from normal fetuses (o) (PT = (0.21 × GA)-0.873; $r = 0.85$, $p < 0.001$), (NBL = –6.927 + (0.830 × GA)-(0.01 × GA^2); $r = 0.87$, $p < 0.001$).

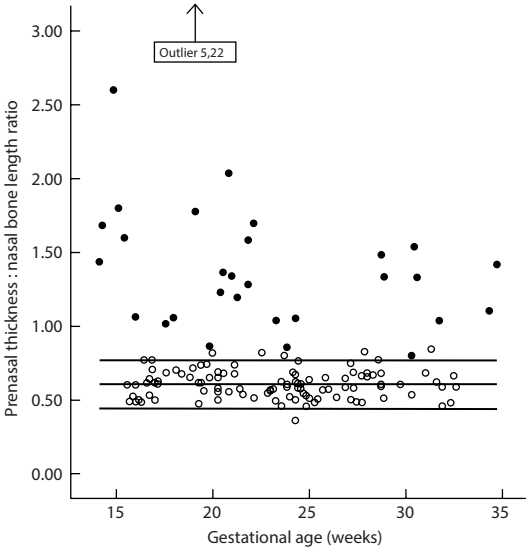

Figure 4 | Scatterplot of prenasal thickness (PT) to nasal bone length (NBL) ratio for 30 DS fetuses (•) plotted on reference curves derived from normal fetuses (o). Mean, 5th and 95th percentile are 0.61, 0.48 and 0.80, respectively.

Nine of the 39 DS fetuses were excluded because of unsatisfactory quality or landmark visualization (all 2D images). Of the remaining 30 DS fetuses, 10 were imaged in 2D, 20 on 3D volumes. The PT, NBL and PT-NBL ratio with the corresponding MoM values for each DS fetus are presented in Table 2. The mean PT and NBL increased between 14 and 34 weeks from 3.0 to 9.2 mm ($r = 0.86$, $p < 0.001$) and from 1.9 to 7.8 mm ($r = 0.85$, $p < 0.001$), respectively. Twenty-three of the 30 (77%) DS fetuses had a PT above the 95th percentile and 20 (67%) had an NBL below the 5th percentile (Figures 2 and 3). In DS fetuses there was a highly significant positive correlation between PT and NBL ($r = 0.81$, $p < 0.001$) whereas the positive correlation between the MoM values did not reach significance ($r = 0.35$, $p = 0.06$) (Table 1). The PT-MoM values did not differ significantly between fetuses with a normal or small (< 5th percentile) NBL (1.51 and 1.42, respectively; $p = 0.47$), whereas the NBL-MoM's between fetuses with a normal or large (> 95th percentile) PT were significantly different (0.72 and 0.48, respectively; $p = 0.003$). In DS fetuses the PT-NBL ratio did not change significantly during gestation, with a mean of 1.50 (95% CI, 1.20 – 1.80 (range, 0.80 – 5.22); $r = -0.35$, $p = 0.07$) (Figure 4). The PT-NBL ratio was significantly higher in DS fetuses ($p < 0.001$). When the 5th and 95th percentiles were used as cut-off values the detection rate, false positive rate and positive likelihood ratio were 100% (95% CI, 89 – 100), 5% (95% CI, 2 – 11)% and 21.2, respectively. Fifteen DS fetuses had both an abnormal PT and NBL, eight had an abnormal PT but normal NBL, five had a normal PT but an abnormal NBL and two fetuses had both PT and NBL within the normal range. However all the DS fetuses had a PT-NBL ratio above the 95th percentile (Figure 4 and Table 1).

Table 2 | Prenasal thickness (PT), nasal bone length (NBL) and PT-NBL ratio with their multiples of the median (MoM) values of the 30 DS cases. GA, gestational age.

GA (weeks)	PT (mm)	PT MoM	NBL (mm)	NBL MoM	PT-NBL ratio	PT-NBL ratio MoM
14+1	2.3	1.10	1.6	0.57	1.44	2.36
14+2	3.0	1.41	1.8	0.62	1.69	2.76
14+6	2.6	1.16	1.0	0.31	2.60	4.26
15+1	3.6	1.56	2.0	0.60	1.80	2.95
15+3	1.7	0.72	1.1	0.30	1.60	2.63
16	2.2	0.88	2.1	0.54	1.07	1.75
17+2	5.2	1.89	1.0	0.23	5.22	8.56
17+4	4.6	1.63	4.5	0.98	1.02	1.68
18	4.0	1.39	3.8	0.80	1.06	1.74
19+1	4.0	1.28	2.3	0.43	1.78	2.92
19+6	5.4	1.63	6.2	1.10	0.87	1.42
20+3	5.3	1.55	4.3	0.73	1.23	2.02
20+4	5.6	1.62	4.1	0.69	1.37	2.24
20+6	5.7	1.63	2.8	0.46	2.04	3.34
21	5.1	1.44	3.8	0.62	1.34	2.20
21+2	5.4	1.50	4.5	0.72	1.20	1.97
21+6	8.6	2.31	5.4	0.84	1.59	2.60
21+6	6.3	1.69	4.9	0.76	1.29	2.11
22+1	6.3	1.67	3.7	0.57	1.70	2.78
23+2	5.2	1.29	5.0	0.72	1.04	1.70
23+6	5.6	1.35	6.5	0.90	0.86	1.41
24+2	5.4	1.28	5.2	0.71	1.04	1.70
28+5	6.4	1.24	4.3	0.50	1.49	2.44
28+6	8.7	1.68	6.5	0.75	1.34	2.19
30+2	6.6	1.20	8.2	0.91	0.80	1.32
30+3	9.7	1.76	6.3	0.69	1.54	2.52
30+4	9.6	1.73	7.2	0.79	1.33	2.19
31+5	6.1	1.04	5.3	0.56	1.14	1.87
34+2	9.1	1.54	8.2	0.87	1.11	1.82
34+5	8.6	1.35	6.1	0.62	1.42	2.33

DISCUSSION

In both euploid and DS fetuses the PT-NBL ratio measured on 3D volumes was stable throughout the second and third trimester, and significantly increased in DS fetuses. When the 95th percentile was used as a cut-off value, the detection rate, false positive rate and positive likelihood ratio were 100% (95% CI, 89 – 100), 5% (95% CI, 2 – 11)% and 21.2, respectively. The PT-NBL ratio therefore qualifies as a strong second- and third-trimester marker for DS. Another important observation is that in euploid fetuses PT is always about 2/3 (0.6) of NBL, a stable relationship that enables easy recognition of normality.

In 1995 Guis et al[6] published a normal range for NBL between 14 and 35 weeks' gestation, and absent nasal bone or hypoplasia of the nasal bone became a widely accepted marker for DS[10-13]. Reference ranges based on a large sample size[14] and on 3D ultrasound have been published[9,15]. In this study screening with NBL achieved a detection rate of 67% for a 5% false-positive rate. Two prospective midtrimester 2D studies, using the 5th percentile as a cut-off value, reported detection rates of 59% and 41%, respectively[16,17]. It is noteworthy that in our study no cases with absent nasal bone were found. Also Bunduki et al[16] and Maymon et al[18] found no absent nasal bones in 22 cases between 16 and 24 weeks and in 25 cases between 15 and 33 weeks, respectively. Cusick et al[19] found only one case of absent nasal bone out of 11 cases studied between 16 and 21 weeks. In other reports absence of nasal bone during the second trimester ranges from 23 to 56%[13,20]. The rigorous selection on image quality, the use of 3D ultrasound and especially the more advanced gestational age are the probable explanation for no cases of absent nasal bone – which would result in a grossly abnormal PT-NBL ratio – being found in our study.

Maymon et al[7] introduced the concept of PT measurement and used PT- and NBL-MoM as a way of enhancing NBL screening performance between 14 and 27 weeks' gestation. In euploid fetuses the PT-NBL ratio was stable at 0.57 and the PT-NBL-MoM in 21 DS fetuses was 1.51. Tables of likelihood ratios based on PT-MoM's were published in 2009[21]. Recently 3D ultrasound-based reference ranges for PT have been constructed[9,22]. Combining second-trimester PT measurement with serum and other markers yields a detection rate comparable with that of first-trimester screening[23].

Our study confirms the diagnostic power of PT measurement. 77% of the 30 DS fetuses had a PT above the 95th percentile, which is similar to the 73% reported in a prospective 3D study by Persico et al[21]. In a meta-analytic study Miguelez et al[23] reported a detection rate of 60% at a 5% false-positive rate.

We found stable PT-NBL ratios in euploid and DS fetuses, but the ratio was significantly higher in the latter. As already mentioned, when 0.8 (the 95th percentile) was used as a cut-off value the sensitivity, specificity and positive likelihood ratio were 100%, 95% and 21.2, respectively. When 1.0 (NBL = PT) was used as the cut-off value the sensitivity and specificity were 90 and 100%, respectively. Maymon et al[7] found a positive likelihood ratio of 13 for a cut-off value of 0.80 for the PT-NBL-MoM. We used absolute values to make recognition of normality simple and the ratio easily applicable in routine settings. Although the results need to be validated by a large prospective study, the PT-NBL ratio appears to be an excellent second- and third-trimester screening test.

In this study 10 DS fetuses were measured with 2D ultrasound although our reference ranges were based on 3D ultrasound. It is known that NBL measurements, obtained by 2D ultrasound, tend to be larger than those obtained by 3D ultrasound[9,15] and that this modality-derived difference happens less for PT[15,22]. Therefore the PT-NBL ratios of the DS fetuses would probably have been even higher had 3D ultrasound been used in all cases.

The ratio shows a better screening performance than does NBL or PT alone. However for risk calculations, the sequential use of the two markers (with two likelihood ratios) may yield better results than combining the two measurements into one ratio (with one likelihood ratio)[7]. However for sequential use it is important that the markers are independent.

In DS interdependency of the two markers is supported by the theory that accumulation of hyaluronic acid (related to chromosome 21 gene-related overexpression of collagen type VI) in the dermis is responsible for excessive hydration of the extracellular matrix. This causes increased skin thickness and may at the same time influence intramembranous ossification of the nasal bone[24-26]. Another theory, suggesting that delayed migration of the neural crest cells alters the membranous ossification of the nasal bones, supports independency of the two markers[27].

Persico et al[22] found no significant difference in delta PT between DS fetuses with and without a nasal bone. Similarly, in this study PT-MoM's were not different between the DS fetuses with a normal or small NBL. Also, the non-significant correlation between PT-MoM and NBL-MoM of DS fetuses indicates independency of the two markers. However the finding of significantly different NBL-MoM's in fetuses with a normal or increased PT contradicts this assumption. Therefore more data are needed to clarify the relationship between the two markers.

In conclusion, the PT-NBL ratio is stable in the second and third trimesters in euploid and DS fetuses. In euploid fetuses PT is consistently about 2/3 of the NBL. All DS fetuses in this series had a PT-NBL ratio above the 95th percentile. The stability and high sensitivity make this ratio a powerful screening tool for DS.

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CHAPTER 4

The facial profile of Down syndrome fetuses in the second and third trimester of pregnancy

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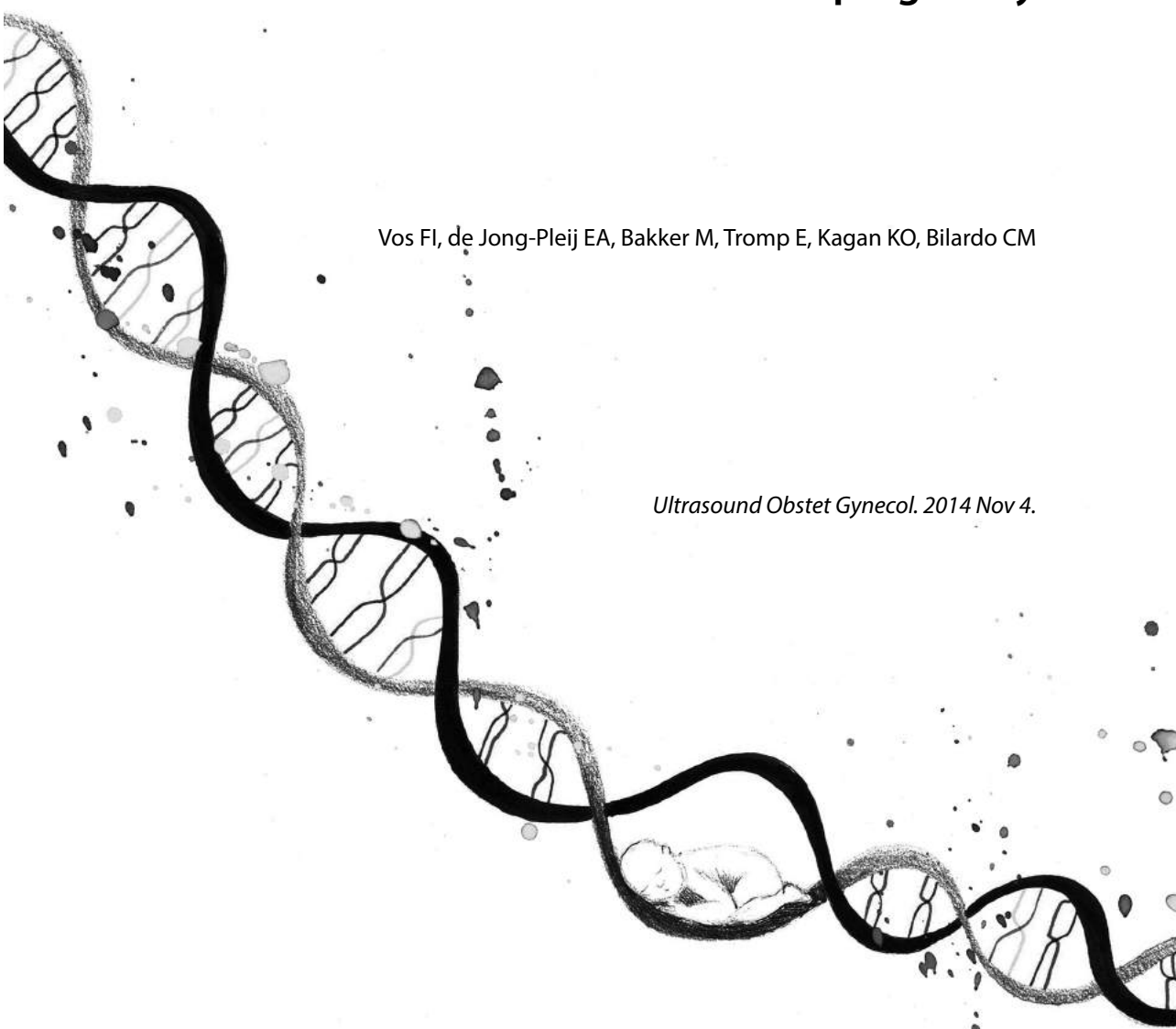

ABSTRACT

Objectives

To investigate the maxilla nasion mandible angle (MNM angle) and fetal profile line (FP line) as methods to assess the degree of midfacial hypoplasia in Down syndrome (DS) fetuses in the second and third trimester of pregnancy.

Methods

The MNM angle and FP line were measured retrospectively in stored 2D pictures or 3D volumes of DS fetuses, corrected to the midsagittal plane. Data, collected from January 2006 to July 2013, were retrieved from the digital databases of the University Medical Centre Utrecht, the Fetal Medicine Unit of the University Medical Centre Groningen and of the Department of Obstetrics and Gynaecology of the University Hospital Tübingen. The MNM angle was expressed in continues values (degrees) and the FP line as positive, negative or zero. Measurements were performed on the stored images by 2 experienced examiners and compared to our previously reported normal ranges. An MNM angle below the 5th percentile of the reference range and a positive or negative FP line were considered abnormal.

Results

A total of 133 Down syndrome fetuses were analyzed. The MNM angle was not influenced by the gestational age ($p = 0.48$) and was significantly smaller in DS fetuses than in euploid fetuses (mean, 12.90; $p = 0.015$). The MNM angle was below the 5th percentile in 16.9% of DS fetuses ($p < 0.01$). In the cohort of DS fetuses, a positive FP line was present in 41.2% of cases (with a false positive rate of 6.3%) and was positively correlated to DS and gestational age ($p < 0.01$). There was no case with a negative FP line. In DS, a positive FP line was correlated with a small MNM angle ($p < 0.01$).

Conclusions

A small MNM angle and a positive FP line can be regarded as novel markers for DS. The FP line is an easy to use marker with a low false positive rate, not requiring knowledge of reference values and the potential to differentiate between DS and trisomy 18, as in the latter the FP line is often negative.

INTRODUCTION

Individuals affected by Down syndrome (DS) are known to have specific facial features[1]. In adult life[2,3] especially the flattened convexity of the profile has been quantified in these individuals. In fetal life these typical craniofacial features[4] have been transformed in measurable markers to improve the detection of DS in pregnancy[5-7]. A short nasal bone length (NBL), for instance, increases the odds of DS by 6- to 7-fold[8]. Prenasal thickness (PT) is above the 95th percentile in about 65% – 75% of the DS cases[9,10].

More recently introduced markers are the prenasal thickness to nasal bone length (PT-NBL) ratio and the prefrontal space ratio (PFSR), both showing detection rates for DS over 80%[7,9].

Recently, we have described two new methods to assess the relationship between mandible and maxilla: the maxilla-nasion-mandible (MNM) angle[11] and the fetal profile (FP) line[12].

The MNM angle, defined as the angle between the maxilla-nasion and mandible-nasion line, is constant at about 13.5 degrees (°) throughout pregnancy, whereas in 3 fetuses with Down syndrome the angle was much smaller (8.2° – 11.2°)[11].

The FP line, consisting of a line that passes through the midpoint of the anterior border of the mandible and the nasion, is always zero or positive in euploid fetuses[12] and often negative in trisomy 18 fetuses[13].

As DS fetuses tend to have midfacial hypoplasia and a rounded forehead, both measurements may be altered in prenatal life.

The aim of this study was to assess whether these two measurements can identify DS fetuses in the second and third trimester of pregnancy.

METHODS

Data were retrieved from the databases of the Fetal Medicine Unit of the following centers: the University Medical Centre Groningen, the University Medical Centre Utrecht and the Eberhard-Karls-Universität Tübingen. The indications for referral to these specialized centers were various: abnormal first trimester serum screening or ultrasound and abnormal second trimester ultrasound findings being the most common. The databases were searched from January 2006 to July 2013 for second and third trimester ultrasound investigations in DS cases from Caucasian parents, confirmed pre- or postnatally by karyotyping.

All ultrasound examinations were performed by experienced sonographers and images were obtained by a General Electric Voluson 730 Expert ultrasound or E8 system equipped with a RAB4-8L probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Images and volumes were stored and examined either on stored images in the General Electric ultrasound system or offline with 4D View software version 7.0 (GE Medical Systems, Kretz Ultrasound, Zipf, Austria).

Only good midsagittal pictures of the fetal profile were selected and considered for further analysis; we considered as such profile pictures showing the forehead, nose, lips and chin and the maxilla as a single horizontal line without the processus frontalis maxillae. Pictures with a visible

zygomatic bone or ramus of the mandible were excluded. For examination, the ultrasound image of the fetal head was enlarged to a maximal image of the fetal profile. In cases where 3D volumes were available, the multiplanar mode was used to depict the exact median plane to improve measurement accuracy[14]. The MNM angle was defined as the angle between the lines maxilla-nasion and mandible-nasion in the median plane (Figure 1). The nasion is defined as the most anterior point at the intersection of the frontal and nasal bone. Jaw landmarks were defined as the middle points of the anterior borders of the maxilla and mandible. When there was a gap between the nasal bone and frontal bone, the landmark nasion was at the point of intersection between the lines tangential to the nasal bone and tangential to the lower part of the frontal bone.

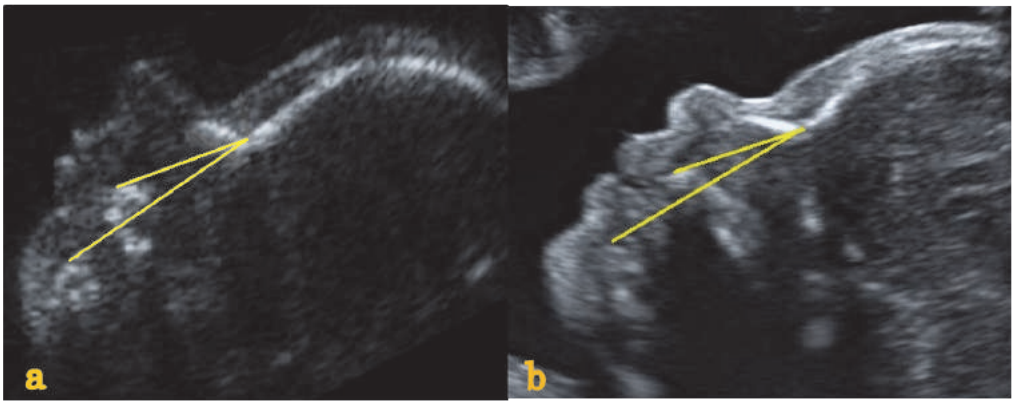

Figure 1 | The MNM angle (**a**) in a euploid fetus at 24+6 weeks gestation and (**b**) in a fetus with Down syndrome at 28+2 weeks gestation.

The FP line was defined as the line that passes through the middle point of the anterior border of the mandible and the nasion. When the FP line passed lengthwise through the frontal bone, this was called 'zero' (Figure 2). When the FP line passed the frontal bone posteriorly, its position was called 'positive'. When the FP line passed the frontal bone anteriorly its position was called 'negative'. The distance between the FP line and the frontal bone was measured perpendicular to the FP line.

For all measurements, calipers were placed on the outermost borders of the skin or bone. The MNM angle and FP line were measured in the same plane.

The FP line and MNM angle values were compared to the reference values derived from our previous reports[11,12], based on 3D measurements of normal fetuses. In the study of the MNM angle in euploid fetuses[12], the MNM angle was not correlated to gestational age and constant throughout gestation (GA) with a mean of 13.53° (5th and 95th percentile were 10.39° and 16.91°, respectively). When studying the FP line in euploid fetuses[11], there were no cases with a negative FP line. The FP line was zero in 93.7% of cases and positive in 6.3%; the latter never occurred before 27 weeks' GA.

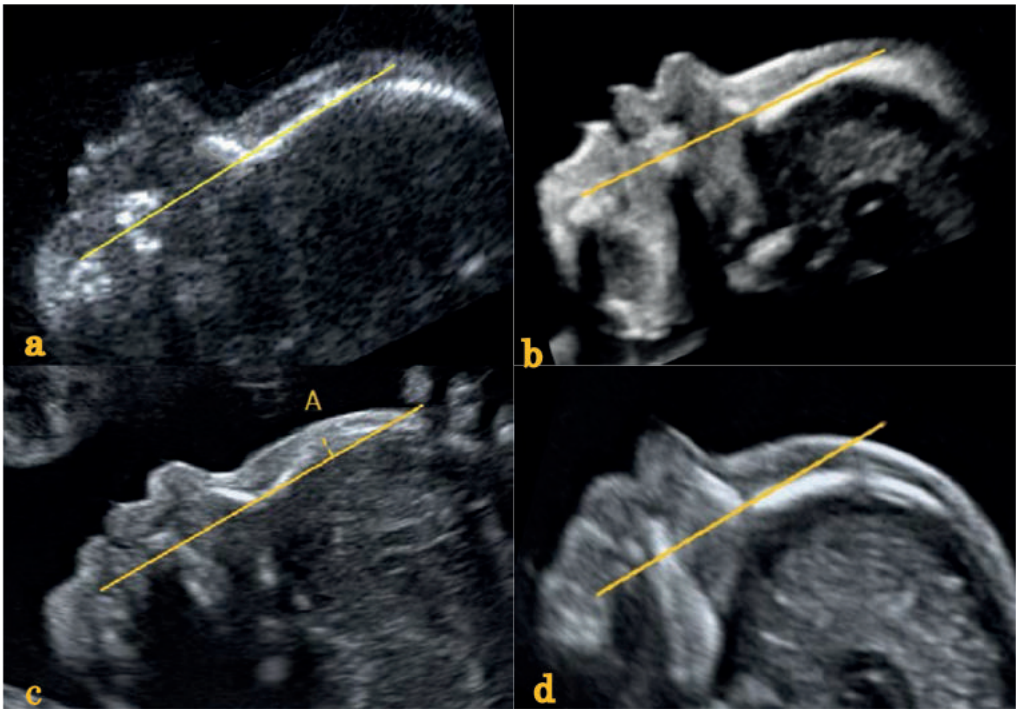

Figure 2 | (**a**) FP line position 'zero' in a euploid fetus at 24+6 weeks gestation, (**b**) FP line position 'zero' in a Down syndrome fetus at 21+3 weeks gestation, (**c**) FP line position 'positive' in a Down syndrome fetus at 28+2 weeks gestation. (A) the distance between FP line and frontal bone, (**d**) FP line position 'negative' in a Edwards syndrome fetus (also known as trisomy 18)at 23+5 weeks gestation.

We compared the results of these two new DS markers with the performance of our previously published markers NBL, PT, PT-NBL ratio and PFSR[9].

For the MNM angle we defined abnormal measurements as being below the 5th or above the 95th percentile of the reference range, whereas the FP line was considered abnormal when it was not zero (positive or negative)[11].

Intra- and inter-observer variability was assessed by Bland-Altman analysis and intraclass correlation coefficient (ICC). Reproducibility of the measurements was assessed in all cases, using stored images (or volumes when available). Markers were measured by two examiners (F.I.V. and E.J.P.), who were blinded to gestational age and to previous measurements, but not to karyotype. Images were chosen at random at different gestational ages, with at least 3 weeks between the assessments. Means with ranges or SD were calculated when appropriate.

Correlations were calculated by Pearson's correlation test and relationship with gestational age by regression analysis. The statistical significance of the difference of the means of two groups was tested with the students t-test. A P-value <0.05 was considered significant. Data were analyzed using the statistical software SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for Windows 2000.

RESULTS

A total of 133 Down syndrome fetuses were included. The median maternal age was 35.8 years (range 23- 46), the median GA was 22+6 (range 14 -38) weeks. Seven cases were excluded because the mandible was not visible, and one image because both the mandible and maxilla were not visible.

The results of the intra- and interobserver variability of measurements are reported in table 1.

Table 1 | Intra- and interobserver variability in Down syndrome fetuses for the MNM angle and FP line. Note that it was not possible to calculate mean differences and LOA for the FP line, as it had a non-continuous outcome. LOA; limits of agreement, ICC; intraclass correlation coefficient.

