Chapter 6

Developmental Disabilities

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Although the classic childhood phenotypes of many developmental disorders have been established for some time, only in the past decade have the genetic etiologies of some of these disorders been identified. Investigations of the molecular basis of these conditions have resulted in the identification of new genes, leading to insights into the function of new proteins and biochemical pathways. In addition, genetic mechanisms previously unknown in humans, such as genomic imprinting, uniparental disomy, expansion of trinucleotide repeats, and facilitation of deletions and duplications by low-copy repeats, were recognized as the causes of some of these conditions.

This chapter reviews the genetic etiologies of several developmental disorders, including the fragile X, Prader-Willi, Angelman, Rett, and Williams syndromes, and disorders due to cryptic unbalanced chromosome rearrangements. The molecular approaches being applied to the diagnosis of these disorders also are reviewed.

FRAGILE X SYNDROME

Molecular Basis

Named for its association with a chromosomal fragile site observed in many patients (*FRAXA* chromosomal locus Xq27.3), fragile X syndrome (FXS) is the most common cause of inherited mental retardation (MR). FXS results from loss or severe reduction of the protein FMRP, encoded by the *FMR1* (fragile X mental retardation) gene.[1] All patients with FXS have mutations in *FMR1*, as no mutations leading to FXS have been identified in other genes. Both males and females may be affected with FXS, but females are typically less severely affected. Thus, FXS is considered to be X-linked dominant with reduced penetrance in females.[2]

The *FMR1* gene encompasses 38 kilobases (kb) of genomic DNA and has 17 exons.[3] The major *FMR1* messenger RNA (mRNA) produced in most tissues is approximately 4 kb, although several protein isoforms are generated by alternative splicing toward the 3′ end of the mRNA in some tissues. While FMRP can be detected in the nucleus, the majority of the protein associates with translating ribosomes in the cytoplasm, where it acts as a negative translational regulator. FMRP also is known to have a role in neuronal synapse maturation and plasticity. Autopsy samples from FXS patients have shown failure of dendritic spines to assume a normal mature size, shape, and distribution.

The molecular genetics of *FMR1* are complex. A repeated trinucleotide sequence, composed primarily of CGG repeats, is located in the untranslated portion of exon 1, ending 69 base pairs upstream of the translational start. Nearly all mutations (>99%) resulting in FXS occur as instability of the trinucleotide repeat, leading to dramatic expansion of the repeat segment (>200 to a few thousand repeats) accompanied by aberrant hypermethylation of CpG dinucleotides within the gene (full mutations). Relatively rare deletions and point mutations in *FMR1* account for the remaining mutations found in patients with FXS. The mechanism of repeat instability in *FMR1* is believed to be DNA polymerase slippage during DNA replication. AGG repeats, spaced at about 10 repeat intervals within the CGG repeat segment, may mitigate potential repeat instability through disruption of higher-order molecular structures formed during DNA replication. These secondary structures contribute to polymerase slippage, and alleles that lack interrupting AGG repeats are at higher risk for expansion.

The *FMR1* repeat region is naturally polymorphic, with variation of the CGG repeats in normal (i.e., stably inherited) alleles ranging from 5 to 40 repeats, and the vast majority of individuals in the general population have 20 to 40 repeats. Intermediate repeat alleles containing 41 to 59 repeats occasionally have minor variations of a few repeats when transmitted from parent to child, producing no clinical consequences. However, in rare instances, transmission of intermediate alleles with 55 to 59 repeats may expand into pathological alleles.

Interestingly, FXS occurs strictly through maternal inheritance. Individuals with full mutations may inherit a

Table 6-1. Normal and Pathological *FMR1* Allele Types

Allele	Repeat Range	Methylation?
Normal	5–40	No
Intermediate	41–59	No
Premutation	60–~200	No
Full mutation	≥200	Yes
Methylation mosaic	≥200	Variable
Premutation/full mutation repeat size mosaic	Mixed premutation and full mutation	Full mutation may be methylated

similar allele from their mothers or, alternatively, their mothers may have a "premutation" allele. *FMR1* alleles with >60 repeats up to approximately 200 repeats are considered premutations because of potential instability. Individuals with premutations do not have typical characteristics associated with FXS but may transmit an unstable repeat, which undergoes extensive repeat expansion. When transmitted by fathers to their daughters, premutations are not dramatically unstable, and as a result full mutations never arise through paternal inheritance.

