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

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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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

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. In adult life, especially the flattened convexity of the profile has been quantified in these individuals. In fetal life, these typical craniofacial features have been transformed into measurable markers to improve the detection of DS in pregnancy. A short nasal bone length (NBL), for instance, increases the odds of DS by 6- to 7-fold. Prenasal thickness (PT) is above the 95th percentile in about 65% – 75% of the DS cases.

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%.

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

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°).

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 and often negative in trisomy 18 fetuses.

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. 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, based on 3D measurements of normal fetuses. In the study of the MNM angle in euploid fetuses, 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, 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 an 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.

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).

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.

<table>
<thead>
<tr>
<th>Intraobserver variability</th>
<th>Interobserver variability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean difference (SD)</td>
</tr>
<tr>
<td>FP line</td>
<td>_</td>
</tr>
<tr>
<td>MNM angle</td>
<td>-0.37 (1.16)</td>
</tr>
</tbody>
</table>
<pre><code>  | -1.94 (-2.6 – 1.3)       |                          |              |                          |                          |
</code></pre>

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\(^1\), 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.

<table>
<thead>
<tr>
<th>FP line</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>FPR (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Second trimester (14 – 27 weeks GA, n = 90)</td>
<td>28.4% (19% – 38%)</td>
<td>100% (98% – 100%)</td>
<td>0% (0% – 2%)</td>
<td>∞</td>
<td>0.7</td>
</tr>
<tr>
<td>Third trimester (≥ 27 weeks GA, n = 35)</td>
<td>76.5% (57% – 87%)</td>
<td>83.1% (73% – 90%)</td>
<td>16.9% (10% – 6%)</td>
<td>4.5</td>
<td>(2.7 – 7.3)</td>
</tr>
<tr>
<td>Combined (n = 125)</td>
<td>41.6% (33% – 51%)</td>
<td>93.7% (89% – 96%)</td>
<td>6.4% (4% – 10%)</td>
<td>6.5</td>
<td>(3.4 – 11.2)</td>
</tr>
</tbody>
</table>

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 5\% percentile (66\%) and in 2 of 8 cases with an MNM angle above the 95\% 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 5\% 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 study⁹.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>FPR (95% CI)</th>
<th>PLR (95% CI)</th>
<th>NLR (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNM angle</td>
<td>16.8% (10.8% – 24.7%)</td>
<td>95.0% (92.9% – 98.2%)</td>
<td>5.0% (1.7% – 11.3%)</td>
<td>4.42 (2.1 – 9.4)</td>
<td>0.86 (0.79 – 0.94)</td>
<td>70.0% (50.6% – 85.2%)</td>
<td>68.7% (63.4% – 73.6%)</td>
</tr>
<tr>
<td>FP line</td>
<td>41.6% (33.1% – 51.2%)</td>
<td>93.7% (89.8% – 96.4%)</td>
<td>6.3% (3.7% – 10.4%)</td>
<td>6.49 (3.4 – 11.1)</td>
<td>0.63 (0.54 – 0.72)</td>
<td>77.6% (65.8% – 86.9%)</td>
<td>75.3% (69.9% – 80.1%)</td>
</tr>
<tr>
<td>NBL</td>
<td>61.9% (53.4% – 69.9%)</td>
<td>95.0% (92.9% – 98.2%)</td>
<td>5.0% (1.7% – 11.3%)</td>
<td>12.32 (5.2 – 29.4)</td>
<td>0.40 (0.31 – 0.52)</td>
<td>94.7% (88.1% – 98.2%)</td>
<td>62.9% (54.7% – 70.6%)</td>
</tr>
<tr>
<td>PT</td>
<td>63.4% (53.4% – 73.1%)</td>
<td>95.0% (92.9% – 98.2%)</td>
<td>5.0% (1.7% – 11.3%)</td>
<td>12.73 (5.4 – 30.3)</td>
<td>0.38 (0.29 – 0.50)</td>
<td>94.8% (88.4% – 98.2%)</td>
<td>64.1% (55.9% – 71.8%)</td>
</tr>
<tr>
<td>PT-NBL ratio</td>
<td>86.2% (79.3% – 91.2%)</td>
<td>95.0% (92.9% – 98.2%)</td>
<td>5.0% (1.7% – 11.3%)</td>
<td>17.37 (7.4 – 41.0)</td>
<td>0.14 (0.08 – 0.23)</td>
<td>96.1% (91.3% – 98.7%)</td>
<td>82.6% (74.4% – 89.0%)</td>
</tr>
<tr>
<td>PFSR</td>
<td>79.7% (71.6% – 86.0%)</td>
<td>95.0% (92.9% – 98.2%)</td>
<td>5.0% (1.7% – 11.3%)</td>
<td>15.96 (6.8 – 37.7)</td>
<td>0.21 (0.14 – 0.32)</td>
<td>95.5% (89.8% – 98.5%)</td>
<td>77.8% (69.5% – 84.8%)</td>
</tr>
</tbody>
</table>

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.

⁹ Down syndrome.
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 5\(^{th}\) and above the 95\(^{th}\) 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 5\(^{th}\) percentile, but 62.5% of angles were above the 50\(^{th}\) 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\textsuperscript{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\%\textsuperscript{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\textsuperscript{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\textsuperscript{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.
REFERENCES
