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Screening for trisomies in dichorionic twins by measurement of fetal nuchal translucency thickness according to the mixture model

David Wright[1], Argyro Syngelaki[2,3], Ismini Staboulidou[2], Jader de Jesus Cruz[2,3] and Kypros H. Nicolaides[2,3]*

[1]*Department of Mathematics and Statistics, University of Plymouth, Plymouth, UK*
[2]*Harris Birthright Research Centre of Fetal Medicine, King's College Hospital, London, UK*
[3]*Department of Fetal Medicine, University College Hospital, London, UK*

Objective To examine the distribution of fetal nuchal translucency (NT) thickness in dichorionic twins and investigate the effect of the correlation between NT measurements in each twin pair on the performance of screening for trisomies.

Methods The distribution of fetal NT for crown–rump length (CRL) was examined in 5646 dichorionic twin pregnancies, including 103 with fetal trisomies 21, 18 or 13. The correlation in fetal NT in each euploid twin pregnancy was estimated.

Results The distribution of NT in both euploid and trisomic fetuses was consistent with the mixture model in singleton pregnancies. In the euploid pregnancies, there was a correlation in log NT measurements in each twin pair ($r = 0.42$, 95% CI: 0.39–0.45) and, after removal of the effect of the operator, this correlation was reduced to 0.34. Allowing for this correlation in risk assessment for trisomies had a major impact on the estimated patient-specific risk but had little effect on the overall performance of screening.

Conclusions In dichorionic twin pregnancies, the mixture model of distributions of NT can be applied as in singletons. In screening for trisomies, the correlation in NT measurements between the fetuses should be taken into account in the estimation of patient-specific risks. Copyright © 2011 John Wiley & Sons, Ltd.

KEY WORDS: first-trimester screening; trisomy 21; trisomy 18; trisomy 13; dichorionic twins; nuchal translucency

INTRODUCTION

In twin pregnancies, effective screening for trisomies is provided by a combination of maternal age and fetal nuchal translucency (NT) thickness (Pandya *et al*., 1995; Sebire *et al*., 1996a,b; Maymon *et al*., 2001). In dichorionic twins, patient-specific risks for trisomy 21 are calculated for each fetus on the basis of maternal age, and the fetal NT and detection rate (75–80%) and false positive rate (5% per fetus or 10% per pregnancy) are similar to those in singleton pregnancies (Sebire *et al*., 1996a). Therefore, effective screening and diagnosis of major aneuploidies can be achieved in the first trimester, allowing the possibility of earlier and therefore safer selective fetocide in cases where one fetus is euploid and the other is abnormal (Sebire *et al*., 1996b).

In the assessment of patient-specific risks for aneuploidies, the *a priori* maternal-age-related risk is multiplied by the likelihood ratio determined from the deviation of the measured NT from the expected median for crown–rump length (CRL). There are essentially two approaches for such quantification of the deviation in NT. One approach is to subtract the normal median from the NT measurement and to produce a deviation in millimetres referred to as *delta* NT (Pandya *et al*., 1995; Spencer *et al*., 2003). The other approach is to divide NT by the normal median to produce a multiple of the median (MoM) value (Nicolaides *et al*., 1998). Recently, a new approach has been proposed, which is based on the observation that in both aneuploid and euploid pregnancies fetal NT follows two distributions, one that is CRL-dependent and another that is CRL-independent (Wright *et al*., 2008). In this mixture model, the distribution in which NT increases with CRL is observed in approximately 95% of euploid fetuses, 5% with trisomy 21, 30% with trisomy 18 and 15% with trisomy 13. The median CRL-independent NT is 2.0 mm for the euploid group and 3.4, 5.5 and 4.0 mm for trisomies 21, 18 and 13 respectively.

In the calculation of risk for trisomies in dichorionic twins, it was assumed that in each pregnancy the measurements of NT for CRL between the two fetuses were independent of each other. However, Wøjdemann *et al*. (2006) examined 150 dichorionic twins with euploid fetuses and reported that there was an association in the measurements of NT in each twin pair with a correlation coefficient of 0.32. Similarly, Cuckle and Maymon (2010), reported a correlation coefficient of 0.43 in 246 dichorionic twins with euploid fetuses.