Hypermethylation of the *FMR1* promoter region, along with repeat expansion, results in decreased or completely absent transcription and the concomitant loss of FMRP. Patients with partial methylation of a full mutation (methylation mosaics) may have some FMRP expression, resulting in a less severe phenotype. In addition, patients with a mixture of cells having either a premutation or full mutation (premutation/full mutation size mosaics) frequently are identified during molecular testing. These patients usually have MR but may perform at the lower end of normal intellect (IQ > 70). Because methylation is not an all-or-none phenomenon within *FMR1*, the FXS phenotype may encompass a spectrum of possible affectations from mild to severe. Table 6-1 summarizes the classification of *FMR1* alleles.

Clinical Utility of Testing

Due to the presence of unrecognized *FMR1* alterations in unaffected, carrier parents, the first indication of FXS within a family is usually the diagnosis of an affected child. Unfortunately, many families do not learn the FXS diagnosis for long periods after first concerns about their child's development or behavior, and many have subsequent pregnancies before diagnosis for their first child. Such situations highlight the importance of early diagnosis so children and families can receive the benefits of genetic counseling and intervention services. Other than the infrequent deletion or point mutation, which often are spontaneous and not inherited from a parent, mothers of *all* FXS patients are either premutation or full-mutation carriers. In turn, at least one of the mother's parents has an *FMR1* alteration. Consequently, *FMR1* mutations may be present in siblings of an affected individual as well as other extended family members. It is important to remember that the daughters of unaffected males with a premutation (transmitting males) are also unaffected carriers, and that their offspring are at risk for FXS. Many families are known in which an *FMR1* mutation has been transmitted through numerous generations and into family branches unknown to one another.

For developmentally delayed children, *FMR1* molecular testing is diagnostic, as FXS affects development from infancy. However, the nonspecific nature of FXS during early development makes the testing approach one of ruling out FXS in most situations. The hallmark finding in almost all patients with FXS is MR, but the physical and behavioral features of males with FXS are variable prior to puberty. Physical features not readily recognizable in preschool-age boys become more obvious with age: long face, prominent forehead, large ears, prominent jaw, and enlarged testicles (macroorchidism). Motor milestones and speech are frequently delayed, and temperament often is affected (e.g., hyperactivity, hand flapping, hand biting, temper tantrums, and occasionally autism). Females with FXS usually have milder manifestations and as a result are more difficult to diagnose clinically. FXS always should be suspected in males with mild to moderate MR and females with mild MR until shown otherwise by negative *FMR1* analysis.

Women who are full-mutation or premutation carriers have a 50% risk of transmitting their abnormal allele in each pregnancy. While transmission of a full mutation always leads to a child with a full mutation, the risk of a premutation transmission resulting in an affected offspring with a full mutation is proportional to the maternal premutation repeat number. Empirically, the 50% risk of a female carrier producing an affected male child is reduced to 7% if the premutation contains 56 to 59 repeats, 10% for 60 to 69 repeats, 29% for 70 to 79 repeats, 36% for 80 to 89 repeats, and 47% for 90 to 99 repeats; it reaches the maximum, 50%, when a premutation has >100 repeats. Because females have approximately 50% penetrance, the risk for producing an affected female is half that of producing an affected male in any premutation repeat interval category.

