The Mid-trimester Genetic Ultrasound: Past, Present and Future

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Down syndrome is the most frequent disease of mental retardation thus many tests have been developed for its prenatal screening. Among these tests, the mid-trimester genetic ultrasound examination which assesses enormous soft markers has proceeded for more than 15 years. Although there is still a debate on the efficiency of the mid-trimester genetic ultrasound, many sonographers still pay much attention to these soft makers. One of the reasons is that as maternal age increase, there are more and more pregnant women over 35 years old and they are suggested to receive an invasive mid-trimester amniocentesis. However, some of them decline to have an invasive procedure and look for a noninvasive screening. With advances in the wide acceptance of first-trimester and second trimester Down syndrome screening, pregnant women with intermediate risk hesitate in deciding whether they should take the risk of abortion and receive further amniocentesis. Many expect other non-invasive methods in evaluating their risk of Down syndrome. In this article, we review the research of all the mid-trimester soft markers including nuchal thickness, the choroid plexus, hypoplasia of the nasal bone, echogenic cardiac focus, echogenic bowel, pyelectesis and shorter femurs or humerus. We also review the history of genetic ultrasound and its efficiency. As more and more non-invasive tests for Down syndrome are established, we compare the use of the genetic ultrasound and other maternal serum marker tests, alone and in combination. The future and perspectives of mid-trimester genetic ultrasound examination are discussed. Sonography examination is still one of the most important non-invasive methods for screening Down syndrome. With more understanding of mid-trimester genetic ultrasound, we can improve the detection rate of Down syndrome and decrease unnecessary invasive procedures such as chorionic villi samplings and amniocentesises. It may also give more assurance and decrease the anxiety of high risk women older than 35 years old or those told they have an intermediate risk after receiving first trimester and second trimester Down syndrome screenings.

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Introduction

Down syndrome is the most frequent cause of mental retardation. In addition to mental retardation, most of Down syndrome affected patients also suffer from congenital heart disease, hearing loss, leukemia and Alzheimer's disease [1]. On average, raising a Down syndrome affected newborn costs around US$612,150 in the United States [2]. Having a child newly diagnosed as having Down syndrome impacts siblings and the family psychologically as well as economically [1]. Down syndrome was known to be caused by an extra chromosome 21, trisomy 21, which occurs with increasing maternal age. Thus, advanced age pregnant women who were 35 years or older have routinely undergone invasive procedures for cytogenetic diagnosis since 1970 [3]. However, 70% of Down syndrome can not be detected using maternal age alone as a screening strategy [4]. Also, there are more and more pregnant women above 35 years old and the invasive procedure related abortions would increase enormously if all pregnant women who are older than 35 years old receive invasive testing. Nowadays, more and more advanced age women search for second non-invasive tests rather than receive routine amniocentesis or chorionic villi samplings. Among the non-invasive tests, genetic ultrasound examinations attract attention of numerous researchers.

Since 1992, multiple serum markers including serum alpha fetoprotein (AFP), free β-human chorionic gonadotropin (free β-hCG), unconjugated estriol (uE3), inhibib A and pregnancy associated plasma protein A (PAPP-A) were developed for Down syndrome screening [4]. The detection rate of Down syndrome has improved to 80–90% in the first trimester Down syndrome screening and the second trimester quadruple test [5]. Although these tests much decrease the false positive rate and thus reduce invasive procedure related abortions compared to the strategy using maternal age alone as a criteria, the results of these tests confuse pregnant women with an intermediate risk. Their risk is between 1/270 and 1/1,000.

Usually, they are advised to accept an intermediate risk and hesitate in deciding whether they should receive further amniocentesis and take the risk of an amniocentesis induced abortion. Some of these patients search for a different and independent method to reevaluate their intermediate risk. Genetic ultrasound reveals its importance in these circumstances. In this article, we review the researches of all mid-trimester soft markers including nuchal thickness, choroid plexus, hypoplasia of nasal bone, echogenic cardiac focus, echogenic bowel, pyelectesis and shorter femurs or humerus. We also review the history of genetic ultrasound and its efficiency.

