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Maternal serum screening in cases of mosaic and translocation Down syndrome

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Objectives To determine if the second-trimester maternal serum markers (MSM) screening for Down syndrome (DS) is efficient in DS mosaicism or structural rearrangement cases.

Method DS mosaic or translocation cases were reviewed from databases of routine MSM DS screening. The control group consisted of 977 trisomy 21 cases included in a series of 854 902 patients (routine screening). DS risk was calculated by combination of maternal age and MSM [alpha-fetoprotein (AFP) and human choriogonadotrophin (hCG) or free β-hCG and/or uE3] expressed in multiples of median (MoM). Mosaic DS cases were divided into three groups, <10%, 10–49%, and ≥50% trisomy 21 cells. Translocation DS cases were divided into three groups, isochromosome, Robertsonian, or reciprocal translocation. Detection rate (DR) and MoMs were evaluated in each group.

Results As many as 76 cases of nonstandard trisomy 21 were collected. For mosaic DS cases ($n = 43$) DR was 69.8% (not significantly different from the 70.8% of control group). When mosaicism was less than 10%, the DR dropped to 25%. For translocation DS cases ($n = 33$) DR was 75.7% (not significantly different from control group) whatever the types of translocation.

Conclusion In the nonstandard DS cases, second-trimester MSMs gave the same detection rate as for standard trisomy 21, except the cases with low-level mosaicism (<10%). Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: trisomy 21; prenatal screening; risk calculation; aneuploidy; detection rate

INTRODUCTION

Maternal serum screening for Down syndrome (DS) is currently performed in most developed countries. In the general population, maternal age, ultrasound findings, and maternal serum markers (first or second trimester) are used alone or in combination for risk calculation. Second-trimester maternal serum marker (MSM) screening allows the detection of 60–70% of DS cases, with a false positive rate of 5% (Cuckle, 2000).

Three types of trisomy 21 are described. In about 95% of DS cases, the anomaly is due to the presence of an entire extra chromosome 21 in all cells (standard trisomy 21). The nondisjunction of chromosome 21 is of maternal origin in 88% of cases, of paternal origin in 8%, and of mitotic origin in 4% (Antonorakis *et al*., 1991; Muller *et al*., 2000). In 2% of DS cases, mosaicism for the trisomic cell line is observed, meaning that the anomaly is located in only some cells of the body, whereas others present the normal chromosomal complement. Mosaicism can occur in two different ways: (1) the initial zygote has three chromosomes 21, which would result in classical trisomy 21, but during the course of cell division one or more cell lines lose one of the three chromosomes 21 leading to the correction of the anomaly in this cell line; (2) the initial zygote has two chromosomes 21, but during the course of cell division one of the chromosomes 21 is duplicated in one cell line. In 3–4% of DS cases, a structural rearrangement involving chromosome 21 is observed. From a cytogenetic point of view, they are now referred to as isochromosome 21 (46,i(21q)) because they usually result from the duplication of the long arm of one chromosome 21 rather than from a true exchange between two different chromosomes 21. However, in a diagnostic setting, the molecular analysis able to differentiate between these two mechanisms is never performed and therefore, we cannot further categorize these cases, and they are all included under the Robertsonian translocation subtype. In about one-fourth of these cases, the translocation is inherited, but this figure varies greatly according to the rearrangement (45% in Robertsonian translocation, 80% in reciprocal translocations and almost never in isochromosome 21) (Gardner and Sutherland, 2004). To the best of our knowledge, none of the published studies-related results of DS MSM screening policies distinguish the three different DS types. Our purpose was to determine if the second-trimester MSM screening usually used in DS cases is as efficient as in standard trisomy 21

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when screening for the rarest chromosomal abnormalities, trisomy 21 mosaicism or structural rearrangement (isochromosomes, Robertsonian or reciprocal translocations).

