SCREENING FOR TRISOMY 21

SCREENING FOR TRISOMY 21

Submitted by

Francisca Sonia Molina García

For the degree of European Doctor in Medicine

University of Granada
January 2009

Supervisors

Professor Kypros Herodotou Nicolaides
King's College Hospital School of Medicine, England

Professor Luis Navarrete López-Cozar
University of Granada, Spain

Professor Jesús Florido Navío
University of Granada, Spain

Editor: Editorial de la Universidad de Granada
Autor: Francisca Sonia Molina García
D.L.: GR 4500-2011
ISBN: 978-84-694-9343-4

Professor Kypros Herodotou Nicolaides
King's College Hospital School of Medicine,
London, England

I confirm that Dr Francisca Sonia Molina García
Has carried out under my supervision the studies presented in the Thesis: Screening for trisomy 21

I have read the Thesis and I am happy for this to be presented to the Tribunal for The Degree of European Doctor in Medicine

Professor Kypros Herodotou Nicolaides
London January 2009

Professor Luis Navarrete López-Cozar
University of Granada,
Granada, Spain

I confirm that Dr Francisca Sonia Molina García
Has carried out under my supervision the studies presented in the Thesis: Screening for trisomy 21

I have read the Thesis and I am happy for this to be presented to the Tribunal for The Degree of European Doctor in Medicine

Professor Luis Navarrete López-Cozar
Granada January 2009

Professor Jesús Florido Navío
University of Granada,
Granada, Spain

I confirm that Dr Francisca Sonia Molina García
Has carried out under my supervision the studies presented in the Thesis: Screening for trisomy 21

I have read the Thesis and I am happy for this to be presented to the Tribunal for The Degree of European Doctor in Medicine

Professor Jesús Florido Navío
Granada January 2009

Acknowledgments

Agradezco al Profesor Nicolaides, padre de la medicina fetal, que me brindara la oportunidad de soñar despierta trabajando bajo su supervisión en el "King´s College Hospital". Su carisma, capacidad de trabajo, profesionalidad y pasión por la medicina fetal son un estímulo para todos los que nos dedicamos a esta parcela de la medicina. Escribir artículos con él es toda una lección de investigación y seriedad científica, pero sobre todo trabajar para él cada día es una lección sobre la vida, sobre las gentes y una reflexión sobre el impacto que tenemos en las embarazadas en momentos a veces difíciles para ellas. Gracias por dirigir esta tesis doctoral y por ser un consejero constante en mis decisiones.

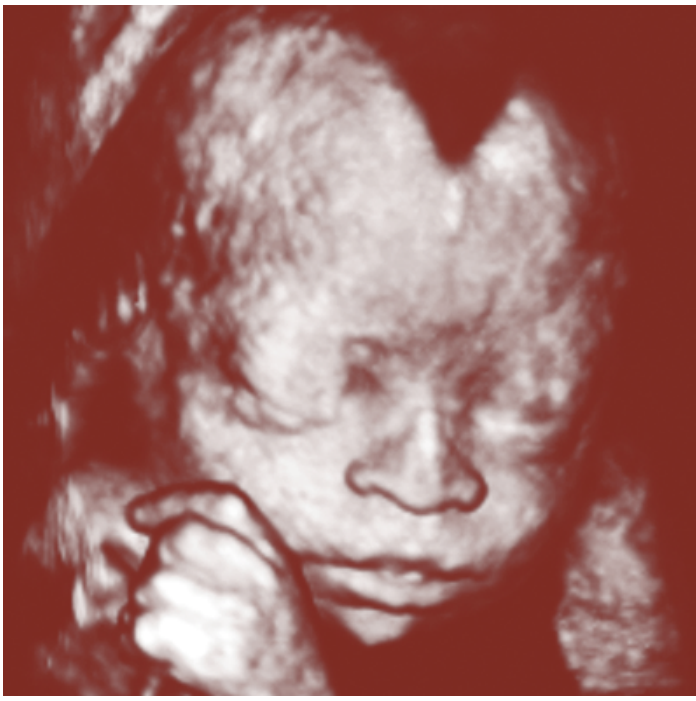
El Profesor Navarrete fue quien me guió desde el principio en el camino a seguir para formarme en Medicina Fetal, apasionado incansable de su trabajo es un ejemplo para todos los que hemos tenido la suerte de compartir trabajo y amistad con él. Me complace enormemente que sea director de esta tesis doctoral compartiendo como hace años inquietudes con Nicolaides.

Me alegra sinceramente que el Profesor Florido aceptara ser el tercer director de este trabajo y le agradezco los consejos y ayuda que siempre he encontrado en él. Ha sido uno de los estímulos más importantes que he encontrado en mi vuelta a Granada y el mejor ejemplo para continuar en el camino de la investigación.

Muchas gracias a todos los autores que han participado en el desarrollo de los artículos que componen esta tesis doctoral, y a los compañeros con los que he tenido oportunidad de trabajar en los distintos hospitales de Londres donde se desarrollaban estos estudios, de todos he aprendido.

Mi amor y agradecimiento especial a mi familia que tanto me ha apoyado siempre en todas las decisiones profesionales que he ido tomando con muchas dudas y miedos, pero también con mucha ilusión, de todos es la satisfacción de presentar este trabajo.

A los compañeros de trabajo que se convirtieron en mejores amigos, Katy, Nerea, M. Dolores, Carmen, Daniela, Juan J. gracias por compartir conmigo los ratos buenos y malos de la profesión y la vida.



Contents

CHAPTER III
AIMS OF THIS THESIS

CHAPTER IV
PUBLISHED STUDIES

STUDY 1 Cystic hygromas, nuchal edema and nuchal translucency at 11–14 weeks of gestation

STUDY 2 Relation between increased fetal nuchal translucency thickness and chromosomal defects

STUDY 3 A mixture model of nuchal translucency thickness in screening for chromosomal defects

STUDY 4 First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact ofmaternal and pregnancy characteristics

STUDY 5 Prenasal thickness in trisomy-21 fetuses at 16–24 weeks of gestation

STUDY 6 Frontomaxillary facial angle in trisomy 21 fetuses at 16–25 weeks of gestation

CHAPTER V
DISCUSSION

CHAPTER VI
CONCLUSIONS
(Spanish and English)

CHAPTER VII
REFERENCES

CHAPTER I

SUMMARY OF THE PROJECT

Los defectos cromosómicos son una de las causas mayores de mortaliad y morbilidad perinatal. La cromosomopatía más prevalente es el síndrome de Down. Su diagnóstico es la indicación más frecuente para realizar un procedimiento invasivo durante el embarazo (amniocentesis o biopsia corial). Estos procedimientos invasivos causan aborto en un 1% de los casos por lo tanto deben de realizarse en embarazos considerados de alto riesgo tras la realización de test de cribado.

El test de cribado establecido en la práctica clínica, con mayor tasa de detección y menores falsos positivos se llama *test combinado del primer trimestre* y engloba parámetros ecográficos como la medida de la translucencia nucal (TN) y parámetros bioquímicos analizados en el suero materno, como la medida de la fracción ß libre de la gonadotrofina coriónica humana (ß-hCG) y la proteína plasmática placentaria A (PAPP-A).

Los objetivos de esta tesis son examinar si la apariencia de la TN aporta información adicional a la medida de la misma en la predicción de cromosomopatías. Examinar la asociación entre el aumento de la TN y las distintas cromosomopatías. Establecer un nuevo modelo de distribución de la TN para describir mejor a los fetos cromosómicamente normales y anormales. Definir la contribución de las variables maternas que influyen en los parámetros bioquímicos, estableciendo factores de corrección de los mismos para el cribado combinado del primer trimestre. Utilizar ecografía de tres dimensiones (3D) para mejorar el estudio de ciertos rasgos (edema pre-nasal y ángulo facial) que aumentarán la tasa de detección de trisomía 21 en el segundo trimestre del embarazo.

Los estudios publicados en revistas científicas de alto impacto, que forman parte de esta tesis, resumen el trabajo que se ha realizado por la doctoranda durante su estancia de formación en el centro de investigación “Harris Birthright Research Center for Fetal Medicine” en el King´s College Hospital en Londres durante un periodo de dos años y medio desde junio de 2004 hasta diciembre de 2006.

El aporte de estas investigaciones permitirá aumentar la tasa de detección de los test de cribado de cromosomopatías durante el embarazo y la disminución de la tasa de falsos positivos, para la realización de procedimientos invasivos diagnósticos de una forma más adecuada en la población de alto riesgo.

Chromosomal abnormalities are major causes of perinatal death and childhood handicap. Down's syndrome is the most prevalent chromosomal defect, and this diagnosis is the most common indication for and invasive procedure during pregnancy (amniocentesis or chorionic villous sampling). These procedures are associated with a risk of miscarriage of about 1% and therefore these tests are carried out only in pregnancies considered to be at high-risk for chromosomal defect after the screening test.

The screening test in practical terms with a better detection and less false positive rates is the combined test in the first trimester of pregnancy and it is made of ultrasound parameters as the nuchal translucency (NT) and maternal serum biochemical parameters as the free fraction of the ß-human chorionic gonadotropin (ß-hCG) and the plasmatic protein A (PAPP-A)

The aims of this thesis are to examine whether the ultrasonographic appearance of nuchal translucency, has any additional contribution to just the measurement of NT thickness in the prediction of chromosomal abnormalities. To examine the association between high fetal NT and chromosomal abnormalities other than trisomy 21. To establish a new model on the distribution of fetal NT in chromosomally normal and abnormal fetuses. To establish a new method for correcting first-trimester maternal serum free ß-hCG and PAPP-A for maternal and pregnancy characteristics in order to improve the description of chromosomally abnormal pregnancies. To use 3D ultrasound examination to help improve the assessment of specific facial features (prenasal thickness, facial angle) that may improve the second trimester ultrasonographic detection of trisomy 21 fetuses.

The studies of this thesis have been published in scientific journals with a high impact and they are part of the work done by the candidate to the degree of doctor during her period in the research center "Harris Birthright Research Center for Fetal Medicine" at King´s College Hospital in London between June 2004 and December 2006.

The results of these investigations will allow us to increase the detection rate of the screening tests for Down's during pregnancy and reduce the false positive tests to select better the high risk population and do less invasive procedures.

CHAPTER II

INTRODUCTION

1.1 BACKGROUND TO SCREENING FOR TRISOMY 21

1.1.1 Overview

Chromosomal abnormalities are major causes of perinatal death and childhood handicap. Consequently, the diagnosis of chromosomal disorders constitutes the most frequent indication for invasive prenatal diagnosis. However, invasive testing by amniocentesis or chorionic villous sampling is associated with a risk of miscarriage of about 1% and therefore these tests are carried out only in pregnancies considered to be at high-risk for chromosomal defects.[1,2] The methods of screening to identify the high-risk group are described in Table 1.1.

Table 1.1 *Screening for Chromosome Abnormalities*

Non-Invasive Screening Methods
Maternal age (35 years or more)
Previous chromosomally abnormal fetus/baby, parental chromosomal rearrangement
Maternal serum biochemistry (15-18 weeks)
Ultrasound at 18-24 weeks

1.1.2 Features and genetics of trisomy 21

Babies with trisomy 21 have a characteristic flattening of the face, slanted palpebral fissures, generalised muscular hypotonia, a large protruding tongue, small ears, short stocky hands and feet, Brushfield's spots, clinodactyly and sandal gap. Heart abnormalities, mainly atrioventricular septal defects are found in about 40% of patients. Other congenital abnormalities include duodenal atresia and hydronephrosis. The degree of mental impairment is variable and the range of intelligence quotient is 25-50. Over half of the patients are expected to survive into their 50s; 20% die by the age of 5 years, mostly from congenital heart disease.

Down's syndrome occurs when either the whole or a segment of the long arm of chromosome 21 is present in three copies instead of two. This can occur as a result of three separate mechanisms: non-dysjunction (94% of cases), translocation (3.6%) and mosaicism (2.4%). In 95% of non-dysjunction trisomy 21 the origin is maternal. Interestingly, non-dysjunction in sperm (5% of trisomy 21) is not dependent on paternal age.

1.1.3 Principles of screening

The critical factors in a screening test are the ability to discriminate between affected and unaffected individuals and this is expressed in terms of the detection rate and the false positive rate.

Detection rate (or sensitivity)
The detection rate is the ability of a test to give a positive result in individuals who have the condition being screened for. It is the proportion of affected individuals yielding a positive result (Figure 1.1).

Screen positive and false positive rate
The screen positive rate is the proportion of affected and unaffected individuals yielding a positive result. The false positive rate is the proportion of unaffected individuals yielding a positive result (Figure 1.1). In screening for chromosomal defects the term screen positive is usually replaced with false positive because the vast majority of screen positive cases are actually normal.

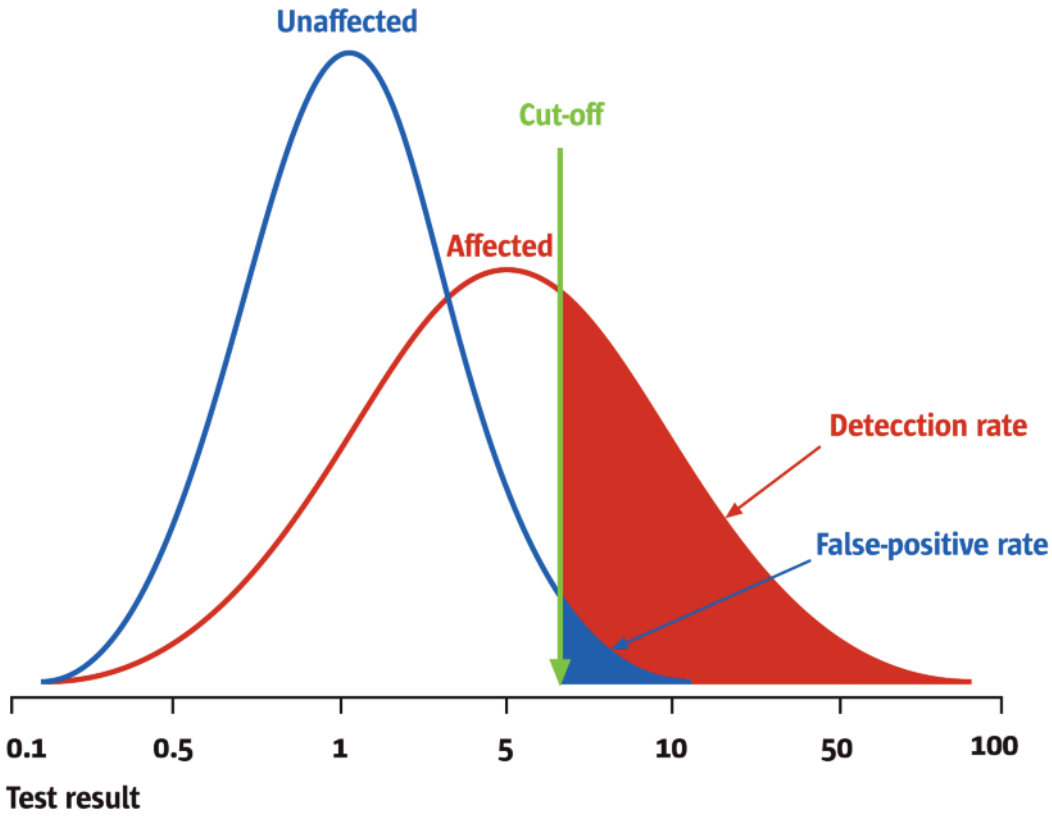


Figure 1.1 *Proportion of affected and unaffected cases above a certain cut-off represent the detection rate and false positive rate, respectively.*

Specificity

Specificity is the ability of a test to give a negative result in individuals who do not have the condition being screened for. It is the proportion of unaffected individuals yielding a negative result. This is the equivalent of (1-false positive rate).

Predictive value

The positive predictive value is the likelihood that the condition is present given a positive result. The predictive value is dependent on the prevalence of the disorder. As the prevalence decreases, the proportion of individuals with a positive test result who actually have the condition will fall and the proportion falsely identified as being at risk will rise. For tests that only yield qualitative results (e.g. fetal karyotype) the detection and false positive rates are fixed proportions.

Performance of screening

This is summarised in Table 1.2.

Table 1.2 *Performance of a screening test*

	Test positive	**Test negative**
Affected	True positive (TP)	False negative (FN)
Unaffected	False positive (FP)	True negative (TN)

Detection rate = TP/TP+FN
False positive rate = FP/FP+TN
Positive predictive value = TP/TP+FP

When a screening test has a continuous variable as the result the detection rate and false positive rate vary and are determined by the chosen cut off level (Figure 1.1). The smaller the overlap between affected and unaffected distributions the better the screening test will be. If the frequency distribution is known it is possible to derive the detection rate for a given false positive rate and vice versa by calculating the area under the curves for affected and unaffected cases.

Risk estimation

The population distributions for a feature, such as nuchal translucency thickness, have to be known for the normal and abnormal (such as trisomy 21) group. The Gaussian height for the normal and abnormal groups can be calculated for any given measurement of nuchal translucency thickness. The ratio between the two calculated heights is the likelihood ratio.
The individual trisomy 21 risk is estimated by multiplying the maternal age-

related risk (prior odds) for trisomy 21 by the likelihood ratio derived from the nuchal translucency thickness. The result is the posterior odds and is used as the risk estimate.

Prior odds x likelihood ratio (LR) = posterior odds
Maternal age related risk x LR = Trisomy 21 risk

In terms of biochemical variables (free ß-hCG and PAPP-A) the concentrations used to calculate the likelihood ratio vary with gestational age. Therefore, in order to be able to calculate the risk estimates for a given gestation, the results of the analytes are expressed as multiple of the medians (MoM). Individual values are divided by the corresponding median for gestational age and expressed as MoM.

Receiver operator characteristic (ROC) curves
The detection and false positive rates of a test compete with one another. The dangers of missing the diagnosis in those that have the condition must be weighed against the hazards of falsely labelling people with a condition that in truth they do not have. This trade-off can be described graphically by plotting the detection rate on the y-axis against the false positive rate on the x-axis. This is the so-called receiver operator characteristic (ROC) curve. An ideal test is one that has high detection rate and a low false positive rate. ROC curves permit comparison of the performance of tests.

1.2 ASSESSMENT OF RISK FOR CHROMOSOMAL DEFECTS

1.2.1 Background or *a priori* risk

While every woman is at risk of having a baby with a chromosome defect, a woman's individual chance will depend on her background or *a priori* risk (which depends on maternal age and gestational age) and the results of any ultrasound or biochemical screening tests carried out during the course of the pregnancy. Every time a test is carried out the *a priori* risk multiplied by the likelihood ratio of the test to calculate a new risk, which then becomes the *a priori* risk for the next test.

1.2.2 Maternal age and gestation

The most common chromosomal defects are trisomies 21, 18 and 13. The risk for all three chromosomal defects increases with maternal age (Tables 1.3, 1.4 and 1.5).[3,4] Additionally, because fetuses with these trisomies are more likely to die in utero than normal fetuses, the risk decreases with gestation. The rates of fetal death between 12 weeks and term is about 30% for trisomy 21 and about 80% for trisomies 18 and 13.

Turner syndrome, unlike trisomies, is unrelated to maternal age. The prevalence is about 1 per 1500 at 12 weeks, 1 per 3000 at 20 weeks and 1 per 4000 at 40 weeks. For the other sex chromosome abnormalities (47,XXX, 47,XXY and 47,XYY), there is no significant change with maternal age and since the rate of intrauterine lethality is not higher than in chromosomally normal fetuses the overall prevalence (about 1 per 500) does not decrease with gestation. Triploidy is highly lethal and thus very rarely observed in live births; the prevalence at 12 and 20 weeks is about 1 per 2000 and 1 per 250, 000, respectively.

In the early 1970's about 5% of pregnant women were 35 years or older and this group contained about 30% of the total number of fetuses with trisomy 21. Therefore, screening on the basis of maternal age was associated with a 5% screen positive rate and a 30% detection rate.

In recent years the trend to delayed childbearing has resulted in a significant increase in the number of pregnant women ≥ 35 years (20%). If all these women were to undergo invasive testing, approximately 50% of the total number of fetuses with trisomy 21 would be detected but at a greatly increased false positive rate.

Table 1.3 *Estimated risk for trisomy 21 in relation to maternal age and gestation*

MATERNAL AGE (YS)	GESTATIONAL AGE					
	10 WEEKS	12 WEEKS	14 WEEKS	16 WEEKS	20 WEEKS	40 WEEKS
20	1/983	1/1068	1/1140	1/1200	1/1295	1/1527
25	1/870	1/946	1/1009	1/1062	1/1147	1/1352
30	1/576	1/626	1/668	1/703	1/759	1/895
31	1/500	1/543	1/580	1/610	1/658	1/776
32	1/424	1/461	1/492	1/518	1/559	1/659
33	1/352	1/383	1/409	1/430	1/464	1/547
34	1/287	1/312	1/333	1/350	1/378	1/446
35	1/229	1/249	1/266	1/280	1/302	1/356
36	1/180	1/196	1/209	1/220	1/238	1/280
37	1/140	1/152	1/163	1/171	1/185	1/218
38	1/108	1/117	1/125	1/131	1/142	1/167
39	1/82	1/89	1/95	1/100	1/108	1/128
40	1/62	1/68	1/72	1/76	1/82	1/97
41	1/47	1/51	1/54	1/57	1/62	1/73
42	1/35	1/38	1/41	1/43	1/46	1/55
43	1/26	1/29	1/30	1/32	1/35	1/41

Table 1.4 *Estimated risk for trisomy 18 in relation to maternal age and gestation*

MATERNAL AGE (YS)	GESTATIONAL AGE					
	10 WEEKS	12 WEEKS	14 WEEKS	16 WEEKS	20 WEEKS	40 WEEKS
20	1/1993	1/2484	1/3015	1/3590	1/4897	1/18013
25	1/1765	1/2200	1/2670	1/3179	1/4336	1/15951
30	1/1168	1/1456	1/1766	1/2103	1/2869	1/10554
31	1/1014	1/1263	1/1533	1/1825	1/2490	1/9160
32	1/860	1/1072	1/1301	1/1549	1/2490	1/7775
33	1/715	1/891	1/1081	1/1287	1/1755	1/6458
34	1/582	1/725	1/880	1/1047	1/1429	1/5256
35	1/465	1/580	1/703	1/837	1/1142	1/4202
36	1/366	1/456	1/553	1/659	1/899	1/3307
37	1/284	1/354	1/430	1/512	1/698	1/2569
38	1/218	1/272	1/330	1/393	1/537	1/1974
39	1/167	1/208	1/252	1/300	1/409	1/1505
40	1/126	1/157	1/191	1/227	1/310	1/1139
41	1/95	1/118	1/144	1/171	1/233	1/858
42	1/71	1/89	1/108	1/128	1/175	1/644
43	1/53	1/66	1/81	1/96	1/131	1/481

Table 1.5 *Estimated risk for trisomy 13 in relation to maternal age and gestation*

MATERNAL AGE (YS)	GESTATIONAL AGE					
	10 WEEKS	12 WEEKS	14 WEEKS	16 WEEKS	20 WEEKS	40 WEEKS
20	1/6347	1/7826	1/9389	1/11042	1/14656	1/42423
25	1/5621	1/6930	1/8314	1/9778	1/12978	1/37567
30	1/3719	1/4585	1/5501	1/6470	1/8587	1/24856
31	1/3228	1/3980	1/4774	1/5615	1/7453	1/21573
32	1/2740	1/3378	1/4052	1/4766	1/6326	1/18311
33	1/2275	1/2806	1/3366	1/3959	1/5254	1/15209
34	1/1852	1/2284	1/2740	1/3222	1/4277	1/12380
35	1/1481	1/1826	1/2190	1/2576	1/3419	1/9876
36	1/1165	1/1437	1/1724	1/2027	1/2691	1/7788
37	1/905	1/1116	1/1339	1/1575	1/2090	1/6050
38	1/696	1/858	1/1029	1/1210	1/1606	1/4650
39	1/530	1/654	1/784	1/922	1/1224	1/3544
40	1/401	1/495	1/594	1/698	1/927	1/2683
41	1/302	1/373	1/447	1/526	1/698	1/2020
42	1/227	1/280	1/335	1/395	1/524	1/1516
43	1/170	1/209	1/251	1/295	1/392	1/1134

1.3 THE 11-13 WEEKS SCAN

1.3.1 Fetal nuchal translucency and trisomy 21

... The hair is not black, as in the real Mongol, but of a brownish colour, straight and scanty. The face is flat and broad, and destitute of prominence. The cheeks are roundish, and extended laterally. The eyes are obliquely placed, and the internal canthi more than normally distant from one another. The palpebral fissure is very narrow. The forehead is wrinkled transversely from the constant assistance which the levatores palpebrarum derive from the occipito-frontalis muscle in opening of the eyes. The lips are large and thick with transverse fissures. The tongue is long, thick, and is much roughened. The nose is small. The skin has a slight dirty yellowish tinge, and is deficient in elasticity, giving the appearance of being too large for the body ...

This is an extract from the paper 'Observations on an ethnic classification of idiots' by J. Langdon Down in 1866.[5]

The observation of Langdon Down that the skin is too large for the body was the basis for the ultrasound observation by Nicolaides in 1992 of increased nuchal translucency (NT) in the third month of intrauterine life (Figures 1.2 and 1.3).[6-8]

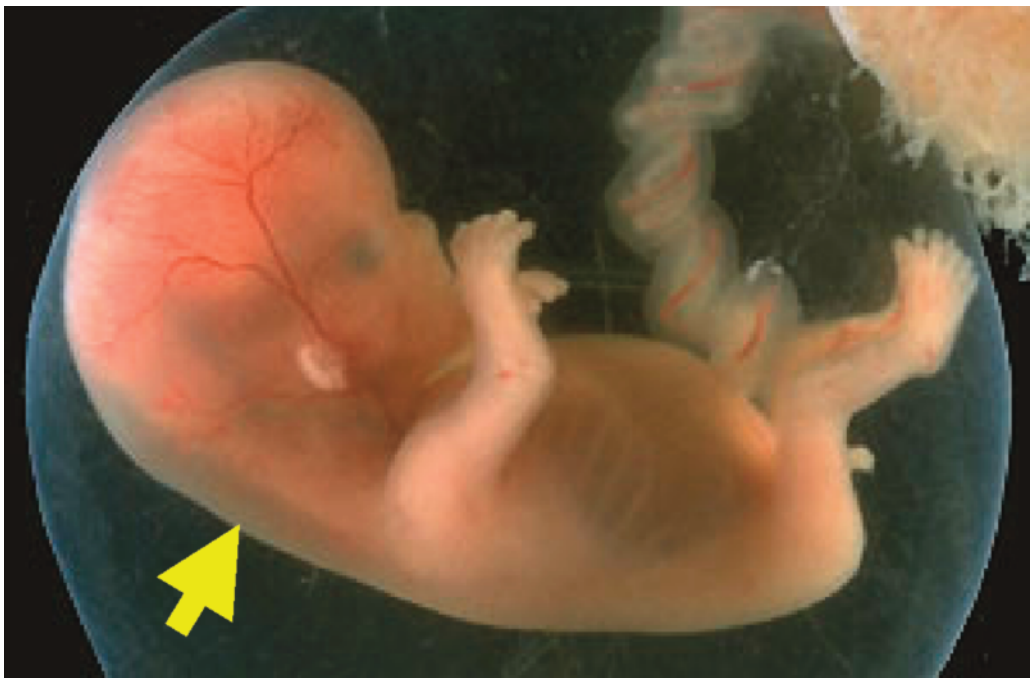


Figure 1.2 *Picture of 11-weeks fetus demonstrating the subcutaneous accumulation of fluid behind the neck.*

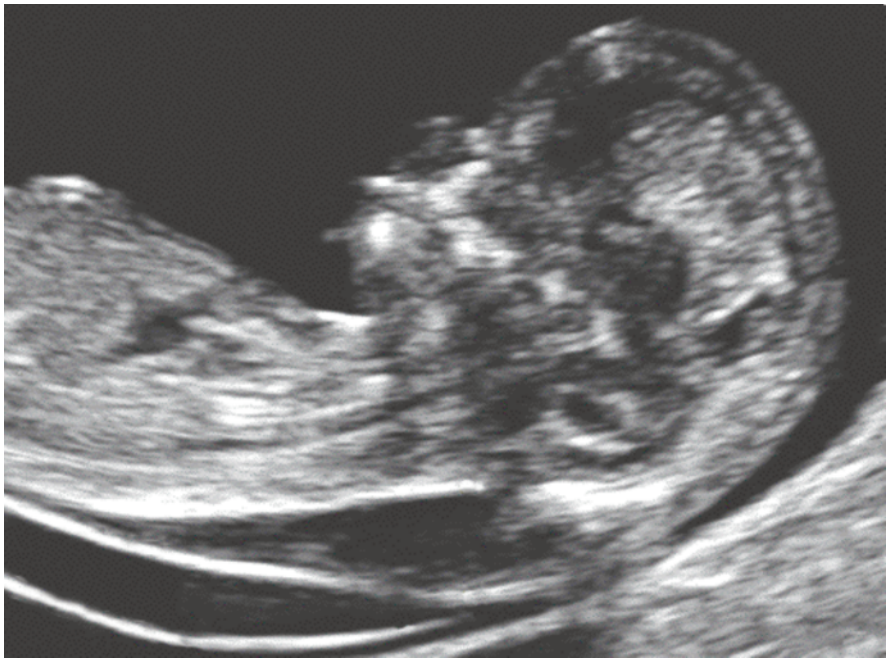


Figure 1.3 *Ultrasound picture of 12-weeks fetus with trisomy 21 demonstrating increased subcutaneous accumulation of fluid behind the neck.*

Studies in the last 10 years involving more than 200,000 pregnancies and including about 900 fetuses with trisomy 21 have demonstrated that screening at 11-13 weeks by a combination of maternal age and fetal NT can detect more than 75% of fetuses with trisomy 21 at an FPR of 5%.[8] The DR is increased to about 90% with the addition of maternal serum free beta-hCG and PAPP-A and to more than 95% with the addition of other ultrasound markers (ductus venosus, tricuspid regurgitation and facial angle) at 11–13 weeks.

Increased NT is also a marker of chromosomal abnormalities other than trisomy 21.[6-8] In addition, in euploid fetuses with high NT, the risk of an adverse outcome, including abnormalities and intrauterine or postnatal death, increases with NT thickness from about 5% for NT between the 95th centile and 3.4 mm, to 30% for NT of 3.5 to 4.4 mm, to 50% for NT of 4.5 to 5.4 mm, and 80% for NT of 5.5 mm or more.[9] In the vast majority of cases with high fetal NT, a series of antenatal investigations, including fetal karyotyping, detailed scans, fetal echocardiography, as well as genetic testing and infection screening, can be completed by 20 weeks of gestation and will distinguish between the pregnancies destined to result in adverse outcome and those leading to the delivery of infants without major defects.[9]

1.3.2 Nuchal translucency measurement technique

It is essential that the same criteria are used to achieve uniformity of results among different operators for the measurement of fetal NT. The criteria,

established by the Fetal Medicine Foundation (FMF) UK which have become the internationally accepted standard are described in Table 1.6.

It should be noted that fetal NT increases with crown–rump length and therefore it is essential to take gestation into account when determining whether a given translucency thickness is increased.

Table 1.6. *Fetal Medicine Foundation criteria for measurement of fetal nuchal translucency*

- The gestation should be between 11 weeks and one day and 13 weeks and six days.
- The fetal crown-rump length should be 45 to 84mm.
- A good sagittal section of the fetus, as for measurement of fetal crown–rump length, should be obtained and the NT should be measured with the fetus in the neutral position The fetal head should be in line with the spine and it should not be hyperextended or flexed.
- Care must be taken to distinguish between fetal skin and amnion. This is achieved by waiting for spontaneous fetal movement away from the amniotic membrane; alternatively the fetus is bounced off the amnion by asking the mother to cough and/or by tapping the maternal abdomen.
- The magnification should be such that the fetus occupies the entire image. Essentially, the aim is that each movement of the callipers produces a 0.1mm change in the measurement.
- The maximum thickness of the black space between the soft tissue overlying the cervical spine and the skin should be measured. During the scan more than one measurement must be taken and the maximum one should be recorded. Do not average the measurements.
- Measurements should be taken with the horizontal lines of the callipers placed ON the lines that define the nuchal translucency thickness (not in the line and not in the nuchal fluid), as shown in the diagram.

Correct **Incorrect**

1.3.3 Deviation in nuchal translucency measurement from normal

Fetal NT increases with crown-rump length; therefore, it is essential to take gestation into account when a determination is made about whether a given NT thickness is increased.[10] In a study that involved 96,127 pregnancies, the median and 95th percentile at a crown-rump length of 45 mm were 1.2 and 2.1 mm; the respective values at a crown-rump length of 84 mm were 1.9 and 2.7 mm.[7] The 99th percentile did not change with crown-rump length and was approximately 3.5 mm.

In a screening for chromosomal abnormalities, patient-specific risks are derived by the multiplication of the *a priori* maternal age and gestation-related risk by a likelihood ratio, which depends on the difference in fetal NT measurement from the expected normal median for the same crown-rump length (Delta value).[7,10] In screening that uses maternal serum biochemical markers, a different approach has been used to take into account the gestational age-related change in marker levels. This method involves the conversion of the measured concentration into a multiple of the median (MoM) of unaffected pregnancies at the same gestation.[11] Essentially, the Gaussian distributions of log10 (MoM) in trisomy 21 and unaffected pregnancies are derived, and the heights of the distributions at a particular MoM, which is the likelihood ratio for trisomy 21, is used to modify the a priori maternal agerelated risk to derive the patient-specific risk.

A study that involved the analysis of data of NT and crown-rump length from 128,030 unaffected and 428 trisomy 21 pregnancies demonstrated that the Delta NT approach provides accurate patient-specific risks.[12] In contrast, the MoM approach was found to be inappropriate for this purpose, because none of the 3 basic assumptions that underpin this method are valid. First, in the unaffected population, the distributions of NT MoM and log10 (NT MoM) were not Gaussian; second, the standard deviations did not remain constant with gestation; and third, the median MoM in the trisomy 21 pregnancies was not a constant proportion of the median for unaffected pregnancies. The MoM approach resulted in women being given an overestimate of risk for trisomy at 11 weeks of gestation and a considerable underestimate of risk at 13 weeks of gestation.