Multiple Soft Aneuploidy Markers

Aneuploidy markers are usually classified into major markers and minor markers (soft markers). The major markers mean the presence of a major fetal anomaly in the brain, heart, lung, gastrointestinal tract, genital organs or the four limbs. Newborns with these major anomalies need further surgery even when their karyotype is normal. These major markers include holoprosencephalies, agenesis of corpuscallosum (ACC), facial clefts, radial clubhand, arthrogyposis, congenital diaphragmatic hernia, congenital heart disease, omphaolocele and duodenal atresia. Theses major markers and their association with aneuploidy are listed in Table 1.

The soft markers are usually non-specific and maybe transient. They did not threaten the health of neonates. However, these findings imply increased risk of chromosome anomalies. The role of soft markers in screening Down syndrome is also controversial. We list all previous reports of these soft markers and define their criteria separately in Table 2.

Nuchal Fold Thickness

In 1985, Benacerraf et al reported that nuchal fold measurement is a sensitive and specific sonographic

Table 1. Major anomalies and their association with aneuploidy

Anormaly	Incidence	Percentage associated with aneuploidy	Aneuploidy
Holoprosencephalies	–	–	Trisomy 13
Agenesis of corpus callosum	0.3–0.7%	–	Trisomy 8, 13, 18
Facial clefts	0.14%	–	Trisomy 10, 13, 18, 22, 9
Radial clubhand	–	–	Trisomy 18 and 21
Arthrogryposis	3/10,000	59% (26/52)	Trisomy 18
Congenital diaphragmatic hernia	1/2,200	10.5%	Trisomy 18
Congenital heart disease	75/1,000	15–50%	Trisomy 13, 18, 21 and triploidy
Omphaolocele	0.74–3.9/10,000	30–40%	Trisomy 13, 18 and 21
Duodenal atresia	1:5,000–10,000	24–35%	Triosmy 21

Table 2. Soft markers and their association with aneuploidy

Soft markers	Prevalence in normal population	Percentage of Down syndrome	Reference
Nuchal thickness (>6 mm)	0 [6]	12% (6/19)	Odibo, 2008 [11]
	0.5% (136/24,325)	34.1% (85/249)	Vintzileos, 1995 [87]*
Nasal hypoplasia (<0.75 MoM)	0.9% (25/2,868)	37% (54/146)	Sonek, 2006 [20]
	2.9% (74/2,515)	85.7% (18/21)	Gianferrari, 2007 [23]
Short femur length (observed/expected <0.91)	5% (616/11,873)	31% (151/483)	Vintzileos, 1995 [87]*
Short femur length (observed/expected <0.9)	4.5% (378/8,385)	33% (54/165)	Vintzileos, 1995 [87]*
Echogenic cardiac focus	240/–	0% (0/27)	Ouzounian, 2007 [54]
	1.5% (359/23,346)	14/–	Goncalves, 2006 [55]
	4.7% (62/1,312)	18% (4/22)	Bromley, 1995 [50]
	4.6% (147/3,192)	30% (16/53)	Winter, 2000 [49]
	2.3% (260/11,135)	20% (5/25)	Anderson, 2003 [52]
	3.9% (311/10,769)	7.1% (1/14)	Lamont, 2004 [53]
	(–/39,230)†	22% (–/130)†	Sotiriadis, 2003 [56]*
Hyperechoic bowel	0.6% (50/8,680)	12.5% (6/48)	Bromley, 1994 [88]
Hypoplasia of the middle phalanx of the fifth digit	18% (–/1,024)	75% (6/8)	Benacerraf, 1990 [65]
	3.1% (13/420)	15.4% (2/13)	Vintzileos, 1996 [66]
Sandal gap	1% (4/42)	25% (1/4)	Vintzileos, 1996 [66]
Pelviectasis	4.7% (20/420)	20% (4/20)	Vintzileos, 1996 [66]
	2.7% (203/7,393)	25% (11/44)	Benacerraf, 1990 [46]

*Meta-analysis study that review all literature in the MedLine; †Isolated finding of echogenic intracardiac focus.

finding for detecting Down syndrome in the second trimester [6–9]. Measurement of nuchal fold thickness was taken by a modified transverse view of the fetal head [9]. Nuchal fold thickness was measured from the outer edge of the occipital bone to the outer edge of the skin fold [9]. The width of the nuchal fold in a normal fetus was consistently between 1 and 5 mm regardless of gestational age (15 to 20 weeks) [6], while 12–75% [9–11] of Down syndrome cases show a nuchal fold thickness more than 6 mm. Although the detection rate (sensitivity) of this marker fails to reach 50% (4% in isolated increased nuchal thickness and 26% in cases with structural anormaly) in the meta-analysis of previous studies, this soft marker is still thought to be valuable due to its high specificity (99%) [12]. It is interesting that this soft marker could be transient and may resolve at the second trimester [13]. Thus, the resolving nuchal fold in second trimester fetuses is not necessarily reassuring [14].

