1 Cytogenetic Abnormalities

Introduction
The diagnosis of a common trisomy by chorionic villus sampling or amniocentesis is the most frequent reason for referral for genetic counseling in the setting of prenatal diagnosis. There is an abundance of information available in the literature about these situations to provide accurate counseling about the spectrum of structural and functional abnormalities that could be present.

This section includes cases which illustrate the challenges in counseling about several of the less common and more vexing results that can arise from prenatal diagnostic testing. Of these, chromosomal mosaicism in chorionic villi or amniotic fluid is among the most troublesome. Prenatally diagnosed chromosomal mosaicism raises the questions of whether the abnormal cell line is also present in the fetus and, if present, whether there will be fetal damage. Although further diagnostic testing can provide more information, the interpretation of additional evaluations is complicated by phenomena such as tissue-specific mosaicism, uniparental disomy, placental mosaicism with adverse effects on the placenta, fetus or both, and the lack of long-term follow-up of surviving children. Another obstacle is that each case is unique; each case has different percentages of abnormal cells in fetal tissues that make extrapolation from the experience of case reports in the literature problematic.

Structural chromosomal rearrangements also present challenges to providing definitive prognostic information. In this situation, questions about whether the normal functioning of gene(s) has been disrupted by a translocation or inversion cannot be answered satisfactorily with current testing methods. Some rearrangements involving chromosomes which have imprinted genes raise concern about uniparental disomy which must also be addressed.

Cases involving a discrepancy between the phenotypic and chromosomal sex illustrate the possibilities of laboratory error, fetal disease states, and the limitations of ultrasonographic imaging.

Uncertainties about recurrence risks are heightened when a woman has had more than one trisomic conception, raising the possibilities of gonadal mosaicism in a parent or a predisposition to non-disjunction. Finally, when a diagnosis of a trisomic fetus is made by pathologic examination alone (i.e., without karyotypic confirmation), providing definitive information about risk of recurrence is problematic. This section presents cases of both common and rare prenatally diagnosed chromosomal abnormalities to illustrate the counseling dilemmas that can arise.

Common aneuploidy – recurrence risks and counseling pitfalls

Case 1 A 38-year-old woman is referred for chorionic villus sampling; her obstetric history is remarkable for a previous pregnancy which resulted in a stillbirth of a female infant at term. The woman relates that she was told that an evaluation of the baby after delivery revealed...
trisomy 18. The woman described her baby as having clenched hands, bilateral club feet, and an absent stomach noted on a prenatal ultrasonographic examination performed shortly before delivery. The medical records were not available for review at this time.

Once a woman has had a pregnancy with trisomy 18, the risk of recurrence is about 2.5 times the risk predicted by her age at the time of next pregnancy. The risk for other aneuploidy is about 1.8 times her age-related risk after one previous trisomy 18 conception. Hypotheses that have been offered for these increased risks include gonadal mosaicism for a trisomic cell line (when there is a recurrence of the same trisomy) and a higher risk of meiotic non-disjunction (when there is a recurrence of a different trisomy). Because trisomy 18 has a low incidence, even among older women, the risk for recurrence of fetal trisomy 18 for this woman would be about 1 in 230 taking into account her age and her obstetric history. The risk for Down syndrome would be about 1 in 65. Chorionic villus sampling or amniocentesis will provide definitive information about the fetal karyotype. Alternatively, the results of first trimester screening or integrated risk assessment can incorporate the woman’s a priori trisomy 18 and trisomy 21 risks based on her history into the risk assessment. Recurrence risks for common aneuploidy are discussed by Warburton et al. (2004).

The woman has chorionic villus sampling at 12 weeks’ gestation. The karyotype of cultured chorionic villus cells is 46,XY. Ultrasonographic examination performed at 28 weeks’ gestation reveals clenched hands, club feet, micrognathia, an absent stomach, and an increased amniotic fluid volume. The fetal karyotype is normal yet the findings on ultrasonographic examination suggest a recurrence of the abnormalities seen in the patient’s stillborn baby. The phenotype of trisomy 18 can sometimes mimic the fetal akinesia deformation sequence, a condition in which multiple joint contractures (arthrogryposis multiplex congenita) are present due to decreased intrauterine fetal movement. Fetal akinesia deformation sequence is an etiologically heterogeneous condition. Causes include underlying abnormalities of the central or peripheral nervous system, of muscle, of connective tissue, intrauterine vascular compromise, maternal disease states, and space constraints within the womb. Although the majority of cases are associated with low recurrence risk, some cases of fetal akinesia deformation sequence are due to an underlying chromosomal abnormality or mutations in a gene coding for inherited disorders with autosomal dominant, autosomal recessive, X-linked, or mitochondrial inheritance.

Review of the patient’s medical records is crucial to providing her with as accurate a recurrence risk as possible. Important information which should be established includes whether a chromosomal analysis was performed or whether the diagnosis of trisomy 18 was made based on physical examination alone.

The medical records from the previous pregnancy become available. The term fetus had contractures at all major joints and a small chin. The internal organs were not examined. A skin biopsy was obtained for chromosomal analysis; cells failed to grow in the laboratory and a karyotype could not be obtained. The medical record states that the differential diagnosis included trisomy 18 and the spectrum of disorders which lead to the fetal akinesia deformation sequence.

Relying on the patient’s own report is hazardous in this situation. While the patient was told that trisomy 18 was a possible explanation for her baby’s abnormalities, she apparently either did not remember or did not understand that other disease states were included in the differential diagnosis. Without documentation that the previous stillbirth had trisomy 18, other diagnostic entities need to be considered.

Referral for genetics evaluation is now indicated. A large number of genetic disorders can lead to the fetal akinesia deformation sequence. An extensive genetic evaluation of the baby after delivery is indicated.

Further questioning of the mother reveals that she and her husband are first cousins.

The history of consanguinity increases the likelihood that an autosomal recessive condition is the underlying basis for the etiology of the fetal abnormalities. This information can help narrow the differential diagnosis and direct the diagnostic evaluation. Even if the mode of inheritance is thought to be secure, the underlying genetic defect present in the family may not be identifiable, due to the genetic heterogeneity of this disorder. The most common autosomal recessive disorder which can present with fetal akinesia is spinal muscular atrophy due to mutations in the SMN1 gene. The incidence of spinal muscular atrophy varies among different ethnic groups. Homozygosity for deletions of exons 7 and 8 of...
the SMN1 gene are found in 95–98% of affected individuals with the remainder being compound heterozygotes for the deletion and a point mutation in the SMN1 gene.

Analysis of DNA obtained from cultured amniocytes revealed that the fetus is homozygous for deletions of exons 7 and 8 in the SMN1 gene.

**Case 2** A 30-year-old woman is referred for genetic counseling because she had a sister who reportedly had Down syndrome and died in the newborn period. The karyotype of the sister is not known. No other family members reportedly have Down syndrome. The woman has a healthy brother.

The risk for having a child with Down syndrome depends on whether the sister had Down syndrome due to trisomy 21, which is the most likely situation, or to an unbalanced inherited chromosomal translocation which may be carried by this patient in the balanced form.

About 95% of cases of Down syndrome are due to trisomy 21. Unaffected siblings of individuals with trisomy 21 Down syndrome do not have an increased risk of having a child with a chromosomal abnormality. About 4% of individuals with Down syndrome have an unbalanced Robertsonian translocation usually involving chromosomes 21 and another acrocentric chromosome (13;21, 14;21, 15;21, 21;22, 21;21 translocations). Unbalanced Robertsonian translocations associated with Down syndrome arise de novo in about two-thirds of cases and the rest are inherited from a parent.

Women who carry Robertsonian translocations involving chromosome 21 have a 10–15% chance of having a fetus with Down syndrome who survives into the second trimester or beyond. The risk of a viable fetus with Down syndrome due to an unbalanced Robertsonian translocation involving chromosome 21 is less than 1% when the translocation is transmitted by a father who is a balanced carrier. Although the risk that our patient carries a Robertsonian translocation is small, definitive information is only available by establishing her peripheral blood karyotype. Array CGH (comparative genomic hybridization) would not provide useful information for this woman because this methodology identifies deletions and duplications of genetic material but does not identify balanced structural rearrangements.

There are some features in a pedigree that heighten concern about a chromosomal rearrangement segregating in a family. These include more than one affected family member with mental retardation and birth defects (or Down syndrome in the case of Robertsonian translocations involving chromosome 21), stillbirths, recurrent pregnancy loss, and subfertility or infertility. These latter problems reflect the decreased viability of chromosomally abnormal conceptuses.