Prenatal testing for *FMR1* mutations is available in many clinical molecular laboratories. Genomic DNA isolated from amniocytes obtained during amniocentesis at 16 to 18 weeks gestation or from chorionic villus sampling (CVS) at 10 to 12 weeks gestation can be used for testing. Prenatal molecular analysis proceeds in much the same fashion as that performed on DNA obtained from adult peripheral blood. However, the DNA analysis of CVS may be more complex, as chorionic villi are extraembryonic. Hypermethylation in CVS may be incomplete and not representative of the true *FMR1* methylation status in fetal tissues. Therefore, occasionally a follow-up amniocentesis may be required to resolve ambiguous CVS test results.

General population screening for *FMR1* mutations has been proposed but remains controversial. In comparison to

most disorders already screened in the newborn period, FXS is more prevalent and testing is highly reliable. However, the relatively high costs and the technical complexities of testing must be resolved before population screening is possible. Protein testing of FMRP may be useful for screening populations with MR.

Available Assays

Routine clinical testing for *FMR1* mutations includes molecular assessment of both the trinucleotide repeat number and the *FMR1* methylation status. Standard technical approaches include (1) double-digest Southern blot analysis using a methylation-sensitive restriction enzyme such as *Eag* I, *BssH* II, or *Nru* I along with a methylation-insensitive restriction enzyme such as *EcoR* I or *Hind* III[4] and (2) polymerase chain reaction (PCR) assays specific for the CGG repeat segment of *FMR1* (Figure 6-1).[5] Specialized fragile X chromosome analysis, using special culture techniques to induce fragile sites, is no longer used for diagnosis of FXS due to low sensitivity. While only a very few FXS patients with point mutations in *FMR1* have been identified, clinical molecular testing does not routinely investigate the gene for point mutations, deletions, insertions, or inversions downstream of the repeat segment.