*Correspondence to: Kypros H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, Denmark Hill, London SE5 9RS, UK.
E-mail: kypros@fetalmedicine.com

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The aims of this study are to examine the distribution of NT measurements in dichorionic twin pregnancies to investigate the correlation between NT measurements in twins, allowing for the effect of CRL and sonographer, and to apply the mixture model to risk assessment independently to each fetus, also allowing for the correlation in the CRL-dependent process.

METHODS

At our centre, early screening for trisomies in twins is based on maternal age and the measurement of fetal NT at 11 to 13^{+6} weeks' gestation (Snijders *et al*., 1998). Essentially, the maternal- and gestational-age-related risk is multiplied by a likelihood ratio corresponding to the deviation in fetal NT thickness from the appropriate normal median for CRL. Fetal NT and CRL are measured in each fetus and, in addition, chorionicity is determined by examining the inter-twin membrane at its junction with the placenta (Sepulveda *et al*., 1996).

Demographic characteristics and ultrasound findings were recorded in a fetal database at the time of the examination. In the cases undergoing chorionic villous sampling or amniocentesis, both fetuses were sampled and the results of fetal karyotype were also entered in the database when they were made available. Data on pregnancy outcome were obtained from the patients themselves, their general practitioners or the maternity units in which they delivered.

A computer search was made to identify all dichorionic twin pregnancies that had NT screening between February 1993 and February 2010. The selection criteria were that, firstly, in each pregnancy both fetuses were alive at the 11 to 13^{+6} weeks' scan and, secondly, the fetal karyotype was determined by prenatal invasive testing or the pregnancy outcome was known.

Statistical analysis

In the twin pregnancies with two euploid fetuses, the distribution of fetal NT for CRL was compared with the percentiles from the mixture model (Wright *et al*., 2008). Two models were developed for the calculation of risk for trisomy 21. In model A, it was assumed that in each pregnancy the measurements of NT for CRL between the two fetuses were independent of each other and the risks were computed according to the mixture model with no modification for twins. The parameters used to determine the risk were the same as previously published (Wright *et al*., 2008). In model B, the correlation of the CRL-dependent NT between the two fetuses in each twin pair was estimated using the method of moments. This correlation was used to modify the mixture model for calculation of risks. Risks were computed under the two models and used to produce estimates of false positive rates and detection rates. For model A, the calculations were identical to those for singletons. For model B, risks were assigned to the pair of twins using Bayes theorem incorporating a bivariate Gaussian distribution for the CRL-dependent process. A technical description of the model is given in the Appendix.

RESULTS

The computer search identified 5646 dichorionic twin pregnancies. The median maternal age was 34.7 (range 16–54) years, the median CRL of the bigger twin was 64.2 (range 45–84) mm and that of the smaller twin was 61.1 (range 45–84) mm. The median NT thickness was 1.7 (range 0.5–12.9) mm. There were 5530 pregnancies with normal fetal karyotype or birth of phenotypically normal babies, 103 with trisomies in one or both fetuses (trisomy 21 in one fetus, $n = 65$; trisomy 18 in one fetus, $n = 22$; trisomy 13 in one fetus, $n = 7$; trisomy 21 in both fetuses, $n = 5$; trisomy 13 in both fetuses, $n = 2$; trisomy 21 in one fetus and trisomy 18 in the other, $n = 1$; trisomy 21 in one fetus and trisomy 13 in the other, $n = 1$) and 13 with other aneuploidies in one fetus that were excluded from further analysis (Turner syndrome, $n = 7$; 47, XYY, $n = 1$; addition or mosaicism, $n = 5$).

The relation between fetal NT and CRL in the euploid and trisomic fetuses is shown in Figures 1 and 2. The empirical estimates of the median and 95th and 99th percentiles for the euploid fetuses, obtained from non-parametric quantile regression, and the distribution of values in the trisomic fetuses are consistent with those from the mixture model in singleton pregnancies (Wright *et al*., 2008).

After removing the effect of CRL, the estimated correlation between log NT measurements in euploid twins was 0.42 (95% CI: 0.39–0.45). After removal of the effect of operator, this correlation was reduced to 0.34.