	Intraobserver variability			Interobserver variability		
	Mean difference *(SD)*	**LOA *(95% CI)***	**ICC**	**Mean difference *(SD)***	**LOA *(95% CI)***	**ICC**
FP line	_	_	1.00	_	_	0.76
MNM angle	-0.37 *(1.16)*	2.68 *(2.0 – 3.4)* -1.94 *(-2.6 – 1.3)*	0.89	-0.57 *(0.57)*	4.92 *(3.7 – 6.2)* -3.78 *(-5.2 – 2.5)*	0.61

The MNM angle was significantly smaller in DS fetuses than in euploid fetuses (mean, 12.90°; SD, 2.84; range, 3.90° – 20.30°, versus mean, 13.53°; SD, 2.00; range, 9.0° – 19.6°, p = 0.015). In comparison with euploid fetuses, 16.8% of DS fetuses had an MNM angle below the 5th percentile (p < 0.01; table 2, figure 3). The MNM was not influenced by the GA (p = 0.48).

In the cohort, no DS fetus had a negative FP line. In DS, the FP line was zero in 73 fetuses (58.4%) and positive in 52 (41.6%, table 3, figure 4).

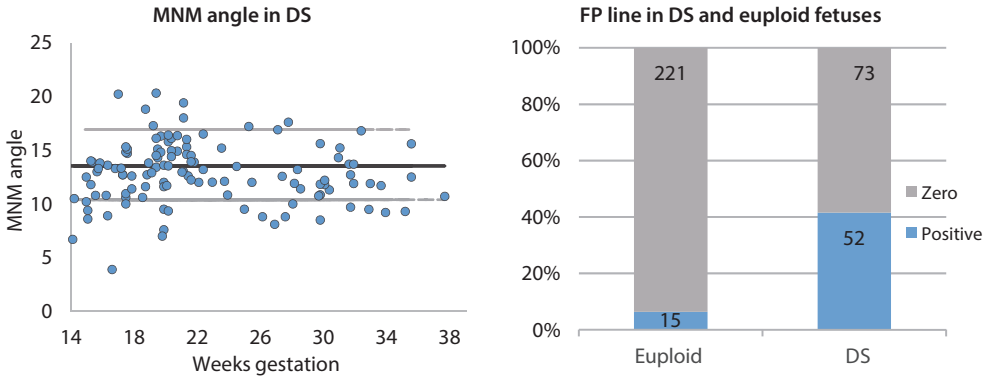

Figure 3 and 4 | The MNM angle and FP line in 125 Down syndrome fetuses compared to euploid fetuses (mean, 5th percentile and 95th percentile for the MNM angle).

A positive FP line was positively correlated with DS and advancing GA ($p < 0.001$). The FP line was never positive in second trimester euploid fetuses[11], which means that a positive FP line in the second trimester has a detection rate (or sensitivity) for DS of 28.4% with a corresponding false positive rate (FPR) of 0%. In the third trimester, the FP line was far more often positive in both DS and euploid fetuses, increasing the detection rate (DR) of a positive FP line for DS to 76.5%, and the FPR to 16.9% (Table 3).

Overall, the distance between the FP line and the frontal bone was not significantly larger in DS fetuses than in euploid fetuses ($p = 0.4$).

Table 3 | For the FP line, a distinction was made into gestational cohorts (second and third trimester) as there was a strong increase in a positive FP line after the second trimester in both Down syndrome and euploid fetuses. Sensitivity was defined as a positive FP line. FPR; false positive rate, PLR; positive likelihood ratio, NLR; negative likelihood ratio, ∞; infinite.

FP line	Sensitivity *(95% CI)*	Specificity *(95% CI)*	FPR *(95% CI)*	PLR *(95% CI)*	NLR *(95% CI)*
Second trimester (14 – 27 weeks GA, n = 90)	28.4% *(19% – 38%)*	100% *(98% – 100%)*	0% *(0% – 2%)*	∞	0.7 *(0.6 – 0.8)*
Third trimester (≥ 27 weeks GA, n = 35)	76.5% *(57% – 87%)*	83.1% *(73% – 90%)*	16.9% *(10% – 6%)*	4.5 *(2.7 – 7.3)*	0.3 *(0.2 – 0.5)*
Combined (n = 125)	41.6% *(33% – 51%)*	93.7% *(89% – 96%)*	6.4% *(4% – 10%)*	6.5 *(3.4 – 11.2)*	0.6 *(0.5 – 0.7)*

The MNM angle was negatively correlated to the FP line ($r = -0.29$, $p < 0.01$) and the detection rates of the two markers were correlated (kappa 0.19, $p = 0.01$) meaning that a small MNM angle is correlated with a positive FP line. The mean difference in MNM angle between fetuses with a positive or zero FP line, was 1.7°. A positive FP line was found in 14 of 21 fetuses with an MNM angle below the 5th percentile (66%) and in 2 of 8 cases with an MNM angle above the 95th percentile (25%). At least one of both markers was abnormal in 47.2% (at a 9.3% FPR).

DISCUSSION

In this study we investigate two new potential second trimester DS markers. We have shown that DS fetuses tend to have a significantly smaller MNM angle and a positive FP line. However, only 16.9% of DS fetuses in this cohort had an MNM angle below the 5th percentile, and 42% had a positive FP line. The latter is particularly interesting, considering that in the second trimester none of the euploid fetuses had a positive FP line (0% FPR). In the third trimester the DR of a positive FP line increases to 75%, however at the cost of a higher FPR (16.9%).

Table 2 | Analysis of the MNM angle and FP line, compared to the outcome of 4 other markers for Down syndrome, assessed in our previous study[9].

	Sensitivity *(95% CI)*	**Specificity** *(95% CI)*	**FPR** *(95% CI)*	**PLR** *(95% CI)*	**NLR** *(95% CI)*	**PPV** *(95% CI)*	**NPV** *(95% CI)*
MNM angle	16.8% *(10.8% – 24.7%)*	95.0% *(92.9% – 98.2%)*	5.0% *(1.7% – 11.3%)*	4.42 *(2.1 – 9.4)*	0.86 *(0.79 – 0.94)*	70.0% *(50.6% – 85.2%)*	68.7% *(63.4% – 73.6%)*
FP line	41.6% *(33.3% – 51.2 %)*	93.7% *(89.8% – 96.4%)*	6.3% *(3.7% – 10.4%)*	6.49 *(3.4 – 11.1)*	0.63 *(0.54 – 0.72)*	77.6% *(65.8% – 86.9%)*	75.3% *(69.9% – 80.1%)*
NBL	61.9% (*53.4% – 69.9 %)*	95.0% *(92.9% – 98.2%)*	5.0% *(1.7% – 11.3%)*	12.32 *(5.2 – 29.4)*	0.40 *(0.31 – 0.52)*	94.74% *(88.1% – 98.2%)*	62.91% *(54.7% – 70.6%)*
PT	63.4% *(53.4% – 73.1%)*	95.0% *(92.9% – 98.2%)*	5.0% *(1.7% – 11.3%)*	12.73 *(5.4 – 30.3)*	0.38 *(0.29 – 0.50)*	94.85% *(88.4% – 98.2%)*	64.19% *(55.9% – 71.8%)*
PT-NBL ratio	86.2% (*79.3% – 91.2%)*	95.0% *(92.9% – 98.2%)*	5.0% *(1.7% – 11.3%)*	17.37 *(7.4 – 41.0)*	0.14 *(0.08 – 0.23)*	96.15% *(91.3% – 98.7%)*	82.6% *(74.4% – 89.0%)*
PFSR	79.7% *(71.6% – 86.0%)*	95.0% *(92.9% – 98.2%)*	5.0% *(1.7% – 11.3%)*	15.96 *(6.8 – 37.7)*	0.21 *(0.14 – 0.32)*	95.50% *(89.8% – 98.5%)*	77.87% *(69.5% – 84.8%)*

MNM angle; maxilla-nasion-mandible angle, FP line; fetal profile line, NBL; nasal bone length, PT; prenasal thickness, PT-NBL ratio; prenasal thickness to nasal bone length ratio, PFSR; prefrontal space ratio, DS; Down syndrome, FPR; false positive rate, PLR; positive likelihood ratio, NLR; negative likelihood ratio, PPV; positive predictive value, NPV; negative predictive value.

In recent literature, attempts have been made to develop markers that objectify the flat profile of DS fetuses, such as the fronto-maxillary facial angle[6,15,16]. Yazdi et al[17] found first trimester DS fetuses to have a larger frontal space measurement (distance between mandibulo-maxillary (MM) line and forehead, measured in the sagittal plane) rather than euploid fetuses. The same group investigated the PFSR in the second trimester[7] and noted that the MM line crosses the forehead posteriorly in 24.2% of DS and 0% of euploid fetuses.

The anatomical position of the fetal mandible or maxilla during the second half of pregnancy has been quantified by a number of angles: the sella-mandibular and sella-maxillary angle[18], the inferior facial angle[19] and, more recently, the MNM angle[11]. These angles are independent of GA. Of these angles, only the inferior facial angle has been investigated in (8) DS fetuses, but no relationship was found[19].

A positive FP line can be caused by a protrusion of the mandible and relative hypoplasia of the nasion (for which reason we were not surprised to find small MNM angles correlated to a positive FP line) combined with the degree of curvature of the frontal bone. However, mandibular protrusion is extremely rare prenatally. The increasing proportion of DS fetuses with a positive FP line with advancing GA was striking. In many DS cases, the growth of the face is disproportional compared to that of the skull. This, and the natural tendency of the forehead to become rounder with advancing gestation result in a higher percentage of a positive FP line in the third trimester. In our recent study[13] of fetuses with Edwards syndrome (ES; also known as trisomy 18), we found the FP line to follow an opposite trend; the FP line was negative in 46.3% of the cases, whilst this was never the case in euploid or DS fetuses. The PT-NBL ratio and PFSR however showed similar trends in ES and DS fetuses. The distance between the FP line and the frontal bone was not different between DS and euploid fetuses and is therefore not of diagnostic value.

The MNM angle does not appear to be a strong DS marker. The MNM angle was below the 5th and above the 95th percentile in 16.9% and 6.5% of DS fetuses, respectively. This suggests that DS fetuses have a wider range in the MNM angle than euploid fetuses. This confirms the finding of Rotten et al[19], where 25% of DS fetuses had an inferior facial angle below the 5th percentile, but 62.5% of angles were above the 50th percentile. A possible explanation for this finding could be that in DS, next to maxillary hypoplasia, there is also mandible hypoplasia[2,3], though in a lesser extent. In this case, the position of both mandible and maxilla could be altered, and this may not be expressed optimally by the MNM angle, as the angle between the two would remain unchanged. This is the first study on the relationship between mandible and maxilla in DS fetuses. Another explanation for the low DR of the MNM angle may be that we are comparing mostly 2D images in DS fetuses with normative data in euploid fetuses, derived from 3D images. In a previous study we found that the MNM angle is significantly larger (by 1.0 degree) when measured on 2D images, whereas the FP line is not influenced by the acquisition method[20]. A limitation of this study is its retrospective nature and the fact that examiners were not blind to karyotype. Another possible bias may be the fact that pregnancies were mostly referred to the Fetal Medicine Units owing to abnormal ultrasound findings. It is possible that dysmorphic facial features are more pronounced in DS fetuses with obvious ultrasound anomalies.

Ideally, in a proper repeatability and reproducibility study also the acquisition of the volume should be repeated. However, a prospective study would require a long inclusion time. We chose instead for a retrospective design including many DS cases giving more statistical power. This has prevented a reproducibility study including volume re-acquisition. The reproducibility figures are therefore assessing the measurement error only. DS screening is preferably carried out in the first trimester in the form of the combined test (CT) because of its superior performance (over 87% at a 5% FPR[21]) but mostly because of ethical and medical reasons.

In this cohort of women carrying a DS fetus, first trimester screening was not performed. It is therefore impossible to calculate the additional value of the MNM angle and FP line as sequential screening. However, we can speculate that in case all the 125 pregnancies included in the study had undergone first trimester screening, at least 109 cases (87%[21]) could have been theoretically detected. In this cohort, the use of the FP line, MNM angle or both (with its DR of 42%, 17% and 47%, respectively) may have led to the additional detection of 7, 2 or 8 cases, respectively. As can be seen in table 2, we have demonstrated that other second trimester facial markers like the PT-NBL ratio and PFSR have a higher detection rate than the MNM angle and FP line (94% for the PT-NBL ratio and PFSR combined[9]). In fact, the use of these novel markers detected only one additional case. However, the FP line has a FPR close to 0% and has the advantage of being useful in identifying other conditions affecting the fetal profile, such as ES in the absence of structural anomalies[13].

In conclusion, in this study we describe the use of the FP line as a simple and novel additional marker for DS with a virtually absent FPR in the second trimester.

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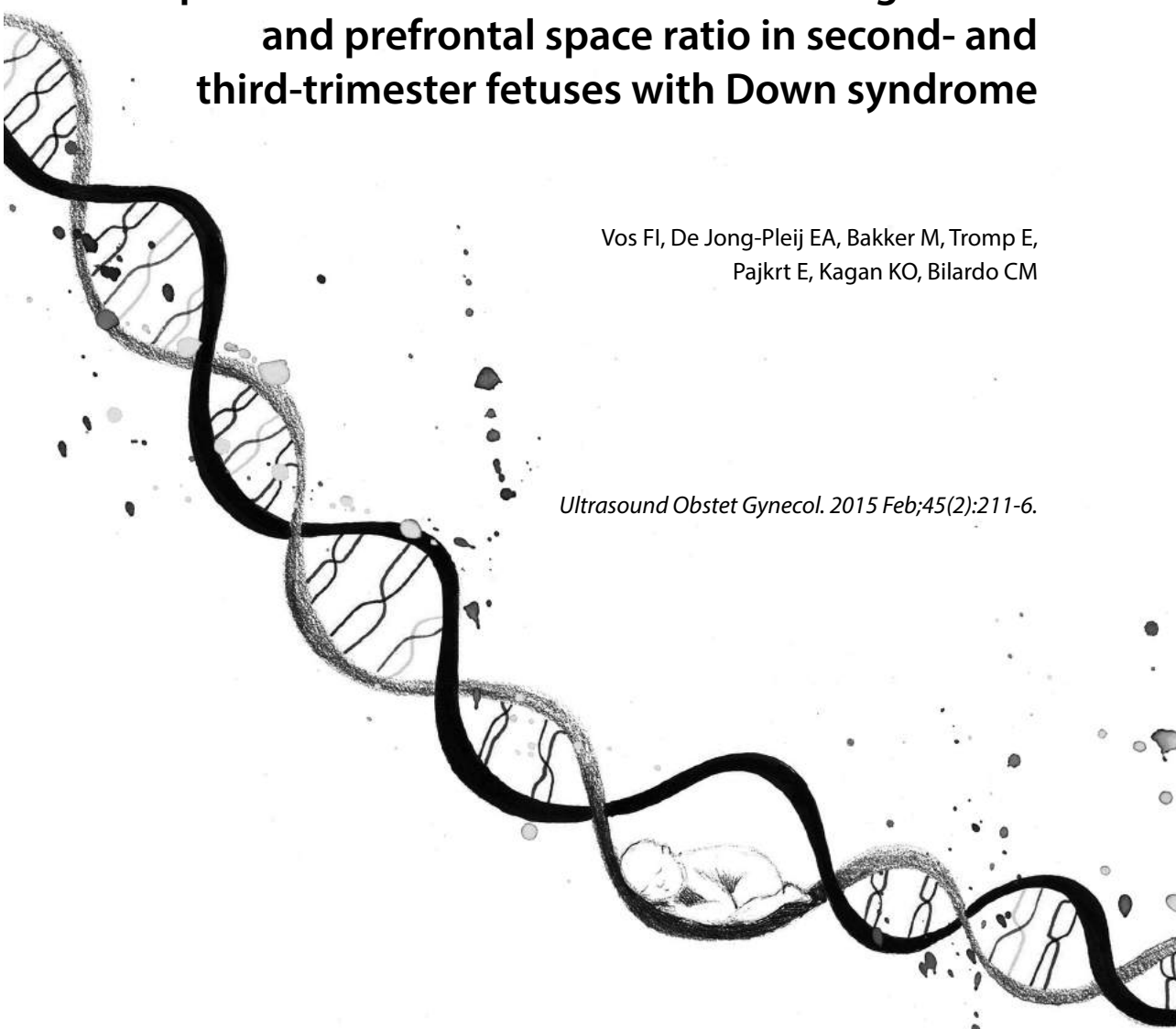
CHAPTER 5

Nasal bone length, prenasal thickness, prenasal thickness-to-nasal bone length ratio and prefrontal space ratio in second- and third-trimester fetuses with Down syndrome

Vos FI, De Jong-Pleij EA, Bakker M, Tromp E,
Pajkrt E, Kagan KO, Bilardo CM

Ultrasound Obstet Gynecol. 2015 Feb;45(2):211-6.



ABSTRACT

Objectives

To evaluate nasal bone length (NBL), prenasal thickness (PT), prenasal thickness-to-nasal bone length (PT-NBL) ratio and prefrontal space ratio (PFSR) as markers for Down syndrome (DS) in the second and third trimesters.

Methods

NBL, PT, PT-NBL ratio and PFSR were measured retrospectively in stored two-dimensional images or three-dimensional volumes (corrected to the mid-sagittal plane) of fetuses with Down syndrome, which were retrieved from the digital databases of participating units. Measurements were performed on the stored images and volumes by two experienced operators, and the values obtained were compared to our previously reported normal ranges for euploid fetuses in order to assess the detection rates for Down syndrome.

Results

A total of 159 fetuses with DS were included in the analysis, six of which were excluded because of inadequate available images. Median maternal age was 36.0 years and median gestational age 23 + 1 weeks. NBL and PT were correlated with gestational age ($P < 0.001$), but the PT-NBL ratio and PFSR were not. Mean NBL, PT, PT-NBL ratio and PFSR were 4.42 mm, 5.56 mm, 1.26 and 0.34, respectively. The nasal bone was absent in 23 (15.4%) cases. As a marker for Down syndrome, the PT-NBL ratio yielded the highest detection rate (86.2%), followed by PFSR (79.7%), PT (63.4%) and NBL (61.9%). All markers were abnormal in 33.6% of cases, whilst all were normal in 4.7%. At least one of the four markers was abnormal in 95.3%, and either the PT-NBL ratio or PFSR was abnormal in 93.8%. Detection rates were not related to gestational age.

Conclusions

The PT-NBL ratio and PFSR are robust second- and third-trimester markers for Down syndrome. Both provide high detection rates and are easy to use, as the cut-off for normality is constant throughout gestation.

INTRODUCTION

In 1866, Langdon Down first described the typical facial features of those affected by the syndrome that received his name[1]: a flat profile, small nose and redundant skin. These typical features, detectable in the profile of fetuses with Down syndrome, are currently used as ultrasound markers for this condition in first- and second-trimester screening. These profile markers are based on the fact that fetuses with Down syndrome (DS) are characterized by different degrees of mid-facial hypoplasia and skin edema[2,3]. Hypoplastic or absent nasal bones, reduced convexity of the fetal profile and thickened skin in the nuchal and prefrontal areas[2-6] have been confirmed in fetuses with DS by postnatal pathological reports and X-ray imaging[7,8]. Prenatally, these features can be quantified by measuring them as fetal profile parameters.

Screening for DS usually is performed in the first trimester of pregnancy, during which nuchal translucency thickness and visualization of the nasal bone are sensitive ultrasound markers[9]. However, in cases in which first-trimester screening is not performed, it is important to define effective second- and third-trimester markers. Fetal profile or neck markers currently are considered by far the most predictive in comparison with other ultrasound markers for Down syndrome, e.g. short femur and humerus, echogenic cardiac focus and hyperechogenic bowel[4,5].

Bony markers such as nasal bone length (NBL) and the frontomaxillary facial angle allow assessment of mid-facial hypoplasia[2,10,11], while prenasal thickness (PT) and nuchal fold measurements allow assessment of skin thickness[3,6]. More recently, combined markers have been proposed, such as the prenasal thickness-to-nasal bone length (PT-NBL) ratio and the prefrontal space ratio (PFSR), which appear to be superior to single markers[12,13]. The theoretical advantage of the PT-NBL ratio and PFSR is that the measurements used to generate these are affected by both thickness of the skin of the forehead and the respective degrees of nasal and mid-facial hypoplasia. Normal reference ranges for these combined markers have also been assessed[12-14].

Despite many reports in the literature concerning single or multiple markers, measured in cohorts of various sizes, a large and comprehensive study in which all profile markers are measured in the same fetus was lacking. The aim of this retrospective study was to assess the performance and interrelation of four fetal profile markers for DS (NBL, PT, PT-NBL ratio and PFSR) measured in the same fetus on images acquired at second- and third-trimester ultrasound examination.

METHODS

Ultrasound records for the study were retrieved from the digital databases of the Fetal Medicine Unit of the following centers: University Medical Centre, Groningen, The Netherlands; Academic Medical Centre, Amsterdam, The Netherlands (until March 2010); University Medical Centre, Utrecht, The Netherlands and the Department of Obstetrics and Gynecology, University Hospital Tübingen, Tübingen, Germany. A search was undertaken for second- and third-trimester midsagittal two-dimensional (2D) ultrasound images or three-dimensional (3D) stored volumes of the profile of fetuses with DS seen between January 2006 and July 2013 at one of the participating institutions.

The diagnosis was confirmed in all cases by prenatal or postnatal karyotyping. Only fetuses of Caucasian parents were included. Ultrasound examinations were performed using GE Voluson 730 Expert ultrasound or E8 systems equipped with a RAB4-8L probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Images and volumes were stored and examined either offline on 4D View software version 7.0 (GE Medical Systems) or on stored images in the GE ultrasound system.

Only good mid-sagittal images of the fetal profile were selected and considered for further analysis; we required that images show the forehead, nose, lips and chin, with the maxilla visible as a single horizontal line without appearance of zygomatic bone. Images with a visible zygomatic bone or ramus of the mandibula were excluded. To perform the measurement, stored 2D ultrasound images of the fetal profile were magnified to fill the entire monitor. In cases for which 3D volumes were available, the multiplanar mode was used to depict the exact median plane of the fetal profile.

The NBL was measured from the nasion to the end of the white distal ossification line (B in Figure 1). The nasion was defined as the most anterior point at the junction between the frontal and nasal bones. Care was taken to avoid adding part of the frontal bone to the measurement[11]. In cases in which there was a gap between the nasal and the frontal bones (disjunction), NBL was measured from the distal to the proximal end of the ossification line. To determine the PFSR, the maxilla-mandible line (MM line) was drawn between the midpoint of the anterior edge of the mandible and the anterior edge of the maxilla. The line was then extended cranially towards the forehead. Subsequently, the skin covering the forehead (C in Figure 1) was measured between the anterior edge of the bony forehead and the anterior edge of the skin in a line parallel to the maxilla and traced from the nasion; this measurement is called prenasal thickness (PT). A second measurement (d) (D in Figure 1), was taken from the anterior edge of the skin (where PT measurement ended) to the point of intercept with the MM line. For cases in which the MM line crossed the prenasal skin posteriorly, PT was measured between the frontal bone and the skin, but d was measured between the MM line and the skin and multiplied by -1. The PFSR was determined by dividing d by PT, and the PT-NBL ratio was calculated by dividing PT by NBL.

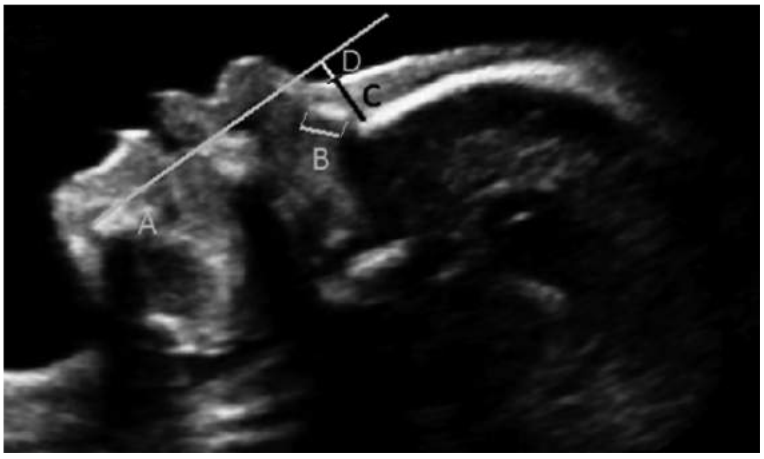


Figure 1 | Ultrasound image of a fetus with Down syndrome at 21+3 weeks' gestation, showing the maxilla–mandible line (**A**), nasal bone length (**B**), prenasal thickness (**C**) and the 'd' measurement (**D**). The prefrontal space ratio was calculated by dividing D by C, and the prenasal thickness-to-nasal bone length ratio was calculated by dividing C by B.

Reproducibility of the fetal profile measurements was assessed in all cases, using stored images (or volumes when available). Markers were measured by two examiners (F.I.V. and E.J.P.) who were blinded to gestational age and to previous measurements, but not to karyotype. Images were chosen at random at different gestational ages, with at least 3 weeks between the two assessments when performed by the same examiner. Only a single measurement was used for the analysis relating to detection rates for DS. Data were compared to reference values derived from our previous studies on euploid fetuses[11,12,14], which found that NBL and PT increased with gestation from 3.3 mm at 15 weeks to 9.6 mm at 33 weeks (NBL= $-6.927 + (0.83 \times GA) - (0.01 \times GA^2)$ and from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks ($PT = 0.212 \times GA - 0.873$), respectively. The PT-NBL ratio and PFSR were stable throughout gestation, with a mean of 0.61 (95th percentile, 0.80) and 0.97 (5th percentile, 0.55), respectively. Measurements of NBL and PFSR below the 5th percentile were considered abnormal, and for PT and PT-NBL ratio values above the 95th percentile were considered abnormal. Multiple of the median (MoM) values were calculated for PT and NBL to correct for gestational age. In cases of absent nasal bone, NBL was set at 1 mm for statistical analysis and was considered to be below the 5th percentile.

Data were analyzed using the statistical software SPSS version 21.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for Windows 2000. Means with ranges or SD were calculated when appropriate. Correlation was determined by Pearson's correlation test. A *P*-value of less than 0.05 was considered statistically significant. Intra- and interobserver variability was assessed by Bland-Altman analysis and intraclass correlation coefficient (ICC).

RESULTS

Images from a total of 159 fetuses with DS were available for analysis, including 33 3D volumes and 126 2D images. Median maternal age was 36.0 (range, 23.1 – 49.5) years and median gestational age was 23 + 1 (range 14 – 38) weeks. We excluded six images from further analysis, five that were not completely mid-sagittal and one that had an unclear nasion. In an additional 22 cases, with only 2D images available, PFSR was not measured because either the mandible or the maxilla was not displayed clearly enough to allow accurate measurement. In 3 cases gestational age was not known. It was possible to obtain all four measurements in 128 cases. Results relating to intra- and interobserver variability in the measurements, assessed by means of Bland-Altman analysis and ICCs, are reported in Table 1. For both intra- and interobserver analysis, ICC values > 0.9 were found for NBL, PT and the PT-NBL ratio, and a lower value of 0.67 was found for the PFSR.