**Case 3** The results of amniocentesis for a 39-year-old woman indicate that the fetus has trisomy 18 (47,XX,+18). Her obstetric history is remarkable for an intrauterine fetal demise at 33 weeks in a fetus who had trisomy 18 diagnosed at 28 weeks' gestation after ultrasonographic examination revealed severe intrauterine growth retardation and congenital heart disease. She was 33 years of age. She also has a healthy son. All pregnancies have been with her husband. No other relatives have had children with birth defects, recurrent miscarriages, or late fetal deaths.

This is the second conception of a fetus with trisomy 18 in this woman. Understanding the reason for the recurrence and predicting a risk for still another occurrence are both unsatisfactory. The two occurrences could be by chance alone given that the woman is 39 years old and is at significant risk for fetal aneuploidy. A second explanation is low-grade mosaicism for trisomy 18 in one member of the couple. The mosaicism would involve an unknowable percentage of germline cells (sperm or ova) and might be demonstrable in peripheral blood lymphocytes or other cell types. There are a small number of persons with identified mosaicism reported in the literature. A third hypothesis raises the possibility of some factor (genetic or otherwise) that increases the rate of meiotic non-disjunction.

**Further reading**


**Reciprocal translocations and structural abnormalities**

**Case 1** A healthy 39-year-old woman had amniocentesis at 16 weeks’ gestation due to maternal age. Her husband is also 39 years old and healthy. The couple has had three early miscarriages without information about the chromosomal status of the conceptions. The amniocyte metaphase karyotype revealed an “apparently balanced” translocation between part of the short arm of chromosome 3 and part of the long arm of chromosome 7 [46,XY, t(3p13.1;q31.2)]. Ultrasonographic examination performed at the time of amniocentesis revealed normal fetal anatomy. The family histories of the patient and her husband were unremarkable for birth defects, mental retardation, classic genetic disease, stillbirths, or miscarriages.

Balanced chromosomal rearrangements are found in a few percent of phenotypically normal individuals who have experienced recurrent spontaneous pregnancy loss. When a woman has had two or three miscarriages, chromosomal analysis of both members of the couple should be performed.

The chromosomal translocation found in the amniotic fluid cells raises concerns about associated damage to the fetus because one or both of the breakpoints could disrupt normal functioning of gene(s) at or near the sites of the breaks. In addition, there might be missing or extra genetic material at the breakpoints that cannot be detected by visual inspection of the chromosomes under the light microscope. An “apparently balanced” chromosomal rearrangement (a translocation or inversion) may therefore actually be associated with duplications or deletions of genetic material. In fact, apparently balanced chromosomal rearrangements are overrepresented in individuals with mental retardation and birth defects, confirming the limitations of routine chromosomal analysis by light microscopy.

A prenatally diagnosed apparently balanced chromosomal rearrangement may have arisen as a de novo event in the sperm or ovum, or may have been transmitted from either the mother or father who carries the same translocation in their somatic and gonadal tissues. The risk of adverse effects on fetal development will depend on whether the translocation is present constitutionally in one of the parents. Therefore, the next step is to establish the peripheral blood karyotypes of both parents.

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**Scenario 1** The father’s peripheral blood karyotype appears identical to that of the fetus: [46,XY, t(3p13.1;q31.2)]. Inherited chromosomal rearrangements involving two chromosomal breakpoints are not associated with a significantly increased risk of birth defects. In this scenario, we have also found the translocation in the 39-year-old father who is in good health. This provides reassurance that the translocation is unlikely to be disrupting crucial genes in him or to be associated with clinically important extra or missing genetic material.

While we can be reassuring that the fetus is unlikely to suffer clinical consequences as a result of the translocation, there are circumstances where two members of the same family have the same “apparently balanced” chromosomal rearrangement but have discordant phenotypes. It is important to acknowledge these unlikely possibilities and why they might occur.

There a number of different reasons which could explain how two individuals in the same family with the same apparently balanced translocation would have different phenotypes.

1. The discordant phenotypes could reflect subtle differences in the translocation (i.e., a duplication or deletion) that occurred during meiosis that could not be detected by routine cytogenetic studies.
2. The translocation might have disrupted a recessive gene in the parent which is compensated for by a normal gene on the chromosomal homolog. For example, in this case, one of the father’s breakpoints is at the cystic fibrosis (CFTR gene) locus on chromosome 7. If this were the case, the father is unaffected by cystic fibrosis because his other CFTR gene (on his homologous chromosome 7) is normal. However, the fetus inherits another chromosome 7 homolog from his mother. If the mother’s CFTR gene on this chromosome has a mutation, the fetus would have cystic fibrosis symptoms after birth due to the presence of two cystic fibrosis mutations.
3. The father is only 39 years old. Whether the gene(s) involved in the breakpoints of his chromosomal translocation are associated with later-onset disorders is not known.
Other genetic or epigenetic influences on genes affected by the translocation, e.g., imprinting, may be present.

To assess the risk of cystic fibrosis, CFTR gene mutation screening or CFTR gene sequencing of the parents and DNA obtained from cultured amniocytes are available, if desired by the couple.

Experience with array CGH in the prenatal diagnosis setting is still limited at the present time. This analysis has the potential to detect chromosomal deletions and insertions that are below the resolution of the metaphase karyotype. Interpretation of array CGH analysis can be complicated by the finding of DNA variants of uncertain clinical importance.

The finding of the translocation in the father also has implications for future pregnancies. The father produces sperm with normal, balanced, and unbalanced amounts of genetic material depending on the segregation of the chromosomes during meiosis. Thus, the couple may face an increased risk of fertility problems due to the chromosomal translocation.

The fertility problems that may occur when a parent has a balanced chromosomal rearrangement include difficulty with conception and recurrent miscarriage occurring due to chromosomally unbalanced conceptions arising from the rearrangement found in the father, and the increased risk of segmental uniparental disomy (for discussion of uniparental disomy see section on Robertsonian translocations).

The chance of an unbalanced viable fetus that survives into later pregnancy or after birth is also increased. Predictions about the likelihood of subfertility or chromosomally unbalanced viable conceptions depend on the size of the unbalanced products of the translocation and the reproductive history of the couple. Identification of the translocation in other family members and their reproductive experience may also help with predictions. In addition, there may be "interchromosomal effects" of the translocation during meiosis in which the translocation interferes with normal pairing of other chromosomes, leading to an increased risk of aneuploidy.

Prenatal diagnosis by chorionic villus sampling or amniocentesis can address the risk of a fetus with an unbalanced translocation or with aneuploidy. Preimplantation genetic diagnosis could also be utilized to identify embryos with unbalanced translocations and introduce only chromosomally normal or balanced embryos to the womb.

The translocation in the father may have been inherited from one of his parents or arisen de novo in the sperm or egg with which he was conceived. If one of his parents is also a translocation carrier, each of the father’s siblings has a significant chance of carrying the translocation. This information would be important to share with the father’s siblings so they can be counseled about possible fertility problems and an increased risk of birth defects.

**Scenario 2** The karyotypes of both parents are normal. Non-paternity is denied by the patient.

Prospective identification and follow-up of other pregnancies with apparently balanced de novo chromosomal rearrangements (translocations and inversions) indicates that risks of obvious birth defects are increased two- to threefold over the background risk of 3% faced by the general population. These increased risks presumably represent unbalanced genetic rearrangements that cannot be ascertained by chromosome analysis. There has been no specific pattern of birth defects in the abnormal fetuses and newborns that have been studied. Risks of learning/behavioral difficulties have not been assessed because there is very limited long-term follow-up of children with de novo apparently balanced chromosomal rearrangements. Because structural birth defects are increased, associated neurodevelopmental problems are also likely.

Array CGH of fetal DNA (obtained from cultured amniocytes or chorionic villi) can detect some deletions and duplications of genetic material that are below the resolution of the light microscope. Array CGH may also be necessary on the parents’ DNA because interpretation of array CGH analysis can be complicated by the finding of DNA variants of uncertain clinical importance. The turnaround time for obtaining results should influence decisions about whether testing of the parents’ samples should be done simultaneously with that of the fetus.

Detailed ultrasonographic examination and fetal echocardiography should be performed to look for anatomic abnormalities in the fetus. It is estimated that about one-third of the defects associated with de novo chromosomal rearrangements would be detectable by prenatal sonography.

**Case 2** A couple is referred for genetic counseling to discuss the results of amniocentesis. The amniocyte karyotype is 46,XY,del(13)(q12q14). This is an unbalanced chromosomal complement in which there is an interstitial deletion of a proximal segment of the long arm of chromosome 13. Array CGH shows a 13 MB deletion including deletion of the retinoblastoma gene. The peripheral blood karyotypes of both members of the couple are normal.
Small deletions of the long arm of chromosome 13 are a rare chromosomal finding and there are only a few case reports of affected individuals. The phenotype described from the case reports includes growth retardation, facial dysmorphology (frontal bossing, bulbous tip of the nose, thick lower lip, large ears and lobes), and mild to moderate mental retardation. Hydrocephalus and neurologic abnormalities may also be part of the phenotype. Absence of one copy of the retinoblastoma gene is predictive of significant increase in risk of malignancy.