The relation of log MoM values for NT relative to the CRL-dependent process between the two fetuses in each twin pair is shown in Figures 3 and 4. The curves show the likehood ratio contours for a trisomy 21 (in twin 1 or twin 2) relative to unaffected pregnancies under the mixture model according to model A, which assumes that the measurements in the two euploid fetuses are independent of each other (Figure 3), and model B, which assumes a correlation in the measurements of the fetuses (Figure 4). In model B, the likelihood ratio for trisomy 21 is relatively low compared to that in model A when the NT is large in both fetuses, and it is high when the NT in one fetus is large and that in the co-twin is normal or small.

The performance of screening with a risk cut-off of 1 in 100 at the time of screening applied to each twin under the two versions of the mixture model was similar (Table 1). The estimated risk for trisomy 21 at 12 weeks' gestation in twin 1 in a dichorionic twin pregnancy where the maternal age is 35 years and the CRL of both fetuses is 60 mm as shown in Table 2. The risk increases with the NT thickness of twin 1 but this is modified by the NT measurement of twin 2.

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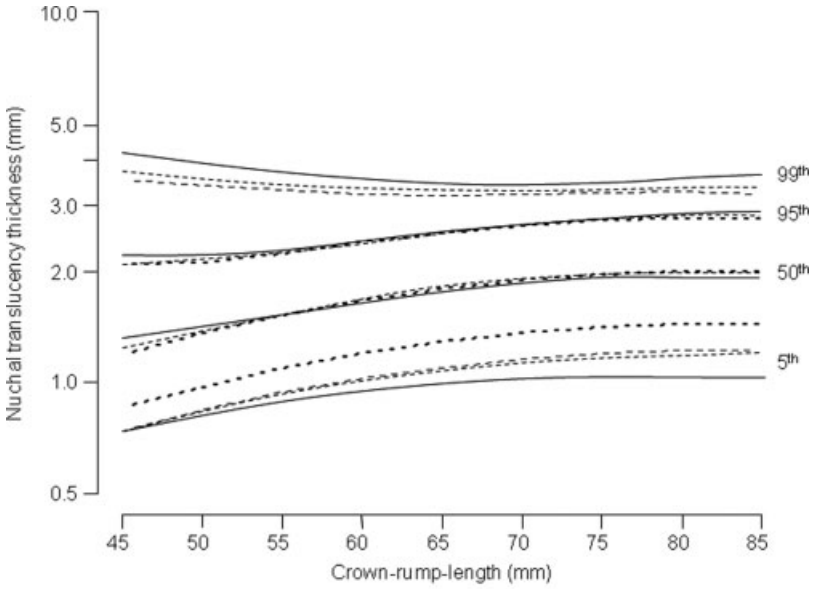

Figure 1—Relation of fetal nuchal translucency thickness with crown–rump length in euploid fetuses. Percentiles obtained from applying non-parametric quantile regression (full lines) and from the mixture model for singleton pregnancies (broken lines)