Measurements of NBL and PT showed a correlation with gestational age ($r = 0.69$; $p < 0.001$ and $r = 0.74$; $p < 0.001$, respectively), but the PT-NBL ratio and PFSR did not. The mean (± SD) values of the NBL, PT, PT-NBL ratio and PFSR were 4.42 ± 2.39 mm, 5.56 ± 1.98 mm, 1.26 ± 0.58 and 0.34 ± 0.31, respectively. The nasal bone was absent in 23 (15.4%) cases. As an absent of nasal bone was significantly more common earlier in pregnancy, as it was negatively correlated with gestational age ($p < 0.01$).

In 43 of 128 (33.6%) DS cases, all markers were abnormal, whilst in 6 of 128 (4.7%) cases all markers were normal. The detection rate, false-positive rate, positive likelihood ratio and negative likelihood ratio of each marker are given in Table 2.

Among the markers the PT-NBL ratio and PFSR yielded the highest detection rates for DS, of 86.2% and 79.7%, respectively. Measurements of the markers in fetuses with DS are plotted against the normal ranges for euploid fetuses[11,12,14] in Figure 2.

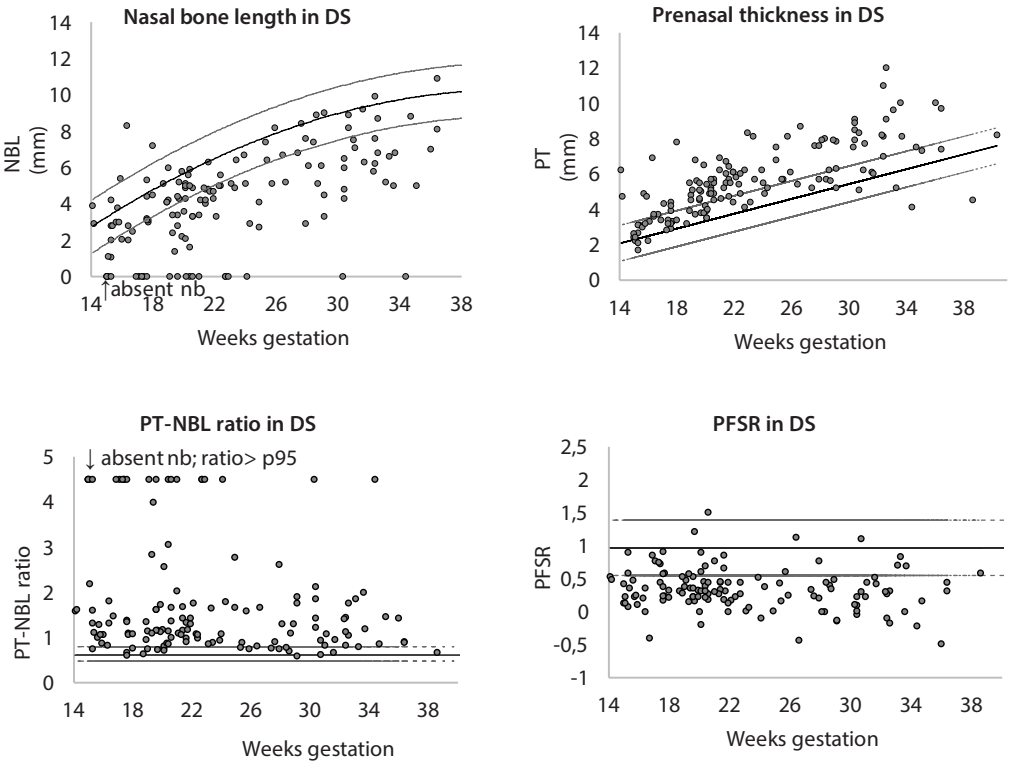


Figure 2 | Measurements of facial profile markers on stored prenatal ultrasound images of fetuses with DS plotted against gestational age and in comparison to the mean, 5th and 95 percentiles as previously reported in euploid fetuses[11,12,14]. Nasal bone length (NBL) (n = 145), prenasal thickness (PT) (n = 145), PT-NBL ratio (145) and prefrontal space ratio (PFSR) (n = 133) fetuses with DS. Fetuses with an absent nasal bone are plotted with an NBL value of zero and a PT-NBL ratio of 4.5. PT and/or NBL measurements could not be obtained in all cases.

At least one of the four markers was abnormal in 95.3% of cases. Abnormality of the PFSR and/or PT-NBL ratio yielded a detection rate of 93.8%. Each individual marker appeared to be equally effective in screening for DS across gestation, since there was no significant correlation between gestational age and the MoM values of NBL and PT or the detection rate of PFSR and PT-NBL ratio.

There was no significant correlation between PFSR and NBL MoM (p = 0.104). All other MoM values of individual markers were significantly correlated with each other (p < 0.01); significance was lowest for PFSR and PT MoM (p = 0.045).

Table 1 | Intra- and interobserver variability in measurements of facial profile markers on stored ultrasound images and volumes of fetuses with DS. ICC, intraclass correlation coefficient; LOA, limits of agreement; NBL, nasal bone length; PT, prenasal thickness; PFSR, prefrontal space ratio.

	Intraobserver variability			**Intraobserver variability**		
	Mean difference *(SD)*	**LOA**	**ICC**	**Mean difference *(SD)***	**LOA**	**ICC**
NBL	-0.14 *(0.40)*	-0.93 – 0.65	0.98	0.28 *(0.77)*	-1.26 – 0.89	0.93
PT	-0.01 *(0.45)*	-0.90 – 0.89	0.98	0.08 *(0.49)*	-0.89 – 1.05	0.97
PT-NBL ratio	0.04 *(0.15)*	-0.26 – 0.34	0.94	-0.01 *0.19)*	-0.39 – 0.38	0.92
PFSR	-0.06 *(0.27)*	-0.60 – 0.48	0.67	-0.17*(0.25)*	-0.67 – 0.33	0.67

Table 2 | Performance of nasal bone length (NBL) , prenasal thickness (PT), prenasal thickness to nasal bone length (PT-NBL) ratio and prefrontal space ratio (PFSR) as markers for DS. 95% CI's are given in parentheses. DR; detection rate, FPR; false positive rate, PLR; positive likelihood ratio, NLR; negative likelihood ratio.

	DR ***(95% CI)***	**FPR** ***(95% CI)***	**PLR** ***(95% CI)***	**NLR** ***(95% CI)***
NBL (mm) (n = 145)	61.9% *(53.4% – 69.9%)*	5.0% *(1.7% – 11.3%)*	12.32 *(5.17 – 29.37)*	0.40 *(0.31 – 0.52)*
PT (mm) (n = 145)	63.4% *(53.4% – 73.1%)*	5.0% *(1.7% – 11.3%)*	12.73 *(5.35 – 30.29)*	0.38 *(0.29 – 0.50)*
PT-NBL ratio (n = 145)	86.2% *(79.3% – 91.2%)*	5.0% *(1.7% – 11.3%)*	17.37 *(7.4 – 41.0)*	0.14 *(0.08 – 0.23)*
PFSR (n = 133)	79.7% *(71.6% – 86.0%)*	5.0% *(1.7% – 11.3%)*	15.96 *(6.75 – 37.72)*	0.21 *(0.14 – 0.32)*

DISCUSSION

This study involves the largest cohort of fetuses with DS thus far, in which all known profile markers have been measured and their detection rate has been established after comparison with normal ranges established by the same study group. The study confirms that screening for DS can be performed effectively in the second and third trimester of pregnancy. The best markers are the PT-NBL ratio and the PSFR, with predicted detection rates of 86% and 80%, respectively. The detection rate further increases to 94% when the PT-NBL ratio and the PFSR are combined, and slightly more (95%) when all facial markers (NBL, PT, PT-NBL ratio and PFSR) are combined. An additional

advantage of using the combined markers (PT-NBL ratio and PFSR) in routine examination is that the 5th (PFSR) and 95th (PT-NBL ratio) percentile cut-offs are constant throughout gestation at 0.55 and 0.80, respectively.

Interest regarding the facial features of individuals with DS dates back to the late 1970's when the cephalic index was proposed as the first ultrasound screening method for DS[15]. Sonek et al[16] were the first to observe the absence of nasal bones as a marker for DS in 2001, whilst the markers PT, PT-NBL ratio and PFSR have been introduced more recently[13,17]. It has been proposed that in DS, changes in the extracellular matrix in the skin and abnormalities of lymphatic vessels lead to a variable increase in skin thickness in the neck and prenasal region[8,18]. Abnormalities in bone growth and development are associated with mid-facial hypoplasia[2,19], resulting in an abnormal profile and small nasal bones. This study investigated the efficacy of various methods of quantification of these abnormalities.

A limitation of this study is its retrospective nature and the fact that examiners were not blinded to the karyotype. However, its strength is to have assessed the value of second-trimester ultrasound markers in a large cohort of fetuses with DS. As expected, the previously reported 100% detection rate for both the PT-NBL ratio[12] and PFSR[20] decreases when the method is applied to a large cohort. However, the combination of both markers leads to a high detection rate (94%), thus far the highest reported in a large study using an algorithm based exclusively on ultrasound measurements.

The PT measurement is part of the PFSR and is referred to as 'd1' in the PFSR studies of Sonek et al[13], Yazdi et al[14] and Chaveeva et al[20]. However, whereas Sonek et al[13] and Yazdi et al[14] calculate d1 as the distance between skull and skin in a line parallel to the maxilla, Chaveeva et al[20] measure it perpendicular to the MM line. In this study we followed the first method, as we suspect that the position of the MM line would be reflected in the length of d1 (PT) when measured perpendicular to it.

Concerning the interdependency of the NBL MoM, PT MoM, PT-NBL ratio and PFSR, we were not surprised to find the PT-NBL ratio significantly correlated to all other markers. The significant, but weaker, correlation ($P = 0.045$) between PT MoM and PFSR suggests that the measurement of d, which represents mid-facial hypoplasia, is independent of PT. Similar to other studies[13,20], the NBL MoM and PFSR were the only markers that were not significantly correlated in this large cohort of fetuses with DS. However, the combination of these two independent markers did not yield a better detection rate than did the combination of the PFSR and the PT-NBL ratio. Ideally, an adequate repeatability and reproducibility study should be performed, not only by remeasuring ultrasound markers on stored pictures, but also by reacquiring the desired image. Due to the retrospective nature of this study, the latter was not possible, and our reproducibility figures therefore relate exclusively to reproducibility of the measurement. Reproducibility was good for all markers, with the exception of the PFSR. An explanation may be found in the fact that when using a marker combining multiple measurements performed in the mid-sagittal plane, such as the PFSR, the slight interobserver variation in each measurement is amplified. Proof of this may be the lower interobserver ICC for PFSR of 0.67, compared to an ICC of 0.98 for PT. In comparison with other studies on the PFSR, reproducibility of our measurements is poorer than that reported by Chaveeva et al.[20] but of the same order as that reported by Sonek et al[13] and Yazdi et al[14].

A number of images (n = 6) and PFSR measurements (n = 22) were excluded from the study either because they were not in a midsagittal view or because of unclear mandible and/or maxilla. These results suggest that when an ultrasound image is obtained with the intention of measuring the nasal bone and prenasal skin thickness, less care is taken in obtaining good visualization of the bony landmarks of the maxilla and mandible. This may not be the case when measurements are taken prospectively, with special attention given to visualization of the bony landmarks of the profile. This assumption is further substantiated by the fact that all discarded ultrasound records were 2D images. If these images had not been excluded then we may have found lower detection rates. Therefore, no firm statements can be made concerning the true detection and false-positive rates until a prospective study is performed.

Studies on DS screening in the second and third trimester are relatively scarce as in countries with well-established DS screening programs, screening occurs preferably in the first trimester (90% in Denmark and France[21,22]). However, well-organized and established first-trimester screening programs are not available in all countries and screening uptake can also be low (20% and 32% in certain areas of England and The Netherlands[23,24]). This means that, whilst non-invasive prenatal testing (NIPT) could potentially replace first-trimester screening, there remains at present a role for the evaluation of DS markers at second-trimester ultrasound examination. A late diagnosis is obviously less desirable when the parents may consider termination of pregnancy. However, in all other cases, a diagnosis may still be important for pregnancy management and for preparation of the birth of an affected child.

In conclusion, according to this large cohort of retrospectively analyzed fetuses with DS, the PT-NBL ratio and PFSR qualify as excellent second-trimester ultrasound markers. The strength of the PT-NBL ratio is that it provides a high detection rate and that it is reproducible. Both markers are easy to use in practice, as no knowledge of gestational age-specific mean values is required.

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CHAPTER 6

Is 3D technique superior to 2D in Down syndrome screening? Evaluation of six second and third trimester fetal profile markers

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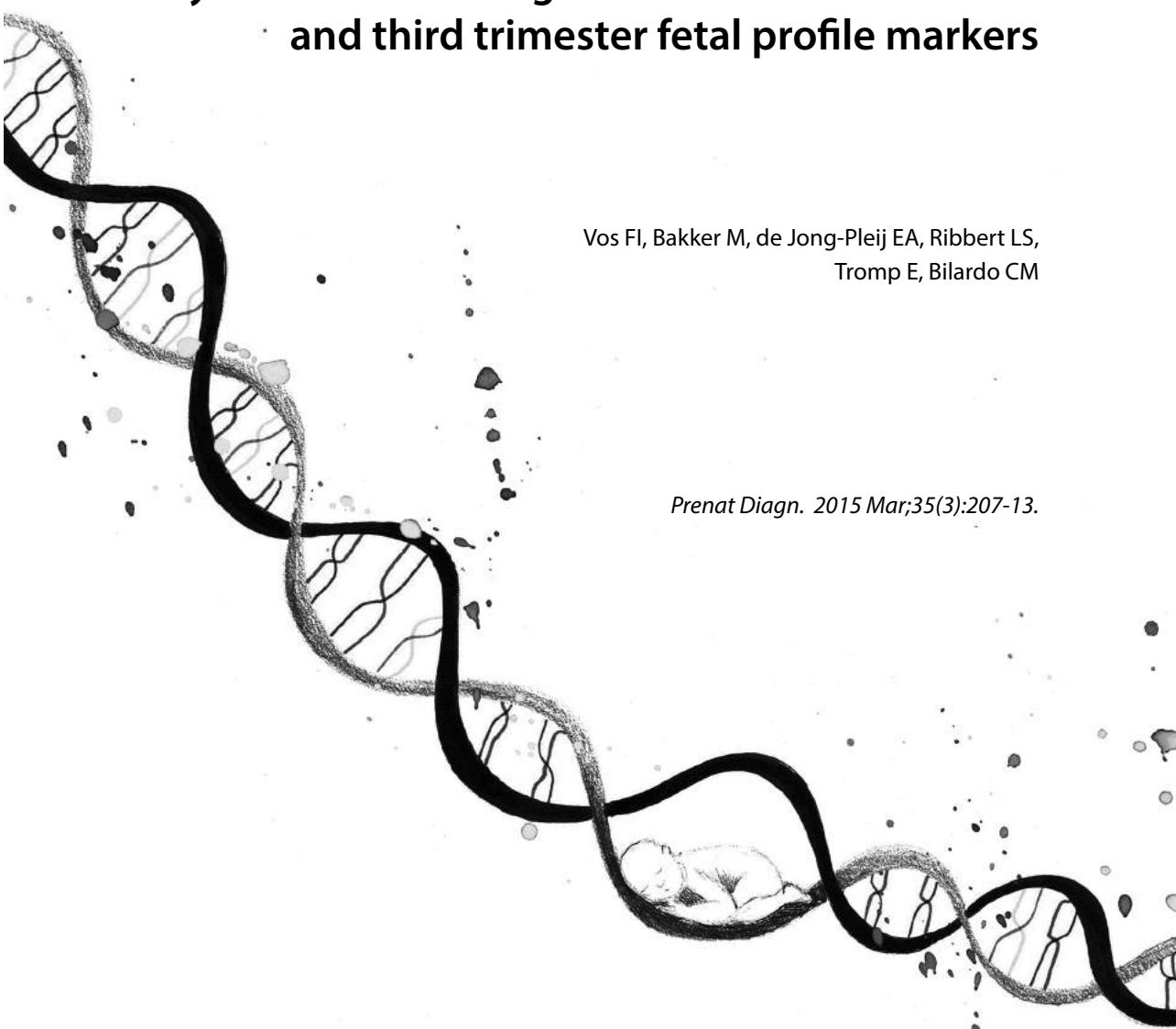

ABSTRACT

Objective

To investigate whether in the clinical setting of second trimester ultrasound (US) investigations 3D multiplanar correction prior to the measurement of Down syndrome (DS) facial markers (nasal bone length (NBL), prenasal thickness (PT), fetal profile (FP) line, maxilla-nasion-mandible (MNM) angle, prenasal thickness to nasal bone length (PT-NBL) ratio and prefrontal space ratio (PFSR)) is superior to subjective judgement of a correct midsagittal plane by 2D technique.

Methods

Measurements were performed on 2D images and 3D volumes (corrected to the midsagittal plane), acquired during the same scanning session.

Results

All six markers were measured in 105 datasets (75 of euploid fetuses and 30 of DS fetuses). The MNM angle measured on 2D images was significantly larger than on 3D volumes ($p < 0.01$). In all other markers there was no significant difference between measurements performed on 2D images or 3D volumes. No statistical difference was found for any marker between measurements performed on images acquired by either 2D or 3D US in their ability to discriminate between euploid and DS fetuses.

Conclusions

NBL, PT, FP line, PT-NBL ratio and PFSR can be confidently used as DS markers in second trimester ultrasound examinations performed by 2D US.

INTRODUCTION

Specific facial profile features of Down syndrome (DS) fetuses have been investigated and used as second and third trimester markers[1-13]. The nasal bone length (NBL) was the first to be extensively investigated, followed by the prenasal thickness (PT). Recent studies have shown that the ratio between these two markers (PT-NBL ratio) and the prefrontal space ratio (PFSR) yields an even better detection rate[6,10]. Furthermore, we have previously investigated the maxilla-nasion-mandible (MNM) angle and fetal profile (FP) line in both euploid and pathological cases[11,14-16]. In countries such as The Netherlands, where participation in first trimester screening is low[17] and many DS fetuses remain undetected until the 20 weeks scan, these markers may be of importance.

Several studies have compared 2D and 3D US imaging during gestation and suggested 3D to be superior by allowing a better identification of anatomical landmarks[18], a higher accuracy and reproducibility in measurements of structures in the fetal face and profile[13,19,20], including the NBL[9,21]. In a previous study[13], we have shown that 2D images judged to be midsagittal in actual fact are not and need 3D multiplanar correction of in average 11.9 (Y-axis) – 4.3 (Z-axis) degrees to become truly midsagittal. Clear landmarks to identify the exact midsagital plane are missing when only 2D imaging is used, making it difficult to be absolutely sure to be in the exact midsagittal plane. However, it is not clear whether in a clinical setting 3D imaging has an additional value in terms of an improved detection rate when compared to 2D.

The aim of this study is to compare the differences in 2D and 3D technique in the measurement and detection rate of facial markers in the second and third trimester.

METHODS

Eligible cases were collected from the databases of the Fetal Medicine Unit of the University Medical Centre Groningen, which acts as a referral center, and of the Ultrasound Unit of the Saint Antonius Hospital in Nieuwegein, which performs US investigations of high risk patients.

Images were obtained by a Voluson 730 Expert ultrasound machine or E8 system equipped with a RAB4-8L probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Three-D volumes of euploid fetuses were retrieved from an available dataset used for a previous study[13] of non-smoking, healthy, low-risk, pregnant Caucasian women with a singleton and uncomplicated pregnancy. The dataset was collected prospectively; after 2D images of the fetal profile were obtained, judged to represent the midsagittal plane and with the fetus facing the transducer, 3D volumes of the fetal face were acquired. Databases of participating centers were searched for second and third trimester DS fetuses of Caucasian parents, collected between January 2006 and July 2013. All cases had been confirmed by karyotyping. The images were collected during clinical investigations and therefore were, in contrast to the images of the euploid fetuses, gathered in a less systematic fashion.

For this study, cases with both a midsagittal 2D image of the fetal profile and a 3D volume, acquired separately in the same scanning session, were included. We excluded 2D images that were obviously not midsagittal by systematically assessing all components of the profile. Images that

showed a body of the mandible, a retrognathic appearance of the chin, a nostril, odd appearance of the nose, a frontal process of the maxilla, a sharp or blunt angle between the nasal and frontal bones, a bossing or sloping appearance of the forehead, sphenoid bone or a lateral ventricle or plexus choroideus were excluded. Visibility of the vomeral bone was considered a very strong indication of the exact midsagittal plane. A square shape of the mandible, normal appearance of lips, philtrum and nose, a flat or only slightly curved forehead and visibility of the corpus callosum were indications for a good midsagittal plane. In order to avoid bias, all the measurements on 2D images were performed first. This was followed by multiplanar correction of the 3D volumes to the exact midsagittal plane with subsequent measurements.

To assess the inter- and intraobserver variability of the measurement error, markers were re-measured after a one-week interval. Markers were measured by two examiners (F.I.V. and E.J.P.), who were blinded to gestational age and to previous measurements, but not to karyotype. The NBL, PT, FP line, MNM angle, PT-NBL ratio and PFSR were all measured as described in previous studies of euploid and DS fetuses[6,10,14,15,22] (Figure 1).

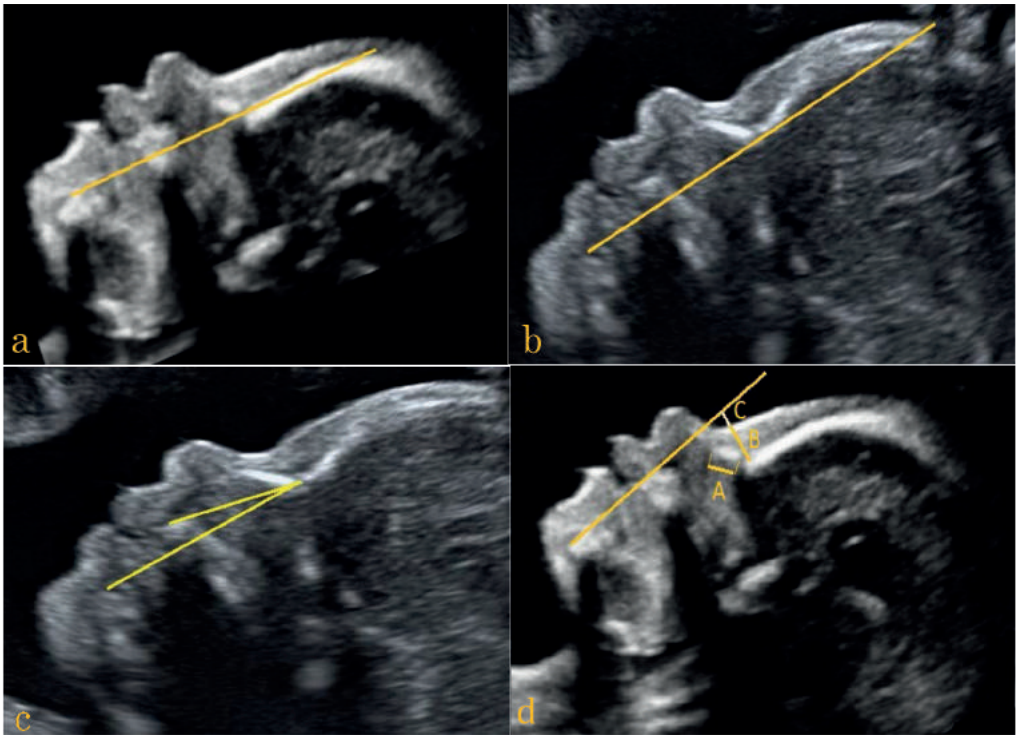

Figure 1 | Ultrasound images of the markers in DS fetuses. (**a**) FP line position 'zero'; (**b**) FP line position 'positive'; (**c**) MNM angle; (**d**) NBL (A), PT (B), PT-NBL ratio (B/A), PFSR (C/B). FP, fetal profile line; MNM angle, maxilla-nasion-mandibula angle; NBL, nasal bone length; PT, prenasal thickness; PT-NBL ratio, prenasal thickness to nasal bone length ratio; PFSR, prefrontal space ratio.

Data were compared to the reference values derived from previous reports on euploid fetuses[6,10,14,22]: the NBL[22] and PT[22] increased with gestation from 3.3 mm at 15 weeks' gestation to 9.6 mm at 33 weeks (NBL = -6.927 + (0.83*GA)-(0.01*GA^2)) and from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks (PT = 0.212 × GA − 0.873), respectively. The MNM angle[14], PT-NBL[6] ratio and PFSR[10] were stable throughout gestation, with a mean of 13.5 degrees (95th percentile = 16.9), 0.61 (95th percentile = 0.80) and 0.97 (5th percentile = 0.55), respectively.

Measurements below the 5th percentile (for NBL and PFSR) or above the 95th percentile (for MNM angle, PT, and PT-NBL ratio) of the reference ranges, were considered abnormal. An FP line that was not 'zero', was considered abnormal[15]. The difference between the 2D and 3D measurement was analyzed in each individual fetus, of which a mean difference was calculated. Differences between measurements were calculated for the whole group and a separate analysis was performed in the group of DS fetuses, in order to assess if the use of one of the two techniques (2D vs 3D) had an impact on the detection rate.

Intraclass correlation coefficients (ICC) were calculated and Bland-Altman analysis was performed to analyze intra- and interobserver variability. The students t-test was used to analyze differences between measurements. A p-value of less than 0.05 was considered statistically significant. Data were analyzed using the statistical software SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for windows 2000.

RESULTS

In the analysis, a total of 105 datasets were included: 75 of euploid fetuses (median gestational age 24, range 15 – 32 weeks) and 30 of DS fetuses (median gestational age 24, range 17 – 34 weeks). Originally, 32 datasets of DS fetuses were available, but 2 were excluded, as the 2D images were judged not to be truly midsagittal.

Mean values, mean differences between 2D and 3D measurements and corresponding ICC of 2D and 3D measurements in a combined cohort of euploid and DS fetuses (n = 105), are shown in table 1. It was not possible to analyze mean differences between measurements of the FP line as the outcome was non-continuous (positive or zero; no FP line was negative).

For the MNM angle, 2D measurements were significantly larger ($p < 0.01$), although the mean difference was small (1.0 degree). For the other markers (NBL, PT, FP line, PT-NBL ratio and PFSR) there was no significant difference in measurements performed in either 2D or 3D US.

Intra- and interobserver variability in 2D and 3D US for each marker (except for the FP line), with their corresponding limits of agreement (LOA) and 95% confidence interval (CI), are shown in figure 2. LOA's were smaller for all 3D measurements, except for the MNM angle.

