Clinical aspects, prenatal diagnosis, and pathogenesis of trisomy 16 mosaicism

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Introduction: Analysis of data from cases of trisomy mosaicism can provide insight for genetic counselling after prenatal diagnosis and for the elucidation of the pathogenesis of trisomy during pregnancy.

Methods: Statistical analysis was carried out on data from 162 cases of pregnancies with prenatal diagnosis of trisomy 16 mosaicism.

Results: The majority of cases resulted in live birth (66%) with an average gestational age of 35.7 weeks and average birth weight of –1.93 standard deviations from the population mean. Among the live births 45% had at least one malformation, the most common being VSD, ASD, and hypospadias. The level of trisomy on direct CVS (cytotrophoblast) was associated with more severe intrauterine growth restriction (IUGR) and higher risk of malformation, while the level of trisomy on cultured CVS (chorionic villous stroma) was associated only with more severe IUGR. Similarly, the presence of trisomy on amniocentesis (amniotic fluid) was associated with both IUGR and malformation, while the presence of trisomy in the amniotic mesenchyme was associated only with IUGR. Surprisingly, the degree of trisomy in placental tissues appeared to be independent of the degree of trisomy in amniotic fluid and amniotic mesenchyme. The sex of the fetus was not associated with any outcome variables, although there was an excess of females (sex ratio = 0.45) that may be explained by selection against male mosaic trisomy 16 embryos before the time of CVS (∼9-12 weeks).

Conclusion: The levels of trisomy in different fetal-placental tissues are significant predictors of some measures of outcome in mosaic trisomy 16 pregnancies.
METHODS
The study sample (n=162 cases) consists of mosaic trisomy 16 pregnancies diagnosed prenatally by CVS or amniocentesis (with or without molecular (PCR) testing for the UPD status of chromosome 16). Rare cases with paternal origin of the trisomy (n=2), partial trisomy (n=3), and concomitant aneuploidy (n=1) were excluded in order not to confound the analysis (Electronic Appendix 1). Some cases are from a continuing study of trisomy mosaicism at the University of British Columbia (UBC) (n=58). This study consists of cases referred from other centres (n=51) and cases initially ascertained at the Children’s and Women’s Health Centre of British Columbia (n=7). Some data from most of these cases have been published previously, and there is overlap with cases published by other research groups (Electronic Appendix 1). The Vancouver study was approved by the ethics committee of the University of British Columbia. An additional 103 cases are from other published reports to date (Electronic Appendix 1). Using the review of mosaic trisomy 16 cases by Benn as a starting point, data were verified from the original sources and care was taken to eliminate duplicated cases. In general, cases were ascertained via mothers undergoing prenatal testing for advanced maternal age, abnormal triple screen, and anomalies or growth restriction noted on ultrasound.

Data were collected on the following variables: (1) pregnancy outcome (live birth, intrauterine death, or termination of pregnancy); (2) gestational age at pregnancy outcome; (3) malformation detected in the fetus/neonate/infant (“malformation” used as general term independent of aetiology, including possible disruptions and deformations); (4) fetal/neonatal weight at pregnancy outcome; (5) percent trisomy on amniocentesis (assessing amniotic fluid, thought to be representative of various fetal tissues); (6) percent trisomy on (semi-) direct CVS (assessing the cytotrophoblast of the placenta); (7) percent trisomy on cultured CVS (assessing the chorionic villous stroma of the placenta); (8) percent trisomy in the chorionic membrane (part of the fetal membranes) or chorionic plate of the placenta (together referred to as “chorion” for simplicity) at postnatal or necropsy examination; (9) percent trisomy in the chorionic villous stroma at postnatal or necropsy examination; (10) percent trisomy in the cytotrophoblast at postnatal or necropsy examination (since postnatal or necropsy examination usually involved FISH without previous culture, both cytotrophoblast and syncytiotrophoblast nuclei were included; thus the general term “trophoblast” is used); (11) percent trisomy in the amniotic mesenchyme at postnatal or necropsy examination of the amnion (part of the fetal membranes) (“amniotic mesenchyme” refers to the layer of the amnion that is studied when the amnion is cultured before analysis); and (12) confirmation of trisomy in various fetal tissues. Since FISH was used to confirm the results from conventional cytogentics only very rarely (n=4) for CVS and amniocentesis, only data from conventional cytogentics were used for variables (5), (6), and (7). As FISH was more common during postnatal or necropsy investigation of the placenta, FISH was given precedence over conventional cytogentics for variables (8), (9), and (10) because FISH samples a greater number of cells. Only data from conventional cytogentics was considered for variable (11). For variable (12), data from conventional cytogentics, FISH, or molecular methods (PCR) were taken into account; if results were contradictory (that is, one was positive for trisomy, the other negative), then the fetal tissue was coded as positive. Molecular detection of trisomy in fetal tissues was only considered for cases from the UBC study where detailed information on chromosome 16 markers was available, and cases from other published reports where there was an explicit statement that PCR showed or excluded trisomy.