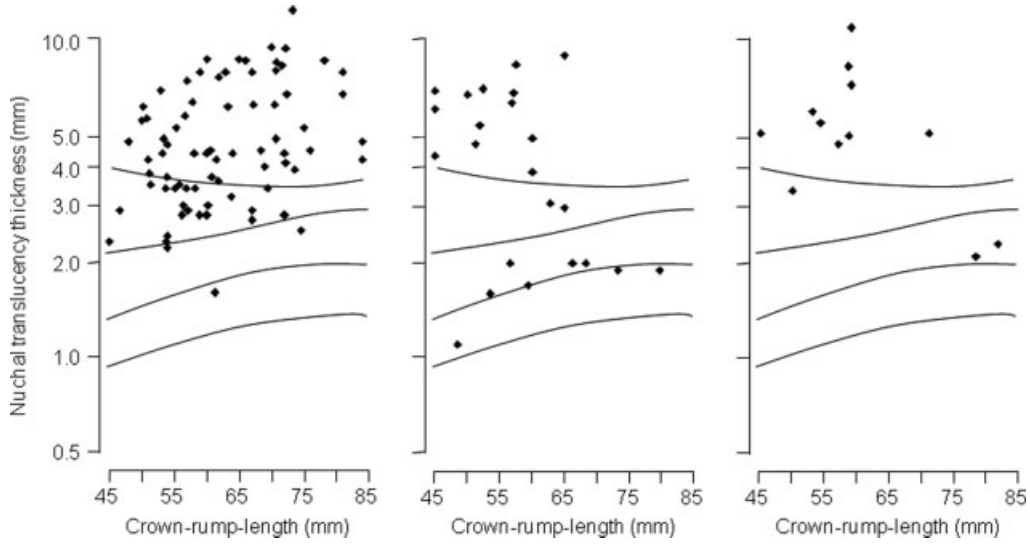

Figure 2—Nuchal translucency thickness in fetuses with trisomy 21 (left), trisomy 18 (middle) and trisomy 13 (right) plotted on the percentiles (5th, 50th, 95th and 99th) for euploid twin fetuses

DISCUSSION

The findings of this study in more than 5500 dichorionic twin pregnancies confirm the results of two previous smaller studies (Wøjdemann *et al.*, 2006; Cuckle and Maymon, 2010) that NT measurements in euploid twins are correlated and this correlation is not a simple reflection of the common effect of sonographers.

High NT is associated with aneuploidies, cardiac defects and a wide range of other fetal malformations and genetic syndromes (Souka *et al.*, 2005). However, in most of the cases the fetuses are normal and it is likely that the individual measurements would be affected by parentally derived genetic factors as well as pregnancy-specific environmental factors. Recent evidence, for example, indicates that there is a significant association between fetal NT and birth weight, with a small NT increasing the risk of delivering small babies and a large NT increasing the risk of delivering macrosomic neonates (Poon *et al.*, 2010, 2011). In this respect, the finding that in normal dichorionic twins there is an association in the NT of the two fetuses is not surprising.

In euploid dichorionic twins, the distribution of NTs was consistent with the mixture model in singleton pregnancies (Wright *et al.*, 2008). In screening for trisomies in twins, the standardised false positive rate for each fetus is very similar to that in singleton pregnancies but the overall false postive rate for twin pregnacies is approximately double that of singleton pregnancies.

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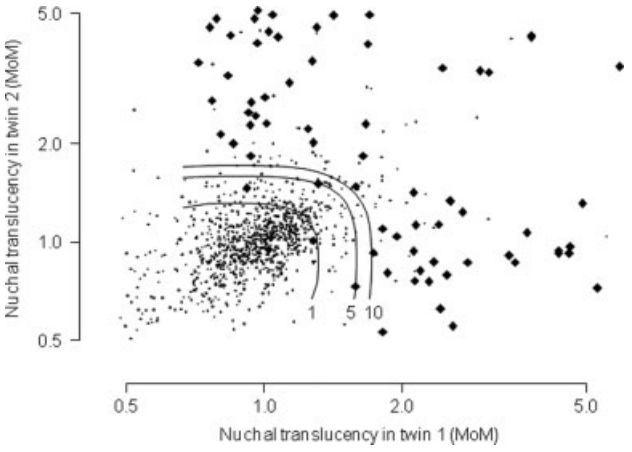

Figure 3—Scatter diagram of fetal nuchal translucency (NT) measurements in twins expressed as multiples of the median (MoM) of the crown–rump-length-dependent component of the mixture model of distributions of NT in euploid fetuses. Sample of 1000 euploid pregnancies (hollow circles) and the full sample of twin pregnancies with a trisomy 21 fetus (full diamonds). The contours are for likelihood ratios (one or more trisomy 21 twins/both euploid twins) of 1, 5 and 10 on the assusmption that the NT measurements in the two fetuses are independent of each other

