I. GENETICS

THE PRENATAL RISK ASSESSMENT OF TRISOMY 21 (DOWN SYNDROME)

Demetra Socolov[1], R Socolov[1], Julieta Maria Puiu[2]
[1]Department of Obstetrics and Gynecology, Iasi University of Medicine- Romania
[2]Department of Genetics, Timisoara University of Medicine- Romania

Abstract

The chromosomal abnormalities (aneuploidies) have a frequency of 1 in 250 live new borns, and 1/3 of them are represented by the Down syndrome (1).

This syndrome was described the first time in 1866 by Langdon Down, and one of the elements his description underlined was the thickened skin ("the skin seems too large for their bodies"). In 1966, 100 years after the original essay of Down, it became possible to diagnose the trisomy 21 prenatally by the karyotype of cultured amniotic fluid cells. Therefore, it was demonstrated that the syndrome is produced when either a whole or a part 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 (95% cases), translocation and mosaicism.

As this aneuploidy not only is the most frequent, but also it causes one third of the severe mental retardation in children, its' screening became of outmost importance. But, as an amniocentesis in every pregnancy would not have the economic efficiency, as well as ethical and medical support, the question of individualising a risk group arises.

This paper tries to evaluate different risk factors, in the perspective of their importance in the clinical decision.

Key words: chromosomal abnormalities (aneuploidies), trisomy 21, mosaicism

1. The basal risk factors

The risk of trisomy 21 increases with the maternal age, as shown by Snijders (2). For example, the risk of a 20 year old woman to give birth to a children with trisomy 21 is 1 in 1527 (1/1527), while a woman over 40 years will have a Down syndrome offspring in 97 cases (1/97). This risk is also related to the gestational age, as fetuses with trisomy 21 are more likely to die in utero than normal ones. Snijders (3, 4) calculated that the in utero death rate was 36% until 10 weeks, 30% at 12 weeks, and 21% at 16 weeks- that means that the estimated risk decreases with the gestational age. In the example above, the risk in a 20 year old woman is 1/983 at 10 weeks, respectively 1/1527 at term, while for a 40 year old woman the risk is 1/62 at 10 weeks and 1/97 at term.

This risk estimation imposed the recommendation adopted by some countries, that a caryotype for trisomy 21 should be performed in all pregnant patients over 38 years (and even 35 years) (5). For 35 year cut-off point, which represents 5% of the pregnant population, there are 30% of trisomy 21 included.

It is important however to remind that, statistically speaking, the number of pregnancies in women over 35 is snaller than the one in younger women. Therefore, using only the maternal age (even correlated with gestational age criteria) could result in missing about 75% of trisomies 21.

Other risk factors, like previous affected pregnancies, modify the age related risk, with an increase of 0.75%. For example, a woman of 35 years, with a basal risk of 0.4% at 12 weeks, would be estimated at 1.15% if a previous Down syndrome case child exists.

2. Ultrasound risk factors

Trisomy 21 is associated with several fetal defects, and some of them could be described by ultrasound prenatal examination.

In the first trimester (upto 14 weeks), the most important ultrasound element for the Down syndrome is the nuchal translucency. It is the school of Nikolaides that showed for the first time the association between the increased nuchal translucency in the first three months of pregnancy (and more precisely, between 11-14 weeks) and Down syndrome. (6) Possible mechanisms for this sign include cardiac failure, venous congestion in the head and neck due to superior mediastinal compression, altered composition of the extracellular matrix, abnormal development of the lymphatic system, failure of lymphatic drainage due to impaired fetal movements, fetal anemia and congenital infection.

The normal nuchal translucency increases with gestation (and with its' most relevant biometric element in this period- the crown-rump length CRL). The Nikolaides ultrasound school demonstrated that the optimal gestational age for this measurement to be accurate and allow risk calculation is 11-13 weeks, with a success rate of 98-100% (corresponding to 45-84 mm CRL). There are several requirements for a correct measurement, but as the experience of that school has shown, a good training makes the procedure entirely reproductible. There are also tables that correlate the nuchal translucency with the CRL, which has been shown to be more accurate than the simple threshold of 3 mm- with a single detection rate of 72%.