PATIENTS AND METHODS

This retrospective multicenter national study was conducted during the period 1997–2006. Databases concerning routine second-trimester maternal DS screening were reviewed, and DS cases with a nonstandard trisomy 21-affected fetus (mosaic or translocation) were studied. Twin pregnancies were excluded. The control group consisted of 977 trisomy 21 cases including a series of 854 902 patients who underwent routine second-trimester maternal serum screening. This multicenter study was published earlier (Muller *et al*., 2002). Briefly, median of maternal age of the trisomy 21 affected pregnancies was 33 years (range 16–47), median of multiple of median (MoM) was 0.71 (range 0.1–36.0) for AFP and 2.35 for β-hCG (range 0.36–55.1). Detection rate was 70.8% for a 6.48% false positive rate.

French DS-screening policy relies on second-trimester MSMs (gestational age between 14 and 18 weeks of amenorrhea). MSM screening is strictly regulated (Muller *et al*., 2002) and 74 laboratories are accredited. They were sent a questionnaire requesting study participation and submission of their results. Not all laboratories participated, and therefore, we do not have an exhaustive record of the types of DS cases, which is why no analysis of the distribution of DS types can be performed.

The markers used were total human choriogonadotrophin (hCG) hormone or its free fraction (β-hCG), alpha-fetoprotein (AFP), and unconjugated estriol (uE3). Results are expressed in MoM. The DS risk was calculated by combination of maternal age and MSMs (double test with AFP and total hCG or free β-hCG or triple test including uE3 in addition). A single cutoff of 1/250 at sampling was used.

Nuchal translucency (NT) was not included in a combined risk calculation because ultrasonographers did not participate in any quality control. However, NT was used as an ultrasound sign of fetal malformation using a single cutoff of 3 mm. For these patients, chorionic villi sampling or amniocentesis was proposed for karyotyping. Therefore, no patient with fetal NT thickness $\geq$3 mm underwent MSM screening, and none of them were included in this study. In addition, in France, as fetal karyotyping is routinely proposed to patients aged 38 years and over, MSM is proposed in only a few of these older patients.

Karyotype formulas were collected from the 76 accredited cytogenetic laboratories. Karyotyping was performed either prenatally for women with an MSM calculated risk $\geq$1/250, for women with abnormal ultrasound findings, and for women $\geq$38 years, or after birth for those refusing amniocentesis, or for those with an MSM calculated risk $<$1/250. According to French law, in the case of severe fetal malformation, termination of pregnancy (TOP) is allowed at the patient's request, after multidisciplinary consultation, whatever the gestational age.

Mosaic DS cases were divided into three groups depending on the percentage of trisomy 21 cells: $<$10%, between 10 and 49%, and $\geq$50%. Translocation DS cases were divided into three groups depending on the type of translocation: isochromosome, Robertsonian, or reciprocal translocation. Results of each MSM were studied in each group. DS detection rate was evaluated in each group. The *F*-test and Chi-square test were used for comparison.

RESULTS

As many as 76 cases of nonstandard trisomy 21 were collected, 43 with mosaicism and 33 with translocation.

Mosaic trisomy 21 cases

The median maternal age at sampling was 32.5 years (range 22–40), a value not significantly different from the age of patients carrying a fetus with standard trisomy 21 ($p = 0.20$) (Tables 1 and 2).

On the basis of second-trimester MSM and maternal age risk calculation (cutoff 1/250), the detection rate was 69.8% (95% CI 56.8–82.8), a percentage not significantly different from the 70.8% achieved in the cases of standard trisomy 21 (Chi-square = 0.14). When

Table 1—Detection rate of trisomy 21 with mosaicism (43 cases) by second-trimester maternal serum markers (MSMs) using a 1/250 cutoff

	n	Detection rate by MSM (%)
Mosaicism (total)	43	69.8
Mosaicism T21 $\geq$50%	18	72.2
Mosaicism T21 10–49%	21	76.2
Mosaicism T21 $<$10%	4	25

Table 2—Median of maternal age and of maternal serum markers in 43 cases of trisomy 21 with mosaicism

	Nb	Maternal age (range)	AFP MoM (range)	hCG or free β-hCG MoM (range)	uE3 MoM (range)
Mosaicism T21	43	32.5 (22–40)	0.89 (0.35–3.12)	1.98 (0.39–8.63)	0.65 (0.44–1.2)
Mosaicism $\geq$50%	18	32 (27–40)	0.83 (0.43–1.66)	2.32 (0.91–7.95)	0.69 (0.44–1.2)
Mosaicism 10–49%	21	33 (22–40)	0.8 (0.35–3.12)	2.22 (0.39–8.63)	0.57 (0.47–1.07)
Mosaicism $<$10%	4	30 (29–39)	0.9 (0.35–1.64)	0.93 (0.68–2.56)	

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the rate of mosaicism was less than 10%, the detection rate dropped to 25%, a percentage significantly different (Chi-square = 2.08) from the two other mosaicism groups.