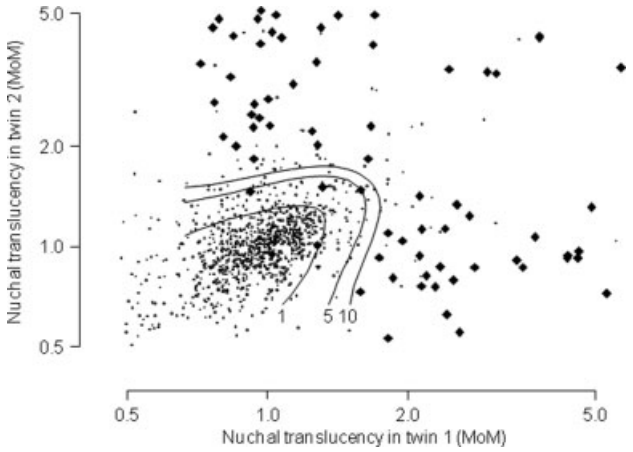

Figure 4—Scatter diagram of fetal nuchal translucency (NT) measurements in twins expressed as multiples of the median (MoM) of the crown–rump-length-dependent component of the mixture model of distributions of NT in euploid fetuses. Sample of 1000 euploid pregnancies (hollow circles) and the full sample of twin pregnancies with a trisomy 21 fetus (full diamonds). The contours are for likelihood ratios (one or more trisomy 21 twins/both euploid twins) of 1, 5 and 10 on the assusmption that the NT measurements in the two fetuses are correlated

In our dataset, the detection rate of trisomies was very high irrespective of whether the model used for screening took into account the correlation in NT measurements between the fetuses or not. Similarly, inclusion of the correlation between NT measurements had a very small impact on the overall false positive rates. However, the correlation in NT measurements in twins had a substantial impact in the estimated patient-specific risk for trisomies. In the example given in Table 2, the estimated risk of trisomy 21 for twin 1 with NT of 2.5 mm was 1 in 116 according to the independent model, whereas with the dependent model the risk increased to 1 in 48 if the NT in twin 2 was 1.0 mm and this decreased to 1 in 255 if the NT of twin 2 was 3.0 mm.

The difference between our model for estimation of fetus-specific risk of aneuploidy, compared to the one proposed by Cuckle and Maymon (2010), relates to the method of estimating both the prior risk and likelihoods. In the estimation of the prior risk, we took the pragmatic approach of assuming that all dichorionic twins are dizygotic and the risk for aneuploidies in each fetus is the same as the maternal- and gestational-age-related risk in singleton pregnancies. Cuckle and Maymon (2010), attempted to adjust the risk according to the estimated proportions of dizygotic and monozygotic twins depending on maternal age, racial origin, method of conception and fetal gender. In the calculation of likelihood ratios, we assumed that as in singleton pregnancies the distribution of NT arises from a mixture of a CRL-dependent and a CRL-independent process, because this provides a better fit to the empirical data on both affected and unaffected pregnancies. Cuckle and Maymon (2010) derived likelihoods using the MoM approach assuming that log MoM values follow Gaussian distributions for affected and unaffected fetuses.

Table 1—Empirical screening performance in dichorionic twins with the mixture model of distributions in nuchal translucency (NT) thickness and a risk cut-off of 1 in 100 at the time of screening applied to each twin

Screen positive	Euploid/euploid, $n = 5530$	Trisomy 21/euploid, $n = 65$	Trisomy 18/euploid, $n = 22$	Trisomy 13/euploid, $n = 7$	Trisomy/trisomy[a], $n = 9$
Model A					
Twin 1	362 (6.5)a	62 (95.4%)	14 (63.6%)	6 (85.7%)	9 (100%)
Twin 2	435 (7.9)b	9 (13.8%)	4 (18.2%)	0	7 (77.8%)
Either twin	618 (11.2%)c	62 (95.4%)	16 (72.7%)	6 (85.7%)	9 (100%)
Model B					
Twin 1	340 (6.1%)d	62 (95.4%)	14 (63.6%)	6 (85.7%)	8 (88.9%)
Twin 2	432 (7.8%)e	8 (12.3%)	4 (18.2%)	0	7 (77.8%)
Either twin	624 (11.3%)f	62 (95.4%)	16 (72.7%)	6 (85.7%)	8 (88.9%)

In model A, it is assumed that the measurements of NT in the two euploid fetuses are independent of each other and, in model B, the correlation in NT between the fetuses is taken into account.
Standardised rate: a = 3.3%, b = 4.7%, c = 6.6%, d = 2.9%, e = 4.5%, f = 6.5%.
[a] Trisomy 21/trisomy 21 ($n = 5$), trisomy 21/trisomy 18 ($n = 1$), trisomy 21/trisomy 13 ($n = 1$), both trisomy 13 ($n = 2$).