The other important alarm sign in the first trimester ultrasound scan is the absence of fetal nasal bone. A recent article (7) shows that 69% of fetuses with trisomy

21 have no nasal bone, while only 0,4% of normal fetuses have this abnormality.

Other signs that were associated with trisomy 21 in different articles are described in table 1.

Tabel 1. Significant ultrasonographic findings in trisomy 21

parameter	gestational age	likelihood ratio for trisomy 21
increased heart rate (over 180/min) (8)	at 14 weeks	NC- 26% of trisomy 21
nuchal edema (> 6 mm)	2nd trimester	19
echogenic foci in the heart (9)	2nd trimester	4
short limbs with femural length < 2 SD (foot/femur>1.1, BIP/femur>1.55)	2nd trimester	2-4
echogenic bowel	2nd trimester	3
mild hydronephrosis (10)	2nd trimester	1.5
choroid plexus cyst	2nd trimester	1.5
nasal bone hypoplasia (under 2 mm, normal > 7 mm)	> 22 weeks	NC- 69% of trisomy 21
facial dysmorphy- flat profile	2nd trimester	NC- in 67% of trisomy 21
illiac bone angle (if >90) (11)	2nd trimester	NC- 60% of trsomy 21
smaller ear length (normal >17 mm)	>22 weeks	NC- 21% of trisomy 21
atrioventricular septal defects	14-24 weeks	NC- 10 to 30% trisomy 21
clinodactily or mid phalanx hypoplasia of the 5th finger	2nd trimester	NC- 4 % of trisomy 21

NC- likelihood ratio not communicated

Polyhydramnios, separation of the amnios and the placenta, and other abnormalities have little specificity for trisomy 21.

Finally these abnormalities have a higher significance if associated, but many of them have low specificity. Therefore, the need of an earlier examination, with the appreciation of the nuchal translucency is obvious.

3. The serum markers

Since the 1980s, several studies answered the need for a screening test for Down syndrome using biochemical markers. Their values, expressed as multiple of median (MoM), have proven to have different significance for the risk of this pathology.

- *human chorionic gonadotrophin* (hCG) is a trophoblastic glycoprotein. In 1987, Bogart (12) showed that in trisomy 21, this evolution is modified, and the serum concentration remains elevated after 16-18 weeks. Although not extremely specific, a level higher than normal at this stage of pregnancy, correlated with other risk factors (increased maternal age, abnormal nuchal translucency) can be significant for this aneuploidy.
- More specifical than the total hCG, the *free beta-hCG* is elevated until 9 weeks, than decreases. In trisomy 21, the level is stable at higher than normal level even after 12 weeks.
- The non-conjugated estriol is lower than normal in trisomy 21, as shown by Canick in 1988 (13). However, the specificity of this marker for this aneuploidy is quite low.
- Alpha feto protein (AFP) is an alpha globuline produced during the embryo development. A higher value is met in multiple pregnancies, intrauterine growth restriction, neural tube deffect, anencephaly, and intrauterine death. Merkatz in 1984 (14) proposed it as a marker for trisomy 21, as its' value is decreased in this aneuploidy- as well as in diabetes.
- The pregnancy associated plasma protein A (PAPP-A) is a trophoblastic protein, which can be evaluated between 7 and 12 weeks. In Down syndrome, it is decreased.
- The inhibines (A and B) are produced by the placenta, with increasing values upto 10 weeks, and then a constant level between 15 and 25 weeks. Their values increases in trisomy 21, and it is strongly correlated with the hCG.
- The specific pregnancy b-1 glycoprotein (SP-1) is also decreased in Down syndrome.

