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MATERNAL-FETAL MEDICINE

New osseous soft markers for trisomy 13, 18 and 21

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Abstract
Introduction For ultrasonographic diagnosis of a fetal trisomy so-called "soft markers" (=ultrasonographically detectable morphological variants) are used. Detection of a certain number of them increases the diagnostic certainty of a fetal trisomy. Up to now there are very few diagnostically accepted osseous soft markers for trisomy. Hence potential osseous soft markers applicable for first and second trimester ultrasound screening for trisomy 21, 18 or 13 were studied.
Methods Postmortal fetal X-rays (ap, lateral) of 358 fetuses (trisomy 21: $n = 109$, trisomy 18: $n = 46$; trisomy 13: $n = 38$, control group: $n = 165$).
Results Not yet described but with trisomy 21 statistically associated soft markers were un-timely os sternale ossification, delayed os sacrum ossification, shortened os maxillare, reduced os maxillare-jaw-corner distance, augmented orbita height, premature os calcaneus ossification, bell-shaped thorax, coronal clefts, trend to wider binocular as well as wider intraocular distances; for trisomy 18: elevated clavicula slope, reduced number of ribs, bell-shaped thorax, coronal clefts, reduced os maxillare-jaw-corner distance, shortened ramus mandibulare, shortened os metacarpale IV and V, augmented ratio between biparietal diameter and (osseus and soft-tissue) shoulder width; for trisomy 13: longer os nasale, elevated clavicula slope, premature sternum, delayed os sacrum ossification, delayed/premature cranium ossification, reduced number of ribs, coronal clefts, reduced os maxillare-jaw-corner distance, shortened ramus mandibulare, augmented orbita height, shortened os metacarpale V and a tendency for a shortened os metacarpale IV.
Conclusion We found several not yet published osseous soft markers statistically associated with trisomy 21, 18 and 13, which can help to ensure sonographically these aneuploidy diagnoses.

Keywords Osseous soft marker · Trisomy 13 · Trisomy 18 · Trisomy 21 · Fetal X-rays

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Introduction

The incidences of the most common and viable trisomies in live births vary from 1:650 (trisomy 21), 1:10000 (trisomy 18) to 7:10,000 (trisomy 13). Today prenatal ultrasound is an accepted diagnostic tool for prenatal detection of trisomy 21, 18 and 13 in first and second trimester. For detection of a potential trisomy prenatal screening ultrasound—generally combined with invasive methods (with a potential lethal complication risk) or serological tests—is the most often used technique [1, 2, 10, 11, 13], permitting

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a risk quantification for fetal aneuploidy (i.e., trisomy 21). Today, ultrasound markers, combined with other biochemical tests have a detection rate of up to 96 % [2]. Depending on the cut-off value the rate of false-positive diagnosis varies from 3 to 5 %. Here the non-invasive prenatal testing (NIPT) cell by cell free DNA analysis—promising a detection rate of 99.5 %—is a new method [11] that seems to be superior to these combined traditional tests and scans. Yet this new method is limited for several reasons. On one hand, it is very cost intensive. Furthermore, its results are difficult to interpret resp false-negative in cases of early fetal loss ("vanishing twin") and in fetuses or mothers with mosaic trisomy. Therefore up to now this test is not used as a general first trimester screening method like the first trimester sonogram offered to all pregnant women checking for any fetal abnormalities. This first trimester sonography still has the advantage of detecting not only trisomies but also other fetal abnormalities and malformations not yet detectable by NIPT. Hence further research for soft markers increasing the diagnostic reliability for trisomies is desirable. So-called "soft markers" are sonographic detectable variants of normal fetal development associated with an increased risk for underlying fetal aneuploidy. Although their verification correlates with an increased risk for fetal aneuploidy they are not diagnostic. Nevertheless, accumulation of them increases the diagnostic certainty of a fetal trisomy.

Up to now these anomalies include individual osseous markers, like absent or hypoplastic nasal bone, shorted os femorale and humerale, smaller digits [9], greater iliac crest angle [8], facial angle, brachycepahlie and strawberry sign, but more often soft-tissue markers, like increased nuchal fold resp. nuchal edema, echogenic bowel or echogenic intracardiac focus, hydronephrosis, ventriculomegaly or ARSA (aberrant right subclavian artery) [1] or—especially in trisomy 21—duodenal obstruction or atrioventricular septum defect. Nevertheless, up to now only single osseous soft markers are used (like missing nasal bone) or studied like coronal clefts [6] and calcaneus ossification [14], and then mainly with regard to only one of the trisomies, most often trisomy 21. Other potential soft markers like a reduce number of ribs—often found in trisomy 13 or 18—have yet not been systematically examined concerning their diagnostic value for a prenatal diagnosis of trisomy 21,18 or 13 (PubMed research 11/2015). Hence, aim of this study was the search for new osseous soft markers that could become valuable sonographic signs for ultrasound screening for trisomy. For determining new osseous soft markers the mean morphological measurements of the studied osseous structures were determined in the varying pregnancy weeks by measuring the structure in the control group.