The median MoM values of the different MSMs were 0.89 for AFP, 1.98 for hCG (total or free β) and 0.65 for uE3 (marker used in 10 cases).

Of the 30 cases at increased risk with MSM, 29 were terminated at the parents' request and 1 was born. Of the 13 cases with calculated risk $<1/250$, six were prenatally diagnosed: two after amniocentesis because of advanced maternal age, 1 because of a previous trisomy 21-affected child, and 3due to the detection of fetal anomalies at second- or third-trimester ultrasound scan. In one of these last cases (craniostenosis detected at third-trimester ultrasonography), amniocentesis led to premature rupture of membranes followed by premature birth (33 weeks) of the trisomy 21-affected child (results of karyotype obtained after delivery). All other seven babies were delivered.

Fetal sex was documented in 40 cases, 18 males, and 22 females.

Translocation trisomy 21 cases

A Robertsonian translocation was observed in the vast majority of cases (31 of the 33 cases), either a 14/21 translocation (16 cases) or 21q;21q Robertsonian translocation (15 cases) (Tables 3–5). Only two cases with reciprocal translocation were observed, both of them inherited: one case involved a maternally inherited extra chromosome 21 due to a 3 : 1 segregation of a reciprocal translocation between chromosome 2 and chromosome 21 (47,XX,*t*(2;21)(p24;q22.2)mat,+21) resulting in partial trisomy 21 (q22qter), while the other one involved a paternally inherited unbalanced reciprocal translocation (46,XY,der(6)*t*(6;21)(q27;q21)pat) resulting in partial trisomy 21 (q21qter) associated with 6q monosomy.

Table 3—Detection rate of translocated trisomy 21 cases (33 cases) by second-trimester maternal serum markers (MSM) using a 1/250 cutoff at sampling

	n	Detection rate by MSM (%)
Translocated T21 (total)	33	75.7
t(14;21)	16	81.2
t(21;21)	15	66.7
Other translocations	2	100

The median maternal age at sampling was 31 years (range 17–41), which was not significantly different from the median of standard trisomy 21 ($p = 0.27$).

The detection rate of the MSM screening was 75.7% (95% CI 61.1–90.3), a percentage not significantly different from the detection rate of standard trisomy 21 (Chi-square = 0.61). No difference was detected among the various types of translocation.

Median MoM values of markers were 0.78 for AFP, 2.2 for hCG (total and free β) and 0.72 for uE3 (marker used in six cases).

Of the 25 at-risk cases with MSM, all were terminated at the parents' request. Of the eight cases with calculated risk $<1/250$, four were prenatally diagnosed due to the detection of fetal anomalies at second- or third-trimester ultrasound examination (one cystic hygroma; one abnormal nuchal thickness; one cardiac malformation; one hydrothorax), and pregnancies were terminated at the parents' request. Four cases went to birth, and three of these infants presented no anomaly at any of the three ultrasound examinations, and in one case *in utero* growth retardation was observed at 32 weeks, but amniocentesis was not proposed to the parents.

Fetal sex was documented in all cases, 13 males and 20 females.

Parental karyotyping was performed in 23 cases and refused in 10 cases. Parental karyotype was normal in 16 cases, corresponding to a *de novo* fetal trisomy 21 translocation, and was inherited in seven cases (from the mother in six cases and from the father in one).