1.4 OTHER FIRST TRIMESTER ULTRASOUND MARKERS

Studies from specialist centers have demonstrated that, in addition to NT, other highly sensitive and specific first-trimester sonographic markers of trisomy 21 are absence of the nasal bone, increased impedance to flow in the ductus venosus, tricuspid regurgitation and maxillary hypoplasia resulting in wide

frontomaxillary facial angle. However, accurate examination for these markers is time-consuming and requires highly skilled operators, and at present it is unlikely that this assessment will be incorporated into the routine first-trimester scan. However, it could be used in specialist centres to re-evaluate the risk in patients with intermediate risk after screening by fetal NT and maternal serum biochemistry.

Absence of the nasal bone is found in about 60% of trisomy 21 fetuses and in 1% of chromosomally normal fetuses.[13,14] Reversed a-wave in the fetal ductus venosus is found in about 65% of trisomy 21 fetuses and in 3% of chromosomally normal fetuses.[15,16] Tricuspid regurgitation was observed in about 55% of trisomy 21 fetuses and 1% of chromosomally normal fetuses.[17-19]

1.5 MATERNAL SERUM BIOCHEMISTRY.

1.5.1 Second trimester

In 1984 Merkatz et al reported low levels of maternal serum alpha fetoprotein (AFP) in trisomy 21 pregnancies.[20]

Subsequently, altered maternal serum levels in affected pregnancies were reported for a series of other feto-placental products, including free ß-hCG, Inhibin A and unconjugated estriol (uE3) between 15 and 20 weeks gestation.[21-23]

To determine a risk for trisomy 21, the likelihood ratios for these substances, after corrections for the interrelations between them, are multiplied by the background maternal age and gestational age-related risk.
The risk of trisomy 21 is increased if the levels of hCG and/or inhibin A are high, and the levels of a-fetoprotein and/or estriol are low. The estimated detection rates are between 50 and 70% for a screen-positive rate of about 5%.[24]

1.5.2 First trimester

In trisomy 21 pregnancies at 11-13 weeks, the maternal serum concentration of free ß-hCG is higher than in chromosomally normal fetuses, whereas PAPP-A is lower (about 2 MoM and about 0.5 MoM, respectively).[25-29] However, in trisomy 21, the deviation from normal in PAPP-A is lower and in free ß-hCG is higher with advancing gestation, and these temporal changes with gestation should be taken into account in the calculation of risk.[30]

There is no significant association between fetal NT and maternal serum free ß-hCG or PAPP-A in either trisomy 21 or chromosomally normal pregnancies, and therefore the ultrasononographic and biochemical markers can be combined to

provide more effective screening than either method individually. Several prospective studies on more than 100,000 pregnancies, including more than 500 fetuses with trisomy 21, have demonstrated that for a 5% false-positive rate the detection rate of trisomy 21 by the first-trimester combined test is 90%.[8,31]

An important development in biochemical analysis is the introduction of new techniques for automated, precise, and reproducible measurements of free ß-hCG and PAPP-A within 30 minutes of obtaining a blood sample. This has made it possible to combine biochemical and ultrasonographic testing as well as counseling in one-stop clinics for early assessment of fetal risk (OSCAR).[28,29] An alternative approach for effective delivery of a first-trimester screening program is serum biochemical testing a few days before the sonographic examination so that the biochemical results are available at the time of the scan to allow the calculation of a combined risk estimate and counseling of the patient.

1.6 SECOND TRIMESTER ULTRASOUND

In the first trimester, a common feature of many chromosomal abnormalities is increased NT thickness. In the second trimester scan, each chromosomal defect has its own syndromal pattern of detectable abnormalities. For example, trisomy 21 is associated with nasal hypoplasia, increased nuchal fold thickness, cardiac defects, intracardiac echogenic foci, duodenal atresia and echogenic bowel, mild hydronephrosis, shortening of the femur and more so of the humerus, sandal gap and clinodactyly, or mid-phalanx hypoplasia of the fifth finger.

Systematic examination of the fetus for anomalies and markers has led to the development of the so-called genetic sonogram. The findings on the genetic sonogram are used to adjust the *a priori* maternal age-related or serum biochemistry-related risk: the *a priori* risk of aneuploidy is multiplied by the likelihood ratios associated with the sonographic findings. The various likelihood ratios are derived by dividing the prevalence of a given defect or marker in chromosomally abnormal fetuses by its prevalence in chromosomally normal fetuses. The resultant likelihood ratio increases as the difference between the two prevalences increases which leads to improvement of the screening test.

The genetic sonogram can be applied in essentially two situations. Firstly, in women who are, either through advanced maternal age or second-trimester biochemical screening, considered to be at a sufficiently high risk for chromosomal defects to be offered an amniocentesis. A significant proportion of such women can be reassured by the absence of any sonographically detectable defects and they choose to not have an amniocentesis. Alternatively, the presence of markers and/or anomalies may increase their risk even further and

may help them to make a decision in the opposite direction. Secondly, the genetic sonogram can also be used in low-risk women. In such cases, the presence of defects/markers will increase the risk of chromosomal defects. If the increase is sufficiently great to place the patient into the increased risk category, a discussion regarding an invasive test should take place.

1.6.1 Major defects

If the second trimester scan demonstrates major defects, it is advisable to offer fetal karyotyping, even if these defects apparently are isolated. The prevalence of such defects is low; therefore, the cost implications are small. If the defects are either lethal or are associated with severe handicap (such as holoprosencephaly), fetal karyotyping constitutes one of a series of investigations to determine the possible cause and thus the risk of recurrence.

If the defect is potentially correctable by intrauterine or postnatal surgery (such as diaphragmatic hernia), the logical course may be to exclude an underlying chromosomal abnormality especially because, for many of these conditions, the usual abnormality is trisomy 18 or 13.

1.6.2 Minor defects

Minor fetal defects or soft markers are common and are not associated usually with any handicap, unless there is an underlying chromosomal abnormality. Routine karyotyping of all pregnancies with these markers would have major implications, both in terms of miscarriage and in economic costs. It is best to base counselling on an individual estimated risk for a chromosomal abnormality, rather than on the arbitrary advice that invasive testing is recommended because the risk is ''high.''

The estimated risk can be derived by multiplying the *a priori* maternal age-related risk by the likelihood ratio of the specific defect. The best estimates of both the positive and negative likelihood ratios for each of the common markers of trisomy 21 are given in Table I.7.[32] These estimates were derived from the combined data from two leading centers of obstetric ultrasound in the United States.[33,34]

Table 1.7 *Incidence of major and minor defects or markers in the second trimester scan in trisomy 21 and chromosomally normal fetuses in the combined data of two major series. From these data the positive and negative likelihood ratios (with 95% confidence interval) for each marker can be calculated. In the last column is the likelihood ratio for each marker found in isolation.*

	Trisomy 21	Normal	+ve LR	-ve LR	LR for isolated marker
Nuchal fold	107/319 (33.5%)	59/9331 (0.6%)	53.05 (39.37-71.26)	0.67 (0.61-0.72)	9.8
Short humerus	102/305 (33.4%)	136/9254 (1.5%)	22.76 (18.04-28.56)	0.68 (0.62-0.73)	4.1
Short femur	132/319 (41.4%)	486/9331 (5.2%)	7.94 (6.77-9.25)	0.62 (0.56-0.67)	1.6
Hydronephrosis	56/319 (17.6%)	242/9331 (2.6%)	6.77 (5.16-8.80)	0.85 (5.16-8.80)	1.0
Echogenic focus	75/266 (28.2%)	401/9119 (4.4%)	6.41 (5.15-7.90)	0.75 (0.69-0.80)	1.1
Echogenic bowel	39/293 (13.3%)	58/9227 (0.6%)	21.17 (14.34-31.06)	0.87 (0.83-0.91)	3.0
Major defect	75/350 (21.4%)	61/9384 (0.65%)	32.96 (23.90-43.28)	0.79 (0.74-0.83)	5.2

Major or minor defects were identified in 74.3% of 350 fetuses with trisomy 21 and in 13.5% of 9384 chromosomally normal fetuses. On the basis of these data, the likelihood ratio for trisomy 21, if there is no detectable defect or marker, is 0.30. In each case, the likelihood ratio is derived by dividing the incidence of a given marker in trisomy 21 pregnancies by its incidence in chromosomally normal pregnancies.

For example, an intracardiac echogenic focus is found in 28.2% of trisomy 21 fetuses and in 4.4% chromosomally normal fetuses, which results in a positive likelihood ratio of 6.41 (28.2/4.4) and a negative likelihood ratio of 0.75 (71.8/95.6). Consequently, the finding of an echogenic focus increases the background risk by a factor of 6.41, but at the same time an absence of this marker should reduce the risk by 25%.

The same logic applies to each one of the six markers in Table I.7. Thus, in a 25-year-old woman who undergoes an ultrasound scan at 20 weeks of gestation, the a *priori risk* is approximately 1 in 1000. If the scan demonstrates an intracardiac echogenic focus, but the nuchal fold is not increased, the humerus and femur are not short, and there is no hydronephrosis, hyperechogenic bowel or major defect,

the combined likelihood ratio should be 1.1 (6.41 x 0.67 x 0.68 x 0.62 x 0.85 x 0.87 x 0.79), and consequently her risk remains at approximately 1 in 1000.

There are no data on the interrelation between these second-trimester ultrasound markers and fetal NT or maternal serum free ß-hCG or PAPP-A at 11-13 weeks of gestation. However, there is no obvious physiologic reason for such an interrelation, and it is reasonable therefore to assume that they are independent. Consequently, in estimating the risk in a pregnancy with a marker, it is logical to take into account the results of previous screening tests.

For example, in a 32-year-old woman at 20 weeks of gestation (background risk, 1 in 507) who had an NT screening that resulted in a 7-fold reduction in risk (to 1 in 3549), after the diagnosis of isolated echogenic bowel at the 20-week scan, the estimated risk would increase by a factor of 3 to 1 in 1183. However, for the same ultrasound finding in the absence of previous NT screening, the risk would increase from 1 in 507 to 1 in 169.

1.6.3 New second trimester markers

Hypoplastic nasal bone

In 1866 Langdon Down, reported that a small nose is one of the common facial features of individuals with the condition that subsequently came to bear his name.[5] Recently, a series of radiologic, histomorphologic and sonographic studies have demonstrated that the nasal bone abnormality associated with trisomy 21 can be detected prenatally:

- Anthropomorphic studies have demonstrated that in postnatal life the nasal root depth is abnormally short in about 50% of affected individuals.[35]
- X-ray studies in aborted fetuses with trisomy reported that the prevalence of short or absent nasal bone in more than 60%.[36-39]
- Ultrasound studies in the first trimester have shown absence of the nasal bone is found in about 60% of trisomy 21 fetuses and in 1% of chromosomally normal fetuses.[13,14]
- Ultrasound studies in the second trimester have shown absence or hypoplasia of the nasal bone in about 60% of trisomy 21 fetuses and in only 1% of normal fetuses (Figure 1.4).[40,41]

Several studies have reported the measurement of nasal bone length in normal fetuses. Their findings are summarized in Table 1.8, which presents the estimated values for various percentiles at 12, 16, and 20 weeks to allow comparison between the studies. The 2.5th percentiles and 5th percentiles in the second trimester are fairly consistent from study to study, and it therefore appears reasonable to define nasal hypoplasia if the length is below 3 mm at 16

weeks and 4.5 mm at 20 weeks. The most likely explanation for the significant divergence in results is that the technique used in theses studies was different. The reference ranges based on a measurement that includes both the hyperechoic central part of the nasal bone and the echogenic extensions at each end[43,44] result in measurements that are greater than the ones where the hyperechoic center only is measured.[46,47]

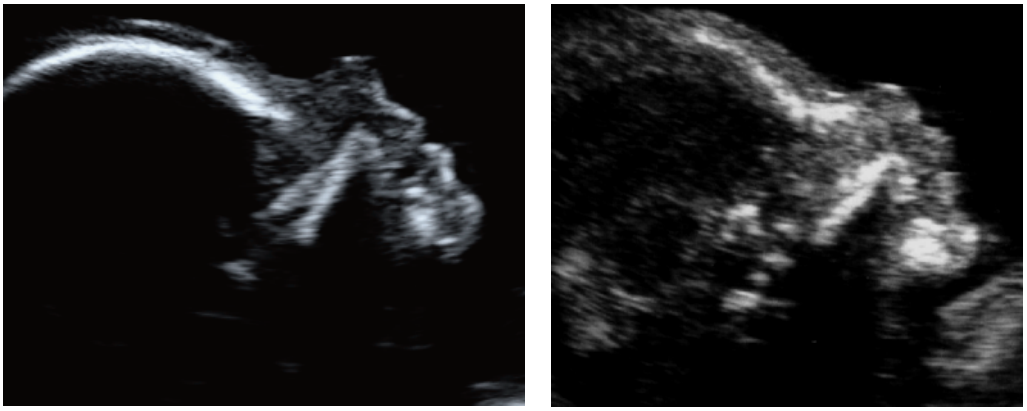


Figure 1.4 *Ultrasound picture of 20-weeks fetuses. On the right the fetus is euploid with normal nasal bone and in the left the fetus with trisomy 21 demonstrates absence of the nasal bone.*

Table 1.8 *Nasal bone length measurements and percentiles at 12, 16, and 20 weeks' gestatin based on data in euploid fetuses.*

Author	N	Nasal bone length (mm)									
		12 wks (centiles)			16 wks (centiles)			20 wks (centiles)			
		2.5th	5.0th	50th	2.5th	5.0th	50th	2.5th	5.0th	50th	
Guis et al[42]	376	—	—	—	3.1	—	5.2	5.7	—	7.6	
Sonek et al[43]	3547	1.7	1.8	2.8	3.2	3.4	4.7	5.0	5.2	6.7	
Orlandi et al[44]	1000	—	2.1*	2.6	—	—	—	—	—	—	
Bunduki et al[45]	1609	—	—	—	3.7	4.1	5.9	4.8	5.2	7.0	
Cicero et al[46]	955	—	1.2	1.5	—	—	—	—	—	—	
Cusick et al[47]	799	—	1.4*	1.9	—	3.3*	4.1	—	5.1*	6.2	
Bromley et al[48]	223	—	—	—	3.4	3.6	4.7	—	—	—	
Tran et al[49]	136	—	—	—	3.1	-	3.4	4.9	—	5.2	
Gamez et al50	1899	—	—	—	—	—	—	5.3	—	6.3	

***10th centile**

Six studies examined the fetal profile for absence of the nasal bone before second-trimester genetic amniocentesis. In the combined data from these studies the nasal bone was absent in 37% of the trisomy 21 fetuses and in 1% of the chromosomally normal fetuses (Table 1.9). In addition, six studies have compared the length of the nasal bone in trisomy 21 and normal fetuses (Table 1.10). In the combined data, the nasal bone was short in 40 of 83 (48.2%) trisomy 21 fetuses and 136 of 5643 (2.4%) normal fetuses. The studies essentially used one of three methods to define nasal bone hypoplasia: firstly, a measurement below the 2.5th, 5th, or 10th percentile of the normal range for gestation,[45,50] secondly, a measurement below a fixed cut-off of 2.5 mm[51] or 3 mm[47], and thirdly, a ratio above specific cut-offs in the ratio of the biparietal diameter to nasal bone length (NBL) ratio.[48,49]

Table 1.9 *Summary of studies reporting on the prevalence of absent nasal bone in chromosomally normal and trisomy 21 fetuses in the second trimester.*

Study	GA(wks)	Abnormal nasal bone			Likelihood ratio	
		Definition	Trisomy 21	Euploid	Positive	Negative
Bromley et al[48]	15-20	Short	5/10 (50.0%)	10/222 (4.5%)	11.1	.52
		Absent or short	11/16 (68.8%)	11/223 (4.9%)	14.0	.32
Cicero et al[51]	15-22	Short	10/23 (43.5%)	6/976 (0.6%)	72.5	.56
		Absent or short	21/34 (61.8%)	12/982 (1.2%)	51.5	.39
Bunduki et al[45]	16-24	Short	13/22 (59.1%)	82/1,600 (5.1%)	11.6	.43
Gamez et al[50]	19-22	Short	5/5 (100%)	34/1899 (1.8%)	55.6	—
Tran et al[49]	14-24	Short	4/20 (20.0%)	4/135 (3.0%)	6.7	.82
		Absent or short	15/31 (48.4%)	5/136 (3.7%)	13.1	.53
Cusick et al[47]	16-19	Short	3/3 (100%)	0/811 (0%)	—	—
		Absent or short	4/4 (100%)	3/814 (0.36%)	278	—
Total		Short	40/83 (48.2%)	136/5643 (2.4%)	20.1	.53
		Absent or short	51/85 (60.0%)	31/2155 (1.4%)	42.8	.40

Table 1.10 *Summary of data from 2-D ultrasound studies comparing the prevalence of nasal bone abnormalities in trisomy 21 and in euploid fetuses.*

Study	Gestation (wks)	Prevalence of absent nasal bone		Likelihood ratios	
		Trisomy 21	Euploid	Positive	Negative
Bromley et al[48]	15-20	6/16 (37.5%)	1/233 (0.4%)	93.8	0.63
Cicero et al[51]	15-22	11/34 (32.4%)	6/982 (0.6%)	54.0	0.68
Vintzileos et al[52]	18-20	12/29 (41.3%)	0/102 (0%)	—	—
Odibo et al[53]	15-22	5/18 (27.8%)	14/583 (2.4%)	11.6	0.74
Cusick et al[47]	16-19	1/4 (25%)	3/814 (0.4%)	69.4	0.75
Tran et al[49]	14-24	11/31 (35.5%)	1/136 (0.7%)	50.7	0.65
Benoit et al[54]	17-26	8/14 (57.1%)	0/18 (0%)	—	—
Total		54/146 (37.0%)	25/2,868 (0.9%)	41.1	0.64

(* data presented here are limited to the second trimester)

The combined prevalence of nasal bone absence and hypoplasia at 14-25 weeks' gestation is 60% in fetuses with trisomy 21 and 1.4% in euploid fetuses. Therefore, on the basis of current evidence it appears that nasal hypoplasia is more important than most of the other second-trimester sonographic features. Since examination of the fetal profile is an integral part of the genetic sonogram assessment of the nasal bone will inevitably become a routine component of such a scan. However, further research is needed into standardization of the measurement and establishment of accurate likelihood ratios.

Three-dimensional (3D) ultrasound studies have confirmed that there is a major difference in the prevalence of nasal bone absence in trisomy 21 fetuses and euploid fetuses. They also showed that this phenomenon is present in all three trimesters of pregnancy.[54-58] Benoit and Chaoui compared the 3-D and 2-D appearance of the nasal bone at 17-33 weeks.[54] The nasal bone was present in all 18 euploid fetuses on 2-D ultrasound and all were found to have both nasal bones present on 3-D ultrasound. In the 20 fetuses with trisomy 21, nine had either an absent or hypoplastic nasal bone on 2-D ultrasound. The 3-D evaluation showed bilateral nasal bone absence in six fetuses and unilateral nasal bone absence in three. Goncalves et al,[58] analyzed 3-D volumes of the nasal bone at 20-25 weeks. Nasal bone absence was detected in 9 of 26 (34.6%) of the trisomy 21 fetuses and in 1 of 27 (3.7%) of the euploid fetuses.

Prenasal skin thickness

An excessive amount of skin is a common feature in individuals with trisomy 21, as originally described by Langdon Down in 1866.[5] Extensive studies have documented this excessive amount of skin as increased nuchal translucency

thickness in the first trimester and increased nuchal fold thickness in the second trimester.[6,7,59,60]

Recently Maymon et al, reported that in second trimester trisomy-21 fetuses the prenasal skin thickness, between the fronto-nasal angle and the outer border of the skin edge, is increased.[61] The image of the fetal profile was taken in a mid-sagittal plane of the fetal head, identifying the nasal bone, lips, maxilla and mandible (Figure 1.5). Care was taken to maintain an angle between the insonation beam and the fetal nose axis close to 45 to avoid any false absence or shortening of the nasal bone. Tilting the transducer was carried out in order to ensure that the skin and the bone are separated. The prenasal thickness was measured between the fronto-nasal angle and the outer part of the skin edge.

In 500 normal fetuses the prenasal thickness increased with gestation. In 58% of 21 fetuses with trisomy 21 at 14–27 weeks of gestation the prenasal thickness was above the 95th centile of the normal fetuses.[61]

One limitation of the study was that the sonographers were aware of the fetal karyotypes at the time of the scan. This could potentially have biased the results of the prenasal thickness. Therefore, the method now needs to be evaluated in a prospective study.

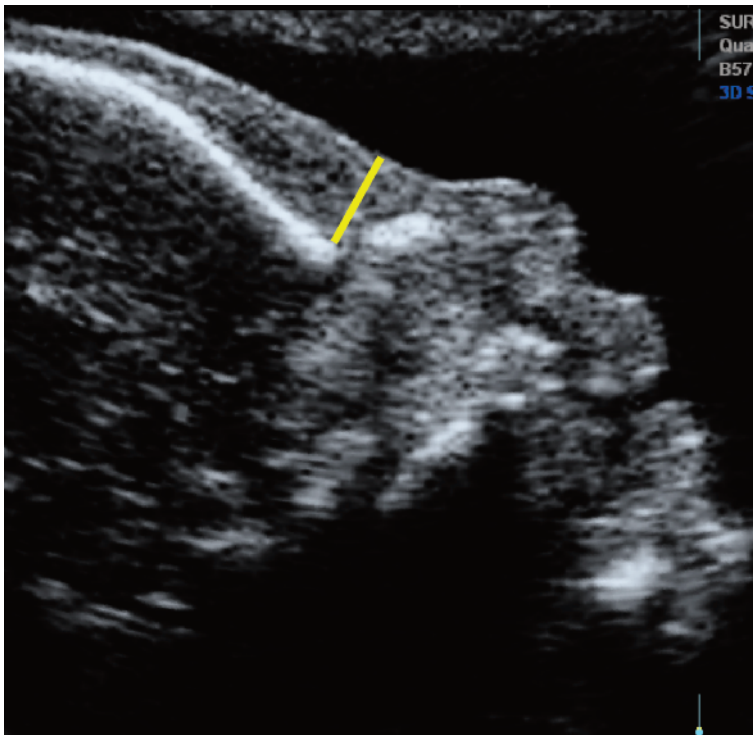


Figure 1.5 *Ultrasound picture of 20-weeks fetus with trisomy 21 demonstrating the measurement of prenasal thickness.*

Frontomaxillary facial angle

The flat face of trisomy 21[5] could be the consequence of nasal hypoplasia but also hypoplasia of the maxilla. Several radiological studies reported underdevelopment of the upper jaw, delayed dental growth, reduction in the number and size of teeth, and absence or hypoplasia of the nasal bone in individuals with trisomy 21.[62-67] Furthermore, prenatal sonographic studies have reported that a significant proportion of fetuses with trisomy 21 have shortening of the maxillary length and maxillary depth.[68-70] However, the reported differences between maxillary measurements in between trisomy 21 and euploid fetuses have not been shown to be large enough to be clinically useful.[68-70] It is possible that the clinically evident flat nature of the profile in trisomy 21 individuals is in part due to not only the size but also the location of the maxilla.

Sonek et al, recently reported a method of determining the position of the front of the maxilla with respect to the fetal forehead.[71,72] This technique involves measuring the angle between the upper edge of the upper palate and the leading edge of the bony forehead, which was termed frontomaxillary facial (FMF) angle (Figure 1.6).

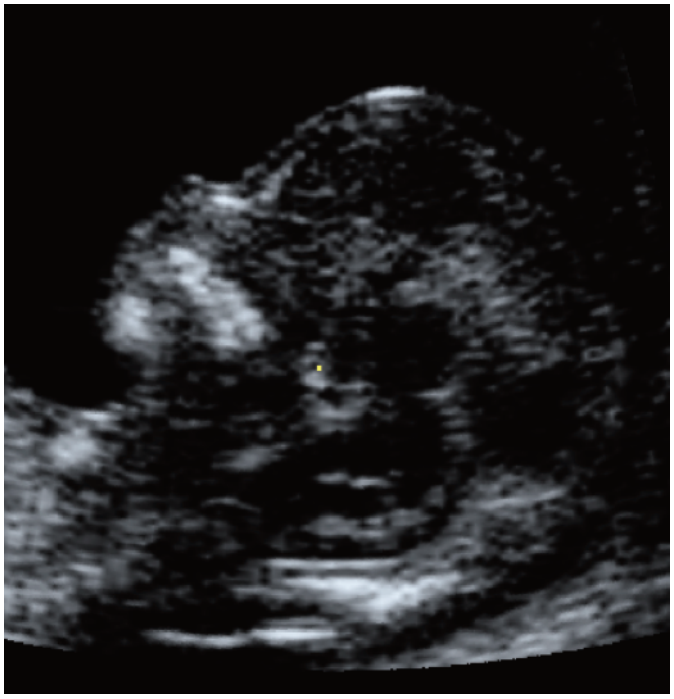


Figure 1.6 *Measurement of the frontomaxillary facial angle*

The FMF angle in stored images of fetal profiles taken before amniocentesis at 14-24 weeks was significantly larger in 34 fetuses with trisomy 21 compared to 100 euploid fetuses.[72]

The impact on first-trimester screening for trisomy 21 by including the measurement of the FMF angle was investigated prospectively.[73] 3D volumes of the fetal head and measurement of the FMF angle were obtained immediately before fetal karyotyping by chorionic villous sampling (CVS) in about 1000 pregnancies including 782 in which the fetus was subsequently found to be euploid and 108 with trisomy 21. The women chose to have CVS after risk assessment by a combination of maternal age, fetal NT thickness and maternal serum free ß-hCG and PAPP-A. It was estimated that incorporating the FMF angle in first-trimester combined screening increased the detection rate of trisomy 21 from 90% to 94% at the false positive rate of 5% and from 85% to 92% at a false positive rate of 3%.

The extent to which measurement of the FMF angle improves the performance of second-trimester screening for trisomy 21 remains to be determined.

CHAPTER III

AIMS OF THIS THESIS

The aims of this thesis are:

To examine whether the ultrasonographic appearance of nuchal translucency, such as the presence of septations within it, has any additional contribution to just the measurement of NT thickness in the prediction of chromosomal abnormalities.

To examine the association between high fetal NT and chromosomal abnormalities other than trisomy 21

To establish a new model on the distribution of fetal NT in chromosomally normal and abnormal fetuses

To establish a new method for correcting first-trimester maternal serum free ß-hCG and PAPP-A for maternal and pregnancy characteristics in order to improve the description of chromosomally abnormal pregnancies

To use 3D ultrasound examination to help improve the assessment of specific facial features (prenasal thickness, facial angle) that may improve the second trimester ultrasonographic detection of trisomy 21 fetuses.

CHAPTER IV

PUBLISHED STUDIES

STUDY 1 Cystic hygromas, nuchal edema and nuchal translucency at 11–14 weeks of gestation

STUDY 2 Relation between increased fetal nuchal translucency thickness and chromosomal defects

STUDY 3 A mixture model of nuchal translucency thickness in screening for chromosomal defects

STUDY 4 First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics

STUDY 5 Prenasal thickness in trisomy-21 fetuses at 16–24 weeks of gestation

STUDY 6 Frontomaxillary facial angle in trisomy 21 fetuses at 16–25 weeks of gestation

Cystic Hygromas, Nuchal Edema, and Nuchal Translucency at 11–14 Weeks of Gestation

Francisca S. Molina, MD, Kyriaki Avgidou, MD, Karl Oliver Kagan, MD, Sara Poggi, MD, and Kypros H. Nicolaides, MD

OBJECTIVE: To estimate the incidence of septations in fetuses with increased nuchal translucency (NT) thickness, and to investigate the relationship between the length and thickness of the translucency and whether the length or septations provide useful information concerning the fetal karyotype in addition to that provided by the NT thickness alone.

METHODS: We examined 386 fetuses with NT thickness equal to or above the 95th percentile for crown-rump length (CRL). A transverse suboccipitobregmatic section of the fetal head was taken to determine whether the sonolucency was septated, and a midsagittal longitudinal section was used to measure NT thickness, CRL, the longitudinal distance between the occiput and the lower end of the sonolucency toward the fetal sacrum (NT length) and the length between the occiput and the sacral tip (spinal length). Logistic regression analysis was used to investigate the effect on abnormal karyotype of CRL, NT thickness, and percentage of NT length to spinal length.

RESULTS: Septations within the translucency were observed in all fetuses. The fetal karyotype was abnormal in 83 (21.5%) pregnancies, and multiple regression showed that the only significant independent predictor of abnormal karyotype was fetal NT thickness.

CONCLUSION: Septations within the translucency can be seen in all fetuses, and therefore this feature cannot be used to distinguish between increased NT and cystic hygromas. The length of the translucency is related to its thickness and does not give useful information concerning the fetal karyotype in addition to that provided by the NT thickness alone.
(Obstet Gynecol 2006;107:678–83)

LEVEL OF EVIDENCE: II-2

From the Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London, United Kingdom.

This study was supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116).

Corresponding author: Professor K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, Denmark Hill, London SE5 8RX; e-mail: kypros@fetalmedicine.com.

ISSN: 0029-7844/06

During the second and third trimesters of pregnancy, abnormal accumulation of fluid behind the fetal neck can be classified as nuchal cystic hygroma or nuchal edema.[1–4] In the case of cystic hygromas, prenatal diagnosis by ultrasonography is based on the demonstration of a bilateral, septated, cystic structure, located in the occipitocervical region.[1,2] They are thought to represent overdistention of the jugular lymphatic sacs as a consequence of failure of communication with the internal jugular vein.[5] Secondary dilatation of the lymphatic channels draining the chest and limbs results in peripheral lymphedema and development of nonimmune hydrops, which is found in about 75% of affected fetuses.[2] In about 75% of fetuses with cystic hygromas, there is a chromosomal abnormality, and in about 95% of cases, the abnormality is Turner syndrome.[2] In the chromosomally normal fetuses, there is a high association with cardiac defects and genetic syndromes, such as multiple pterygium syndrome.[2] The overall prognosis in fetuses with cystic hygromas is poor, and the survival rate is less than 5%.[2] Nuchal edema is considered present if, in the midsagittal plane of the neck, there is subcutaneous edema that produces a characteristic tremor on ballotment of the fetal head.[4] This constitutes the severe end of the spectrum of increased nuchal fold thickness, which is defined as soft-tissue thickening of 6 mm or more, seen in the suboccipitobregmatic view of the fetal head.[3] Nuchal edema may be confined to the neck or it may be generalized, as part of hydrops fetalis. Chromosomal abnormalities are found in about one third of the fetuses, and in about 80% of these, the abnormality is trisomy 21, 18, or 13.[4] Edema is also associated with fetal cardiovascular and pulmonary defects, skeletal dysplasias, congenital infection, and metabolic and hematological disorders; consequently, the prognosis for chromosomally normal fetuses with nuchal edema is poor.[4]

In the late 1980s and early 1990s, with the introduction of first trimester screening, several terms were used to describe the abnormal accumulation of

fluid behind the fetal neck, including cystic hygroma and nuchal edema with or without internal septations. However, in the published reports on first-trimester cystic hygroma, the pattern of associated chromosomal defects, both trisomies and Turners syndrome, was suggestive that the term did not have the same pathophysiological significance as second-trimester cystic hygroma (Table 1).[6–15] Furthermore, there was a wide range in the incidence of chromosomal defects from 28% to 100%.

In 1992, we introduced the term nuchal translucency (NT) thickness to standardize the technique of measuring the fluid, irrespective of whether it is septated and whether it is confined to the neck or envelopes the whole fetus.[16] The rationale was that it is possible to standardize and audit the results of a measurement but not those of a subjective appearance. The NT thickness is usually measured with the fetus in the midsagittal position, in which case the appearance of the fluid is homogeneously translucent. Furthermore, increased NT is associated with trisomy 21, Turner syndrome, and other chromosomal abnormalities, as well as many fetal malformations and genetic syndromes,[17,18] and the incidence of these abnormalities is related to the thickness rather than the appearance of NT.[19–21]

Recently, the term cystic hygroma has been reintroduced, to apparently describe a distinct entity from NT. Cystic hygroma was defined as an enlarged sonolucency with clearly visible septations extending along the fetal body axis, in contrast to NT, which was described as a nonseptated sonolucent area confined to the fetal neck.[22]

The aims of this study are, first, to estimate the incidence of septations in fetuses with increased NT thickness, and second, to investigate the relationship between the length and thickness of the translucency and whether the length and septations provide useful information concerning the fetal karyotype in addition to that provided by the NT thickness alone.

PATIENTS AND METHODS

In the Fetal Medicine Centre, first-trimester screening for chromosomal defects, since 1999, is performed considering a combination of maternal age, fetal NT thickness, and maternal serum–free ß-hCG and pregnancy associated plasma protein A (PAPP-A) at $11^{0/7}$ to $13^{6/7}$ weeks of gestation.[23] Patient-specific risks are calculated by a multivariable approach in which the maternal age–related risk is multiplied with each likelihood ratio derived from the fetal NT and maternal weight–adjusted serum-free β-hCG and PAPP-A. The parents are counseled regarding the estimated risk for trisomy 21, and if they consider this risk to be high, they have an invasive diagnostic test– chorionic villus sampling or amniocentesis.