Materials and methods

358 postmortem X-rays (ap and lateral) of still born fetuses and fetuses after termination of pregnancy from 9 to 39 weeks of gestation were included. In detail 109 cases of trisomy 21, 46 cases of trisomy 18, 38 cases of trisomy 13 and 165 cases of a control group could be analyzed. The number of fetuses before the thirteenth pregnancy week and beyond the twentieth in the trisomy groups was very small to influence the study of the non-dichotomic soft markers, so we excluded them. For this reason, we analyzed 89 cases of trisomy 21, 35 cases of trisomy 18, 28 cases of trisomy 13 and 92 cases of the control group for the non-dichotomic soft markers.

All diagnoses of a trisomy had been confirmed by chromosomal analysis.

The control group consisted of euploid fetuses with intrauterine fetal death due to placental insufficiency, chorioamnionitis or abortion due to maternal medical indication and cardiac defect (Table 1).

Conventional fetal X-rays (ap and lateral) had been taken as a routine procedure before diagnostic fetal autopsy. All autopsies including X-rays were approved by the parents. In this retrospective study based on postmortem pathological material where no changes in the clinical decision have been deduced by its design, there were no ethical dilemmas. Therefore, ethics committee decided that their approval was not necessary. We conducted meticulous quantitative geometrical measurements (by digital caliper) and structural analysis in search of the

Table 1 Control group

	Sex	Cause of death
1st Trimester	$f = 9$, $m = 5$	Chorioamnionitis ($n = 8$) abruptio placentae ($n = 6$)
2nd Trimester	$f = 35$, $m = 31$	Maternal medical indication ($n = 2$) chorioamnionitis ($n = 33$) cardiac failure ($n = 6$) abrubtio placentae ($n = 10$) placental insufficiency ($n = 15$)
3rd Trimester	$f = 17$, $m = 18$	Abruptio placentae ($n = 4$) maternal medical indication ($n = 3$) complication of the umbilical cord ($n = 2$) chorinamnionitis ($n = 4$) placental insuffiency ($n = 22$)

N 165, *f* female, *m* male

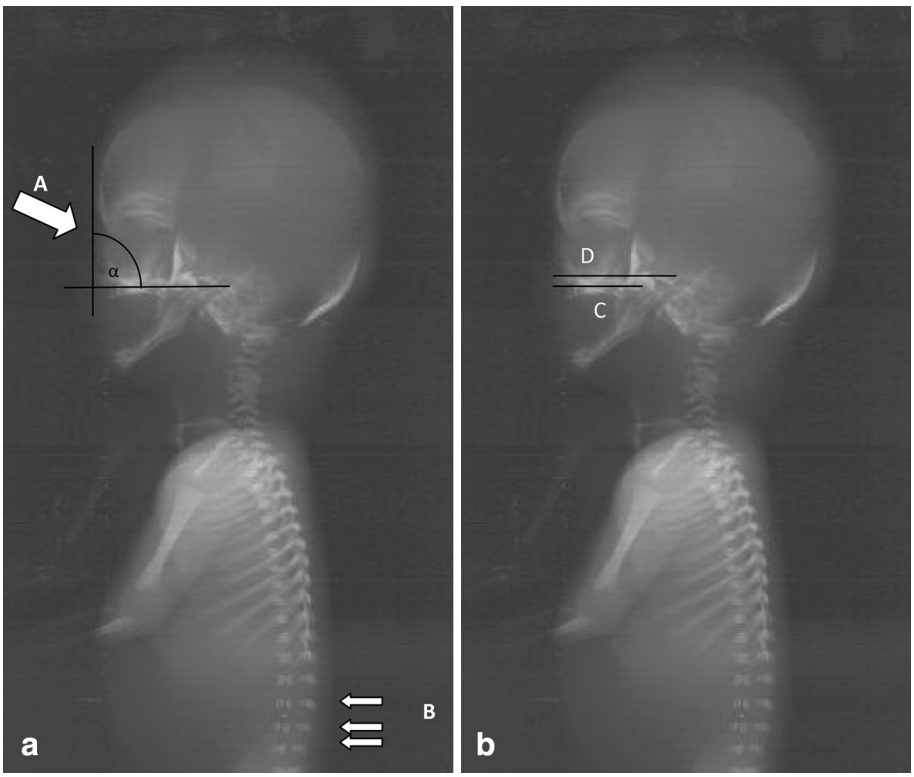

Fig. 1 Lateral x-ray of a fetus with trisomy 21 at 17 weeks of pregnancy. *A* Absence of os nasale, *B* coronal clefts, α frontomaxillary angle, *C* length of os maxillare, *D* distance between os maxillare and jaw corner