As shown in the table, adapted from Uzan 1998 (1), there are only few markers which have significant variations in trisomy 21 (values > 2 MoM, or less than 0.5 MoM- significantly increased or decreased):

Table 2. Serum markers for Down syndrome screening (1)

Marker	Origins	Maximal concentration	Optimal screening period	Concentration (MoM)
hCG	Trophoblast	9 weeks	15-18 weeks	2.1
Free beta hCG	Trophoblast	9 weeks	11-18 weeks	2.4
PAPP-A	Trophoblast	20 weeks	7-12 weeks	0.3-0.5

AFP	Fetal	30 weeks	15-18 weeks	0.7
E3	Fetal, placental	37 weeks	15-18	0.73

If available, the best markers are therefore free hCG and PAPP-A, and they can be determined even earlier in the pregnancy- up to 14-15 weeks. The previous "classical" triple markers (AFP, hCG and E3) were determined at 16-18 weeks, and therefore needed a longer waiting period after the first risk assessment, at 12-14 weeks by the maternal age and nuchal translucency.

The other markers (for example, inhibine A or B) have concordant values with the main ones above, and therefore are not suitable for a multiple testing, as they will be similar in most of cases.

4. Integrating the different elements for the risk calculation

The first estimation of risk, done by statistical studies, regarded only the maternal age, corrected after the gestational age (table 3). Yet, the risks associated with amniocentesis (6% fetal loss) or chorionic trophoblastic biopsy (7.6% fetal loss) required a further limitation for the risk groups. (15)

Table 3. The risk for trisomy 21, depending on maternal and gestational age, adapted from (2)

Maternal/ gestational age	12 weeks (1 in … cases)	16 weeks (1 in … cases)	20 weeks (1 in … cases)	40 weeks (1 in … cases)
20	1068	1200	1295	1527
25	946	1062	1147	1352
30	626	703	759	895
35	249	280	302	356
36	196	220	238	280
37	152	171	185	218
38	117	131	142	167
39	89	100	108	128
40	68	76	82	97
41	51	57	62	73
42	51	57	62	73
43	29	32	35	41
44	21	24	26	30
45	16	18	19	23

Depending on the national health policies, availability of caryotyping for aneuploidy, and the desire of the patient, a chorionic villus sampling or amniocenthesis could be proposed. In France for example, the risk threshold is 1/250 since 1997 (16).

The risk factosr associated with ultrasound markers- especially the nuchal translucency at 12-14 weeks, is also depending on the gestational age (or crown-rump length- CRL). If we decide upon a cut-off value (2.5 mm at 12 weeks, or 3 mm at 14 weeks were proposed by most of the authors), the risk ratio will be increased 3.5 times if the difference is 1 mm, 10 times if 1.5 mm, 30 times if 2 mm and 55 times if 2.5 mm. (17, 18).

There are two different approaches towards the integrated use of markers:

1). nuchal translucency with immediate biochemical assessment (of free beta hCG and PAPP-A) at 12-14 weeks. This has the advantage of shortening the "waiting" period, with less trauma for the patient. Unfortunately, not everywhere these tests are available. The rate of detection is 90% of affected fetuses, and the further development of analysis allow a short 30 min interval results, practically as one-step clinic for assessment of risk (also called OSCAR) (19)

2). nuchal translucency at 12-14 weeks, and biochemical markers at 16-18 weeks. It is the most common methodology, multiplying the basal maternal age risk with the likelihood ratio for these 2 elements. For the biochemistry markers, the estimated detection rates are 50-70%, for a false positive rate of 5%. As nuchal translucency will detect almost 90% of trisomy 21 at 12-14 weeks, the efficiency of the 2nd trimester markers is 6% (60% of the remaining 10%), Also, in interpreting the results, we should be careful as 1 trisomy in 3 will have normal serum markers (1).

In women who had the 1st trimester screening by ultrasound plus biochemical markers, the 2nd trimester testing could be avoided, as recommended by Bizot and Nicolaides (20), because:

- the sensitivities of the 1st and 2nd trimester biochemical screening are similar

the main component of the 2nd trimester screening is beta hCG, and there is a good correlation between first and second trimester maternal hCG levels.

After calculating the integrated risk, by multiplying the risk (or dividing the coefficient described above as in relation to the maternal age), one can therefore decide what is the next step- amniocentesis or chorionic villi sampling,

depending on the gestational age. Then, it could be up to the specialist and the parents if another serum marker is used, or if one could wait for a reevaluation at 22 weeks for the minor ultrasound signs cited before.