Nasal Hypoplasia

Sonek and Nicolaides first described absence or hypoplasia of the nasal bone in three mid-trimester fetuses with prenataly diagnosed Down syndrome [15]. They suggested a slightly parasagittal view to measure the length of fetal nasal bone, at the optimal angles of 45 degrees or 135 degrees. If this angle is less than 45 degrees or greater than 135 degrees, the nasal bone may artificially appear to be absent [15]. As the angle of insonation approaches 90 degrees, the edges of the nasal bone may become difficult to delineate precisely because of echo scatter, and the nasal bone measurement may therefore become artificially large [15]. Figure 1 shows the face profile of a fetus with prenataly diagnosed Down syndrome in our hospital. A slightly parasagittal view reveals absence of the nasal bone and this characteristic changes the appearance of the fetal face profile.

After this report, a lot of research was conducted to evaluate efficiency of the nasal bone

Fig. 1. *The face profile of a fetus with prenataly diagnosed Down syndrome. Slightly parasagittal view reveals an absence of the nasal bone (asterisk) and this characteristic changes the appearance of fetal face profile.*

hypoplasia for screening Down syndrome [11, 16–31]. The measurement of the nasal bone was performed in the first trimester and the second trimester. Absence of the fetal nasal bone in the first trimester was proved to improve the detection rate of Down syndrome if nuchal transluceny thickness and serum biochemistry (PAPP-A and free b-hCG) were combined to assess risk [26]. However, this marker is still thought to be secondary and only should be evaluated by sufficient adequately trained sonographer [26]. In the second trimester, four different methods to define nasal hypoplasia have been used in previous studies. (1) A measurement below the 2.5th, 5th, or 10th percentile of the normal range for gestation [32–34]; (2) A measurement below a fixed cutoff of 2.5 mm or 3 mm [16]; and (3) A ratio above a specific criteria in the ratio of the biparietal diameter to nasal bone length ratio [35, 36]. (4) Evaluating nasal bone length by multiples of the median (MoM) [23,25]. Recently, Odibo et al reported that defining nasal bone hypopasia in the second trimester as less than 0.75 MoM has better specificity [25]. After this report, evaluating nasal bone length by MoM and taking account of ethnicity and gestational ages are most widely accepted in many centers [28,31].

Hyperechoic Bowel

Hyperechoic bowel was defined as an increase of the echogenicity of the fetal bowel to that of equal to bone [37]. This finding is associated with several conditions including aneuploidy, infection, growth restriction, cystic fibrosis and Rhesus sensitization [38–45]. This may be due to bowel hypotonia with excessive desiccation of the meconiusm such as in fetuses with Down syndrome, viscid meconium such as intrauterine growth restriction, cystic fibrosis, or fetal swallowing of blood in the amniotic fluid and external compression of the bowel by a tumor or ascites [39]. Because the echogenicity of the fetal bowel can be subjective and may vary according to the ultrasound machine and the frequency of the transducer used, and the majority of fetuses with hyperechoic bowel have a normal outcome [37], it is controversial to use this marker as a tool to screen Down syndrome. In the Strocker et al's study, only 8% (5/62) of fetuses with isolated hyperechoic bowel had aneuploidy.

Renal Pyelectasis

Renal pyelectasis was defined by Beacerraf et al as an anteroposterior diameter of the fetal renal pelvis of ≥ 4 mm at 16–20 weeks, ≥ 5 mm at 20–30 weeks or ≥ 7 mm at 30–40 weeks gestation (Fig. 2) [46]. In their study, 25% of affected fetuses had renal pyelectasis compared with 2% to 3% of unaffected fetuses [46]. In previous publications, many authors found that the likelihood ratios of renal pyelectasis for Down syndrome is around 1.5 to 1.9 [12,47]. However, just like other soft markers, isolated renal pyelectasis was found frequently among normal fetuses.