following abnormalities—always studying anterior–posterior and lateral radiograph: presence and length of the os nasale (Fig. 1), frontomaxillary angle (Fig. 1a), palatine cleft, length of os maxillare [15], distance between os maxillare and jaw corner (Fig. 1b), length of os mandibulare and ramus mandibulare, height and width of the orbita (Fig. 2), intraoccular and bioccular diameter, dental development and cranial ossification, biparietal diameter, polydactylia, number of hand and foot rays, length of os metacarpale IV and V, aplasia of the radial bone [12], deformation of the thorax [7], shoulder width (osseous and soft-tissue), ratio between biparietal diameter and shoulder width (osseus and soft-tissue) in frontal plane, number of ribs, sternal ossification, coronal clefts, patellar ossification, time adequate ossification of os calcaneus and length of os femorale. Reference value of the anatomical details was the fetal radiology atlas of Schumacher et al. [14]. For statistical analysis these signs were divided into dichotomic soft markers (i.e., presences/absence of the os nasale [4]), tenary soft markers (timely, early or delayed development) and non-dichotomic development-associated markers (length dependent on pregnancy week). As potential dichotomic soft markers—premature ossification of the os calcaneus, bell-shaped thorax, coronal clefts [6], premature or delayed sternum ossification and elevated clavicula slope, as potential tenary soft markers timely/not timely development of os sacrum, premature or delayed

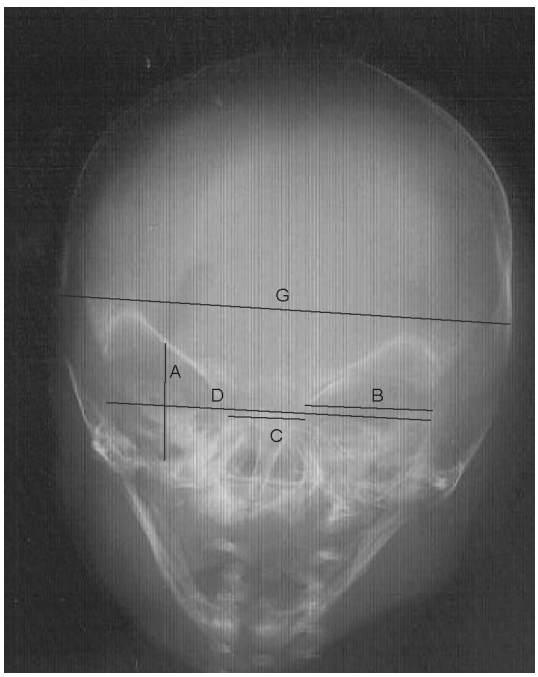

Fig. 2 ap-X-ray of a euploid fetus of 18 weeks of pregnancy: *A* height of orbita, *B* width of orbita, *C* intraocular distance, *D* interoccular distance, *G* biparietal distance

ossification of the cranium or numbers of ribs in relation to gestational age—were regarded. As potential non-dichotomic soft markers—length of os nasale, length of os

maxillare, distance between os maxillare and jaw corner, length of ramus mandibulare, height of orbita, binocculary diameter, binocular distance, length of os metacarpale IV and V, ratio between biparietal diameter and shoulder width (osseous and soft-tissue)—were considered. Statistics itself was conducted with IBM SPSS Statistic® (Version 21.0. Armonk, NY: IBM Corp 2012) and R®(R Foundation for Statistical Computing, Vienna, Austria, 2012), assisted by the Institute of Mathematical Statistics and Actuarial Science. For dichotomous soft markers odds ratios were calculated as cross product and p values were derived from Fisher's exact test. For continuous soft marker odds ratios and p values were taken from logistic regression.

Results

The age of the examined fetuses ranged from 9 to 39 weeks of gestation. Fetuses with trisomy 21 represented the largest group ($n = 109$), followed by trisomy 18 ($n = 46$) and trisomy 13 ($n = 38$). For the non-dichotomic soft marker trisomy 21 still represented the largest group ($n = 89$), followed by trisomy 18 ($n = 35$) and trisomy 13 ($n = 28$).