It should be emphasised that the study sample may be biased towards cases with poorer outcomes, since such cases are more likely to be ascertained (for example, because of anomalies observed on ultrasound), referred for research purposes, and/or submitted for publication. Therefore, purely descriptive statistics should not be considered estimates of the actual values in the general population, but are intended as descriptions of the study sample specifically. Statistical associations between variables are more likely to be unbiased, but any potential biases should be considered when interpreting results. For example, postnatal or necropsy examination of the amniotic mesenchyme and fetal tissues may have been more thorough in pregnancies with abnormal outcome or with trisomy detected on amniocentesis. Furthermore, it should be noted that there was variation in the quantity and quality of data available among the cases (for example, in the descriptions of malformations).

Associations between variables were tested for statistically (for example, t test, Fisher exact test, Yates chi-square test) using SPSS 10.0 and the VassarStats Web Site for Statistical Computation (http://faculty.vassar.edu/lowry/VassarStats.html). All tests were one tailed (except for the Yates chi-square test). Data were grouped or categorised for analysis to maximise power (for instance, the percent trisomy on CVS was grouped into 100% and <100%). As an example, 50% and 60% trisomy on CVS will be detected as a true difference on a non-grouped analysis, but may be biologically equivalent because the level of trisomy on CVS is affected by stochastic processes (such as the area of the placenta that is sampled and culture of the sample before cytogentics).

RESULTS
Clinical outcome of prenatally diagnosed mosaic trisomy 16 pregnancies
Even with an expected bias towards poor outcome, the majority of cases (66%) resulted in live births, of which 93% survived beyond the neonatal period. Eleven percent of pregnancies ended in intrauterine death (IUD), while 2% of the pregnancies were terminated. Results are for 157 cases informative for pregnancy outcome. Fig 1 shows the distribution of gestational ages for the live births (including both those that survived beyond the neonatal period and neonatal deaths); the average gestational age was 35.7 (0.41) (mean (SE)) weeks. The distribution of birth weights (number of SDs

Figure 1

Histogram of gestational ages for live births, including seven cases that resulted in neonatal death and 70 cases that survived beyond the neonatal period.
anomalies in the general population at birth (2–3% cases, equivalent to at least twice the risk of major structural present in eight or more cases (that is, in >6% of informative considered sporadic or random. Several malformations were present in three or fewer cases in the study sample (of 129 cases informative for malformation status), and were considered more likely to have a true association with trisomy 16 mosaicism: VSD 16.3% (n=15), ASD 9.8% (n=9), and hypoplasias 26.9% (n=7, of 26 informative male cases). All of these malformations were significantly more frequent when compared to the corresponding prevalences among neonates in the general population (Electronic Appendix 2, p<0.0001).

Figure 2
Histogram of birth weights (number of SDs from the gestational age corrected general population mean birth weights) for live births (including both those that survived beyond the neonatal period and neonatal deaths). Results are for 72 informative cases.

from gestational age corrected general population mean birth weights) for the live births is illustrated in fig 2. Virtually all birth weights (90%) were below the general population mean birth weights (that is, 0 standard deviations), with an average birth weight of –1.92 (0.16) (mean (SE)) SD from the mean.

Considering all cases regardless of pregnancy outcome, at least one malformation was present in 49% of the 129 cases where malformation status was known. Malformations were classified anatomically into cardiac, pulmonary, genitourinary, gastrointestinal, craniofacial, extra-craniofacial musculoskeletal, and miscellaneous dermatological. Cardiac malformations were most prevalent (62% of cases positive for malformation), with the other malformation classes present in ∼20–45% of positive cases.