Echogenic Cardiac Focus

In 1994, Brown et al described left ventricular echogenic focus in the fetal heart and one of the three fetuses had trisomy 21 [48]. These intracardiac
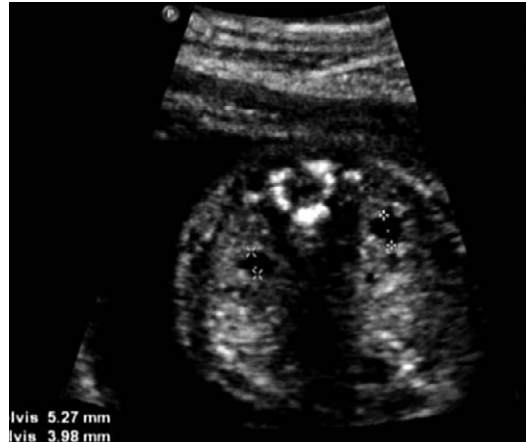

Fig. 2. *Renal pyelectasis was defined by Beacerraf et al as an anteroposterior diameter of the fetal renal pelvis of ≥ 4 mm at 16–20 weeks and ≥ 5 mm at 20–30 weeks.*

echogenic foci or "golf balls" represent mineralization within the fetal papillary muscle [48]. Under sonographic examination an echogenic intracradiac focus is a bright area as bright as bone generally in the left ventricle but occasionally bilateral or right-sided, located just below the mitral or tricuspid valves [13]. After this report, many studies have been reported including those that were positive [49,50] and those that has no association [51,52] with trisomy 21. Although many authors recognized this finding as a normal variant [53] and found no difference when these neonates are follow up 3 months later [51,52,54], many authors still find that the incidence of this marker among trisomy 21 cases is higher than in normal populations [49,50,55]. These studies are listed in the Table 2. Among these studies, Sotiriadis et al performed a meta-analysis including 11 studies published from 1995 to 2001 [56]. There were totally 39,230 normal cases and 130 cases of Down syndrome in the isolated settings in which echogenic intracardiac focus was an isolated finding [56]. The weighted sensitivity and specificity for isolated finding was 0.22 (95% confidence interval, 0.1–0.33) and 0.959 (95% confidence interval, 0.91–0.982) [56]. Based on the random effects estimates, the positive likelihood ratio was 5.4 in the "isolated" setting and the negative likelihood ratio value was 0.81 [56]. Thus the author concluded that echogenic

intracardiac echogenic foci have modest diagnostic performance [56].

However, Caughey et al reported that using echogenic intracardiac focus as a screening tool in low risk populations would lead to a large number of amniocenteses and miscarriages to identify a small number of Down syndrome fetuses based on their model [57]. This marker seems to be noted more frequently in the normal Asian populations [58,59]. Thus, amniocenteses due to isolated ultrasound finding should not be encouraged especially in the Asian population. However, some authors found the echogenic intracardiac focus is associated with cardiac anomalies and suggest women carrying fetuses with the echogenic intracardiac focus should be offered detailed fetal echocardiography [55].

Hypoplasia of the Middle Phalanx of the Fifth Digit (Clinodactyly) and Sandal Gap

Hypoplasia of the middle phalanx of the fifth digit and simian crease were thought to be one of the characteristics in the Down syndrome hand and was noted in 60% of Down syndrome affected infants (Fig. 3A) [60]. Unlike simian crease which is difficult to identify, prenatal ultrasound screening of hypoplasia of the middle phalanx of the fifth digit was performed by many authors in their genetic sonogram [61–64]. In the second trimester, a Down syndrome affected fetus showed hypoplasia or absence of the middle phalanx of the fifth digit (Fig. 3B) and the fifth digit also curved abnormally inward [62]. Benacerraf et al measured the ratio of the middle phalanx of the fifth digit over the middle phalanx of the fourth digit [65]. If the cut-off of ratio was defined as 0.7, 75% (6/8) of Down syndrome cases could be detected while 18% (2/13) of Down syndrome affected fetuses showed this characteristic [66]. However, it is important to remember that the middle phalanx of the fifth digit was only visualized in 14.3% (2/14) of cases at 13 weeks, 70.3% (154/219) at 14 weeks, 82.2% (240/292) at 15 weeks, 97.4% (111/114) at 16 weeks and 100% (43/43) at 17 weeks of gestation according to Zalel et al's study [64]. This emphasizes the limited role of non-ossification of the middle phalanx of the fifth digit as a sonographic marker of Down syndrome before 17 weeks of gestation [64]. Absence of the middle phalanx of the fifth digit at 14 weeks may disappear at 20 weeks (Fig. 4A and 4B).