In the separate analysis of DS fetuses only, no statistical difference was found for any marker between measurements performed in images acquired by either 2D or 3D US in their ability to discriminate between euploid and DS fetuses (table 2, figure 3-8).

Table 1 | Mean values of 2D and 3D measurements (n = 105) in a combined cohort of euploid and DS fetuses. Corresponding mean difference with limits of agreement (LOA) and intra class correlation coefficients (ICC's) are reported. It was not possible to analyze mean differences between measurements of the FP line, as the outcome was non-continues. MNM, maxilla-nasion-mandible angle; PT, prenasal thickness; PT-NBL ratio, prenasal thickness to nasal bone length ratio; PFSR, prefrontal space ratio. *FP line in 3D: 77.5% positive, 22.5% zero. **FP line in 2D: 79.8% positive, 20.2% zero. ***Significant difference between 2D and 3D measurements in the MNM angle ($p < 0.01$).

	Mean		Mean difference (LOA)	ICC
	3D	2D		
NBL	6.29	6.20	0.08 *(-2.48 – 2.57)*	0.84
FP line	*	**	–	0.68
MNM angle	14.77	13.75	***1.03 *(-5,14 – 6.13)*	0.40
PT	5.41	5.09	0.33 *(-1.50 – 1.83)*	0.83
PT-NBL ratio	0.91	0.89	0.03 *(-0.45 – 0.49)*	0.83
PFSR	1.04	1.09	-0.06 *(-0.87 – 0.79)*	0.68

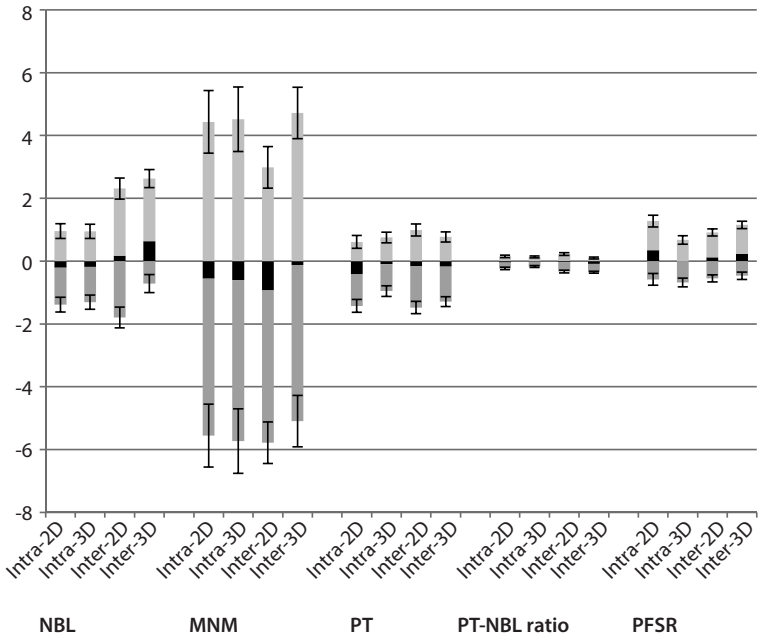

Figure 2 | Box plot showing mean difference (black bars) and 95% limits of agreement (boxes) with their confidence intervals (whiskers), for intra- and inter-observer variability in 2D and 3D measurements. The nasal bone length (NBL) and prenasal thickness (PT) are expressed in millimeters, the maxilla-nasion-mandible (MNM) angle in degrees. PT-NBL ratio, prenasal thickness to nasal bone length ratio; PFSR, prefrontal space ratio.

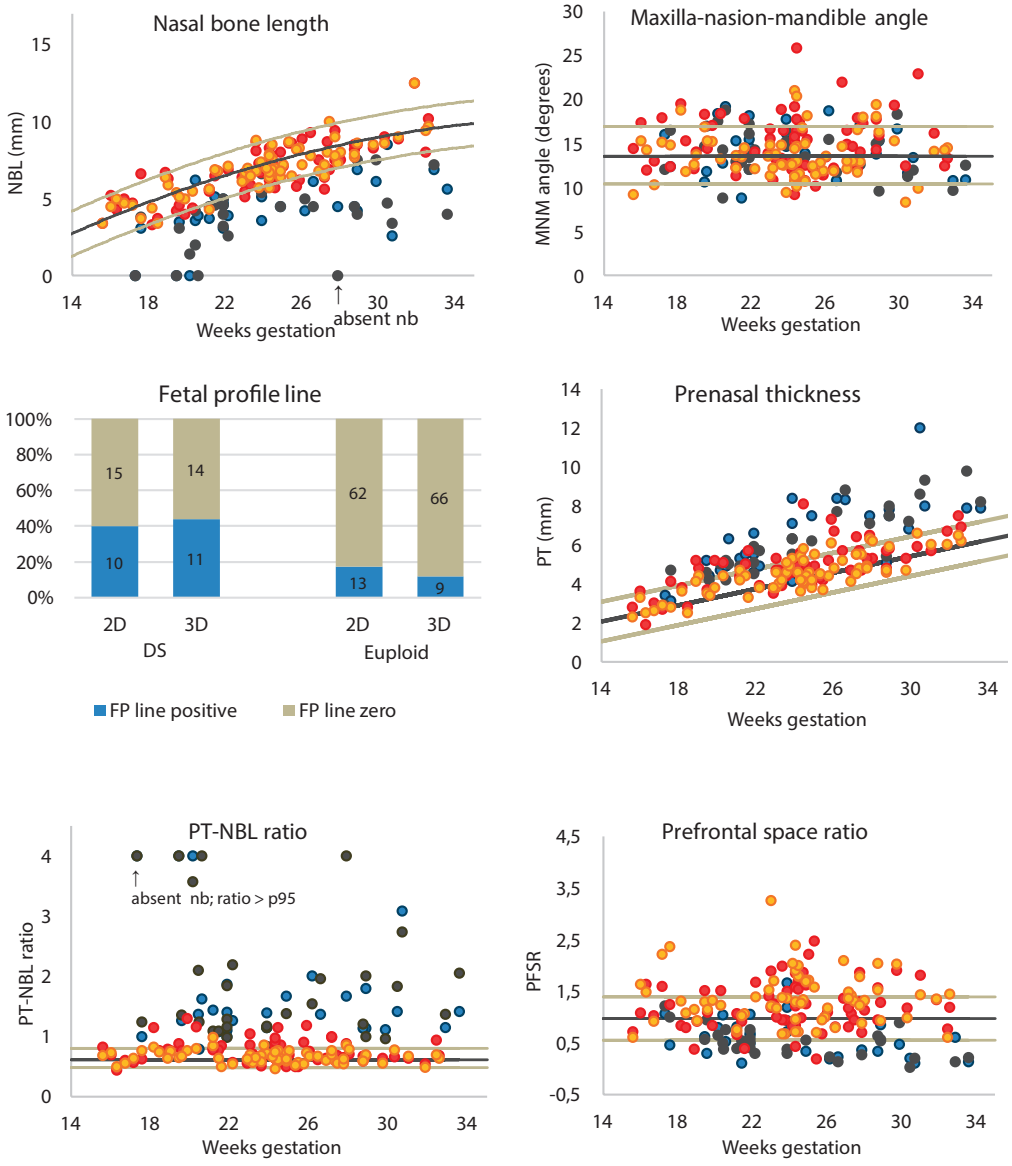

Figure 3 | Nasal bone length (NBL), Maxilla-nasion-mandible (MNM) angle, Fetal profile (FP) line, prenasal thickness (PT), PT-NBL ratio, prefrontal space ratio (PFSR) measurements performed on 2D images and 3D volumes in euploid and Down syndrome (DS) fetuses. For NBL, MNM angle, PT, PT-NBL ratio and PFSR plotted on normal ranges (mean, 5th percentile and 95th percentile). ● euploid 3D, ● euploid 2D, ● DS 3D, ● DS 2D.

Table 2 | Mean values for 30 datasets of DS fetuses with their corresponding detection rate. No statistical significant differences were observed between 2D and 3D measurements.

	Mean		Detection rate	
	3D	**2D**	**2D**	**3D**
NBL	4.37	3.91	83.3%	82.6%
FP line	_	_	37.0%	41.4%
MNM angle	14.35	14.07	3.7%	10%
PT	6.31	6.15	83.3%	86.7%
PT-NBL ratio	1.40	1.55	96.7%	100%
PFSR	0.60	0.51	50%	56.7%

DISCUSSION

This study demonstrates that when strict criteria are applied, subjective judgment of a good midsagittal plane on 2D images is sufficient to ensure a good performance of facial markers for DS. In a cohort of euploid and DS fetuses, no significant difference was found in NBL, PT, FP line, PT-NBL ratio and PFSR, measured in midsagittal images obtained by 2D or 3D US. Only for the MNM angle 2D measurements were slightly, but significantly, larger. Both 2D and 3D technique can perform equally well in identifying DS fetuses, without significant difference between measurements.

The clinical implication of these findings is that these markers can be used effectively in routine screening settings relying on 2D technique using strict criteria, without missing out on the additional benefit of 3D US. This finding has great implications in a moment of worldwide financial constraints and growing medico-legal problems, where the general opinion is that 3D US is superior to 2D US[13,18-21].

In literature, the role of 3D technique in the measurement of fetal facial biometrical parameters has been underlined by many studies. A volume obtained starting from an oblique scanning plane can be corrected to the exact midsagittal plane, allowing accurate and reproducible measurements[13]. Moreover, a stored 3D volume can be analyzed off-line retrospectively, possibly shortening the time of investigation.

Suggested disadvantages of using 3D volume corrections are that it requires costly equipment, specialized personnel and it may be more time consuming[23]. However, other studies found no difference in time[13,24], or found 3D to be even faster[25-27].

Following our previous report[13], this is the first study that evaluates the use of 2D versus 3D acquired images in the evaluation of profile markers.

Benoit et al[21] demonstrated that, in case of suspicion of an absent nasal bone on 2D images, the nasal bone can be better visualized in 3D volumes. Persico et al[9] showed that 3D NBL measurements tend to be larger when the scanning plane is not exactly midsagittal, which decreased the detection rate for DS. In our previous study[13], we found no difference between measurement modality, but reported narrower limits of agreement in 3D performed measurements.

A possible criticism of this study is that for comparison, we performed a selection of 2D pictures likely to represent the true midsagittal plane. All 2D ultrasound measurements are taken on planes

subjectively judged as correct according to anatomical landmarks. However, in a previous study[13] we showed that after volume acquisition, even when the image on the A plane was subjectively judged to be midsagittal, variable degrees of correction by multiplanar mode were required in order to obtain the true midsagittal plane. Based on the results of the present study, the measurements of facial markers in the initial 2D image are highly comparable to those measured in 3D corrected planes.

Limitations of this study are its retrospective nature and the fact that examiners were not blinded to the karyotype. An ideal repeatability and reproducibility study would require reacquisition of the same images by two observers. Due to the retrospective nature of this study this was not possible, however reproducibility of the markers is established in the original publications[6,14,22].

Influence of circumstances like reduced amniotic fluid or the fetus facing down were not taken into account, however, these circumstances would equally affect 2D and 3D performance[13,28].

We expressed differences in measurements in ICC, as the markers are quantified by different metric parameters (degrees, mm and ratio's). The MNM angle, FP line and PFSR had a relatively low ICC when 2D and 3D measurements were compared. One explanation may be that the above mentioned markers, in contrast to the PT and NBL, require multiple landmarks which may enhance the variability in the measurement.

For the MNM angle, 2D performed measurements were significantly larger, the ICC of 2D versus 3D measurements was low and LOA's of intra- and inter-observer variability were relatively wide. This could be due to the fact that the reproducibility of the MNM angle is in general more challenging and that especially in this case, the bony structures used as a landmark for the measurement are better identified by 3D US.

Conversely, the PFSR has a sub-optimal ICC when 2D and 3D measurements are compared, but the mean difference between 2D and 3D measurements is not significant. The LOA's of the PFSR are larger when compared to that of the PT-NBL ratio (also expressed as a ratio), especially in 2D. These findings show that the reproducibility of the PFSR (in 2D) is lower, but the actual measurement is not influenced by the technique of image acquisition.

In spite of these findings, no impact of acquisition modality could be found in detection rate and measurements of all markers, performed in DS fetuses. This is reassuring, as the goal of these measurements is to discriminate between DS and euploid cases.

The best moment for DS screening is undisputable the late first trimester. However, uptake of first trimester screening varies among countries. In The Netherlands, for instance, where the combined test is only covered by insurance beyond 36 years of age, the uptake is low[29] . In contrast, uptake of second trimester ultrasound screening for structural anomalies, which is covered for all women[30], is over 90%[31]. This means that a considerable number of DS pregnancies remain undetected. Also when late termination of pregnancy is not available, a late diagnosis of a chromosomal anomaly is important to prepare future parents and establish the appropriate obstetrical management.

In conclusion, we have shown that, with exception of larger MNM angles in 2D images, no significant differences were found between 2D and 3D imaging in a number of facial DS markers. In particular, NBL, PT, FP line, PT-NBL ratio and PFSR can be confidently used as DS markers in ultrasound examinations performed by 2D US, provided the markers are measured in a midsagittal image, acquired according to strict criteria.

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CHAPTER 7

Trends in serial measurements of ultrasound markers in second and third trimester Down syndrome fetuses

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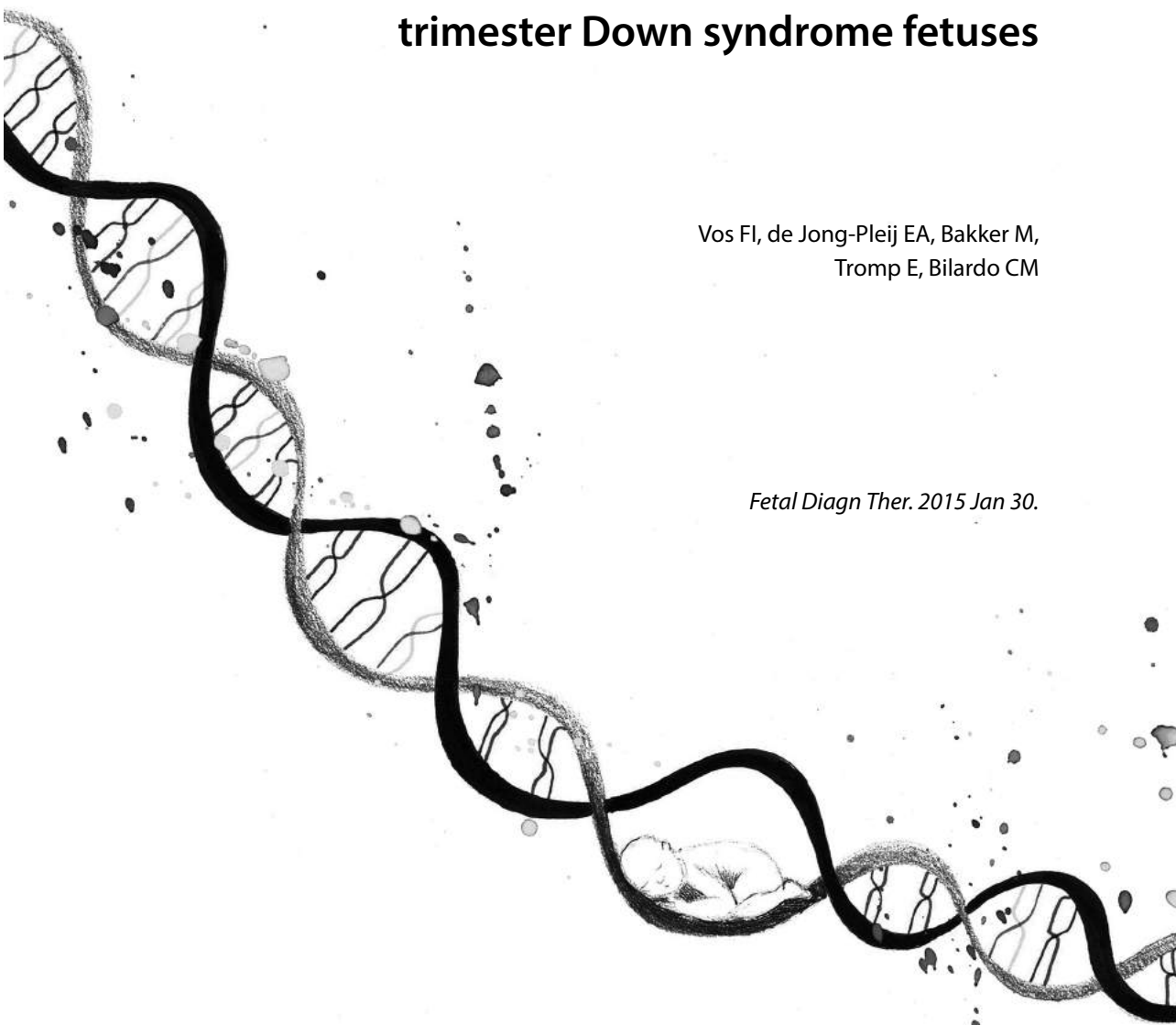
Fetal Diagn Ther. 2015 Jan 30.



ABSTRACT

Objectives

To evaluate trends of nasal bone length (NBL), prenasal thickness (PT), nuchal fold (NF), prenasal thickness to nasal bone length (PT-NBL) ratio and prefrontal space ratio (PFSR), measured serially in second and third trimesters Down syndrome (DS) fetuses.

Methods

Prenatal databases were searched for cases of continuing DS pregnancies with serial measurements, taken at least two weeks apart. Trends were plotted on previously reported normal ranges.

Results

Serial measurements were available in 25 Down syndrome fetuses. Median gestational age (GA) was 25 weeks; average number of visits per case was 2.44, with a median interval of 39 days between investigations. In DS fetuses, NBL and PT showed fairly stable trends with gestation. PFSR, but especially NF, had a more unpredictable trend. The PT-NBL ratio was the most stable marker, remaining the same value in 95% of cases. NBL, PT and NF showed more deviance from the normal range with advancing gestation, but Multiple of the Median values remained stable. All but two fetuses had common markers or structural anomalies, especially heart defects.

Conclusions

The PT-NBL ratio is the most constant DS marker throughout gestation, following a predictable trend.

INTRODUCTION

Beyond the first trimester of pregnancy, prenatal ultrasound assessment of Down Syndrome (DS), has focused, among other things, on markers located in the fetal profile and neck. Short nasal bone length (NBL), increased prenasal thickness (PT) and increased nuchal fold (NF) thickness are the most frequently investigated[1-12]. Recent studies have indicated that the use of ratio's, such as the prenasal thickness to nasal bone length (PT-NBL) ratio and the prefrontal space ratio (PFSR), are strong and easy to use second trimester markers[13-15].

In the Netherlands, uptake of first trimester screening for DS is low[16]. The combined test is free only for women of 36 years and older, whereas second trimester ultrasound screening for structural anomalies at around 20 weeks' gestation is fully covered by medical insurance[17] and chosen by over 95% of women[18]. This means that a considerable number of DS pregnancies remains undetected. In case of occasional detection of DS markers at the 20 weeks scan, not all women choose karyotyping and, even if they do, not all decide to terminate the pregnancy. As a result, a number of DS fetuses can be followed-up during pregnancy and trends in DS markers can be observed. In a recent study, we have demonstrated that the NBL, PT, PT-NBL ratio and PFSR are valuable and reproducible DS markers[13,14,19] and assessed the detection rates, which appear to be evenly distributed throughout the second and third trimester. However, these studies were based on cross-sectional measurements in both normal and DS fetuses.

Aim of this study was to assess individual trends in a number of DS markers measured serially in the same affected fetus.

METHODS

The Fetal Medicine Units of the University Medical Center Groningen and of the Saint Antonius Hospital in Nieuwegein act as referral centers. Databases were searched (from January 2006 to October 2013) for second and third trimester ultrasound investigations in DS cases from Caucasian parents, confirmed pre- or postnatally by karyotyping. At our institutions, all ongoing pregnancies with (suspected) chromosomal abnormalities receive follow up at regular intervals. All patients consented to the serial measurement of facial markers. Cases with at least two measurements taken with a minimum interval of two weeks, were included in the study. When possible, the measurements were performed on 3D volumes after multiplanar mode correction to the exact median view in order to improve measurement accuracy[20]. NF was measured on stored 2D images or, in case no images were available, the measurement was retrieved from ultrasound reports. The NBL, PT, PT-NBL ratio and PFSR were measured as described previously[13]. The NF was measured on a fronto-occipital transverse view - including the cavum septum pellucidum, cerebellum and the posterior fossa- as the distance between the median point of the outer curve of the occipital bone and the outer skin edge[20].

Data were compared to the reference values derived from our previous reports on euploid fetuses[14,19] or compared to reference values derived from the literature[8,9,21,22,23]; the NBL and PT

increased with gestation from 3.3 mm at 15 weeks' gestation to 9.6 mm at 33 weeks (NBL = -6.927 + (0.83*GA)-(0.01*GA^2)) and from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks (PT = 0.212 × GA – 0.873), respectively. The PT-NBL ratio and PFSR were stable throughout gestation, with a mean of 0.61 (95th percentile = 0.80) and 0.97 (5th percentile = 0.55), respectively.

Measurements below the 5th percentile (for NBL and PFSR) or above the 95th percentile (for NF, PT and PT-NBL ratio) of the reference ranges, were considered abnormal. Measurements below the 5th percentile (for the NBL and PFSR) or above the 95th percentile (for PT, NF, and the PT-NBL ratio) of the reference ranges were considered abnormal. Multiple of the Median (MoM) values were created for the NBL, PT and NF in order to correct for gestational age (GA). In a previous study of both euploid and DS fetuses[13,19] we have investigated intra- and interobserver variability. Additional ultrasound findings at the examination in the participating referral centers were documented when available and classified as structural and non-structural anomalies (not including the profile markers NBL, PT, NF, PT-NBL ratio and PFSR).

Images were obtained by a General Electric Voluson 730 Expert ultrasound or E8 system equipped with a RAB4-8L probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria). Images and volumes were stored and examined either offline on 4D View software version 7.0 (GE Medical Systems, Kretz Ultrasound, Zipf, Austria) or on stored images in the General Electric ultrasound system. Markers were measured by two examiners (F.I.V. and E.J.P.), who were blinded to gestational age and to previous measurements, but not to karyotype.

Correlation coefficients were calculated by Pearson's correlation test. A p-value of less than 0.05 was considered statistically significant. Averaged trendlines for serial measurements in individual fetuses were calculated by the mixed models analysis in SPSS. This analysis models the covariance structure of data and is the best model to create a trendline from repeated measurements. It expresses the relationship with time, corrects for random effects, deals with missing data and is especially suitable for small samples. The data were analyzed using the statistical software SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for windows 2000.

RESULTS

A total of 25 Down syndrome fetuses were included in the analysis. The median GA was 25^{+}0 weeks; 20^{+}4 weeks (range 14 – 26 weeks) at initial measurement and 29^{+}2 weeks (range 22 – 36 weeks) at final measurement. Median interval between measurements was 39 days (range 14 – 98 days) with an average number of 2.44 visits per case; in 14 fetuses measurements were performed twice, in 10 three times and in one fetus four times. Of all the measurements (except for the NF measurements), 54% was performed on 2D images and 46% on 3D volumes. The percentage of DS fetuses with an abnormal first measurement, last measurement or the same outcome (both normal or abnormal) at both measurements is displayed in table 1.

Overall NBL, PT and NF measurements increased significantly with GA ($p < 0.01$). However in 41.7% of cases, the NF did not increase in at least one consecutive measurement. NBL and PT did not increase in at least one consecutive measurement in 4.8% and 13.6%, respectively.

Longitudinal trends in individual markers measured in DS fetuses, are presented in figure 1, together with the mean measurement in normal fetuses[8,14,19,23].

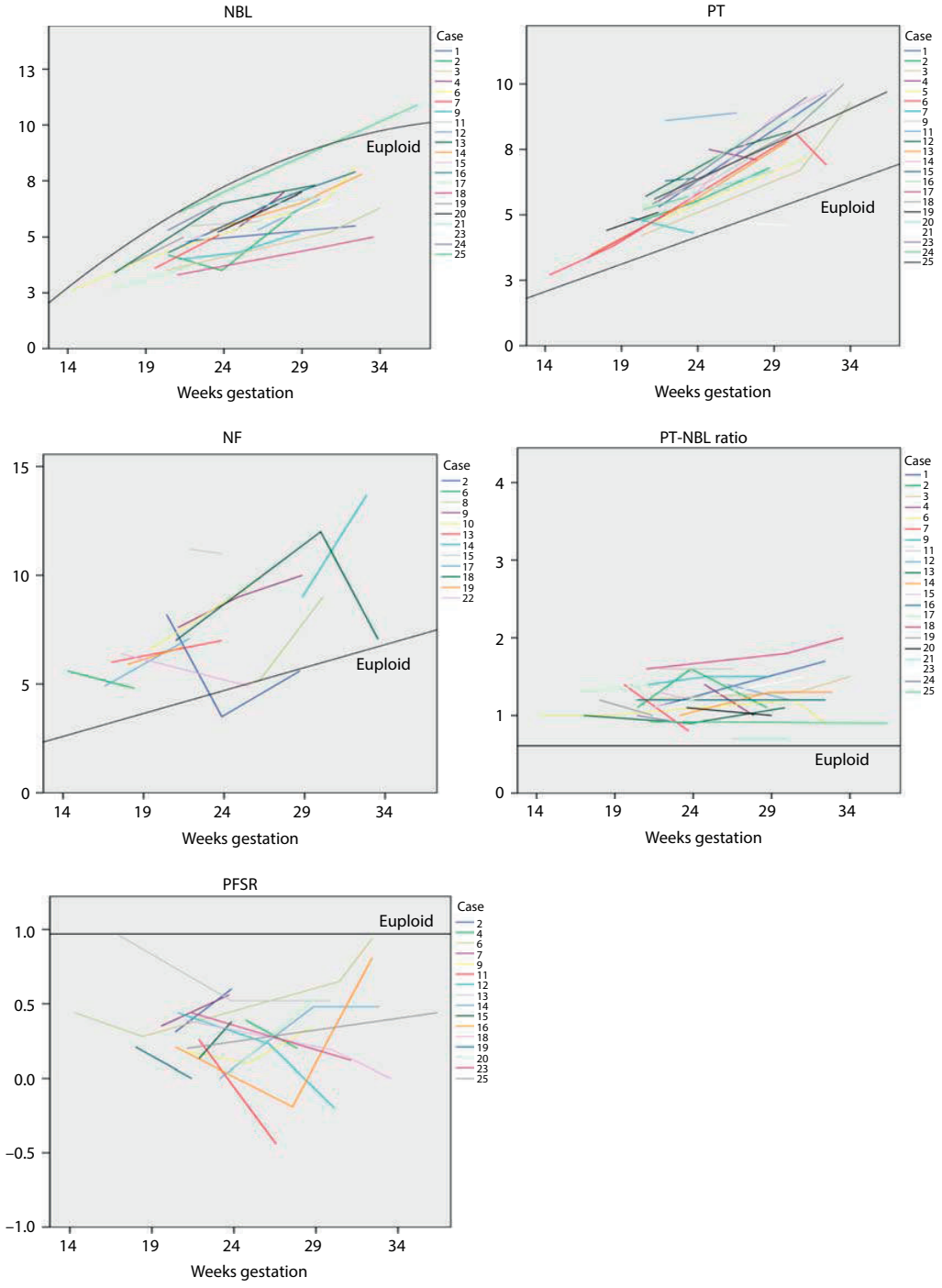


Figure 1 | Serial measurements of ultrasound markers in individual DS cases, compared to the mean of euploid fetuses. DS, down syndrome; NBL, nasal bone length; PT, prenasal thickness; NF, nuchal fold; PFSR, prefrontal space ratio.