Considering individual types of malformations, almost all were present in three or fewer cases in the study sample (of 129 cases informative for malformation status), and were considered sporadic or random. Several malformations were present in eight or more cases (that is, in >6% of informative cases, equivalent to at least twice the risk of major structural anomalies in the general population at birth (2–3%)%), and thus were considered more likely to have a true association with trisomy 16 mosaicism: VSD 17.8% (n=23), two vessel cord 10.9% (n=14), ASD 7.8% (n=10), clinodactyly 7.8% (n=10), and pulmonary hypoplasia 7.0% (n=9). It should be noted that at least five of the cases of pulmonary hypoplasia were probably secondary to other malformations (for example, diaphragmatic hernia, renal anomalies, premature rup-

T able 1 Outcome variables of interest

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>OUT*</td>
<td>1</td>
<td>Intrauterine or neonatal death</td>
</tr>
<tr>
<td>0</td>
<td>Live birth with survival beyond the neonatal period</td>
<td></td>
</tr>
<tr>
<td>GEST*</td>
<td>1</td>
<td>Gestational age at live birth or intrauterine death</td>
</tr>
<tr>
<td>0</td>
<td>Weight of the fetus/neonate in terms of the number of SDs from gestational age corrected general population mean birth weights</td>
<td></td>
</tr>
<tr>
<td>MALF†</td>
<td>1</td>
<td>Presence of malformation in the fetus/neonate/infant</td>
</tr>
<tr>
<td>0</td>
<td>Absence of malformation in the fetus/neonate/infant</td>
<td></td>
</tr>
</tbody>
</table>

*Excluding cases with termination of pregnancy. Including all cases regardless of pregnancy outcome (live birth, intrauterine death, or termination of pregnancy).

Trisomy 16 in the placenta
Since most trisomy 16 cases show 100% trisomy in both direct CVS (cytotrophoblast) and cultured CVS (chorionic villous stroma), more meaningful binary “indicator” variables, referred to as dCVS and cCVS respectively, were set up: the presence of euploidy (≤100% trisomy) was coded as “0”, while the presence of non-mosaic trisomy (100% trisomy) was coded as “1”. Associations between dCVS and cCVS, and four outcome variables of interest (table 1) were then tested for. Non-mosaic trisomy on direct CVS (cytotrophoblast) (dCVS=1) was associated with lower fetal/neonatal weight in terms of the number of SDs from the gestational age corrected general population mean birth weights (t=2.22, df=n–2 = 36, p = 0.016; table 2) and higher risk of malformation (MALF) (Fisher exact test, n=50, p=0.015, RR=∞; table 3), but was not associated with the risk of intrauterine or neonatal death (OUT) (Fisher exact test, n=51, p=0.589) or the gestational age at live birth or intrauterine death (GEST) (t=0.67, df=41, p=0.255). In contrast, non-mosaic trisomy on cultured CVS (chorionic villous stroma) (cCVS=1) was associated with only lower BW (t=1.91 df=34, p=0.032; table 2), and less significantly so compared to non-mosaic trisomy on direct CVS (cytotrophoblast).

Statistical analysis involving postnatal or necropsy investigation of the different placental tissues (trophoblast, chorionic villous stroma, and chorion) should be viewed more cautiously since the number of informative cases for any given outcome was small (n=15). To determine whether there is potentially selection against trisomic cells during development, the percent trisomy in cultured and direct CVS, and at postnatal or necropsy assessment of the placental tissues, were coded so that “0” refers to 0%, “1” refers to 1%–10%, “2” refers to 11%–20%, etc. For those cases with both cultured CVS and postnatal or necropsy examination of the chorionic villous stroma, the chorionic villous stroma had significantly lower levels of trisomy: 6.05 (0.79) (mean (SE)) versus 9.23 (0.39) (mean (SE)) (paired sample t=3.89, df=21, p=0.0004). For those cases with both direct CVS and postnatal or necropsy examination of the trophoblast, there was a trend towards a lower level of trisomy at postnatal or necropsy examination of the trophoblast: 7.75 (0.90) (mean (SE)) versus 9.42 (0.58) (mean (SE)) (paired sample t=1.62, df=11, p=0.067). It should be noted that the latter may not be significant because the paired sample size (n=df+1=12) was smaller than the
paired sample size for the comparison between cultured CVS and chorionic villous stroma (n=df+1= 22).