Risk of recurrence of another child with a chromosome 13q deletion is small, although higher than that for other couples in the general population. The small increase in risk reflects the unlikely possibility that one of the parents carries a chromosomal rearrangement or an interstitial deletion involving chromosome 13 in their gonadal cells. The fetal karyotype could be established by chorionic villus sampling beginning at 10 weeks’ gestation in a future pregnancy if the couple desired early prenatal diagnosis. Ultrasonographic examination has little, if any, utility in the diagnosis of this chromosomal abnormality.

Further reading

Robertsonian translocations refer to a specific class of chromosomal rearrangements in which there is fusion of the long arms of two acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22). Robertsonian translocations can be homologous in which there is fusion of the long arms of the same acrocentric chromosome, or non-homologous, i.e., fusion of the long arms of two different acrocentric chromosomes. Balanced Robertsonian translocations are the most common human chromosomal translocation with an incidence of 1 in 900 (Table 1.1). About 4% of liveborns with Down syndrome are due to unbalanced Robertsonian translocations involving chromosome 21.

The large majority of Robertsonian translocations are between non-homologous acrocentric chromosomes, and the 13;14 Robertsonian translocation is the most common. The short arm of an acrocentric chromosome is comprised of the satellite, the satellite stalk, and the proximal short arm. The satellite stalk, also known as the nucleolar organizing region, contains multiple copies of genes coding for ribosomal RNA. An individual with one Robertsonian translocation has only eight satellite stalks instead of the usual ten. However, this reduction is not detrimental although, presumably, a minimum number of stalks with active genes is necessary for normal cellular function.

Table 1.1 Robertsonian translocations

<table>
<thead>
<tr>
<th>Non-homologous Robertsonian translocations</th>
<th>Homologous Robertsonian translocations</th>
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<tbody>
<tr>
<td>t(13;14)*</td>
<td>t(13;13)</td>
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<tr>
<td>t(14;15)</td>
<td>t(14;14)</td>
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<tr>
<td>t(15;21)**</td>
<td>t(15;15)</td>
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<td>t(21;22)**</td>
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<td>t(13;15)</td>
<td>t(14;22)</td>
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<tr>
<td>t(14;21)**</td>
<td>t(15;22)</td>
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</tbody>
</table>

*Most common, **less common, remainder very rare.

Further reading

Case 1 A healthy 31-year-old woman is referred for genetic counseling. After having one healthy child, she had three miscarriages in early pregnancy. All her pregnancies have been with the same partner. Her husband's peripheral blood karyotype is normal (46,XY). She has a balanced Robertsonian translocation between chromosomes 14 and 21 [45,XX,der(14q;21q)].

Constitutional balanced chromosomal rearrangements, which are seen in about 1 in 400 phenotypically normal individuals, are associated with an increased risk of spontaneous pregnancy loss, chromosomally unbalanced liveborns, and occasionally, infertility. Empiric data suggest that after three or more miscarriages, the probability that one member of a couple has a balanced chromosomal rearrangement, either a chromosomal translocation or chromosomal inversion, is 3–5%. Identification of a balanced chromosomal rearrangement in an individual provides the couple with the likely explanation for their fertility problems, forewarns them about the possibility of a liveborn with an unbalanced chromosomal rearrangement, and affords them the opportunity for preimplantation or prenatal genetic diagnosis.

Robertsonian translocations refer to a specific class of chromosomal rearrangements in which there is fusion of the long arms of two acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22). Robertsonian translocations can be homologous in which there is fusion of the long arms of the same acrocentric chromosome, or non-homologous, i.e., fusion of the long arms of two different acrocentric chromosomes. Balanced Robertsonian translocations are the most common human chromosomal translocation with an incidence of 1 in 900 (Table 1.1). About 4% of liveborns with Down syndrome are due to unbalanced Robertsonian translocations involving chromosome 21.

The large majority of Robertsonian translocations are between non-homologous acrocentric chromosomes, and the 13;14 Robertsonian translocation is the most common.

The short arm of an acrocentric chromosome is comprised of the satellite, the satellite stalk, and the proximal short arm. The satellite stalk, also known as the nucleolar organizing region, contains multiple copies of genes coding for ribosomal RNA. An individual with one Robertsonian translocation has only eight satellite stalks instead of the usual ten. However, this reduction is not detrimental although, presumably, a minimum number of stalks with active genes is necessary for normal cellular function.
With rare exceptions, balanced Robertsonian translocations are not associated with adverse effects on health or development. Individuals with balanced Robertsonian translocations do have an increased risk of infertility, recurrent spontaneous abortions, and, depending on the chromosomes involved in the translocation, an increased risk of chromosomally unbalanced viable fetuses and children. These problems occur because the Robertsonian translocation causes abnormal segregation of the chromosomes during meiosis, resulting in gametes with six different possible chromosomal configurations, only two of which are balanced, as shown in Figure 1.1. Thus, at fertilization, the zygote could have an entirely normal chromosomal complement, the balanced translocation, or various combinations of unbalanced products as illustrated in Figure 1.2.
The risk that a balanced Robertsonian translocation carrier will have a viable chromosomally unbalanced fetus at the time of amniocentesis or later in pregnancy is shown in Table 1.2. Although two-thirds of zygotes shown in Figure 1.2 are chromosomally unbalanced, most fetuses of Robertsonian translocation carriers – i.e., all the monosomic fetuses, most fetuses who have three copies of chromosomes 13, and virtually all fetuses with three copies of chromosomes 14, 15, or 22 – will not survive for more than a few days or weeks following fertilization. This accounts for the overall low risk of viable unbalanced fetuses among parents who carry a non-homologous Robertsonian translocation.

The risk of unbalanced embryos surviving into the second trimester or after birth depends on the chromosomes involved in the translocation and which parent carries the Robertsonian translocation. As shown in Table 1.2, the risk is highest for women who carry a non-homologous Robertsonian translocation.

<table>
<thead>
<tr>
<th>Robertsonian translocation</th>
<th>Balanced carrier mother: % unbalanced at amniocentesis</th>
<th>Balanced carrier father: % unbalanced at amniocentesis</th>
</tr>
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<tbody>
<tr>
<td>13q14q</td>
<td>1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>13q15q</td>
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<td>14q21q</td>
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<td>15q21q</td>
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<tr>
<td>15q22q</td>
<td>&lt;1</td>
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1Frequency at livebirth is less than in the second trimester. Estimates for rare Robertsonian translocations are extrapolated from data for the common Robertsonian translocations.

Risk of uniparental disomy 14 or 15 is about 0.65% (CI 0.2–2.3%) for Robertsonian translocations involving chromosomes 14 and 15. Carriers of homologous Robertsonian translocations (13q13q, 14q14q, 15q15q, 21q21q, 22q22q) have a remote chance of having a chromosomally normal conceptus because their gametes will either be nullisomic or disomic for the translocation chromosome. Monosomy for other than the X chromosome results in embryonic death. Trisomy for chromosomes 14 and 15 results in early embryonic death. Fetuses with trisomy for chromosomes 13 or 22 rarely survive into the early second trimester or beyond. 21q21q homologous translocation carriers have a significant risk of a viable fetus with trisomy 21.

The significantly lower risk of viable chromosomally unbalanced fetuses in pregnancies where the father transmits a Robertsonian translocation involving chromosome 21 might reflect selection against chromosomally unbalanced sperm during gametogenesis or fertilization. In contrast to the high risk of Down syndrome in fetuses of women with Robertsonian translocations involving chromosome 21, individuals with non-homologous Robertsonian translocations involving chromosome 13, regardless of their sex, have only a small chance of having a fetus with three copies of chromosome 13 who survives into the second trimester of pregnancy. Individuals with non-homologous Robertsonian translocations involving chromosomes other than 13 and 21 have a very small risk of having a viable fetus with a non-mosaic trisomy even late in the first trimester.

Some individuals with balanced non-homologous Robertsonian translocations experience recurrent early pregnancy loss while others do not. These variations may exist even between members of the same family and do not have a satisfactory explanation. Preimplantation genetic diagnosis might be considered for a couple who have had recurrent miscarriages with unbalanced fetuses.

Due to their rarity, no empiric data are available about the risks of viable unbalanced fetuses for individuals who carry 13;21 and 15;21 Robertsonian translocations. However, we believe the reproductive experience probably would parallel that of the carriers of 14;21 and 21;22 Robertsonian translocations as shown in Table 1.2.

