Prefrontal space ratio in second- and third-trimester screening for trisomy 21

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KEYWORDS: prefrontal space ratio; screening; second trimester; trisomy 21

ABSTRACT

Objective To evaluate the prefrontal space ratio (PFSR) in second- and third-trimester euploid fetuses and fetuses with trisomy 21.

Methods This was a retrospective study utilizing stored mid-sagittal two-dimensional images of second- and third-trimester fetal faces that were recorded during prenatal ultrasound examinations at the Department of Prenatal Medicine at the University of Tuebingen, Germany and at a private center for prenatal medicine in Nuremberg, Germany. For the normal range, 279 euploid pregnancies between 15 and 40 weeks’ gestation were included. The results were compared with 91 cases with trisomy 21 between 15 and 40 weeks. For the ratio measurement, a line was drawn between the leading edge of the mandible and the maxilla (MM line) and extended in front of the forehead. The ratio of the distance between the leading edge of the skull and the leading edge of the skin (d1) to the distance between the skin and the point where the MM line was intercepted (d2) was calculated. The PFSR was determined by dividing d2 by d1.

Results In the euploid and trisomy 21 groups, the median gestational age at the time of ultrasound examination was 21.1 (range, 15.0–40.0) and 21.4 (range, 15.0–40.3) weeks, respectively. Multiple regression analysis showed that PFSR was independent of maternal and gestational age. In the euploid group, the mean PFSR was 0.97 ± 0.29. In fetuses with trisomy 21, the mean PFSR was 0.2 ± 0.38 (P < 0.0001). The PFSR was below the 5th centile in 14 (5.0%) euploid fetuses and in 72 (79.1%) fetuses with trisomy 21.

Conclusion The PFSR is a simple and effective marker in second- and third-trimester screening for trisomy 21.

INTRODUCTION

Screening for aneuploidy using ultrasound relies on the search for certain fetal anomalies and markers. It has been well established that in the second trimester of pregnancy anomalies such as cardiac defects, duodenal atresia and ventriculomegaly, as well as markers such as nuchal edema, echogenic bowel, mild hydronephrosis, shortening of the humerus and femur, sandal gap, clinodactyly and hypoplasia of the middle phalanx of the fifth digit are all seen more commonly in fetuses with trisomy 21 than in euploid fetuses.

Differences in facial morphological characteristics between euploid individuals and those with trisomy 21 are especially common. The areas for which the most extensive investigation in screening for trisomy 21 has been done are the following: presence or absence and size of nasal bone, thickness of prenasal skin and size of maxilla and/or its position within the fetal face. All appear to yield high detection rates. For example, absence or hypoplasia of the nasal bone is found in 60–70% of first-trimester fetuses with trisomy 21 but in only 1–2% of euploid fetuses.

The prenasal skin tends to be thicker in fetuses with trisomy 21, and in the second trimester approximately 70% of fetuses with trisomy 21 have a prenasal skin thickness above the 95th percentile.

The size of the maxilla tends to be smaller in trisomy 21 than in euploid fetuses. Cicero et al. demonstrated that in the first trimester the maxillary length was below the 5th centile in 14 (5.0%) euploid fetuses and in 72 (79.1%) fetuses with trisomy 21. However, the differences in maxillary length were so small (mean = 0.7 mm) that this finding has limited clinical utility. The exact reason for the relative shortness of the maxilla in trisomy 21 is unclear, though oligodontia, a well-recognized facial feature of this
syndrome, may be a contributory factor. The flat face, which is commonly seen in individuals with trisomy 21, may be due to hypoplasia of the maxilla and, in addition, dorsal displacement of the maxilla. These features are probably a result of an inherent problem with connective tissue formation and a concomitant decrease in movement of the tongue, which is thought to play a role in normal development of the upper palate.

Prefrontal space ratio (PFSR) is a novel parameter that exploits the dorsal displacement of the anterior edge of the maxilla and thickening of the prenasal skin in trisomy 21. In a retrospective study by Sonnek et al., a precise midline view of the fetal face was established by manipulating stored three-dimensional (3D) volumes of fetal faces between 16 and 25 weeks’ gestation. A line (the mandibulomaxillary (MM) line) was drawn between the anterior aspect of both the fetal chin and the maxilla and extended in front of the fetal forehead. The PFSR was established by dividing the following two distances: leading edge of skull to prenasal skin (d1) and prenasal skin to the point where the MM line is intercepted (d2). The PFSR, d2/d1, was found to be significantly smaller in fetuses with trisomy 21 than in euploid fetuses. Not only was this ratio found to be highly discriminative but the measurements were also highly reproducible.