**Trisomy 16 in the amniotic mesenchyme**

Since about half of postnatal or necropsy investigations of the amniotic mesenchyme (that is, sampling of the amnion followed by culture before analysis) yielded a euploid karyotype, percent trisomy in the amniotic mesenchyme was coded into an indicator variable AMN: “0” refers to the presence of non-mosaic euploidy (0% trisomy) and “1” refers to the presence of trisomy (>0% trisomy). The presence of trisomy in the amniotic mesenchyme (AMN=1) was only associated with lower BW (Welch’s approximate t =2.98, df=15, p<0.005; table 2).

**Trisomy 16 in the fetus**

For the cases where percent trisomy on amniocentesis (in amniotic fluid) was known, about half also had a non-mosaic euploid karyotype. Therefore, another indicator variable (AF) was set up; the presence of non-mosaic euploidy (0% trisomy) on amniocentesis was coded as “0”, while the presence of trisomy (>0% trisomy) was coded as “1”. The presence of trisomy on amniocentesis (AF=1) was associated with lower BW (t=3.55, df=68, p=0.0003; table 2) and higher risk of malformation (Yates chi-square = 13.90, n=111, p=0.0002, RR = 2.10; table 3). Removing cases with AF=0 (that is, considering only cases where the amniotic fluid is positive for trisomy), the level of trisomy in amniotic fluid (coded so that “1” refers to 1%-10%, “2” refers to 11%-20%, etc) was not associated with BW (r=0.13, n=20, p=0.297) or malformation (t=0.58, df=43, p=0.282). Therefore, the presence of trisomy in amniotic fluid, but not the actual level above 0%, is predictive of outcome. It should be noted, however, that there was considerable variability in outcomes even for a given result on amniocentesis. Table 2 shows that there is a large standard deviation for the BW distribution for cases with trisomy present on amniocentesis (SD 1.07), and for the BW distribution for cases with no trisomy present on amniocentesis (SD 1.33). In addition, table 3 shows that although the risk of malformation increases when trisomy is present on amniocentesis, the risk of malformation is still considerable (34%) even when no trisomy is detected on amniocentesis.

In addition, the presence of trisomy on amniocentesis (AF=1) was also associated with the presence of trisomy in amniotic mesenchyme (AMN=1) (Fisher exact test, n=21, 7.70 p=0.007, RR=7.70; table 4). However, there were four discordant cases, with three cases being positive for trisomy in amniotic fluid but negative in the amniotic mesenchyme, and

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### Table 2: Associations with BW*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tissue</th>
<th>Procedure</th>
<th>Value</th>
<th>Level of trisomy</th>
<th>No Mean BW*</th>
<th>SD</th>
<th>t statistic, df, and p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>dCVS</td>
<td>Cytotrophoblast</td>
<td>Direct CVS</td>
<td>1</td>
<td>100%</td>
<td>34</td>
<td>−2.05</td>
<td>t = 2.22, df = 36, p = 0.016</td>
</tr>
<tr>
<td>cCVS</td>
<td>Chorionic villosus stroma</td>
<td>Cultured CVS</td>
<td>1</td>
<td>100%</td>
<td>31</td>
<td>−1.80</td>
<td>t = 1.91, df = 34, p = 0.032</td>
</tr>
<tr>
<td>AF</td>
<td>Amniotic fluid</td>
<td>Amniocentesis</td>
<td>1</td>
<td>&gt;0%</td>
<td>8</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>AMN</td>
<td>Amniotic mesenchyme</td>
<td>Postnatal or necropsy sampling of amnion followed by culture</td>
<td>1</td>
<td>&gt;0%</td>
<td>0</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

*Weight of the fetus/neonate in terms of the number of SDs from gestational age corrected general population mean birth weights.

### Table 3: Associations with risk of malformation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tissue</th>
<th>Procedure</th>
<th>Value</th>
<th>Level of trisomy</th>
<th>Absence of malformation</th>
<th>Presence of malformation</th>
<th>Risk</th>
<th>Statistic, RR and p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>dCVS</td>
<td>Cytotrophoblast</td>
<td>Direct CVS</td>
<td>1</td>
<td>100%</td>
<td>23</td>
<td>19</td>
<td>45%</td>
<td>Fisher exact test, RR = 0.015</td>
</tr>
<tr>
<td>AF</td>
<td>Amniotic fluid</td>
<td>Amniocentesis</td>
<td>1</td>
<td>&gt;0%</td>
<td>8</td>
<td>0</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

