

Outcome of pregnancies with trisomy 2 cells in chorionic villi

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Objective To describe the outcome of pregnancies with trisomy 2 in cultures of first-trimester chorionic villous samples (CVS) and determine whether amniocentesis is necessary in the management of such cases.

Methods Cultures of chorionic villi were performed at 11–13 weeks in 37 474 pregnancies. In those with trisomy 2 cells, amniocentesis was performed at 16 weeks. Pregnancy outcome was obtained from maternity records.

Results Trisomy 2 cells in CVS cultures were observed in 45 of 37 474 pregnancies (1.2 per 1000). In 43 cases ultrasound examination at 16–20 weeks showed no fetal abnormalities, amniocentesis demonstrated the presence of only normal cells, and all 43 pregnancies ended in normal healthy live births. The birth weight was below the 5th centile in six neonates (13.9%). There was a significant association between the birth weight centile and the percentage of trisomic cells in the CVS culture ($r = 0.409$, $p = 0.010$). In one case, there was fetal death at 15 weeks. In a second case, amniocentesis showed one cell with trisomy 2 in a total of 53 cells, and ultrasound examination at 18 weeks showed severe fetal growth restriction and coarctation of the aorta.

Conclusion In at least 95% of cases with trisomy 2 in CVS cultures there is confined placental mosaicism (CPM). The prognosis is good, but in about 15% of cases there is fetal growth restriction. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: trisomy 2; confined placental mosaicism; chorionic villous sampling; fetal growth restriction

INTRODUCTION

Complete trisomy 2 is a lethal chromosomal abnormality found in about 1% of first-trimester spontaneous miscarriages (Wang, 2000), while trisomy 2 is one of the most frequently involved trisomies in pseudomosaicism both in amniocytes and chorionic villi samples (CVS) cultures (Hahnemann and Vejerslev, 1997; Benn and Hsu, 2004). In contrast, true trisomy 2 mosaicism is rare and has been reported in only one in 58 000 cases of second-trimester amniocenteses (Sago *et al.*, 1997). The outcome of fetuses with true trisomy 2 mosaicism is poor (Hsu *et al.*, 1997; Sago *et al.*, 1997). In 11 reported cases there were three fetal deaths with no obvious defects, four terminations of pregnancy (with one case each of spina bifida, hemimelia, ambiguous genitalia and oligo-hydramnios) and four live births, including one with no abnormalities, one with growth restriction, one with subtle dysmorphic features and one with a cardiac defect, facial dysmorphism and pectus excavatum (Hsu *et al.*, 1997).

Chromosome mosaicism in CVS is detected in 1–2% of cases (Hahnemann and Vejerslev, 1997; Grati *et al.*, 2006) and the reported prevalence of trisomy 2 mosaicism in the combined data from three studies on a total of 144 103 cases of CVS was 1 in 2000 (Wolstenholme, 1996; Hahnemann and Vejerslev, 1997; Grati *et al.*, 2006). Since the prevalence of true fetal trisomy

2 mosaicism is significantly lower (Sago *et al.*, 1997) than that observed in CVS cultures the vast majority of the latter must represent confined placental mosaicism (CPM). The outcome of pregnancies with trisomy 2 CPM is uncertain because current knowledge is essentially based on case reports (Webb *et al.*, 1996; Ariel *et al.*, 1997; Gibbons *et al.*, 1997; Sifakis *et al.*, 2004).

The aim of this study is to define the outcome of 45 pregnancies with trisomy 2 in first-trimester CVS cultures and determine whether amniocentesis is necessary in the management of such cases.

METHODS

At King's College Hospital, London, UK and Mitera Hospital, Athens, Greece CVS is carried out at 11–13 weeks for fetal karyotyping at the request of the parents after screening for trisomies either by maternal age or maternal age, fetal nuchal translucency thickness and maternal serum free β -hCG and PAPP-A (Kagan *et al.*, 2008). In both centers, in the majority of samples a direct preparation was not performed because it was replaced by rapid analysis of the most frequent aneuploidies by quantitative fluorescence PCR (QF-PCR) (Christopoulou *et al.*, 2009). Cytogenetic analysis of chorionic villi was performed by standard techniques which involve the setting up of two to three primary long-term cultures, metaphase chromosome preparations and analysis of 10–20 cells from two different cultures by GTG banding. True trisomy 2 or mosaicism in the chorionic villi was diagnosed if the trisomy was detected

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in at least two separate primary cultures (ACC, 2007, guidelines for CVS).