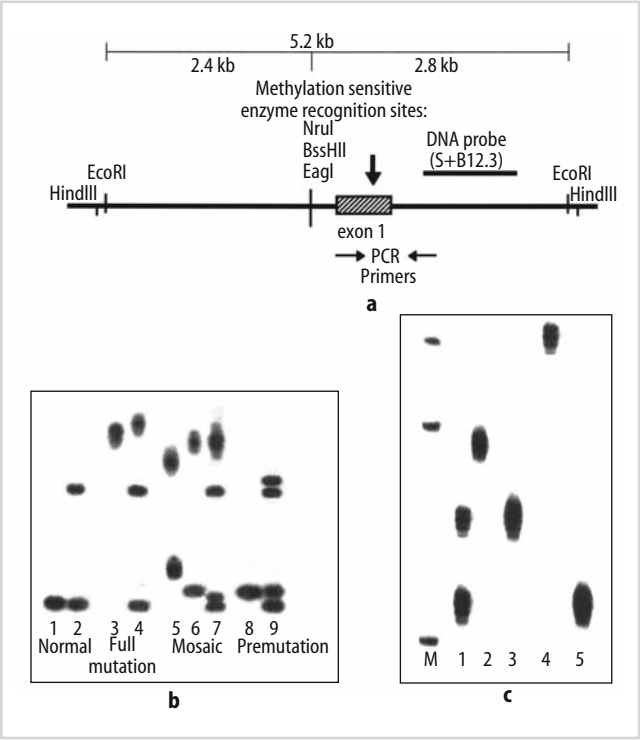

Figure 6-1. Repeat expansion and methylation in *FMR1*. (a) Restriction enzyme map of *FMR1*, with locations of restriction enzyme sites, DNA probe, and PCR primers used in molecular testing. Top line indicates DNA fragments generated using *EcoR* I and *Eag* I as depicted in the Southern blot analysis in Figure 6-1b (2.8 kb and 5.2 kb fragments are detected by the DNA probe StB12.3). The vertical arrow indicates the location of the CGG repeat in exon 1. (b) Southern blot analysis of *FMR1*. Only the 2.8 kb fragment is detected in normal males (lane 1), while both the 2.8 kb and 5.2 kb fragments are detected in normal females (lane 2) due to methylation associated with normal X inactivation. Completely methylated full mutations are depicted in lane 3 (affected male with full mutation) and lane 4 (affected female with full mutation contained on one of her X chromosomes; normal allele on her other X chromosome). Smeary signals occur due to variable repeat expansion within peripheral lymphocytes used for DNA isolation. Mosaic patterns are illustrated in lane 5 (male with partial methylation of full mutation), lane 6 (male with premutation/full mutation mosaicism), and lane 7 (female with premutation/full mutation mosaicism). Lane 8 illustrates a transmitting male with a premutation and lane 9 illustrates a female with a premutation. Both premutations contain approximately 75 repeats. (c) PCR analysis of *FMR1* repeats from five individuals separated on a 6% polyacrylamide gel. Lane 1 contains PCR products from a female with 20 and 30 repeats, respectively, contained within her two normal alleles. Lanes 2, 3, and 5 are males with normal repeat alleles (40, 30, and 20 repeats, respectively), while lane 4 illustrates a male with a 65-repeat premutation allele. Smeary signals result from DNA polymerase stuttering during the PCR amplification.

In most laboratory settings PCR is used to size normal and premutation alleles with typical sensitivity up to 120 to 150 repeats. PCR product yield is inversely proportional to the number of trinucleotide repeats such that little or no product can be obtained when larger repeats are present. Some PCR-based testing protocols may have higher sensitivity regarding detection of larger repeats, yet few laboratories have adopted these practices due to technical difficulties. When used in conjunction with PCR, Southern blot analysis provides a more complete inspection of the gene by detecting multiple possible molecular events, including repeat expansion, DNA methylation, and the relatively rare *FMR1* deletions around the trinucleotide repeat segment. Although it is not routinely performed in most clinical laboratory settings, a few laboratories utilize protein-based testing for FMRP. Since severity of the FXS phenotype appears to inversely correlate with FMRP expression, assessment of FMRP production in patients with methylation mosaicism may be a useful prognostic indicator of disease severity.[6]

Interpretation of Test Results

Because *FMR1* appears to be the only disease-causing gene for FXS, test specificity is 100%. Using both Southern blot analysis and PCR specific for *FMR1*, test sensitivity for repeat expansion can be estimated to be nearly 99%, as only rare point mutations, small deletions/insertions remote from the repeat segment, or gene inversions would be missed. However, these nonrepeat expansion molecular alterations may be underascertained in *FMR1* since gene regions downstream of the repeat segment in exon 1 are rarely investigated, even if repeat expansion is not present. As detected by testing, the presence of cellular mosaicism, in either repeats or methylation, presents potential problems for prediction of FXS severity. Essentially all patients with mutations resulting in reduction of FMRP are impaired, but expression of variable amounts of FMRP may allow some individuals to function at a higher level than expected. These individuals may occasionally have intelligence quotients that are not in the MR range. Prognostication of severity based on testing of a young child should be

predicated with great caution during genetic counseling, because no long-term study exists following the development of individuals with methylation mosaicism.

Laboratory Issues

Due to unusual complexity in *FMR1*, molecular testing should be performed by an experienced molecular pathologist. If the etiology of MR in an individual is unknown, DNA analysis for FXS should be performed as part of a comprehensive genetic evaluation that includes routine cytogenetic analysis. Cytogenetic abnormalities have been identified as frequently as or more frequently than *FMR1* mutations in individuals with MR who are referred for FXS testing. In addition, the use of Southern blotting on DNA isolated from amniocytes for prenatal *FMR1* analysis, with typical 2- to 3-week turnaround times, may lead to stressful situations on occasion regarding the timing of possible pregnancy termination. Utilization of CVS provides additional time for possible pregnancy termination, but equivocal results sometimes occur due to incomplete methylation when a full mutation is present.

PCR-based commercial kits used to estimate repeat copy number are available through some suppliers but not widely utilized. Many laboratories use laboratory-developed methods for both *FMR1* Southern blot analysis and PCR. Patient control cell lines may be purchased from the Coriell Institute (http://coriell.umdnj.edu/). Testing for FXS is routinely included in proficiency tests administered by the College of American Pathologists (CAP).