### Table 4: Associations between trisomy on amniocentesis and trisomy in amniotic mesenchyme and fetal/neonatal/infant tissues

<table>
<thead>
<tr>
<th>Variable</th>
<th>Tissue</th>
<th>Procedure</th>
<th>Value</th>
<th>Level of trisomy</th>
<th>0% trisomy on amniocentesis</th>
<th>&gt;0% trisomy on amniocentesis</th>
<th>RR and p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMN</td>
<td>Amniotic mesenchyme</td>
<td>Postnatal or necropsy sampling of amnion followed by culture</td>
<td>1</td>
<td>&gt;0%</td>
<td>1</td>
<td>7</td>
<td>RR = 7.70, p = 0.007</td>
</tr>
<tr>
<td>FETUS</td>
<td>Fetal/neonatal/infant tissues</td>
<td>Postnatal or necropsy sampling</td>
<td>1</td>
<td>&gt;0%</td>
<td>3</td>
<td>17</td>
<td>RR = 4.10, p = 0.004</td>
</tr>
<tr>
<td>BLOOD</td>
<td>Fetal/neonatal/infant blood</td>
<td>Cordocentesis or postnatal or necropsy sampling</td>
<td>1</td>
<td>&gt;0%</td>
<td>0</td>
<td>27</td>
<td>RR = 2.40, p = 0.396</td>
</tr>
</tbody>
</table>

*RR calculated with AF as “exposure” variable from which AMN, FETUS, and BLOOD were ascertained, and p value derived from Fisher exact test.
one case being negative in amniotic fluid but positive in the amniotic mesenchyme. Moreover, a variety of fetal tissues were examined for trisomy at postnatal or necropsy examination, including blood (n=73) and skin (n=45) as well as a number of other tissues (kidney, lung, liver, brain, heart, gonad, spleen, adrenal, thymus, intestine, buccal, cartilage, connective tissue, diaphragm, eye, fascia, muscle, tendon, and rectum) that were examined less often (all n<10 except for kidney (n=16) and lung (n=15)). The presence of trisomy in tissues of the fetus/neonate/infant detected at postnatal or necropsy examination was coded into an indicator variable FETUS: “0” refers to non-mosaic euploidy (0% trisomy) in all tissues examined and “1” refers to the presence of trisomy (>0% trisomy) in at least one tissue. The presence of trisomy in at least one fetal tissue (FETUS=1) was not associated with the presence of trisomy in the amniotic mesenchyme (AMN=1) (Fisher exact test, n=23, p=0.596), but was associated with the presence of trisomy on amniocentesis (AF=1) (Fisher exact test, n=81, p=0.004, RR=4.10, table 4). Again, it should be noted that there were 33 discordant cases, with 30 cases being negative for trisomy in amniotic fluid but positive in fetal/neonatal/infant tissues, and three cases being negative in amniotic fluid but positive in fetal/neonatal/infant tissues.

Since trisomy in fetal blood can be tested for prenatally (cordocentesis), the clinical value of trisomy detected in blood lymphocytes was of interest. As was already established,3 trisomy 16 was rarely found in blood (trisomy was detected in only 5.5% of cases where the blood was tested for trisomy via cordocentesis, or postnatal or necropsy sampling), and so cordocentesis is not of prenatal diagnostic value in this context. The presence of trisomy in the blood (coded into an indicator variable BLOOD) was not significantly associated with the presence of trisomy on amniocentesis (AF=1) (Fisher exact test, n=63, p=0.396, RR=2.40, table 4), although this result is probably influenced by the small number of cases positive for trisomy in blood.