A screening study involving 96,127 pregnancies, established that the 95th percentile of vertical thickness of NT, measured in the midsagittal section of the fetus, increased linearly with fetal crown-rump length (CRL) from 2.1 mm at a CRL of 45 mm to 2.7 mm for CRL of 84 mm.[19]

In this study, which was performed between January 2003 and June 2005, in the cases undergoing chorionic villous sampling and in which the fetal NT thickness was equal to or above the 95th percentile for CRL, in addition to the measurement of the fetal NT thickness and CRL, we also measured NT length (Fig. 1). This was defined as the longitudinal distance between the occiput and the lower end of the sonolucency toward the fetal sacrum (NT length) and expressed as a percentage of the length between the

Table 1. Reports on First-Trimester Cystic Hygroma Describing the Pattern of Associated Chromosomal Defects

			Chromosomal Defect			
Author	Gestational Age (wk)	N	Total (%)	Trisomy 21, 18, or 13	Turner Syndrome	Other
Pons et al[6] (1989)	11–14	4	4 (100.0)	3	1	–
Cullen et al[7] (1990)	10–13	29	15 (51.7)	8	4	3
MacLeod and Hugo[8] (1991)	10–14	5	4 (80.0)	2	2	–
Shulman et al[9] (1992)	10–13	18	9 (50.0)	7	2	–
Suchet et al[10] (1992)	9–14	13	8 (61.5)	–	7	1
van Zalen-Sprock et al[11] (1992)	10–14	4	2 (50.0)	2	–	–
Ville et al[12] (1992)	9–14	56	16 (28.6)	11	4	1
Johnson et al[13] (1993)	10–14	68	41 (60.3)	27	9	5
Nadel et al[14] (1993)	10–15	22	19 (86.4)	10	9	–
Trauffer et al[15] (1994)	10–14	43	21 (48.8)	14	4	3
Total		262	139 (53.1)	84	42	13

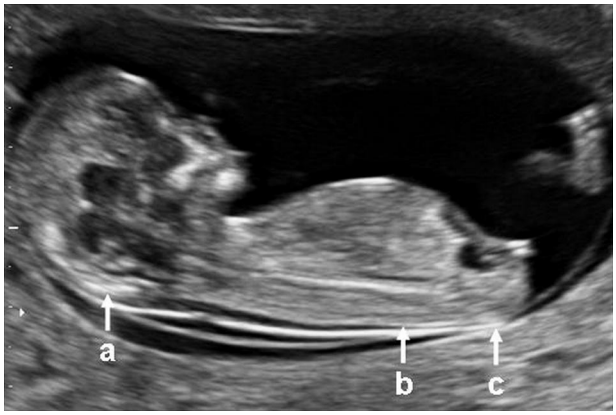


Fig. 1. Longitudinal section of a 12-week-old fetus demonstrating the measurement of the fetal nuchal translucency length, between the occiput *(a)* and the lower end of the sonolucency *(b)* and the spinal length, between the occiput *(a)* and the sacral tip *(c)*.

Molina. Nuchal Translucency and Cystic Hygromas. Obstet Gynecol 2006.

occiput and the sacral tip (spinal length). A transverse suboccipitobregmatic section of the fetal head was also obtained to determine whether or not the sonolucency was septated.

Approval for the study was obtained from King's College Hospital Research Ethics Committee.

Regression analysis was used to examine the significance of the association between NT thickness and percentage of NT length to spinal length. The prevalence and distribution of chromosomal defects were estimated for each NT category: between the 95th percentile for CRL and 3.4 mm, 3.5–4.4 mm, 4.5–5.4 mm, 5.5–6.4 mm, and 6.5 mm or more. The patients were subdivided into those with abnormal and normal karyotype, and logistic regression analysis was used to investigate the effect on abnormal karyotype of gestational age in days, fetal CRL in millimeters, fetal NT in millimeters, NT length to spinal length as a percentage, and presence or absence of septations. Multiple logistic regression analysis was subsequently performed to determine the significant independent contribution of those variables yielding a $P < .05$ in the univariable analysis.

RESULTS

Fetal karyotyping was carried in 386 singleton pregnancies with increased NT thickness. The median maternal age was 36 (range 22–46) years and the median fetal CRL was 65 (range 45–84) mm. A transverse suboccipitobregmatic plane of the fetal head was successfully imaged in 378 (97.9%) fetuses, and septations within the translucency were observed in all cases (Fig. 2).

The NT length and spinal length were measured in all 386 fetuses. There was a significant association between NT thickness and percentage of NT length to spinal length ($r = 0.791$, $P < .001$; Fig. 3). In all fetuses with NT thickness of 6 mm or more, the

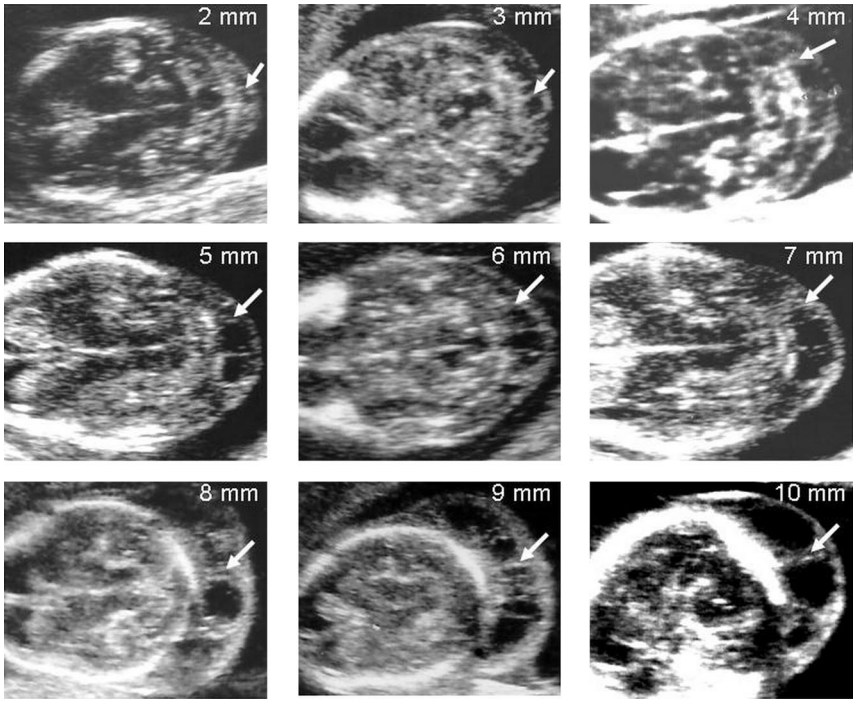


Fig. 2. Transverse suboccipitobregmatic sections of the fetal head demonstrating the presence of septated sonolucency in fetuses with nuchal translucency thickness between 2 mm and 10 mm in thickness at 11–$13^{6/7}$ weeks of gestation. The *arrows* point to the septations.

Molina. Nuchal Translucency and Cystic Hygromas. Obstet Gynecol 2006.

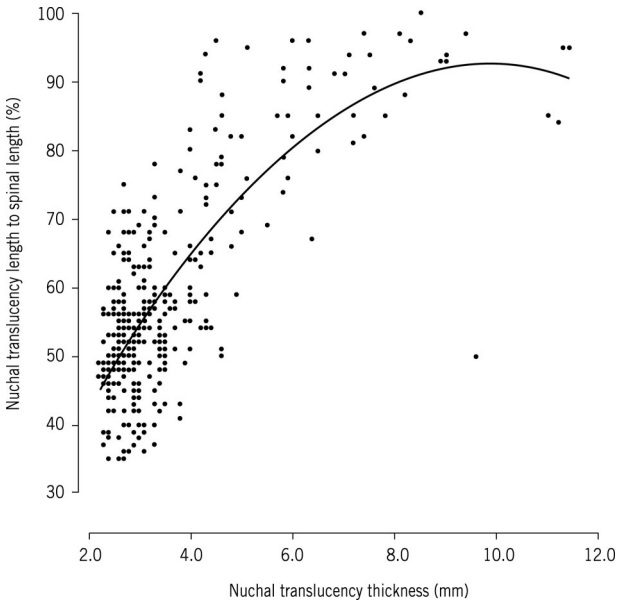


Fig. 3. Relation between percentage of nuchal translucency length to spinal length and nuchal translucency thickness.
Molina. Nuchal Translucency and Cystic Hygromas. Obstet Gynecol 2006.

percentage of NT length to spinal length was 80% or more, except in two of the fetuses with Turner syndrome, in which the increased NT thickness was confined to the neck and thorax (Fig. 4).

The fetal karyotype was abnormal in 83 (21.5%, 95% confidence interval 17.4–25.6%) pregnancies, including 52 cases of trisomy 21 (Table 2). Univariable regression analysis demonstrated that significant predictors of abnormal karyotype were gestational age, fetal CRL, fetal NT, and NT length to spinal length, but multiple regression showed that the only significant independent predictor was fetal NT (Table 3).

DISCUSSION

The findings of this study confirm that, first, there is a high association between increased NT and trisomy 21 as well as other chromosomal defects, and second, the incidence of chromosomal defects increases with NT thickness.[16,19–21] In a previous, more extended study of 11,315 pregnancies with increased NT, the fetal karyotype was abnormal in 2,168 (19.2%), and the incidence of chromosomal defects increased with NT thickness from about 7% for those with NT between the 95th percentile for CRL and 3.4 mm to 20% for NT of 3.5–4.4 mm, 50% for NT of 4.5–6.4 mm, and 75% for NT of 6.5 mm or more.[21] Furthermore, the distribution of NT was different for each type of chromosomal defect; in the majority of fetuses with trisomy 21 the NT thickness was below 4.5 mm, whereas in the majority of fetuses with trisomies 13 or 18 it was 4.5–8.4 mm, and in those with Turner syndrome it was 8.5 mm or more.

The data demonstrate that septations within the translucency can be seen in all fetuses with increased NT, provided the fetal neck is examined in the transverse suboccipitobregmatic plane. The ability to observe such septations is simply dependent on, first, obtaining the correct transverse plane of the fetal head and neck with the septations being parallel to the transducer, and second, on the use of the appropriate gain on the settings of the machine.

The data also demonstrate that the length of extension of NT from the cervical toward the sacral region is related to the thickness of NT. This is not surprising and it is comparable to ankle edema in postnatal life, which like increased NT, has a multifactorial etiology; the more the edema, the higher up the legs it can be demonstrated. The study has also shown that, whatever association there is between the length of NT and abnormal fetal karyotype, this association is a mere consequence of the fact that a higher NT thickness corresponds to a greater length.

In the early 1990s, the term "increased NT thickness" was introduced to describe the abnormal accumulation of fluid under the skin behind the fetal neck. The methodology for measuring NT thickness is

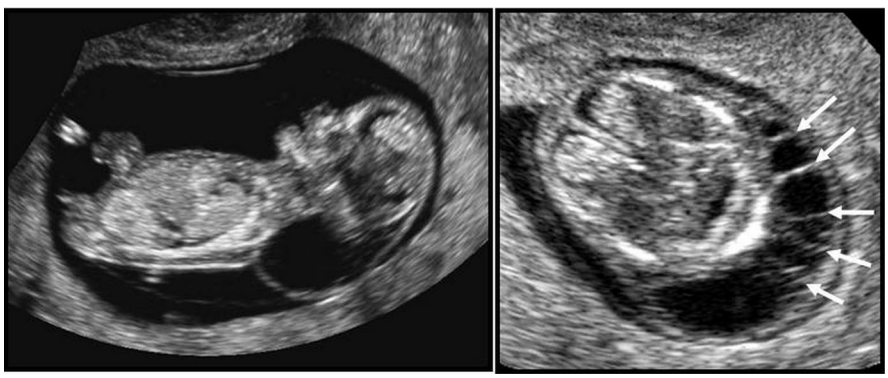


Fig. 4. Transverse and longitudinal sections of a 12-week-old fetus with Turner syndrome demonstrating septated sonolucency, 9 mm in thickness, which was confined to the neck and upper thorax. The *arrows* on the right point to the septations.
Molina. Nuchal Translucency and Cystic Hygromas. Obstet Gynecol 2006.

Table 2. Incidence of Chromosomal Defects in fetuses With Increased Nuchal Translucency Thickness

		Abnormal Karyotype				
Nuchal Translucency (mm)	N	Total*	Trisomy 21†	Trisomy 18 or 13†	Turner Syndrome†	Other†
95th percentile–3.4	268	23 (8.6, 5.2–12.0)	15 (28.9)	4 (20.0)	–	4 (57.1)
3.5–4.4	60	20 (33.3, 21.1–45.6)	15 (28.9)	4 (20.0)	–	1 (14.3)
4.5–5.4	18	11 (61.1, 36.2–86.1)	7 (13.4)	3 (15.0)	1 (25.0)	–
5.5–6.4	14	9 (64.3, 35.6–93.7)	5 (9.6)	3 (15.0)	–	1 (14.3)
≥ 6.5	26	20 (76.9, 59.6–94.3)	10 (19.2)	6 (30.0)	3 (75.0)	1 (14.3)
Total	386	83 (21.5, 17.4–25.6)	52 (100)	20 (100)	4 (100)	7 (100)

* Percentages in parentheses with 95% confidence interval are based on row totals.
† Percentages in parentheses are based on column totals.

Table 3. Regression Analysis in the Prediction of Abnormal Karyotype in Fetuses with Increased Nuchal Translucency Thickness

		Chromosomal Abnormality			
		Univariable Analysis		Multivariable Analysis	
Variable	Median (range)	OR (95% CI)	*P*	OR (95% CI)	*P*
Gestational age (day)	88 (77–97)	0.92 (0.87–0.97)	< .002	0.97 (0.88–1.07)	.562
Crown-rump length (mm)	65 (45–84)	0.95 (0.92–0.97)	< .001	0.98 (0.93–1.04)	.532
Nuchal translucency thickness (mm)	3.0 (2.2–11.4)	2.07 (1.69–2.52)	< .001	1.69 (1.25–2.28)	< .001
Nuchal translucency length to spinal length (%)	55 (35–100)	1.07 (1.05–1.09)	< .001	1.02 (0.99–1.05)	.179

OR, odds ratio; 95% CI, 95% confidence interval.

reproducible, and the measurement can be subjected to external quality assurance.[20] However, those performing the NT scan should receive training and acquire expertise in doing so.[20] Extensive research in the last 15 years has established that increased NT is associated with chromosomal defects, many fetal malformations, and genetic syndromes, and the incidence of these abnormalities is related to the thickness, rather than the appearance, of NT.[17–21] The likelihood ratios for trisomies 21, 18, and 13, for deviations in the measured NT from the normal median for CRL, have been validated by prospective screening studies involving many hundreds of thousands of patients.[20] The measurement of fetal NT can be combined with maternal serum–free ß-hCG and PAPP-A at 11–13$^{6/7}$ weeks to give accurate patient-specific risks and provide the most effective method of screening for trisomy 21, with a detection rate of 90% for a false positive rate of 5%.[20,23] Further improvement in first-trimester screening can be achieved by the inclusion of assessment of the nasal bone and flow in the tricuspid valve and ductus venosus, with a detection rate of more than 90% for a false-positive rate of less than 3%.[24] During the second trimester, increased NT usually resolves, but in a few cases, it evolves into nuchal edema or true cystic hygromas.[1,2]

It was recently suggested that first-trimester cystic hygroma constitutes such a unique and easily identifiable marker that, in contrast to NT, it can be implemented in population screening without the need for sonographers to receive special training and ongoing surveillance to confirm accuracy of diagnosis.[22] Furthermore, it was recommended that, after the diagnosis of cystic hygromas, it is unnecessary for maternal serum–free ß-hCG and PAPP-A to be measured and for software to be used for calculation of patient-specific risk of chromosomal defects. Instead, the parents should be counseled that 50% of fetuses will have a chromosomal abnormality.[22] However, the findings of the present study have demonstrated that, first, when visualized appropriately all increased NTs contain septae, second, the length of NT correlates with its thickness, and third, the risk of a chromosome abnormality is not dependent on the length of the translucent area once its thickness is accounted for. Consequently, cystic hygroma does not constitute a distinct entity in the first trimester that confers a special risk status independent of the NT thickness.

REFERENCES

1. Chervenak FA, Isaacson G, Blakemore KJ, Breg RW, Hobbins JC, Berkowitz RL, et al. Fetal cystic hygroma: cause and natural history. N Engl J Med 1983;309:822–5.

2. Azar G, Snijders RJM, Gosden CM, Nicolaides KH. Fetal nuchal cystic hygromata: associated malformations and chromosomal defects. Fetal Diagn Ther 1991;6:46–57.
3. Benacerraf BR, Gelman R, Frigoletto FD. Sonographic identification of second-trimester fetuses with Down's syndrome. N Engl J Med 1987;317:1371–6.
4. Nicolaides KH, Azar G, Snijders RJM, Gosden CM. Fetal nuchal edema: associated malformations and chromosomal defects. Fetal Diagn Ther 1992;7:123–31.
5. Van der Putte SCJ. Lymphatic malformation in human fetuses: a study of fetuses with Turner's syndrome or status Bonnevie-Ullrich. Virchows Arch A Pathol Anat Histol 1977;376: 233–46.
6. Pons JC, Diallo AA, Eydoux P, Rais S, Doumerc S, Frydman R, Papiernik E. Chorionic villus sampling after first trimester diagnosis of fetal cystic hygroma Colli. Eur J Obstet Gynecol Reprod Biol 1989;33:141–6.
7. Cullen MT, Gabrielli S, Green JJ, Rizzo N, Mahoney MJ, Salafia C, et al. Diagnosis and significance of cystic hygroma in the first trimester. Prenat Diagn 1990;10:643–51.
8. MacLeod AM, McHugo JM. Prenatal diagnosis of nuchal cystic hygroma. Br J Radiol 1991;64:802–7.
9. Shulman LP, Emerson D, Felker R, Phillips O, Simpson J, Elias S. High frequency of cytogenetic abnormalities with cystic hygroma diagnosed in the first trimester. Obstet Gynecol 1992;80:80–2.
10. Suchet IB, van der Westhuizen NG, Labatte MF. Fetal cystic hygromas: further insights into their natural history. Can Assoc Radiol J 1992;43:420–4.
11. Van Zalen-Sprock MM, van Vugt JMG, van Geijn HP. First-trimester diagnosis of cystic hygroma: course and outcome. Am J Obstet Gynecol 1992;167:94–8.
12. Ville Y, Borghi E, Pons JC, Lelorc'h M. Fetal karyotype from cystic hygroma fluid. Prenat Diagn 1992;12:139–43.
13. Johnson MP, Johnson A, Holzgreve W, Isada NB, Wapner RJ, Treadwell MC, et al. First-trimester simple hygroma: cause and outcome. Am J Obstet Gynecol 1993;168:156–61.
14. Nadel A, Bromley B, Benacerraf BR. Nuchal thickening or cystic hygromas in first- and early second-trimester fetuses: prognosis and outcome. Obstet Gynecol 1993;82:43–8.
15. Trauffer ML, Anderson CE, Johnson A, Heeger S, Morgan P, Wapner RJ. The natural history of euploid pregnancies with first-trimester cystic hygromas. Am J Obstet Gynecol 1994; 170:1279–84.
16. Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. Br Med J 1992;304: 867–89.
17. Souka AP, Snidjers RJM, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10–14 weeks of gestation. Ultrasound Obstet Gynecol 1998;11:391–400.
18. Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol 2005;192:1005–21.
19. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation: Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998;351:343–6.
20. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191:45–67.
21. Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation between increased fetal nuchal translucency thickness and chromosomal defects. Obstet Gynecol 2006; 107:6–10.
22. Malone FD, Ball RH, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, et al. First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome. Obstet Gynecol 2005;106:288–94.
23. Avgidou K, Papageorghiou A, Bindra R, Spencer K, Nicolaides KH. Prospective first-trimester screening for trisomy 21 in 30,564 pregnancies. Am J Obstet Gynecol 2005;192:1761–7.
24. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75,821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. Ultrasound Obstet Gynecol 2005;25:221–6.

Original Research

Relation Between Increased Fetal Nuchal Translucency Thickness and Chromosomal Defects

Karl Oliver Kagan, MD, Kyriaki Avgidou, MD, Francisca S. Molina, MD, Katarzyna Gajewska, MD, Kypros H. Nicolaides, MD

OBJECTIVE: To examine the prevalence and distribution of all chromosomal defects in fetuses with increased nuchal translucency thickness.

METHODS: Assessment of risk for trisomy 21 was carried out by a combination of maternal age and fetal nuchal translucency thickness at 11–13 + 6 weeks. A search of the database was made to identify, first, all singleton pregnancies in which fetal karyotyping was carried out and, second, the cases where the fetal nuchal translucency was equal to or above the 95th centile for fetal crown-rump length. The prevalence and distribution of chromosomal defects were determined for each nuchal translucency category: between the 95th centile for crown-rump length and 3.4 mm, 3.5–4.4 mm, 4.5–5.4 mm, 5.5–6.4 mm, 6.5–7.4 mm, 7.5–8.4 mm, 8.5–9.4 mm, 9.5–10.4 mm, 10.5–11.4 mm, and 11.5 mm or more.

RESULTS: The search identified 11,315 pregnancies. The median maternal age was 34.5 (range 15–50) years, and the median fetal crown-rump length was 64 (range 45–84) mm. The fetal karyotype was abnormal in 2,168 (19.2%) pregnancies, and the incidence of chromosomal defects increased with nuchal translucency thickness from approximately 7% for those with nuchal translucency between the 95th centile for crown-rump length and 3.4 mm to 75% for nuchal translucency of 8.5 mm or more. In the majority of fetuses with trisomy 21, the nuchal translucency thickness was less then 4.5 mm, whereas in the majority of fetuses with trisomies 13 or 18 it was 4.5–8.4 mm, and in those with Turner syndrome it was 8.5 mm or more.

CONCLUSION: In fetuses with increased nuchal translucency, approximately one half of the chromosomally abnormal group is affected by defects other than trisomy 21. The distribution of nuchal translucency is different for each type of chromosomal defect.
(Obstet Gynecol 2006;107:6–10)

LEVEL OF EVIDENCE: II-3

See related editorial on page 2.

From the Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, London, United Kingdom.

Supported by a grant from the Fetal Medicine Foundation (Charity No. 1037116).

Corresponding author: Professor K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, Denmark Hill, London SE5 8RX, United Kingdom; e-mail: kypros@technocom.com.

ISSN: 0029-7844/05

Fetal nuchal translucency refers to the sonographic finding of a subcutaneous collection of fluid behind the fetal neck in the first trimester of pregnancy, and the term is used irrespective of whether the fluid is septated and whether it is confined to the neck or envelopes the whole fetus.[1] Nuchal translucency is considered to be increased if the vertical thickness, measured in the midsagittal section of the fetus, is equal to or above the 95th centile of a reference range established in a screening study involving 96,127 pregnancies.[2] The 95th centile of nuchal translucency increased linearly with fetal crown-rump length (CRL) from 2.1 mm at a CRL of 45 mm to 2.7 mm for CRL of 84 mm, whereas the 99th centile did not change with CRL, and it was approximately 3.5 mm.[2]

Increased nuchal translucency is associated with trisomy 21 and other chromosomal abnormalities as well as many fetal malformations and genetic syndromes.[3–5] Several studies have established that, first, increased nuchal translucency, both on its own and in combination with other sonographic or maternal serum biochemical markers, is effective in first trimester screening for trisomy 21, and second, the incidence of trisomy 21 increases with fetal nuchal translucency thickness.[3] The aim of this study was to examine the prevalence

and distribution of all chromosomal defects in fetuses with increased nuchal translucency thickness.

PATIENTS AND METHODS

This was a retrospective study examining the relation between nuchal translucency thickness and chromosomal abnormalities in singleton pregnancies with increased nuchal translucency at 11–13 + 6 weeks of gestation. In our center assessment of risk for trisomy 21 by a combination of maternal age and fetal nuchal translucency has been carried out since 1992.[1–3] In all patients attending for the 11–13 + 6 weeks scan, maternal demographic characteristics and ultrasound findings, including nuchal translucency thickness and CRL, were recorded in a computer database. The patient-specific risk for trisomy 21 was calculated by multiplying the maternal age and gestational age–related risk by the likelihood ratio for fetal nuchal translucency, which is dependent on the degree of deviation in the measured nuchal translucency from the normal median for the same CRL.[2] The parents were counseled regarding the estimated risk for trisomy 21, and if they considered this risk to be high, they were offered the option of an invasive diagnostic test—chorionic villus sampling or amniocentesis. Karyotype results and details on pregnancy outcomes were added into the computer database as soon as these became available.

A search of the database was made to identify, first, all pregnancies in which fetal karyotyping was carried out between January 1992 and April 2005, and second, the cases where the fetal nuchal translucency was equal to or above the 95th centile for fetal CRL.[2] Approval for the study was obtained from King's College Hospital Research Ethics Committee.

The prevalence and distribution of chromosomal defects were estimated for each nuchal translucency category: between the 95th centile for CRL and 3.4 mm, 3.5–4.4 mm, 4.5–5.4 mm, 5.5–6.4 mm, 6.5–7.4 mm, 7.5–8.4 mm, 8.5–9.4 mm, 9.5–10.4 mm, 10.5–11.4 mm, and 11.5 mm or more. The prevalence of trisomies 21, 18, and 13 increases with maternal age and decreases with gestational age.[6,7] The prevalence of Turner syndrome, other sex chromosome aneuploidies, and triploidy does not change with maternal age but decreases with gestation, and at 12 weeks the respective prevalences are approximately 1 in 1,500, 1 in 500, and 1 in 2,000.[7] For each nuchal translucency category, the maternal and gestational age distribution and the previously published risk for each aneuploidy were used[6,7] to estimate the expected number of fetuses with trisomies 21, 18, and 13, Turner syndrome, other sex chromosome aneuploidies, and triploidy. The observed-to-expected ratio was then calculated, and regression analysis was used to determine the significance of the association between the ratio and nuchal translucency thickness.

RESULTS

Fetal karyotyping was performed in 11,315 singleton pregnancies with high nuchal translucency thickness. The median maternal age was 34.5 (range 15–50) years, and the median fetal CRL was 64 (range 45–84) mm. The fetal karyotype was abnormal in 2,168 (19.2%) pregnancies, including 1,170 cases of trisomy 21 (Table 1). The overall incidence of chromosomal defects increased with nuchal translucency thickness from approximately 7% for those with nuchal translucency between the 95th centile for CRL and 3.4 mm to 20% for nuchal translucency of 3.5–4.4

Table 1. Incidence of Chromosomal Defects in Fetuses With Increased Nuchal Translucency Thickness

Nuchal Translucency (mm)	Median Maternal Age [Range (y)]	N	Abnormal Karyotype							
			Total	Trisomy 21	Trisomy 18	Trisomy 13	Turner Syndrome	Other Sex	Triploidy	Other*
95th–3.4	35.0 (15.7–49.9)	7,109	507 (7.1)	335 (66.1)	49 (9.7)	37 (7.3)	6 (1.2)	30 (5.9)	10 (2.0)	40 (7.9)
3.5–4.4	33.5 (15.0–47.1)	2,101	423 (20.1)	290 (68.6)	47 (11.1)	30 (7.1)	6 (1.4)	13 (3.1)	12 (2.8)	25 (5.9)
4.5–5.4	34.1 (16.1–48.6)	707	321 (45.4)	185 (57.6)	67 (20.9)	28 (8.7)	9 (2.8)	5 (1.6)	9 (2.8)	18 (5.6)
5.5–6.4	33.5 (16.5–47.4)	437	219 (50.1)	108 (49.3)	62 (28.3)	27 (12.3)	9 (4.1)	2 (0.9)	4 (1.8)	7 (3.2)
6.5–7.4	35.2 (17.0–47.0)	309	218 (70.6)	99 (45.4)	65 (29.8)	17 (7.8)	23 (10.6)	1 (0.5)	6 (2.8)	7 (3.2)
7.5–8.4	35.0 (17.7–48.0)	209	148 (70.8)	59 (39.9)	55 (37.2)	14 (9.5)	12 (8.1)	1 (0.7)	2 (1.4)	5 (3.4)
8.5–9.4	33.9 (18.7–46.3)	168	126 (75.0)	45 (35.7)	35 (27.8)	4 (3.2)	38 (30.2)	1 (0.8)	1 (0.8)	2 (1.6)
9.5–10.4	33.3 (18.1–44.7)	88	74 (74.9)	22 (29.7)	18 (24.3)	5 (2.9)	27 (36.5)	0 (0.0)	1 (1.4)	1 (1.4)
10.5 – 11.4	34.6 (17.8-45.5)	64	45 (70.3)	14 (31.1)	9 (20.0)	1 (1.6)	19 (42.2)	0 (0.0)	1 (2.2)	1 (2.2)
>11.5	32.6 (17.3–46.9)	123	87 (70.7)	13 (14.9)	11 (12.6)	0 (0.0)	61 (70.1)	0 (0.0)	0 (0.0)	2 (2.3)
Total	34.5 (15.0–49.9)	11,315	2,168 (19.2)	1,170 (54.0)	418 (19.3)	163 (7.5)	210 (9.7)	53 (2.4)	46 (2.1)	108 (5.0)

Values are n (%) unless otherwise specified.
* Unbalanced translocations (n = 49), marker chromosomes (n = 6), mosaicism (n = 8), and duplications and deletions (n = 45).

Table 2. Observed-to-Expected Ratio of Each Chromosomal Defect in Fetuses With Increased Nuchal Translucency Thickness

Nuchal Translucency (mm)			Trisomy 21			Trisomy 18			Trisomy 13			Turner Syndrome			Other Sex			Triploidy		
Class	Median	N	Exp	Obs	Obs/ Exp	Exp	Obs	Obs/ Exp	Exp	Obs	Obs/ Exp	Exp	Obs	Obs/ Exp	Exp	Obs	Obs/ Exp	Exp	Obs	Obs/ Exp
Total	3.1	11,315	80.8	1,170	14.47	34.7	418	12.04	11.0	163	14.77	7.5	210	27.84	22.6	53	2.34	5.7	46	8.13
95th–3.4	2.8	7,109	52.4	335	6.39	22.5	49	2.18	7.1	37	5.18	4.7	6	1.27	14.2	30	2.11	3.6	10	2.81
3.5–4.4	3.8	2,101	11.7	290	24.80	5.0	47	9.36	1.6	30	18.80	1.4	6	4.28	4.2	13	3.09	1.1	12	11.42
4.5–5.4	4.9	707	5.6	185	32.77	2.4	67	27.64	0.8	28	36.34	0.5	9	19.09	1.4	5	3.54	0.4	9	25.46
5.5–6.4	6	437	3.5	108	30.55	1.5	62	40.85	0.5	27	55.97	0.3	9	30.89	0.9	2	2.29	0.2	4	18.31
6.5–7.4	7	309	2.7	99	36.55	1.2	65	55.88	0.4	17	46.00	0.2	23	111.65	0.6	1	1.62	0.2	6	38.83
7.5–8.4	7.9	209	1.9	59	30.78	0.8	55	66.82	0.3	14	53.50	0.1	12	86.12	0.4	1	2.39	0.1	2	19.14
8.5–9.4	8.9	168	1.1	45	39.61	0.5	35	71.75	0.2	4	25.71	0.1	38	339.29	0.3	1	2.98	0.1	1	11.90
9.5-10.4	10	92	0.7	22	32.45	0.3	18	59.94	0.1	5	53.51	0.1	27	440.22	0.2	0	0.00	0.0	1	21.74
10.5–11.4	11	58	0.4	14	31.31	0.2	9	46.88	0.1	1	16.39	0.0	19	491.38	0.1	0	0.00	0.0	1	34.48
> 11.5	13.2	125	0.7	13	18.82	0.3	11	37.08	0.1	0	0.00	0.1	61	732.00	0.3	0	0.00	0.1	0	0.00

Obs, observed; Exp, expected.
Values are n unless otherwise specified.

mm, 50% for nuchal translucency of 5.5–6.4 mm, and 75% for nuchal translucency of 8.5 mm or more. In the majority of fetuses with trisomy 21, the nuchal translucency thickness was less than 4.5 mm, whereas in the majority of fetuses with trisomies 13 or 18, it was 4.5–8.4 mm, and in those with Turner syndrome, it was 8.5 mm or more.

The observed prevalence of trisomies 21, 18, and 13, Turner syndrome, other sex chromosome aneuploidies, and triploidy was higher than the respective prevalences estimated on the basis of the maternal age and gestational age distribution of the population (Table 2). The observed-to-expected ratio increased significantly with nuchal translucency thickness for trisomy 21 ($r = 0.919$, $P = .008$), trisomy 18 ($r = 0.970$, $P < .001$), trisomy 13 ($r = 0.870$, $P = .007$), Turner syndrome ($r = 0.987$, $P < .001$) and other sex chromosome abnormalities ($r = 0.759$, $P = .011$) but not for triploidy ($r = 0.684$, $P = .255$) (Fig. 1).

DISCUSSION

The findings of this study confirm the high association between increased nuchal translucency and trisomy 21 as well as other chromosomal defects.[1–3] Thus, the incidence of chromosomal defects increases with nuchal translucency thickness from approximately 7% for those with nuchal translucency between the 95th centile for CRL and 3.4 mm to 75% for nuchal translucency of 8.5 mm or more.

The data demonstrate that in fetuses with increased nuchal translucency approximately one half of the chromosomally abnormal group is affected by defects other than trisomy 21. Furthermore, the distribution of nuchal translucency is different for each type of chromosomal defect. Thus, the nuchal translucency thickness was less than 4.5 mm in approximately 50% of fetuses with trisomy 21 and those with triploidy. In contrast, the nuchal translucency thickness was 4.5 mm or more in approximately 60% of fetuses with trisomy 13, 75% of those with trisomy 18, and 90% of fetuses with Turner syndrome. Additionally, the observed-to-expected ratio of trisomies 21, 18, and 13 increases with nuchal translucency thickness to a peak at approximately 8–9 mm and thereafter decreases, whereas in the case of Turner syndrome, the ratio increases exponentially with fetal nuchal translucency. For other sex chromosome defects the ratio decreases with nuchal translucency, and for triploidy it does not change significantly with nuchal translucency.