The sandal gap deformity was first described by Wilkins et al in 1994 [67,68]. This deformity is
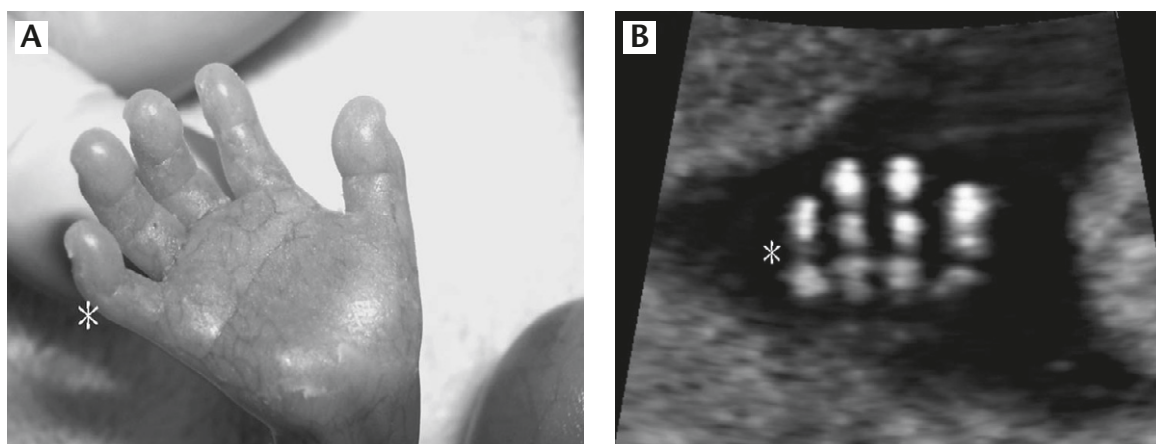

Fig. 3. *(A) Hypoplasia of the middle phalanx of the fifth digit (asterisk) and a simian crease were thought to be one of the characteristics in the Down syndrome's hand and was noted in 60% of Down syndrome affected infants. (B) In the second trimester, a Down syndrome affected fetus showed hypoplasia or an absence of the middle phalanx of the fifth digit.*
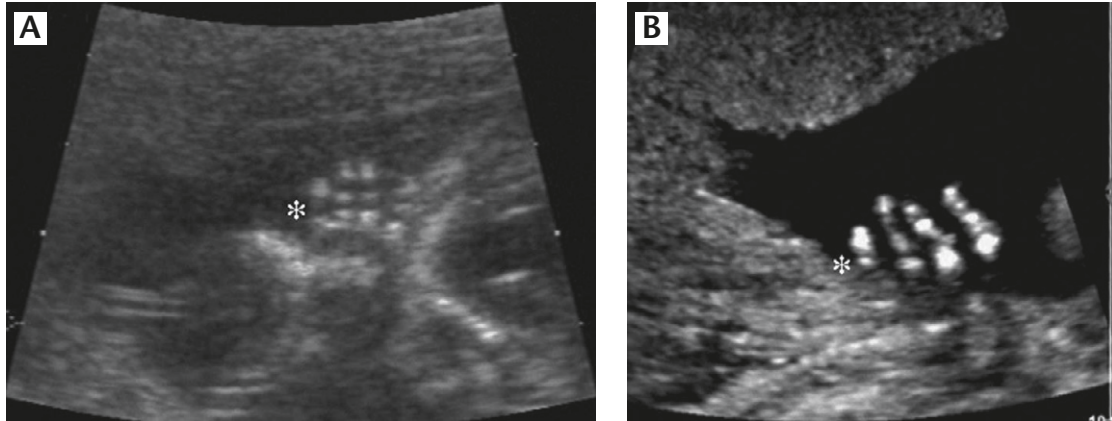
Fig. 4. *(A) Absence of the middle phalanx of the fifth digit at 14 weeks (B) may disappear at 20 weeks.*
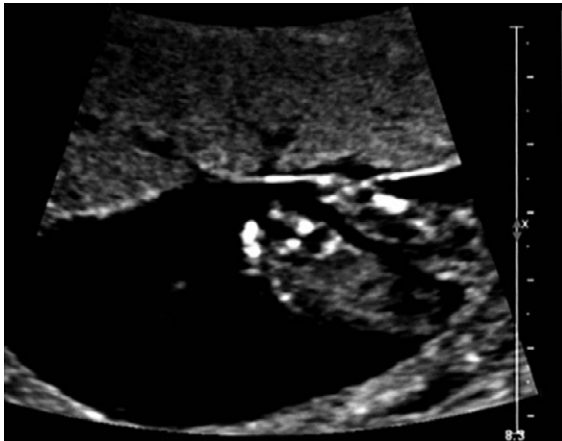