The overall trend in serial measurements in DS fetuses was calculated by mixed model analysis and compared to the corresponding normal range for each marker[8,14,19,23] (figure 2).

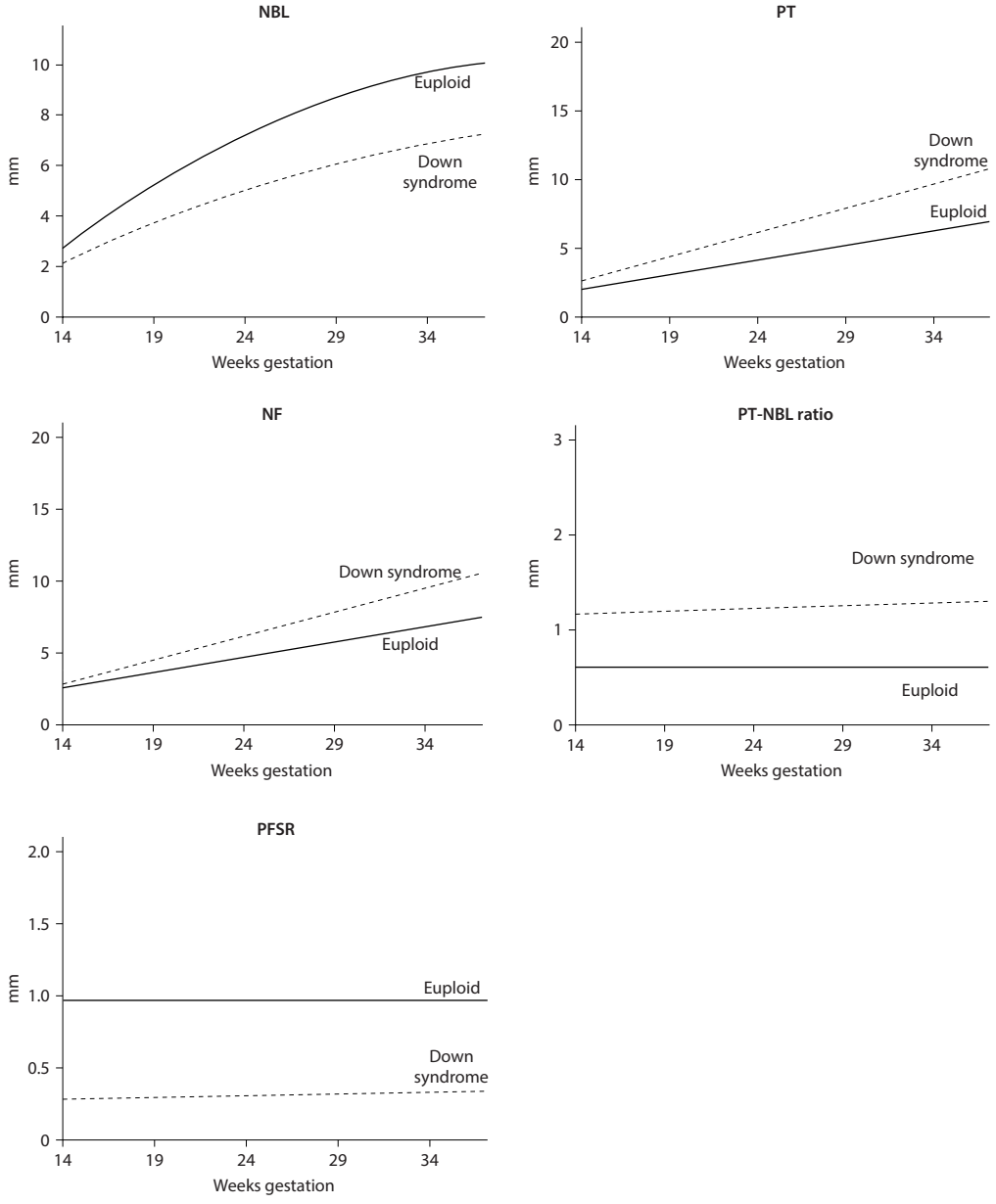


Figure 2 | Mixed model analysis showing the trend line of serial measurements in 25 DS foetuses (longitudinal), compared to the corresponding mean in euploid fetuses. DS, down syndrome; NBL, nasal bone length; PT, prenasal thickness; NF, nuchal fold; PFSR, prefrontal space ratio.

Table 1 | Percentage of DS fetuses with an abnormal first and last measurements or with the same outcome at first and last measurement (both normal and abnormal). NBL, nasal bone length; PT, prenasal thickness; NF, nuchal fold; PFSR, prefrontal space ratio.

	Abnormal at first measurement	Abnormal at last measurement	Same trend at first and last measurement
NBL	66%	76%	81%
PT	82%	86%	86%
NF	83%	66%	50%
PT-NBL ratio	95%	95%	95%
PFSR	94%	75%	69%

Median PT-NBL ratio and PFSR were 1.30 and 0.32, respectively. MoM values for the NF, NBL, and PT, were 1.60, 0.71 and 1.50, respectively. There were no significant correlations between GA and NF MoM, NBL MoM, PT MoM, PT-NBL and PFSR. NBL was the only marker which became increasingly more abnormal with GA ($p = 0.035$).

An overview of the soft markers (besides profile markers) and structural anomalies observed in 22/25 fetuses is presented in table 2. No structural anomalies were observed in 3 (14%) fetuses and no soft markers were observed in 3 (14%) of fetuses. Two (9%) fetuses did not have any soft marker or structural anomaly. When the NBL, PT, NF, PT-NBL ratio and PFSR were added as markers, all fetuses were identified. All 3 fetuses which underwent first trimester combined testing had an increased risk for DS. In one case, non-invasive prenatal testing (NIPT) was performed.

Table 2 | Additional ultrasound findings in 22 DS fetuses at the ultrasound exam. In 3 cases it was not possible to retrieve information from the database. *Soft markers and abnormal findings: ventriculomegaly, aberrant right subclavian artery, mild hydronephrosis, echogenic intracardiac focus, brachycephaly, echogenic bowel, mild pyelectasis, sandal gap, short humerus and femur. ** Profile markers: NBL, PT, NF, PT-NBL ratio and PFSR.

DS fetuses (n = 22)	
Soft markers and abnormal findings* (besides profile markers**)	86%
Structural anomalies	86%
Congenital heart defects	55%
Both soft markers and structural anomalies present	91%
One or more profile markers	100%
Median number of soft markers observed per fetus	1 (average 1.8, range 0 – 5)
Median number of structural anomalies observed per fetus	1 (average 1.4, range 0 – 3)
Previous first trimester combined screening	14%

DISCUSSION

In this study we report longitudinal trends in 5 ultrasound markers measured serially in 25 DS fetuses. NBL, PT, and PT-NBL ratio seem to follow a constant trend with proportional increase of the first two and stability of the third with gestation, whereas NF and PFSR show a great variability and no clear trend. The constant trends observed in NBL, PT and PT-NBL ratio confirm the robustness of these ultrasound markers as also inferred by their high reproducibility in affected fetuses[13]. Conversely, no longitudinal trends were observed in NF measurements, where, in spite of a large number of abnormal first measurements (83%), only 50% of fetuses followed the same trend at subsequent measurements and in 42% no increase in measurements was observed with gestation.

Unfortunately, large studies investigating the reproducibility of NF measurements in both normal and DS fetuses are lacking[8,24,25]. We speculate that the reason for the great variation in NF and the apparent lack of trend in the measurement with gestation is probably the consequence of the difficulty in standardizing the scanning plane where the measurement is taken. A slight change in the angulation of the probe used to obtain the view where the NF is measured, can in fact produce a great variation in the measurement. Furthermore, the position of the fetus in utero can also influence the NF measurement. Especially at later gestational ages, when the fetal head is more often flexed, it can be particularly challenging to visualize the NF and impossible to measure it with the neck in a neutral position.

A limitation of this study and possible cause for the variation in NF, is the fact that some of the NF values were not measured on stored pictures, but derived from the data stored in the database, whereas all other facial markers were (re) measured off-line by the examiners in the same stored picture of a fetal profile. Albeit this limitation, we decided to include the NF in the study, as this is a widely used DS marker.

Three underlying pathological mechanisms have been advocated for the presence of nuchal skin edema in DS fetuses: changes in the extracellular matrix, abnormalities of lymphatic vessels and cardiac dysfunction[26-29]. In this cohort, a cardiac anomaly was present in 55% of the fetuses with known additional ultrasound findings. This was mostly an atrio-ventricular septal defect that is normally not associated with cardiac failure[29] or other kinds of edema or fluid retention. In DS fetuses, an altered venous-lymphatic differentiation of the endothelial cells of the jugular lymphatic sacs has been proposed to occur in the late first or beginning second trimester, causing nuchal edema[28,30]. Fewer studies on pathological examinations of NF in the late second trimester are available. Its pathophysiological background should probably be sought in the altered hydrophilic property of the skin, in combination with the evolution in the second trimester of an enlarged nuchal translucency (NT) in the first trimester. However, the exact relationship between the neck edema present in the first trimester as enlarged NT and in the second trimester as thickened NF, remains controversial[8,31-33]. Unfortunately, the majority (85%) of the fetuses in this study had no combined first trimester screening (including NT measurement).

Also the PFSR showed a considerable variation in longitudinal trends, with an abnormal first measurement in 94%, abnormal last measurement in 75%, but constant in only 69% of the cases. Also for the PSFR, this "instability" may be attributable to the vulnerability of a ratio combining

measurements influenced by the assessment of an angle dependent on good visualization of bony landmarks[13]. Thus, slight variations in one of the components may be heavily reflected in the accuracy of the "combined" marker. However, despite the considerable variation in consecutive measurements, the marker was below the 5th percentile in the majority of cases.

Of all investigated markers the PT-NBL ratio confirms its superiority. Ninety-five percent of DS fetuses had an abnormal ratio at the first and last measurement and remained constant throughout gestation.

The mixed model analysis expresses how one would expect a marker to evolve within time after being measured at a certain point during gestation. The trendlines in figure 2 show that the PT-NBL ratio and PFSR diverge from their corresponding normal ranges, but follow exactly the same trend. In the other markers (NBL, PT, NF) more divergence from the normal range with advancing gestation is observed, suggesting that the degree of abnormality of the marker increases with advancing age. However, their relative deviation from the normal range remains unchanged, as confirmed by the fact that MoM values for these markers remained stable throughout gestation. These findings are confirmed by Maymon et al[6] and Cusick et al[10], who found constant MoM values for NBL and PT-NBL ratio during gestation, whereas Persico et al[7] and Miguelez et al[34] reported an increase in delta PT and MoM PT during gestation.

In terms of discriminative power for DS, the only marker in this study showing a potentially statistically significant increase in detection rate with advancing gestation is the NBL. This is at variance with the findings of our previous study on a cross-sectional cohort of 159 DS cases, where the detection rate of all markers, including NBL, did not change with gestation[13].

Another limitation of this study is that part of the measurements was performed on 2D images and part on 3D volumes. However, in another study we found that measurements of the NBL, PT, PT-NBL ratio and PFSR were not significantly influenced by the acquisition method[35].

Furthermore, GA at time of measurement and number of measurements vary per case, making comparison among cases more challenging.

In conclusion, this study offers insight in the natural history of 5 ultrasound markers in DS fetuses and confirms the strength of the PT-NBL ratio. The PT-NBL ratio follows a stable trend during gestation when measured in the same fetus, with little deviation between measurements in the second and third trimester of pregnancy.

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CHAPTER 8

Fetal facial profile markers in second and third trimester fetuses with Edwards syndrome

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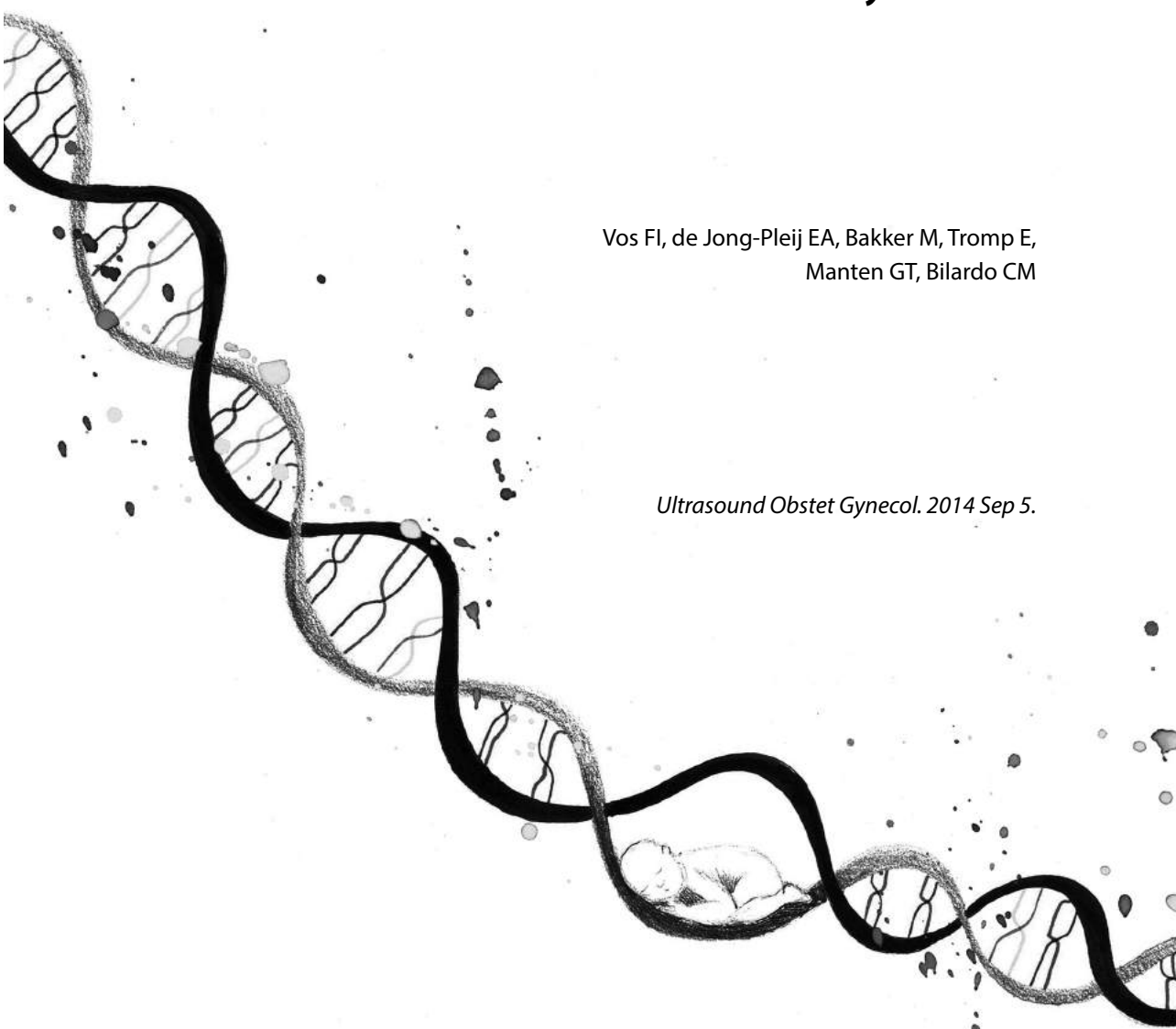

ABSTRACT

Objectives

To evaluate the nasal bone length (NBL), the maxilla-nasion-mandible (MNM) angle, the fetal profile (FP) line, the prenasal thickness (PT), the prenasal thickness to nasal bone length (PT-NBL) ratio and the prefrontal space ratio (PFSR) as second and third trimester markers for Edwards syndrome (also known as trisomy 18).

Methods

The NBL, MNM angle, FP line, PT, PT-NBL ratio and PFSR were measured retrospectively in stored 2D pictures or 3D volumes corrected to the midsagittal plane of fetuses with Edwards syndrome (ES). Data were collected from March 2007 to January 2014. Measurements were performed by 2 examinors and compared to previously reported normal ranges. Additional ultrasound findings (markers, structural anomalies, IUGR) were noted, specifying whether they were detected at the initial routine second trimester scan or at the subsequent advanced ultrasound examination after referral for karyotyping.

Results

43 ES fetuses were included. Median maternal age was 37 years and median gestational age 21^{+2} weeks. NBL and PT were correlated to gestational age ($p < 0.001$), the other markers were not. The mean NBL, MNM angle, PT, PT-NBL ratio and PFSR were 3.76, 16.67, 4.25, 1.39 and 0.87, respectively. The FP line was zero (normal) in 53.7% of cases and negative (abnormal) in 46.3%. All markers were significantly correlated to ES. In the detection rate for ES, the PT-NBL ratio yielded the highest detection rate (88.4%), followed by the NBL (83.7%), MNM angle (56.4%), FP line (46.3%), PT (27.9%) and the PFSR (20.5%). The false positive rate was 5%, except for the FP line, where it was 0%. Various combinations of the 4 best markers (NBL, FP line, MNM angle and PT-NBL ratio) yielded detection rates ranging between 90% and 95%. No structural anomalies were detected in 22% of fetuses at the initial scan and in 2% at the advanced scan.

Conclusions

The PT-NBL ratio and NBL are strong second and third trimester markers for ES. A negative FP line has a 0% false positive rate and the potential to differentiate between ES and Down syndrome, as in the latter the FP line is often positive. No major anomaly was observed at the initial scan in about 1/4 fetuses, underlining the role of second trimester facial marker evaluation.

INTRODUCTION

After trisomy 21, commonly known as Down syndrome (DS), Edwards syndrome (ES; also known as trisomy 18) is the second most common autosomal trisomic disorder in live born babies[1]. The prevalence of live born ES babies varies between countries from 1.0 per 10,000 registered births in 2003 – 2007 in the UK[2], to 2.66 between 2004 – 2006 in the USA[3]. As the risk of fetal loss is high (72% at 12 weeks gestation and 65% at 18 weeks[4]) and termination of pregnancy is carried out in a large percentage of affected pregnancies (83% – 86%[2,4]), the number of affected pregnancies is much higher (an estimated 6.5 in 10,000 registries[2]) than recorded live births.

In the late first trimester, the combined test is used, next to DS screening, as screening for ES, providing individual risk calculations in pregnancy[5]. Not all women undergo this early form of aneuploidy screening, with wide differences in uptake reported across Europe, varying from 90% in Denmark and France[6,7] to 20% and 32% in parts of England and The Netherlands, respectively[8,9]. This means that a substantial group of ES fetuses remains undetected until the routine 20-weeks scan. In the Netherlands, more than 90% of the pregnant population undergoes this routine anomaly scan[10]. Some of the major and minor structural anomalies associated with ES[11] can already be observed in the first trimester[12,13]. However, the sensitivity of ultrasound examination is higher at the time of the 20-weeks scan[11]. Among other anomalies, typical subtle ultrasound features, located in the head and neck region are reported in ES fetuses. These are: absent/hypoplastic nasal bone[14,15], thickened nuchal fold[14,16,17], abnormal facial features (micrognathia, flat profile, sloping forehead)[15,18], abnormal shape of the skull[15,17], and micrognathia[19,20]. We have previously investigated the performance of the profile markers nasal bone length (NBL), maxilla-nasion-mandible (MNM) angle, fetal profile (FP) line, prenasal thickness (PT), prenasal thickness to nasal bone length (PT-NBL) ratio and prefrontal space ratio (PFSR) in euploid and DS fetuses[21,22].

Aim of this retrospective study is to investigate the performance of the same markers in ES fetuses.

METHODS

All cases where ES was suspected and later diagnosed at the mid trimester scan or at later scans, were selected from the databases of the University Medical Centre Groningen, of the University Medical Centre Utrecht and of the Saint Antonius Hospital in Nieuwegein, which act as referral centers. Databases of the participating centers were searched for good quality 3D volumes and 2D images of ES cases from Caucasian parents (as our population was mainly Caucasian). Images were acquired in the second and third trimester, between March 2007 and January 2014. All diagnoses were confirmed by karyotyping (pre- or postnatally).

Only true midsagittal pictures of the fetal profile were selected and considered for further analysis; we considered as such profile pictures showing the forehead, nose, lips and chin, the maxilla as a single horizontal line without zygomatic bone. Pictures with a visible zygomatic bone or ramus of the mandibula were excluded. Volumes were acquired during periods of quiescence from

fetuses facing the transducer, starting from as close as possible to the exact mid-sagittal profile view and with an insonation angle of less than 45º with respect to the nasal bone.

The NBL, PT, PT-NBL ratio, MNM angle, FP line and PFSR were measured as described in our previous studies[23-27] (Figure 1) .

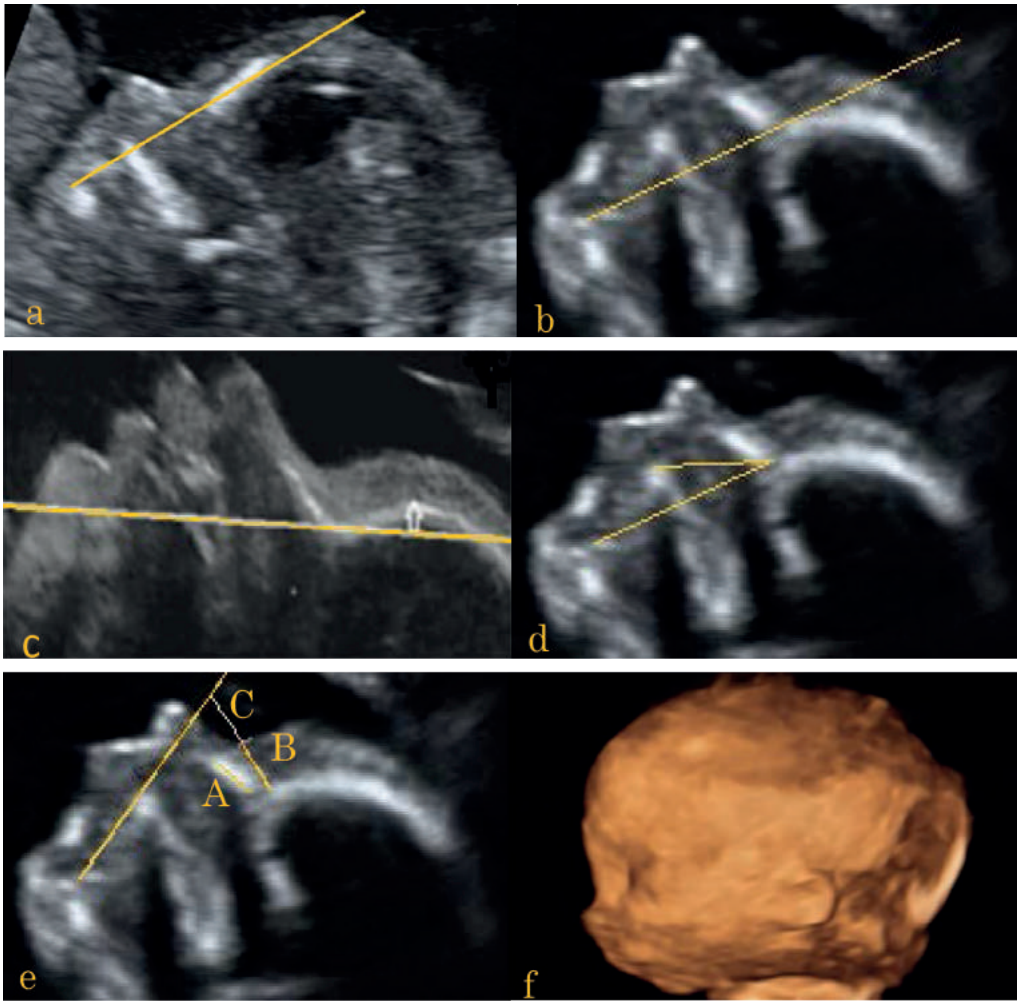

Figure 1 | Ultrasound images of the markers in T18 fetuses, except for the fetus in image c, which is euploid. (**a**) FP line position 'zero'; (**b**) FP line position 'negative'; (**c**), FP line position 'positive'; (**d**) MNM angle; (**e**) NBL (A), PT (B), PT-NBL ratio (B/A), PFSR (C/B); (**f**) 3D reconstruction of T18 fetus. FP, fetal profile; MNM, maxilla-nasion-mandible; NBL, nasal bone length; PT, prenasal thickness; PFSR, prefrontal space ratio.

The FP line was defined as the line that passes through the middle point of the anterior border of the mandible and the nasion. The nasion was defined as the most anterior point in the junction between the frontal and nasal bones. When the FP line passed lengthwise through the frontal bone, this was called 'zero' (Figure 1,a). When the FP line passed the frontal bone anteriorly its position was called 'negative' (Figure 1, b). When the FP line passed the frontal bone posteriorly, its position was called 'positive' (Figure 1, c). The MNM angle was defined as the angle in the median plane between the lines maxilla-nasion and mandible-nasion (Figure 1, d). NBL was measured from the nasion to the end of the white distal ossification line (Figure 1, e A). In cases in which there was a gap between the nasal and the frontal bones (disjunction), the NBL was measured from the distal to the proximal end of the ossification line. To measure the PFSR, first the maxilla-mandible line was drawn between the midpoint of the anterior edge of the mandible and the anterior edge of the maxilla. The line was then extended cranially towards the forehead. Subsequently, the skin covering the forehead was measured between the anterior edge of the bony forehead and the anterior edge of the skin in a line that is parallel to the maxilla and that is traced from the nasion. This measurement is called the prenasal thickness (PT, Figure 1, e B). A second measurement d (Figure 1, e C), was taken starting from the anterior edge of the skin (where PT ended), to the point of interception with the MM line. The PFSR was determined by dividing d by PT. The PT-NBL ratio was constructed by dividing PT by NBL. All markers were measured in the same plane.

All ultrasound examinations were performed by experienced sonographers and images were obtained by a General Electric Voluson 730 Expert ultrasound or E8 system equipped with a RAB4-8L probe (GE Medical Systems, Kretz Ultrasound, Zipf, Austria).