The difference in phenotypic pattern of nuchal translucency thickness characterizing each chromosomal defect presumably reflects the heterogeneity in causes for the abnormal accumulation of subcutaneous fluid in the nuchal region. Suggested mechanisms for increased nuchal translucency include cardiac dysfunction in association with abnormalities of the heart and great arteries;[8,9] superior mediastinal compression due to diaphragmatic hernia, which is commonly found in fetuses with trisomy 18;[10,11] failure of lymphatic drainage due to impaired development of the lymphatic system, which has been demonstrated by immunohistochemical studies in nuchal skin tissue from fetuses with Turner syndrome;[12] and altered composition of the subcutaneous connective tissue, leading to the accumulation of subcutaneous edema.[13,14] Although cardiac defects are commonly found in association with all major chromosomal

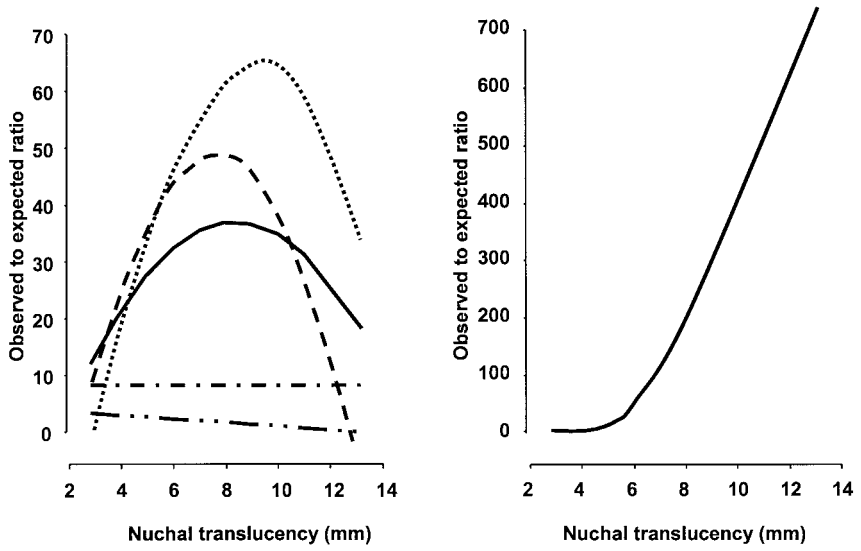


Fig. 1. Relation between fetal nuchal translucency thickness and the observed-to-expected ratio for trisomy 21 *(——)*, trisomy 18 *(•••••••••••••)*, trisomy 13 *(– – – –)*, triploidy *(• – • – • –)*, and other sex chromosome defects *(•• – •• – •• –)* on the left and Turner syndrome on the right.
Kagan. Increased Nuchal Translucency. Obstet Gynecol 2006.

abnormalities, there are differences in the pattern of cardiac defects and consequently different severities of cardiac dysfunction.[8,9] Many of the component proteins of the extracellular matrix are encoded on chromosomes 21, 18, or 13, and immunohistochemical studies of the skin of chromosomally abnormal fetuses have demonstrated specific alterations of the extracellular matrix that may be attributed to gene dosage effects. Thus, the dermis of trisomy 21 fetuses is rich in collagen type VI, whereas dermal fibroblasts of trisomy 13 fetuses demonstrate an abundance of collagen type IV and those of trisomy 18 fetuses an abundance of laminin.[13,14]

The clinical implications of our findings are, first, increased nuchal translucency is an effective marker not only of trisomy 21 but also of all major chromosomal defects and, second, in nuchal translucency screening for trisomy 21, the finding of increased nuchal translucency should prompt ultrasonographers to consider the possibility of other chromosomal defects and undertake a systematic examination of the fetus for detectable features of such defects. These include nasal bone hypoplasia and atrioventricular septal defect in trisomy 21, fetal growth restriction, diaphragmatic hernia, exomphalos, overlapping fingers and single umbilical artery in trisomy 18, holoprosencephaly, facial cleft, megacystis, polydactyly and tachycardia in trisomy 13, fetal growth restriction and tachycardia in Turner syndrome, and severe asymmetrical growth restriction with either molar or hypoplastic placenta in diandric and digynic triploidy respectively.[15–23]

REFERENCES

1. Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. BMJ 1992;304:867–9.
2. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. Lancet 1998;352:343–6.
3. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191:45–67.
4. Souka AP, Snijders RJ, Novakov A, Soares W, Nicolaides KH. Defects and syndromes in chromosomally normal fetuses with increased nuchal translucency thickness at 10–14 weeks of gestation. Ultrasound Obstet Gynecol 1998;11:391–400.
5. Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol 2005;192:1005–21.
6. Snijders RJ, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age- and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol 1999;13:167–70.
7. Snijders RJ, Sebire NJ, Nicolaides KH. Maternal age and gestational age-specific risks for chromosomal defects. Fetal Diag Ther 1995;10:356–67.
8. Hyett J, Perdu M, Sharland G, Snijders R, Nicolaides KH. Using fetal nuchal translucency to screen for major congenital cardiac defects at 10-14 weeks of gestation: population based cohort study. BMJ 1999;318:81–5.
9. Hyett J, Moscoso G, Nicolaides K. Abnormalities of the heart and great arteries in first trimester chromosomally abnormal fetuses. Am J Med Genet 1997;69:207–16.
10. Sebire NJ, Snijders RJ, Davenport M, Greenough A, Nicolaides KH. Fetal nuchal translucency thickness at 10-14 weeks' gestation and congenital diaphragmatic hernia. Obstet Gynecol 1997;90:943–6.
11. Thorpe-Beeston JG, Gosden CM, Nicolaides KH. Prenatal diagnosis of congenital diaphragmatic hernia: associated malformations and chromosomal defects. Fetal Ther 1989;4:21–8.
12. von Kaisenberg CS, Nicolaides KH, Brand-Saberi B. Lymphatic vessel hypoplasia in fetuses with Turner syndrome. Hum Reprod 1999;14:823–6.
13. von Kaisenberg CS, Krenn V, Ludwig M, Nicolaides KH, Brand-Saberi B. Morphological classification of nuchal skin in fetuses with trisomy 21, 18, and 13 at 12–18 weeks and in a trisomy 16 mouse. Anat Embryol (Berl) 1998;197:105–24.
14. von Kaisenberg CS, Brand-Saberi B, Christ B, Vallian S, Farzaneh F, Nicolaides KH. Collagen type VI gene expression in the skin of trisomy 21 fetuses. Obstet Gynecol 1998;91: 319–23.
15. Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: an observational study. Lancet 2001;358: 1665–7.
16. Kuhn P, Brizot ML, Pandya PP, Snijders RJ, Nicolaides KH. Crown-rump length in chromosomally abnormal fetuses at 10 to 13 weeks' gestation. Am J Obstet Gynecol 1995;172:32–5.
17. Sherod C, Sebire NJ, Soares W, Snijders RJ, Nicolaides KH. Prenatal diagnosis of trisomy 18 at the 10–14-week ultrasound scan. Ultrasound Obstet Gynecol 1997;10:387–90.
18. Jauniaux E, Brown R, Snijders RJ, Noble P, Nicolaides KH. Early prenatal diagnosis of triploidy. Am J Obstet Gynecol 1997;176:550–4.
19. Rembouskos G, Cicero S, Longo D, Sacchini C, Nicolaides KH. Single Umbilical Artery at 11-14 weeks' gestation: relation to chromosomal defects. Ultrasound Obstet Gynecol 2003;22: 567–70.
20. Sebire NJ, Von Kaisenberg C, Rubio C, Nicolaides KH. Fetal megacystis at 10-14 weeks of gestation. Ultrasound Obstet Gynecol 1996;8:387–90.
21. Liao AW, Sebire NJ, Geerts L, Cicero S, Nicolaides KH. Megacystis at 10-14 weeks of gestation: chromosomal defects and outcome according to bladder length. Ultrasound Obstet Gynecol 2003;21:338–41.
22. Snijders RJ, Sebire NJ, Souka A, Santiago C, Nicolaides KH. Fetal exomphalos and chromosomal defects: relationship to maternal age and gestation. Ultrasound Obstet Gynecol 1995; 6:250–5.
23. Liao AW, Snijders R, Geerts L, Spencer K, Nicolaides KH. Fetal heart rate in chromosomally abnormal fetuses. Ultrasound Obstet Gynecol 2000;16:610–3.

Ultrasound Obstet Gynecol 2008; **31**: 376–383
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5299

A mixture model of nuchal translucency thickness in screening for chromosomal defects

D. WRIGHT*, K. O. KAGAN†‡, F. S. MOLINA†§, A. GAZZONI† and K. H. NICOLAIDES†

**Department of Mathematics and Statistics, University of Plymouth, Plymouth and †Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK, ‡Department of Obstetrics and Gynecology, University of Tuebingen, Tuebingen, Germany and §Hospital Universitario Virgen de las Nieves, Granada, Spain*

KEYWORDS: chromosomal defects; first trimester; nuchal translucency; risk assessment; screening; trisomy 21

ABSTRACT

Objective *Fetal nuchal translucency (NT) thickness increases with crown–rump length (CRL). In screening for chromosomal defects patient-specific risks are derived by multiplying the* a priori *maternal age-related risk by a likelihood ratio, determined from the deviation of the measured NT from the expected median. To quantify this deviation the measured NT is either subtracted (delta NT) or divided by the expected median (multiple of the median method, MoM). This study examines the validity of these methods.*

Methods *NT was prospectively measured at 11 + 0 to 13 + 6 weeks in screening for chromosomal defects. The distribution of NT in euploid and chromosomally abnormal fetuses was examined.*

Results *There were 37 078 normal pregnancies and 264 with trisomy 21, 81 with trisomy 18, 38 with trisomy 13 and 27 with Turner syndrome. We found that firstly, contrary to the assumption underlying the delta NT method, the distribution of delta NT changes with CRL and secondly, contrary to the assumption underlying the MoM method the distribution of NT was not Gaussian. Fetal NT followed two distributions, one that was dependent on CRL and one that was independent of CRL. The distribution in which NT increases with CRL was observed in about 95% of euploid fetuses, 5% with trisomy 21, 30% with trisomy 18, 15% with trisomy 13 and 10% with Turner syndrome. The median CRL-independent NT was 2.0 mm for the euploid group and 3.4, 5.5, 4.0 and 7.8 mm for trisomies 21, 18, 13 and Turner syndrome, respectively.*

Conclusions *The NT thickness in chromosomally normal and abnormal fetuses follows a mixture of a gestation-dependent and gestation-independent distribution. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Fetal nuchal translucency (NT) thickness is the single most effective marker of trisomy 21 and all other major chromosomal defects. It increases with crown–rump length (CRL), and it is important to take gestational age into account when determining whether a given NT thickness is increased[1]. In screening for chromosomal defects patient-specific risks are derived by multiplying the *a priori* maternal age and gestation-related risk by a likelihood ratio, determined from the deviation of the fetal NT measurement from the normal median for CRL.

There are essentially two approaches to quantifying the deviation of NT from the normal median. One approach is to subtract the normal median from the NT measurement and to produce a deviation in mm referred to as delta NT. The other approach is to divide NT by the normal median to produce a multiple of the median (MoM) value[2,3]. In the calculation of patient-specific risks for trisomy 21 the *a priori* maternal age-related risk is multiplied by the likelihood ratio for a measured NT, which is the ratio of the heights of distributions of measurements in trisomy 21 and unaffected pregnancies. In the delta method it is assumed that there is a common distribution of NT delta values independent of CRL in the trisomy 21 pregnancies and another common distribution in unaffected pregnancies. In the MoM method it is assumed that the distributions of the log transformed MoM values in trisomy 21 and unaffected pregnancies are Gaussian.

In this paper we examine departures from the assumptions underlying the delta and MoM approaches

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, Denmark Hill, London SE5 8RX, UK (e-mail: fmf@fetalmedicine.com)

Accepted: 8 February 2008

and suggest a model based on two-component mixtures of distributions. We propose that in unaffected pregnancies the majority of fetuses demonstrate an increase in NT with CRL and in a minority of cases NT tends to be relatively large and is independent of CRL. In the chromosomally abnormal pregnancies there is a different mixture of NT distributions, with a minority of cases following the same CRL-dependent distribution as in the unaffected pregnancies, but in the majority of cases there is a distribution that is independent of CRL, with a higher mean and standard deviation (SD) than in unaffected pregnancies.

METHODS

At the Fetal Medicine Centre, London, screening for trisomy 21 is carried out by a combination of maternal age, fetal NT thickness and maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) in a one-stop-clinic for first-trimester assessment of risk (OSCAR) at 11 to 13 + 6 weeks of gestation[4]. Transabdominal ultrasound examination is performed to diagnose any major fetal defects and for measurement of CRL and fetal NT thickness[1]. The Kryptor system (Brahms AG, Berlin, Germany) is used to measure PAPP-A and free β-hCG. Maternal demographic characteristics, ultrasononographic measurements and biochemical results are recorded in a computer database, and karyotype results and details on pregnancy outcomes are added into the database as soon as they become available.

A search of the database was done to identify all singleton pregnancies in which first-trimester screening by fetal NT, PAPP-A and free β-hCG was carried out from July 1999 to July 2005. In this study we examine the distribution of NT in chromosomally abnormal fetuses and in unaffected pregnancies.

Statistical analysis

Distribution of logMoM (NT) and delta NT

The adequacy of the MoM approach was assessed firstly by examining non-parametric centile curves to show how the distribution of log(NT) changes with CRL, and secondly by examining Gaussian probability plots of logMoM (NT), stratified by gestational age, to determine whether there are departures from the assumed Gaussian form; throughout this paper logs are to base 10.

The delta NT approach does not assume a Gaussian distribution, but it is implicit in this approach that the CRL-specific centile curves in the trisomy 21 and unaffected pregnancies should be parallel. We assessed the adequacy of this method by examining non-parametric centile curves for NT by CRL.

Two-component mixture model

In the unaffected pregnancies it was assumed that log(NT) arises from a mixture of two Gaussian distributions: firstly, a proportion $(1 - p)$ in which there is a quadratic relationship between mean log(NT) (μ_o) and CRL ($\mu_0 = \beta_0 + (\beta_1 \times \mathrm{CRL}) + (\beta_2 \times \mathrm{CRL}^2)$) with an SD that is constant (σ_0), and secondly, a proportion (p) in which NT is CRL-independent with a mean of μ_1 and SD of σ_1.

In each chromosomally abnormal pregnancy (trisomies 21, 18 and 13 and Turner syndrome) it was assumed that log(NT) also arises from a mixture of two Gaussian distributions: firstly, a proportion $(1 - p_{abnormal})$ with the same CRL-dependent distribution as in unaffected pregnancies, and secondly, a chromosomal abnormality-specific proportion ($p_{abnormal}$) in which NT is CRL-independent with a mean log(NT) of $\mu_{abnormal}$ and SD of $\sigma_{abnormal}$. The proportions $p_{abnormal}$ defining the mixture, and the mean and standard deviation of the CRL-independent component differ according to abnormality (trisomies 21, 18 and 13 and Turner syndrome).

For unaffected pregnancies the CRL-dependent component dominates and p is close to 0, while for the chromosomal abnormalities the CRL-dependent component is uncommon and p is close to 1. Allowing p in unaffected pregnancies to depend on CRL, according to a logistic regression model, improves the fit of the model and has a clinically meaningful interpretation that we shall discuss. In this model $p = 1/(1 + \exp(-\alpha_0 - \alpha_1 \mathrm{CRL}))$.

To complete the model we assumed that variation between operators is represented by random effects acting additively on log(NT) with mean 0 and standard deviation σ_{op}. The degree of heterogeneity between operators is reflected in the magnitude of σ_{op}. The mixture model was fitted within a Bayesian framework using Markov chain Monte Carlo (MCMC) implemented in WinBUGS[5]. Copies of the WinBUGS model specification are available on request from the authors.

Assessment of screening performance

Crude detection rates and false positive rates were calculated by taking the proportions with risks above a given risk threshold. Standardized rates were produced by first calculating age-specific detection and false positive rates and then weighting them according to the maternal age distributions of affected and unaffected pregnancies in England and Wales in 2000–2002[6]. These standardized rates were compared with detection and false positive rates estimated using Monte Carlo methods to sample from the modeled mixtures of Gaussian distributions.

RESULTS

The search of the database identified 38 791 singleton pregnancies but 1303 (3.4%) were excluded from further analysis because the fetal karyotype or pregnancy outcome was not available ($n = 1231$) or was due to a chromosomal abnormality other than trisomy 21, 18, 13

or Turner syndrome ($n = 72$). In the remaining 37 488 cases there were 37 078 pregnancies with a normal karyotype or delivery of a phenotypically normal baby (unaffected group), 264 with trisomy 21, 81 with trisomy 18, 38 with trisomy 13 and 27 with Turner syndrome. The characteristics of the women included in this study are shown in Table 1. According to the maternal age distribution of our population and the gestational age at the time of screening we would have expected 274 (95% prediction interval: 241–306) cases with trisomy 21[7–9].

Distribution of logMoM (NT)

Gaussian probability plots of logMoM (NT) in unaffected pregnancies at 11, 12 and 13 weeks' gestation are shown in Figure 1. Contrary to the assumptions of the MoM approach there are departures from the Gaussian form owing to the upper tail, especially at 11 weeks' gestation.

Non-parametric centile curves for NT by CRL

Non-parametric centile estimates for NT by CRL at 1%, 5%, 50%, 95% and 99% fitted to the data of unaffected pregnancies on a linear axis and on a logarithmic axis are shown in Figure 2. Under the assumptions of the delta NT approach the centiles in Figure 2a should be parallel, but instead the plot shows substantial deviations from parallelism. Most notably, the 99th centile is almost horizontal and increases with smaller CRLs, while the other centiles increase with CRL. This means that the distribution of delta NT is changing with gestation in a way that cannot be captured by a common non-parametric density estimate fitted to the data combined across all gestations.

Under the assumptions of the MoM approach, the centiles on the log scale in Figure 2a should be symmetric about the median according to a Gaussian distribution. Although they are reasonably symmetric for CRLs above 60 mm – corresponding to gestational ages above the middle of week 12 – there are marked departures from symmetry at lower CRLs.

Table 1 Characteristics of the study population

Parameter	*Median (range) or* n *(%)*
Maternal characteristics	
Age (years, median (range)	35.2 (16.0–52.0)
Weight (kg, median (range)	63.6 (39.0–150.0)
Spontaneous conception (*n* (%))	35 628 (95.0)
Smoker (*n* (%))	1458 (3.9)
Ethnicity (*n* (%))	
Caucasian	35 366 (94.3)
Afro-Caribbean	283 (0.8)
East Asian	378 (1.0)
South Asian	1191 (3.2)
Mixed	270 (0.7)
Gestational age (*n* (%))	
11 + 0 to 11 + 6 weeks	4413 (11.8)
12 + 0 to 12 + 6 weeks	20 602 (55.0)
13 + 0 to 13 + 6 weeks	12 473 (33.3)
Crown–rump length (mm, median (range))	62.6 (45.0–84.0)
Karyotype (*n* (%))	
Normal	37 078 (98.9)
Trisomy 21	264 (0.7)
Trisomy 18	81 (0.2)
Trisomy 13	38 (0.1)
Turner syndrome	27 (0.1)
Total	37 488

Mixture model

The fitted mixture model is described in Table 2. In the unaffected pregnancies the dominant part of the mixture is the CRL-dependent Gaussian distribution, which accounts for about 95% of cases. In contrast, in all chromosomally abnormal pregnancies the dominant part of the mixture is the CRL-independent Gaussian distribution, which accounts for about 95% of cases with trisomy 21, 70% of trisomy 18, 85% of trisomy 13 and 80% of Turner syndrome (Figure 3). The SD of logMoM (NT) from the fitted mixture model in unaffected pregnancies decreases with gestation (Figure 4). The SDs are substantially lower than in previous studies[10,11].

The estimated SD of the operator effects was 0.0289, which is small relative to the SD of the CRL-dependent distribution (0.079). Operator effects account for an estimated 12% [$0.0289^2/(0.079^2 + 0.0289^2) \times 100\%$] of the total variation from the CRL-dependent process with operator effects added.

In the mixture model, as in the MoM and delta NT methods, it is necessary to incorporate truncation limits to prevent misleading results at extremes and ensure that the likelihood ratio is a monotonic function of NT. The upper truncation limit in our model was 10 mm and the lower increased with CRL from 1.2 mm at 45 mm to 1.8 at 70 mm and then remained constant until 84 mm.

Detection rates and false positive rates

Crude, standardized and modeled detection rates for fixed false positive rates are shown in Table 3, and the accuracy of estimated risk for trisomy 21 by a combination of maternal age and fetal NT is shown in Table 4.

DISCUSSION

The findings of this study demonstrate that fetal NT follows two distributions, one of which is dependent on CRL while the other is independent of CRL. The distribution in which NT increases with CRL is the same for chromosomally abnormal and unaffected pregnancies but the proportion that follows this distribution is large in the unaffected group (about 95%) and small in the abnormal group, being about 5%, 30%, 15% and 10% for trisomies 21, 18, 13 and Turner syndrome, respectively. In contrast, the proportion of cases in which NT does not change with gestation is small

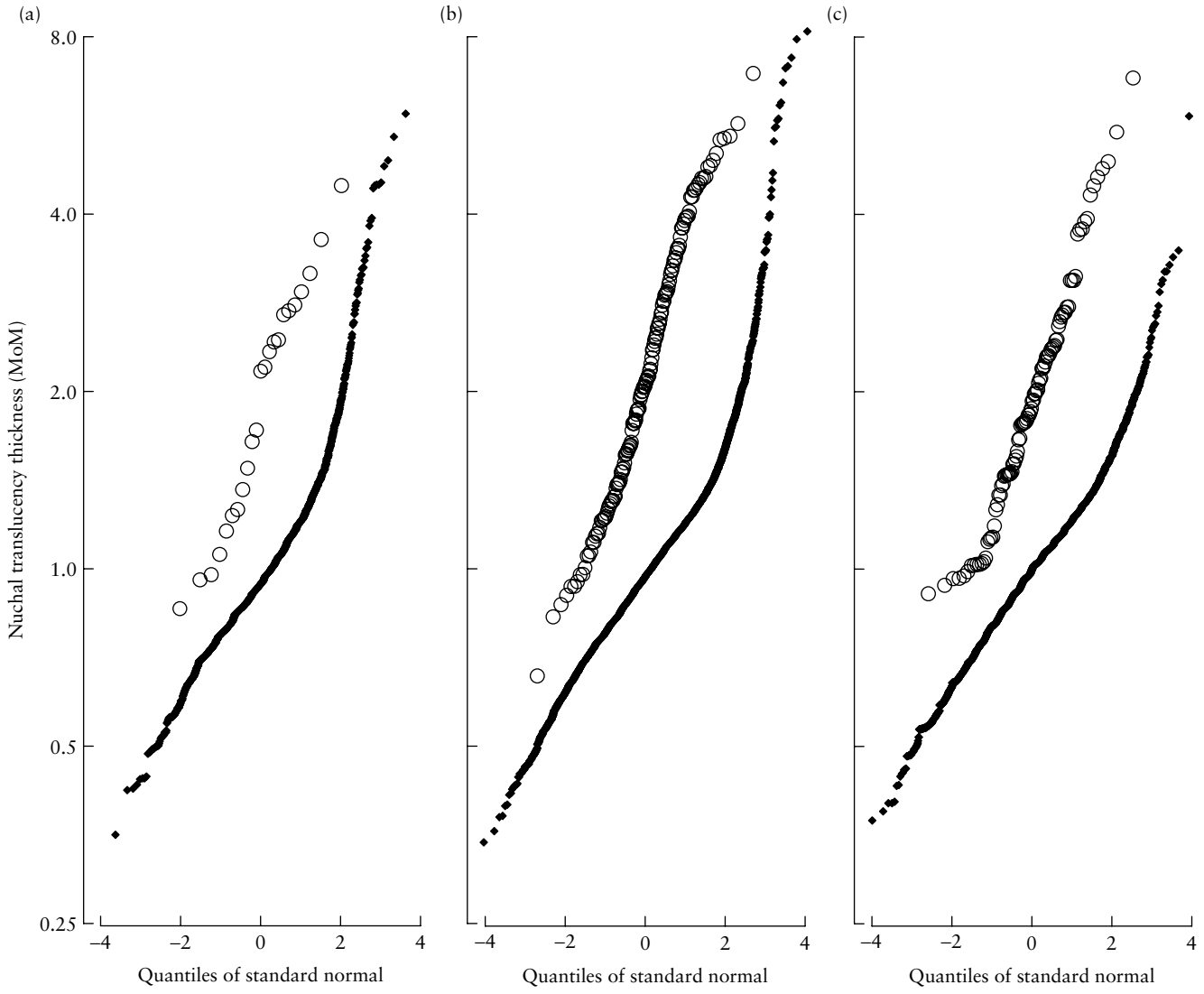


Figure 1 Gaussian probability plots of nuchal translucency thickness (multiples of the median (MoM)) in unaffected (♦) and trisomy 21 (○) pregnancies at 11 (a), 12 (b) and 13 (c) weeks of gestation. The vertical axis is a logarithmic scale.

for unaffected pregnancies and large for the abnormal group. Furthermore, the median NT is different, being 2.0 mm for the unaffected group and 3.4, 5.5, 4.0 and 7.8 mm for trisomies 21, 18, 13 and Turner syndrome, respectively.

The study has also highlighted the limitations of the two previous methods of assessing NT thickness. We found that, contrary to the assumption underlying the delta NT method, the non-parametric centile estimates for NT by CRL show that the distribution of delta NT changes with CRL. Similarly, the assumption of a Gaussian distribution that underpins the MoM method is not valid because there are departures from such a form in the logMoM (NT), and the centiles on the log scale are not symmetric about the median. The delta NT and MoM methods provide a measure of the deviation of an observed NT from its expected value. However, to produce accurate risks, the above assumptions need to be taken into account.

The mixture model of the form we propose is useful in situations where a single Gaussian or other distribution fails to provide an adequate fit. One of the earliest applications, published in 1894, was a mixture of two Gaussian distributions fitted by Karl Pearson on the ratio of forehead to body length in crabs sampled from the bay of Naples. This led to the conjecture that the crabs came from different species[12].

The mixture model of NT distributions is compatible with our understanding of the pathophysiology of increased NT in both chromosomally normal and abnormal fetuses[13–21]. The CRL-dependent NT distribution in unaffected pregnancies presumably reflects a physiological development of the fetal nuchal region during the gestational range of $11+0$ to $13+6$ weeks. The increased CRL-independent NT observed in the chromosomally normal fetuses could at least in some cases be a consequence of a wide range of well-reported non-chromosomal defects, such as cardiac, skeletal and other malformations, genetic syndromes and hematological disorders. The proportion of NT measurements arising from the CRL-independent process in the mixture model decreased with CRL from around 10% at a CRL of 45 mm to around 3%

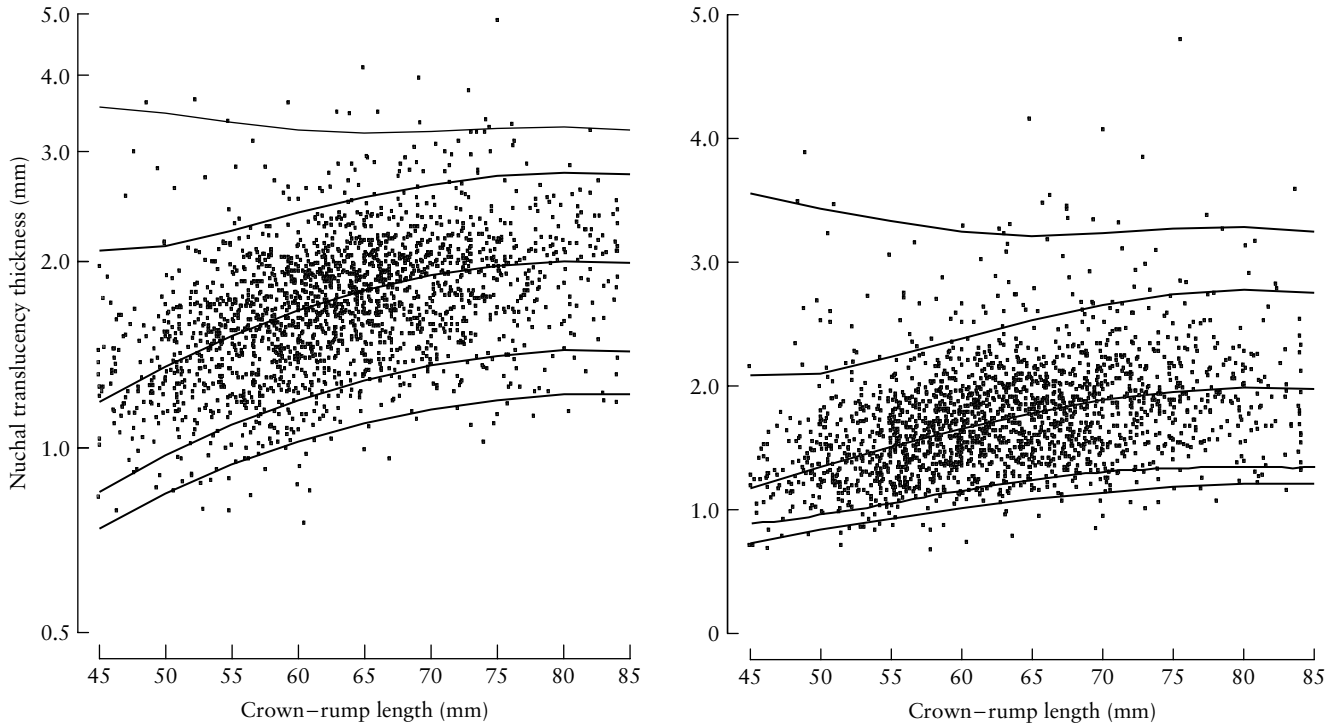


Figure 2 Distribution of nuchal translucency thickness with crown–rump length in unaffected pregnancies together with the modeled median and 1st, 5th, 50th, 95th and 99th centiles on a logarithmic scale (a) and on a linear scale (b).

Table 2 Fitted mixture model for nuchal translucency thickness

Parameter	*Estimate*	*90% CI*
CRL-dependent		
Intercept (β_0)	−0.8951	−0.9460 to −0.8423
Coefficient of CRL (β_1)	0.02940	0.02781 to 0.03101
Coefficient of CRL^2 (β_2)	−0.0001812	−0.0001935 to −0.0001686
Standard deviation (σ_0)	0.07900	0.07792 to 0.08008
CRL-independent: normal		
Logistic model intercept for mixture proportion		
Intercept (α_0)	−0.3319	−1.1220 to 0.4707
Coefficient of CRL (α_1)	−0.03790	−0.05208 to −0.02423
Mean (μ_1)	0.3019	0.2860 to 0.3195
Standard deviation (σ_1)	0.1945	0.1852 to 0.2045
CRL-independent: trisomy 21		
Proportion (p_{T21})	0.9406	0.0116 to 0.9913
Mean (μ_{T21})	0.5330	0.5052 to 0.5623
Standard deviation (σ_{T21})	0.2093	0.1925 to 0.2271
CRL-independent: trisomy 18		
Proportion (p_{T18})	0.7096	0.6188 to 0.7956
Mean (μ_{T18})	0.7439	0.6990 to 0.7875
Standard deviation (σ_{T18})	0.1658	0.1414 to 0.1972
CRL-independent: trisomy 13		
Proportion (p_{T13})	0.8376	0.6603 to 0.9804
Mean (μ_{T13})	0.6018	0.5100 to 0.6969
Standard deviation (σ_{T13})	0.2032	0.1414 to 0.2693
CRL-independent: Turner syndrome		
Proportion (p_{Turner})	0.8090	0.7793 to 0.9894
Mean (μ_{Turner})	0.9629	0.7624 to 1.0030
Standard deviation (σ_{Turner})	0.1316	0.1929 to 0.3905
Operator standard deviation (σ_{op})	0.02890	0.02337 to 0.3573

CRL, crown–rump length.

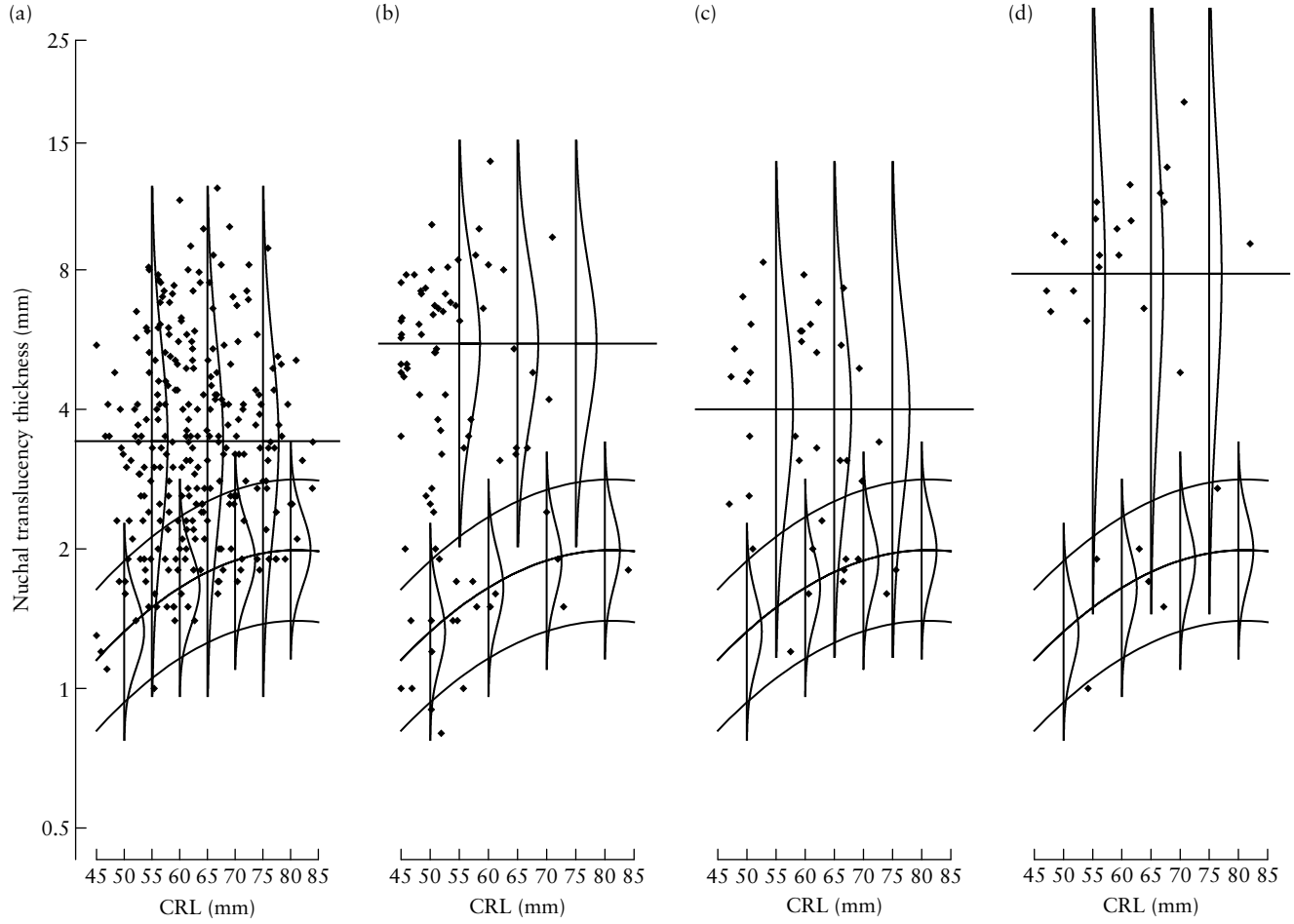


Figure 3 Mixture model with crown–rump length-independent (upper) and crown–rump length-dependent (lower) distribution in trisomies 21 (a), 18 (b) and 13 (c) and Turner syndrome (d).