The risk of birth defects in the balanced offspring of a parent who has a balanced Robertsonian translocation involving chromosome 21. For 14;21 and 21;22 translocation carriers, the risk at the time of amniocentesis of a viable fetus with Down syndrome is about 15% for a female Robertsonian translocation carrier. In contrast, this risk is only about 1% for a male Robertsonian translocation carrier.

Data adapted from Gardner and Sutherland (2004) and Silverstein et al. (2002).
the number 14 or 15 chromosomes. Both maternal and paternal uniparental disomy 14 and 15 are associated with deleterious effects on normal development. Maternal disomy for chromosome 14 has effects on development of variable severity including prenatal and postnatal growth restriction and facial dysmorphology. Cognitive functioning ranges from normal to severe retardation. Paternal disomy 14 is associated with polyhydramnios, prenatal and postnatal growth restriction, a small thorax, and kyphosis. Maternal disomy 15 is one mechanism that leads to Prader–Willi syndrome and paternal disomy 15 to Angelman syndrome. There is no evidence for deleterious effects of maternal or paternal imprinting of genes on chromosomes 13, 21, and 22 as neither maternal nor paternal uniparental disomy for these chromosomes has been associated with an abnormal phenotype.

In addition to trisomy rescue two other recognized mechanisms, gamete complementation and gamete compensation, can lead to uniparental disomy. Gamete complementation occurs when an ovum missing a specific chromosome is fertilized by a sperm which by chance has two copies of that same chromosome. Gamete compensation refers to the situation where a zygote which is monosomic for a certain chromosome is rescued from embryonic death by a mitotic nondisjunction event leading to duplication of that chromosome.

When a parent carries a Robertsonian translocation involving chromosomes 14 or 15, the risk of uniparental disomy is about 0.65% (95% CI 0.2–2.3%).

Uniparental disomy may cause problems in the fetus due to the presence of recessive alleles or deletions at a gene locus or due to the effects of genetic imprinting. Imprinting refers to the situation in which genes behave differently depending on whether they are inherited from the mother or the father. For example, some genes will only be expressed on a maternally inherited chromosome and others only on a paternally inherited chromosome. For some, but not all genes, absence of a maternal or paternal contribution will result in adverse effects on development or function. Chromosomes containing genes which are imprinted and the clinical consequences of imprinting are listed in Table 1.3.

Trisomy rescue during embryogenesis can also lead to the situation in which a fetus is mosaic for a disomic and a chromosomally unbalanced cell line. Phenotypic effects are highly variable and depend on the percentage and distribution of the chromosomally abnormal cells. Chorionic villus sampling or amniocentesis should identify most situations in which the fetus has a large percentage of chromosomally unbalanced cells. Low-level mosaicism, however, may go undetected. The experience with prenatal diagnosis with Robertsonian translocation carriers indicates that if the chorionic villus or amniocyte karyotype is normal, the probability of deleterious underlying mosaicism for a chromosomally unbalanced cell line is very small.

Testing for whole chromosome uniparental disomy 14 or 15 can be accomplished by analysis of DNA obtained from chorionic villi or amniocytes and the peripheral blood cells of the parents. DNA polymorphisms associated with the specific chromosome are analyzed to determine whether there is chromosomal material from both parents. Detection of some cases of uniparental disomy is also becoming available by analysis of the fetal DNA alone.

**Case 2** Chromosomal analysis is performed on a newborn after a pediatrician suspects that he has Down syndrome. The karyotype of the baby is 46,XY,-21, þt(21q;21q). The baby has Down syndrome because he has a homologous Robertsonian translocation involving chromosome 21 with two copies of chromosome 21 genes, in addition to a free-standing chromosome 21. Analysis of the parents’ peripheral blood karyotypes is indicated.

Available data show that 90% of unbalanced homologous Robertsonian translocations causing Down syndrome arise as de novo events. In 10% of cases, one of the parents is found to carry the homologous Robertsonian translocation. Homologous translocations for the other acrocentric chromosomes in fetuses arising de novo is not known.

If the translocation has arisen de novo in the baby, the risk of recurrence is very small, only slightly increased over the background risk faced by all couples. This slightly increased risk reflects the unlikely possibility that one of the parents has mosaicism for the homologous Robertsonian translocation in his or her germ cells.

If a parent carries the translocation, the chance of having a chromosomally normal child is remote. The gametes of the parent with the homologous Robertsonian translocation contain either two copies of chromosome 21 or no copy. The former situation results in conceptuses with three copies of chromosome 21 and the latter results in conceptuses with monosomy 21 which is lethal during early embryogenesis.
There have been rare reports of chromosomally normal or chromosomally balanced children born to individuals who carry homologous Robertsonian translocations. This can be due to underlying gonadal mosaicism for a normal cell line in the carrier parent, or, by trisomy rescue, gamete compensation or gamete complementation.

Couples in which one member of the couple carries a homologous Robertsonian translocation should be counseled that the chance of a chromosomally balanced fetus is remote. Infertility, recurrent miscarriage, or in the case of individuals with 21;21 and 13;13 Robertsonian translocations, viable fetuses with Down syndrome or trisomy 13 would be expected. Pregnancy could be achieved by either egg or sperm donation.

### Further reading


### Table 1.3 Abnormal phenotypes associated with whole chromosome uniparental disomy (UPD)

<table>
<thead>
<tr>
<th>Maternal UPD</th>
<th>Chromosome</th>
<th>Paternal UPD</th>
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<tr>
<td>1</td>
<td>6</td>
<td>Transient neonatal diabetes, IUGR, macroglossia</td>
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<td>6</td>
<td>7</td>
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<tr>
<td>Pre- and postnatal growth retardation; relative macrocephaly; facial dysmorphism resembling Russell–Silver syndrome; developmental delay in some cases, retarded bone age</td>
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<tr>
<td>8</td>
<td></td>
<td>Beckwith–Wiedemann syndrome</td>
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<tr>
<td>12</td>
<td></td>
<td>Polycythaemia and premature labor; pre- and postnatal growth retardation; small bell-shaped thorax with short, curved ribs, kyphosis, all of variable severity</td>
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<tr>
<td>13</td>
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<tr>
<td>Pre- and postnatal growth retardation; facial dysmorphology (large and broad forehead, high palate, fleshy nasal tip, slight blepharophimosis), muscular hypotonia; precocious puberty; obesity, wide range of intellectual deficits</td>
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<tr>
<td>14</td>
<td></td>
<td>Prader–Willi syndrome</td>
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<td>X</td>
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<tr>
<td>patXY</td>
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Adapted from Kotzot D, Utermann G (2005) *American Journal of Medical Genetics* 136A:287–305. The chromosomes with no clinical descriptions have not been associated with a clinical phenotype at this time.
Chromosomal mosaicism – prenatal diagnosis

Chromosomal mosaicism refers to the situation in which there are cells with different chromosomal complements. It may occur in an individual or in the in vitro culture of cells in a laboratory sample. In liveborns, there is a phenotypic continuum associated with chromosomal mosaicism which ranges from normal or near normal to the classic manifestations of the chromosomal abnormality. Chromosomal mosaicism is often found in amniotic fluid and chorionic villus cell culture. How we determine whether chromosomally abnormal cells found in chorionic villi or amniotic fluid pose a significant risk of underlying mosaicism in fetal tissues will be illustrated by the following cases.

The clinical importance of prenatally diagnosed chromosomal mosaicism depends on a number of factors including the level of mosaicism and the chromosomal abnormality involved.

Three levels of prenatally diagnosed chromosomal mosaicism are defined as follows.

- **Level I mosaicism** (single cell pseudomosaicism): a single abnormal cell in an otherwise normal study.
- **Level II mosaicism** (multiple cell pseudomosaicism): the presence of two or more abnormal cells with the same abnormality, which are restricted to a single culture vessel.
- **Level III mosaicism** (true mosaicism): the presence of abnormal cells with the same abnormality which are found in different culture vessels. True mosaicism means that the abnormal cell line is very unlikely to have arisen in cell culture after chorionic villus sampling or amniocentesis was performed. This conclusion is warranted because the same chromosomal abnormality is present in cells recovered from different culture vessels into which the original, uncultured sample had been placed.

Table 1.4 summarizes relevant information when considering the diagnostic problem of prenatally diagnosed chromosomal mosaicism.

Mosaicism in amniotic fluid for a common chromosomal abnormality (trisomy 21)

A woman elected amniocentesis based on her age of 35 years. The amniocyte karyotype is 47,XX,+21/46,XX. Cells with trisomy 21 are recovered from three different cover slips. About 20% of the cells have trisomy 21. Because cells with a trisomy 21 karyotype were recovered from more than one culture vessel, level III mosaicism (true mosaicism) is present in this study.