**Independence of variables**

To determine whether the effects of the variables (dCVS, cCVS, AF, and AMN) on outcome are independent, ideally regression modelling should be used. For instance, it might be hypothesized that dCVS and AF may be positively correlated; that is, cases with non-mosaic trisomy on direct CVS (dCVS=1) may be more likely to have the presence of trisomy on amniocentesis (AF=1). However, sample sizes were too small so that significance was lost even in simple regression models between one explanatory variable and one outcome variable when only cases informative for a particular outcome were considered. Therefore, in lieu of regression modelling, pairwise associations between the variables were determined. dCVS (level of trisomy in the cytrophoblast determined by direct CVS) and cCVS (level of trisomy in the chorionic villous stroma determined by cultured CVS) were associated (that is, were not independent) (Fisher exact test, n=35, p=0.002, RR=3.17). As noted previously, AF (presence of trisomy in the amniotic fluid determined by amniocentesis) and AMN (presence of trisomy in the amniotic mesenchyme determined by postnatal or necropsy sampling followed by culture) were not independent (Fisher exact test, n=21, p=0.007, RR=7.70). In contrast, both dCVS (Fisher exact test, n=42, p=0.557) and cCVS (Fisher exact test, n=43, p=0.523) were not associated with (that is, were independent of) AF. Similarly, both dCVS (Fisher exact test, n=10, p=0.133) and cCVS (Fisher exact test, n=13, p=0.577) were independent of AMN. Although the sample sizes for the latter associations were small (n=10 and n=13), there were no clear trends, which is consistent with the lack of association between dCVS/cCVS and AF. Electronic Appendix 3 summarises which variables were (and were not) independent of each other.

**DISCUSSION**

**Clinical outcome of prenatally diagnosed mosaic trisomy 16 pregnancies**

Although this sample is likely to be biased towards poorer outcome, the majority of prenatally diagnosed mosaic trisomy 16 pregnancies resulted in live births with survival beyond the neonatal period. The distribution of gestational ages for all the live births suggests that trisomy 16 pregnancies may be at higher risk for preterm delivery (fig 1). Further research is needed to determine whether there is truly a higher risk of preterm delivery in an unbiased population, and, if so, whether it is primarily the result of preterm labour or induction or caesarean section secondary to other complications. In addition, virtually all birth weights for the live births were below the gestational age corrected mean birth weights in the general population14 (fig 2). This indicates that some level of below average growth is a nearly universal phenomenon in trisomy 16 mosaicism, and supports the hypothesis of undetected trisomy mosaicism as an aetiological factor in both severe and mild idiopathic intrauterine growth restriction. Anatomical classes of malformations were present at approximately the same frequency (~20-45%), with cardiac malformations most commonly seen (62%). However, some individual malformations (VSD, ASD, and hypospadias) were particularly frequent among live births (~6% of informative cases) and were significantly more frequent compared to the
Trisomy 16 in the placenta

It has previously been shown that the clinical outcome of trisomy predominantly or completely confined to the placenta was strongly associated with the level of trisomy in the term trophoblast but not with the level of trisomy on CVS. However, the data were confounded by the inclusion of a mixture of trisomies involving different chromosomes and different origins. In this study sample, non-mosaic trisomy on direct CVS (cytotrophoblast) was found to be associated with low BW (table 2) and increased risk of malformation (table 3). Cytotrophoblast function is important for implantation and for differentiation to the hormone secreting syncytiotrophoblast and invasive extravillous cytotrophoblast. Placental insufficiency is a cause of intrauterine growth restriction and can be caused by poor remodelling of maternal spiral arteries, which is thought to depend on normal extravillous cytotrophoblast function. Increased apoptosis of syncytiotrophoblast has been observed in pregnancies complicated by growth restriction, while decreased villous trophoblast proliferation has been seen in spontaneous abortions with chromosome abnormalities (mostly single autosomal trisomies). Notably, it has been shown that trisomy 21 placentas have a defect in cytotrophoblast differentiation to syncytiotrophoblast. Non-mosaic trisomy on cultured CVS (chorionic villous stroma) was also found to be associated with BW (table 2). This may be because of correlation with high levels of trisomy in the cytotrophoblast or malfunction of the chorionic villous stroma. For instance, the chorionic villous stroma is thought to be an inducer of overlying cytotrophoblast proliferation. Increased apoptosis and decreased proliferation of chorionic villous stromal cells have also been observed in spontaneous abortions with chromosome abnormalities (mostly single autosomal trisomies).

Trisomy 16 in the amniotic mesenchyme

The presence of trisomy in the amniotic mesenchyme was associated with lower BW (table 2). This may be because of a correlation between trisomy in the amniotic mesenchyme and trisomy in the amniotic fluid (and therefore, fetal tissues) (Electronic Appendix 3). The association between trisomy in the amniotic mesenchyme and outcome may also be the result of functional effects of the trisomy. For example, the amniochorionic membrane is part of the “transmembrane-ous” pathway of fluid exchange between the amniotic fluid and maternal blood that is involved in amniotic fluid regulation.