We searched our databases to identify all cases of trisomy 2 diagnosed after CVS between 1991 and 2008 and then examined their medical records to determine the pregnancy outcome. In each case, the birth weight was expressed as a percentile after correction for gestation at delivery and sex, maternal racial origin, weight, height and parity and the newborn was considered small for gestation if the percentile was less than 5% (Gardosi and Francis, 2006).

RESULTS

During the study period fetal karyotyping by CVS was carried out in a total of 37 474 pregnancies and trisomy 2 cells were observed in 45 (1.2 per 1000). In two cases all cells examined were trisomic and in 43 cases there was mosaicism with the percentage of trisomy 2 cells ranging from 7 to 94% (median 38%). Amniocentesis was performed in 44 cases but no uniparental disomy studies were carried out.

The median maternal age of the 45 cases with trisomy 2 cells in the placenta was 38 (range 20–45) years, the median gestation at CVS was 12 (range 11–13) weeks, the median fetal crown-rump length was 68 (range 45–84) mm and the median fetal nuchal translucency thickness was 2.0 (range 1.0–8.1) mm. The gender of the infant was female in 18 (40%) and male in 27 (60%). Maternal serum free β -hCG and PAPP-A was measured in 18 of the cases and the respective median values were 0.84 (range 0.31–4.83) MoM and 0.92 (range 0.04–3.2) MoM.

In 43 cases, including 42 in which the CVS showed trisomy 2 mosaicism and one full trisomy 2, detailed ultrasound examination at 16–20 weeks showed no fetal abnormalities, and in all of these cases amniocentesis at 16–18 weeks demonstrated the presence of only normal cells. All pregnancies resulted in live birth of normal healthy infants. The median gestation at delivery was 38 (range 32–42) weeks, and the median birth weight was on the 36th centile (range from below the 1st centile to the 99th centile), and in six (13.9%) cases it was below the 5th centile. There was a significant association between the birth weight centile and the percentage of trisomic cells in the CVS culture ($r = 0.440$, $p = 0.003$; Figure 1).

In one case in which the CVS showed trisomy 2 in 22% of the cells examined, ultrasound examination at 17 weeks demonstrated a dead fetus and the measurements were compatible with 15 weeks. At 13 weeks, the crown-rump length was on the 50th centile, the nuchal translucency was 2.2 mm, and the maternal serum free β -hCG and PAPP-A were 0.58 and 0.81 MoM, respectively.

In another case in which the CVS cultures showed full trisomy 2, at amniocentesis there was one trisomy 2 cell in a total of 53 cells from two cultures. At 12 weeks the fetal crown-rump length was on the 50th centile, the nuchal translucency was 1.0 mm and the maternal

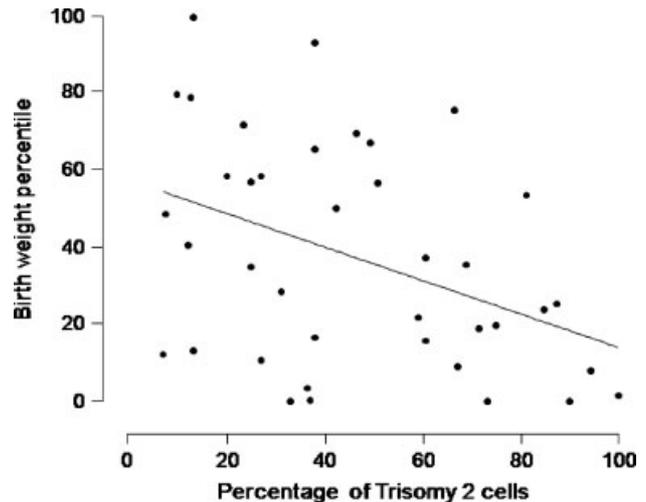


Figure 1—Significant association between the birth weight centile and the percentage of trisomic cells in the chorionic villi culture ($r = 0.440$, $p = 0.003$)

serum free β -hCG and PAPP-A was 0.55 and 0.04 MoM, respectively. At 18 weeks, there was severe fetal growth restriction with both the fetal head and abdominal circumference below the 3rd centile and measurements corresponding to 16 weeks. There was oligohydramnios and Doppler assessment of the umbilical arteries showed absent end-diastolic flow. Specialist fetal echocardiography demonstrated coarctation of the aorta. In view of the likely poor prognosis, the pregnancy was terminated at the request of the parents and the autopsy examination confirmed the fetal cardiac defect.