As up to now not published but significantly with trisomy 21 associated soft marker for trisomy 21 turned out bell-shaped thorax (OR 0.38, 95 % CI [0.17, 0.87], $p = 0.0191$), un-timely os calcaneus ossification (OR 2.61 [1.52, 4.47], $p = 0.001$), coronal cleft (OR 6.55 [3.37, 12.8], $p < 0.001$), un-timely ossification of os sternale (OR 0.38 [0.19, 0.79], $p = 0.009$) and os sacrum (delayed OR 0.40 [0.20, 0.82], $p = 0.014$, preterm OR 3.60 [1.16, 11.1], $p = 0.024$), shortened length of os nasale (OR 0.66 per mm length [0.53, 0.81], $p < 0.001$), shortened os maxillare (OR 0.93 [0.88, 0.98], $p = 0.006$), shortened distance between os maxillare and jaw corner (OR 0.95 [0.91, 0.99, $p = 0.027$), orbita height (OR 0.97, [0.90, 1.04], $p = 0.339$) (Fig. 2) and larger ratio between biparietal diameter and tissue shoulder width (OR 5.16, [3.89, –], $p = 0.011$) (Table 2).

With trisomy 18 the following soft markers were statistically significantly associated: bell-shaped thorax (OR 0.10 [0.01, 0.77], $p = 0.007$), coronal cleft (OR 2.98 [1.19, 7.48], $p = 0.025$), elevated clavicula slope (OR 22.3 [2.52, 197], $p = 0.001$), delayed cranium ossification (OR 2.47 [1.18, 5.15], $p = 0.017$), reduced number of ribs (OR 21.9, [9.41, 51.1], $p < 0.001$), length of os nasale (OR 0.71[0.54, 0.94], $p = 0.018$), distance between os maxillare and jaw corner (OR 0.92 [0.86, 0.99], $p = 0.016$), ramus mandibulare (OR 0.93, [0.87, 0.98], $p = 0.012$), os metacarpale IV (OR 0.77, [0.64, 0.93], $p = 0.006$), os metacarpale V (OR 0.78, [0.64,0.95], $p = 0.013$), ratio between biparietal diameter and osseous shoulder width (OR 2884, [33.5, –], $p < 0.001$), ratio between biparietal diameter and soft-tissue shoulder width (OR 59,991 [77.4, –], $p = 0.001$) and larger biparietal diameter (OR 0.993, [0.96, 1.02], $p = 0.665$) (Table 2).

For trisomy 13 we found the following soft markers: coronal clefts (OR 3.56, [1.45, 8.79], $p = 0.007$) (Fig. 1a), os sternale ossification (OR 0.09, [0.01, 0.69] $p = 0.004$), elevated clavicula slope (OR 30.2, [3.51, 260], $p < 0,001$), delayed development of os sacrum (OR 0.30, [0.12, 0.76], $p = 0.0013$), delayed cranium ossification (OR 3.62, [1.65, 7.97] $p = 0.001$), reduced number of ribs (OR 20.6 [8.45, 50.0] $p < 0.001$) (Fig. 3), larger os nasale length (OR 1.08, [0.88, 1.34], $p = 0.464$), shortened distance between os maxillare and jaw corner (OR 0.98, [0.92, 1.05], $p = 0.609$), shortened ramus mandibulare (OR 0.98, [0.93, 1.03], $p = 0.423$), larger orbita height (OR 0.93, [0.94, 1.03], $p = 0,145$) and shortened os metacarpale IV (OR 0.88, [0.75, 1.04] $p = 0.0126$) (Table 2).

The measurements of potential osseous soft markers in correlation with the gestational age and the measurements of the control group (=reference values) are summarized in Table 3 and illustrated in Fig. 2.

As soft markers for the studied trisomies could be excluded: delayed (T13: $p = 0.4813$, T18: $p = 0.5343$, T2: $p = 0.2951$) or premature dentition (T13: $p = 1$, T18: $p = 0.7414$, T21 $p = 1$), altered length of os mandibulare (T13: $p = 0.244$, T18 $p = 0.292$, T21: $p = 0.440$), altered diameter of orbita (T13: $p = 0.2303$, T18: $p = 0.721$, T21: $p = 0,393$), altered number of hand (6 rays T13: $p = 0.0667$, T18: $p = 1.0$, T21: $p = 1.0$; 4 rays T13: $p = 1.0$, T18: $p = 0.997$, T21: $p = 0.4435$) and/or foot rays (6 rays T13: $p = 0.0767$, T18: $p = 1$, T21: $p = 1$, 4 rays T13: $p = 1$, T18: $p = 1$, T21: $p = 1$), fused vertebras (T13: $p = 0.1819$, there were no fused vertebras in the other trisomies), premature patella ossification (T13: $p = 1$, T18: $p = 1$, T21: $p = 0.6434$), altered bone based (T13: $p = 0.086$, T18: $p = 0.083$, T21: $p = 0.652$) resp. soft tissue based shoulder width diameter (T13: $p = 0.343$, T18: $p = 0.948$, T21: $p = 0.137$).

Discussion

Today in industrialized countries there is a strong tendency to an advanced maternal age, especially for the first pregnancy. As these patients have an improved risk for trisomy the need for exact non-invasive prediction of a fetal trisomy becomes more and more important. Hence, for prenatal non-invasive diagnostic of a fetal trisomy, especially trisomy 21, 18 or 13, soft markers detectable by ultrasound are indispensable.