For assessing reproducibility, all markers were measured by two examiners in all cases (F.I.V. and E.J.P.), who were blinded to gestational age and to previous measurements, but not to karyotype. Data were compared to the reference values derived from previous reports on euploid fetuses[23-25,27]: the NBL and PT increased with gestation from 3.3 mm at 15 weeks' gestation to 9.6 mm at 33 weeks (NBL = -6.927 + (0.83*GA)-(0.01*GA^2)) and from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks (PT = 0.212 × GA − 0.873), respectively. The MNM angle, PT-NBL ratio and PFSR were stable throughout gestation, with a mean of 13.5 degrees (95^{th} percentile = 16.9), 0.61 (95^{th} percentile = 0.80) and 0.97 (5^{th} percentile = 0.55), respectively. Measurements below the 5^{th} percentile (for NBL and PFSR) or above the 95^{th} percentile (for MNM angle, PT, and PT-NBL ratio) of the reference ranges, were considered abnormal. An FP line that was not 'zero', was considered abnormal[26,28]. Multiple of the Median (MoM) values were created for the PT and NBL, in order to correct for gestational age.

In all cases, intraclass correlation coefficients (ICC's) were calculated to analyze intra- and interobserver variability. The students t-test was used to analyze differences between measurements. A p-value of less than 0.05 was considered statistically significant. MoM values were calculated for gestation dependent markers. Data were analyzed using the statistical software SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA) and Excel for windows 2000.

Additional ultrasound findings such as markers[29] and/or structural anomalies were documented, specifying whether they were described at the initial routine 20-weeks scan or during subsequent advanced morphological ultrasound examination after referral for karyotyping.

RESULTS

A total of 45 ES cases were available for analysis (6 on stored 3D volumes, 39 on stored 2D images). Median maternal age was 37 (range 26 – 46) years, and median gestational age $21^{+}2$ (range $14^{+}5$ – $31^{+}5$) weeks. Two cases were excluded because the profile view was not midsagittal. In 2 cases, the fetal mandible was not optimally visualized, and consequently the FP line, MNM angle and PFSR could not be analyzed. In another case, the maxilla was not optimally visualized and in another case the fetus had an oro-facial cleft, therefore the MNM angle and PFSR could not be measured. All markers could be successfully measured in the same fetus in 39 cases.

The intra- and inter-observer variability of the measurements is presented in table 1.

Table 1 | Intra- and interobserver variability in ES fetuses.

	Intraobserver variability		**Interobserver variability**	
	Mean difference ***(SD, 95% CI)***	**ICC**	**Mean difference** ***(SD, 95% CI)***	**ICC**
NBL	-0.17 *(0.59, -0.4 – 0.1)*	0.95 *(0.9 – 1.0)*	-0.00 *(0.66, -0.3 – 0.3)*	0.93 *(0.9 – 1.0)*
MNM angle	-0.54 *(3.24, -1.9 – 0.8)*	0.70 *(0.4 – 0.9)*	1.36 *(2.65, 0.3 – 2.4)*	0.73 *(0.5 – 0.9)*
FP line	*	0.74 *(0.5 – 0.9)*	*	0.92 *(0.8 – 1.0)*
PT	-0.09 *(0.40, -0.2 – 0.1)*	0.96 *(0.9 – 1.0)*	0.03 *(0.39, -0.1 – 0.2)*	0.96 *(0.9 – 1.0)*
PT-NBL ratio	-0.05 *(0.28, -0.1 – 0.2)*	0.97 *(0.9 – 1.0)*	0.13 *(0.62, -0.1 – 0.4)*	0.80 *(0.6 – 0.9)*
PFSR	-0.01 *(0.24, -0.1 – 0.1)*	0.83 *(0.7 – 0.9)*	-0.06 *(0.25, -1.2 – 0.0)*	0.77 *(0.5 – 0.9)*

CI, confidence interval; ICC, intraclass correlation coefficient; NBL, nasal bone length; MNM, maxilla-nasion-mandible; FP, fetal profile; PT, prenasal thickness; PFSR, prefrontal space ratio. * As the outcome of the FP line was not continues (negative, zero or positive), it was not possible to calculate mean differences.

The mean (+- SD) NBL, MNM angle, PT, PT-NBL ratio and PFSR were 3.76 (1.62), 16.67 (3.61), 4.25 (1.33), 1.39 (1.00) and 0.87 (0.40), respectively. The nasal bone was absent in 3 (7.0%) cases. The FP line was negative in 46.3% of cases, zero in 53.7%, and in no case positive. The MNM angle, FP line, PT-NBL ratio and PFSR did not change significantly with gestational age, whereas NBL and PT were significantly correlated with gestational age ($p < 0.001$). All markers were correlated with ES. All showed a p-value below 0.001, except for the PSFR ($p = 0.044$).

The detection rate (DR), false-positive rate (FPR), positive likelihood ratio and negative likelihood ratio of all markers are shown in table 2.

Table 2 | The performance of the NBL, MNM angle, FP line, PT, PT-NBL ratio and PFSR.

	DR *(95% CI)*	**FPR** *(95% CI)*	**PLR** *(95% CI)*	**NLR** *(95% CI)*
NBL (mm)	83.7% *(68.6% – 93.0%)*	5.0% *(1.7% – 11.3%)*	16.7 *(7.0 – 39.6)*	0.17 *(0.09 – 0.35)*
MNM angle (degrees)	56.4% *(38.3% – 71.4%)*	5.0% *(1.7% – 11.3%)*	11.3 *(4.5 – 27.2)*	0.46 *(0.33 – 0.67)*
FP line	46.3% *(29.3% – 61.5%)*	0% *(0% – 3.7%)*	∞	0.54 *(0.42 – 0.73)*
PT (mm)	27.9% *(13.9% – 42.0%)*	5.0% *(1.7% – 11.3%)*	5.6 *(1.9 – 14.2)*	0.76 *(0.65 – 0.94)*
PT-NBL ratio	88.4% *(74.4% – 96.0%)*	5.0% *(1.7% – 11.3%)*	17.7 *(7.4 – 41.7)*	0.12 *(0.05 – 0.29)*
PFSR	20.5% *(9.6% – 37.3%)*	5.0% *(1.7% – 11.3%)*	4.1 *(1.5 – 12.1)*	0.84 *(0.70 – 0.99)*

NBL; nasal bone length, MNM angle, maxilla-nasion-mandible angle; FP line, fetal profile line; PT; prenasal thickness, PT-NBL ratio; prenasal thickness to nasal bone length ratio, PFSR; prefrontal space ratio, DR; detection rate, FPR; false positive rate, PLR; positive likelihood ratio, NLR; negative likelihood ratio, ∞; infinity.

Of the six markers, the PT-NBL ratio had the best screening performance with a DR of 88%, followed by the NBL with a DR of 84%.

There was no case in which the 6 markers were all normal or all abnormal. In all cases at least 1 of the six markers was abnormal. Various combinations of the 4 strongest markers (NBL, FP line, MNM angle and PT-NBL ratio) yielded similar DR's ranging between 90% and 95% (Table 3).

Table 3 | Detection rates of various combinations of ES markers.

	NBL	**FP line**	**MNM angle**	**PT-NBL ratio**
NBL	X			
FP line	90%	X		
MNM angle	95%	72%	X	
PT-NBL ratio	93%	93%	93%	X

NBL; nasal bone length, MNM angle, maxilla-nasion-mandible angle; FP line, fetal profile line; PT-NBL ratio; prenasal thickness to nasal bone length ratio

When MoM NBL, MNM angle, FP line, MoM PT, PT-NBL ratio and PFSR were compared, the PT-NBL ratio was significantly correlated to MoM NBL and MoM PT ($p < 0.01$). The MNM angle was correlated to the FP line and PFSR ($p = 0.015$ and $p < 0.01$, respectively). Gestational age at the time of detection did not influence DR in any of the markers, with the exception of PT, where DR was significantly higher with advancing gestation ($p < 0.01$). Figures 2-7 show the six individual markers plotted against their normal ranges throughout gestation[23-27].

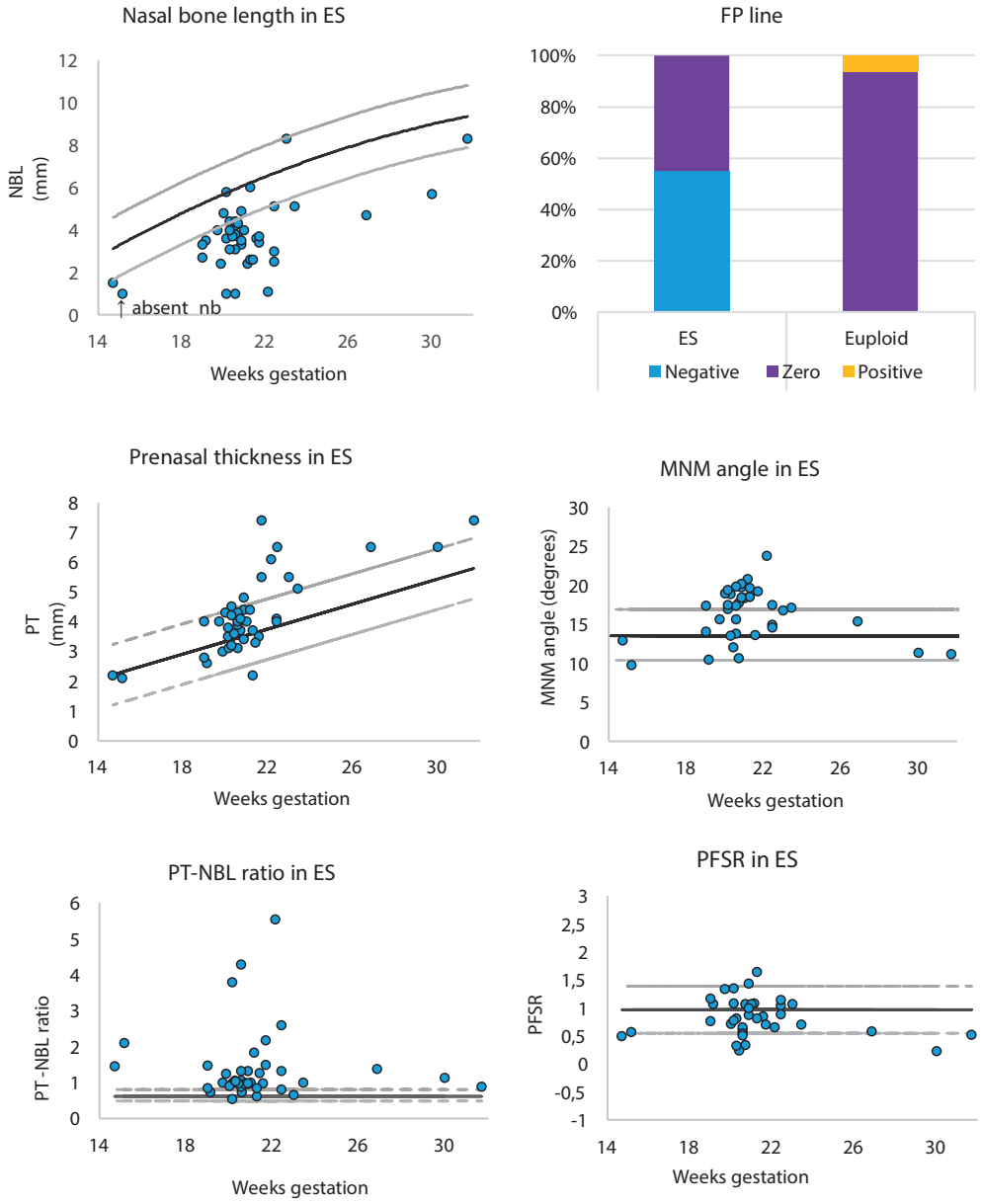

Figure 2-7 | NBL (n = 43), FP line (n = 41), MNM angle (n = 39), PT (n = 43), PT-NBL ratio (n = 43) and PFSR (n = 39) in ES fetuses, plotted on normal ranges[24,26,27] (mean, 5th centile and 95th centile).

NBL; nasal bone length, MNM angle, maxilla-nasion-mandible angle; FP line, fetal profile line; PT; prenasal thickness, PT-NBL ratio; prenasal thickness to nasal bone length ratio, PFSR; prefrontal space ratio,

Table 4 | Abnormal ultrasound findings in T18 fetuses at initial and advanced second trimester ultrasound scan. Nuchal fold: > 5 mm before 20 weeks GA and > 6mm over 20 weeks GA. Renal pyelectasis: 5 – 10 mm in the second trimester and 10 – 15 mm in the third trimester, Short humerus: below the 5th percentile, growth restriction: below the 5th percentile.

	Initial 20-weeks scan (n =27)	Advanced scan (n = 43)
Soft markers[29] and abnormal findings (besides profile markers)		
1. Choroid plexus cyst	44%	70%
2. Single umbilical artery	26%	33%
3. Short femur	11%	12%
4. NF	11%	11%
5. Overlapping fingers	7%	60%
6. Renal pyelectasis	4%	5%
7. Echogenic bowel	-	7%
8. Clinodactyly	-	5%
9. Echogenic intracardiac focus	-	-
10. Short humerus	-	-
11. Other	4%	26%
Structural anomalies		
1. Heart	52%	77%
2. Growth restriction	11%	37%
3. Skeletal (including facial cleft and anomalies of the feet)	7%	67%
4. Central Nervous system	7%	35%
5. Chest	7%	16%
6. Abdomen	7%	16%
7. Genitourinary	-	9%
8. Cystic hygroma	-	-
Average number of soft markers observed	1.4	2.6
Average number of structural anomalies observed	0.9	2.6
≥ 1 soft marker	100%	100%
No structural anomaly observed	22%	2%

Table 4 shows the percentage of ES fetuses showing abnormal features (markers other than profile markers, pathological conditions or structural anomalies) at the initial ultrasound scan and at the advanced ultrasound examination at the referral center. It was not possible to retrieve data of the initial scan in all fetuses. In this cohort a mean of 1.4 'soft' ultrasound markers or other abnormal

findings (such as choroid plexus cyst or polyhydramnios) and 0.9 structural anomalies were observed at the 20-weeks scan and 2.6 soft markers and structural anomalies at the advanced referral scan (Table 4). All fetuses had at least one soft marker at both the initial 20-weeks scan and advanced scan. In 22% of fetuses no structural anomalies were observed at the initial 20-weeks scan compared to 2% at the advanced scan.

DISCUSSION

In this study we report for the first time of the use of profile markers already extensively investigated in DS, in another trisomy, namely ES. We show that the highest detection rate is obtained when the PT-NBL ratio (88%) is used, closely followed by the NBL (84%).

One of the main findings of this study is that in second trimester ES fetuses, the NBL is exceptionally small, even smaller than in DS[21]. Nasal hypoplasia has been reported in several chromosomal disorders including DS, ES, T13 and Turner syndrome, of which DS is the most extensively investigated[21,30-33]. A short nasal bone has been reported in about 53%[13,32] of first trimester ES fetuses and in 67% of second trimester ES fetuses when combined with an enlarged nuchal fold[14].

This study indicates that nasal bone hypoplasia in ES seems to become more pronounced with advancing gestation. Growth restriction, a very common feature in ES[34], may be an explanation for this finding. Another common feature in ES is micrognathia. Micrognathia is also a common finding in triploidy and Turners' syndrome and it is suggested to be associated with an abnormal karyotype in 66% of the cases when observed prenatally[31,35,36]. It is not surprising that markers taking into account micrognathia, such as the MNM angle and the FP line, have a better performance in ES than in DS[21].

This is the first study investigating the MNM angle and the FP line in ES. Two other facial angles, the fronto-maxillary-facial (FMF) angle and the mandibulo-maxillary-facial (MMF) angle, are described by Borenstein in first trimester ES fetuses[37]. The FMF angle reflects mid-facial hypoplasia and the MMF the relationship between mandible and maxilla. The DR of the MMF angle in ES (33%) is lower than the DR of 56% of the MNM angle, reported in this study (at 5% FPR). However, The MNM angle has a wide standard deviation and a high inter- and intraobserver-variability. A negative FP line is caused by micrognathia and/or a sloping forehead, both common in ES[20]. In this cohort we found a negative FP line in 46% of cases. This is a modest DR compared to NBL and PT-NBL ratio. The additional value of this marker in ES is the fact that the FP line is never negative in euploid fetuses[26], implying a 0% FPR. Moreover, DS fetuses show more frequently a positive FP line[22]. Hence, in the presence of nasal hypoplasia, a negative FP line of is suggestive of ES and a positive FP line of DS. Prenasal edema, a common feature in DS, is far less common in ES, as reflected by the poor performance of PT and PSFR. However PT did slightly improve the DR of NBL when combined in a ratio.

The PFSR is a marker taking into account the position of the mandible and prenasal thickening. Micrognathia increases the PFSR value, however prenasal thickening reduces it (as it is the case in DS[21]). The DR of the PFSR in ES was 21% (PFSR value below the 5th percentile). We therefore

hypothesize that in ES fetuses, the effect of micrognathia on the PFSR may be counterbalanced by the presence of prenasal thickening.

In reporting additional ultrasound findings in this cohort we make a distinction between findings observed at the initial (usually routine) second trimester ultrasound examination, and findings at the advanced ultrasound examination carried out by Fetal Medicine experts after referral. In women who did not undergo first trimester screening, a systematic evaluation of facial markers at the 20-weeks scan may alert the ultrasonographer about a possible aneuploidy, especially when obvious structural anomalies are not observed. This is substantially different than when (subtle) anomalies are observed when there is already a suspicion of aneuploidy that has warranted referral to a Fetal Medicine Unit.

At the routine 20-weeks scan, an average of 1.4 soft markers and abnormal findings- mostly choroid plexus cysts (CPC) and single umbilical artery – and 1 structural anomaly were seen. Interestingly, next to congenital heart disease and major skeletal defects, CPC and overlapping fingers were the most frequently observed minor anomalies at the advanced ultrasound examination (70% and 60%, respectively). Overlapping fingers are highly associated with ES[38], in contrast to CPC[39] (as an isolated finding[38,40]). In almost 1/4 ES fetuses, no major anomaly was observed at the initial scan. This strengthens our belief that there may be a role for the systematic and routine evaluation of facial markers at the 20-weeks scan. In fact, in our experience, even women who have declined DS screening value to be informed about the chance of their fetus to be affected by a lethal condition, such as ES .

A limitation of this study is its retrospective nature and the fact that examiners were not blinded to the karyotype. Ideally, a repeatability and reproducibility study should be performed not only by re-measuring ultrasound markers on stored pictures, but also by re-acquiring the desired image. Due to the retrospective nature of this study the latter was not possible, and the reproducibility figures therefore relate exclusively to the reproducibility of the measurement. Furthermore, it was not possible to retrieve data on additional ultrasound findings at the initial scan in all fetuses.

In conclusion, this study shows that when at second trimester ultrasound gross anomalies are not observed, ES can be can effectively detected by the combination of markers for micrognathia (MNM angle and FP line) and by a small nasal bone (NBL and PT-NBL ratio). We prefer a combination of PT-NBL ratio and FP line; the PT-NBL ratio is in fact the strongest marker for ES (and DS), while the FP line can differentiate between ES and DS. Furthermore, both markers are independent of gestation and therefore a fixed cut-off can be used.

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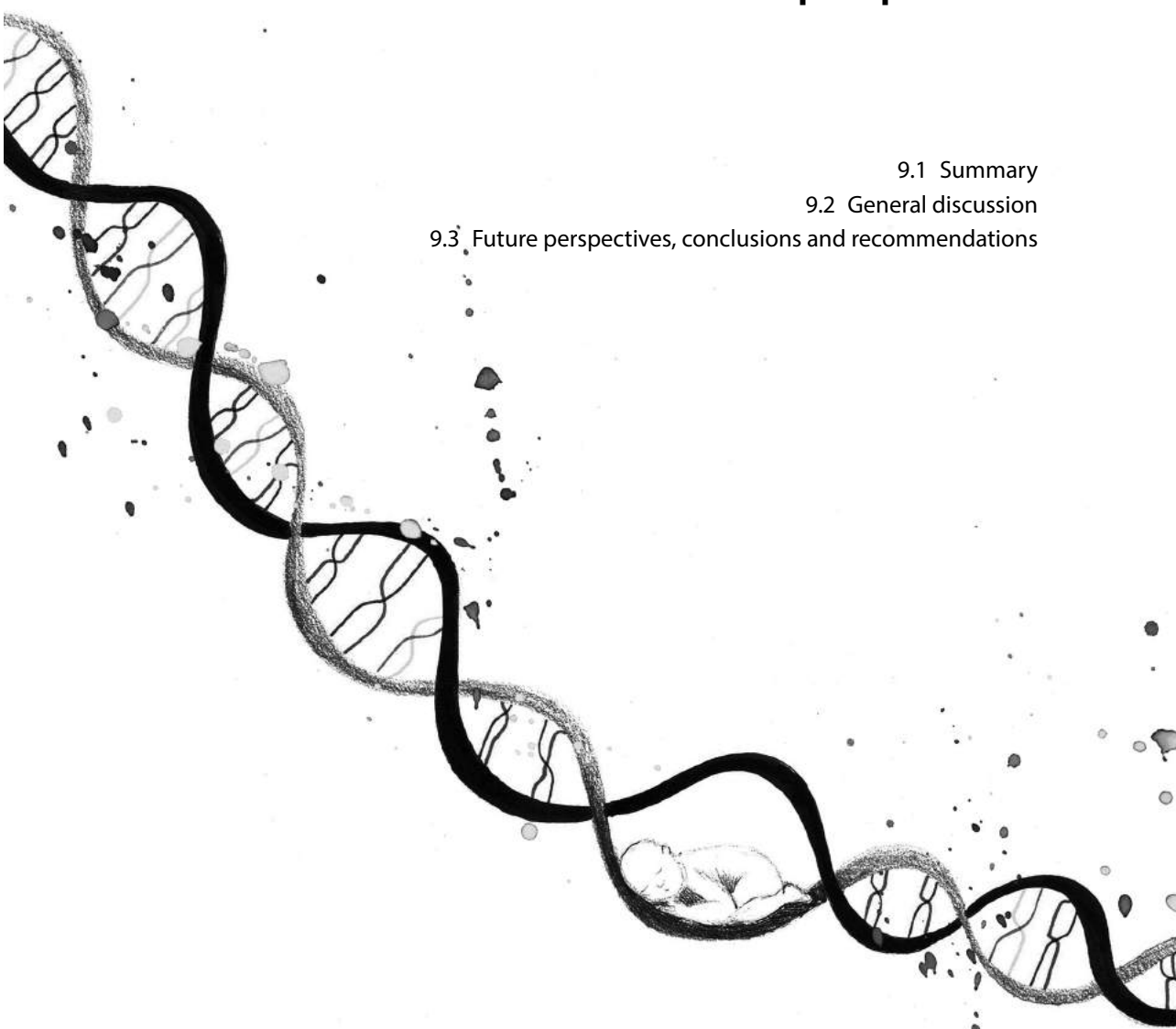
CHAPTER 9

Summary, general discussion and future perspectives

9.1 Summary
9.2 General discussion
9.3 Future perspectives, conclusions and recommendations



9.1 SUMMARY

Chapter 1 gives a brief insight in the history of ultrasound (US) as a tool to examine the fetus. US technique finds its roots in the early 1960's. The transition of US examination from a rudimentary technique, to the highly advanced screening and diagnostic tool that it is today is reviewed. Furthermore, the new development of three-dimensional (3D) US is discussed, with its merits and limitations compared to conventional two-dimensional (2D) US. Screening for Down syndrome (DS), the most common autosomal trisomy in life born infants is discussed. Also, an overview is given of the current screening options available in the first and second trimester. Special attention is paid to markers that can be found in the fetal profile during US examination in the second trimester. Furthermore, a brief introduction of Edwards syndrome (ES) is provided with information on incidence, major malformations observed during pregnancy and at birth, survival and screening possibilities.

In **chapter 2 and 3**, four structures located in the fetal profile, that were introduced recently as DS markers, are assessed in a group of euploid fetuses. Novel 3D based reference ranges are constructed. Subsequently, one of these markers, the prenasal thickness to nasal bone length (PT-NBL) ratio is tested in a small cohort of DS fetuses.

Chapter 2 assesses the feasibility of measurements of the nasal bone length (NBL), prenasal thickness (PT) and fronto-maxillary facial (FMF) angle, performed on the same 3D multiplanar corrected profile view in healthy second and third trimester fetuses. A three points scoring system was used to grade the images in terms of contrast and clarity. Only images with the highest two scores were used for further analysis. Measurements of each marker were repeated three times and the average was taken as the final measurement. It was significantly more often possible to achieve a high quality visualization of the NBL and PT (98% and 97%, respectively), than of the FMF angle (26%, $p < 0.001$). Both intra- and inter observer variability were superior in the first two markers. NBL increased significantly with gestation, from 3.3 mm at 15 weeks to 9.6 mm at 33 weeks gestation. PT was also correlated to gestational age, and increased from 2.3 mm at 15 weeks to 6.1 mm at 33 weeks gestation. Reference ranges for both markers are presented. The FMF angle did not seem to be correlated to gestational age, but owing to the paucity of high quality FMF angle measurements, extensive analysis was not performed with this angle and no reference range was constructed. An interesting observation was that after we had redefined the measurement technique for NBL (carefully excluding the frontal bone from the measurement by measuring along the superior surface of the bone and not mid-way through), our reference range for the NBL showed a systematically smaller length than other 2D US based publications, whilst following the same curve. In conclusion, NBL and PT, measured on 3D rendered volumes, are easily applicable markers, whereas the FMF-angle is more challenging. Furthermore, care should be taken in excluding the frontal bone from the measurement.

In **chapter 3**, we have studied the ratio of the PT to NBL, the PT-NBL ratio, in normal and DS fetuses in the second and third trimester of pregnancy. The measurements of the study mentioned in chapter 2 were used to calculate the PT-NBL ratio in normal fetuses. The PT-NBL ratio did not

increase with gestational age (mean 0.61, 95% CI, 0.59 – 0.63; r = -0.04, P = 0.7). The 5th and 95th percentiles were 0.48 and 0.80, respectively. This reference range was used to compare to a small cohort of DS fetuses. The PT-NBL ratio was significantly higher in DS fetuses than in normal fetuses (P < 0.001) but was also stable throughout gestation, with a mean of 1.50 (95% CI, 1.20 – 1.80; r = -0.35, P = 0.07). All DS fetuses had a PT-NBL ratio above the 95th percentile. When the 95th percentile of the PT-NBL ratio was used as a cut-off value, the detection and false positive rates for DS were 100% (95% CI, 89 – 100%) and 5% (95% CI, 2 – 11%), respectively. The positive likelihood ratio was 21.2. The conclusion of this study is that the PT-NBL ratio is a stable marker for DS in the second and third trimester of pregnancy. Most importantly, all DS fetuses in this series had a PT-NBL ratio above the 95th percentile, making it a very promising marker for DS.