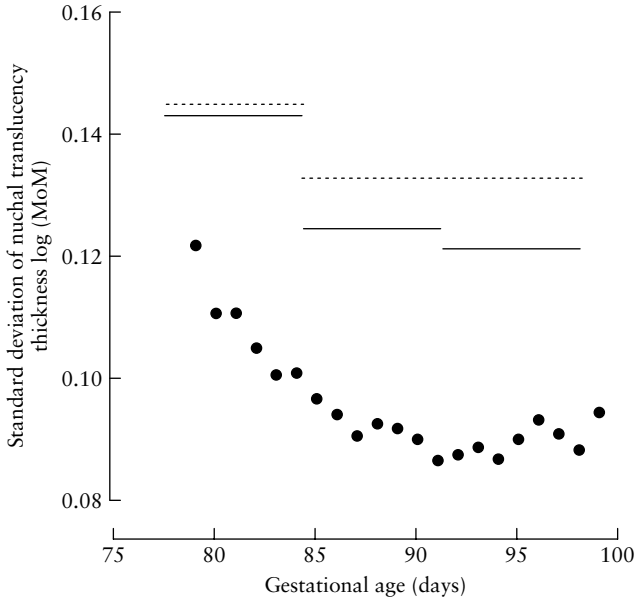


Figure 4 Relationship between the standard deviations of nuchal translucency thickness (log multiples of the median (MoM)) with gestational age in unaffected pregnancies in this study (●) and in two previous studies (SURUSS[10] (-------) and FASTER[11] (———)).

at a CRL of 85 mm. This most likely reflects the falling pregnancy loss rate with increasing gestational age.

The findings in the chromosomally abnormal fetuses are compatible with the wide range of phenotypic expression in these abnormalities. Presumably, in the 5–30% of cases with CRL-dependent NT distribution the development of the fetal nuchal region is normal. In the ones with increased CRL-independent NT there is abnormal nuchal development due either to chromosomal abnormality-specific primary alterations in the composition of the dermis and lymphatic channels or to secondary accumulation of subcutaneous fluid caused by associated cardiovascular, thoracic and other malformations.

The data confirm the high association between increased fetal NT and trisomy 21 as well as the other major chromosomal abnormalities. The detection rates for given false positive rates expected in population screening using the mixture model are similar to those observed in our cases after the appropriate adjustments to take into account the maternal age distribution of pregnancies in England and Wales (Table 3). Additionally, as shown in Table 4, the patient-specific risks derived from the new mixture model are accurate and valid for counseling. The high detection rates achieved by NT screening and the low SDs in this study re-emphasize the importance

Table 3 Detection rates of trisomy 21 for given false-positive rates

False-positive rate (%)	Detection rate (%)											
	Overall (n = 264)			*11 weeks* (n = 23)			*12 weeks* (n = 145)			*13 weeks* (n = 96)		
	Crude	*STD*	*Modeled*	*Crude*	*STD*	*Modeled*	*Crude*	*STD*	*Modeled*	*Crude*	*STD*	*Modeled*
1	58	54	55	44	36	56	61	60	56	61	61	53
2	64	65	64	48	48	68	66	71	66	66	68	61
3	69	72	70	52	51	74	72	76	71	71	70	65
4	76	77	73	57	63	78	79	83	75	75	74	68
5	77	80	75	57	63	81	81	84	77	78	76	70
10	83	87	83	70	89	89	87	88	84	82	86	77

The crude rates are the ones observed in our population with mean maternal age of 35 years, the standardized (STD) rates are the ones after adjustments to the maternal age distribution of pregnancies in England and Wales in 2000–2002[6] and the modeled rates are the standardized rates predicted from the mixture model in this study.

Table 4 Accuracy of estimated risk for trisomy 21 by a combination of maternal age and fetal nuchal translucency thickness

Estimated risk (range (median))	*Trisomy 21 (n (%))*	*Unaffected (n (%))*	*Observed risk*
≥ 1 in 10 (1 in 3)	129 (48.9)	218 (0.6)	1 in 3
1 in 10 to 1 in 100 (1 in 49)	66 (25.0)	1396 (3.8)	1 in 22
1 in 100 to 1 in 250 (1 in 185)	25 (9.5)	2684 (7.2)	1 in 108
1 in 250 to 1 in 1000 (1 in 583)	34 (12.9)	13 196 (35.6)	1 in 389
1 in 1000 to 1 in 5000 (1 in 1837)	10 (3.8)	17 636 (47.6)	1 in 1765
< 1 in 5000 (1 in 5200)	0	1948 (5.3)	—

of appropriate training and certification of competence of sonographers as well as regular audit of images and distributions of measurements[14].

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116).

REFERENCES

1. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First-Trimester Screening Group. *Lancet* 1998; **352**: 343–346.
2. Spencer K, Bindra R, Nix AB, Heath V, Nicolaides KH. Delta-NT or NT MoM: which is the most appropriate method for calculating accurate patient-specific risks for trisomy 21 in the first trimester? *Ultrasound Obstet Gynecol* 2003; **22**: 142–148.
3. Nicolaides KH, Snijders RJ, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. *Prenat Diagn* 1998; **18**: 519–523.
4. Avgidou K, Papageorghiou A, Bindra R, Spencer K, Nicolaides KH. Prospective first-trimester screening for trisomy 21 in 30,564 pregnancies. *Am J Obstet Gynecol* 2005; **192**: 1761–1767.
5. Gilks WR, Thomas A, Spiegelhalter DJ. A language and program for complex Bayesian modelling. *The Statistician* 1994; **43**: 169–177.
6. Office for National Statistics. *Birth Statistics. Review of the Registrar General on Births and Patterns of Family Building in England and Wales*. 2000–2002. Series FM1, Nos 29–31. Stationary Office: London.
7. Cuckle HS, Wald NJ, Thompson SG. Estimating a woman's risk of having a pregnancy associated with Down's syndrome using her age and serum alpha-fetoprotein level. *Br J Obstet Gynaecol* 1987; **94**: 387–402.
8. Bray IC, Wright DE. Estimating the spontaneous loss of Down syndrome fetuses between the times of chorionic villus sampling, amniocentesis and livebirth. *Prenat Diagn* 1998; **18**: 1045–1054.
9. Snijders RJM, Sebire NJ, Nicolaides KH. Maternal age and gestational age-specific risk for chromosomal defects. *Fetal Diagn Ther* 1995; **10**: 356–367.
10. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM; SURUSS Research Group. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *Health Technol Assess* 2003; **7**: 1–77.
11. Canick JA, Lambert-Messerlian GM, Palomaki GE, Neveux LM, Malone FD, Ball RH, Nyberg DA, Comstock CH, Bukowski R, Saade GR, Berkowitz RL, Dar P, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME. Comparison of serum markers in first-trimester Down syndrome screening. *Obstet Gynecol* 2006; **108**: 1192–1199.
12. Pearson K. Contributions to the mathematical theory of evolution. *Phil Trans Roy Soc London* 1894; **185**: 71–110.
13. Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ* 1992; **304**: 867–869.
14. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. *Am J Obstet Gynecol* 2004; **191**: 45–67.
15. Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation between increased fetal nuchal translucency thickness and chromosomal defects. *Obstet Gynecol* 2006; **107**: 6–10.
16. Molina FS, Avgidou K, Kagan KO, Poggi S, Nicolaides KH. Cystic hygromas, nuchal edema, and nuchal translucency

at 11–14 weeks of gestation. *Obstet Gynecol* 2006; **107**: 678–683.
17. Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. *Am J Obstet Gynecol* 2005; **192**: 1005–1021.
18. Hyett J, Perdu M, Sharland G, Snijders R, Nicolaides KH. Using fetal nuchal translucency to screen for major congenital cardiac defects at 10–14 weeks of gestation: population based cohort study. *BMJ* 1999; **318**: 81–85.
19. von Kaisenberg CS, Nicolaides KH, Brand-Saberi B. Lymphatic vessel hypoplasia in fetuses with Turner syndrome. *Hum Reprod* 1999; **14**: 823–826.
20. von Kaisenberg CS, Krenn V, Ludwig M, Nicolaides KH, Brand-Saberi B. Morphological classification of nuchal skin in fetuses with trisomy 21, 18 and 13 at 12–18 weeks and in a trisomy 16 mouse. *Anat Embryol* 1998; **197**: 105–124.
21. von Kaisenberg CS, Brand-Saberi B, Christ B, Vallian S, Farzaneh F, Nicolaides KH. Collagen type VI gene expression in the skin of trisomy 21 fetuses. *Obstet Gynecol* 1998; **91**: 319–323.

APPENDIX

Illustration of calculations

This paper describes a two-component mixture model for the distribution of log(NT) in chromosomally normal pregnancies and in pregnancies with trisomies 21, 18 and 13. We illustrate the calculations for a pregnancy with fetal CRL of 60 mm and NT of 2.5 mm (see Table 2).

CRL-dependent component (normal pregnancies)

- Estimated mean $= -0.8951 + (0.02940 \times 60) - (0.0001812 \times 60^2) = 0.2166$.
- Estimated standard deviation $= (0.07900^2 + 0.02890^2)^{0.5} = 0.08412$.
- The median NT for the CRL dependent process is $10^{0.2166} = 1.6466$
- The measured NT of 2.5 mm is equivalent to $2.5/1.6466 = 1.518$ MoM.
- The probability density at $\log(2.5) = 0.3979$ for the fitted Gaussian distribution is 0.4642.

CRL-independent component (normal pregnancies)

- Estimated mean = 0.3019
- Estimated standard deviation $= (0.1945^2 + 0.02890^2)^{0.5} = 0.1966$.
- The probability density at $\log(2.5) = 0.3979$ for the fitted Gaussian distribution is 1.8007.

Mixture model (normal pregnancies)

- According to the mixture for unaffected pregnancies, the fitted logit of the proportion arising from the CRL-independent process is given by $-0.3319 - (0.03790 \times 60) = -2.6059$. The fitted proportion is then given by $1/(1 + \exp(-(-2.6059))) = 0.06878$ (approximately 7% of observations of NT arise from the CRL-independent component).
- The probability density for unaffected pregnancies is given by a weighted average of two Gaussian densities: the CRL-independent process (weight = 0.06878) and the CRL-dependent process (weight $= 1 - 0.06878 = 0.93122$). This gives the fitted mixture model probability density of $(0.06878 \times 1.8007) + (0.93122 \times 0.4642) = 0.5561$.

CRL-independent component (trisomy 21 pregnancies)

- Estimated mean = 0.5330
- Estimated standard deviation $= (0.2093^2 + 0.02890^2)^{0.5} = 0.2113$.
- The probability density at $\log(2.5) = 0.3979$ for the fitted Gaussian distribution is 1.5393.

Mixture model (trisomy 21 pregnancies)

- According to the mixture model the estimated proportion of trisomy 21 pregnancies arising from the CRL-independent component is 0.9406. This gives the fitted mixture model density of $(0.9406 \times 1.5393) + (0.0594 \times 0.4642) = 1.4754$.

Likelihood ratio

The likelihood ratio of trisomy 21 to normal pregnancies is given by the probability density of trisomy 21 pregnancies divided by the probability density for normal pregnancies $= 1.4754/0.5561 = 2.653$.

Figure 5 shows the behavior of the likelihood ratio for the pregnancy with CRL = 60 mm and illustrates how with the mixture model the likelihood ratio rises steeply and then flattens as NT increases. This reflects the CRL independent component of the mixture model.

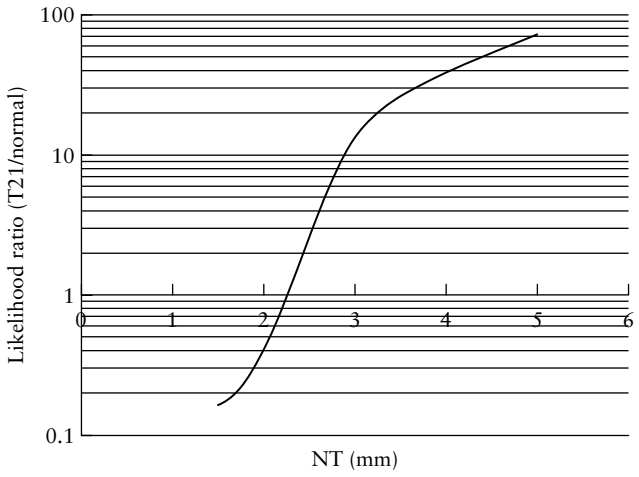


Figure 5 Relationship between likelihood ratio and nuchal translucency (NT) thickness for a crown–rump length of 60 mm.

Ultrasound Obstet Gynecol 2008; **31**: 493–502
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5332

First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics

K. O. KAGAN*†, D. WRIGHT‡, K. SPENCER*§, F. S. MOLINA*¶ and K. H. NICOLAIDES*

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, ‡Department of Mathematics and Statistics, University of Plymouth, Plymouth, §Prenatal Screening Unit, Clinical Biochemistry Department, King George Hospital, Goodmayes, UK, †Department of Obstetrics and Gynecology, University of Tuebingen, Tuebingen, Germany and ¶Hospital Universitario Virgen de las Nievas, Granada, Spain*

KEYWORDS: first-trimester screening; free β-hCG; PAPP-A; trisomy 21

ABSTRACT

Objectives *To use multiple regression analysis to define the contribution of maternal variables that influence the measured concentration of free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), and the interaction between these covariates, in first-trimester biochemical screening for trisomy 21.*

Methods *This was a multicenter study of prospective screening for trisomy 21 by a combination of fetal nuchal translucency thickness, and maternal serum free β-hCG and PAPP-A at 11 + 0 to 13 + 6 weeks of gestation. In the pregnancies subsequently found to have trisomy 21 and in those with no obvious chromosomal abnormality, we used multiple regression analysis to account for pregnancy characteristics that influence the measured concentrations of free β-hCG and PAPP-A. We fitted Gaussian distributions to the distribution of log multiples of the median (MoM) values in trisomy 21 and in unaffected pregnancies.*

Results *There were 491 cases of trisomy 21 and 96 803 chromosomally normal pregnancies. Compared with values in Caucasian women, those who were parous, non-smokers and those who conceived spontaneously, PAPP-A was 57% higher in women of Afro-Caribbean origin, 3% higher in South Asians, 9% higher in East Asians, 2% higher in nulliparous women, 17% lower in smokers and 10% lower in those conceiving by* in-vitro *fertilization (IVF). Free β-hCG was 12% higher in women of Afro-Caribbean origin, 9% lower in South Asians, 8% higher in East Asians, 2% higher in nulliparous women, 4% lower in smokers and 9% higher in those conceiving by IVF. In screening for trisomy 21 by maternal age and serum free β-hCG and PAPP-A the estimated detection rate was 65% for a false-positive rate of 5%.*

Conclusions *In first-trimester biochemical screening for trisomy 21 it is essential to adjust the measured values of free β-hCG and PAPP-A for maternal and pregnancy characteristics. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

Screening for trisomy 21 by fetal nuchal translucency (NT) thickness and maternal serum free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11 + 0 to 13 + 6 weeks of gestation detects about 90% of affected pregnancies for a false-positive rate of 5%[1–4].

In trisomy 21 pregnancies maternal serum free β-hCG is about twice as high and PAPP-A is reduced to about half compared with values in chromosomally normal pregnancies. In the development of risk algorithms for combined screening the estimation of accurate patient-specific risks necessitates adjustments in the measured free β-hCG and PAPP-A to take into account their association with gestational age, maternal weight, ethnicity, smoking status and method of conception[1,5–11]. Essentially, each measured level is first converted to a multiple of the expected normal median (MoM) specific to a pregnancy of the same gestational age, maternal weight, smoking

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital Medical School, Denmark Hill, London SE5 8RX, UK (e-mail: fmf@fetalmedicine.com)

Accepted: 7 March 2008

status, ethnicity and method of conception. The MoM distribution of each metabolite in unaffected and trisomy 21 pregnancies is assumed to be Gaussian, specified in terms of means, SD and correlations.

In this study of 491 pregnancies with trisomy 21 and 96 803 unaffected pregnancies we used multiple regression analysis, instead of a sequential approach, to take into account the pregnancy characteristics that influence the measured concentration of free β-hCG and PAPP-A. The multiple regression model was then used to estimate likelihood ratios for the biochemical markers that can be combined with maternal age to produce patient-specific risks for each case.

METHODS

The data for this study were derived from prospective assessment of risk for trisomy 21 by a combination of maternal age, fetal NT thickness, and maternal serum PAPP-A and free β-hCG at 11 + 0 to 13 + 6 weeks of gestation[3]. Two groups of UK hospitals were included. In Group A (Fetal Medicine Centre, London; King's College Hospital, London) the ultrasound and biochemical measurements and assessment of risk were carried out in the same hospital visit. In Group B (Harold Wood Hospital, Romford; King George Hospital, Goodmayes; Canterbury Hospital, Canterbury; William Harvey Hospital, Ashford; Queen Elizabeth The Queen Mother's Hospital, Margate), the biochemical measurements and assessment of risk were carried out either in the same hospital visit, on the same day, or 1 day before or after the ultrasound scan.

Transabdominal ultrasound examination was performed to diagnose any major fetal defects and for measurement of the crown–rump length (CRL) and fetal NT thickness[12]. Measurements of PAPP-A and free β-hCG were carried out by an automated machine that provides reproducible results within 30 min (Kryptor system, Brahms AG, Berlin, Germany; or Delfia Express system, Perkin Elmer, Waltham, MA, USA). Gestational age was based on the CRL at the time of the screening, and was calculated using the formula obtained from Robinson and Fleming[13].

Maternal weight, measured at the time of the scan, demographic characteristics, ultrasonographic measurements and biochemical results were recorded in computer databases. Ethnic origin was assessed by self-classification and included Caucasian, South Asian (Indian, Pakistani, Bangladeshi), Afro-Caribbean, East Asian (Chinese, Korean, Japanese), and mixed ethnicity, which mainly consisted of those of Caucasian and Afro-Caribbean origin. Smoking status was similarly based on a self-completed questionnaire, and each woman was classified as either a non-smoker or smoker irrespective of the individual cigarette consumption. Women were grouped as parous if they had previous deliveries beyond 23 weeks and nulliparous if they had no pregnancies resulting in delivery beyond 23 weeks. Mode of conception was either spontaneous, including those receiving ovulation induction drugs, or assisted reproduction by *in-vitro* fertilization (IVF). Karyotype results and details of pregnancy outcomes were added to the databases as soon as they became available.

A search of the databases was done to identify all singleton pregnancies in which first-trimester screening by NT, PAPP-A and free β-hCG was carried out between June 1999 and December 2006.

Statistical analysis

The statistical analysis was performed on the separate datasets A and B, and on the combined data. Forest plots showing point-wise 95% CI for effects from the two sources and the combined source were produced.

Multiple regression analysis of log-transformed marker values was carried out to provide estimates of parameters required to produce log MoM values for PAPP-A and free β-hCG[14]. Quadratic terms were used to model the effect of gestational age and maternal weight. Effects of the machine used for biochemical analysis, maternal ethnic origin, smoking status and method of conception were included as factors in the multiple regression models. Parity was analyzed separately owing to incomplete data on this factor. The mean log MoM in trisomy 21 was represented as a linear function of gestational age. Bivariate Gaussian models were fitted to the distribution of log MoM PAPP-A and log MoM free β-hCG in trisomy 21. In the analyses observations falling outside the 99.99th contour were considered as outliers and removed. For the analysis of the pooled dataset, a term was used to model the difference between sources.

Likelihood ratios were computed from the fitted distribution and used with maternal age to produce patient-specific risks for each case. Crude detection rates and false-positive rates were calculated by taking the proportions with risks above a given risk threshold. Maternal age-specific detection and false-positive rates were then produced, and adjusted according to the maternal age distribution of pregnancies in England and Wales in 2000–2002[15]. These standardized rates were compared with detection and false-positive rates estimated using Monte Carlo methods to sample from the modeled Gaussian distributions.

RESULTS

Data description

In Group A, the search of the databases identified 45 668 singleton pregnancies in which first-trimester combined screening was carried out, and data on maternal weight, ethnic origin, smoking status and mode of conception were available. We excluded 1869 (4.1%) cases from further analysis because there were no data on fetal karyotype or pregnancy outcome ($n = 1605$) or there was an abnormal karyotype other than trisomy 21 ($n = 264$). The remaining data included 43 478 unaffected cases and 321 cases with trisomy 21. In Group B, the search of

the database identified 55 482 singleton pregnancies in which first-trimester combined screening was carried out, and data on maternal weight, ethnic origin, smoking status and mode of conception were available. We excluded 1987 (3.6%) cases from further analysis because there were no data on fetal karyotype or pregnancy outcome ($n = 1866$) or there was an abnormal karyotype other than trisomy 21 ($n = 121$). The remaining data included 53 325 unaffected pregnancies and 170 cases with trisomy 21. In summary, the pooled dataset consisted of 96 803 unaffected pregnancies and 491 cases with trisomy 21.

In the pooled dataset the median maternal age was 32.8 (range, 14–53; mean (SD), 32.1 (5.7)) years, the median gestational age at screening was 12 + 5 (range, 11 + 0 to 13 + 6) weeks and the median CRL was 62.8 (range, 45.0–84.0) mm (Table 1). The median maternal age of pregnancies in England and Wales in 2000–2002 was 29.0 years.

Distribution of log MoM in unaffected and trisomy 21 pregnancies

Free β-hCG and PAPP-A values were outside the 99.99th contours for unaffected and trisomy 21 pregnancies in 84/96 803 (0.09%) and 1/491 (0.2%) cases, respectively and these were excluded from further analysis.

In the unaffected pregnancies (pooled dataset) multiple regression analysis demonstrated that, compared with values in Caucasian women who were parous, non-smokers and conceived spontaneously, PAPP-A MoM was 57% higher in women of Afro-Caribbean origin, 3% higher in South Asians, 9% higher in East Asians, 2%

Table 2 Adjustment factors for multiples of the median values of pregnancy-associated plasma protein-A (PAPP-A) and serum free beta-human chorionic gonadotropin (β-hCG) derived from the pooled dataset

	Coefficient			
	Log_{10} *scale*		*Original scale*	
Parameter	*Estimate*	*Standard error*	*Estimate*	*95% CI*
PAPP-A				
Afro-Caribbean	0.195	0.004	1.566	1.540–1.592
South Asian	0.012	0.003	1.028	1.014–1.042
Mixed	0.036	0.010	1.085	1.038–1.135
East Asian	0.039	0.009	1.093	1.048–1.140
Smoker	−0.082	0.002	0.828	0.819–0.836
Conception by IVF	−0.047	0.005	0.897	0.878–0.918
Nulliparous	0.009	0.002	1.020	1.012–1.028
Delfia Express	−0.123	0.003	0.753	0.743–0.763
Group B effect	0.006	0.002	1.015	1.007–1.022
Free β-hCG				
Afro-Caribbean	0.049	0.004	1.121	1.099–1.143
South Asian	−0.043	0.004	0.905	0.891–0.920
Mixed	−0.025	0.011	0.945	0.898–0.994
East Asian	0.032	0.011	1.076	1.024–1.129
Smoker	−0.018	0.003	0.959	0.948–0.971
Conception by IVF	0.037	0.006	1.088	1.061–1.116
Nulliparous	0.007	0.002	1.016	1.007–1.026
Delfia Express	0.022	0.003	1.052	1.036–1.068
Group B effect	0.006	0.002	1.013	1.004–1.021

Adjustments were for other races relative to Caucasian, smokers relative to non-smokers, *in-vitro* fertilization (IVF) relative to spontaneous conception, nulliparous relative to parous, Delfia Express relative to Kryptor system, and Group B relative to Group A.

Table 1 Characteristics of the study population

Parameter	*Group A* (n = *43 799*)	*Group B* (n = *53 495*)
Maternal characteristics		
Age (years)	35.4 (16.5–52.1)	30.5 (13.5–53.0)
Weight (kg)	63.6 (34.0–150.0)	65.4 (29.6–192.0)
Spontaneous conception	41 879 (95.6)	53 300 (99.6)
Smoker	2044 (4.7)	9288 (17.4)
Nulliparous	19 419 (44.3)	7596 (14.2)
Ethnicity		
Caucasian	39 661 (90.6)	46 803 (87.5)
Afro-Caribbean	1614 (3.7)	2319 (4.3)
East Asian	471 (1.1)	91 (0.2)
South Asian	1538 (3.5)	4282 (8.0)
Mixed	515 (1.2)	0 (0)
Gestational age		
11 + 0 to 11 + 6 weeks	4628 (10.6)	6752 (12.6)
12 + 0 to 12 + 6 weeks	24 303 (55.5)	29 098 (54.4)
13 + 0 to 13 + 6 weeks	14 868 (33.9)	17 645 (33.0)
Crown–rump length (mm)	62.9 (45.0–84.0)	62.7 (45.0–84.0)
Karyotype		
Normal	43 478 (99.3)	53 325 (99.7)
Trisomy 21	321 (0.7)	170 (0.3)

Values are median (range) or *n* (%). Group A, Fetal Medicine Centre and King's College Hospital; Group B, Harold Wood Hospital, King George Hospital, Canterbury Hospital, William Harvey Hospital and Queen Elizabeth The Queen Mother's Hospital.

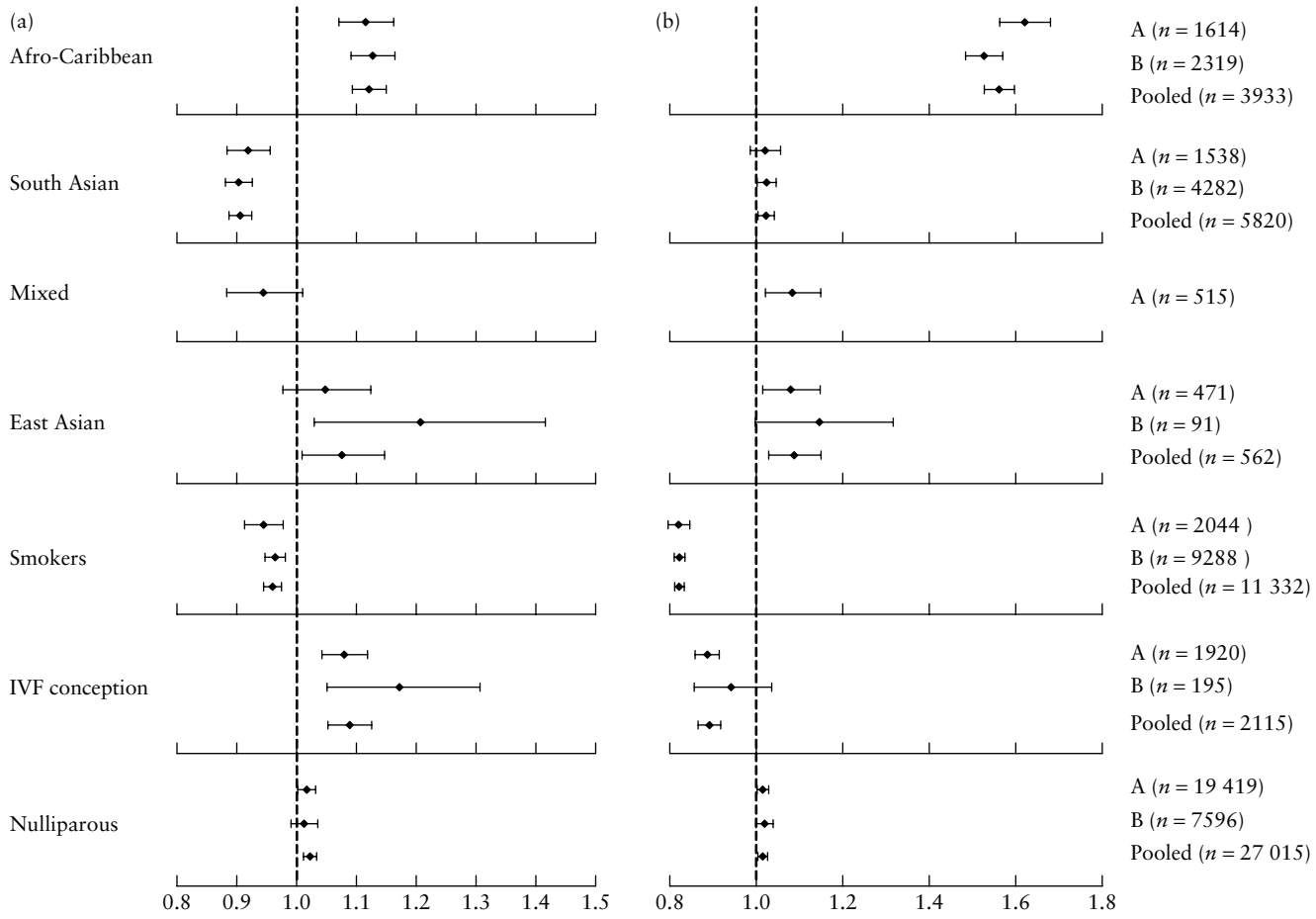


Figure 1 Estimated effects on free beta-human chorionic gonadotropin (a) and pregnancy-associated plasma protein-A (b) of different ethnic groups relative to Caucasian, of smokers relative to non-smokers, of *in-vitro* fertilization (IVF) relative to spontaneous conception, and of nulliparous relative to parous women. Effects are shown with 95% CI.

Table 3 Parameter estimates and correlation for unaffected and trisomy 21 cases

Parameter	*Karyotype*	n	*Estimate*	*95% CI*
SD of log MoM PAPP-A	Normal	96 719	0.2203	0.2190–0.2216
	Trisomy 21	490	0.2359	0.2179–0.2570
SD of log MoM free β-hCG	Normal	96 719	0.2544	0.2529–0.2559
	Trisomy 21	490	0.2699	0.2493–0.2940
Correlation	Normal	96 719	0.2143	0.2064–0.2222
	Trisomy 21	490	0.0821	−0.0344 to 0.1964

β-hCG, beta-human chorionic gonadotropin; MoM, multiples of the median; PAPP-A, pregnancy-associated plasma protein-A.

higher in nulliparous women, 17% lower in smokers and 10% lower in those conceiving by IVF. Free β-hCG MoM was 12% higher in women of Afro-Caribbean origin, 9% lower in South Asians, 8% higher in East Asians, 2% higher in nulliparous women, 4% lower in smokers and 9% higher in those conceiving by IVF. The results are shown in Figure 1 and Table 2. Parameter estimates for the fitted Gaussian distributions are shown in Figure 2 and Table 3.

The fitted equation for estimating median log metabolite values in chromosomally normal pregnancies from gestation (in days) and weight (in kg) were:

$$\begin{aligned}\text{Median } \log_{10} \text{ free } \beta\text{-hCG} &= 1.64931 - 0.0057856 \times (\text{gestation} - 77) \\ &\quad - 0.00023901 \times (\text{gestation} - 77)^2 - 0.0045501 \\ &\quad \times (\text{weight} - 69) + 0.000028909 \times (\text{weight} - 69)^2\end{aligned}$$

$$\begin{aligned}\text{Median } \log_{10} \text{ PAPP-A} &= 0.18992 + 0.026102 \times (\text{gestation} - 77) - 0.0074642 \\ &\quad \times (\text{weight} - 69) + 0.000030669 \times (\text{weight} - 69)^2.\end{aligned}$$

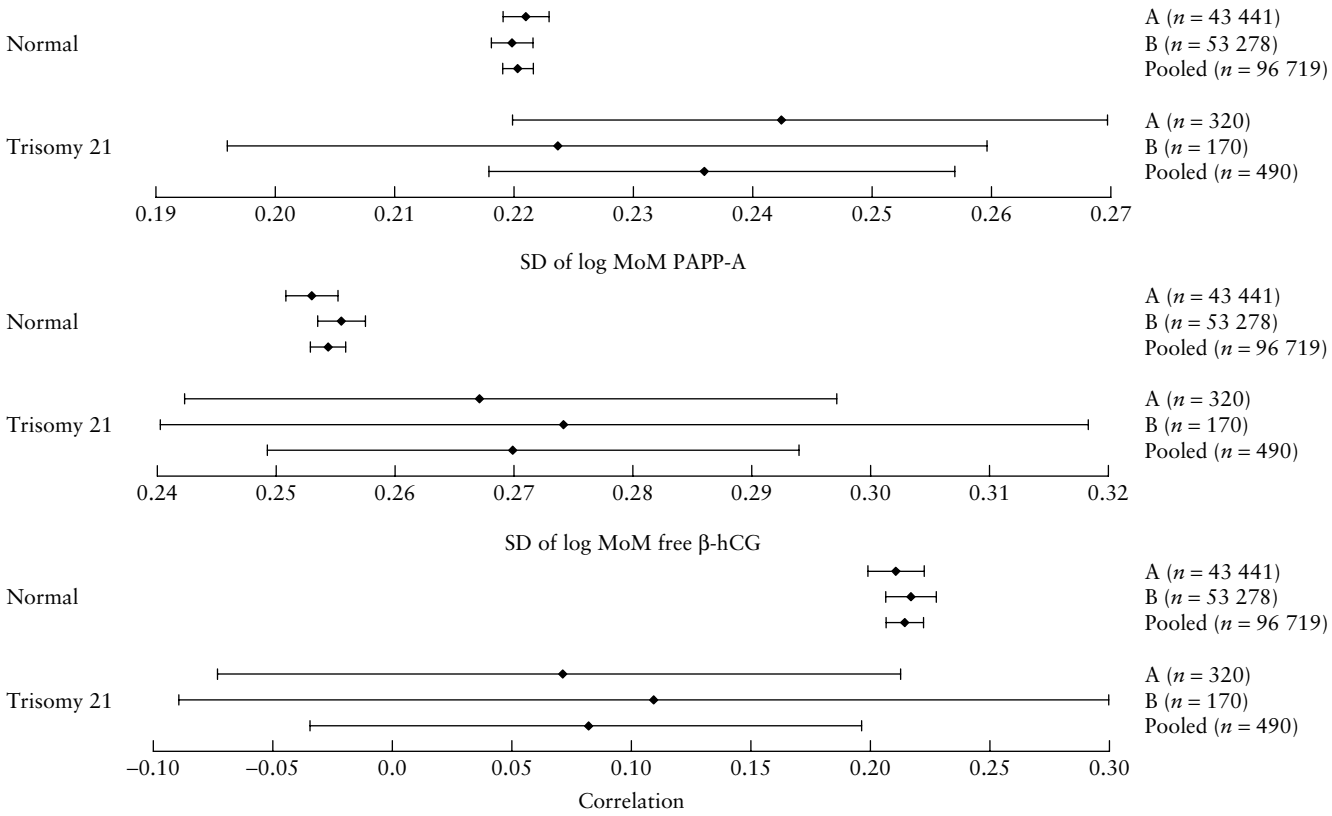


Figure 2 Parameter estimated SDs and correlations with 95% CIs for trisomy 21 and unaffected pregnancies. β-hCG, beta-human chorionic gonadotropin; MoM, multiple of the median; PAPP-A, pregnancy-associated plasma protein-A.