Trisomy 16 in the fetus

The presence of trisomy on amniocentesis (that is, in amniotic fluid) was associated with lower BW (table 2) and increased risk of malformation (table 3) confirming the importance of trisomy in fetal tissues for outcome. Interestingly, it is the simple presence of trisomy at amniocentesis, but not the level above 0%, that appears to be important. This finding is similar to the observation that the level of trisomy 18 in lymphocytes does not appear to correlate with outcome in mosaic trisomy 18 subjects. It also suggests that the level of trisomy on amniocentesis is influenced by stochastic processes during cell culture and during development, and should be considered a random “snapshot” of the degree and distribution of trisomy in the fetus.

Implications for the prenatal diagnosis of trisomy 16 mosaicism

Tables 2 and 3 describe the predictive value of amniocentesis and direct and cultured CVS for outcome. Also, the risk of finding trisomy on amniocentesis is independent of the levels of trisomy on direct and cultured CVS. In other words, cases with non-mosaic trisomy on either direct or cultured CVS were not more likely to have trisomy detected at amniocentesis compared to cases with <100% trisomy on either direct or cultured CVS. It is also important to re-emphasise that most cases of trisomy 16 are initially diagnosed with non-mosaic trisomy on CVS. However, as noted previously, virtually all embryos that survive to the time of prenatal diagnosis are mosaic. Thus, even with non-mosaic (100%) trisomy detected on CVS, the clinical outcomes of the pregnancy will correspond to outcomes seen in the population of mosaic trisomy 16 pregnancies. Moreover, although several variables have been shown to be predictive of outcome, there is variation in...
outcome even for a given value of a variable (tables 2 and 3). It should also be noted that ultrasound is one prenatal variable that has not been assessed in this study, but is likely to be important for identifying those cases with the worse prognoses.11

Implications for the pathogenesis of trisomy 16 mosaicism

Fig 3 summarises the results pertinent to the pathogenesis of trisomy 16 mosaicism. Trisomy in the cytotrophoblast (detected by direct CVS), chorionic villous stroma (detected by cultured CVS), amniotic fluid (detected by amniocentesis), and amniotic mesenchyme (detected by postnatal or necropsy sampling followed by culture) appear to have an effect on risk of intrauterine growth restriction, while only trisomy in the cytotrophoblast and amniotic fluid appear to have an effect on risk of malformation. Since the levels of trisomy in the placental tissues may be independent of the levels of trisomy in the amniotic fluid and amniotic mesenchyme (Electronic Appendix 3), it is possible that the effect of placental trisomy on outcome seen in this study sample may be independent (at least in part) of the effect of trisomy present in the fetus. Thus, it is theoretically possible that prenatal therapeutic interventions to improve placental function may improve outcome in trisomic pregnancies.

Sex of the fetus in trisomy 16 mosaicism

The sex ratio in the study sample (0.45) was biased towards females, as initially noted by Benn, and was significantly different from the expected sex ratio calculated from prenatal controls.17 This is similar to a report of a significant excess of females in prenatally diagnosed trisomy 21 mosaicism (sex ratio = 0.72) compared to an excess of males in prenatally diagnosed non-mosaic trisomy 21.18 The same phenomenon was found in another report,19 in addition to a significant excess of females in prenatally diagnosed trisomy 18 mosaicism (sex ratio = 0.52) and a trend towards excess females in prenatally diagnosed trisomy 13 mosaicism (sex ratio = 0.76) compared to prenatal controls.

We found no evidence for selection against male fetuses after the time of CVS, indicating that the bias in sex ratio is set in the first trimester (before ∼9–12 weeks). As previously suggested,20 two possible explanations are (1) increased probability of rescue in female non-mosaic trisomy 16 embryos and/or (2) increased selection against male mosaic trisomy 16 embryos before the time of CVS.

Future directions

Although this study has focused on intrauterine growth restriction and malformation, the most important outcome, long term postnatal prognosis, has been not been assessed because adequate follow up data are not yet available. The most common malformations in neonates (hydropsplasias, and ASD/VSD if clinically insignificant) may not have an impact on long term outcome, and of the few cases with long term follow up (S Langlois, unpublished data),21 22 postnatal development seems to progress quite well in general. We encourage clinical geneticists world wide formally to follow up cases of prenatal diagnosis of trisomy mosaicism at their centres and to publish this information for the improvement of genetic counselling.

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