Follow-up of pregnancies in which chromosomal mosaicism has been observed in cultured amnioncytes suggests that the chromosomal mosaicism may be confined to cells shed from the placenta without phenotypic effects in the fetus. Amniotic fluid cells originate from both the fetus and the placenta although placentally derived cells are a small minority. Nonetheless, abnormal cells recovered in an amniotic fluid sample could be restricted to those originating in the placenta or membranes. Follow-up of pregnancies in which level III (true) mosaicism has been observed in amniotic fluid cells indicate a 70% or higher chance of mosaicism subsequently being confirmed in the fetus, or in the baby after birth. Predictions about the likelihood of demonstrable abnormalities that are associated with the abnormal cells are more difficult to make.

Caution must be exercised when counseling about the overall published concordance rate between amniotic fluid cell mosaicism and the underlying fetal karyotype because the above concordance rate of 70% underestimates the likelihood of a population of abnormal cells in the fetus. Follow-up studies of prenatally diagnosed cases of chromosomal mosaicism have relied mostly on an analysis of peripheral blood cells (and occasionally skin cells) of a liveborn baby or of skin cells from an aborted fetus. This is a major limitation of these studies because mosaicism for rare trisomies (e.g., trisomy 15 – see case below) is often restricted to certain somatic tissues and may be selected against in tissues that are usually submitted for cytogenetic analysis. In addition, the follow-up reports of the liveborns usually discuss the phenotypes present in the newborn period or early infancy. Later-onset developmental problems would not be recognized or reported in the literature. Thus, in the setting of an amniotic fluid study with level III mosaicism for trisomy 21, the likelihood of underlying mosaicism in the fetus is at least 70% and probably higher.

Further testing of the pregnancy to provide more information about the fetal karyotype could be obtained by fetal blood sampling and fetal skin biopsy. In contrast to
amniocytes, which may contain a mixture of fetal and placental cells, there is no ambiguity about the origin of cells obtained from fetal blood or skin. If the fetal lymphocyte karyotype is normal, the risk of clinically important mosaicism will be significantly reduced, to a few percent or less. However, normal results of invasive testing cannot completely exclude the possibility of underlying mosaicism in the fetus as the abnormal cell line has a chance of being present in fetal tissues, such as the brain or heart, which are not sampled either before or after birth.

Although some association may exist, correlations between the percentage of abnormal cells recovered in amniocytes or a fetal tissue and the severity of effects on normal development must be made cautiously. It is impossible to know the percentage of abnormal cells in critical body organs.

Detailed ultrasonographic imaging including fetal echocardiography is recommended. The identification of anatomic or growth problems in the fetus, regardless of the results of further invasive testing of the pregnancy, will further raise concerns that the fetus has a significant fraction of abnormal cells. However, normal fetal imaging cannot significantly diminish the risk of clinically underlying mosaicism in the fetus. Even in the non-mosaic form, only about half of fetuses with Down syndrome have detectable signs on ultrasonographic examination.

In the setting of mosaicism, in which a normal cell line will moderate or minimize the Down syndrome

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**Table 1.4 Prenatally diagnosed chromosomal mosaicism**

<table>
<thead>
<tr>
<th>Level of mosaicism</th>
<th>Amniocentesis</th>
<th>Chorionic villus sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level I mosaicism</td>
<td>5%</td>
<td>&gt;5%</td>
</tr>
<tr>
<td>Level III mosaicism</td>
<td>0.25%</td>
<td>1%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>III Confirmation of mosaicism in fetus or baby</th>
<th>Amniocentesis</th>
<th>Chorionic villus sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levels I and II</td>
<td>Unknown, but infrequent</td>
<td>Unknown, but infrequent</td>
</tr>
<tr>
<td>Level III mosaicism</td>
<td>70%</td>
<td>40%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>IV Factors influencing assessment of risk to fetus/pregnancy and recommendations, if any, for further testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Level of mosaicism and adequacy of the study</td>
</tr>
<tr>
<td>2. Chromosomal abnormality involved</td>
</tr>
<tr>
<td>3. Potential for phenotypic expression of uniparental disomy</td>
</tr>
<tr>
<td>4. Phenomenon of tissue-limited mosaicism</td>
</tr>
<tr>
<td>5. Association of confined placental mosaicism with increased risk of poor pregnancy outcome</td>
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</table>

<table>
<thead>
<tr>
<th>V Options for further testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Amniocentesis after mosaicism found at CVS or repeat amniocentesis</td>
</tr>
<tr>
<td>2. Fetal blood sampling</td>
</tr>
<tr>
<td>3. Fetal skin biopsy</td>
</tr>
<tr>
<td>4. Uniparental disomy studies if amniocytes are diploid</td>
</tr>
<tr>
<td>5. Interphase cell analysis by FISH</td>
</tr>
<tr>
<td>6. Targeted ultrasonographic examination including fetal echocardiography</td>
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<tr>
<td>7. Chromosome studies in the newborn or infant periods</td>
</tr>
</tbody>
</table>

*Very few attempts have been made to perform postnatal cytogenetic studies in level I and level II cases to look for evidence of mosaicism; no excess risk of pregnancy loss or birth defects has been reported in this group; no long-term follow-up is available; anecdotal reports exist of level I and II prenatal mosaic findings subsequently being associated with recovery of the abnormal cell line from liveborns.

*Represents overall risk of all aneuploidies; actual risk in a given pregnancy must take into the account specific aneuploidy which is present.
phenotype, the likelihood of visible anatomic variants or defects is even smaller. It is also important to recognize that a sonographic abnormality might have a totally independent cause unassociated with the mosaicism.

Maternal cell contamination of amniotic fluid is extremely unlikely in the cytogenetics laboratory when the amniocentesis has been uncomplicated and the first few milliliters of fluid are not used for the cytogenetic studies.

When there is no sonographic evidence at the time of amniocentesis of a co-twin, now deceased, who was noted on a first-trimester ultrasonographic examination, cells from the vanishing twin have a very low chance of being present in the amniotic fluid sample.

If the pregnancy continues to term, a karyotype could be obtained from cord blood or from peripheral blood, or from a skin biopsy in the newborn period, or no further testing be performed at all. FISH (fluorescence in situ hybridization) studies of interphase cells such as buccal mucosal cells could also be done. Normal results from testing during the pregnancy and, if desired, of the newborn would provide reasonable inference that the abnormal cell line was unassociated with adverse phenotypic effects in the fetus, but would not completely exclude them.

Mosaicism in chorionic villus cells for a rare chromosomal abnormality (trisomy 15)

A woman elected chorionic villus sampling based on her age of 40 years. The chorionic villus cell karyotype is 47, XX, +15/46,XX. Cells with trisomy 15 are found on different cover slips.

Cells with a trisomy 15 karyotype were recovered from two different initial cultures. Level III mosaicism (true mosaicism) is present in this study.

Follow-up of pregnancies in which chromosomal mosaicism has been observed in chorionic villi indicate that the chromosomal mosaicism may be confined to the placenta without phenotypic effects in the fetus. A rough estimate of its presence in the fetus is 40%.

Liveborns with birth defects have been reported who have trisomy 15 mosaicism. Recognition of the entity has been very infrequent, however. The probability of adverse phenotypic effects in the setting of chromosomal mosaicism is related to the percentage and distribution of the cells with abnormal chromosomes. Depending on the percentage and distribution of the abnormal cell line, effects could range from none or mild, to significant abnormalities including mental retardation and structural birth defects. The presence of the abnormal cell line could easily lead to early fetal death.

As with amniotic fluid cell studies, caution must be exercised when counseling about the published overall concordance rate between chorionic villus cell mosaicism and the fetal karyotype. Most studies which attempt to confirm the results of chorionic villi in a liveborn rely only on an analysis of peripheral blood cells and occasionally skin cells. In the past, most studies examined metaphase cells and did not examine interphase cells. More recent studies are including interphase cells. Especially for rare trisomies, e.g., trisomy 15, it is possible that mosaicism for the abnormal cell line is restricted to only certain fetal tissues and may be selected against in tissues that are usually submitted for cytogenetic analysis. The published studies reporting on concordance rates between mosaicism found at the time of chorionic villus sampling and subsequent recovery of abnormal cells in fetal tissue or liveborns are also limited by the fact that relatively little data are available about any specific trisomy.

Further testing of the pregnancy to provide more information about the fetal karyotype could be obtained by amniocentesis, fetal blood sampling, and fetal skin sampling. In addition to standard metaphase analysis of amniocytes, it would be useful to study the amniotic fluid cell population by FISH using a chromosome 15 probe to look for trisomy 15 cells that would not be found by metaphase analysis.