Table 4 Detection rates of trisomy 21 for given false-positive rates (FPR)

	Detection rate (%)											
	Overall (n = *491)*			*11 weeks* (n = *52)*			*12 weeks* (n = *273)*			*13 weeks* (n = *166)*		
FPR (%)	*Crude*	*Standardized*	*Modeled*	*Crude*	*Standardized*	*Modeled*	*Crude*	*Standardized*	*Modeled*	*Crude*	*Standardized*	*Modeled*
1	40	37	45	37	38	54	43	43	46	34	33	40
2	50	51	55	52	69	64	54	56	56	43	44	49
3	58	57	61	67	79	70	60	61	62	52	49	55
4	63	60	65	73	79	74	65	64	66	54	53	60
5	66	65	68	73	86	77	70	68	69	58	57	62
10	78	79	78	90	98	85	79	80	79	70	79	74

The crude rates are those observed in our population, the standardized rates are rates after adjustments for the maternal age distribution of pregnancies in England and Wales in 2000–2002, and the modeled rates are the standardized rates predicted from the fitted Gaussian model in this study.

Table 5 Accuracy of estimated risk for trisomy 21 by a combination of maternal age, and serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A

Estimated risk				
Range	*Median*	*Trisomy 21* (n *(%))*	*Chromosomally normal* (n *(%))*	*Observed risk*
1 in 10 or higher	1 in 5	158 (32.2)	757 (0.8)	1 in 6
1 in 10 to 1 in 100	1 in 52	185 (37.7)	5711 (5.9)	1 in 32
1 in 100 to 1 in 250	1 in 169	68 (13.8)	7105 (7.3)	1 in 105
1 in 250 to 1 in 1000	1 in 555	56 (11.4)	21 589 (22.3)	1 in 387
1 in 1000 to 1 in 5000	1 in 2249	20 (4.1)	35 102 (36.3)	1 in 1756
1 in 5000 or lower	1 in 10 628	4 (0.8)	26 539 (27.4)	1 in 6636

These apply to non-smoking, parous, Caucasian women who conceived spontaneously and whose assays were performed using the Kryptor machine. Adjustment factors for other groups are given in Table 2. Forest plots for the effects of ethnicity, smoking, IVF and parity are shown in Figure 1.

The distributions of log MoM values in unaffected and trisomy 21 pregnancies were well approximated by a bivariate Gaussian model with a mean of zero for unaffected pregnancies. For trisomy 21 pregnancies the mean log MoM depended on gestation according to the fitted regression models:

$$\text{Free } \beta\text{-hCG}(\log_{10}\text{MoM}) = 0.2468 + 0.004267 \times (\text{gestation} - 77)$$

$$\text{PAPP-A}(\log_{10}\text{MoM} = -0.4668 + 0.01642 \times (\text{gestation} - 77)$$

The fitted regression lines with 95% confidence limits are shown with the log MoM values in Figure 3. SD values and correlations for the fitted bivariate Gaussian distributions are given in Table 3. Gaussian probability plots for log MoM values of free β-hCG and PAPP-A are shown in Figure 4.

Empirical and model-based predictions

Table 4 shows the crude, standardized (for the maternal age distribution in England and Wales in 2000–2002) and modeled false-positive and detection rates. The observed prevalence of trisomy 21 according to the predicted risk, based on maternal age and serum free β-hCG and PAPP-A, is shown in Table 5.

Effect of maternal weight and fetal crown–rump length on patient-specific risk

The effect of changes in weight on computed MoM values and trisomy 21 risk is shown in Figure 5. In the example it is assumed that the pregnant woman is 35 years old, Caucasian, parous and a non-smoker who conceived spontaneously, with a fetal CRL of 65 mm and serum concentrations of PAPP-A and free β-hCG of 3.7 U/L and 40 U/L respectively measured by the Kryptor system at 12 weeks of gestation. The maternal serum concentrations of PAPP-A and free β-hCG expressed as MoMs increase with maternal weight and the patient-specific risk of trisomy 21 decreases with maternal weight.

The effect of fetal CRL on patient-specific risk for trisomy 21 is illustrated in Figure 6. The assumptions are the same as in the example in Figure 5 but, in addition, the maternal weight is 65 kg. The maternal serum concentrations of PAPP-A and free β-hCG expressed as MoMs decrease and increase respectively with fetal CRL, and the patient-specific risk of trisomy 21 increases with fetal CRL.

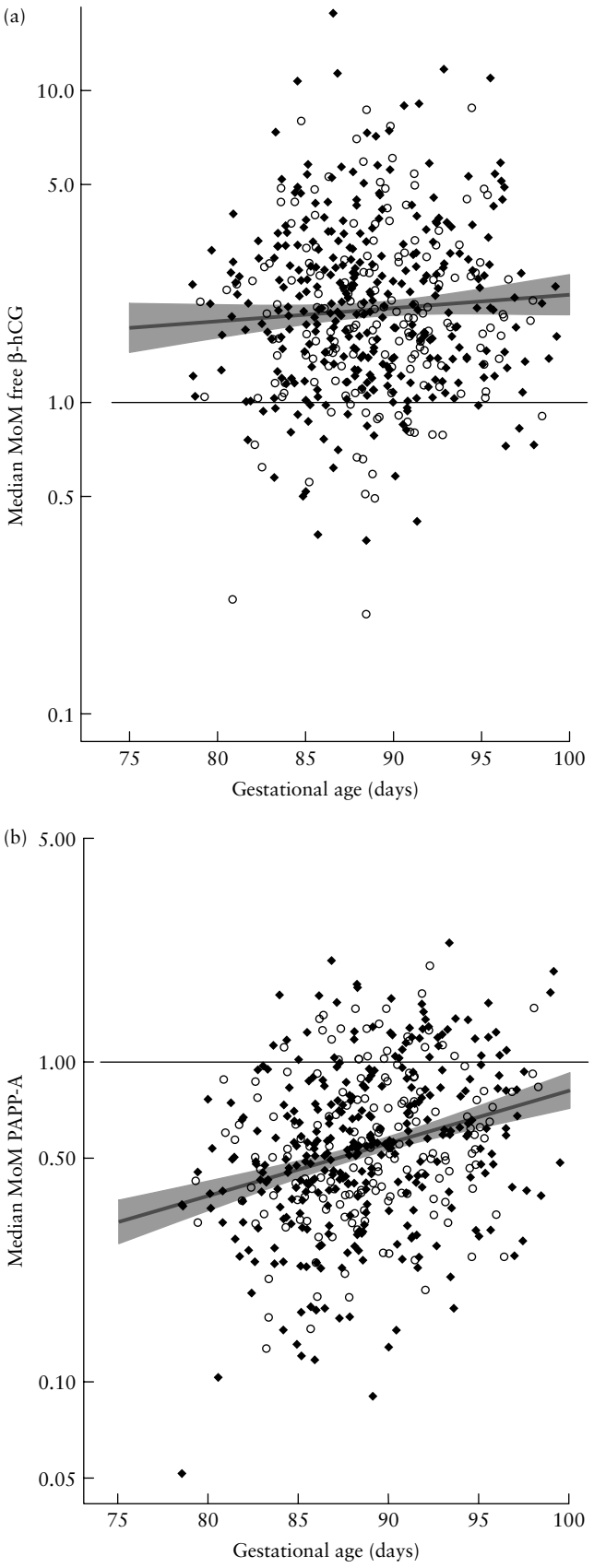


Figure 3 Multiples of the median (MoM) values of free beta-human chorionic gonadotropin (β-hCG) (a) and pregnancy-associated plasma protein-A (PAPP-A) (b) for trisomy 21 pregnancies, with fitted regression line and 95% CI (shaded area). ♦, Group A; ○, Group B.

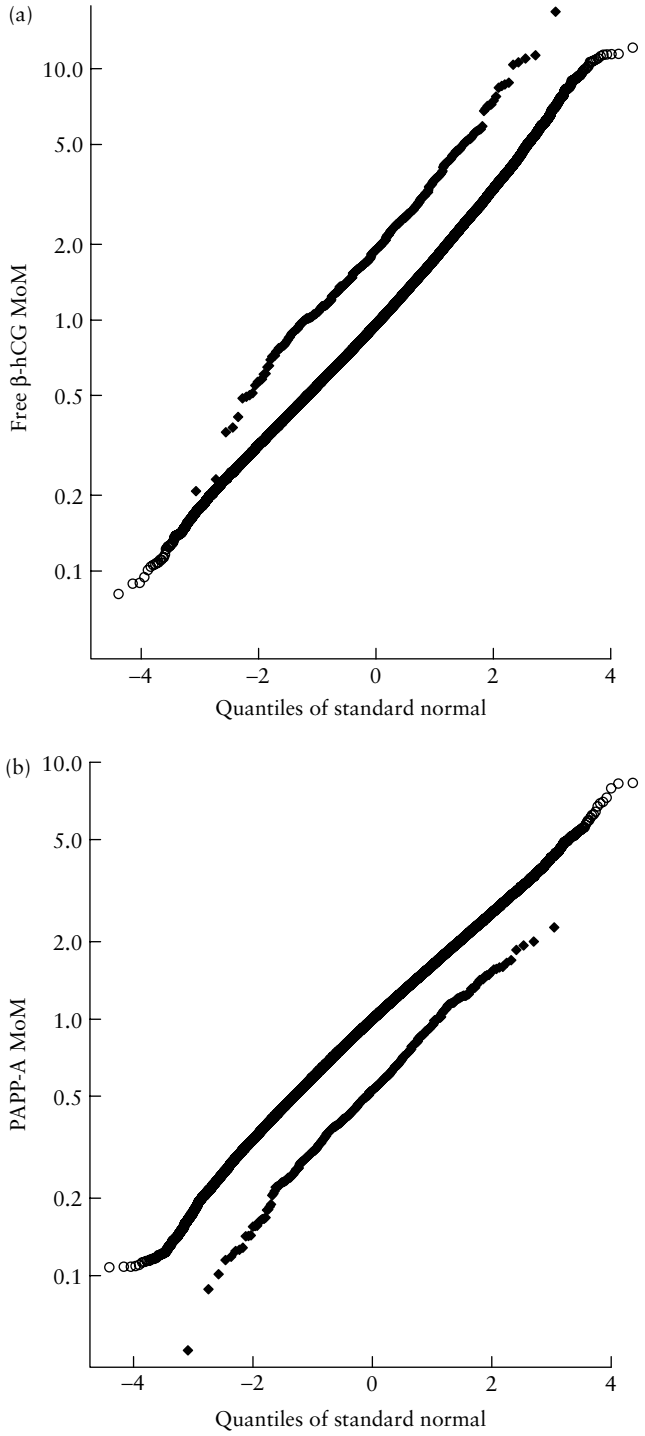


Figure 4 Gaussian probability plots for log multiples of the median (MoM) values of free beta-human chorionic gonadotropin (β-hCG) (a) and pregnancy-associated plasma protein-A (PAPP-A) (b), showing unaffected cases (○) and trisomy 21 cases (♦).

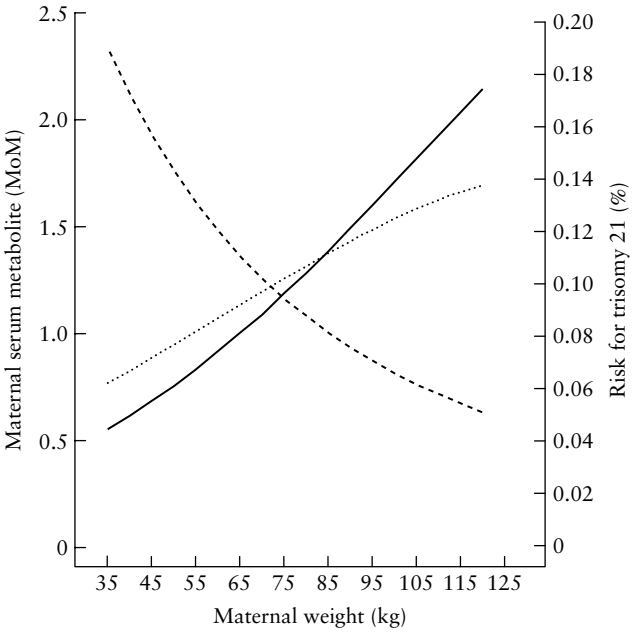


Figure 5 Effect of maternal weight on computed multiples of the median (MoM) values for pregnancy-associated plasma protein-A (PAPP-A) (———) and free beta-human chorionic gonadotropin (β-hCG) (·····) and on computed risk for trisomy 21 (- - - - - - -). These results apply to a pregnancy in a 35-year-old woman, with fetal crown–rump length 65 mm and with concentrations of PAPP-A and free β-hCG of 3.7 U/L and 40 U/L, respectively.

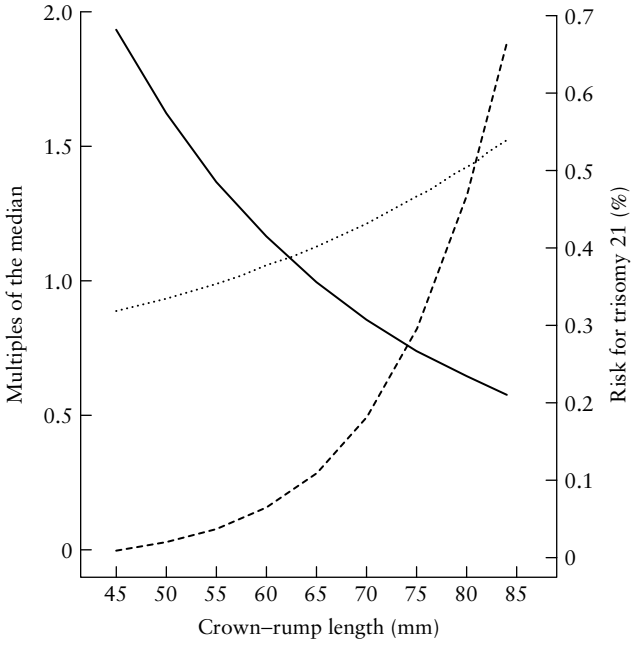


Figure 6 Effect of crown–rump length on computed multiples of the median (MoM) values for pregnancy-associated plasma protein-A (PAPP-A) (———) and free beta-human chorionic gonadotropin (β-hCG) (·····) and on computed risk for trisomy 21 (- - - - - - -). These results apply to a pregnancy in a 35-year-old woman with maternal weight 65 kg and with concentrations of PAPP-A and free β-hCG of 3.7 U/L and 40 U/L, respectively.

Failure to adjust for maternal characteristics

Table 6 shows the false-positive and detection rates of screening by maternal serum biochemistry for trisomy 21 if no adjustments are made for maternal characteristics, other than maternal weight and gestation. At a risk cut-off of 1 in 100 at 12 weeks of gestation in a reference group of Caucasian, parous, non-smoking women who conceived spontaneously, and with measurement of analytes by the Kryptor system, the false-positive and detection rates would be 4.6% and 68%, respectively. If the women were of Afro-Caribbean origin and the appropriate adjustments

Table 6 False-positive and detection rates of trisomy 21 at a risk cut-off of 1 in 100 in screening by a combination of maternal age, and maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A at 12 weeks of gestation

Group	*False-positive rate (%)*	*Detection rate (%)*
Reference group	4.6	68
Afro-Caribbean	1.4	51
South Asian	3.3	63
East Asian	4.2	66
Smoker	7.0	74
IVF conception	7.6	75
Nulliparous	4.5	68
Delfia Express	11	80

These are modeled performance measures under the assumption that no adjustments are made for maternal characteristics. The reference group comprises Caucasian, parous, non-smoking women with spontaneous conception and measurement of analytes by the Kryptor system. IVF, *in-vitro* fertilization.

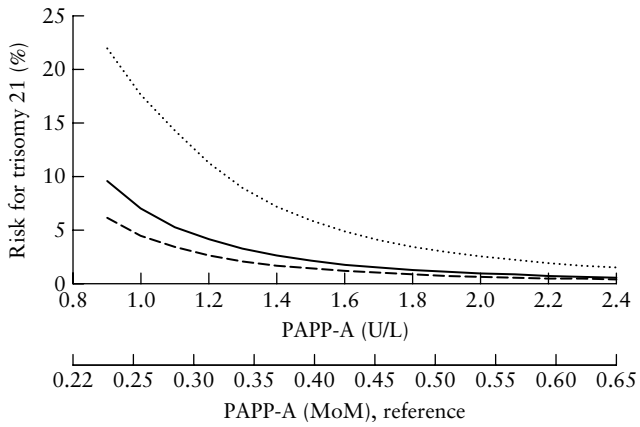


Figure 7 Patient-specific risk for trisomy 21 in a reference woman (———), an Afro-Caribbean woman (·····) and a cigarette smoker (- - - - - - -) in relation to the measured maternal serum concentration of pregnancy-associated plasma protein-A (PAPP-A). The reference woman is a 35-year-old Caucasian, who is a non-smoker, parous, weighs 65 kg and conceived spontaneously; the fetal crown–rump length is 65 mm, and maternal serum free beta-human chorionic gonadotropin (β-hCG) and PAPP-A were measured with the Kryptor analyzer. The free β-hCG concentration was 53 U/L, which corresponds to 1.5 multiples of the median (MoM) in the reference woman, 1.34 MoM in the Afro-Caribbean woman and 1.56 MoM in the cigarette smoker. The lower *x*-axis shows the PAPP-A MoM values corresponding to the measured concentration in the reference woman.

were made, at the same risk cut-off of 1 in 100, the false-positive and detection rates would also be 4.6% and 68%, but without adjustments the respective values would be 1.4% and 51%. Similarly, without appropriate adjustments the false-positive rates for cigarette smokers and women conceiving by IVF would be 7.0% and 7.6%, with respective detection rates of 74% and 75%.

Table 7 illustrates the effect of maternal characteristics on the patient-specific estimated risk of trisomy 21. For example, an Afro-Caribbean woman with a risk estimate of 1 in 40 would have been given a risk of 1 in 100 if her ethnic origin was not taken into account.

The correct estimated patient-specific risk for trisomy 21 for the same measured maternal serum concentrations of free β-hCG and PAPP-A depends on the maternal characteristics. This is illustrated in Figure 7, which shows the patient-specific risk in a reference woman, an Afro-Caribbean and a cigarette smoker in relation to various serum concentrations of PAPP-A and a fixed concentration of free β-hCG.

DISCUSSION

This study confirms that in trisomy 21 pregnancies at 11 to 13 + 6 weeks' gestation the level of free β-hCG is higher and that of PAPP-A is lower than in unaffected pregnancies. It has also demonstrated that assessment of accurate patient-specific risks necessitates making adjustments in the measured maternal serum

Table 7 Differences in multiples of the median (MoM) values and risks for trisomy 21 in women with different maternal characteristics

Case	*Characteristics*	*β-hCG (MoM)*	*PAPP-A (MoM)*	*Risk*
1	Caucasian, parous, non-smoker, weight 85 kg, aged 38 years, spontaneous conception, crown–rump length 65 mm	1.20	0.56	1 in 100
2	Same as Case 1 but crown–rump length 55 mm	1.05	0.77	1 in 347
3	Same as Case 1 but maternal weight 50 kg	0.83	0.31	1 in 48
4	Same as Case 1 but Afro-Caribbean	1.07	0.36	1 in 40
5	Same as Case 1 but East Asian	1.12	0.51	1 in 93
6	Same as Case 1 but South Asian	1.33	0.55	1 in 73
7	Same as Case 1 but smoker	1.25	0.68	1 in 145
8	Same as Case 1 but nulliparous	1.18	0.55	1 in 99
9	Same as Case 1 but conception by IVF	1.10	0.63	1 in 158
10	Same as Case 1 but analytes measured by Delfia Express	1.14	0.75	1 in 224
11	Same as Case 1 but Afro-Caribbean, weight 50 kg, smoker	0.77	0.24	1 in 29

Case 1 is a Caucasian woman, who is 38 years old, parous, a non-smoker, weighs 85 kg and conceived spontaneously, with a maternal serum free beta-human chorionic gonadotropin (β-hCG) level of 35 U/mL and pregnancy-associated plasma protein-A (PAPP-A) level of 1.5 U/mL measured by the Kryptor system. If the fetal crown–rump length is 65 mm, the free β-hCG and PAPP-A correspond to 1.20 MoM and 0.56 MoM respectively, and the estimated maternal and gestational age-related risk for trisomy 21 is 1 in 136. Cases 2–11 are similar to Case 1 but with variations in some of the characteristics.

concentration of free β-hCG and PAPP-A to correct for gestational age, maternal weight, ethnicity, smoking status, method of conception and parity, as well as the machine and reagents used for the assays.

The advantages of our study are the large number of cases examined (491 cases of trisomy 21 and 96 803 unaffected pregnancies) and the use of multiple regression analysis to define the contribution of maternal variables that influence the measured concentration of free β-hCG and PAPP-A, and the interaction between these covariates. The alternative method of sequential adjustment for each individual parameter fails to take into account the interaction between the covariates[14].

The levels of both PAPP-A and β-hCG were influenced by the ethnic origin of the women. The most striking finding was that there was a 57% increase in the levels of PAPP-A and a 12% increase in β-hCG in women of Afro-Caribbean origin compared with Caucasians. In biochemical screening of women of Afro-Caribbean origin failure to take into account ethnic origin would result in a substantial underestimate of the true risk of trisomy 21. As illustrated in Table 7, an Afro-Caribbean woman with a risk estimate of 1 in 40 would have been given a risk of 1 in 100 if her ethnic origin was not taken into account. Similarly, erroneous risks would be given to women who smoke and those conceiving by IVF because the associated decrease in serum PAPP-A could be misinterpreted as an increased risk for trisomy 21 and a substantial increase in false-positive rate. Another important factor in biochemical screening highlighted by our results is the need to make adjustments for the reagents and machines used for the measurement of free β-hCG and PAPP-A.

Likelihood ratios were established from the Gaussian distributions in trisomy 21 and unaffected pregnancies, and these were then used together with maternal age to produce patient-specific risks and to calculate detection and false-positive rates. The modeled performance of first-trimester biochemical screening was similar to that observed and, for a false-positive rate of 5%, the detection rate of trisomy 21 was 68%.

The overall performance of screening by maternal age and serum free β-hCG and PAPP-A was better at 11 weeks than at 13 weeks, with a greater relative contribution from PAPP-A at 11 weeks and from free β-hCG at 13 weeks. In trisomy 21 pregnancies the median MoM free β-hCG increased from 1.80 at 11 weeks to 2.09 at 13 weeks, and the respective values for PAPP-A were 0.38 and 0.65 MoMs. These values derived from our large prospective study, in which the biochemical tests were carried out in all 96 803 cases, are different from those reported in two previous multicenter studies in the UK and USA[16,17]. The Serum, Urine and Ultrasound Screening Study (SURUSS) recruited 47 053 pregnancies, including 101 with trisomy 21, but measured free β-hCG and PAPP-A retrospectively in stored samples from 98 trisomy 21 pregnancies and 1090 matched unaffected controls[16]. Similarly, 38 167 pregnancies were recruited in the First- and Second-Trimester Evaluation of Risk (FASTER) study, including 117 with trisomy 21, and free β-hCG and PAPP-A were measured retrospectively in stored samples from 79 trisomy 21 pregnancies and 395 matched unaffected controls[17]. Compared with our findings, the difference between trisomy 21 and unaffected pregnancies in free β-hCG in the SURUSS was overestimated by 8% at 11 weeks and by 19% at 13 weeks, and in the FASTER study it was underestimated by 34% and 19%, respectively[16,17]. The difference in PAPP-A in both previous studies was underestimated by 11% at 11 weeks and overestimated by 18% at 13 weeks[16,17]. In our study, the modeled SD of PAPP-A in unaffected pregnancies was 3–12% lower and in trisomy 21 cases it was 16–20% lower than in previous reports[16,17]. The respective values for free β-hCG in our study were lower by 4–17% in unaffected pregnancies. The SD for free β-hCG in the trisomy 21 pregnancies in our study was 26% lower than in the FASTER study and similar to that in the SURUSS[16,17].

Our findings have two implications. First, in first-trimester biochemical screening for calculation of accurate patient-specific risks for trisomy 21, it is essential to take into account gestational age, maternal weight, ethnicity, smoking status, method of conception and machine used for the assays. Second, the performance of the test is substantially better at 11–12 weeks than at 13 weeks.

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (charity number 1037116).

REFERENCES

1. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999; **13**: 231–237.
2. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one stop clinic: a review of three years prospective experience. *BJOG* 2003; **110**: 281–286.
3. Avgidou K, Papageorghiou A, Bindra R, Spencer K, Nicolaides KH. Prospective first-trimester screening for trisomy 21 in 30 564 pregnancies. *Am J Obstet Gynecol* 2005; **192**: 1761–1767.
4. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005; **25**: 221–226.
5. Spencer K, Bindra R, Nicolaides KH. Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. *Prenat Diagn* 2003; **23**: 851–855.
6. Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy. *Ann Clin Biochem* 2002; **39**: 567–568.
7. Spencer K, Ong CY, Liao AW, Nicolaides KH. The influence of ethnic origin on first trimester biochemical markers of chromosomal abnormalities. *Prenat Diagn* 2000; **20**: 491–494.
8. Spencer K, Heath V, El-Sheikhah A, Ong CYT, Nicolaides KH. Ethnicity and the need for correction of biochemical and ultrasound markers of chromosomal anomalies in the first

trimester: a study of Oriental, Asian and Afro-Caribbean populations. *Prenat Diagn* 2005; **25**: 365–369.
9. Spencer K, Bindra R, Cacho AM, Nicolaides KH. The impact of correcting for smoking status when screening for chromosomal anomalies using maternal serum biochemistry and fetal nuchal translucency thickness in the first trimester of pregnancy. *Prenat Diagn* 2004; **24**: 169–173.
10. Kagan KO, Frisova V, Nicolaides KH, Spencer K. Dose dependency between cigarette consumption and reduced maternal serum PAPP-A levels at 11–13 + 6 weeks of gestation. *Prenat Diagn* 2007; **27**: 849–853.
11. Liao AW, Heath V, Kametas N, Spencer K, Nicolaides KH. First-trimester screening for trisomy 21 in singleton pregnancies achieved by assisted reproduction. *Hum Reprod* 2001; **16**: 1501–1504.
12. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Fetal Medicine Foundation First Trimester Screening Group. *Lancet* 1998; **352**: 343–346.
13. Robinson HP, Fleming JE. A critical evaluation of sonar crown–rump length measurements. *Br J Obstet Gynaecol* 1975; **82**: 702–710.
14. Draper N, Smith R. *Applied Regression Analysis* (3rd edn). Wiley: New York, NY, 1998.
15. Office for National Statistics. *Birth Statistics. Review of the Registrar General on births and patterns of family building in England and Wales.* Series FM1, Nos 29–31. Stationary Office: London, 2002.
16. Wald NJ, Rodeck C, Hackshaw AK, Walters J, Chitty L, Mackinson AM. First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS). *J Med Screen* 2003; **10**: 56–104.
17. Canick JA, Lambert-Messerlian GM, Palomaki GE, Neveux LM, Malone FD, Ball RH, Nyberg DA, Comstock CH, Bukowski R, Saade GR, Berkowitz RL, Dar P, Dugoff L, Craigo SD, Timor-Tritsch IE, Carr SR, Wolfe HM, D'Alton ME. Comparison of serum markers in first-trimester Down syndrome screening. *Obstet Gynecol* 2006; **108**: 1192–1199.

Ultrasound Obstet Gynecol 2008; **32**: 751–754
Published online 29 July 2008 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5404

Prenasal thickness in trisomy-21 fetuses at 16–24 weeks of gestation

N. PERSICO*, M. BORENSTEIN*, F. MOLINA†, G. AZUMENDI† and K. H. NICOLAIDES*

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK and †Unidad de Ecografia, Centro Gutenberg, Malaga, Spain*

KEYWORDS: 3D ultrasound; prenasal thickness; second-trimester screening; trisomy 21

ABSTRACT

Objective *To construct a reference range for fetal prenasal thickness between 16 and 24 weeks of gestation and to evaluate the thickness in fetuses with trisomy 21.*

Methods *We acquired three-dimensional (3D) volumes of the fetal profile from 135 normal fetuses and 26 fetuses with trisomy 21 at 16–24 weeks' gestation. We used the multiplanar mode to obtain the exact mid-sagittal plane and measured the prenasal thickness as the shortest distance between the anterior edge of the lowest part of the frontal bone (at the junction with the nasal bone when present) and the skin anteriorly.*

Results *In the normal group prenasal thickness increased with gestation from a mean of 2.4 mm at 16 weeks to 4.6 mm at 24 weeks. Repeatability studies demonstrated that in 95% of the cases the difference between two measurements of prenasal thickness by the same operator and by different operators was less than 1 mm. In the trisomy-21 fetuses the mean prenasal thickness was significantly larger than in normal fetuses and in 19 (73.1%) cases it was above the 95th centile of the normal range. There was no significant difference in prenasal thickness between the trisomic fetuses with and without ventriculomegaly, nuchal edema, absent nasal bone or a cardiac defect.*

Conclusions *The fetal profile is routinely examined during the second-trimester scan and therefore the skill needed to obtain the view necessary for the measurement of prenasal thickness is widely available. If the finding of our study – that in more than 70% of fetuses with trisomy 21 prenasal thickness is above the 95th centile – is confirmed in prospective screening studies this measurement alone could prove a highly sensitive method of second-trimester screening for trisomy 21. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

An excessive amount of skin is a common feature in individuals with trisomy 21, as originally described by Langdon Down in 1866[1]. Extensive studies have documented this excessive amount of skin as increased nuchal translucency thickness in the first trimester and increased nuchal fold thickness in the second trimester[2–5]. More recently Maymon *et al.* reported that in second-trimester trisomy-21 fetuses the prenasal skin thickness, between the frontonasal angle and the outer border of the skin edge, is increased[6]; in 58% of 21 fetuses with trisomy 21 at 14–27 weeks of gestation the prenasal thickness was above the 95th centile of 500 normal fetuses.

The aim of this three-dimensional (3D) ultrasound study was to construct a reference range for fetal prenasal thickness between 16 and 24 weeks' gestation and to evaluate further the thickness in fetuses with trisomy 21.

METHODS

We measured prenasal thickness using 3D volumes of the fetal face that had been acquired from two groups of patients. The first group comprised 135 singleton pregnancies with appropriately growing fetuses and no sonographic evidence of fetal abnormality. These patients were attending our fetal medicine centers for routine ultrasound examination at 16–24 weeks, and for this study we prospectively selected 15 consecutive cases per gestational week. The second group comprised 26 fetuses with trisomy 21 confirmed by chorionic villus sampling or amniocentesis, which were carried out because of a high suspicion for a chromosomal defect. In 14 (53.8%) cases the maternal age was 35 years or more and in all but one case there was at least one fetal abnormality or sonographic marker of chromosomal defect, including mild ventriculomegaly ($n = 5$), nuchal edema ($n = 9$), absent

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 8RX, UK (e-mail: fmf@fetalmedicine.com)

Accepted: 7 March 2008

nasal bone ($n = 7$), cardiac defect ($n = 11$), intracardiac echogenic focus ($n = 4$), hyperechogenic bowel ($n = 3$), collapsed stomach ($n = 2$), duodenal atresia ($n = 1$), mild hydronephrosis ($n = 3$), short femur ($n = 4$), talipes ($n = 2$), clinodactyly ($n = 2$), cleft lip and palate ($n = 1$), choroid plexus cyst ($n = 1$) and sandal gap ($n = 1$).

In each case transabdominal ultrasonography was carried out by sonographers with extensive experience in 3D ultrasound, using a RAB 4-8L probe (Voluson 730 Expert, GE Medical Systems, Milwaukee, WI, USA). A 3D volume of the fetal head was acquired in the mid-sagittal plane of the face with the transducer parallel to or within 30° of the long axis of the nose. The 3D volumes were examined off-line using the multiplanar mode to confirm the exact mid-sagittal plane and to make minor corrections from the original acquisition plane when necessary. The exact mid-sagittal plane was defined by the presence of the tip of the nose, the palate without the zygomatic bone and the translucent diencephalon at 16 weeks and the corpus callosum at 17–24 weeks. Prenasal thickness was defined as the shortest distance between the anterior edge of the lowest part of the frontal bone (at the junction with the nasal bone when present) and the facial skin anteriorly (Figure 1). This is essentially the same technique as that used in the study by Maymon *et al.*, who reported the distance between the skin and the junction between the frontal and nasal bone[6], but we selected the lowest part of the frontal bone because the nasal bone is absent in about one-third of fetuses with trisomy 21.