UNIPARENTAL DISOMY

Molecular Basis

Several developmental disorders arise not just from classical gene mutations but also from the effects brought to bear on gene expression by chromosomal aneuploidy. Aneuploidy occurs in a substantial percentage of all recognized pregnancies, yet most instances result in embryonic lethality with spontaneous abortion during early pregnancy. This high rate of observed aneuploidy suggests the existence of numerous abnormal gametes, either nullisomic or disomic for a particular chromosome, due to meiotic nondisjunction events. Considering the relatively high frequency of aneuploidies, Eric Engel in 1980 hypothesized the potential for rare "gametic complementation" between a gamete nullisomic for a particular chromosome and a gamete disomic for the same chromosome.[7] Although derived from two separate "mistakes," such a union would lead to an apparently normal (2 n or disomic) individual with inheritance of two copies of a chromosome pair (or a chromosomal segment) from one parent and no copy from the other parent, or uniparental disomy (UPD). Maternal UPD occurs when a child has two copies of one of the mother's chromosomes and no copies of that particular chromosome from the father. Paternal UPD occurs when a child inherits two copies of a specific chromosome from the father and no copies of that chromosome from the mother.

Uniparental disomy may or may not cause developmental problems, depending on which chromosome is involved. However, patients identified with UPD indicate that the possible clinical consequences include (1) expression of recessive disorders when only one parent carries a recessive trait, (2) disorders related to parent-of-origin effects (imprinted genes), and (3) residential effects of chromosome aneuploidy (mosaicism). The inheritance of two identical chromosomes, or isodisomy, may occur due to meiosis II nondisjunction, formation of isochromosomes through centromeric misdivision, or mitotic nondisjunction in a monosomic diploid cell. Isodisomy is of particular concern due to the potential expression of recessive disorders when one parent is a carrier of a recessive trait, and for imprinting disorders. Inheritance of two homologous, but nonidentical, chromosomes from one parent is termed heterodisomy, and occurs as a result of a meiosis I nondisjunction. The presence of heterodisomy raises concern related to expression of imprinting disorders.

Gametic complementation is one of several possible mechanisms producing UPD (Table 6-2). The most common mechanism leading to UPD appears to be trisomy rescue. Observations of mosaicism for normal and trisomic karyotypes confined to extraembryonic (placental) tissue obtained by CVS, or confined placental mosaicism (CPM), led to recognition of trisomy rescue. Upon later cytogenetic examination of fetal or neonatal tissue, this mosaicism is not detected and has resolved into an apparently normal disomy. A trisomy may be "rescued" by loss of one trisomic set member through nondisjunction, anaphase lag, or chromosome degradation mediated by centromeric loss. Because the chromosome loss is random, the incidence of UPD in a diploid fetus with known CPM is theoretically 1 in 3. Correction or rescue of a monosomic cell line may occur through early mitotic nondisjunction or endoreduplication of a whole chromosome in a monosomic conception. In addition, chromosomal transloca-

Table 6-2. Mechanisms Leading to UPD

Trisomy rescue
Monosomy rescue
Gametic complementation
Chromosomal translocation
• Centric fusions of acrocentric chromosomes
• Familial heterologous Robertsonian translocation
• Familial homologous Robertsonian translocation
• Heterologous de novo centric fusions
• Homologous de novo centric fusions
• Reciprocal balanced translocations
De novo somatic recombination
Pericentric and paracentric inversions in imprinted domains
Small marker chromosomes containing imprinted genes

Source: Reference 8.

tions, somatic recombination, inversions in imprinted domains, and marker chromosomes also may lead to UPD.

When Engel first conceptualized UPD, he calculated that perhaps 3 in 10,000 individuals have UPD for one of the chromosomes (15, 16, 21, 22, or the sex chromosomes) commonly observed in aneuploidy.[7] Immediately recognized was the potential consequence of isodisomy, resulting in duplication of recessive alleles from a single carrier parent. In 1988, the discovery of cystic fibrosis (CF) in a young girl with maternal UPD for chromosome 7 was the first report of UPD resulting in a recessive condition.[9] The girl's mother was a CF carrier but her father was not. Recessive conditions caused by UPD have been reported for UPD involving chromosomes 1, 2, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 16, and X (Table 6-3).