Fig. 5. *The sandal gap deformity (asterisk) was noted in a Down syndrome affected fetus. There is a separation of the great toe from the second toe.*

a noticeably large separation of the great toe and the second toe (Fig. 5). Little research of the sandal gap was found in the previous study. This may be due to difficult definition of this deformity and high false positive rate among the normal fetuses.

Short Femur and Short Humerus

Since 1987, because children affected with Down syndrome were noted to have a shorter stature, the link with a shorter femur and shorter humerus was studied and proved to be associated with Down syndrome [69,70]. Locwood et al chose an upper limit of 1.5 standard deviations above the mean in the biparietal diameter (BPD)/femur length ratio [68,69]. While Benaceraf et al calculated the observed-to-expected femur length ratio and found an increased risk of Down syndrome if the ratio was less than 0.91 [70]. Based on 192 normal fetuses, the expected femur length can be estimated as $(0.9028 \times BPD) - 9.3105$ [70]. In previous studies, the detection rate of BPD/femur ratio for Down syndrome ranged from 18% to 70% with positive screen rate of 2% to 23% [68] and the detection rate of observed to expected femur length ratio for Down syndrome ranged from 12% to 68% with positive screen rate of 2% to 15% [68]. Conversely, the expected humerus length was calculated as $(0.8492 \times BPD) - 7.9404$ based on 400 normal fetuses [71]. The detection rate of Down syndrome by using the criteria of observed to expected humerus ratio less than 0.9 alone was 50% with a positive screen rate of 6.25% [71]. If a shorter humerus and femur were both used in combination, Biagiotti et al reported that the detection rate was 44.4% in 27 cases of Down syndrome and the positive screen rate could be decreased to 7.6% [72].

Like the marker of nasal hypoplasia, a shorter femur and humerus were noted more frequently in the Asian population [73]. And there were also reports that the difference of gender influenced the length of femur and humerus of Down syndrome affected fetuses [74].
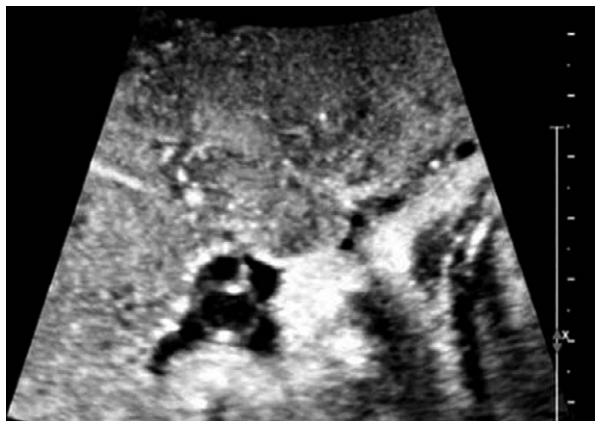
Fig. 6. *Usually, the normal umbilical cord has one vein and two arteries, while a fetus with a single umbilical artery has only one artery and one vein.*

Other Markers

Choroid plexus cysts in the mid-trimester were thought to be associated with aneuploidy, especially with trisomy 18 [75]. However, invasive procedures such as amniocentesis should not be offered for cases with isolated choroids plexus cysts because of its low cost-effectiveness [75]. Some authors suggest estimating the patient's risk of trisomy 18 by multiplying the prior risk (the risk assigned by serum screening or the age-related risk if no serum screening done) by the likelihood ratio of 9 [75,76].