In our study, we set out to evaluate the PFSR as a method of screening for trisomy 21 in the second and third trimesters of pregnancy using two-dimensional (2D) ultrasound.

METHODS

This was a retrospective study utilizing stored 2D images of second- and third-trimester fetal profiles. The prenatal ultrasound examinations used in this study were performed at the Department of Prenatal Medicine at the University of Tuebingen, Germany, between 2004 and 2012 and at the Center for Prenatal Diagnosis and Genetics in Nuremberg, Germany, between 2000 and 2012. First, we searched the databases at both centers for pregnancies in which the pre- and postnatal diagnosis of trisomy 21 had been made and that had had an ultrasound examination after 16 weeks’ gestation. Only those images that represented a true mid-sagittal section of the face were used in the study. Eighty such cases were identified at the Tuebingen center and 13 at the Nuremberg center. Two cases were excluded, as in these it was not possible to identify the borders of the mandible clearly enough, leaving 91 cases for analysis.

Second, we searched the database for euploid pregnancies that were known to have resulted in neonates with normal outcomes. For this purpose, only the database at the Tuebingen center was used. For each pregnancy with trisomy, we randomly selected three euploid cases between 15 and 40 weeks that were referred for reasons other than the suspicion of a fetal anomaly, thus we had a cohort of 279 (i.e. 93 × 3) euploid fetuses. In both the euploid pregnancies and those with trisomy 21 in which more than one examination was performed, only the images from the earliest suitable examination were used in our analysis.

The following data were recorded in each case: maternal history, gestational age, fetal head biometry, presence or absence of nasal bone, length of nasal bone, prenasal thickness and fetal karyotype.

In order for an image to be acceptable for the PFSR measurement, it had to meet the following criteria: true mid-sagittal section (preferably with the corpus callosum visible) and clearly identifiable anterior edges of the mandible and maxilla as well as the leading edge of the bony forehead and the skin over the forehead. The magnification was such that the profile filled the majority of the image.

The reference point was placed on the anterior edge of the mental protuberance. Care was taken to avoid the tooth buds, which are located just superiorly to this point. This was done because tooth buds change in size as the fetus develops; placing the reference point here would introduce an unpredictable confounding variable. A line was then drawn between the reference point and the anterior edge of the maxilla (the MM line) and extended in front of the fetal forehead. A measurement (d1) was taken between the leading edge of the bony forehead to the leading edge of the skin in a line that is parallel to the inferior edge of the maxilla. The direction of this line is also approximately perpendicular to the frontal bone. The level at which this measurement was taken was located just superiorly to the point where the skin over the forehead turns anteriorly over the fetal nose. A second measurement (d2) was performed at the same level as the first one and along the same line. This measurement started at the leading edge of the skin at the same point as the d1 measurement and ended at the point of intercept with the MM line. When the MM line crossed behind the edge of the prenasal skin, the d1 measurement was still taken between the frontal bone and the skin but the d2 measurement was then taken between the MM line and the skin and multiplied by −1. The PFSR was determined by dividing d2 by d1 (Figure 1).

Two operators measured the PFSR. Both were blinded to their own results, the measurements of the other operator and the karyotype. Operator 2 measured the PFSR in each case twice to determine interobserver repeatability.

Statistical analysis

Intra- and interobserver repeatability was examined using 95% limits of agreement. The normal range in euploid fetuses was computed based on gestational age by applying univariate regression analysis. Multiple regression analysis was used to determine the relevant covariates among maternal and gestational age, nasal bone length and prenasal thickness (in multiples of the median (MoM)), biparietal diameter, head circumference and abnormal karyotype.

The PFSR distribution in fetuses with trisomy 21 was compared with the distribution in the euploid group using Student’s t-test after having verified that both distributions were normal by the Kolmogorov–Smirnov test.
Measurements of the nasal bone and prenasal thickness were transformed into MoM values according to gestational age at the time of examination. The respective formulae were:

- Nasal bone length: $L_{\text{NB}} = -0.65 + (0.28 \times GA)$, $P < 0.0001$, $r = 0.853$ and
- Prenasal thickness: $T_{\text{PN}} = -0.63 + (0.23 \times GA)$, $P < 0.0001$, $r = 0.785$,

where $GA$ is gestational age in weeks.

