Prenatal Diagnosis: Cases & Clinical Challenges

Miriam S. DiMaio
MSW
Senior Genetic Counselor
Department of Genetics
Yale University School of Medicine
New Haven, CT
USA

Joyce E. Fox
MD
Chief, Division of Medical Genetics
Schneider Children's Hospital
Professor of Clinical Pediatrics
Albert Einstein College of Medicine
New Hyde Park, NY
USA

Maurice J. Mahoney
MD, JD
Professor of Genetics, Pediatrics, Obstetrics,
Gynecology and Reproductive Sciences
Department of Genetics
Yale University School of Medicine
New Haven, CT
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Preface

The advent of mid-trimester amniocentesis in the mid-1960s initiated the era of prenatal diagnosis, a new window into fetal development, health and disease. At that time, the molecular basis of almost all Mendelian disorders was unknown, and few genetic disorders could be tested for prenatally. Initially, fetal diagnosis was largely restricted to chromosomal abnormalities, the few single gene disorders for which molecular or biochemical testing could be performed on amniocytes or amniotic fluid supernatant, and fetal abnormalities that could be identified by ultrasound examination. For some rare disorders, more invasive and riskier testing by fetal blood or skin sampling or fetoscopy could provide information about the fetus.

In the ensuing decades, the explosion of knowledge about the human genome and the molecular pathogenesis of many human diseases, the availability of rapid and highly accurate molecular diagnostic techniques, and the refinement of ultrasound imaging techniques have transformed the field of prenatal diagnosis. Furthermore, maternal serum analyte testing and carrier screening for genetic disorders based on ethnic background, family history or population risk have improved our ability to identify women who are appropriate candidates for diagnostic testing. Next on the horizon will be the diagnosis of fetal disease states using fetal nucleic acids (RNA or DNA) recovered from the maternal circulation. This will markedly alter the current state of prenatal diagnosis and will probably supplant many of our current approaches.

The rapid advances in understanding the molecular basis of human disease have also revealed genetic complexities and mechanisms that were only postulated or even unimagined a generation ago. We now recognize that for some disorders, different mutations in a single gene can result in markedly disparate clinical presentations. Such disorders, once defined by narrow clinical criteria, are now known to have remarkable variation in their manifestations and age of onset depending on the nature of specific mutation(s) in a single gene. Conversely, the same or similar clinical phenotype can result from mutations in more than one gene. In addition, non-Mendelian mechanisms such as uniparental disomy, trinucleotide repeat expansions, and epigenetic phenomenona such as imprinting add another level of complexity when considering an underlying diagnosis.

A problem that often complicates counseling in prenatal diagnosis is the difficulty of making precise predictions about the severity of a disorder that has been diagnosed in utero. This is most common when chromosomal mosaicism is diagnosed in chorionic villi or amniotic fluid and where the possible outcomes range from a disabling condition to normal or near normal. Counseling is also difficult for disorders which have highly variable severity among members of the same family, are of mid-life onset, have a wide range in age of onset, or have reduced penetrance.

For some fetal abnormalities diagnosed on ultrasound examination, there is insufficient information to establish a diagnosis. Questions about the etiology of the fetal abnormalities and their recurrence in subsequent children may have to be resolved after delivery following examination of the baby or by the results of pathological examination that allow a more focused approach to molecular or other testing. Sometimes, however, an underlying diagnosis will not be established, and providing precise information about risk of recurrence is not possible. Empiric data may be available and provide some guidance. Such data, however, reflect the experience of many families and represent an average risk with some families having a much higher or lower risk.

Exposure to common and unusual clinical problems in prenatal diagnosis should be an integral component in the training of obstetricians, medical geneticists, and genetic counselors. A major shortcoming of such training is that the clinical experience is usually limited to a short period of time in which few complex cases will arise. Physicians and genetic counselors in training are therefore not exposed to the broad range of diagnostic problems and dilemmas that occur in the field of prenatal diagnosis, and they finish their training programs with only superficial clinical exposure. We hope that this book will serve as a supplement to clinical training in the field of prenatal diagnosis.