Usually, the normal umbilical cord has one vein and two arteries, while a fetus with single umbilical artery has only one artery and one vein (Fig. 6). There is a well-known association between single umbilical artery and cytogenetic abnormly, especially trisomy 18 [77]. Other abnormal karyotypes include Turner syndrome, trisomy 13 and triploidy [77]. Although no obvious association with Down syndrome was reported, some author finds it is associated with congenital anormalies including the heart, the gastrointestinal system and the central nervous system. The incidence for cytogenetic abnormalities among fetuses demonstrating single umbilical artery has been reported at 17%, with nearly one half of these fetuses having major anomalies [77].

Combination of Multiple Soft Markers in the Mid-trimester Genetic Sonogram

As more and more soft makers are proved to be associated with trisomy 21, many authors are trying to find an effective strategy to combine all of the useful soft makers. The goal of this research was to increase the detection rate of Down syndrome affected fetuses and decrease the false positive rate as much as possible. Since 1992, the outcomes of this research was highly variable and the sensitivity ranging from 50% to 93% (Table 3) [78–86].

This is because the studied subject, number of soft markers, definition of soft markers, combined serum markers, ethnicity, trained sonographers, samples sizes and method of analysis were all different. Also, it is difficult to obtain a meta-analysis which combines all the data from these different groups. Before 1998, all studied subjects were high risk pregnancies including those with an advanced maternal age, high risk of serum double test or triple test, or previous affected offspring. For these cases a, sonographic score index system which was developed by Benacerraf et al was the most popular [78]. However, most of these studies have a high false positive rate which is over 10%. In 1998, Nyberg et al developed a different way to combine these soft markers [84]. They used age-adjusted ultrasound risk assessment (AAURA) which assesses the individual risk for fetal Down's syndrome based on maternal age and second-trimester ultrasound findings. All soft markers were given an individual likelihood ratio (LR) such as structural abnormality (LR 25), nuchal thickening (LR 18.6), echogenic bowel (LR 5.5), shortened humerus (LR 2.5), shortened femur (LR 2.2), echogenic intracardiac focus (LR 2), and renal pyelectasis (LR 1.6) [84]. The advantage of this method is that multiple markers including maternal age, serum markers and sonographic soft markers can be taken into account and give an individual risk for different patients. This risk can be weighed against the risk of invasive procedures such as amniocentesis and the patient can make a decision more easily. Unfortunately,

Table 3. Combination of multiple soft markers in the mid-trimester

Author/Year	Materials	Sensitivity	False positive rate	Methods
Benacerraf, 1992 [78]	Advanced age and low alpa fetoprotein	81% (26/32) when sum of score ≥2	13% (77/588)	Femur and humerus lengths (score=1), nuchal fold (score=2), renal pelvic dimension (score=1), and major structural defects (score=2)
Nadel, 1995 [79]	Advanced age	86% (83/97) when sum of score ≥1	13% (88/694)	Femur and humerus lengths (score=1), nuchal fold (score=2), renal pelvic dimension (score=1), and major structural defects (score=2)
DeVore, 1995 [89]	Risk>1:270	87% (13/15)	–	Cardiovascular abnormalities identified by color Doppler and real-time 2D ultrasound
Nyberg, 1995 [80]	Risk>1:195 (based on triple test)	50% (9/18) (One or more markers)	7.2% (27/374)	Structural defects, nuchal thickening or cystic hygroma, echogenic bowel, cerebral ventricular dilatation, pyelectasis, and shortened femur
Vintzileos, 1996 [66]	Risk>1:274	85.7% (12/14) (Two or more markers)	3.2% (15/392)	Structural anomalies (including face, hands, and cardiac), short femur, short humerus, pyelectasis, nuchal fold thickening, echogenic bowel, choroid plexus cysts, hypoplastic middle phalanx of the fifth digit, wide space between the first and second toes, and two-vessel umbilical cord
Bahado-Singh, 1996 [81]	Risk>1:270 (based on triple test)	90%	14% (132/962)	Abnormal nuchal thickness or observed/expected humerus length <0.92
Bromley, 1997 [82]	Advanced age and high risk of triple test	83% (44/53) (score ≥1) 75.5% (40/53) (score ≥2)	17.5% (31/177) 5.7% (10/177)	Femur and humerus lengths (score=1), nuchal fold (score=2), renal pelvic dimension (score=1), and major structural defects (score=2), echogenic intracardiac focus (score=1), maternal age ≥35 (score=1), maternal age ≥40 (score=2)
Verdin, 1998 [83]	Risk>1:250 (based on double test)	81% (9/11)	9.8% (44/449)	Structural defects, shortened femur length, echogenic bowel, dilation of the renal pelvis and choroid plexus cysts
Nyberg, 1998 [84]	Normal population	74% (105/142) (risk>1:200) 68.3% (97/142) (one or more markers)	14.7% 12.5%	Age-adjusted ultrasound risk assessment (AAURA) based on maternal age risk; Structural abnormality (LR 25), nuchal thickening (LR 18.6), echogenic bowel (LR 5.5), shortened humerus (LR 2.5), shortened femur (LR 2.2), echogenic intracardiac focus (LR 2), and renal pyelectasis (LR 1.6)
Sohl, 1999 [85]	Advanced age and high risk of triple test	68%	17%	Echogenic cardiac foci, echogenic bowel, cerebral ventricular dilatation, choroids plexus cyst, choroidal separation, renal pyelectasis, 2-vessel umbilical cord and shortened femur