The screening performance of a protocol based on maternal age in combination with the PFSR was investigated by simulating values for maternal age and PFSR for 20,000 euploid and 20,000 trisomy 21 pregnancies at 20 weeks’ gestation. For this simulation, we used SDs and correlation coefficients for PFSR from the present study, and maternal age distribution of pregnancies in England and Wales in 2000–2002. In each case, the maternal age-related risk for trisomy 21 at term was computed and adjusted according to the gestational age at the time of screening. A likelihood ratio for trisomy 21 was multiplied by the maternal and gestational age-related risk factors. Detection and false-positive rates were calculated as proportion of cases with risks above certain thresholds. Statistical analysis was performed with Microsoft Excel for Mac 2011 (Redmond, WA, USA) and IBM SPSS20 (Armonk, NY, USA), and $P < 0.05$ was considered to be statistically significant.

**RESULTS**

A total of 370 fetuses were available for analysis. Of those, 279 were euploid and 91 were affected by trisomy 21. In the euploid group, the median maternal age was 30.5 years and the median gestational age was 21.1 weeks, while in the trisomy 21 group the median maternal age was 36.3 years and the median gestational age was 21.4 weeks. Further characteristics of the study population are shown in Table 1.

The mean difference between the first and second PFSR measurements of Operator 2 was $-0.01$, and in 95% of the cases the difference was between $-0.19$ and 0.17. The mean difference between the measurements of Operators 1 and 2 was $-0.01$, with 95% of the difference of the ratios being between $-0.59$ and 0.58.

Multiple regression analysis showed that the PFSR was statistically correlated with an abnormal karyotype ($P < 0.0001$), nasal bone length ($P = 0.001$) and prenasal thickness ($P < 0.0001$), but was independent of maternal age ($P = 0.331$), gestational age ($P = 0.672$), biparietal diameter ($P = 0.652$) and head circumference ($P = 0.259$).

The PFSRs, $d_1$ (frontal bone to skin distance) and $d_2$ (skin to MM line distance) measurements in euploid and in trisomy 21 fetuses are shown in Table 2. In the euploid group, the mean PFSR was $0.97 \pm 0.29$ (range, $0.20-2.02$). In fetuses with trisomy 21, the mean PFSR was $0.21 \pm 0.38$ (range, $-0.56$ to 1.42), which is significantly lower than in the euploid group ($P < 0.0001$). The independence of the PFSR with respect to gestational age that was noted on multiple regression

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**Table 1** Maternal and pregnancy characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Euploid ($n = 279$)</th>
<th>Trisomy 21 ($n = 91$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>30.5 (19.0–45.9)</td>
<td>36.3 (17.2–49.5)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>21.1 (15.0–40.0)</td>
<td>21.4 (15.0–40.3)</td>
</tr>
<tr>
<td>Second-trimester US (14–25 weeks)</td>
<td>211 (75.6)</td>
<td>63 (69.2)</td>
</tr>
<tr>
<td>Third-trimester US (26–40 weeks)</td>
<td>68 (24.4)</td>
<td>28 (30.8)</td>
</tr>
<tr>
<td>Caucasian ethnicity</td>
<td>279 (100)</td>
<td>91 (100)</td>
</tr>
</tbody>
</table>

Data given as median (range) or $n$ (%). US, ultrasound examination.
In this study, we have shown, using 2D ultrasound, that there is a significant difference in the PFSR between euploid fetuses and those with trisomy 21. In our study group of 91 fetuses with trisomy 21, almost 80% had an abnormally low PFSR, irrespective of gestational age. Using mathematical modeling, when our results are applied to general population screening based on maternal age, gestational age and PFSR, it is estimated that an 83% detection rate for a false-positive rate of 5% may be achieved. At the 5th percentile of the normal distribution, the PFSR is 0.55. In other words, d1 (the frontal bone–skin distance) is about twice as long as d2 (the skin–MM line distance) at the 5th percentile. The mean PFSR in euploid fetuses is around 1 while in trisomy 21 it is about 0.2. The MM line courses behind the surface of the prenasal skin in approximately 25% of trisomy 21 fetuses and in none of the euploid fetuses, making this finding highly predictive of fetal aneuploidy.