Triggered by the finding that in fetal radiographs some osseous markers like coronal clefts [6] are helpful as diagnostic markers for trisomies the rational for this study

Table 2 Incidence of osseous soft markers in trisomy 13, 18 and 21

	Trisomy 13		Trisomy 18		Trisomy 21	
	Odds ratio *p* value	95 % CL	Odds ratio *p* value	95 % CL	Odds ratio *p* value	95 % CL
Bell shaped thorax	0.53 *0.337*	[0.17, 1.61]	0.10 *0.007*	[0.01, 0.77]	0.38 *0.019*	[0.17, 0.87]
Ossification of calcaneus	0.32 *0.064*	[0.09, 1.09]	1.58 *0.327*	[0.73, 3.16]	2.61 *0.001*	[1.52,4.47]
Coronal cleft	3.56 *0.007*	[1.45, 8.79]	2.98 *0.025*	[1.19, 7.48]	6.55 *<0.001*	[3.37, 12.8]
Ossification of the sternale	0.09 *0.004*	[0.01, 0.69]	0.47 *0.191*	[0.17, 1.29]	0.38 *0.009*	[0.19, 0.79]
Elevated clavicula slope	30.2 *<0.001*	[3.51, 260]	22.3 *0.001*	[2.52, 197]	3.21 *0.561*	[0.29, 35.9]
Dev. of sacrum advanced	2.37 *0.596*	[0.43, 12.9]	2.94 *0.154*	[0.67, 13.0]	3.60 *0.024*	[1.16, 11.1]
Dev. of sacrum delayed	0.30 *0.013*	[0.12, 0.76]	0.47 *0.150*	[0.18, 1.24]	0.40 *0.014*	[0.20, 0.82]
Ossific. of cranium delayed	3.62 *0.001*	[1.65, 7.97]	2.47 *0. 017*	[1.18, 5.15]	1.38 *0.301*	[0.78, 2.46]
Count of ribs reduced	20.6 *<0.001*	[8.45, 50.0]	21.9 *<0. 001*	[9.41, 51.1]	1.71 *0.108*	[0.92, 3.18]
Length of nasale 1 mm	1.08 *0.464*	[0.88, 1.34]	0.71 *0. 018*	[0.54, 0.94]	0.66 *<0.001*	[0.53, 0.81]
Os maxillare 1 mm	0.98 *0.672*	[0.91, 1.07]	0.91 *0. 026*	[0.84, 0.99]	0.93 *0.006*	[0.88, 0.98]
Os max. to jaw 1 mm	0.98 *0.609*	[0.92, 1.05]	0.92 *0. 016*	[0.86, 0.99]	0.95 *0.027*	[0.91, 0.99]
Ramus madibulare 1 mm	0.98 *0.423*	[0.93, 1.03]	0.93 *0. 012*	[0.87, 0.98]	0.95 *0.019*	[0.92, 0.99]
Orbita hight 1 mm	0.93 *0.145*	[0.94, 1.03]	0.91 *0. 070*	[0.92, 1.01]	0.97 *0.339*	[0.90, 1.04]
Binocculary diam.	0.95 *0.023*	[0.91, 0.99]	0.96 *0. 104*	[0.92, 1.01]	0.97 *0.053*	[0.94, 1.00]
Intraoccular dist.	0.90 *0.227*	[0.74, 1.09]	1.04 *0. 333*	[0.96, 1.11]	0.86 *0.042*	[0.75, 0.99]
Os metacarpale IV	0.88 *0.126*	[0.75, 1.04]	0.77 *0. 006*	[0.64, 0.93]	0.86 *0.012*	[0.76, 0.97]
Os metacarpale V	0.87 *0.115*	[0.73, 1.04]	0.78 *0. 013*	[0.64, 0.95]	0.88 *0.045*	[0.77, 1.00]
Oss. shoulder diam.	0.98 *0.042*	[0.95, 1.00]	0.97 *0.005*	[0.94, 0.99]	0.98 *0.013*	[0.96, 1.00]
Bip. diamd osseus shoulder width	1.42 *0.835*	[0.05, 38.6]	2884 *<0.001*	[33.5, -]	4.22 *0.303*	[0.27, 65.3]
Bip. diam. tissued shoulder width	1.95 *0.802*	[0.01, 354]	59,991 *0.001*	[77.4, -]	5.16 *0.011*	[3.89, -]
Bip. diameter	0.97 *0.056*	[0.94, 1.00]	0.993 *0.665*	[0.96, 1.02]	0.98 *0.115*	[0.96, 1.00]

