CHAPTER

General introduction

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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 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) - 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 dysmorphology 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. 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 intrauterine growth retardation.

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. Halfway through the 1990s, articles concerning 3D reconstruction of the fetal face were published. 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. Rotten was the first to describe the use of 3D ultrasound in DS fetuses in 2002.
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. (2) With respect to the relationship between parents and fetus, the expectation is that depicting the fetus by ultrasound would increase parental bonding. 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)). 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 marker2. 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 France16,19 to 20 – 30% in parts of England and The Netherlands20-21. 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 professional20-23.
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 21\(^{st}\) 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 an extra copy of the 21st chromosome is explained through the biological mechanism of gametogenesis. Gametogenesis 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.

![Figure of meiosis I and II and corresponding non-disjunctions.](image)

The gamete with the additional chromosome is of maternal origin in an estimated 95%, opposed to paternal origin in 5%. The only well documented risk factor for DS remains advanced maternal age. 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. Several studies have suggested a male predominance of DS baby’s 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 babies36.

A limited increase in DS live births has been observed in the Netherlands during the last 18 years29,37: 10 out of 10,000 live born babies were diagnosed as having DS in 1996 versus 16 in 10,000 live births currently. Penrose38 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 201039, while the number of terminations of DS pregnancies has also increased, but in a less pronounced way37. 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 5040 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 babies29,41,42. 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 dysplasia41. Other structural anomalies that affect DS babies are gastro-intestinal atresia, cleft lip and palate, megacolon and cataract43.

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 group44 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 countries44. In (the north of) The Netherlands, Cocchi et al37 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 population29). The neonatal mortality rate in DS (< 28 days after birth) is 1.65%, opposed to 0.36% for a control group of healthy neonates29. In a recent study45, newborn DS babies who died in the post-neonatal period had significantly more heart-related causes of death. These findings were largely confirmed in other studies46,47, 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 2046. However, in the past 40 years, the life expectancy of DS individuals has increased drastically (to an estimated 60 years48), amongst other things due the safe and widespread availability of cardiac surgical treatments49,50. 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 residency45. 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. As is the case in DS, gametes containing the extra chromosome are of maternal origin in the vast majority (> 95%).

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, to 2.66 per 10,000 registered births between 2004 – 2006 in the US. As the risk of fetal loss or stillbirth is high (72% at 12 weeks gestation and 65% at 18 weeks) and TOP is carried out in a large percentage of affected pregnancies (83% – 86%), the number of affected pregnancies is much higher (an estimated 6.5 in 10,000 registries) than the amount of live births.

As for DS, maternal age is a risk factor for an ES pregnancy. 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). 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, with 1-year survival rates ranging from 6% – 8.1%. Female babies with ES are reported to have a better chance at survival both pre- and postnatally.

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). More subtle malformations are odd shaped skull, choroid plexus cysts, single umbilical artery, absent nasal bone and increased nuchal thickness.

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

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 and absent nasal bone are often encountered abnormalities. 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 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. At this time a thickened NT and a short femur length 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.

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. 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).

**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. 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. 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 dysfunction. Abnormalities in bone growth and development is thought to be a contributing factor to the abnormal facial anatomy observed in DS. Several pathological reports have confirmed these conclusions by post-mortem examination and X-ray imaging.

**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, as continuous values, as percentiles, as multiple of the mean, in a ratio as biparietal diameter to nasal bone length ratio and as the PT-NBL ratio.