Conclusion

Ten years ago, the English ultrasound school provided the specialists with an important element in their struggle to screen for the most common aneuploidy- the nuchal translucency. This way the ultrasound scan at 11-14 weeks of pregnancy became an extremely important exam, justifying the recommendation for three ultrasound scans during the pregnancy.

Since then, the screening for trisomy 21 re-evaluated the biochemical markers, and we should now be able to integrate the different elements into a risk assessment strategy, able to detect therefore most of the Down syndromes in time to allow an informed parental decision and medical care.

References:

1. Uzan M, Pharisien I, Vedovato C, Theodore V. [Marqueurs seriques et nuque. Indications du caryotype. Cout social et financier]. CD-Rom of the Journees Trans Alpines, 1998; 46
2. Snijders RJM, Sundberg K, Holzgreve W et al. Maternal age and gestation-specific risk for trisomy 21. Ultrasound Obstet Gynecol 1999; 13: 167-170
3. Snijders RJM, Holzgreve W, Cuckle H, Nicolaides KH. Maternal age-specific risks for trisomies at 9-14 weeks gestation. Prenat Diagn 1994; 14: 543-52
4. Snijders RJM, Sebire NJ, Cuckle H, Nicolaides KH. Maternal age and gestational age specific risks for chromosomal defects. Fetal Diag Ther 1995; 10: 356-67
5. Bernard JP, senat MV, Ville Y. [Depistage de la trisomie 21: tests successifs ou tests sequentiels?]. CD-Rom of the Journees Trans Alpines, 2001
6. Nicolaides KH, Azar G, Snijders RJM, Gosden CM. Fetal nuchal edema: associated malformations and chromosomal defects. Fetal diag Ther 1992; 7: 123-31
7. Cicero S, Bindra R, Rembouskos G, et al. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta hCG and PAPP-A at 11 to 14 weeks. Prenat Diagn 2003; 23: 306-10
8. Hyett JA. [La frequence cardiaque comme marqueur d'une anomalie chromosomique precoce du 1er trimestre]. LUS 1996; 5: 21-2
9. Bromley B, Lieberman E, Laboda I, Benacerraf BR. Echogenic intracardiac focus: a sonographic sign for fetal Down syndrome. Obstet Gynecol 1995; 86: 998-1001
10. Vintzileos AM, Egan JF Adjusting the risk for trisomy 21 on the basis of the second trimester ultrasonography. Am J Obstet Gynecol 1995; 172: 837-44
11. Belics Z, Beke A, Csabay L et al. Sonographic measurement of the fetal iliac angle in trisomy 21, 18 and 13. Fetal Diag Ther 2003; 18: 47-50
12. Bogart MH, Pandian MR, Jones OW. Abnormal maternal serum chorionic gonadotrophin levels in pregnancies with fetal chromosome abnormalities. Prenatal Diag 1987; 7: 623-30
13. Canick JA, Knight GJ, Palomaki GE et al. Low second trimester maternal serum unconjugated oestriol in pregnancies with Down's syndrome. Br J Obstet Gynecol 1988; 95: 330-3
14. Merkatz IR, Nitowsky HM, Jmacri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol 1984; 148: 886-94
15. CEMAT Group. Randomised trial to assess safety and outcome of early and mid-trimester amniocentesis. Lancet 1998; 351: 242-7
16. Talmant C. [La trisomie 21 en 1998. Signes d'appel echographiques, difficultes et resultats]. CD-Rom of Journees Trans-Alpines 1998: 35
17. Snijders RJM, Noble P, Sebire N, Ssouka 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
18. Bourgeois JM. [Clarte nucale et risque de trisomie 21]. EGEO 2000; 5: 308-9
19. Bindra R, Heath V, Liao A et al. 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
20. Bizot M, Noble P. Nuchal translucency and maternal serum biochemistry. In: Nicolaides KH, Sebire NJ, Snijders RJM (eds). The 11-14 week scan. The diagnosis of fetal abnormalities. Parthenon Publ, New York; 1999: 38-41