DISCUSSION

The prevalence of trisomy 2 in chorionic villi in our study was 1.2 per 1000 (0.12%), while in previous CVS studies it was significantly lower ranging from 0.03% to 0.07% (Wolstenholme, 1996; Hahnemann and Vejerslev, 1997; Grati *et al.*, 2006). Since the overall prevalence of CPM in first-trimester CVS is about 1% (Hahnemann and Vejerslev, 1997) it appears that trisomy 2 accounts for 3–12% of all cases of CPM. It is not possible to determine whether this represents the overall prevalence in the general pregnant population, or in the subgroup undergoing first-trimester CVS for the specific indications of advanced maternal age and abnormal first-trimester results after screening by fetal nuchal translucency thickness and serum biochemistry. It is possible that the higher prevalence in our study compared to that in previous reports is the consequence of a shift in the indications for CVS from essentially maternal age in previous studies to abnormal sonographic and serum biochemistry results in our study. However, in our pregnancies complicated by the presence of trisomy 2 in CVS cultures there was no characteristic first-trimester sonographic or biochemical profile. The median fetal nuchal translucency of 2.0 mm in those with trisomy 2 was identical to the value in our total population of pregnancies undergoing CVS, and the values of maternal serum

free β -hCG and PAPP-A were both about one MoM. Thus, it is possible that the high prevalence of trisomy 2 in chorionic villi in our study could be attributed to the fact that in earlier studies many cases were included in which only direct preparations had been performed and as a result trisomy 2 was underestimated (Wolstenholme, 1996; Hahnemann and Vejerslev, 1997).

One of the inherent limitations of this study is that data from the direct preparations were not available. Therefore, this does not allow us to hypothesize if the additional chromosome 2 is the result of a mitotic or meiotic error (Wolstenholme, 1996). This is important because when the error is mitotic, a small fraction of mesenchymal cells is trisomic only in the culture and the pregnancy outcome is normal with no discernible effect upon placental function (Robinson *et al.*, 1997; Sago *et al.*, 1997; Wolstenholme *et al.*, 2001). On the other hand, in the case of trisomy rescue of a 47, +2 conceptus from either a maternal or paternal error, the level of trisomic cells in the CVS is typically high with the involvement of both the cytotrophoblast (direct) and the mesenchymal core (culture). In these cases, fetal growth restriction (FGR) is a frequent observation with poor outcome (Wolstenholme, 1996; Shaffer *et al.*, 1996; Wolstenholme *et al.*, 2001).

It is interesting that in our pregnancies with CPM there was a significant association between the percentage of trisomic cells in the CVS culture and the birth weight centile of the neonates and in about 15% of cases the birth weight was below the 5th centile. However, there was a wide scatter of results around the regression line describing this association and it will therefore find no clinical application in the management of affected pregnancies. Previous molecular studies examined the association between trisomy 2 CPM and FGR and concluded that the cause of impaired growth is the placental trisomy itself rather than fetal uniparental disomy (Albrecht *et al.*, 2001; Wolstenholme *et al.*, 2001). Consequently, uniparental disomy analysis is unlikely to allow accurate differentiation between pregnancies resulting in the birth of appropriately grown neonates and those complicated by FGR and fetal or perinatal death. On the other hand, demonstration or exclusion of a corrected trisomy with molecular methods would improve differentiation between high and low risk cases. However, it is important to point out that at CVS only a small sample from one site of the placenta is made available for analysis that may not be representative of the percentage of trisomic cells elsewhere. So, it is important that the pregnancy is evaluated after detailed ultrasonographic examination.

In one of our cases, there was fetal death before planned amniocentesis. In 43 of the 44 cases undergoing amniocentesis there were no trisomic cells in the amniotic fluid culture. In all these cases, ultrasound examination at 16–20 weeks showed no fetal abnormalities and the pregnancies resulted in the live birth of phenotypically normal neonates. Similarly, Grati *et al.*, reported 11 pregnancies with trisomy 2 cells in CVS cultures and in all cases amniocentesis showed that the abnormal cell line did not extend to the fetus. In another of our cases with full trisomy 2 in the chorionic villi amniocentesis

showed one trisomy 2 cell in a total of 53 cells from two cultures and the fetus had a cardiac defect and growth restriction. According to guidelines, this represents pseudomosaicism in the amniotic fluid and may be derived from the trisomic placenta, although low true fetal trisomy 2 cannot be excluded (ACC, 2005, guidelines for amniotic fluid; Benn and Hsu, 2004). So, one implication of our findings is that the presence of trisomy 2 in CVS cultures may not require further investigation by amniocentesis at least in those cases where detailed ultrasound examination does not demonstrate any defects.

In conclusion, the finding of trisomy 2 cells in CVS cultures is usually the consequence of CPM and the trisomic cell line does not extend to the fetus. The management of affected pregnancies should include ultrasound examinations to exclude fetal defects and in such cases amniocentesis may be unnecessary. However, in about 15% of cases there is FGR necessitating serial ultrasound scans to monitor fetal growth.

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