All the measurements were made by sonographers who were not aware of the fetal karyotype. In addition one of the sonographers measured 30 randomly selected cases on two occasions, and in 30 cases the measurement was performed by two different operators to assess repeatability.

Statistical analysis

Regression analysis was used to determine the significance of the association between prenasal thickness and gestational age. The Kolmogorov–Smirnov test was used to confirm the normality of the distribution of the prenasal thickness measurement in chromosomally normal and trisomy-21 fetuses. The values of prenasal thickness were then expressed as a difference from the appropriate expected mean for gestation (delta value). The Kolmogorov–Smirnov test was used to confirm the normality of the distribution of the delta values in the normal and trisomy-21 fetuses. The Mann–Whitney *U*-test for independent samples was used to compare mean prenasal thickness delta values between normal fetuses and trisomy-21 fetuses, and it was also used to compare, within the trisomy-21 group, those with and without common defects (ventriculomegaly, nuchal edema, absent nasal bone and cardiac defect). Bland–Altman analysis was used to compare the measurement agreement and bias for a single examiner and between two examiners[7]. The data were analyzed using the statistical software SPSS 12.0 (Chicago, IL, USA) and Excel for Windows 2000 (Microsoft Corp., Redmond, WA, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

In the 135 normal fetuses the median maternal age was 32 (range, 16–44) years and the median gestational age (GA) was 20 (range, 16–24) weeks. Prenasal thickness increased with gestational age following a second order polynomial trend from 2.4 mm at 16 weeks to 4.6 mm at 24 weeks (prenasal thickness $= -12.000 + (1.315 \times \text{GA}) - (0.026 \times \text{GA}^2)$; $r = 0.781$, $P < 0.01$, SD $= 0.993$;

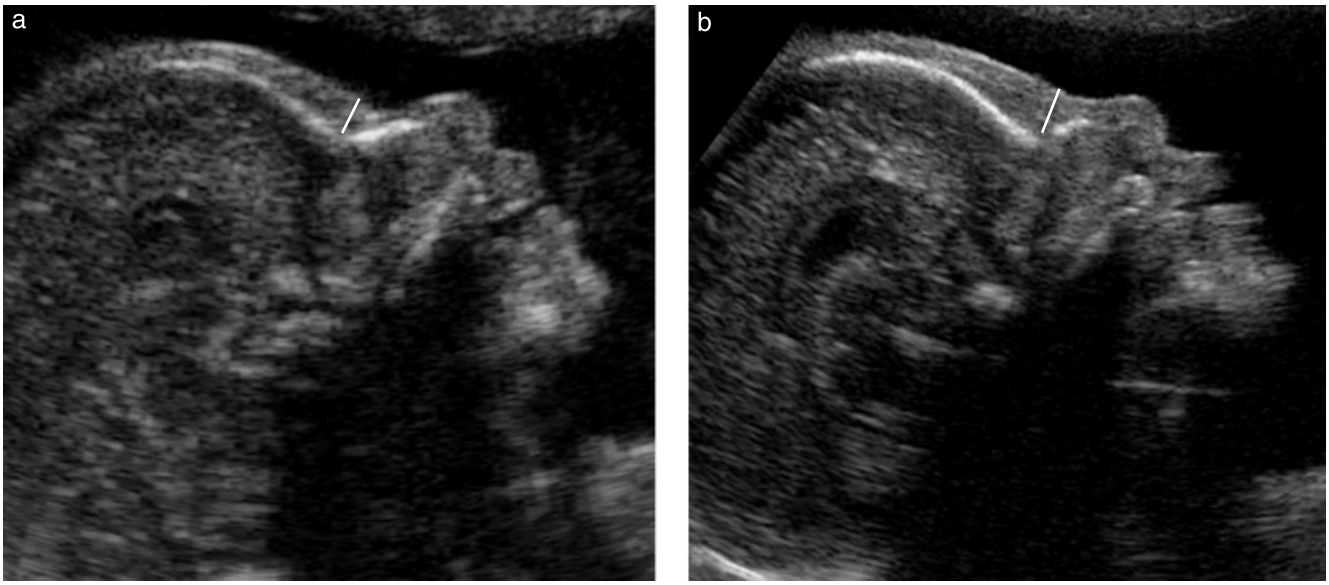


Figure 1 Ultrasound images of normal (a) and trisomy-21 (b) fetuses demonstrating the measurement of prenasal thickness as the shortest distance between the anterior edge of the lowest part of the frontal bone and the facial skin. Note that in the mid-sagittal plane the tip of the nose, the palate without the zygomatic bone and the corpus callosum are visible.

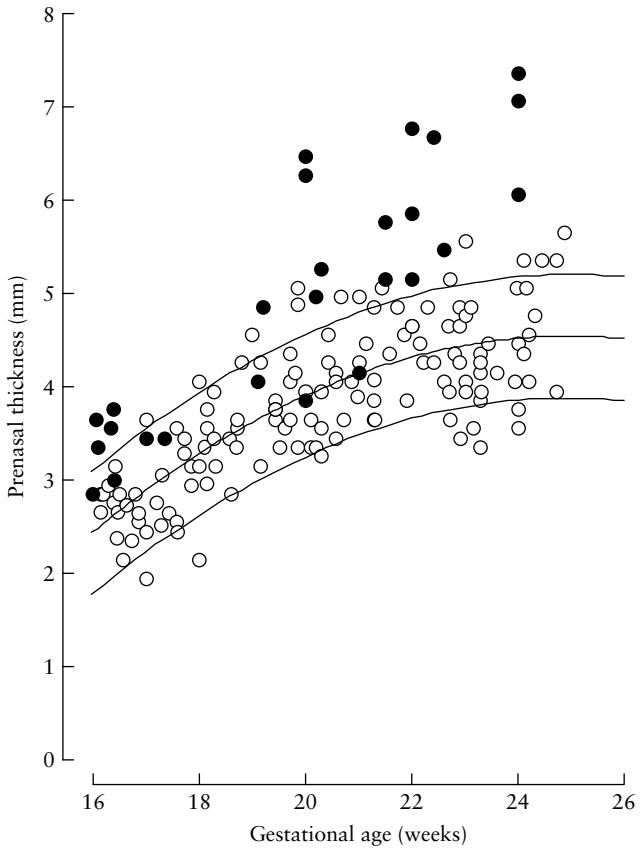


Figure 2 Variation of prenasal thickness with gestation in 135 normal fetuses (○) and 26 fetuses with trisomy 21 (●), plotted on the reference range (mean, 5th and 95th percentiles) of the normal fetuses.

Figure 2). The mean delta prenasal thickness was −0.166 (95% CI, −0.251 to −0.080) mm.

In the 26 fetuses with trisomy 21, the median maternal age was 35 (range, 20–44) years and the median gestational age was 20 (range, 16–24) weeks. Prenasal thickness was above the mean of the normal range in 25 (96.2%) and above the 95th centile of the normal range in 19 (73.1%) fetuses, respectively (Figure 2). In the trisomy-21 fetuses delta prenasal thickness was significantly greater than in the normal fetuses (mean 1.089 (95% CI, 0.767–1.412) mm; $P < 0.01$) and the delta prenasal thickness increased significantly with gestation ($r = 0.500$, $P < 0.01$). The nasal bone was present in all the 135 normal fetuses and absent in 7 (26.9%) of the 26 fetuses with trisomy 21. There was no significant difference in the mean delta prenasal thickness between those with and without ventriculomegaly ($P = 0.705$), nuchal edema ($P = 0.261$), absent nasal bone ($P = 0.279$) or cardiac defects ($P = 0.938$).

The mean difference and the 95% limits of agreement between paired measurements of prenasal thickness (mm) by the same observer were −0.063 (−0.489 (95% CI, −0.558 to −0.421) to 0.363 (95% CI, 0.294 to 0.431)) and the respective values in paired measurements by the two different observers were 0.090 (−0.360 (95% CI, −0.432 to −0.287) to 0.540 (95% CI, 0.467 to 0.612)) (Figure 3).

DISCUSSION

The findings of this study confirm that in fetuses with trisomy 21 during the second trimester of pregnancy there is increased prenasal thickness. In the normal fetuses prenasal thickness increased with gestation from a mean of 2.4 mm at 16 weeks to 4.6 mm at 24 weeks, which is similar to the reference range reported by Maymon *et al.*[6]. In their study the distribution of prenasal thickness was skewed and the values in both the normal and trisomy-21 groups were reported as multiples of the median. In our study the distribution of prenasal thickness with gestation was Gaussian, the SD did not change with gestation and we expressed the values in the normal and trisomy-21 groups as deltas (differences in mm from the normal mean for gestation). In the trisomy-21 group prenasal thickness was above the 95th centile of our normal range in 73.1% of our 26 fetuses, compared with 72% of the 18 fetuses reported by Maymon *et al.*[6] at 18–24 weeks. The measurement of prenasal thickness is reproducible and in 95% of the cases the difference between two measurements by the same operator and by different operators was less than 1 mm.

In this study all the cases of trisomy 21 had been identified by prior screening through maternal age, second-trimester serum biochemistry or routine ultrasound examination, and in 25 of the 26 cases there was a fetal abnormality or sonographic marker of chromosomal defect. However, there was no significant association between prenasal thickness and any of the commonly detected defects, including nuchal edema. It is therefore reasonable to assume that our findings on prenasal thickness are representative of all fetuses with trisomy 21.

In the first trimester of pregnancy effective screening for trisomy 21 is provided by a combination of fetal nuchal translucency thickness and maternal serum free β-human chorionic gonadotropin and pregnancy-associated plasma protein-A, with a detection rate of 90% for a false positive rate of 5%[8]. The incorporation of additional sonographic markers, such as nasal bone, frontomaxillary facial angle, and tricuspid and ductus venosus flow could increase the detection rate to more than 95% with a simultaneous reduction in false positive rate to less than 3%[8]. In the second trimester screening by maternal age and maternal serum biochemistry has detection rates of 30% and 65%, respectively, for a false positive rate of 5%[9,10]. Although many reports have highlighted the association between trisomy 21 and several defects or sonographic markers, such as cardiac abnormalities, increased nuchal fold thickness, short femur, echogenic intracardiac focus, hyperechogenic bowel or hydronephrosis, each one of these features are observed in a minority of affected fetuses[11]. If the finding of our study – that at 16–24 weeks prenasal thickness is above the 95th centile in more than 70% of trisomy-21 fetuses – is confirmed in prospective screening studies this measurement alone could prove the most sensitive method of second-trimester screening for trisomy 21.

The fetal profile is routinely examined during the second-trimester scan and therefore the skill needed to

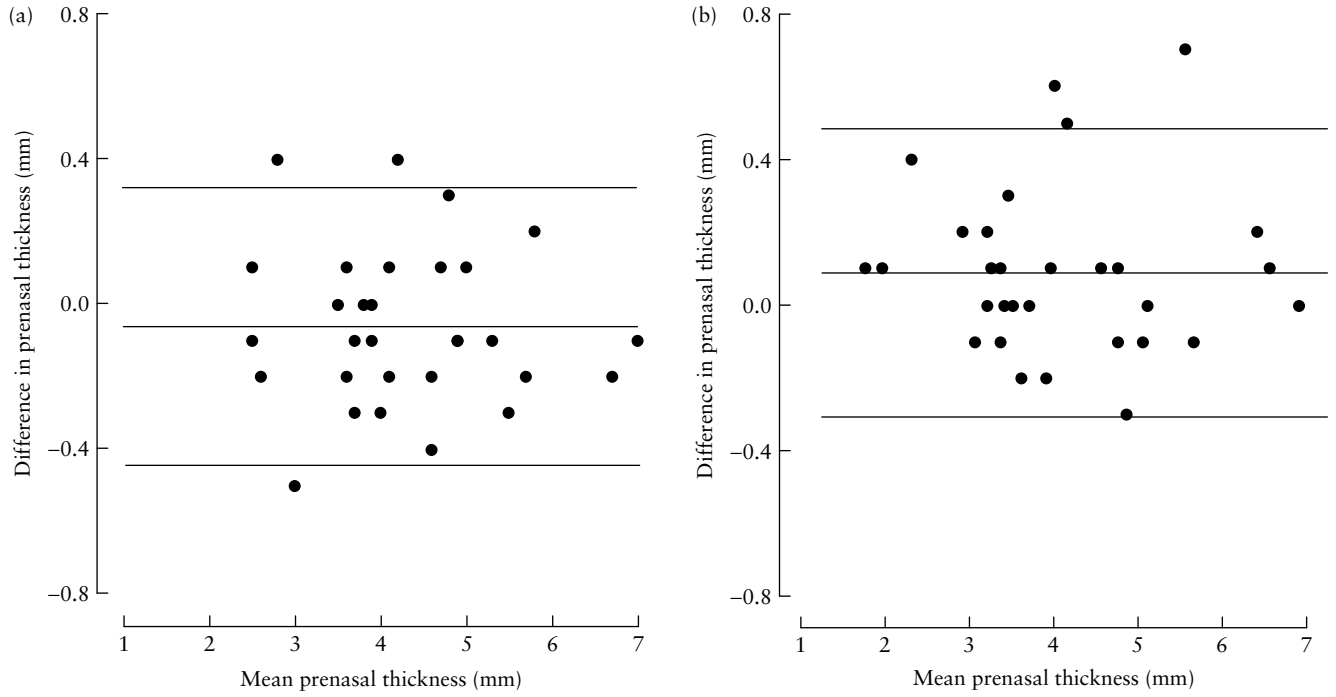


Figure 3 Mean difference and the 95% limits of agreement between paired measurements of the prenasal thickness by the same sonographer (a) and between paired measurements by two different observers (b).

obtain the view necessary for the measurement of prenasal thickness is widely available. Furthermore, the same view can be used for assessment of the nasal bone. The finding of this study that the nasal bone is absent in about 27% of trisomy-21 fetuses is compatible with the results of previous studies[12]. The additional finding that in trisomy-21 fetuses prenasal thickness appears to be independent of the presence or absence of the nasal bone suggests that the two sonographic markers could be combined for an even higher detection rate of affected fetuses. A third promising sonographic marker that can be assessed in the same profile view is the frontomaxillary facial angle, which has recently been reported to be above the 95th centile in about 65% of fetuses with trisomy 21[13].

In this study we used the multiplanar mode of 3D ultrasound to ensure accurate measurement of prenasal thickness in the exact mid-sagittal plane. However, the landmarks defining this plane, including the tip of the nose, the palate without the zygomatic bone and the diencephalon or corpus callosum, can easily be identified by two-dimensional ultrasonography. Large-scale prospective studies are now needed to determine the performance of second-trimester screening for trisomy 21 by prenasal thickness and combinations with the nasal bone and frontomaxillary facial angle as well as serum biochemistry.

REFERENCES

1. Down LJ. Observations on an ethnic classification of idiots. *Clinical Lectures and Reports, London Hospital* 1866; **3**: 259–262.
2. Benacerraf BR, Gelman R, Frigoletto FD. Sonographic identification of second-trimester fetuses with Down's syndrome. *N Engl J Med* 1987; **317**: 1371–1376.
3. Nicolaides KH, Azar G, Snijders RJM, Gosden CM. Fetal nuchal edema: associated malformations and chromosomal defects. *Fetal Diagn Ther* 1992; **7**: 123–131.
4. Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. *BMJ* 1992; **304**: 867–869.
5. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. *Lancet* 1998; **351**: 343–346.
6. Maymon R, Levinsohn-Tavor O, Cuckle H, Tovbin Y, Dreazen E, Wiener Y, Herman A. Second trimester ultrasound prenasal thickness combined with nasal bone length: a new method of Down syndrome screening. *Prenat Diagn* 2005; **25**: 906–911.
7. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**: 307–310.
8. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005; **25**: 221–226.
9. Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. *Health Technol Assess* 1998; **2**: 1–112.
10. Cuckle H, Benn P, Wright D. Down syndrome screening in the first and/or second trimester: model predicted performance using meta-analysis parameters. *Semin Perinatol* 2005; **29**: 252–257.
11. Smith-Bindman R, Hosmer W, Feldstein V, Deeks J, Goldberg J. Second-trimester ultrasound to detect fetuses with Down syndrome: a meta-analysis. *JAMA* 2001; **285**: 1044–1055.
12. Sonek JD, Cicero S, Neiger R, Nicolaides KH. Nasal bone assessment in prenatal screening for trisomy 21. *Am J Obstet Gynecol* 2006; **195**: 1219–1230.
13. Molina F, Persico N, Borenstein M, Sonek J, Nicolaides KH. Frontomaxillary facial angle in trisomy 21 fetuses at 16–24 weeks of gestation. *Ultrasound Obstet Gynecol* 2008; **31**: 384–387.

Ultrasound Obstet Gynecol 2008; **31**: 384–387
Published online 5 March 2008 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/uog.5288

Frontomaxillary facial angle in trisomy 21 fetuses at 16–24 weeks of gestation

F. MOLINA*†, N. PERSICO*, M. BORENSTEIN*, J. SONEK‡ and K. H. NICOLAIDES*

**Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London, UK, †Unidad de Ecografia, Centro Gutenberg, Malaga, Spain and ‡Department of Obstetrics and Gynecology, Wright State University, Dayton, OH, USA*

KEYWORDS: frontomaxillary facial angle; maxilla; second-trimester screening; three-dimensional ultrasound; trisomy 21

ABSTRACT

Objectives *To establish a normal range for the frontomaxillary facial (FMF) angle by three-dimensional (3D) ultrasound imaging and to examine the FMF angle in trisomy 21 fetuses at 16–24 weeks of gestation.*

Methods *We measured the FMF angle using 3D volumes of the fetal profile obtained with the transducer parallel to the long axis of the nose and at 45° to the palate, which had been acquired from 150 normal fetuses and 23 fetuses with trisomy 21.*

Results *In the normal group there was no significant association between the FMF angle and gestational age; the mean FMF angle was 83.9° (range, 76.9–90.2°) and the 95th centile was 88.5°. In 15 (65.2%) of the fetuses with trisomy 21 the FMF angle was greater than 88.5°. Repeatability studies demonstrated that in 95% of cases the difference between two measurements of FMF angle by the same operator and different operators was less than 5°.*

Conclusions *In the majority of second-trimester fetuses with trisomy 21 the FMF angle is increased. Copyright © 2008 ISUOG. Published by John Wiley & Sons, Ltd.*

INTRODUCTION

A common phenotypic feature of individuals with trisomy 21 is a flat face[1]. We have recently reported a method for quantifying the flat face of fetuses with trisomy 21 and demonstrated an increase in the frontomaxillary facial (FMF) angle, which is the angle between the upper surface of the palate and the leading edge of the forehead[2,3]. A three-dimensional (3D) ultrasound study of fetuses at 11 + 0 to 13 + 6 weeks of gestation reported that the FMF angle was above the 95th centile of the normal range in more than 60% of fetuses with trisomy 21[2]. Similarly, a study of stored images of fetal profiles taken by two-dimensional (2D) ultrasound examination before amniocentesis at 14–24 weeks reported that the FMF angle was above the 95th centile of the normal range in 79% of 34 fetuses with trisomy 21[3].

In our continuing 3D ultrasound studies of the FMF angle we found that the palate is visible as a single homogeneously hyperechogenic rectangular structure in the mid-sagittal view of the fetal face at 11 to 13 + 6 weeks[4]. In the second and third trimesters we noticed that there are at least two echogenic structures, the inferior one representing the palate and the superior ones the vomer (Figure 1). The vomer is a thin bone that forms the posterior and inferior portion of the nasal septum. It extends from the midline to the sphenoid, ethmoid, left and right palatine bones, and left and right maxillary bones.

The aim of this prospective 3D ultrasound study was to present a reference range of the FMF angle and to evaluate the angle in fetuses with trisomy 21 at between 16 and 24 weeks of gestation.

METHODS

We measured the FMF angle using 3D volumes of the fetal face, which had been successfully acquired in the mid-sagittal plane of the face from two groups of patients. The first group comprised 150 singleton pregnancies with appropriately growing fetuses and no sonographic evidence of fetal abnormality. These patients were attending our fetal medicine centers for routine ultrasound examination at 16–25 weeks and for this study we prospectively recruited 15 consecutive cases per gestational week. The

Correspondence to: Prof. K. H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 8RX, UK (e-mail: fmf@fetalmedicine.com)

Accepted: 30 January 2008

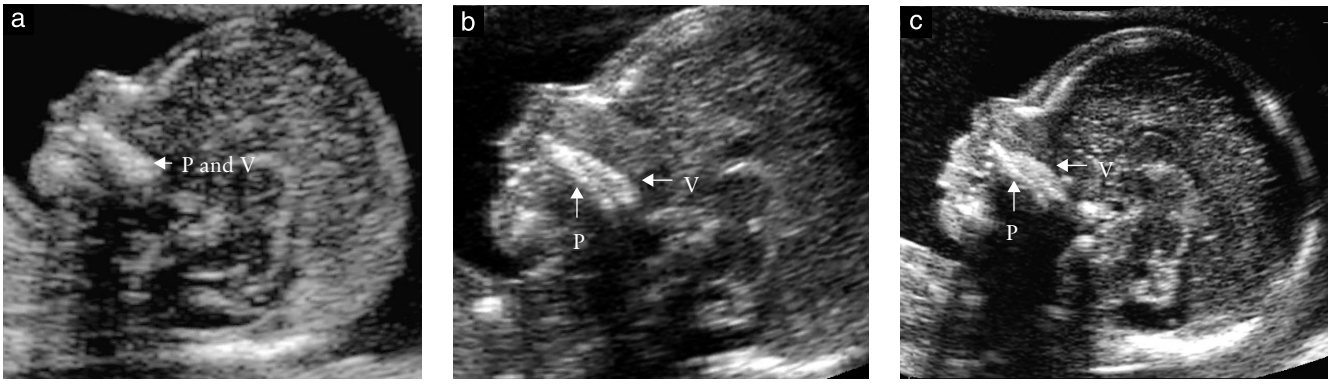


Figure 1 Ultrasound images of a normal fetal profile at 12 weeks (a), 16 weeks (b) and 20 weeks (c) of gestation. At 12 weeks the palate (P) and vomer (V) appear as a single hyperechogenic rectangular structure, but in the second trimester there are two echogenic structures. The inferior one, which is directed towards the basilar portion of the occipital bone posteriorly, represents the palate. The vomer is the superior one, with an irregular convex shape on the top, and is directed towards the sphenoid bone posteriorly.

second group comprised 23 fetuses with trisomy 21 confirmed by chorionic villus sampling or amniocentesis carried out because of a high risk of a chromosomal defect. In 13 (56.5%) cases the maternal age was 35 years or more, and in all but one case there was at least one fetal abnormality or sonographic marker of a chromosomal defect, including mild ventriculomegaly ($n = 5$), nuchal edema ($n = 8$), absent nasal bone ($n = 6$), cardiac defect ($n = 8$), intracardiac echogenic focus ($n = 4$), hyperechogenic bowel ($n = 2$), collapsed stomach ($n = 2$), duodenal atresia ($n = 1$), mild hydronephrosis ($n = 3$), short femur ($n = 4$), talipes ($n = 1$) and clinodactyly ($n = 1$).

In each case transabdominal ultrasound examination (RAB 4-8L probe, Voluson 730 Expert, GE Medical Systems, Milwaukee, WI, USA) was carried out by sonographers with extensive experience in 3D ultrasound imaging. A 3D volume of the fetal head had been acquired in the mid-sagittal plane of the face with the transducer parallel to or within 30° of the long axis of the nose and 45° to the palate. In this plane, the palate is visualized as an echogenic line, with a downwards diagonal direction from the maxillary bone anteriorly towards the basilar portion of the occipital bone posteriorly (Figure 2). Care was taken to distinguish between the palate and the overlying vomeral bone, which is less echogenic and has an irregular convex shape on the top. The vomeral bone courses diagonally from the maxilla anteriorly towards the sphenoid bone posteriorly. The 3D volumes were examined offline using the multiplanar mode to verify the exact mid-sagittal plane and to make minor corrections from the original acquisition plane when necessary. For measurement of the FMF angle the first ray was drawn along the superior edge of the palate and the second ray from the upper anterior corner of the maxilla extending to the external surface of the frontal bone. This technique differs from that used at 11 to 13 + 6 weeks and the one used in our previous second-trimester retrospective study[2,3]. At 11 to 13 + 6 weeks the vomer and palate are indistinguishable, and so the first ray of the FMF angle is drawn in the upper part of the palate–vomer complex. As far as the second trimester is concerned, the vomer and palate can be seen separately and in our previous study we drew the first ray of the angle in the upper part of the vomer[3]. In the present study we selected the palate rather than the vomer because the upper surface of the former is straight whereas that in the vomer is usually convex.

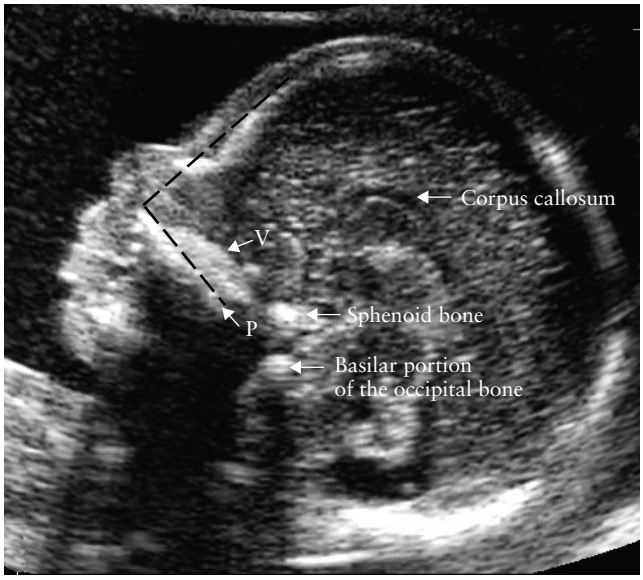


Figure 2 Ultrasound image of a normal fetal profile at 20 weeks demonstrating the measurement of the frontomaxillary facial angle.

The FMF angle measurements were made independently by two sonographers who were not aware of the fetal karyotype. Intraobserver variability in measurements was assessed based on FMF angle measurements by two observers in 100 cases. Intraobserver variability was assessed by one sonographer measuring 50 randomly selected cases on two occasions.

Statistical analysis

Regression analysis was used to determine the significance of the association between the FMF angle and gestational age. Mann–Whitney *U*-test was used to compare the FMF angle between the normal group and the trisomy

21 fetuses, and between those with and without common defects (ventriculomegaly, nuchal edema, absent nasal bone, cardiac defect) within the trisomy 21 group.

Bland–Altman analysis was used to examine the measurement agreement and bias for a single examiner and between two examiners[5]. The data were analyzed using the statistical software SPSS 12.0 (Chicago, IL, USA) and Excel for Windows 2000 (Microsoft Corp., Redmond, WA, USA). $P < 0.05$ was considered statistically significant.

RESULTS

In the group of 150 normal fetuses the median maternal age was 32 (range, 16–44) years and the median gestational age was 21 (range, 16–25) weeks. The maternal ethnicity was Caucasian in 97 (64.7%), Afro–Caribbean in 39 (26.0%), Indian or Pakistani in eight (5.3%), and Chinese or Japanese in six (4.0%). There was no significant association between the FMF angle and gestational age ($r = 0.002$, $P = 0.981$) (Figure 3). The mean FMF angle was 83.9° (range, 76.9–90.2°). The Kolmogorov–Smirnov test confirmed the normality of the distribution, and the 5th and 95th centiles were 79.3° and 88.5°, respectively.

In the 23 fetuses with trisomy 21 the median maternal age was 35 (range, 20–44) years and the median gestational age was 20 (range, 16–24) weeks. There was no significant association between the FMF angle and gestational age ($r = 0.189$, $P = 0.387$). The mean FMF angle was 89.4° (range, 83.1–95.6°) and was significantly larger than that in the normal fetuses ($P < 0.001$). The measurement was above the 95th centile of the normal range in 15 (65.2%) of the trisomy 21 fetuses (Figure 3). There was no significant difference in mean FMF angle between those with and without ventriculomegaly (90.9° vs. 89.1°, $P = 0.491$), with and without nuchal edema (90.1° vs. 88.4°, $P = 0.294$), or with and without cardiac defect (89.4° vs. 89.3°, $P = 0.946$). The mean FMF angle was significantly higher in those with an absent rather than present nasal bone (92.0° vs. 88.6°, $P = 0.036$) and it was above the 95th centile in all six cases with an absent nasal bone.

The mean difference and 95% limits of agreement (with their 95% CI) between paired measurements by the same observer were −0.06° (−2.57° (−2.88 to −2.25) to 2.45° (2.13–2.57)) and the respective values in paired measurements by the two different observers were −0.321° (−2.70° (−2.96 to −2.52) to 2.10° (1.88–2.31)).

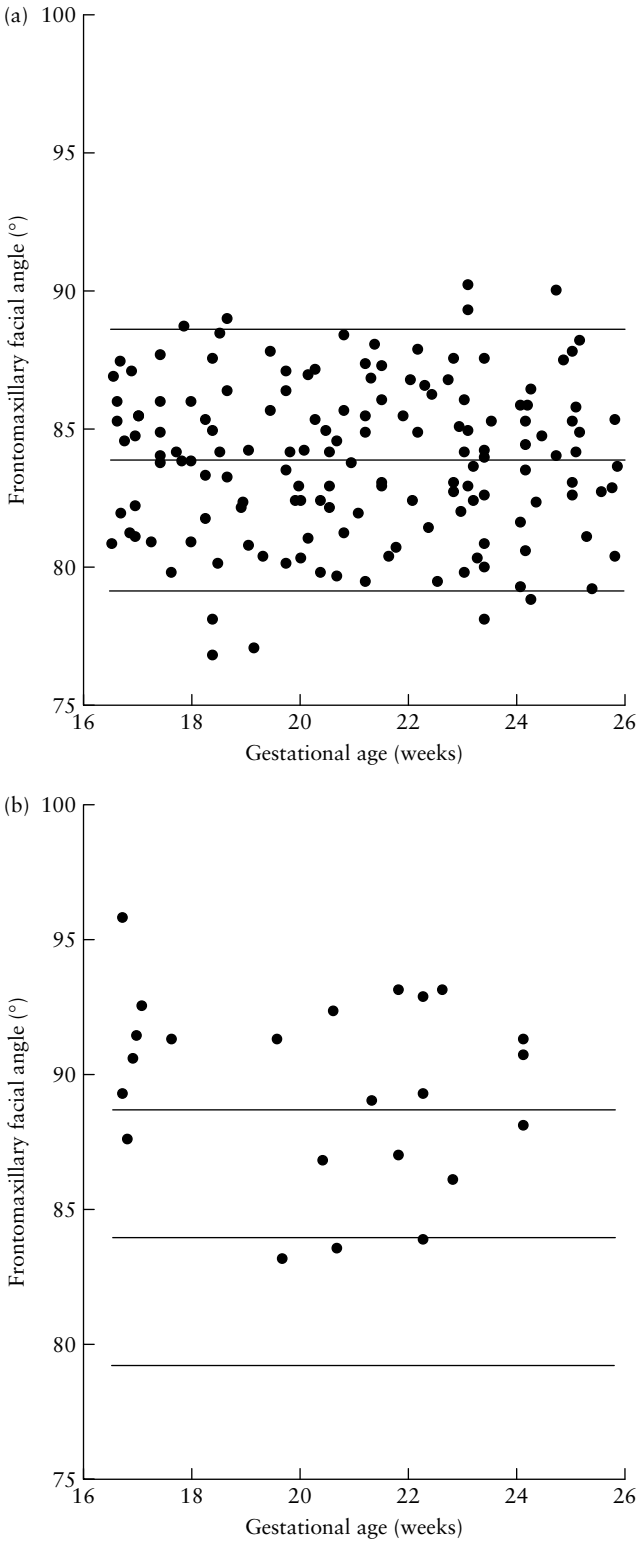


Figure 3 Frontomaxillary facial angle in 150 normal fetuses (a) and 23 fetuses with trisomy 21 (b), plotted on the reference range (mean, 95th and 5th percentiles) for normal fetuses with gestation.

DISCUSSION

The data in this prospective 3D ultrasound study confirm our observations from a previous retrospective 2D ultrasound study that the FMF angle during the second trimester in fetuses with trisomy 21 is substantially bigger than that in normal fetuses. We established that the FMF angle does not change between 16 and 25 weeks of gestation in normal fetuses, and that the 95th centile is 88.5°. However, in about 65% of fetuses with trisomy 21 the FMF angle is above this value. The measurement of the FMF angle in the second and third trimesters is reproducible, and in 95% of the cases the difference between two measurements by the same operator and different operators is less than 5°.

When measuring the FMF angle it is important to appreciate that the palate and vomer appear as a single rectangular structure in the scan performed at 11 to 13 + 6 weeks, whereas in the second and third trimesters the two structures are joined anteriorly at the maxilla but diverge posteriorly to produce two separate echogenic lines. In a previous second-trimester study we drew the first ray of the FMF angle in the upper part of the vomer and reported that the FMF angle decreases with gestation[3]. In the present study we used the palate rather than the vomer because the upper surface of the former is straight whereas that of the vomer is usually convex.

The wide FMF angle in fetuses with trisomy 21 could explain the flat face, which is a common phenotypic expression characteristic of this chromosomal defect[1]. Farkas *et al.* examined 120 patients with trisomy 21 at 7 months to 36 years of age, and reported an abnormally short distance between the nostril and ear in 62% of the cases[6]. Allanson *et al.* examined 199 patients with trisomy 21 at 6 months to 61 years of age and reported that maxillary growth was reduced in comparison to mandibular growth[7]. Shapiro *et al.* examined 153 patients with trisomy 21 at 7–66 years of age and reported that the length of the palate was below the 2.5th centile of the normal range in 95%[8]. Lauridsen *et al.* examined 31 aborted fetuses with trisomy 21 at 16–25 weeks of gestation, and reported that both components of the hard palate, the maxilla and the palatine bones were shorter than in chromosomally normal fetuses[9].