A low risk of adverse phenotypic effects will be predicted if results of amniocentesis are normal, and if detailed ultrasonographic examination documents normal fetal growth and development. However, once mosaicism has been documented in chorionic villi or amniotic fluid, normal results of further imaging or invasive testing cannot completely exclude the possibility of underlying mosaicism in the fetus as the abnormal cell line may be present in fetal tissues that cannot be sampled, and the abnormal cells may not cause an abnormal sonographic phenotype. Trisomy 15 has rarely been recovered from metaphase analysis of peripheral blood lymphocytes, suggesting that these cells may not mature in the marrow or survive in the peripheral circulation very well. In the setting of trisomy 15 mosaicism, normal results of fetal blood sampling would provide limited useful information.

If trisomy 15 cells are found in cultured amnioncytes, this substantially increases the likelihood that they are populating fetal tissues. However, amniocytes are derived from both the placenta and the fetus. Recovery of trisomy 15 cells in amniotic fluid does not prove that the fetus has underlying mosaicism as the abnormal cells...
could still be restricted to the placenta although this subsequent finding would substantially increase the risk of true fetal mosaicism. The overall concordance rate between amniocyte mosaicism and subsequent recovery of the abnormal cells in fetal tissue or a liveborn is suggested to be about 70%. As stated earlier, this is probably an underestimate. It is likely that if trisomy 15 cells are recovered in amniotic fluid, even if ultrasonographic examination reveals normal fetal anatomy, there would be a high probability (>80%) of underlying trisomy 15 mosaicism in the fetus with a wide spectrum of possible effects.

Recovery of trisomy 15 cells from a fetal tissue, e.g., skin or blood, will further raise concerns that the fetus has a significant fraction of abnormal cells. Correlations between the percentage of abnormal cells recovered in amniocytes or a fetal tissue and the severity of effects on normal development must be made cautiously. Particularly for rare trisomies, it may not be appropriate to generalize from the karyotype of fetal lymphocytes or skin fibroblasts to other tissues such as the brain. Also, normal fetal imaging should not be used to conclude that the risk of clinically underlying mosaicism in the fetus is negligible. Karyotypically abnormal cells can have major deleterious effects on organ functioning without gross anatomic defects.

Detailed ultrasonographic imaging including fetal echocardiography is recommended. The identification of anatomic or growth problems in the fetus, regardless of the results of further invasive testing of the pregnancy, will raise concerns considerably that the fetus has suffered phenotypic effects from trisomy 15 mosaicism.

The cells with trisomy 15 could have arisen due to a chromosomal non-disjunction event which occurred in the egg or sperm with which the fetus was conceived. Alternatively, the cells with trisomy 15 could have arisen after conception by a mitotic non-disjunction event. The former explanation for the presence of the trisomy 15 cell line in chorionic villi poses some additional risk of abnormality in the fetus or placenta due to the possibility that the diploid cell line, the “normal” cells, has both of its number 15 chromosomes coming from one parent, a situation known as uniparental disomy. Uniparental disomy arises when a trisomic conceptus, trisomy 15 in this situation, is “rescued” from embryonic death by loss of one of the number 15 chromosomes to generate the mosaic condition with the diploid cell line in addition to the trisomic cell line. If there is uniparental disomy, both members of the homologous chromosome pair in the diploid cell line will have come from the same parent.

Uniparental disomy causes an abnormal phenotype due to imprinting effects or homozygosity at recessive disease loci. Recognizable genetic syndromes have been reported in association with uniparental disomy for chromosome 15 and for uniparental disomy for other chromosomes (see section on Robertsonian translocations). Prader–Willi syndrome occurs when an individual has two chromosome 15s which are maternally inherited. When both chromosome 15s are inherited from a father, this results in Angelman syndrome. Both disorders are associated with significant mental retardation, characteristic physical findings and aberrant behavior. Mosaicism due to trisomy rescue situations arise more frequently, but not exclusively, in the setting of advanced maternal age where there is an increased risk of trisomic conceptuses. The presence or absence of uniparental disomy can be established by analysis of DNA extracted from amniocytes unless there is a major fraction of trisomic cells present. Current uniparental disomy studies often require fetal DNA and DNA from both parents. New methods may make it possible to use fetal DNA only.

Unrecognized maternal cell contamination of cultured chorionic villi is a negligible problem for cytogenetic analysis when chorionic villi are expertly dissected from maternal decidua in an experienced laboratory and the cells are harvested after only a few days in culture.

For twin gestations, it is unlikely that a chorionic villus sample will be contaminated with cells from a living or deceased twin but this possibility cannot be ruled out. The obstetrician’s documentation of the positional relationship of the placentas and the approach of the sampling instrument during the chorionic villus sampling procedure will be helpful in considering the likelihood of contamination.

The trisomy 15 cell line could be confined to the placenta and membranes and still pose problems for the fetus. Placental chromosomal mosaicism may lead to placental dysfunction and poor pregnancy outcome, including intrauterine growth restriction, preterm delivery and intrauterine fetal death. Even if there are no abnormal cells in the fetus, harmful effects could occur because of mosaicism in the placenta.

If the pregnancy continues to term, postnatal chromosome studies could be obtained. If they are normal, and earlier studies of the fetus had been normal, there is a reasonable inference that the abnormal cell line would be unassociated with adverse phenotypic effects in the child, although the evidence would not be conclusive.
Mosaicism in chorionic villus cells for a different rare chromosomal abnormality (trisomy 16)

The karyotype of cultured chorionic villi for a 38-year-old woman is 47,XX, +16. No cell with a normal karyotype was identified. The fetus appeared to have normal growth and development at the time of chorionic villus sampling at 12 weeks’ gestation.

Trisomy 16 is one of the most common chromosomal abnormalities identified in first-trimester spontaneous miscarriages. Identification of a placenta with full or mosaic trisomy 16 is an uncommon finding. Although mosaicism with a normal cell line is not present in this case, only a small fraction of the placenta was sampled.

Liveborns with birth defects due to trisomy 16 mosaicism have been reported. This condition is very rare. Given trisomy 16 cells in the placenta, a rough estimate of its presence in the fetus is 40%. Fetal mosaicism could be associated with a wide range of anatomic and/or functional problems depending on the distribution and number of the trisomy 16 cells. From the limited literature about fetal and placental trisomy 16, up to half of fetuses would either not survive in utero or be abnormal when there is a trisomy 16 placenta. This may be due to placental insufficiency or abnormalities intrinsic to the fetus or both.

Most cases of trisomy 16 mosaicism arise following a trisomy rescue event, increasing the chance of uniparental disomy for trisomy 16. There have been a few reports of chromosomally normal infants with maternal disomy for chromosome 16. Intrauterine growth restriction has been documented in some of these cases. Rarely, birth defects or mental retardation have been present. The scarcity of cases does not allow conclusions as to whether there is a consistent pattern. It is not possible in these cases to determine whether the adverse effects on growth were attributable to imprinting problems associated with uniparental disomy, to dysfunction of a mosaic placenta, or to underlying trisomy 16 mosaicism in tissues which were not analyzed.

Further invasive testing of the pregnancy to provide more information about the fetal karyotype could be obtained by amniocentesis and fetal skin biopsy. Non-invasive testing would include detailed ultrasonographic examination including fetal echocardiography. Low risks of phenotypic effects would be predicted if results of further testing are normal and the fetus appears to have normal anatomy and growth. However, normal results of further testing cannot completely exclude the possibility of underlying mosaicism in the fetus. Trisomy 16 is rarely recovered from metaphase analysis of peripheral blood lymphocytes, suggesting that fetal blood sampling would be unlikely to provide useful information. Future studies of interphase cells in fetal blood using chromosome 16 probes may alter that conclusion, however.

Recovery of trisomy 16 cells from additional invasive testing and/or the identification of anatomic or growth problems in the fetus will considerably raise concerns that the fetus has suffered damaging effects. If growth restriction is the only finding, this might reflect an abnormal placenta with a chromosomally normal fetus. For the latter situation, there would still be uncertainty about long-term deficits for a child. Trisomy 16 cells confined to the placenta are associated with a significant risk of intrauterine growth restriction and perinatal loss.

Mosaicism in amniotic fluid for a rare chromosomal abnormality (trisomy 8)

A 37-year-old woman elects amniocentesis. The karyotype of cultured amniotic fluid cells is 46,XX. One colony contained two cells with a 47,XX, +8 karyotype. Only cells with normal karyotypes were recovered from a large number of other colonies which were examined.

Two cells with trisomy 8 from a single colony were recovered in an otherwise normal study. This is level II mosaicism (multiple cell pseudomosaicism).