Chapter 4 and 5 deal with the introduction of two markers for DS, the maxilla-nasion-mandible (MNM) angle and the fetal profile (FP) line, and assess four other markers for DS, the NBL, PT, PT-NBL ratio and prefrontal space ratio (PFSR). These markers are located in the fetal profile and aim to quantify the shape of the profile. The measurability and reproducibility of the MNM angle and FP line with its corresponding FP distance (the shortest distance between the FP line and frontal bone) is assessed in a group of euploid fetuses, **chapter 4** introduces them as markers for DS. This was done retrospectively in a cohort of 138 DS fetuses in the second and third trimester of pregnancy. Measurements were compared to our previously reported normal ranges. The MNM angle was significantly smaller in DS fetuses (mean 12.9º) than in euploid fetuses (mean 13.5º , p = 0.015). However, in only 16.9% of DS fetuses, the MNM angle was below the 5th percentile, although this was significantly more often than in euploid fetuses (p < 0.01). The MNM angle was not influenced by the gestational age (p = 0.48). Intra- and inter-observer reproducibility was expressed as intra-class correlation coefficient (ICC) with values of 0.89 and 0.61 for the MNM angle and 1.0 and 0.76 for the FP line, respectively. In the cohort of DS fetuses, none had a negative FP line. In the entire DS group, the FP line was positive in 41.1% of fetuses and correlated to both DS and gestational age (p < 0.001). As in a previously studied group of euploid fetuses, the FP line was never positive before 27 weeks gestation, we decided to divide the DS group in two cohorts: the second and third trimester. Their respective detection rates with false positive rates (FPR) were 28.4% with 0% FPR and 76.5% with 16.9% FPR for the second and third trimester, respectively. The FP distance increased with gestational age (p<0.001), with a mean distance of 3.1 mm. The FP distance was not significantly larger in DS fetuses than in euploid fetuses (p = 0.4). Small MNM angles were correlated with a positive FP line (p < 0.001). By means of this study we propose the FP line as a novel marker for DS with an extremely low false positive rate in the second trimester. As the FP line requires no reference values (as it is positive, negative or zero), its use is very easy.

In **chapter 5**, the four markers NBL, PT, PT-NBL ratio and PFSR are evaluated in a large cohort of second and third trimester DS fetuses. The markers were measured in 159 DS fetuses and compared to previously reported reference ranges. The median maternal age was 35.8 years, the median gestational age was $23^{+}1$ weeks. Intra- and inter-observer variability were best in NBL, PT and PT-NBL ratio, with intra-class correlation coefficients (ICC) of 0.98 and 0.93, 0.98 and 0.97, 0.94 and 0.92, respectively. The PFSR had ICC's of 0.67 and 0.67, respectively. NBL and PT were correlated to gestational age (p < 0.001), PT-NBL ratio and PFSR were not. All markers were correlated to DS

($p < 0.001$). The mean NBL, PT, PT-NBL ratio and PFSR were 5.08, 5.56, 1.26 and 0.34, respectively. The nasal bone was absent in 23 (15.4%) cases. In screening by all four markers, the PT-NBL ratio yielded the highest detection rate of 86.2%, followed by the PFSR (79.7%), PT (63.4%) and NBL (61.9%). In 33.6%, all markers were abnormal. In 4.7% of cases, all markers were normal. The combination of all four markers yielded the best detection rate of 95.3%, followed by a combination of PFSR and PT-NBL ratio with 93.8%. As the PT and NBL are used to calculate the PT-NBL ratio and PT is also used to calculate the PFSR, we were not surprised to find only the Multiple of the Median (MoM) NBL and PFSR to be independent of each other ($p = 0.10$). Both the DR for all markers as well as MoM NBL, MoM PT, PT-NBL ratio and PFSR, were not correlated to gestational age. In conclusion we propagate the use of the PT-NBL ratio and PFSR in the second trimester of pregnancy. Not only do these markers achieve a high detection rate of 94%, they are also very user-friendly in the fact that they require no knowledge of gestational age specific means.

Chapter 6 deals with the assessment of differences between measurements when using either 2D images or 3D volumes. Differences between six markers (NBL, PT, FP line, MNM angle, PT-NBL ratio and PFSR) when measured on 2D images (acquired with strict criteria) or 3D rendered volumes were analyzed, as well as their clinical application in screening settings. All six markers were measured in 105 datasets: 75 of euploid fetuses and 30 of DS fetuses. 2D images and 3D volumes were both derived separately in the same scanning session. The MNM angle measurements in 2D US were significantly larger by 1.0 degree ($p < 0.01$). In all other markers there was no significant difference between 2D or 3D US. Limits of agreement (LOA) for intra- and inter-observer variability were smaller in 3D measurements for all markers, except for the MNM angle. When the group of DS fetuses was analyzed separately, no statistical difference was found for any of the markers measured in 2D or 3D US, in their ability to discriminate between normal and DS fetuses. With this study, we have shown that, with exception of larger MNM angles in 2D images, no significant differences are found in a number of facial profile parameters and DS markers. In particular, NBL, PT, FP line, PT-NBL ratio and PFSR can be confidently used as DS screening markers in US examinations performed by 2D US, without missing out on the additional benefit of 3D US, provided the markers are measured in an as good as possible mid-sagittal image of the fetal profile.

Chapter 7 evaluates repeated measurements of the NBL, PT, nuchal fold (NF), PT-NBL ratio and PFSR in second and third trimester DS fetuses. Markers were studied retrospectively and compared to previously reported normal ranges. A total of 24 DS fetuses were analyzed. The median gestational age was 25 weeks. Median gestational age at initial examination was $20^{+}4$ weeks, and at final examination $29^{+}2$ weeks. The median interval between measurements was 39 days (range 14 – 98 days), with an average number of 2.6 visits per case. NBL, PT and NF increased significantly with gestational age ($p < 0.01$), PT-NBL ratio and PFSR did not. In 42% of DS cases, NF did not increase in at least one consecutive measurement, opposed to 4.8% and 13.6% for NBL and PT, respectively. The PT-NBL ratio was the most stable marker, remaining the same value in 95% of cases. In a 'mixed model' format, a corresponding trendline for repeated measurements was compared to the mean of euploid fetuses. In this format, the gestational age dependent markers (NBL, PT, NF) expressed more deviance with advancing gestation, but MoM values remained stable. The NF and PFSR showed the largest differences between measurements when every case was individually depicted. However for

the PFSR, most measurements were still far below the normal range. In this study we conclude that repeated measures of the NF and PFSR are the least likely to follow an expected and steady trend in a single DS fetus, probably due to challenging reproducibility. However, PFSR still remains a valuable marker, as most measurements are still far from normal.

In **chapter 8** we assess six markers that were initially introduced as markers for DS, as potential markers for Edwards syndrome (also known as trisomy 18) in the second and third trimester. The markers tested include the PT, NBL, PT-NBL ratio, PFSR, MNM angle and FP line. Measurements were compared to previously published normal ranges. In order to further investigate the clinical relevance of US markers for Edwards syndrome (ES), additional US findings (markers, structural anomalies, growth retardation) were noted, specifying whether they were detected at the initial routine 20-weeks scan or at the subsequent advanced US examination after referral for karyotyping. Fourty-three ES fetuses were included. Median maternal age was 37 years and median gestational age 21^{+2} weeks. As in DS, the NBL and PT were correlated to gestational age ($p < 0.001$), the other markers were not. The mean NBL, MNM angle, PT, PT-NBL ratio and PFSR were 3.76, 16.67, 4.25, 1.39 and 0.87, respectively. The FP line was zero (normal) in 53.7% of cases and negative (abnormal) in 46.3%. All markers were significantly correlated to ES. A short nasal bone was a prominent feature in ES fetuses, opposed to an enlarged PT. This was illustrated in the performance of the separate markers: in the detection rate for ES, the PT-NBL ratio yielded the highest detection rate (88.4%), followed by the NBL (83.7%), MNM angle (56.4%), FP line (46.3%), PT (27.9%) and the PFSR (20.5%). The false positive rate was 5%, except for the FP line, where it was 0%. Various combinations of the 4 best markers (NBL, FP line, MNM angle and PT-NBL ratio) yielded detection rates ranging between 90% and 95%. No structural anomalies were detected in 22% of fetuses at the initial scan and in 2% at the advanced scan. The main conclusions of this chapter are that the PT-NBL ratio and NBL are strong second and third trimester markers for ES. Furthermore, a negative FP line has a 0% false positive rate and the potential to differentiate between ES and DS, as in the latter the FP line is often positive. No major anomaly was observed at the initial scan in about 1 in 4 ES fetuses, underlining the role of second trimester facial marker evaluation.

Summary of the most important findings

Examination of markers in euploid fetuses:

- The PT, NBL and PT-NBL ratio are reproducible markers that are easy to measure.
- The FMF angle is often difficult to assess (in retrospect), as the landmarks which are used to construct this marker (palate, vomer) are often not clearly visible in the second and third trimester.
- It is important not to include part of the frontal bone when the NBL is measured, as our reference range showed a systematically smaller measurement, when compared to other publications.
- The PT-NBL ratio in euploid fetuses has a constant mean value of 0.61 throughout the second and third trimester. The 95^{th} percentile is 0.80.

Screening performance of markers in DS fetuses:

- The PT-NBL ratio yields the highest detection rate of 86.2%.
- The two best-performing DS markers in terms of detection rate, the PT-NBL ratio and PFSR, together detect 94% of DS fetuses and require no knowledge of a reference range.
- A positive FP line has an extremely low false positive rate in the second trimester of 0%.

Comparison of 2D and 3D US:

- When measurements in NBL, FP line, MNM angle, PT, PT-NBL ratio and PFSR are compared in 2D (required according strict criteria) and 3D images, only the MNM angle shows a small difference.
- No statistical difference was found between 2D and 3D acquired measurements for any of the six markers in their ability to discriminate between normal and DS fetuses.

Longitudinal analysis of DS markers:

- The reliability of NF as a second trimester DS marker is disputable.
- Repeated PFSR measurements in the same fetus are subject to considerable variation.
- The PT-NBL ratio is a very stable marker.

Screening performance of markers in ES fetuses:

- The PT-NBL ratio and NBL are strong second and third trimester markers for ES.
- A negative FP line showed a 0% false positive rate in this study and offers the potential to differentiate between ES and DS.
- No major structural anomaly was observed at the initial US examination in about 1 in 4 fetuses, opposed to 2% at the advanced US exam.

9.2 GENERAL DISCUSSION

This thesis has explored the potential of facial profile markers for identifying aneuploid fetuses in ultrasound (US) investigations performed beyond the first trimester.

The result of the thesis can be summarized as follows: of all markers that have been explored, the prenasal thickness (PT) to nasal bone length (NBL) ratio (PT-NBL ratio), is a strong second and third trimester US marker for both Down syndrome (DS) and Edwards syndrome (ES), whereas the fetal profile (FP) line is often positive in DS and negative in ES. For clinical practice, the great advantage of the PT-NBL ratio lies in the fact that the ratio is stable during pregnancy with the PT being about 2/3 of the NBL with the 95th percentile stable at 0.80. Moreover, this thesis demonstrates that, although 3D correction of the profile by 3D multiplanar mode allows definition of the correct midsagittal plane, the use of this correction is not essential when applying the markers in current clinical practice. This is an important issue, as it implies that the profile markers can theoretically be part of routine US investigation, even when the used US equipment does not include a 3D mode.

Of all the other studied facial profile markers in DS, the second best was the PSFR ratio. Its sensitivity was however slightly inferior to that of the PT-NBL ratio and the measurement might

be more time consuming. Moreover, it could be difficult to master and sensitivity in ES is low. The other markers, such as the PT and NBL (as separate markers) and the MNM angle, appear less effective. However, even if these markers do not seem to play an important role in the identification of DS and ES, the merit of this study is to have reinforced their use as instruments to study the fetal face. Familiarity with their use and application may be of great value when the ultrasonographer suspects an abnormal profile and needs this finding to be supported by an objective evaluation of facial proportions and relations. The less effective markers therefore still qualify as important instruments in the hands of the ultrasonographer, as they can be applied in the emerging field of fetal dysmorphology.

The studies included in this thesis were all retrospective. This has enabled inclusion of a large number of DS and ES fetuses, retrieved from databases of more than one centre. In case of a prospective study design, a lot of time would have been necessary to collect an equal number of cases. A clear limitation of a retrospective design – whereby cases are selected after the karyotype is known and the profile markers are measured on stored pictures – is that the sensitivity of the markers may be overestimated. However, with the rapid advent of cell free fetal DNA techniques in maternal blood as early screening for trisomies, we assume that the number of fetuses with trisomies at the second trimester scan may in the future be drastically reduced and therefore future validation of the data in prospective studies may become extremely unlikely.

A legitimate question regarding this study could be how it has been possible to collect so many cases of chromosomally abnormal fetuses reaching the second trimester undetected. This was possible, as many women in The Netherlands do not choose to undergo first trimester screening for aneuploidies. Later in pregnancy, they might be referred to a prenatal diagnostic center because of the finding of structural anomalies or other pathologies, such as growth retardation, detected at the second or even at a third trimester scan. This, in turn, prompted karyotyping before or after birth and from these cases, stored pictures of second and/or third trimester prenatal facial features of the chromosomally abnormal fetuses were retrieved. Moreover, the series of studies in this thesis should be seen as a logic continuation of the work our group started about ten years ago by applying the advantages of 3D US to the study of the fetal face[1]. These studies pointed out that 3D multiplanar mode technique could be of help in standardizing the planes for a morphometric evaluation of the fetal face. Conditions such as micrognathia, sloping forehead, bossing forehead, facial clefts etc. could be objectively measured[2-4]. In the first series of studies, we also reported for the first time on the detection of DS by using a combination of facial markers; the PT-NBL ratio[5]. In this study we reported a DR of 100% in 30 fetuses. This exceptionally high detection rate stimulated our group to focus on further studies concerning the application of all the previously defined facial markers in fetal trisomy screening. By extending the number of DS cases the performance of the PT-NBL ratio in the second study was, as expected, less than 100%. It is likely that, in a prospective design, this would become even lower. However, this ratio remains an exceptionally good marker for fetal trisomies in the second and third trimester and, if combined with other markers such as the PFSR, can reach detection rates up to 94%. This is better than all other previously known and used markers in the so called "genetic sonogram".

It is undisputed that for all kinds of practical and ethical reasons the preferred moment for screening for trisomies is in the first trimester. This is especially the case as the scenery of prenatal screening for trisomies is rapidly changing due to the introduction of non-invasive prenatal testing (NIPT). However, it is also true that for all kinds of other reasons it will never be possible to ensure that first trimester screening takes place in all pregnancies. Therefore it is very important that also in the second and third trimester of pregnancy, strong markers for trisomies are available. Participation in first trimester screening for trisomies in The Netherlands is low in comparison to other European countries, not reaching more than 30% of the pregnant population and even less in rural areas. A negative attitude towards termination of pregnancy (TOP) and acceptation of DS have been reported as reasons for the low uptake[6]. However, other factors, such as not being fully aware of the fact that even young women have a chance of having a DS baby or that by declining the CT a choice with clear consequences is being made, also play a role. A regrettable factor that may have influenced counselling and the attitude of women and care givers with respect to the CT is the fact that first trimester screening was free of charge until the end of 2014 only for women of 36 years and older. Unfortunately, in spite of pressure from various professional organizations, the Dutch Ministry of Health has decided to eliminate the inequality between older and younger women by establishing that all women, irrespective of their age, have to pay for the CT[7]. In case of increased risk, access to NIPT and invasive procedures will be free, whereas access to invasive procedures based purely on maternal age is not reimbursed anymore. The community of professionals involved in counselling and screening of pregnant women is anxious to see what the effects will be of such a new change in course of the Dutch policy makers. One may speculate that the uptake of the CT may decrease in general or increase only among older women, but the opposite may also occur, with more women choosing NIPT directly, irrespective of the Dutch regulations. Furthermore, we hope that the traditional first trimester scan will stay preserved in the future, as the goal of this scan is not only to screen for trisomies, but also for other anomalies. One way or another, we expect that for the time being, many cases of chromosomal anomalies will remain undetected until the moment of the 20-weeks scan which is part of routine prenatal care.

An ethical objection to screening for DS by means of the 20-weeks scan might be that most women undergo the scan with the expectation to see their baby, not realizing that it may also reveal unexpected malformations and even malformations related to chromosomal anomalies[8]. This was assessed by a Dutch study[9], indicating discordance between medical experts and pregnant woman's attitude towards the 20-weeks scan. The first group regarded the scan primarily as a mean to detect anomalies and, opposed to the pregnant women, considered the 20-weeks scan to have a similar value as first trimester screening for congenital anomalies. These findings raise the impression that women may not be sufficiently informed about screening for anomalies in general and not be fully aware of the implications for opting in or out. Another objection that has been raised is that women who decline the CT, also indirectly decline screening for trisomies at the 20-weeks scan. This suggests that women should be informed about the fact that detection of chromosomal anomalies, although less effectively, can also occur in the second trimester of pregnancy. Accordingly, one may even consider explicitly asking women whether they want specific measurements such as the NBL and PT to be carried out.

In conclusion, we consider the studies reported in this thesis as an important contribution in filling the gaps in the (Dutch) prenatal screening system and to provide measurement tools for objectifying fetal facial dysmorphology.

9.3 FUTURE PERSPECTIVES, CONCLUSIONS AND RECOMMENDATIONS

The fetal facial markers discussed in this thesis seem to be very promising adjuncts in prompting a strong suspicion of a chromosomal anomaly. Even when they are isolated, they can warrant further investigation. The results of this thesis are based on a retrospective analysis of prospectively collected data. This aspect, and the fact that examiners were not blinded to the karyotype, could be regarded as limitations of the study. To adequately confirm the findings of this thesis, a large prospective study would be necessary. This was also suggested by a recent meta-analysis on the nasal bone as a marker for aneuploidy. The authors Moreno-Cid et al[10] found retrospective studies to report structurally higher rates of abnormal nasal bones in Down syndrome (DS) fetuses than in prospective studies. When collecting data retrospectively, a selection bias in favour of including abnormal cases in the analysis is inevitable.

Most profile markers discussed in this thesis are clearly different in chromosomally abnormal fetuses. However, it is still unclear whether these markers, when isolated, can be used to discriminate between different chromosomal anomalies or if they can be used to identify other genetic syndromes. For instance, the PT-NBL ratio is enlarged in both DS and in Edwards syndrome (ES). On the other hand the FP line, influenced by a flat profile and retrognathia, can potentially discriminate DS from ES. Ideally it would possible to create an algorithm able to identify and discriminate different chromosomal anomalies and genetic syndromes, similarly to first trimester screening.

In the literature, several methods to quantify facial features are reported. The possibilities to draw lines and measure angles in the face are limitless, and the quest for the best marker is not over yet. The recently proposed idea of combining the fetal profile line and the mandibulo-maxillary line (of the PFSR) into a reverse MNM-angle is a good example of the evolution of existing markers into a sensitive clinical tool[11].

In this thesis, only fetuses of Caucasian parents were examined. For some markers like the nasal bone, it has already been established that ethnic variation influences the markers and their performance and should therefore be taken into account[12]. Further investigation of ethnic influences on all markers reported in this thesis is therefore recommended.

Ideally, we would hope that these relatively easy to use markers would be able to extend our insight in the pathophysiological mechanisms leading to facial dysmorphic features. It would also be desirable to establish a link with the severity of expression of the condition. If it would be possible to connect subtle anatomic variations like facial markers to postnatal outcome, it might be possible to offer a more specific prognosis in case of an affected pregnancy.

At this moment, the subject of intra-uterine dysmorphology is only at an early stage. The next big challenge will be, next to extending the diagnostic ability to identify more genetic syndromes,

to be able to use this diagnostic tool in earlier stages in pregnancy. This would allow more time for targeted genetic investigations where possible, and for parents to be optimally informed on the condition affecting their fetus, as to make a well informed decision concerning the future of the pregnancy.

Most markers assessed in this thesis, such as the PT-NBL ratio, are easily performed after simple training when a good profile view is obtained by 2D ultrasound. Therefore, we expect that in the future the measurement of this marker will become part of the routine 20-weeks scan, on condition that the mother wants to be informed about the likelihood of an aneuploidy. Finally, it is not unthinkable that these markers may already find an application in the first trimester of pregnancy.

Conclusions and recommendations

- 3D US enables visualization of the fetal face in utero.
- Typical facial features can be quantified in the profile view. 3D can be of help in defining the exact midsagittal profile view.
- The first trimester is the best moment for screening for aneuploidies. However not all women undergo first trimester screening. This means that cases of aneuploidies can still be identified at later scans
- The 'genetic sonogram' has recently been enriched by new fetal profile markers for aneuploidies.
- The most sensitive markers for second trimester DS screening are the PT-NBL ratio and the PFSR.
- The most sensitive marker for second trimester ES screening is the PT-NBL ratio.
- The FP line makes it easier to differentiate between DS and ES.
- Other markers investigated in this thesis are less prone to routine clinical application.
- Even in the event that non-invasive prenatal testing (NIPT) may transform the current aneuploidy screening policy, fetal dysmorphology will continue to exist and to expand. This thesis must be regarded as a further step in that direction.

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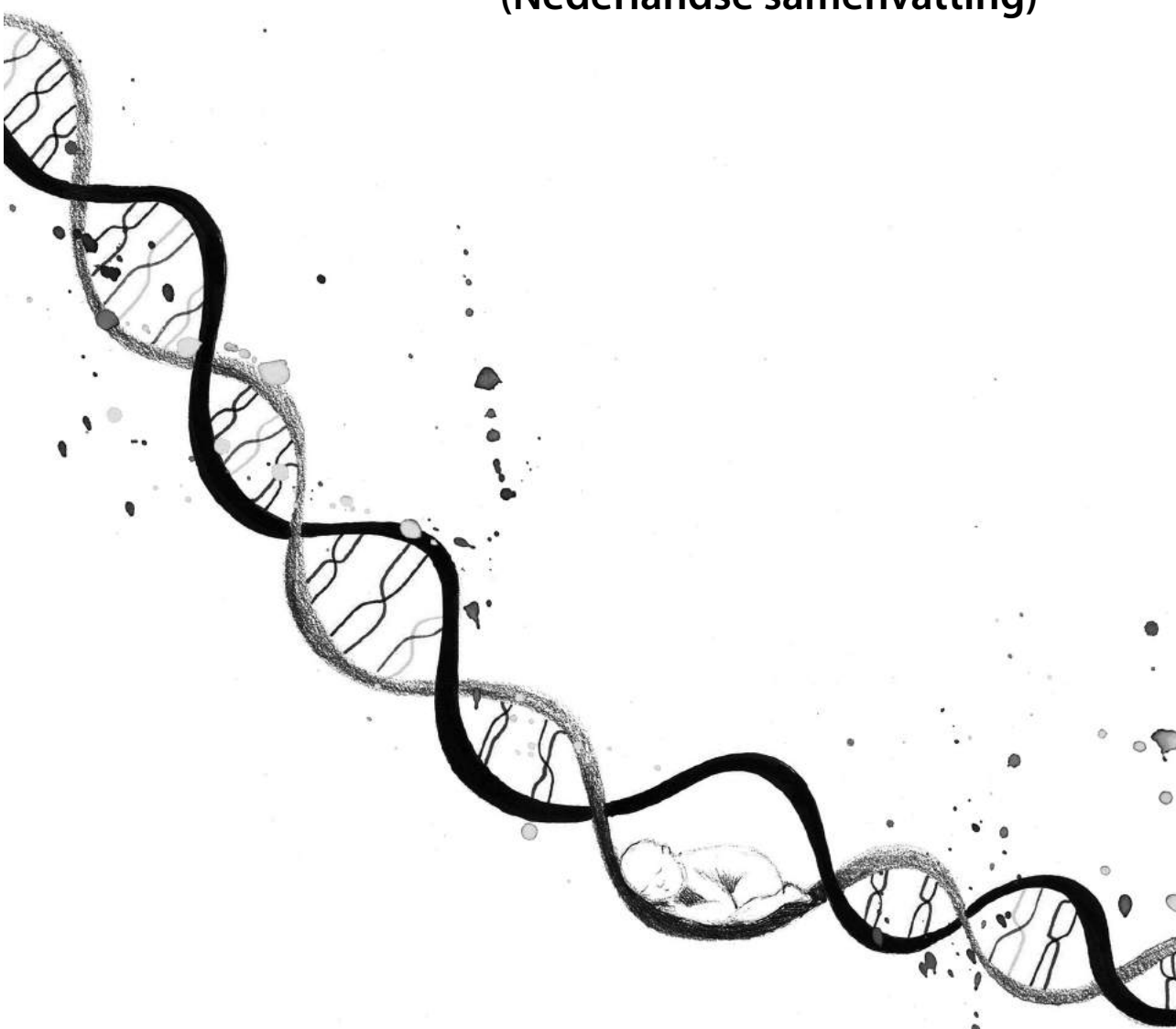
CHAPTER 10

Summary in Dutch
(Nederlandse samenvatting)



Hoofdstuk 1 biedt een korte geschiedenis van echografie als methode om de foetus te onderzoeken. De techniek van prenatale echografie werd voor het eerst in de vroege jaren zestig gebruikt. Vervolgens wordt de overgang van echografie als onderzoeksmethode met veel beperkingen, tot het huidige gebruik als geavanceerde screenings- en diagnostische techniek besproken. Daarnaast wordt de recente ontwikkeling van driedimensionale (3D) echografie besproken, met de voordelen en beperkingen ten opzichte van de conventionele tweedimensionale (2D) techniek. Ook wordt de screeningsmethode voor het syndroom van Down (DS), de meest voorkomende trisomie in levend geboren baby's, besproken. Daarnaast wordt een overzicht gegeven van de huidige screeningsmogelijkheden in het eerste en tweede trimester. In het bijzonder wordt ingegaan op markers die in het foetale profiel te zien zijn tijdens echografisch onderzoek in het tweede trimester. Tenslotte wordt het Edwards-syndroom (ES) geïntroduceerd waarbij ingegaan wordt op incidentie, grote malformaties in de pre- en postnatale periode, prognose en screeningsopties.

In **hoofdstuk 2 en 3**, worden vier structuren in het foetale profiel, die recent als markers voor DS zijn geïntroduceerd, bekeken in een groep euploïde (chromosomaal normale) foetussen. Normaalwaarden op basis van 3D-metingen van de markers worden gemaakt, waarna vervolgens één marker, de "prenasal thickness to nasal bone length ratio" (PT-NBL ratio; verhouding huiddikte voor de neus en lengte neusbeentje), wordt getest in een kleine groep DS-foetussen.