DISCUSSION

Second-trimester maternal serum screening for trisomy 21 is based on the presence of abnormal levels of different markers, of which hCG (or its free β subunit), with a median value at 2.02 MoM (2.30 for free β-hCG) (Cuckle, 2000). These anomalies were demonstrated in standard trisomy 21 cases, but even though a huge number of publications concerning maternal serum screening have been published in the last decade, no differentiation has ever been made concerning the precise type of trisomy 21. In a previous study based on 110 cases, we demonstrated that no difference in second-trimester maternal serum screening was observed depending on the parental origin of the additional chromosome 21 (Muller *et al.*, 2000). Even if mosaicism and translocation trisomy 21 represent only 5–8% of DS cases (Mutton *et al.*, 1996), it would be of interest to know if second-trimester MSMs have the same

Table 4—Median maternal serum markers in 33 cases of translocated trisomy 21 cases. The uE3 measured in 6 cases

	Nb	Maternal age (range)	AFP MoM (range)	hCG or free β-hCG MoM (range)	uE3 MoM (range)
Translocation	33	31 (17–41)	0.78 (0.39–1.91)	2.20 (0.54–6.05)	0.72 (0.36–1.14)*
Translocation 14–21	16	28 (17–41)	0.57 (0.39–1.31)	2.28 (0.75–4.91)	
Translocation 21–21	15	31 (24–38)	0.81 (0.53–1.28)	2.20 (0.54–3.59)	
Other translocations	2	(32–40)	(0.57–1.91)	(4.46–6.05)	

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Table 5—Detection rate of inherited versus *de novo* translocated trisomy 21 cases (33 cases) by second-trimester maternal serum markers (MSMs) using a 1/250 cutoff at sampling

	n	Detection rate by MSM (%)
Robertsonian translocation	33	75.7
De novo	16	75
Inherited	7	71.4
Unknown	10	80

detection rate as for standard trisomy 21. Since trisomy 21 maternal serum screening is organized on a national basis with strict regulation in France (Muller *et al*., 2002), it was possible to contact the 76 accredited laboratories to obtain the information concerning the DS-chromosomal formula. We obtained answers in 76 cases (43 of mosaicism and 33 of translocation).

In this study, no statistical difference in MSM detection rate was observed between standard trisomy 21 and mosaicism or translocation cases (70.8, 69.8 and 75.7%, respectively). The only difference noted was for the particular group of four cases with less than 10% mosaicism, for which a 25% detection rate was observed. This low-detection rate is due to a normal median MoM hCG value (0.93 vs 2.27). Due to the small number of cases, these results should be carefully assessed even though these data are not fully illogical. The phenotype in the mosaic state depends upon which tissue and how much of that tissue is abnormal. Although mosaic cases prenatally detected were diagnosed through amniotic fluid sampling, and although there is no correlation between the percentage of abnormal cells in the placenta and amniotic fluid, a possible explanation is that the normal hCG values observed in $<10\%$ mosaicism DS cases can be related to the low percentage of trisomy 21 cells in the placenta, and therefore to a reduced trophoblast dysfunction. In a retrospective observational study based on 208 postnatal cases of DS, 8 (3.85%) mosaic cases were observed, of which 3 were diagnosed after day 7 and 3 as adults, which underscores the variability in the degree of severity in mosaic cases (Devlin and Morrison, 2004). In the same way, Casado *et al*. (2007) observed a significant difference in the level of erythrocyte malondialdehyde (MDA, an oxidative stress marker) between DS individuals with standard and translocation trisomy 21, but in DS with mosaicism MDA levels depend on the percentage of diploid and trisomic cells.

In translocations, isochromosome 21 and Robertsonian translocations were observed in 31 of the 33 cases, a proportion similar to that described in large series (Gardner and Sutherland, 2004). Trisomy by translocation is present in all cells of all tissues, including placenta, explaining the similar results of MSM in translocations and in standard trisomy 21 (75.7% vs 70.8%). No difference was noted depending on the partner chromosome of the translocation, although this result has no statistical significance due to the low number of cases. Trisomy 21 by Robertsonian translocation or isochromosome 21 have the same triple amount of chromosome 21 as standard trisomy 21, therefore, explaining the absence of difference in MSM screening. In our survey, parental karyotyping was obtained in 23 of the 33 cases with translocations. An inherited translocation was observed in 7 cases (with a maternal origin in 6) and a *de novo* translocation was observed in 16 cases. This proportion is similar to that reported elsewhere (Mutton *et al*., 1996; Schinzel, 2001).