Follow-up of pregnancies in which abnormal amniotic fluid cells are restricted to a single colony or culture vessel do not demonstrate an increased risk of birth defects above the background risk. These studies have significant limitations because they report the experience of level I and level II mosaicism for all chromosomal abnormalities. There is a paucity of follow-up data about mosaicism for specific chromosomes. Thus, the published studies, while reassuring, do not exclude the possibility of a small increased risk of abnormalities associated with underlying mosaicism in the fetus. Furthermore, if detrimental effects on fetal development are present, they may not be recognized at birth. There are numerous anecdotal reports of level I and level II mosaicism being associated with underlying mosaicism in the fetus or liveborn, and, at times, with adverse effects on development.

Especially for rare trisomies, e.g., trisomy 8, it is possible that mosaicism for the abnormal cell line is restricted to only certain fetal tissues and is not present...
In the setting of only two cells with trisomy 8 in an otherwise normal amniotic fluid cell study, the magnitude of risk is unknown but may be higher than for other trisomies. There are a few case reports of babies diagnosed with trisomy 8 mosaicism syndrome for whom there was only a single cell or even no abnormal cell in amniocyte culture. These reports indicate that trisomy 8 cells may not enter or survive in amniotic fluid as readily as other types of cells. The abnormalities identified in the children with trisomy 8 mosaicism but normal amniocyte karyotypes include mental retardation, cleft palate, and ophthalmologic, cardiac and renal abnormalities. Trisomy 8 mosaicism is also associated with an increased risk of hematopoietic cell malignancies.

Further testing of the pregnancy to determine whether the fetus has trisomy 8 mosaicism is complicated by the knowledge that in affected liveborns the abnormal cell line is often not present in lymphocytes. Therefore, normal results of fetal blood sampling may not be informative with respect to the possibility of underlying trisomy 8 mosaicism although studies of interphase analysis of blood cells by FISH have not been reported. Fetal skin biopsy may have the best chance of detecting trisomy 8 mosaicism if the fetus is truly mosaic. Any further cytogenetic studies should use FISH on interphase cells as well as standard metaphase analysis. If further extensive cytogenetic studies are performed and normal, there may still be a small chance of underlying trisomy 8 mosaicism in the fetus that is higher than for other chromosomes. Abnormal phenotypes have not been identified with uniparental disomy for chromosome 8.

Detailed ultrasonographic imaging including fetal echocardiography is also recommended. However, normal fetal imaging should not be used to conclude that the risk of clinically underlying mosaicism in the fetus is negligible. Karyotypically abnormal cells can have major deleterious effects on organ functioning without gross anatomic defects being present, with the brain being the most vulnerable organ. After birth, chromosome studies can be pursued from both blood and solid tissues.

General discussion on prenatally diagnosed chromosomal mosaicism

Further invasive testing of the pregnancy to provide more information about the fetal karyotype exposes the pregnancy to the risks of those procedures. Thus, whether to proceed with further invasive testing must be balanced against the likelihood of finding clinically important results that are related to underlying mosaicism in the fetus. While level III mosaicism for a numerical chromosomal abnormality raises significant concerns about underlying fetal pathology, assessing the risk of abnormalities in a fetus in the setting of level I and level II mosaicism is usually much more problematic. In experienced hands, the risk of pregnancy loss associated with fetal blood sampling and fetal skin sampling is about 1% and 3%, respectively. How to balance these procedure-related risks and the limited information they provide with the uncertain risk of fetal disease is one of the challenges faced by clinicians counseling about prenatally diagnosed mosaicism.

The decision to proceed with further invasive testing of the pregnancy should also be influenced by whether the results, normal or abnormal, will allow revision of prognosis for the fetus. For example, in the setting of level III mosaicism in amniotic fluid for the sex chromosome abnormalities, 47,XXX, 47,XXY, and 47,XYY, recovery of the abnormal cell line in fetal blood would not usually change the uncertainties of prognosis. In the setting of level III mosaicism for 47,XXX (46,XY/47,XXX), the presence of a significant fraction of chromosomally normal cells would be expected to moderate the expression of Klinefelter syndrome and the likelihood for near normal development is increased. However, important aspects of the expression of the symptoms of Klinefelter syndrome depend on the percentage and location of the cells with the extra X chromosome, e.g., brain and testes, information that is not available either prenatally or after birth, regardless of whether 47,XXX cells are recovered in a fetal blood sample.

Mosaicism for a 45,X cell line, either 45,X/46,XX or 45,X/46,XY, has a wide phenotypic spectrum. For 45,X/46,XX mosaicism, there is a phenotypic continuum ranging from the common findings of Turner syndrome to a normal or near normal female. Similarly, males with 45,X/46,XY mosaicism may have varying degrees of ambiguous genitalia or abnormal male genitalia of variable severity, structural abnormalities of the heart and kidneys seen in Turner syndrome, or have a normal or near normal male phenotype. Retrospective data collected about individuals diagnosed postnatally with 45,X/46,XY mosaicism include mainly those individuals who come to medical attention because of structural and/or functional abnormalities and thus cannot be used to provide prognostic information prospectively.
when the diagnosis of 45,X/46,XY mosaicism is made prenatally.

Predictions about adverse effects on development when 45,X/46,XY mosaicism is present in chorionic villi or amniocytes can be best made by detailed ultrasonographic examination. If fetal anatomy appears normal and the fetus has normally formed male genitalia, there is a high probability (>95%) of a normal appearing baby at birth although there is a presumed increased risk of abnormal gonadal histology and germ cell tumors. Unfortunately, information about pubertal development, fertility, and risks of malignancy are not yet available for phenotypically normal boys who have been prenatally diagnosed with 45,X/46,XY mosaicism. Because of the possible increased risk of germ cell tumors in this group of boys, careful surveillance is indicated.

For prenatally diagnosed 45,X/46,XX mosaicism, if ultrasonographic examination shows absence of increased nuchal thickening, cystic hygroma, and heart and renal malformations, the risks of lymphatic abnormalities and other structural birth defects often seen in Turner syndrome will be small, but not eliminated. The risks of short stature and ovarian failure are increased but their degree cannot be predicted; there may be gonadal dysgenesis typical of Turner syndrome, less severe effects on ovarian function and fertility, or no apparent effects at all of the 45,X cell line.

In summary, when 45,X/46,XX and 45,X/46,XY mosaicism is detected in chorionic villi or amniotic fluid cells, the recovery of 45,X cells from a fetal tissue is unlikely to add information above that obtained from ultrasonographic examination. Mosaicism for a 45,X cell line can be explored after birth by chromosome studies of peripheral or cord blood cells, skin, including foreskin, or buccal mucosa.

Further reading

16 http://mosaicism.cfri.ca/index.htm
Chromosomal mosaicism – postnatal diagnosis

A 27-year-old woman and her husband are referred for genetic counseling because her peripheral blood karyotype obtained after the woman’s second early miscarriage showed mosaicism for a 45,X cell line in 43% of her cells. The husband has a normal karyotype. The woman is 5’7” tall, has no external stigmata of Turner syndrome and has had normal renal and cardiac ultrasonographic examinations. She has a normal follicle stimulating hormone level.

Individuals with Turner syndrome mosaicism may have no signs of the syndrome, may have some manifestations of Turner syndrome, or may have all of the problems typically associated with non-mosaic Turner syndrome. The wide range of severity of findings in Turner syndrome mosaicism reflects the percentage and distribution of cells missing an X chromosome in various tissues. Individuals with Turner syndrome mosaicism who have functioning ovaries are at increased risk for premature ovarian failure. It is not possible to predict whether or when this might occur.

If the woman is able to conceive another pregnancy, she would be at increased risk for conceiving an embryo which is missing an X chromosome; almost all of these embryos would not survive beyond a few weeks’ gestation. Thus, individuals with Turner syndrome mosaicism often have an increased risk of early pregnancy loss. There are conflicting data about whether she would also have an increased risk for conceptuses with other chromosomal abnormalities. If the woman can successfully conceive and carry a pregnancy through the end of the first trimester, there is a good chance of a normal outcome.

Prenatal diagnosis by chorionic villus sampling or amniocentesis could be performed to address the risk of chromosomal abnormalities. If the woman wishes to avoid an invasive procedure, first and second trimester serum screening and ultrasonography would also be reasonable approaches to provide information about the risk of common chromosomal abnormalities and other birth defects. Most fetuses with Turner syndrome would have visible stigmata on ultrasonographic examination.

Whether to recommend in vitro fertilization with preimplantation genetic diagnosis to increase the chance of implantation of a chromosomally normal embryo is unclear for this woman’s situation. Her risk of fetal chromosomal abnormalities is unknown although the risk is probably increased over her age-related risks.