In this study all cases of trisomy 21 had been identified by previous screening through maternal age, second-trimester serum biochemistry or routine ultrasound examination, and in 22/23 cases there was a fetal abnormality or sonographic marker of a chromosomal defect. However, there was no significant association between the FMF angle and any of the commonly detected defects. It is therefore reasonable to assume that our findings concerning the FMF angle are representative of all fetuses with trisomy 21.

In the first trimester of pregnancy effective screening for trisomy 21 is provided by a combination of fetal nuchal translucency thickness and maternal serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A levels, with a detection rate of 90% for a false-positive rate of 5%[10]. The incorporation of additional sonographic markers, such as nasal bone, FMF angle, and tricuspid and ductus venosus flow, could increase the detection rate to more than 95% with a simultaneous reduction in false-positive rate to less than 3%[10].

In the second trimester, screening for trisomy 21 by maternal age or maternal serum biochemistry have detection rates of 30% and 65%, respectively, for a false-positive rate of 5%[11]. Although many reports have highlighted the association between trisomy 21 and several defects or sonographic markers, such as cardiac abnormalities, increased nuchal fold thickness, short femur, echogenic intracardiac focus, hyperechogenic bowel, or hydronephrosis, each one of these features is observed in a minority of affected fetuses[12]. In contrast, sonographic assessment of the fetal profile for the FMF angle is likely to prove a sensitive method of second-trimester screening for trisomy 21. The fetal profile is examined routinely during the second-trimester scan and so the skill necessary to obtain this view is widely available. When the profile is obtained with the transducer parallel to the long axis of the nose and at 45° to the palate, it is possible to examine in the same view the FMF angle as well as the nasal bones and prenasal skin thickness, the other two promising second-trimester markers[13,14].

ACKNOWLEDGMENT

This study was supported by a grant from The Fetal Medicine Foundation (Charity No: 1037116).

REFERENCES

1. Down LJ. Observations on an ethnic classification of idiots. *Clinical Lectures and Reports, London Hospital* 1866; **3**: 259–262.
2. Sonek J, Borenstein M, Dagklis T, Persico N, Nicolaides KH. Fronto maxillary facial angle in fetuses with trisomy 21 at 11–13 + 6 weeks. *Am J Obstet Gynecol* 2007; **196**: 271.e1–e4.
3. Sonek J, Borenstein M, Downing C, McKenna D, Neiger R, Croom C, Gengrich T, Nicolaides KH. Fronto-maxillary facial angles in screening for trisomy 21 between 14 and 23 weeks' gestation. *Am J Obstet Gynecol* 2007; (in press).
4. Plasencia W, Dagklis T, Sotiriadis A, Borenstein M, Nicolaides KH. Fronto-maxillary facial angle at 11 + 0 to 13 + 6 weeks – reproducibility of measurement. *Ultrasound Obstet Gynecol* 2007; **29**: 18–21.
5. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**: 307–310.
6. Farkas LG, Katic MJ, Forrest CR, Litsas L. Surface anatomy of the face in Down's syndrome: linear and angular measurements in the craniofacial regions. *J Craniofac Surg* 2001; **12**: 373–379.
7. Allanson JE, O'Hara P, Farkas LG, Nair RC. Anthropometric craniofacial pattern profiles in Down syndrome. *Am J Med Genet* 1993; **47**: 748–752.
8. Shapiro BL, Gorlin RJ, Redman RS, Bruhl HH. The palate and Down's syndrome. *N Engl J Med* 1967; **276**: 1460–1463.
9. Lauridsen H, Hansen BF, Reintoft I, Keeling JW, Skovgaard LT, Kjær I. Short hard palate in prenatal trisomy 21. *Orthod Craniofac Res* 2005; **8**: 91–95.
10. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. *Ultrasound Obstet Gynecol* 2005; **25**: 221–226.
11. Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. *Health Technol Assess* 1998; **2**: 1–112.
12. Smith-Bindman R, Hosmer W, Feldstein V, Deeks J, Goldberg J. Second-trimester ultrasound to detect fetuses with Down syndrome: a meta-analysis. *JAMA* 2001; **285**: 1044–1055.
13. Sonek JD, Cicero S, Neiger R, Nicolaides KH. Nasal bone assessment in prenatal screening for trisomy 21. *Am J Obstet Gynecol* 2006; **195**: 1219–1230.
14. Maymon R, Levinsohn-Tavor O, Cuckle H, Tovbin Y, Dreazen E, Wiener Y, Herman A. Second trimester ultrasound prenasal thickness combined with nasal bone length: a new method of Down syndrome screening. *Prenat Diagn* 2005; **25**: 906–911.

CHAPTER V

DISCUSSION

The studies in this thesis have to a large extent fulfilled the aims as outlined in chapter III.

3.1 Measurement of nuchal translucency

The first study investigated whether the ultrasonographic appearance of nuchal translucency, such as the presence of septations within it, has any additional contribution to just the measurement of NT thickness in the prediction of chromosomal abnormalities.[74]

Nicolaides in 1992 introduced the term NT thickness to standardize the technique of measuring the fluid behind the fetal neck, irrespective of whether it is septated and whether it is confined to the neck or envelopes the whole fetus.[6] The rationale was that it is possible to standardize and audit the results of a measurement but not those of a subjective appearance.

In 2005, Malone et al introduced the term cystic hygroma to apparently describe a distinct entity from NT.[75] Cystic hygroma was defined as an enlarged sonolucency with clearly visible septations extending along the fetal body axis, in contrast to NT, which was described as a nonseptated sonolucent area confined to the fetal neck. It was recommended that, after the diagnosis of cystic hygromas, it is unnecessary for maternal serum free ß-hCG and PAPP-A to be measured and for software to be used for calculation of patient-specific risk of chromosomal defects. Instead, the parents should be counseled that 50% of fetuses will have a chromosomal abnormality.
The findings of the study in this thesis have demonstrated that, first, when visualized appropriately all increased NTs contain septae, second, the length of NT correlates with its thickness, and third, the risk of a chromosome abnormality is not dependent on the length of the translucent area once its thickness is accounted for.[74] Consequently, cystic hygroma does not constitute a distinct entity in the first trimester that confers a special risk status independent of the NT thickness.

3.2 Distribution of nuchal translucency in different chromosomal defects

Increased NT is associated with trisomy 21 and other chromosomal abnormalities as well as many fetal malformations and genetic syndromes.[8,9] The aim of the second study in this thesis was to examine the prevalence and distribution of all chromosomal defects in fetuses with increased NT thickness.[76] The study examined 11,315 pregnancies with increased NT and the fetal karyotype was abnormal in 2,168 (19.2%). The incidence of chromosomal defects

increased with NT thickness and approximately one half of the chromosomally abnormal group was affected by defects other than trisomy 21. The distribution of NT was different for each type of chromosomal defect. In the majority of fetuses with trisomy 21 the NT thickness was below 4.5 mm, whereas in the majority of fetuses with trisomies 13 or 18 it was 4.5-8.4 mm, and in those with Turner syndrome it was 8.5 mm or more.

The difference in phenotypic pattern of NT thickness characterizing each chromosomal defect presumably reflects the heterogeneity in causes for the abnormal accumulation of subcutaneous fluid in the nuchal region.

The clinical implications of the findings of the study[76] are, first, increased NT is an effective marker not only of trisomy 21 but also of all major chromosomal defects and, second, in NT screening for trisomy 21, the finding of increased NT should prompt ultrasonographers to consider the possibility of other chromosomal defects and undertake a systematic examination of the fetus for detectable features of such defects.

3.3 Mixture model of the distribution of nuchal translucency

In screening for chromosomal defects patient-specific risks are derived by multiplying the *a priori* maternal age and gestation-related risk by a likelihood ratio, determined from the deviation of the fetal NT measurement from the normal median for crown-rump length (CRL).

There are essentially two approaches to quantifying the deviation of NT from the normal median.[12,77] One approach is to subtract the normal median from the NT measurement and to produce a deviation in mm referred to as delta NT. The other approach is to divide NT by the normal median to produce a multiple of the median (MoM) value.

In the calculation of patient-specific risks for trisomy 21 the *a priori* maternal age-related risk is multiplied by the likelihood ratio for a measured NT, which is the ratio of the heights of distributions of measurements in trisomy 21 and unaffected pregnancies. In the delta method it is assumed that there is a common distribution of NT delta values independent of CRL in the trisomy 21 pregnancies and another common distribution in unaffected pregnancies. In the MoM method it is assumed that the distributions of the log transformed MoM values in trisomy 21 and unaffected pregnancies are Gaussian.
The third study of this Thesis examined 37,078 normal pregnancies and 264 with trisomy 21, 81 with trisomy 18, 38 with trisomy 13 and 27 with Turner syndrome.[78] We found that firstly, contrary to the assumption underlying the delta NT method, the distribution of delta NT changes with CRL and secondly,

contrary to the assumption underlying the MoM method the distribution of NT was not Gaussian.

Fetal NT followed two distributions, one that was dependent on CRL and one that was independent of CRL. The distribution in which NT increases with CRL was observed in about 95% of euploid fetuses, 5% with trisomy 21, 30% with trisomy 18, 15% with trisomy 13 and 10% with Turner syndrome. The median CRL-independent NT was 2.0 mm for the euploid group and 3.4, 5.5, 4.0 and 7.8 mm for trisomies 21, 18, 13 and Turner syndrome, respectively.

The findings in the chromosomally abnormal fetuses are compatible with the wide range of phenotypic expression in these abnormalities. Presumably, in the 5–30% of cases with CRL-dependent NT distribution the development of the fetal nuchal region is normal. In the ones with increased CRL-independent NT there is abnormal nuchal development due either to chromosomal abnormality specific primary alterations in the composition of the dermis and lymphatic channels or to secondary accumulation of subcutaneous fluid caused by associated cardiovascular, thoracic and other malformations.

The study also demonstrated that the patient-specific risks derived from the new mixture model are accurate and valid for counselling.

3.4 First-trimester biochemical screening

In trisomy 21 pregnancies maternal serum free -hCG is about twice as high and PAPP-A is reduced to about half compared with values in chromosomally normal pregnancies. In the development of risk algorithms for combined screening the estimation of accurate patient specific risks necessitates adjustments in the measured free -hCG and PAPP-A to take into account their association with gestational age, maternal weight, ethnicity, smoking status and method of conception. Essentially, each measured level is first converted to a multiple of the expected normal median (MoM) specific to a pregnancy of the same gestational age, maternal weight, smoking status, ethnicity and method of conception. The MoM distribution of each metabolite in unaffected and trisomy 21 pregnancies is assumed to be Gaussian, specified in terms of means, SD and correlations.

The fourth study in this Thesis examined 491 pregnancies with trisomy 21 and 96,803 unaffected pregnancies.[79] We used multiple regression analysis, instead of a sequential approach, to take into account the pregnancy characteristics that influence the measured concentration of free -hCG and PAPP-A and the interaction between these covariates. The multiple regression model was then used to estimate likelihood ratios for the biochemical markers that can be

combined with maternal age to produce patient-specific risks for each case.

The study made two important contributions. First, it demonstrated that in first trimester biochemical screening for calculation of accurate patient-specific risks for trisomy 21, it is essential to take into account gestational age, maternal weight, ethnicity, smoking status, method of conception and machine used for the assays. Second, the performance of screening by maternal age and serum free -hCG and PAPP-A was better at 11 weeks than at 13 weeks, with a greater relative contribution from PAPP-A at 11 weeks and from free -hCG at 13 weeks.

3.5 Second-trimester prenasal thickness

This study used the multiplanar mode of 3D ultrasound to obtain the exact mid-sagittal planeof the fetal face and measure the prenasal thickness in 135 normal fetuses and 26 fetuses with trisomy 21 at 16–24 weeks' gestation.[80]
In the normal group prenasal thickness increased with gestation from a mean of 2.4 mm at 16 weeks to 4.6 mm at 24 weeks. In the trisomy-21 fetuses the mean prenasal thickness was significantly larger than in normal fetuses and in 19 (73.1%) cases it was above the 95th centile of the normal range.

In this study all the cases of trisomy 21 had been identified by prior screening through maternal age, second-trimester serum biochemistry or routine ultrasound examination, and in 25 of the 26 cases there was a fetal abnormality or sonographic marker of chromosomal defect. However, there was no significant association between prenasal thickness and any of the commonly detected defects, including nuchal edema. It is therefore reasonable to assume that the findings on prenasal thickness are representative of all fetuses with trisomy 21.

The fetal profile is routinely examined during the second-trimester scan and therefore the skill needed to obtain the view necessary for the measurement of prenasal thickness is widely available. If the finding of the study – that in more than 70% of fetuses with trisomy 21 prenasal thickness is above the 95th centile is confirmed in prospective screening studies this measurement alone could prove a highly sensitive method of second-trimester screening for trisomy 21.

3.6 Second-trimester frontomaxillary facial angle

Measurement of the frontomaxillary facial angle provides an objective measure of the flat face which is a common phenotypic feature of individuals with trisomy 21. Studies in the first-trimester demonstrated that in trisomy 21 fetuses the FMF angle is increased and that incorporating this measurement in

combined screening by fetal NT and serum biochemistry improves the performance of screening.[73]

A study in the second-trimester measured the FMF angle in stored images of fetal profiles taken before amniocentesis at 14-24 weeks and reported the angle was significantly larger in 34 fetuses with trisomy 21 compared to 100 euploid fetuses.[72]

In the first-trimester the palate is visible as a single homogeneously hyperechogenic rectangular structure in the mid-sagittal view of the fetal face. The study of this Thesis demonstrated that in the second trimester there are at least two echogenic structures, the inferior one representing the palate and the superior ones the vomer.[81]

The study used 3D ultrasound to examine prospectively 150 normal fetuses and 23 fetuses with trisomy 21 at 16 and 25 weeks of gestation.[81] In normal fetuses the FMF angle did not change with gestation and the 95th centile was 88.5°. In 15 (65.2%) of the fetuses with trisomy 21 the FMF angle was greater than 88°. In the second trimester, screening for trisomy 21 by maternal age or maternal serum biochemistry have detection rates of 30% and 65%, respectively, for a false-positive rate of 5%.[82] Although many reports have highlighted the association between trisomy 21 and several defects or sonographic markers, such as cardiac abnormalities, increased nuchal fold thickness, short femur, echogenic intracardiac focus, hyperechogenic bowel, or hydronephrosis, each one of these features is observed in a minority of affected fetuses.[32] In contrast, sonographic assessment of the fetal profile for the FMF angle is likely to prove a sensitive method of second trimester screening for trisomy 21.

The two second-trimester ultrasound studies have demonstrated that the fetal profile, which is examined routinely during ultrasound examination can identify two important markers of trisomy 21: prenasal edema and increased FMF angle. Major prospective screening studies are now needed to define the performance of screening for trisomy 21 of these sonographic markers both on their own and in combination with second-trimester biochemical testing.

CHAPTER VI

CONCLUSIONS

El Higroma Quístico no constituye una entidad distinta, ni confiere un riesgo especial de forma independiente al que proporciona el grosor de la TN en el primer trimestre.

El aumento de la TN es un marcador efectivo no sólo para trisomía 21 sino para las otras cromosomopatías mayores y cada cromosomopatía se caracteriza por un patrón distinto de grosor de TN.

La TN fetal sigue dos distribuciones, una que es dependiente de la LCC y otra que es independiente de la LCC. La distribución de TN que aumenta con la LCC se observa en el 95% de los fetos euploides y en el 5% de las trisomías 21. La TN media, independiente de la LCC que caracteriza a las trisomías 21 es de 3.4 mm.

En el cribado bioquímico del primer trimestre para calcular el riesgo de trisomía 21 es esencial tener en cuenta la edad gestacional, el peso materno, grupo étnico, hábito tabáquico, método de concepción y tipo de analizador bioquímico.

La medida del edema prenasal es un método muy sensible para el cribado de trisomía 21 en el segundo trimestre del embarazo.
En 65.2% de los fetos con trisomía 21 el ángulo FMF fue mayor del percentil 95 de la normalidad.

Cystic hygroma does not constitute a distinct entity in the first trimester that confers a special risk status independent of the NT thickness.

Increased NT is an effective marker not only of trisomy 21 but also of all major chromosomal defects and the difference in phenotypic pattern of NT thickness characterize each chromosomal defect.

Fetal NT followed two distributions, one that was dependent on CRL and one that was independent of CRL. The distribution in which NT increases with CRL was observed in about 95% of euploid fetuses, 5% with trisomy 21. The median CRL-independent NT was 3.4 mm for the trisomy 21 group.

In first trimester biochemical screening for calculation of accurate patient-specific risks for trisomy 21, it is essential to take into account gestational age, maternal weight, ethnicity, smoking status, method of conception and machine used for the assays.

The measurement of prenasal thickness is a highly sensitive method of second-trimester screening for trisomy 21.

In 65.2% of the fetuses with trisomy 21 the FMF angle was greater than the 95th centile of the normal range.

CHAPTER VII

REFERENCES

1. Tabor A, Philip J, Madsen M, Bang J, Obel EB, Norgaard-Pedersen B. Randomised controlled trial of genetic amniocentesis in 4,606 low-risk women. Lancet 1986;i:1287–93

2. Alfirevic Z, Sundberg K, Brigham S. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Database Syst Rev. 2003;(3):CD003252

3. Snijders RJM, Sundberg K, Holzgreve W, Henry G, Nicolaides KH. Maternal age and gestation- specific risk for trisomy 21. Ultrasound Obstet Gynecol 1999;13:167–70

4. Snijders RJM, Sebire NJ, Cuckle H, Nicolaides KH. Maternal age and gestational age-specific risks for chromosomal defects. Fetal Diagn Therapy 1995;10:356–67

5. Down LJ. Observations on an ethnic classification of idiots. Clinical Lectures and Reports, London Hospital 1866;3:259-62

6. Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy. Br Med J 1992;304:867–9

7. Snijders RJM, Noble P, Sebire N, Souka A, Nicolaides KH. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. Lancet 1998;351:343–6

8. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol 2004;191:45-67

9. Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. Increased nuchal translucency with normal karyotype. Am J Obstet Gynecol 2005;192:1005-21

10. Pandya PP, Snijders RJM, Johnson SJ, Brizot M, Nicolaides KH. Screening for fetal trisomies by maternal age and fetal nuchal translucency thickness at 10 to 14 weeks of gestation. BJOG 1995;102:957-62

11. Reynolds T, Penney M. The mathematical basis of multivariate risk analysis with special reference to Down syndrome associated pregnancy. Ann Clin Biochem 1990;27:452-8

12. Spencer K, Bindra R, Nix ABJ, Heath V, Nicolaides KH. Delta- NT or NT MoM: Which is the most appropriate method for calculating accurate patient-specific risks for trisomy 21 in the first trimester? Ultrasound Obstet Gynecol 2003;22:142-8

13. Cicero S, Curcio P, Papageorghiou A, Sonek J, Nicolaides K. Absence of nasal bone in fetuses with trisomy 21 at 11-14 weeks of gestation: an observational study. Lancet 2001;358:1665-7

14. Cicero S, Avgidou K, Rembouskos G, Kagan KO, Nicolaides KH. Nasal bone in first-trimester screening for trisomy 21. Am J Obstet Gynecol 2006;195:109-14

15. Matias A, Gomes C, Flack N, Montenegro N, Nicolaides KH. Screening for chromosomal abnormalities at 11–14 weeks: the role of ductus venosus blood flow. Ultrasound Obstet Gynecol 1998;12:380–4

16. Maiz N, Valencia C, Kagan KO, Wright D, Nicolaides KH. Ductus venosus Doppler in screening for trisomies 21, 18 and 13 and Turner syndrome at 11+0-13+6 weeks of gestation. Ultrasound Obstet Gynecol 2009

17. Faiola S, Tsoi E, Huggon IC, Allan LD, Nicolaides KH. Likelihood ratio for trisomy 21 in fetuses with tricuspid regurgitation at the 11 to 13 + 6-week scan. Ultrasound Obstet Gynecol 2005;26:22–7

18. Falcon O, Faiola S, Huggon I, Allan L, Nicolaides KH. Fetal tricuspid regurgitation at the 11 + 0 to 13 + 6-week scan: association with chromosomal defects and reproducibility of the method. Ultrasound Obstet Gynecol 2006;27:609-12

19. Kagan KO, Valencia C, Livanos P, Wright D, Nicolaides KH. Tricuspid regurgitation in screening for trisomies 21, 18 and 13 and Turner syndrome at 11 + 0 to 13 + 6 weeks of gestation. Ultrasound Obstet Gynecol 2009;33:18-22

20. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984;148:886-94

21. Macri JN, Kasturi RV, Krantz DA, Cook EJ, Moore ND, Young JA, Romero K, Laresen JW. Maternal serum Down syndrome screening: free beta protein is a more effective marker than human chorionic gonadotrophin. Am J Obstet Gynecol 1990;163: 1248-53

22. Canick J, Knight GJ, Palomaki GE, Haddow JE, Cuckle HS, Wald NJ. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J Obstet Gynaecol 1988;95:330-3

23. Van Lith JM, Pratt JJ, Beekhuis JR, Mantingh A. Second trimester maternal serum immuno-reactive inhibin as a marker for fetal Down's syndrome. Prenat Diagn 1992;12:801-6

24. Cuckle H. Integrating Down's syndrome screening. Curr Opin Obstet Gynaecol 2001;13:175-81

25. Brizot, M.L., Snijders, R.J.M., Bersinger, N.A., Kuhn, P., Nicolaides, K.H. Maternal serum pregnancy associated placental protein A and fetal nuchal translucency thickness for the prediction of fetal trisomies in early pregnancy. Obstet Gynecol 1994;84:918–22

26. Brizot ML, Snijders RJM, Butler J, Bersinger NA, Nicolaides KH. Maternal serum hCG and fetal nuchal translucency thickness for the prediction of fetal trisomies in the first trimester of pregnancy. Br J Obstet Gynaecol 1995;102:1227–32

27. Spencer K, Souter V, Tul N, Snijders R, Nicolaides KH. A screening program for trisomy 21 at 10–14 weeks using fetal nuchal translucency, maternal serum free ß-human chorionic gonado- tropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 1999;13:231–7

28. Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. One Stop Clinic for Assessment of Risk for Trisomy 21 at 11-14 weeks: A Prospective Study of 15,030 Pregnancies. Ultrasound Obstet Gynecol 2002;20:219-25

29. Spencer K, Spencer CE, Power M, Dawson C, Nicolaides KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one stop clinic: A review of three years prospective experience. Br J Obstet Gynaecol 2003;110:281-6

30. Spencer K, Crossley JA, Aitken DA, Nix AB, Dunstan FD, Williams K. Temporal changes in maternal serum biochemical markers of trisomy 21 across the first and second trimester of pregnancy. Ann Clin Biochem 2002;39:567-576

31. Nicolaides KH, Spencer K, Avgidou K, Faiola S, Falcon O. Multicenter study of first-trimester screening for trisomy 21 in 75 821 pregnancies: results and estimation of the potential impact of individual risk-orientated two-stage first-trimester screening. Ultrasound Obstet Gynecol 2005;25:221-6

32. Nicolaides KH. Screening for chromosomal defects. Ultrasound Obstet Gynecol 2003;21:313-21

33. Nyberg DA, Souter VL, El-Bastawissi A, Young S, Luthhardt F, Luthy DA. Isolated sonographic markers for detection of fetal Down syndrome in the second trimester of pregnancy. J Ultrasound Med 2001;20:1053-63

34. Bromley B, Lieberman E, Shipp TD, Benacerraf BR. The genetic sonogram: a method of risk assessment for Down syndrome in the second trimester. J Ultrasound Med 2002;21:1087-96

35. Farkas LG, Katic MJ, Forrest CR, Litsas L. Surface anatomy of the face in Down's syndrome: linear and angular measurements in the craniofacial regions. J Craniofac Surg 2001;12:373-9

36. Keeling JW, Hansen BF, Kjaer I. Pattern of malformation in the axial skeleton in human trisomy 21 fetuses. Am J Med Genet 1997; 68:466-71

37. Stempfle N, Huten Y, Fredouille C, Brisse H, Nessmann C. Skeletal bnormalities in fetuses with Down's syndrome: A radiologic postmortem study. Pediatr Radiol 1999;29:682-8

38. Tuxen A, Keeling JW, Reintoft I, Fischer Hansen B, Nolting D, Kjaer I. A histological and radiological investigation of the nasal bone in fetuses with Down syndrome. Ultrasound Obstet Gynecol 2003;22:22-6

39. Larose et al Minderer S, Gloning KP, Henrich W, Stoger H. The nasal bone in fetuses with trisomy 21: sonographic versus pathomorphological findings. Ultrasound Obstet Gynecol 2003;22:16-21

40. Sonek J, Nicolaides K. Prenatal ultrasonographic diagnosis of nasal bone abnormalities in three fetuses with Down syndrome. Am J Obstet Gynecol 2002;186:139-41

41. Sonek JD, Cicero S, Neiger R, Nicolaides KH. Nasal bone assessment in prenatal screening for trisomy 21. Am J Obstet Gynecol 2006;195:1219-30

42. Guis F, Ville Y, Doumerc S, Pons J, Frydman R. Ultrasound evaluation of the length of the fetal nasal bones throughout gestation. Ultrasound Obstet Gynecol 1995;5:304-7

43. Sonek J, McKenna D, Webb D, Croom C, Nicolaides K. Nasal bone length throughout gestation: Normal ranges based on 3537 fetal ultrasound measurements. Ultrasound Obstet Gynecol 2003;21:152-5

44. Orlandi F, Bilardo CM, Campogrande M, Krantz D, Hallahan T, Rossi C, Viora E. Measurement of nasal bone length at 11-14 weeks of pregnancy and its potential role in Down syndrome risk assessment. Ultrasound Obstet Gynecol 2003;22:36-9

45. Bunduki V, Ruano J, Miguelez J, Yoshizaki C, Kahhale S, Zugaib M. Fetal bone length: Reference range and clinical application in ultrasound screening for Trisomy 21. Ultrasound Obstet Gynecol 2003;21:156-60

46. Cicero S, Bindra R, Rembouskos G, Tripsanas C, Nicolaides K. Fetal nasal bone length in chromosomally normal and abnormal fetuses at 11-14 weeks of gestation. J Matern Fetal Neo Med 2002;11:400-2

47. Cusick W, Provenzano J, Sullivan C, Gallousis F, Rodis J. Fetal nasal bone length in euploid and aneuploid fetuses between 11 and 20 weeks' gestation: a prospective study. J Ultrasound Med 2004; 23:1327-33

48. Bromley B, Lieberman E, Shipp T, Benaceraff B. Fetal nasal bone length: A marker for Down syndrome in the second trimester. J Ultrasound Med 2002;21:1387-94

49. Tran L, Carr D, Mitsumori L, Uhrich S, Shields L. Second-trimester biparietal diameter /nasal bone length ratio as an independent predictor of trisomy 21. J Ultrasound Med 2005; 24:805-10

50. Gamez F, Ferreiro P, Salmean JM. Ultrasonographic measurement of fetal nasal bone in a low risk population at 19-22 gestational weeks. Ultrasound Obstet Gynecol 2003;22:152-3

51. Cicero S, Sonek J, McKenna D, Croom C, Johnson L, Nicolaides K. Nasal bone hypoplasia in fetuses with Trisomy 21 at 15-22 weeks' gestation. Ultrasound Obstet Gynecol 2003;21:15-8

52. Vintzileos A, Walters C, Yeo L. Absent nasal bone in the prenatal detection of fetuses with trisomy 21 in a high-risk population. Obstet Gynecol 2003;101:905-8

53. Odibo AO, Sehdev HM, Dunn L, McDonald R, Macones GA. The association between fetal nasal bone hyplasia and aneuploidy. Obstet Gynecol 2004;104:1229-33

54. Benoit B, Chaoui R. Three-dimensional ultrasound with maximal mode rendering: a novel technique for the diagnosis of bilateral or unilateral absence or hypoplasia of nasal bones in second-trimester screening for Down syndrome. Ultrasound Obstet Gynecol 2005;25:19-24

55. Rembouskos G, Cicero S, Longo D, Vandecruys H, Nicolaides KH. Assessment of the fetal nasal bone at 11-14 weeks of gestation by three-dimensional ultrasound. Ultrasound Obstet Gynecol 2004;23:232-6

56. Peralta CF, Falcon O, Wegrzyn P, Faro C, Nicolaides KH. Assessment of the gap between the fetal nasal bones at 11 to 13 + 6 weeks of gestation by three-dimensional ultrasound. Ultrasound Obstet Gynecol 2005;25:464-7

57. Lee W, DeVore GR, Comstock CH, Kalache KD, McNie B, Chaiworapongsa T, Conoscenti G, Treadwell MC, Johnson A, Huang R, Romero R. Nasal bone evaluation in fetuses with Down syndrome during the second and third trimesters of pregnancy. J Ultrasound Med 2003;22:55-60

58. Goncalves LF, Espinoza J, Lee W, Schoen ML, Devers P, Mazor M, Chaiworapongsa T, DeVore GR, Romero R. Phenotypic characteristics of absent and hypoplastic nasal bones in fetuses with Down syndrome: description by 3-dimensional ultrasonography and clinical significance. J Ultrasound Med 2004;23:1619-27

59. Benacerraf BR, Gelman R, Frigoletto FD. Sonographic identification of second-trimester fetuses with Down's syndrome. N Engl JMed 1987;317:1371–6

60. Nicolaides KH, Azar G, Snijders RJM, Gosden CM. Fetal nuchal edema: associated malformations and chromosomal defects. Fetal Diagn Ther 1992;7:123–31

61. Maymon R, Levinsohn-Tavor O, Cuckle H, Tovbin Y, Dreazen E, Wiener Y, Herman A. Second trimester ultrasound prenasal thickness combined with nasal bone length: a new method of Down syndrome screening. Prenat Diagn 2005;25:906–11

62. Farkas LG, Katic MJ, Forrest CR, Litsas L. Surface anatomy of the face in Down's syndrome: linear and angular measurements in the craniofacial regions. J Craniofac Surg 2001;12:373-9

63. Allanson JE, O'Hara P, Farkas LG, Nair RC. Anthropometric craniofacial pattern profiles in Down syndrome. Am J Med Genet 1993;47:748-52

64. Shapira J, Chaushu S, Becker A. Prevalence of tooth transposition, third molar agenesis, and maxillary canine impaction in individuals with Down syndrome. Orthod 2000;70:290-6

65. Mestrovic SR, Rajic Z, Papic JS. Hypodontia in patients with Down's syndrome. Coll Antropol 1998;22:69-72

66. Kumasaka S, Miyagi A, Sakai N, Shindo J, Kashima I. Oligodontia: a radiographic comparison of subjects with Down syndrome and normal subjects. Spec Care Dentist 1997;17:137-41

67. Russell BG, Kjaer I. Tooth agenesis in Down syndrome. Am J Med Genet 1995;55:466-71

68. Cicero S, Curcio P, Rembouskos G, Sonek JD, Nicolaides KH. Maxillary length at 11-14 weeks of gestation in fetuses with trisomy 21. Ultrasound Obstet Gynecol 2004;24:19-22

69. Dagklis T, Borenstein M, Peralta CFA, Faro C, Nicolaides KH. Three-dimensional evaluation of mid-facial hypoplasia in fetuses with trisomy 21 at 11-13+6 weeks. Ultrasound Obstet Gynecol 2006;28:261-5

70. Berganni A, Bamberg C, Ederl K, Proquitt'e, Hartung JP, Bollman R, Kalache KD. Mid-facial antrhropometry in second-trimester fetuses with trisomy 21: a three dimensional ultrasound study. Prenatal Diagnosis 2006;26:158-62

71. Sonek J, Borenstein M, Dagklis T, Persico N, Nicolaides K. Frontomaxillary facial angle in fetuses with trisomy 21 at 11-13 weeks. Am J Obstet Gynecol 2007;196:271.e1-4

72. Sonek J, Borenstein M, Downing C, McKenna D, Neiger R, Croom C, Gengrich T, Nicolaides KH. Fronto-maxillary facial angles in screening for trisomy 21 between 14 and 23 weeks' gestation. Am J Obstet Gynecol 2007;197:160,e1-5

73. Borenstein M, Persico N, Kagan KO, Gazzoni A, Nicolaides KH. Frontomaxillary facial angle in screening for trisomy 21 at 11+0 to 13+6 weeks. Ultrasound Obstet Gynecol 2008;32:5-11

74. Molina FS, Avgidou K, Kagan KO, Poggi S, Nicolaides KH. Cystic hygromas, nuchal edema and nuchal translucency at 11–14 weeks of gestation. Obstet Gynecol 2006;107:1-6

75. Malone FD, Ball RH, Nyberg DA, Comstock CH, Saade GR, Berkowitz RL, et al. First-trimester septated cystic hygroma: prevalence, natural history, and pediatric outcome. Obstet Gynecol 2005;106:288–94

76. Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation between increased fetal nuchal translucency thickness and chromosomal defects. Obstet Gynecol 2006;107:6-10

77. Nicolaides KH, Snijders RJ, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. Prenat Diagn 1998; 18: 519–523.

78. Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. A mixture model of nuchal translucency thickness in screening for chromosomal defects.Ultrasound Obstet Gynecol 2008;31:376-83

79. Kagan KO, Wright D, Spencer K, Molina FS, Nicolaides KH. First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. Ultrasound Obstet Gynecol 2008;31:493-502

80. Persico N, Borenstein M, Molina F, Azumendi G, Nicolaides KH. Prenasal thickness in trisomy-21 fetuses at 16-24 weeks of gestation. Ultrasound Obstet Gynecol 2008;32:751-4

81. Molina F, Persico N, Borenstein M, Sonek J, Nicolaides KH. Frontomaxillary facial angle in trisomy 21 fetuses at 16-24 weeks of gestation. Ultrasound Obstet Gynecol 2008;31:384-7

82. Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. Health Technol Assess 1998;2: 1–112