The later discovery of genomic imprinting revealed additional pathological consequences related to UPD.[10] While most genes are expressed from functional alleles derived from both parents, a small minority of genes are normally expressed only from one allele, either the maternal or paternal allele. This differential gene expression depending on the parent of origin results from imprinting, a process initiated in germinal tissue and maintained in somatic tissue by methylation of DNA. Imprinting may be tissue specific and is a normal process for regulating dosage of gene products when normal, biparental inheritance occurs. As a result of DNA methylation, control elements regulate expression of specific individual genes, or whole segments of chromosomes containing several genes, exclusively from either the maternal or paternal alleles. Uniparental disomy for chromosomes containing imprinted genes results in functional loss of gene expression even when no change to the DNA sequence has occurred. Although a small number of genes are affected, several disorders result from imprinting defects or loss of gene expression related to UPD of a chromosome containing imprinted genes (Table 6-4).

Table 6-3. Recessive Disorders Associated with UPD

Disorder	Chromosome	Parent of Origin
Junctional epidermolysis bullosa	1	M, P
Chédiak-Higashi syndrome	1	M
Pycnodysostosis	1	P
Congenital pain insensitivity with anhidrosis	1	P
5-alpha-reductase deficiency	2	P
Abetalipoproteinemia	4	M
Spinal muscular atrophy	5	P
Methylmalonic acidemia	6	P
21-hydroxylase deficiency	6	P
Complement deficiency	6	P
Cystic fibrosis	7	M
Osteogenesis imperfecta	7	M
Congenital chloride diarrhea	7	P
Lipoprotein lipase deficiency	8	P
Leigh syndrome	9	M
Cartilage hair hypoplasia	9	M
β-thalassemia	11	M
Retinoblastoma	13	P
Rod monochromacy	14	M
Bloom syndrome	15	M
α-thalassemia	16	P
Familial Mediterranean fever	16	P
Hemophilia	X	P
Duchenne muscular dystrophy	X	M

Sources: References 8, 10.
M, maternal; P, paternal.

Table 6-4. Disorders Associated with Imprinted Genes

Disorder/Phenotype	Gene(s)	Locus	Maternal or Paternal UPD
Transient neonatal diabetes mellitus	*IGF2R*	6q25–q27	Paternal
Russell-Silver syndrome	*PEG1/MEST*	7q32	Maternal
Beckwith-Wiedemann syndrome	*IGF2*, *H19*, *P57KIP2*, *KVLQT*	11p15	Paternal
Maternal UPD14 syndrome (precocious puberty/ short stature)	unknown	14	Maternal
Paternal UPD14 syndrome (abnormal thorax, short stature)	unknown	14	Paternal
Prader-Willi syndrome	*SNRPN*, *ZNF127*, *FZN127*, *IPW*, *NDN*, *PAR1*, *PAR5*	15q11–q13	Maternal
Angelman syndrome	*UBE3A*	15q11–q13	Paternal
Intrauterine growth retardation	unknown	16	Maternal

Sources: References 8, 10.

Clinical Utility of Testing

Molecular testing is valuable in uncovering UPD as an explanation for imprinting disorders and some recessive diseases. Testing is typically performed for diagnostic purposes on infants, children, or adults when UPD is suspected. Prenatal testing is indicated when confined placental mosaicism is detected. In addition to the clarity a diagnosis brings in most situations, genetic counseling and risk assessments for disorders caused by UPD are greatly affected because recurrence risks are quite different in UPD situations. The recurrence risk for UPD is negligible (<1%) except for that caused by the presence of balanced translocations carried by a parent (perhaps unknown to the parent). Recurrence risks in these situations may be as high as 25%. Uniparental disomy testing after an observation of confined placental mosaicism also

allows informed reproductive choices or may prepare parents for the birth of an affected child.