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Table 2—Estimated risk for trisomy 21 at 87 days of gestation in the presenting fetus in a dichorionic twin pregnancy where the maternal age was 35 years and the crown–rump length of both fetuses was 60 mm

	Estimated risk (1 in number below) for twin 1			
		Model B with different measurements of NT in twin 2		
NT twin 1 (mm)	Model A	NT 1.0 mm (0.61)	NT 2.0 mm (1.21)	NT 3.0 mm (1.82)
1.6 (0.97)	1685	1388	1598	1476
1.7 (1.03)	1488	996	1552	1353
1.8 (1.09)	1246	682	1418	1199
1.9 (1.15)	991	454	1224	1031
2.0 (1.21)	750	299	999	864
2.1 (1.27)	543	198	773	707
2.2 (1.33)	380	133	568	566
2.3 (1.39)	259	91	400	443
2.4 (1.46)	174	65	273	339
2.5 (1.52)	116	48	182	255
2.6 (1.58)	79	37	120	189
2.7 (1.64)	55	30	80	139
2.8 (1.70)	40	25	54	101
2.9 (1.76)	30	22	38	74
3.0 (1.82)	24	19	29	54
3.1 (1.88)	20	17	22	40
3.2 (1.94)	17	16	19	31
3.3 (2.00)	15	14	16	24
3.4 (2.06)	14	13	14	19
3.5 (2.12)	13	12	13	16
3.6 (2.18)	12	11	12	14
3.7 (2.24)	11	11	11	12
3.8 (2.30)	10	10	10	11
3.9 (2.37)	9	9	9	10
4.0 (2.43)	9	9	9	9

The risk increases with the nuchal translucency (NT) thickness of twin 1. In model A, it is assumed that there is independence in the measurements of NT in the two fetuses. In model B, there is a correlation between the measurements of NT in the two fetuses. The prior risk for a singe fetus with maternal age of 35 years is 1 in 305. MoM values relative to the crown–rump-length-dependent process are shown in brackets.

The implications of the results of this study in dichorionic twins are that, firstly, the mixture model of distributions of NT can be applied as in singleton pregnancies and, secondly, in screening for trisomies the correlation in NT measurements between the fetuses should be taken into account in the estimation of patient-specific risks.

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APPENDIX: RISK CALCULATIONS FOR DICHORIONIC TWIN PREGNANCIES

If we assume that each fetus either has trisomy 21 or is euploid, the possible outcomes for twin 1 and twin 2 can be represented by the four cells in a 2×2 table. The final or posterior risk of the four possible outcomes are obtained from applying Bayes theorem to combine prior probabilities with the likelihoods for the four table entries. If another condition, such as trisomy 18, is to be included, then a 3×3 table is necessary.

Prior probabilities

In the calculation of the prior probabilities, it is assumed that trisomy 21 arises independently and the probability of each twin being affected is the same as that of a singleton pregnancy and depends on the maternal age and gestation. This leads to a prior probability distribution over the possible outcomes given in Table A1, where π denotes the maternal- and gestational-age-specific risk of trisomy 21.

Likelihoods

The likelihoods for the four cell entries are obtained from the mixture model. Each cell is a mixture of Gaussian probability densities according to whether or not the NT measurement for the fetus arises from the CRL-dependent or CRL-independent component as described in Wright *et al*. (2008). The mixing probabilities, which we denote by p_1 and p_2 for twin 1 and twin 2 respectively, that the measurement arises from the CRL-independent component give rise to the entries in Table A2.

The likelihood for the four possible outcomes is all of the form

$$(1-p_1)(1-p_2)\varphi_{\mathrm{D1D2}} + p_1(1-p_2)\varphi_{\mathrm{I1}}\varphi_{\mathrm{D2}} + (1-p_1)\varphi_2\varphi_{\mathrm{D1}}\varphi_{\mathrm{I2}} + p_1p_2\varphi_{\mathrm{I1}}\varphi_{\mathrm{I2}} \quad (1)$$

In the above expression, φ_{D1D2} is a bivariate Gaussian probability density for the situation where both NT measurements arise from the CRL-dependent process. The mean and standard deviations describing this distribution are the same as those of Wright *et al*. (2008). In model B, it is assumed that the bivariate Gaussian distribution has a non-zero correlation coefficient.