In the event that the woman experiences premature ovarian failure, pregnancy could be achieved via in vitro fertilization using an egg donated by another woman.

Further reading


Sex discrepancies

| Case 1 | A 38-year-old woman elects amniocentesis based on maternal age. First and second trimester aneuploidy screening had not been performed. The amniocyte karyotype was 46,XY and there was a normal amniotic fluid alpha-fetoprotein concentration. A follow-up ultrasonographic examination at 20 weeks’ gestation revealed female-appearing genitalia. |

There are a number of explanations for a discrepancy between the chromosomal and phenotypic sex in this case.
- **Errors in ultrasonographic designation of the fetal sex.** At this gestational age, ultrasonographic anatomy is almost always correct in gender identification but circumstances such as ambiguous genitalia or micropenis and bifid scrotum might lead to the impression of female external genitalia when, in fact, some other conclusion would be made on direct visual examination.
- **Misidentified laboratory specimens.** Prevention of sample mix-ups should be of highest priority for any prenatal diagnosis program. Confirming proper sample labeling with the patient immediately following an invasive procedure and meticulous and redundant laboratory procedures are crucial components to preventing sample mix-ups. However, despite the most careful safeguards, occasional mistakes occur. Large studies of sample identification errors in clinical laboratory testing, indicate that the frequency of mislabeled
samples ranges widely among different laboratories around the world.

- **Fetal pathology.** Fetal pathology must be considered as a possibility in this circumstance.

Another amniocentesis was recommended to confirm the chromosomal sex.

The karyotype of amniotic fluid cells obtained from the second amniocentesis was 46,XY, confirming the results of the first amniocentesis, allowing the possibility of a misidentified sample to be dismissed.

In this situation there is discordance between the female external appearance of the fetus and the 46,XY karyotype, and the differential diagnosis includes several entities. These would include SRY mutations or deletions, androgen receptor mutations and other causes of androgen insensitivity, Smith–Lemli–Opitz syndrome, early errors in steroidogenesis, gonadal dysgenesis, several autosomal chromosome deletions or duplications, SOX9 mutations that would usually show findings of campomelic dysplasia, Leydig cell hypoplasia, DAX1 duplications at Xp21, true hermaphroditism, Denys–Drash syndrome, Wilms tumor syndromes, and others. Many of these are uncommon causes of the key findings and none of them makes up a large fraction of the differential.

Further diagnostic studies could include comparative genomic hybridization for chromosomal deletions and duplications and androgen receptor gene sequencing, which finds most autosomal recessive mutations in that gene. These molecular tests utilize DNA obtained from available cultured amniocytes, repeat amniocentesis, or placental biopsy. Measurement of 7-dehydrocholesterol, the abnormal metabolite diagnostic of Smith–Lemli–Opitz syndrome, could be performed on amniotic fluid supernatant. Further anatomic information might be possible with additional ultrasonographic examinations over subsequent weeks.

The patient had another detailed ultrasonographic examination and fetal echocardiography which revealed a complete atrioventricular canal defect and long bone measurements which lagged behind predicted gestational age.

When the fetus is a chromosomal male, a discrepancy between the chromosomal and phenotypic sex, regardless of the presence or absence of other birth defects, should prompt strong consideration of Smith–Lemli–Opitz syndrome. Smith–Lemli–Opitz syndrome is an autosomal recessive disorder of cholesterol biosynthesis caused by a deficiency of 7-dehydrocholesterol reductase with an incidence in northern Europeans as high as 1 in 10 000. It is characterized by prenatal and postnatal growth retardation, ambiguous genitalia in males, microcephaly, moderate to severe mental retardation, and multiple major and minor malformations. Affected individuals have markedly elevated levels of 7-dehydrocholesterol and may have hypocholesterolemia. The prenatal diagnosis of Smith–Lemli–Opitz syndrome is accomplished by measurement of 7-dehydrocholesterol in amniotic fluid supernatant or chorionic villus cells.

In this patient’s pregnancy, 7-dehydrocholesterol was assayed in frozen amniotic fluid supernatant from both amniocenteses and was markedly elevated, confirming the diagnosis of Smith–Lemli–Opitz syndrome. Establishing a definitive diagnosis allowed for the provision of accurate information about prognosis, risk of recurrence, and the availability of early prenatal diagnosis in future pregnancies by measurement of 7-dehydrocholesterol in chorionic villus cells. Mutations in only one gene, DHCR7, are known to cause Smith–Lemli–Opitz syndrome. If the disease-causing mutation(s) are identified in this conception, preimplantation genetic diagnosis and DNA-based diagnosis using chorionic villus or amniotic fluid cells would also be possible in future pregnancies.

**Case 2** A 41-year-old primiparous woman elected chorionic villus sampling at 11 weeks' gestation. The karyotype from cultured chorionic villus cells was 46,XX. Detailed ultrasonographic examination was performed at 18 weeks' gestation and revealed normal appearing fetal anatomy. The fetal genitalia appeared male and this was confirmed on a subsequent ultrasonogram.

There are a number of explanations for a discrepancy between the chromosomal and phenotypic sex in this case.

- **Errors in ultrasonographic designation of the fetal sex.** At this gestational age, ultrasonographic anatomy is almost always correct in gender identification.
- **Misidentified laboratory specimens.** Despite the most careful safeguards, sample error can still occur at the time of the procedure or in the laboratory, as discussed in the previous case of XY genotype and female phenotype.
- **Maternal cell contamination.** Unrecognized maternal cell contamination is a negligible concern when villi are
expertly dissected and harvested for cytogenetic analysis after a few days in culture.

- **Fetal pathology.** Fetal pathology must be considered as a possibility in this circumstance.

  Amniocentesis was recommended to confirm the chromosomal sex.

  *The karyotype of amniotic fluid cells obtained from amniotic fluid cells was 46,XX, confirming the results of chorionic villus sampling, dismissing the possibility of a misidentified laboratory specimen as an explanation.*

The most likely explanations for the discrepancy between the fetal karyotype and phenotype include:

1. a male fetus with the SRY gene that encodes the testis-determining factor. This may result from a translocation between the SRY gene from a Y chromosome and the pseudoautosomal region of the X chromosome, or translocation between the SRY gene and an autosome; and
2. a female fetus with excessive androgen synthesis due to an enzyme deficiency in the pathways of steroidogenesis. These enzyme deficiencies all have autosomal recessive inheritance. The most common is 21-hydroxylase deficiency which has a high incidence in Ashkenazi Jews and is caused by mutations in the CYP21A2 gene. In 46,XX females of any ethnic group, genetic disorders associated with excessive androgen production are a common cause of virilization. In contrast, in 46,XY males, abnormalities in the androgen synthesis pathway are a rare cause of sexual ambiguity.

  Further diagnostic studies could include fluorescence in situ hybridization (FISH) analysis of DNA from cultured amniocytes to look for the presence of the SRY gene. Results of FISH analysis are usually available within one to two days.

  If FISH analysis of DNA obtained from amniotic fluid cells were negative for the SRY gene, mutation analysis of the parents’ 21-hydroxylase genes could be undertaken. Mutation analysis has a greater than 95% sensitivity in the detection of disease-causing mutations but may take several weeks to accomplish. If both parents have identifiable mutations, the a priori risk of 21-hydroxylase deficiency in the fetus would be 25% and definitive testing of DNA obtained from cultured amniocytes to confirm 21-hydroxylase deficiency as the explanation of the virilized fetus could be accomplished. If neither or only one parent has an identifiable mutation in their 21-hydroxylase gene, the chance that 21-hydroxylase deficiency is the explanation for the fetal problems would be small.

There are major limitations to prenatal diagnosis of other disorders of steroidogenesis that could result in a virilized female fetus. Other genes coding for steroidogenic enzymes not involved in androgen biosynthesis, such as P450aro, must also be considered, as mutations in these can result in overproduction of androgens, resulting in disorders of sex determination. All are rare conditions. Enzyme testing of amniotic fluid is not available because these enzymes are not expressed in amniotic fluid cells or present in amniotic fluid supernatant. Unfortunately, mutation testing of known genes is not likely to be practical.

*In this patient’s pregnancy, FISH analysis of amniotic fluid cells identified the presence of the SRY gene, the sex-determining region of the Y chromosome. This occurred presumably due to an abnormal X-Y or Y-autosome interchange during spermatogenesis in the patient’s husband who has a normal (46,XY) karyotype. He has three children (two daughters and one son) from a previous marriage. The X-Y or Y-autosome interchange arose as a de novo event in the pregnancy and has a low risk of recurrence in future conceptions.*

**Further reading**