UPD involving most chromosomes does not cause obvious abnormalities related to imprinting defects, as only a few chromosomes (6, 7, 11, 14, and 15) are known to contain genes that undergo imprinting. Of these, maternally imprinted genes are present on chromosomes 7, 14, 15 and 16, while paternally imprinted genes are contained on chromosomes 6, 11, 14, and 15. Maternally imprinted genes also are suspected, but not confirmed, to be present on chromosomes 2 and 16. No cases are known of either maternal or paternal UPD for chromosomes 3, 12, 18, or 19. In addition, neither paternal UPD for chromosomes 4, 9, or 10, nor maternal UPD for chromosomes 5 or 11, has been reported. Paternal UPD for both the X and Y chromosomes together can only be heterodisomic, of paternal origin, and detected when a paternal X-linked recessive disease is inherited from the father. For many chromosomes, too few examples have been observed to determine a possible phenotype related to UPD.

Available Assays

When UPD is suspected, confirmatory testing is based on detection of polymorphic DNA markers in both parents and the child (Figure 6-2). The markers of choice typically are single nucleotide polymorphisms (SNPs), di-, tri-, or tetranucleotide repeat polymorphisms (short tandem repeats [STRs], microsatellites), or variable number of tandem repeats (VNTRs, minisatellites). These natural variants occur throughout the human genome, and characterized DNA markers are available for all chromosomes.

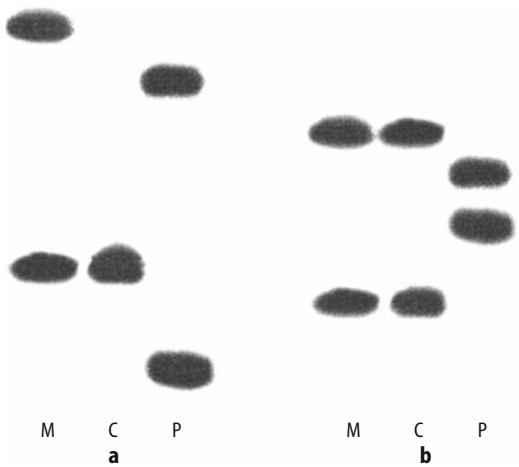

Figure 6-2. DNA marker analysis for isodisomy and heterodisomy. Polyacrylamide gel electrophoresis of a DNA marker indicating typical patterns found when maternal isodisomy (a) or heterodisomy (b) occurs. Parental patterns are opposite if paternal UPD is present (not shown). Autoradiographic signals were produced using PCR incorporating a radioactive label. Maternal DNA is designated as "M," paternal DNA as "P," and DNA from their child with UPD as "C." (a) Maternal isodisomy is indicated by the absence of paternal alleles and the presence of a single maternal allele in the child. (b) Maternal heterodisomy is indicated by the absence of paternal alleles and the presence of both maternal alleles in the child.

While DNA markers can be detected by several laboratory techniques, almost all use PCR amplification to produce sufficient material for analysis. Normal biparental inheritance will result in one of each parental allele for each DNA marker being present in the offspring. Uniparental disomy is present when no alleles are detected from one parent. When no paternal markers are detected, resulting from maternal UPD, paternity can be confirmed by using DNA markers from other chromosomes. Once a marker identifies UPD, additional markers in the chromosomal region can be used to fully explore potential segmental hetero- or isodisomy.

Interpretation of Test Results

DNA marker analysis has a sensitivity approaching 100%, if sufficient markers are informative. However, with the large number of available DNA markers for each chromosome, finding informative markers usually is only a matter of using higher marker density. Segmental disomy may potentially be missed if it involves a chromosomal region that coincidentally is not investigated. The specificity of DNA marker analysis should be 100%, assuming the markers have been correctly mapped to the appropriate chromosome.