Hoofdstuk 2 behandelt de haalbaarheid om drie markers, de nasal bone length (NBL; neusbeenlengte), prenasal thickness (PT; huiddikte voor de neus) en fronto-maxillary facial (FMF) hoek (voorhoofd-kaak-gezichtshoek), te meten in hetzelfde 3D-gecorrigeerde profiel van euploïde tweede en derde trimester foetussen. Een scoringssysteem wordt gebruikt waarbij de beelden van de profielen worden beoordeeld op contrast en helderheid. Alleen beelden met een hoge score worden gebruikt voor verdere analyse. Elke marker werd drie keer gemeten. Het gemiddelde werd als uiteindelijke meting gebruikt. Het was significant vaker mogelijk om de NBL en PT duidelijk en volledig in beeld te krijgen (98% en 97%, respectievelijk) dan om de FMF hoek in beeld te krijgen (26%, $p < 0.001$). Tevens was de intra- en interobserver variabiliteit voor de NBL en PT superieur ten opzichte van de FMF hoek. De NBL groeide significant gedurende de zwangerschap, van 3.3 mm bij 15 weken tot 9.6 mm bij 33 weken. De PT werd ook groter gedurende de zwangerschap, van 2.3 mm bij 15 weken tot 6.1 mm bij 33 weken. Normaalwaarden voor beide markers werden opgesteld. De FMF hoek lijkt niet gecorreleerd aan de zwangerschapsduur, maar door de slechte meetbaarheid van deze marker werd geen verdere analyse verricht en werd geen normaalwaarde geconstrueerd. Een interessante observatie was dat nadat we de definitie van de NBL hadden aangepast (waarbij de doorsnede van het os frontale (voorhoofdsbot) niet bij de NBL meting wordt betrokken) onze normaalwaarden bij elke zwagerschapsduur kleiner waren dan in andere gepubliceerde studies over de NBL, terwijl er gedurende de zwangerschap een gelijkvormige curve te zien was. Geconcludeerd kan worden dat de NBL en PT, gemeten op met 3D aangepaste beelden, makkelijk te gebruiken markers zijn, terwijl de FMF hoek een grotere uitdaging vormt. Daarnaast moet men er op bedacht zijn niet een deel van het os frontale in de NBL meting te includeren.

In **hoofdstuk 3** bestuderen we de PT-NBL ratio. Dit is de ratio tussen de PT en de NBL. De PT-NBL ratio werd bestudeerd in zowel DS- als euploïde foetussen in het tweede en derde trimester. De normaalwaarden voor NBL en PT resulterend uit het onderzoek in hoofdstuk 2 werden gebruikt om normaalwaarden voor de PT-NBL ratio op te stellen. De PT-NBL ratio bleef gelijk gedurende de zwangerschapsduur (gemiddelde waarde 0.61, 95% CI, 0.59 – 0.63; $r = -0.04$, $P = 0.7$). Het 5^{e} en 95^{e}

percentiel waren respectievelijk 0.48 en 0.80. Deze normaalwaarden werden vervolgens gebruikt om een kleine groep DS-foetussen mee te vergelijken. De PT-NBL ratio was significant groter in DS-foetussen ($P < 0.001$), maar bleef net zoals in de groep euploïde foetussen gelijk gedurende de zwangerschap met een gemiddelde waarde van 1.50 (95% CI, 1.20 – 1.80; $r = -0.35$, $P = 0.07$). Alle DS-foetussen hadden een PT-NBL ratio boven het 95e percentiel. Als dit 95e percentiel werd aangehouden als afkapwaarde, dan was de detectiegraad van de PT-NBL ratio 100% (95% CI, 89 –100%) met een fout positief percentage van 5% (95% CI, 2 – 11%). De positieve likelihood ratio was 21.2. De conclusie van deze studie is dat de PT-NBL ratio een stabiele marker is om DS op te sporen gedurende het tweede en derde trimester. Bovendien hadden alle DS-foetussen in deze studie een PT-NBL ratio boven het 95e percentiel, wat het tot een veelbelovende marker voor het opsporen van DS maakt.

In **Hoofdstuk 4 en 5** worden twee markers voor DS geïntroduceerd, de maxilla-nasion-mandible (MNM) hoek (hoek tussen mandibula, neusbrug en maxilla) en de fetal profile (FP) lijn (voorhoofdslijn). Daarnaast worden vier andere markers, de NBL, PT, PT-NBL ratio en prefrontal space ratio (PFSR; verhouding tussen mandibula en maxilla gecombineerd met de huiddikte voor de neus), getest als DS-markers in een grote groep DS-foetussen. Al deze markers zijn gelokaliseerd in het foetale profiel, met als doel de vorm van het foetale profiel te kwantificeren.

De meetbaarheid en reproduceerbaarheid van de MNM hoek en de FP lijn met de bijbehorende FP afstand (de kortste afstand tussen de FP lijn en het os frontale) wordt onderzocht in **hoofdstuk 4**. Het onderzoek werd retrospectief verricht in een groep van 138 tweede en derde trimester DS-foetussen. Metingen werden vergeleken met de normaalwaarden die in eerdere studies waren opgesteld. De MNM hoek was significant kleiner in DS-foetussen (gemiddeld 12.9^0) dan in euploïde foetussen (gemiddeld 13.5^0, $p = 0.015$). In slechts in 16.9% van de DS-foetussen was de MNM hoek onder het 5e percentiel, hoewel dit wel significant vaker het geval was dan in euploïde foetussen ($p < 0.01$). De MNM hoek bleef gelijk gedurende de zwangerschap ($p = 0.48$). Intra- en interobserver variabiliteit werd uitgedrukt als de intra-class correlation coefficient (ICC) met waarden van 0.89 en 0.61 voor respectievelijk de MNM hoek en 1.0 en 0.76 voor de FP lijn. In het cohort met DS-foetussen had geen enkele foetus een negatieve FP lijn. 41.1% had een positieve FP lijn (significant vaker dan in euploïde foetussen, $p < 0.001$) en dit was significant vaker het geval in een latere fase in de zwangerschap ($p < 0.001$). Aangezien uit een eerdere studie van deze groep was gebleken dat de FP lijn nooit positief was in de periode voor 27 weken zwangerschapsduur, besloten we het cohort DS-foetussen in tweeën op te splitsen in het tweede en derde trimester. In deze twee groepen hadden respectievelijk 28.4% en 76.5% van de DS-foetussen een positieve FP lijn met een fout positieve waarde van respectievelijk 0% en 16.9%. De FP afstand nam toe gedurende de zwangerschap ($p < 0.001$), met een gemiddelde van 3.1 mm. De FP afstand was niet significant groter in DS-foetussen dan in euploïde foetussen ($p = 0.4$). Een kleine MNM hoek was significant gecorreleerd met een positieve FP lijn ($p < 0.001$). Naar aanleiding van deze studie stellen wij voor om de FP lijn te gebruiken als nieuwe marker voor DS, waarbij in het tweede trimester een zeer lage fout positieve incidentie wordt geconstateerd. Omdat er voor de FP lijn geen kennis van referentiewaarden nodig is (de FP lijn is positief, negatief of zero), is het een gebruiksvriendelijke marker voor het opsporen van DS.

In **hoofdstuk 5**, worden de vier recent geintroduceerde DS-markers NBL, PT, PT-NBL ratio en PFSR geëvalueerd in een grote groep tweede en derde trimester DS-foetussen. De markers zijn gemeten

in 159 DS-foetussen en uitkomsten werden vergeleken met eerder gepubliceerde normaalwaarden. De gemiddelde maternale leeftijd was 35.8 jaar, de gemiddelde zwangerschapsduur was 23+1 weken. Intra- en interobserver variabiliteit waren het beste in de NBL, PT en PT-NBL ratio, met ICC's van respectievelijk 0.98 en 0.93, 0.98 en 0.97, en 0.94 en 0.92. De PFSR had ICC's van respectievelijk 0.67 en 0.67. De grootte van de NBL en PT waren gecorreleerd aan de zwangerschapsduur ($p < 0.001$), maar niet aan de PT-NBL-ratio en PFSR. Alle markers waren gecorreleerd met DS ($p < 0.001$). De gemiddelde waarde van de NBL, PT, PT-NBL ratio en PFSR waren respectievelijk 5.08, 5.56, 1.26 en 0.34. Het neusbotje was afwezig in 23 (15.4%) foetussen. Als methode voor DS-screening, behaalde de PT-NBL ratio de hoogste detectiegraad met 86.2%, gevolgd door de PFSR (79.7%), PT (63.4%) en NBL (61.9%). In 33.6% van de foetussen waren alle markers afwijkend. In 4.7% van de foetussen waren alle markers normaal. De combinatie van alle vier markers samen behaalde een detectiegraad van 95.3%, gevolgd door een combinatie van de PFSR met de PT-NBL-ratio met 93.8%. Aangezien de PT en NBL worden gebruikt om de PT-NBL-ratio te berekenen, en de PT ook wordt gebruikt om de PFSR te berekenen, waren we niet verbaasd dat alleen de multiple of the median (MoM) metingen van de NBL en PFSR onafhankelijk waren van elkaar ($p = 0.10$). De detectiegraad van alle markers als de MoM NBL, MoM PT, PT-NBL ratio en PFSR, waren niet gecorreleerd aan de zwangerschapsduur. Concluderend adviseren wij om in het tweede en derde trimester van de zwangerschap de PT-NBL ratio en PFSR te gebruiken in het kader van onderzoek naar DS. Niet alleen omdat deze markers een hoge detectiegraad hebben, gecombineerd 94%, maar ook omdat ze makkelijk zijn in het gebruik aangezien ze geen kennis vereisen van specifieke normaalwaarden afhankelijk van de zwangerschapsduur.

Hoofdstuk 6 behandelt de verschillen tussen metingen die worden gemaakt met behulp van 2D-beelden of 3D-volumes. Verschillen tussen metingen van zes markers (NBL, PT, FP lijn, MNM hoek, PT-NBL ratio en PFSR) gemaakt op basis van 2D-beelden (gemaakt na het volgen van strikte criteria) of door middel van 3D-aangepaste volumes, werden geanalyseerd, alsmede hun klinische applicatie. Alle zes markers werden gemeten in 105 datasets: 75 van euploïde foetussen en 30 van DS-foetussen. 2D-beelden en 3D- volumes waren beide vervaardigd tijdens hetzelfde onderzoek van de foetus. De MNM hoek was significant één graad groter wanneer deze op 2D-beelden werd gemeten ($p < 0.01$). Voor alle andere metingen was er geen significant verschil of de meting met behulp van 2D- of 3D-echografie was gemaakt. De limits of agreement (LOA) voor intra- and inter-observer variabiliteit waren kleiner na 3D-metingen voor alle markers, behalve voor de MNM hoek. Wanneer de groep DS-foetussen apart werd geanalyseerd was er geen significant verschil in detectiegraad voor DS, wanneer ze met behulp van 2D- of 3D-beelden waren geanalyseerd. Met deze studie laten we zien dat, de MNM hoek uitgezonderd, er geen significante verschillen worden gezien tussen 2D- en 3D-metingen van een aantal DS-markers. Specifiek de NBL, PT, FP lijn, PT-NBL-ratio en PFSR kunnen worden gebruikt als DS-markers in 2D-beelden, voorbehouden dat de 2D-beelden zijn gemaakt van goede mid-sagittale beelden van het foetale profiel.

Hoofdstuk 7 evalueert herhaalde metingen van de NBL, PT, nuchal fold (NF; nekplooi), PT-NBL-ratio en PFSR in tweede en derde trimester DS-foetussen. De markers werden retrospectief geanalyseerd en vergeleken met eerder gepubliceerde normaalwaarden. In totaal werden 24 DS-foetussen geanalyseerd. De gemiddelde zwangerschapsduur was 25 weken. De gemiddelde zwangerschapsduur bij het eerste onderzoek was 20+4 weken en bij het laatste onderzoek 29+2

weken. Het gemiddelde interval tussen de metingen was 39 dagen (spreiding 14 – 98 dagen), met een gemiddeld aantal onderzoeken van 2.6 per foetus. De NBL, PT en NF namen significant toe tijdens de zwangerschap ($p < 0.01$), in tegenstelling tot de PT-NBL-ratio en PFSR. In 42% van de DS-foetussen, steeg de grootte van de NF niet in minstens één opvolgende meting, tegenover 4.8% en 13.6% in het geval van respectievelijk de NBL en PT. De PT-NBL ratio was de meest stabiele marker, waarbij in 95% van de DS-foetussen de waarde stabiel bleef. In een mixed model-format werd een corresponderende trend voor herhaalde metingen vergeleken met de gemiddelde waarde van een groep euploïde foetussen. In dit format toonden de zwangerschapsduurafhankelijke markers (NBL, PT, NF) meer variatie bij een langere zwangerschapsduur. MoM-waarden bleven echter stabiel. De NF en PFSR toonden de grootste verschillen tussen metingen wanneer alle foetussen met een individuele lijn werden afgebeeld. Niettemin waren de meeste PFSR-metingen ver onder de normaallijn. Uit deze studie concluderen wij dat herhaalde metingen van de NF en PFSR een grotendeels onvoorspelbaar verloop hebben wanneer ze in één en dezelfde foetus worden gemeten. Dit wordt hoogst waarschijnlijk veroorzaakt door een lastige reproduceerbaarheid. De PFSR lijkt echter toch zijn waarde als marker voor DS te behouden, aangezien de meeste metingen ver onder de normaallijn blijven.

In **hoofdstuk 8** worden zes markers die eerder als markers voor DS zijn gebruikt, geïntroduceerd als markers voor het detecteren van het Edwards-syndroom (ES) in het tweede en derde trimester. De markers die worden beoordeeld zijn de PT, NBL, PT-NBL ratio, PFSR, MNM hoek en FP lijn. De metingen werden vergeleken met eerder gepubliceerde normaalwaarden. Om de mogelijke klinische relevantie van markers voor ES te beoordelen, werden tevens additionele bevindingen (andere markers, structurele anomalieën, groeirestrictie) gedocumenteerd. Aanvullend werd bekeken of deze additionele bevindingen reeds bij de initiële routine 20-weken echo waren ontdekt, of pas bij het uitgebreide echografische onderzoek na verwijzing (en soms ook karyotypering). 43 ES-foetussen werden geïncludeerd. De gemiddelde maternale leeftijd was 37 jaar en de gemiddelde zwangerschapsduur was 21+2 weken. Net als in DS bestond er een significante correlatie tussen de zwangerschapsduur en de grootte van de NBL en PT ($p < 0.001$), dit gold niet voor de andere markers. Alle markers waren significant gecorreleerd aan ES. De gemiddelde NBL, MNM hoek, PT, PT-NBL ratio en PFSR waren respectievelijk 3.76, 16.67, 4.25, 1.39 en 0.87. De FP lijn was zero (normaal) in 53.7% van de foetussen en negatief (abnormaal) in 46.3% van de gevallen. Een kort neusbotje kwam vaker voor dan een verdikte PT. Dit kwam naar voren in de uiteenlopende effectiviteit van de markers: de PT-NBL ratio had de hoogste detectiegraad (88.4%), gevolgd door de NBL (83.7%), MNM angle (56.4%), FP lijn (46.3%), PT (27.9%) en de PFSR (20.5%). Het fout positieve percentage was 5% voor alle markers, behalve voor de FP lijn, waarbij het fout positieve percentage 0% was. Verschillende combinaties van de vier beste markers (NBL, FP lijn, MNM angle and PT-NBL ratio) behaalden een detectiegraad tussen de 90% en 95%. Voorts waren er geen structurele anomalieën gedocumenteerd in 22% van de foetussen bij de initiële 20-weken echo, terwijl dat maar 2% was bij het uitgebreide echografische onderzoek. De belangrijkste conclusies die kunnen worden getrokken naar aanleiding van dit onderzoek zijn dat de PT-NBL ratio en de NBL duidelijke markers zijn voor ES in het tweede en derde trimester. Daarnaast is het ook opvallend dat een negatieve FP lijn geen fout positieve waarden heeft en dat de FP lijn de mogelijkheid heeft te differentiëren tussen ES en DS, aangezien in de laatste groep de FP lijn nooit negatief is. Tenslotte werd in bijna een

kwart van de foetussen bij de 20-weken echo geen grote anomalie geïdentificeerd, wat het belang van echografische markers voor ES onderstreept.

Opsomming van de meest belangrijke bevindingen

Onderzoek van markers in euploïde foetussen:

- De PT, NBL en PT-NBL ratio zijn relatief makkelijk te beoordelen en leveren betrouwbare metingen op. De FMF hoek is vaak moeilijker om retrospectief te beoordelen, mogelijk omdat de herkenningspunten die worden gebruikt (palatum, vomer), in het tweede en derde trimester van de zwangerschap vaak niet goed zijn te onderscheiden.
- Het is van belang om bij het meten van de NBL niet een deel van het os frontale in de meting te betrekken.
- De PT-NBL ratio heeft in euploïde foetussen een constante gemiddelde waarde van 0.61 en 95^{e} percentiel van 0.80 gedurende het tweede en derde trimester van de zwangerschap.

Prestatie van markers ten opzichte van Down syndroom (DS):

- In een groep retrospectief geanalyseerde DS-foetussen, heeft 86.2% een PT-NBL waarde boven het 95^{e} percentiel. In 94% van de DS-foetussen heeft een PT-NBL ratio boven het 95^{e} percentiel én/of een PFSR waarde onder het 5e percentiel.
- De PT-NBL ratio en PFSR vereisen geen kennis van normaalwaarden specifiek voor de zwangerschapsduur.
- Een positieve FP lijn heeft geen fout positieve waarden in het tweede trimester.

Vergelijking tussen 2D- en 3D-echografie:

- Wanneer metingen van de markers NBL, FP lijn, MNM hoek, PT, PT-NBL ratio en PFSR, gemaakt met behulp van 2D-beelden (vervaardigd op basis van strikte criteria), worden vergeleken met metingen verkregen door middel van 3D-volumes, is er alleen voor de MNM hoek een significant verschil.
- De markers hadden in DS-foetussen niet vaker een abnormale waarde wanneer ze door middel van 2D of 3D-echografie waren gemeten.

Longitudinale analyse van DS-markers:

- De betrouwbaarheid van de NF als tweede trimester-marker is discutabel.
- Herhaalde metingen van de PFSR in dezelfde foetus is onderhevig aan substantiële verschillen.
- De PT-NBL ratio is een stabiele marker wanneer die op verschillende momenten tijdens de zwangerschap wordt gemeten.

Prestatie van markers in relatie tot Edwards syndroom (ES):

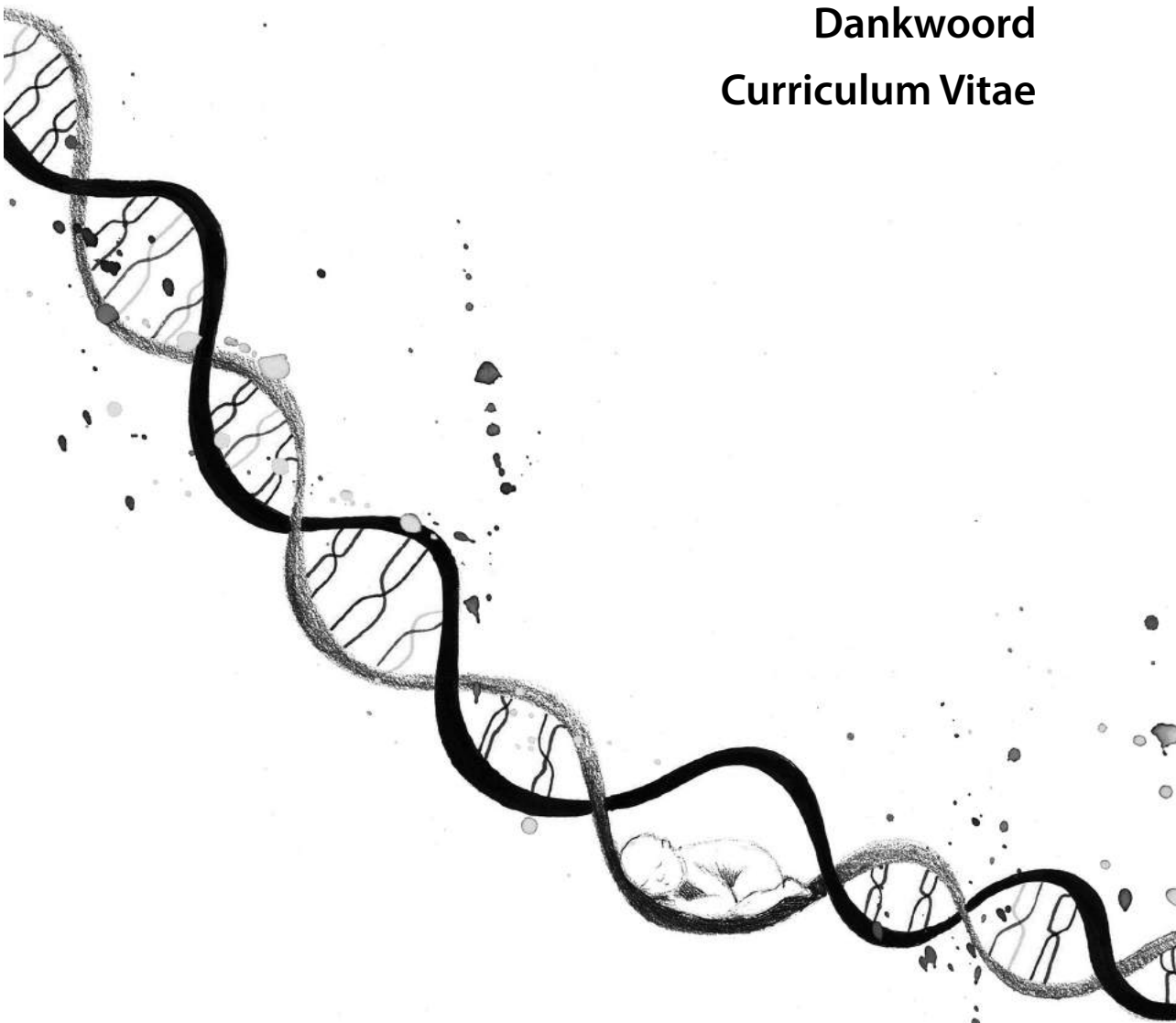
- De PT-NBL ratio en NBL zijn sterke tweede en derde trimester markers voor ES.
- Een negatieve FP lijn had in dit onderzoek geen fout positieve resultaten en biedt eventueel de mogelijkheid te differentiëren tussen ES en DS.
- Er werden in ongeveer een kwart van de ES foetussen geen structurele afwijkingen geobserveerd bij de initiële 20-weken echo, terwijl dit bij het uitgebreide echografisch onderzoek slechts 2% was.

APPENDICES

List of Publications

Dankwoord

Curriculum Vitae



LIST OF PUBLICATIONS

Prenasal thickness-to-nasal bone length ratio: a strong and simple second- and third-trimester marker for trisomy 21. De Jong-Pleij EA, **Vos FI**, Ribbert LS, Pistorius LR, Tromp E, Bilardo CM. Ultrasound Obstet Gynecol. 2012 Feb;39(2):185-90.

Three-dimensional ultrasound imaging and measurement of nasal bone length, prenasal thickness and frontomaxillary facial angle in normal second- and third-trimester fetuses. **Vos FI**, De Jong-Pleij EA, Ribbert LS, Tromp E, Bilardo CM. Ultrasound Obstet Gynecol. 2012 Jun;39(6):636-41

Fetal facial profile markers in second and third trimester trisomy 18 fetuses. **Vos FI**, de Jong-Pleij EA, Bakker M, Tromp E, Manten GT, Bilardo CM. Ultrasound Obstet Gynecol. 2014 Sep 5.

The facial profile of Down syndrome fetuses in the second and third trimester of pregnancy. **Vos FI**, de Jong-Pleij EA, Bakker M, Tromp E, Kagan KO, Bilardo CM. Ultrasound Obstet Gynecol. 2014 Nov 4.

Trends in serial measurements of ultrasound markers in second and third trimester Down syndrome fetuses. **Vos FI**, de Jong-Pleij EA, Bakker M, Tromp E, Bilardo CM. Fetal Diagn Ther. 2015 Jan 30.

Nasal bone length, prenasal thickness, prenasal thickness-to-nasal bone length ratio and prefrontal space ratio in second- and third-trimester fetuses with Down syndrome. **Vos FI**, De Jong-Pleij EA, Bakker M, Tromp E, Pajkrt E, Kagan KO, Bilardo CM. Ultrasound Obstet Gynecol. 2015 Feb;45(2):211-6.

Is 3D technique superior to 2D in Down syndrome screening? Evaluation of six second and third trimester fetal profile markers. **Vos FI**, Bakker M, de Jong-Pleij EA, Ribbert LS, Tromp E, Bilardo CM. Prenat Diagn. 2015 Mar;35(3):207-13.

DANKWOORD

Graag zou ik de volgende personen bedanken die mij van steun zijn geweest tijdens het schrijven van dit proefschrift.

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Lieve Gerard, wat ben ik trots dat ik me jouw dochter mag noemen. Ook al zitten we professioneel in een andere 'tak van sport', jouw werkethos, doorzettings- en aanpassingsvermogen zijn altijd een voorbeeld voor mij geweest, en hebben mij gevormd tot de persoon die ik vandaag ben.

Ten slotte mijn gezin: René, Cosima en …..?

Cosi, je bent een fantastisch, slim, mooi en lief meisje. Maar allerbelangrijkst is, je bent óns lieve meisje. Je broertje is op het moment dat ik dit dankwoord schrijf flink aan het schoppen in mijn buik, volgens mij is dat een teken dat wij met z'n allen een prachtig leven en een vliegende start tegemoet gaan!

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CURRICULUM VITAE

Fedia Isabella Vos werd op 24 januari 1986 geboren in de Amsterdamse Jordaan en groeide daar ook op. Zij behaalde in 2004 haar diploma aan het Vossius Gymnasium te Amsterdam. Na te hebben gereisd in Zuid-Amerika begon zij haar studie geneeskunde aan de Universiteit van Amsterdam. In het kader van haar wetenschappelijke stage startte zij een onderzoek onder begeleiding van (thans professor) dr. C.M. Bilardo op de afdeling prenatale diagnostiek van het Academisch Medisch Centrum te Amsterdam. Hieruit volgden de eerste twee publicaties in dit proefschrift. Na haar studie geneeskunde te hebben afgerond in 2013 breidde zij, in samenwerking met prof. dr. C.M. Bilardo en dr. E.A.P. de Jong-Pleij, het eerdere onderzoek uit tot het proefschrift 'Ultrasonography of the fetal nose, maxilla, mandible and forehead as markers for aneuploidy'. Begin 2014 begon zij met de opleiding tot specialist Keel-, Neus-, Oorheelkunde / Hoofd-halschirurgie in het VU Medisch Centrum te Amsterdam.

Sinds haar 17e jaar is Fedja samen met haar geliefde, René. Zij wonen in Amsterdam samen met hun dochter Cosima, en er zal binnenkort nog een kleintje volgen.