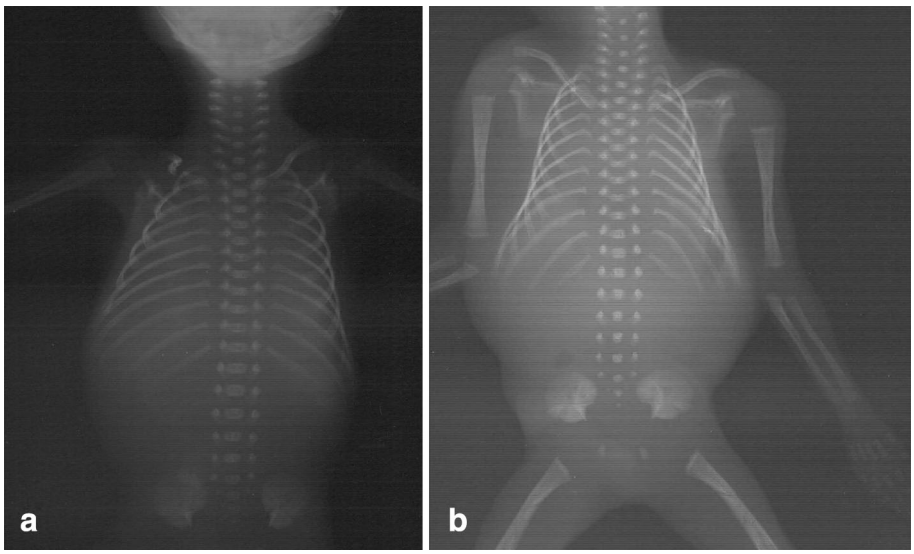

Fig. 3 Number of ribs: **a** Fetus at 20 weeks of gestation with reduced number of ribs (11 pairs), **b** euploid fetus at 17 weeks of gestation with normal number of ribs (12 pairs)

was the search for statistically reliable trisomy markers that could be implemented in ultrasound diagnostic thereby improving the sensitivity and specificity of ultrasound screening for trisomy.

In our studies we could prove deviations for several fetal osseous structures making them applicable as soft makers for the three most common and viable trisomies. Relying on our statistical analysis we would recommend the length of os nasale, os maxillare, os maxillare to jaw corner, ramus mandibulare, orbital measures and the length of os metacarpale IV and V as reliable new osseous soft markers for trisomy 21, 18 and 13.

While the absence of nasal bone in trisomy 21 is a well-known and well-published soft marker [4], there is a scarce number of ultrasound studies on nasal bone length resp hypoplasia in this trisomy [4, 15]. But—according to our literature review (pub med 11/2015)—there are none for trisomy 18 or 13. Likewise, latter aspect holds true for studies on the iliac crest angel and the frontomaxillary facial angle especially in trisomy 13 and 18. The few studies on iliac crest angle [8] and frontomaxillary facial angle [3, 8] concentrate on trisomy 21. Although both osseous markers are estimated as osseous markers for trisomy 21 the lacking reproducibility of the published results is stressed in the discussions [8]. This might be an explanation for the scarce number of further studies on these osseous markers and the even meager number of studies on, e.g., maxillary length [15].

The bell-shaped thorax, last time studied in 1988 [7], is one of the soft markers which could be used easily for detection of trisomy 18 and 21 as well as the preterm ossification of os calcaneus for trisomy 21 [14]. To our knowledge there are no published studies describing these soft markers as diagnostic helpful markers. These markers as well as a reduced number of ribs or coronal clefts are more easily detectable than time dependent markers like development of the os sacrum or the question of timely ossification of the cranium, ossification of os sternale or elevated clavicula slope which needs much experience on the side of the examiners. As far as published literature (PubMed 11/2015) these soft markers, too, are not yet described in studies.

The only marker positive for all three trisomies was "coronal cleft", defined as radiolucent band running through at least one vertebral body is a physiological variation of the fetal vertebral ossification pattern. Although this marker is found almost exclusively in fetuses with chromosomal aberrations and here especially trisomies [6] it is fairly unknown and hence not yet used as diagnostic marker. One reason could be that it can only be detected in the lateral spinal projection [6, 14].

Without question it is difficult to detect sonographically a timely inappropriate sternal ossification, an elevated clavicula slope or a deviating intraocular distance especially as standard post mortal radiographic fetal pathologic studies of the structures studied here are very rare. Furthermore, the calculation of the ratio between the biparietal diameter and the shoulder width (osseous and tissue) could be difficult because of the difficulty of an accurate measurement of these two distances in a moving fetus.