**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, multiple of the mean and as the PT-NBL ratio. 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)\cite{92,94}, nasofrontal angle\cite{95} and frontonasal facial angle\cite{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\cite{97}, the inferior facial angle\cite{98} and the MNM angle\cite{99}. All reports mention the angles to be independent of gestational age. To our knowledge only Rotten et al\cite{99} 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)\cite{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\textsuperscript{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.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Study</th>
<th>Design</th>
<th>Dimension</th>
<th>Gestation</th>
<th>Euploid fetuses</th>
<th>DS fetuses</th>
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<td># FP</td>
<td># DR</td>
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<td>prospective</td>
<td>2D</td>
<td>14 – 27</td>
<td>500</td>
<td>5%</td>
<td>21</td>
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<td>Jung, 2007(^{58})</td>
<td>prospective</td>
<td>2D</td>
<td>16 – 28</td>
<td>2833</td>
<td>3,1%</td>
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<td>Cusick, 2007(^{59})</td>
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<td>16 – 21</td>
<td>371</td>
<td>3,5%</td>
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<td>Gianferrari, 2007(^{50})</td>
<td>retrospective</td>
<td>2D</td>
<td>15 – 25</td>
<td>2515</td>
<td>2,9%</td>
<td>21</td>
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<td>Hung, 2008(^{100})</td>
<td>retrospective</td>
<td>2D</td>
<td>13 – 29</td>
<td>342</td>
<td>3,2%</td>
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<td>Odibo, 2008(^{46})</td>
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<td>16 – 22</td>
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<td>Persico, 2012(^{101})</td>
<td>retrospective</td>
<td>3D</td>
<td>16 – 24</td>
<td>135</td>
<td>3,0%</td>
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<td>PT</td>
<td>Persico, 2008(^{79})</td>
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<td>16 – 24</td>
<td>135</td>
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<td>Definition of PT: *</td>
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<td>Migueliz, 2010(^{202})</td>
<td>retrospective</td>
<td>2D and 3D</td>
<td>14 – 27</td>
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<td>PT-NBL ratio</td>
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<td>prospective</td>
<td>2D</td>
<td>14 – 27</td>
<td>500 5%</td>
<td>21 63%</td>
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<td>De Jong-Pleij, 2012&lt;sup&gt;**&lt;/sup&gt;</td>
<td>retrospective</td>
<td>2D and 3D</td>
<td>15 – 33</td>
<td>219 2,3%</td>
<td>30 100%</td>
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<tr>
<td>PFSR</td>
<td>Sonek, 2012&lt;sup&gt;**&lt;/sup&gt;</td>
<td>retrospective</td>
<td>3D</td>
<td>15 – 25</td>
<td>90 5%</td>
<td>26 100%</td>
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<tr>
<td></td>
<td>Yazdi, 2013&lt;sup&gt;**&lt;/sup&gt;</td>
<td>retrospective</td>
<td>2D</td>
<td>15 – 40</td>
<td>279 5%</td>
<td>91 79.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chaveeva, 2013&lt;sup&gt;**&lt;/sup&gt;</td>
<td>retrospective</td>
<td>2D</td>
<td>16 – 24</td>
<td>240 5%</td>
<td>45 100%</td>
<td></td>
</tr>
<tr>
<td>FMF angle</td>
<td>Sonek, 2007&lt;sup&gt;**&lt;/sup&gt;</td>
<td>retrospective</td>
<td>2D</td>
<td>14 – 24</td>
<td>100 9%</td>
<td>34 87.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Molina, 2008&lt;sup&gt;**&lt;/sup&gt;</td>
<td>retrospective</td>
<td>3D</td>
<td>16 – 25</td>
<td>150 3,3%</td>
<td>23 65.2%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Odibo, 2009&lt;sup&gt;**&lt;/sup&gt;</td>
<td>retrospective</td>
<td>2D</td>
<td>16 – 22</td>
<td>201 5,6%</td>
<td>21 14.3%</td>
<td>Uses &gt; 30° above the mean as a cutoff. Definition of FMF: ‡‡</td>
</tr>
<tr>
<td></td>
<td>Sooklim, 2010&lt;sup&gt;**&lt;/sup&gt;</td>
<td>prospective</td>
<td>2D</td>
<td>17 – 19</td>
<td>386 3,9%</td>
<td>10 30,0%</td>
<td>Uses FMF values &gt; 90° a cutoff. Definition of FMF: ‡‡</td>
</tr>
</tbody>
</table>

<sup>*As the shortest distance between lowest part of the frontal bone and the facial skin anteriorly.</sup><br/>
<sup>**As the distance between skull and skin, tangential to Mandibula-maxillary line.</sup><br/>
<sup>*** As a line parallel to maxilla.</sup><br/>
<sup>‡ 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.</sup><br/>
<sup>‡‡ The second ray from the upper anterior corner of the maxilla to the external surface of the frontal bone.</sup><br/>
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.
REFERENCES