In all our seven inherited cases, parental translocation was not previously diagnosed. The discovery of such a chromosomal parental anomaly must prompt the referral of the carrier individual to a genetic counselor. When a translocation is present in a family, despite the good results we observed here with DS maternal serum screening, fetal karyotyping must be proposed due to the high risk of unbalanced segregation.

Of the 21 cases of trisomy 21 with MSM risk calculation under the cutoff of 1/250 (13 mosaicism cases and 8 translocation cases), second-trimester ultrasound examination was abnormal in 8 (38%). This percentage is lower than for standard trisomy 21: 55% according to Vintzileos *et al*. (1997) and 57% according to Smith-Bindman *et al*. (2007).

The median MoM hCG values observed here (1.98 for mosaicism and 2.2 for translocation), were not different from the 2.2 value observed in standard trisomy 21. Although our series can be biased because cases with increased NT at first-trimester ultrasound examination had fetal karyotyping and were not included in the maternal serum screening program, due to the independence of MSMs and NT (Brizot *et al*., 1995), it is unlikely that this selection bias would have biased hCG values.

It is established that hCG is of placental origin, synthesized by the trophoblast, mainly by the syncytiotrophoblast (Muyan and Boime, 1997). In trisomy 21, cultured cytotrophoblasts isolated from trisomy 21-affected placentas aggregate but fuse poorly or late (Frendo *et al*., 2000), leading to a dramatic decrease in the synthesis and secretion of syncytiotrophoblastic pregnancy-associated hormones such as hCG (Massin *et al*., 2001). In addition, the hCG produced by T21-affected trophoblasts had a low bioactivity, resulting in a poor ability to stimulate the hCG receptors, and therefore to be internalized, which explains the higher value observed in maternal serum despite a lower production (Pidoux *et al*., 2007). These anomalies can be related to the high proportion of hyperglycosylated hCG (H-hCG), also called invasive trophoblast antigen (ITA), found in trisomy 21-affected pregnancies (Cole *et al*., 1997). These anomalies were demonstrated in standard trisomy 21 cases, but were not studied in nonstandard trisomy 21 cases.

In conclusion, in the nonstandard cases of trisomy 21 (mosaicism and translocation), second-trimester MSMs gave the same detection rate as for standard trisomy 21. The only statistically significant exception concerns cases with low-level mosaicism, but the frequency of this particular karyotype is very low and does not call into question the value of MSM screening.

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APPENDIX

Study group

French laboratories authorized by the Ministry of Health to carry out prenatal cytogenetics and/or DS screening: Amiens-CHU (C Lemay); Angers-CHU (H Puissant); Bordeaux (A Liquier,E Ruedas, J Souby); Brest (MP Moineau, JP Codet); Chambéry (J Lespinasse); Clermont-Ferrand-CHU (P Vago); Dijon (MF Frigère, S Ewing, M Guiguet); Dreux (C Finot, MH Ramaorasy); Evreux (S Séréro); Le Blanc-Mesnil (P Clément, L Lohman, M Mintz); Le Mans-CH (D Martin); Lille-CHU (G Renom, JM Perini); Lille-CHU (B Delobel); Limoges (C Yardin); Lyon (C Boisson, F Poloce); Lyon Mérieux (C Sault); Metz (ME Larcher, M Wasel); Nîmes (M Cabrol); Paris-Necker (G Quenum-Miraillet, N Morichon); Paris-Pitié (M Bernard); Saint-Brieuc (B Le Fiblec); Saint-Etienne (P Guiardiola, P Antoine, G Belot); Strasbourg (G Coumaros, C Koehl, L Lehr); Toulouse (JF Rousselle, P Demas); Toulouse-CHU Rangueil (F Fortenfant, A Blancher); Toulouse-CHU Purpan (G Bourrouillou); Tours (D Dudragne, B Cara); Tours-Chambray (D Galliano); Versailles SESEP (B Simon-Bouy).

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