The PFSR appears to provide a higher detection rate than do other facial markers for chromosomal abnormalities. Using nasal bone absence or hypoplasia as a marker for trisomy 21, Cicero et al.3 reported a detection rate of about 60% for a false-positive rate of 1.2%. However, other second-trimester studies with substantially lower detection rates have been reported. This may be due in part to the use of different definitions of nasal bone hypoplasia.12,13 In a 3D study of 26 fetuses with trisomy 21 and 135 euploid fetuses between 16 and 24 weeks, Persico et al.4 examined prenasal skin thickness. Using this method, they reported a detection rate of about 70% for a 5% false-positive rate. Similarly, Miguelez et al.14 examined 135 fetuses with trisomy 21 between 14 and 27 weeks’ gestation and found a 60% detection rate for the same false-positive rate.

The negative likelihood ratio of a normal PFSR was 0.22, which is substantially lower than the negative likelihood ratio of the common soft markers such as echogenic focus and nuchal fold, both of which have a negative likelihood ratio of 0.801. In this respect, the PFSR may help to further refine the risk for trisomy 21 if other soft markers are present. However, further studies need to examine the interdependency of the PFSR and other soft markers.

An alternative method of prenatal screening for trisomy 21, which exploits the maxillary abnormalities and increased prenatal skin thickness, is measurement of the frontomaxillary angle in the second trimester.15 The vertical ray of the angle is placed on the surface of the prenasal skin and the apex is at the anterior-most edge of the maxilla. In trisomy 21, thickening of the prenasal skin and dorsal displacement of the maxilla result in an increase in this angle. Even though a detection rate of 88% for a false-positive rate of 5% was found in the initial report, low reproducibility (possibly due to difficulty with obtaining the correct view using 2D ultrasound) has limited its introduction into general screening protocols.

In contrast, the PFSR seems to be an easy, effective and reproducible measurement in screening for trisomy 21. Similarly to the facial angle, it combines two distinct facial characteristics of fetuses with trisomy 21: increased prenatal thickness and mid-face hypoplasia. The increased prenatal thickness inevitably leads to an increase in the denominator of the ratio, i.e. the distance between the frontal bone and the skin edge (d1). The short and dorsally displaced maxilla tilts the MM line closer to the forehead, reducing the numerator of the ratio, i.e. the distance between the skin edge and the MM line (d2). Therefore, both these factors act to reduce the PFSR in fetuses with trisomy 21.

Our results are consistent with the previously published 3D study that introduced the PFSR as a possible tool in screening for trisomy 21.8 In this study, the authors examined 26 second-trimester fetuses with trisomy 21 and found a mean PFSR of 0.36, which is consistent with 0.21 in our study. However, their mean PFSR in

<table>
<thead>
<tr>
<th>Karyotype</th>
<th>n</th>
<th>d1 (Frontal os–skin)</th>
<th>d2 (Skin–MM line)</th>
<th>Prefrontal space ratio (d2/d1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euploid</td>
<td>279</td>
<td>4.6 ± 1.4</td>
<td>4.4 ± 1.6</td>
<td>0.97 ± 0.29</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>91</td>
<td>5.1 ± 2.0†</td>
<td>0.9 ± 1.7†</td>
<td>0.21 ± 0.38†</td>
</tr>
</tbody>
</table>

Data given as mean ± SD. Significant differences between euploid and trisomy 21 fetuses (t-test) are indicated: *P = 0.008; †P < 0.0001. MM, mandibulomaxillary.
the euploid population was 1.48, which is substantially larger than the one seen in our study (0.97). Some of this difference may be explained by the fact that they used 3D blocks to generate the precise midline view. Although we collected good 2D images showing the fetal face in a mid-sagittal section, it is probable that a better mid-sagittal section could be generated by manipulating a 3D volume. This in turn may result in a better separation of euploid and aneuploid fetuses. To some extent, the difference could also be explained by ethnic differences and by the gestational age distribution in the study groups. The 3D study only included fetuses up to 25 weeks’ gestation, whereas our study extended into the third trimester. However, both studies have shown that the ratio is independent of gestational age. As such, the inclusion of third-trimester pregnancies in our study is not likely to be responsible for this difference.

The main disadvantage of our study is that some of the affected fetuses were referred to us because of a previously identified increased risk for trisomy 21. It is possible that some of these referrals were based on increased prenasal thickness or a subjective suspicion of a flat fetal face. Consequently, the finding of an abnormal PFSR in those affected fetuses may be overrepresented. Further studies need to focus on PFSR in prospective second-trimester screening for trisomy 21.

REFERENCES