There are limitations of this study. First, is that our control group ($n = 165$) was comparatively small in relation to other sonography-based studies, more often performed in large prenatal centers [3, 5]. These studies included large control groups (over 750 cases), but only a small number of trisomies. In contrast to this our study was a retrospective study carried out in a prenatal reference

Table 3 Mean measurements of statistically significant soft markers associated with trisomy 21, 18, 13

OS nasale	13–14 wp	15–16 wp	17–18 wp	19–20 wp
Control group	1.91 mm (±0.6 mm)	2.56 mm (±0.69 mm)	3.29 mm (±0.54 mm)	4.17 mm (±0.83 mm)
Trisomy 21	1.78 mm (±0.47 mm)	2.11 mm (±0.76 mm)	2.75 mm (±0.68 mm)	3.28 mm (±0.86 mm)
Trisomy 18	1.76 mm (±0.38 mm)	2.5 mm (±0.66 mm)	2.67 mm (±0.94 mm)	3.47 mm (±2.01 mm)
Trisomy 13	2.3 mm (±0.39 mm)	2.9 mm (±1.70 mm)	4.27 mm (±0.82 mm)	4.33 mm (±0.68 mm)
OS maxillare				
Control group	7.13 mm (±1.87 mm)	9.49 mm (±1.45 mm)	12.37 mm (±1.62 mm)	14.63 mm (±1.26 mm)
Trisomy 21	6.73 mm (±1.07 mm)	8.45 mm (±1.71 mm)	12.05 mm (±1.36 mm)	14.11 mm (±1.32 mm)
Trisomy 18	6.62 mm (±0.68 mm)	9.47 mm (±0.73 mm)	12.09 mm (±1.2 mm)	13.53 mm (±1.72 mm)
Trisomy 13	8.27 mm (±1.9 mm)	7.3 mm (±0.85 mm)	11.6 mm (±1.56 mm)	14.3 mm (±2.35 mm)
OS maxillare to jaw angle				
Control group	10.4 mm (±2.28 mm)	13.64 mm (±2.09 mm)	17.14 mm (±1.96 mm)	18.4 mm (±0.96 mm)
Trisomy 21	9.89 mm (±1.24 mm)	13.06 mm (±1.67 mm)	14.79 mm (±1.99 mm)	18.6 mm (±2.53 mm)
Trisomy 18	8.9 mm (±1.62 mm)	12.72 mm (±1.59 mm)	15.32 mm (±2.6 mm)	19.87 mm (±1.63 mm)
Trisomy 13	10.73 mm (±2.97 mm)	9.9 mm (±0.64 mm)	14.71 mm (±2.04 mm)	19.87 mm (±1.69 mm)
Ramus mandibulare				
Control group	6.99 mm (±1.81 mm)	9.27 mm (±1.39 mm)	11.87 mm (±2.05 mm)	13.13 mm (±1.52 mm)
Trisomy 21	7.26 mm (±1.0 mm)	9.66 mm (±2.64 mm)	11.46 mm (±1.63 mm)	13.37 mm (±1.91 mm)
Trisomy 18	6.73 mm (±0.64 mm)	9.85 mm (±1.88 mm)	10.84 mm (±1.01 mm)	12.33 mm (±1.32 mm)
Trisomy 13	7.4 mm (±0.82 mm)	8.35 mm (±0.07 mm)	10.18 mm (±1.17 mm)	11.9 mm (±1.04 mm)
Orbita high				
Control group	7.75 mm (±1.31 mm)	9.79 mm (±1.05 mm)	12.11 mm (±1.02 mm)	13.55 mm (±1.97 mm)
Trisomy 21	8.55 mm (±0.90 mm)	10.22 mm (±1.25 mm)	12.45 mm (±0.83 mm)	13.64 mm (±0.92 mm)
Trisomy 18	8.18 mm (±1.49 mm)	9.83 mm (±1.29 mm)	12.31 mm (±1.23 mm)	13.7 mm (±1.64 mm)
Trisomy 13	9.2 mm (±0.75 mm)	9.6 mm (single value)	11.11 mm (±3.83 mm)	14.13 mm (±0.83 mm)
Binoccular diameter				
Control group	15.83 mm (±1.1 mm)	23.46 mm (±2.45 mm)	28.07 mm (±3.45 mm)	31.73 mm (±3.24 mm)
Trisomy 21	17.9 mm (±2.40 mm)	23.01 mm (±3.35 mm)	27.78 mm (±2.12 mm)	31.04 mm (±4.54 mm)
Trisomy 18	19.03 mm	21.2 mm	29.4 mm	34.4 mm