(Contd.)

Table 3. Continued

Author/Year	Materials	Sensitivity	False positive rate	Methods
Vintzileos, 1999 [86]	Risk > 1:274	82% (28/34)	9% (159/1,792)	Cardiac anomalies, shorter humerus, shorter tibia, nuchal fold, short ear length, hypoplastic middle phalanx of the fifth digit, sandal gap, Echogenic cardiac foci, echogenic bowel, cerebral ventricular dilatation, choroids plexus cyst, choroidal separation, renal pyelectasis, 2-vessel umbilical cord and shortened femur
Benn, 2002 [90]	High risk pregnancy	90% (72)	6.7% (7,063)	Quadruple test, nuchal fold thickness, proximal long bone length
Parra-cordero, 2007 [91]	Normal population	77.8% (14/18)	3.1% (3,071)	Absent nasal bone, nuchal edema, short femur, echogenic foci and cardiac defect
Vergani, 2008 [92]	High risk pregnancies	83.3 (5/6)	28.5% (159/558)	Nuchal fold thickness, presence of renal pelvic dilation, absent mid-phalanx of the 5th finger, presence of noncardiac malformations or isolated heart defects
Bottalico, 2009 [93]	High risk (n = 581) and low risk (n = 79) pregnancies	66.6% (8/12)	19.3%	Increased nuchal skinfold, short humerus, short femur, echogenic bowel, pyelectasis, echogenic intracardiac focus, absence or hypoplasia of fifth mid phalanx or choroid plexus cyst

the detection rate of this method did not appear superior to the sonographic scoring index system (Table 3).

Evidence has showed that first trimester Down syndrome screening which includes measurement of nuchal thickness, pregnancy-associated plasma protein-A(PAPP-A) and free beta-human chorionic gonadotropin (free β-hCG), can reach a high detection rate of 85% with a low false positive rate of 5% [5]. Because of a lack of an adequate facility and trained sonographers, some areas use a second trimester quadruple test which includes measurements of alpha-fetoprotein, total human chorionic gonadotropin, unconjugated estriol, and inhibin A, and can reach a similar detection rate and false positive rate compared to first trimester Down syndrome screening [5]. We suggest that the second trimester genetic sonogram cannot replace Down syndrome screening due to a lack of large trials and a stable detection rate of previous studies. However, there are more and more researchers using genetic sonogram as the second line of Down syndrome screening, especially when women with high risk pregnancies decline invasive procedures and search for non-invasive methods. In some studies, although a genetic sonogram cannot reach high detection rates, it can decrease the risk of Down syndrome if all soft makers can not been seen.

Future Perspectives

At present, it is hard to improve upon the detection rates of mid-trimester genetic sonogram for Down syndrome among the low risk pregnancies and this possibly leads to more invasive procedures inducing abortion due to high false positive rates. More evidence is still needed to prove that a second trimester genetic sonogram can decrease unnecessary invasive procedures among high risk pregnancies and give greater assurance to women with high risk pregnancies. Before the genetic sonogram can be accepted worldwide, unifying the definition of soft makers, auditing adequate sonographers and giving adequate genetic counseling is very important.

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