Table A1—Prior probabilities under the independence model

	Twin 2	
Twin 1	Euploid	Trisomy 21
Euploid	$(1-\pi)(1-\pi)$	$\pi(1-\pi)$
Trisomy 21	$(1-\pi)\pi$	π^2

Table A2—Mixture probabilities

	Twin 2	
Twin 1	CRL-dependent	CRL-independent
CRL-dependent	$(1-p_1)(1-p_2)$	$p_1(1-p_2)$
CRL-independent	$(1-p_1)p_2$	p_1p_2

ϕ_{D1} and ϕ_{D2} are univariate Gaussian probability densities for twin 1 and twin 2 under the CRL-dependent model. Similarly, ϕ_{I1} and ϕ_{I2} are univariate Gaussian probability densities for twin 1 and twin 2 under the CRL-independent model. These are identical to those that would pertain in singleton pregnancies from the mixture model of Wright *et al*. (2008).

Posterior probabilities

Posterior probabilities are obtained first by multiplying the prior probabilities in Table A1 with the corresponding likelihoods in Equation (1) to produce a table of prior × likelihoods. These are then normalised by dividing by their total to produce a set of four probabilities that add to 1.0. This gives the probability distribution of the outcomes of the pregnancy.

REFERENCES

Cuckle H, Maymon R. 2010. Down syndrome risk calculation for a twin fetus taking account of the nuchal translucency in the co-twin. *Prenat Diagn* **30**: 827–833.

Maymon R, Jauniaux E, Holmes A, *et al*. 2001. Nuchal translucency measurement and pregnancy outcome after assisted conception versus spontaneously conceived twins. *Hum Reprod* **16**: 1999–2004.

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

Pandya PP, Hilbert F, Snijders RJ, Nicolaides KH. 1995. Nuchal translucency thickness and crown-rump length in twin pregnancies with chromosomally abnormal fetuses. *J Ultrasound Med* **14**: 565–568.

Poon LC, Karagiannis G, Staboulidou I, Shafiei A, Nicolaides KH. 2011. Reference range of birth weight with gestation and first-trimester prediction of small for gestation neonates. *Prenat Diagn* **31**(1): 58–65.

Poon LCY, Karagiannis G, Stratieva V, Syngelaki A, Nicolaides KH. 2010. First-trimester prediction of macrosomia. *Fetal Diagn Ther in press*.

Sebire NJ, Snijders RJM, Hughes K, Sepulveda W, Nicolaides KH. 1996a. Screening for trisomy 21 in twin pregnancies by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. *BJOG* **103**: 999–1003.

Sebire NJ, Noble PL, Psarra A, Papapanagiotou G, Nicolaides KH. 1996b. Fetal karyotyping in twin pregnancies: selection of technique by measurement of fetal nuchal translucency. *BJOG* **103**: 887–890.

Sepulveda W, Sebire NJ, Hughes K, Odibo A, Nicolaides KH. 1996. The lambda sign at 10–14 weeks of gestation as a predictor of chorionicity in twin pregnancies. *Ultrasound Obstet Gynecol* **7**: 421–423.

Snijders RMJ, Noble P, Sebire N, Souka A, Nicolaides KH. 1998. 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* **351**: 343–346.

Souka AP, Von Kaisenberg CS, Hyett JA, Sonek JD, Nicolaides KH. 2005. Increased nuchal translucency with normal karyotype. *Am J Obstet Gynecol* **192**: 1005–1021.

Spencer K, Bindra R, Nix AB, Heath V, Nicolaides KH. 2003. 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* **22**: 142–148.

Wøjdemann KR, Larsen SO, Shalmi AC, *et al*. 2006. Nuchal translucency measurements are highly correlated in both mono- and dichorionic twin pairs. *Prenat Diagn* **26**: 218–220.

Wright D, Kagan KO, Molina FS, Gazzoni A, Nicolaides KH. 2008. A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound Obstet Gynecol* **31**: 376–383.

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