Table 3 continued

OS nasale	13–14 wp (±6.5 mm)	15–16 wp (±2.82 mm)	17–18 wp (±2.8 mm)	19–20 wp (±3.68 mm)
Trisomy 13	17.04 mm (±4.5 mm)	26.2 mm (single value)	28.03 mm (±1.99 mm)	31.3 mm (single value)
Intraoccular distance				
Control group	3.02 mm (±0.67 mm)	4.61 mm (±0.94 mm)	5.51 mm (±0.8 mm)	6.01 mm (±1.00 mm)
Trisomy 21	3.03 mm (±0.93 mm)	4.37 mm (±0.98 mm)	5.74 mm (±0.97 mm)	6.4 mm (±1.24 mm)
Trisomy 18	4.37 mm (±1.35 mm)	4.33 mm (±0.21 mm)	5.92 mm (±0.81 mm)	6.05 mm (±0.07 mm)
Trisomy 13	3.74 mm (±1.37 mm)	7.8 mm (single value)	5.76 mm (±1.71 mm)	5.4 mm (single value)
Os metacarpale IV				
Control group	1.53 mm (±0.68 mm)	2.24 mm (±0.38 mm)	3.33 mm (±0.56 mm)	4.27 mm (±0.58 mm)
Trisomy 21	1.38 mm (±0.26 mm)	2.35 mm (±0.41 mm)	2.95 mm (±0.41 mm)	4.18 mm (±0.27 mm)
Trisomy 18	1.23 mm (±0.38 mm)	1.88 mm (±0.52 mm)	3.05 mm (±0.42 mm)	4.18 mm (±0.33 mm)
Trisomy 13	1.19 mm (±0.42 mm)	1.93 mm (±0.06 mm)	3.13 mm (±0.4 mm)	3.73 mm (±0.43 mm)
Os metacarpale V				
Control group	1.16 mm (±0.56 mm)	1.91 mm (±0.35 mm)	2.88 mm (±0.54 mm)	3.74 mm (±0.52 mm)
Trisomy 21	1.14 mm (±0.25 mm)	2.42 mm (±1.27 mm)	2.74 mm (±0.37 mm)	3.87 mm (±0.43 mm)
Trisomy 18	1.05 mm (±0.42 mm)	1.71 mm (±0.51 mm)	2.73 mm (±0.4 mm)	3.68 mm (±0.26 mm)
Trisomy 13	0.95 mm (±0.39 mm)	1.57 mm (±0.15 mm)	2.74 mm (±0.39 mm)	3.5 mm (±0.29 mm)
Osseous shoulder diameter				
Control group	24.36 mm (±7.46 mm)	28.84 mm (±3.92 mm)	37.66 mm (±3.87 mm)	44.35 mm (±4.41 mm)
Trisomy 21	21.03 mm (±4.20 mm)	30.22 mm (±4.73 mm)	34.83 mm (±3.79 mm)	43.4 mm (±2.34 mm)
Trisomy 18	21.7 mm (±5.39 mm)	27.59 mm (±5.96 mm)	37.05 mm (±4.5 mm)	45.23 mm (±4.5 mm)
Trisomy 13	19.15 mm (±2.52 mm)	–	36.71 mm (±4.31 mm)	40.58 mm (±1.74 mm)

wp Week of pregnancy

center with a high percentage of induced abortions due to chromosomal aberrations.

Secondly the composition of the control group consisting of fetuses that died due to maternal medical indications, chorioamnionitis, placental insufficiency and abortion because of cardiac malformations has to be discussed. It could be argued that the osseous length of the studied structures is reduced in fetuses with intrauterine death due to placental insufficiency. On the other hand, this diagnosis ensures a regular and proportional skeletal development, especially in the first and second trimester, and hence all theses fetuses included in the control group had regular skeletal proportions.

Another limitation of this study is the missing comparability of the method used here (fetal radiology) and the method used for trimester scan (ultrasound) especially when a skeletal mode is used. It has to be studied whether the diagnostic signs found by radiography will be

applicable to these ultrasound techniques. Hence, although this study indicates a number of potential new osseous soft markers allowing a more precise diagnosis of trisomy 21, 18 and 13 the transfer of our results into actual every day diagnostic and hence their verification is limited, e.g., due to technical limitations of the ultrasound device and the ultrasonic testing. Although we put together a fairly high number of trisomies the found diagnostic signs should be studied with larger groups and then specifically for each week of gestation.

On the other hand, the method used by us provides a standardized positioning of the fetuses on the radiographic plate and hence ensures a high reproducibility of our results.

Conclusion

We detected several new osseous markers for trisomy 13, 18 and 21 while excluding other promising osseous candidates like altered number of hand/foot rays. On the basis of these findings prospective ultrasound examinations with new, even more sensitive generations of ultrasonic devices showing more delicate (osseous) structures should prove the practicability of the here described soft markers. Thereby these new markers could become additional reliable diagnostic signs, complementing the up to now used sonographic and serological trisomy markers. Hence they could help to predict more precisely a suspected trisomy, evade invasive tissue sampling for chromosomal analysis, reduce by this morbidity and mortality and offer a reliable diagnostic alternative to the cost intensive NIPT.

Compliance with ethical standards

Conflict of interest We declare that we have no conflict of interest.

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