This book is a product of our own clinical experience over several decades. We have used cases from our own practice and from colleagues elsewhere, some of which have been modified, and present them as vignettes to portray diagnostic problems in prenatal diagnosis. We recognize that our case material reflects predominantly the experience of prenatal diagnosis in the United States and Canada and that medical centers in other parts of the world may have a different experience. Our presentations also reflect, to some degree, protocols that have been developed at our own medical centers.

The format of the book includes a brief synopsis of each case followed by a discussion of the problem, an explanation of the underlying biology, the available testing options, and the results that might be obtained. These cases illustrate approaches to management, including pedigree interpretation, probability, laboratory and technical analysis, and counseling. This book is not a comprehensive reference about prenatal diagnosis and is not intended to provide in-depth information about the genetic disorders that are discussed. In the interest of presenting cases in a straightforward way, our discussions may lack some of the complexities and nuances that would be found in more comprehensive sources. Some of the cases presented in the book include clinical situations or laboratory results that are rarely encountered in a general prenatal genetics practice. We have chosen to use these unusual cases because they illustrate important concepts about disease causation which have applicability to other more common problems in prenatal diagnosis. As we experience the rapid changes in laboratory methods of genetic diagnosis and in imaging technology, it is easy to predict that diagnostic approaches described herein will become outdated and replaced by newer methods.

The cases emphasize three types of clinical problems which are currently the primary focus of prenatal diagnosis: chromosomal abnormalities, Mendelian disorders, and fetal structural abnormalities that can be diagnosed by ultrasound examination. Multifactorial disorders, other than those associated with structural birth defects, are neglected because their etiology is, at present, not well understood. As our understanding of the molecular and other bases of this class of disorders increases, we anticipate that there will be interest in the prenatal diagnosis of severely disabling conditions.

We have not focused on the counseling aspects of prenatal testing and the psychological impact of abnormal test results. Whether to interrupt or continue a pregnancy is one of the most wrenching decisions that a couple can face. Recognition of the different choices that parents make when confronted with the same fetal disease state reinforces the importance of impartial and non-directive counseling after a diagnosis has been established.

There are excellent web-based resources that are available and provide comprehensive information about the field. Information about many of the genetic disorders which are discussed in this book were obtained from GeneTests, which is a web based medical genetics information resource for health care providers. GeneTests provides authoritative and comprehensive peer reviewed articles that are written by experts in the field and are updated frequently. GeneTests also contains a directory of clinical and research based genetics laboratories worldwide and the genetic disorders for which testing is available. Another indispensable web based resource is Online Mendelian Inheritance in Man (OMIM), an online catalog of Mendelian traits and disorders, now numbering over 12,000 that includes their clinical presentations and underlying molecular and biochemical bases.

1 Cytogenetic Abnormalities

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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*

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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 chromosome 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

1 Hook EB, Cross PK, Jackson L *et al.* (1988) Maternal age-specific rate of 47, + 21 and other cytogenetic abnormalities diagnosed in the first trimester of pregnancy in chorionic villus biopsy specimens: comparison with rates expected from observations at amniocentesis. *American Journal of Human Genetics* **42**(6):797–807.

2 Snijders RJM, Holzgreve W, Cuckle H *et al.* (1994) Maternal age-specific risk for trisomies at 9–14 weeks gestation. *Prenatal Diagnosis* **14**:543–552.

3 Snijders RJ, Sundberg K, Holzgreve W *et al.* (1999) Maternal age- and gestation-specific risk for trisomy 21. *Ultrasound in Obstetrics and Gynecology* **13**:167–170.

4 Warburton D, Dallaire L, Thangavelu M *et al*. (2004) Trisomy recurrence: a reconsideration based on North American data. *American Journal of Human Genetics* **75** (3):376–385.

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(3;7)(p13.1;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.

Scenario 1 *The father's peripheral blood karyotype appears identical to that of the fetus: [46,XY,t(3p13.1;7q31.2)].*

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.