Because meiosis I nondisjunction occurs more frequently than meiosis II nondisjunction, heterodisomy will be detected more often than isodisomy. The centromeric regions of heterodisomic homologues nearly always retain heterozygous regions but, crossing over in distal segments may result in homozygosity or heterozygosity. Therefore, informative DNA markers from the centromeric region will be heterozygous, but more distal markers may be heterozygous or homozygous due to recombination events. Isodisomy results in homozygous markers near the centromere, while more distal markers may be heterozygous (partial isodisomy) or homozygous (complete isodisomy). Isodisomy for one chromosomal segment and heterodisomy for another is not uncommon and results from meiotic recombination. In addition, UPD may be detected in only one chromosomal segment in juxtaposition to segments inherited biparentally due to mitotic recombination. Thus, it is important to study markers located near the centromere as well as more distal markers to fully clarify the origins of UPD.

Laboratory Issues

While UPD is readily detected by DNA marker analysis, both parents may not be available for testing. In such cases, analysis of one parent and the child still may be successful for identification of UPD for a particular chromosome. For example, if a mother-child pair is analyzed, the absence of maternal alleles suggests paternal UPD for a specific chromosome, while the converse situation is true with a father-

child pair. Markers from other chromosomes may be used to confirm parentage.

In addition to UPD, absence of DNA marker alleles inherited from a parent may arise as the result of submicroscopic chromosome deletion. Fluorescent in situ hybridization (FISH) analysis is used to confirm microscopic deletions in cases where multiple DNA markers from the same chromosome indicate an absence of marker contribution from one parent in one chromosomal segment yet the presence of normal biparental inheritance in another segment. Routine chromosome analysis (karyotyping) should be used to rule out large deletions as part of a full patient study.

Commercial kits are not available for DNA marker analysis of all chromosomes. However, oligonucleotide primers for amplification of marker loci easily can be synthesized and obtained from numerous companies. Depending on the disorder, cell lines and DNA from patients with specific UPD-associated disorders may be available from the Coriell Cell Repositories (http://coriell.umdnj.edu/). Although proficiency testing for Prader-Willi and Angelman syndromes is available, to date no proficiency test is available specifically to assess proficiency in UPD testing.

PRADER-WILLI AND ANGELMAN SYNDROMES

Molecular Basis

Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are neurodevelopmental disorders caused by a variety of genetic abnormalities involving the proximal part of the long arm of chromosome 15 (q11–q13). Within a 2 megabase (Mb) domain, gene expression depends on the parent of origin resulting from imprinting. Several genes defined above in the region (*SNRPN* [small nuclear ribonuclear protein N], *ZNF127*, *IPW*, *PAR1*, *PAR5*, *PW71*, *NECDIN*) are expressed exclusively from the paternal chromosome in all tissues studied, whereas another gene, *UBE3A* (ubiquitin-protein ligase E3A), is expressed only from the maternal chromosome in brain but has biparental expression in other tissues. Evidence suggests that PWS is caused by loss of expression of one or more of the C/D box small nucleolar RNAs (snoRNAs) encoded within the *SNRPN* locus.[11] On the other hand, AS results from loss of expression of *UBE3A*, which is involved in the ubiquitination pathway targeting certain proteins for degradation.[12] Imprinting of genes in this domain is coordinately controlled by a bipartite imprinting center (IC) overlapping the *SNRPN* promoter and extending 35 kb upstream. Flanking the imprinted region and approximately 4 Mb apart are several low-copy repeats derived from an ancestral *HERC2* gene and other sequences that predispose the region to chromosomal rearrangement by unequal crossing over.

All the genetic mechanisms identified in PWS interfere with the expression of paternally expressed genes in the 15q11–q13 domain.[13] Approximately 70% of PWS patients have a 4 Mb deletion of the paternal chromosome 15q11–13, which occurs sporadically through unequal crossing over. Maternal UPD for chromosome 15 accounts for about 20% of PWS and is usually the consequence of abnormal chromosome segregation during meiosis. In 1% to 5% of cases, microdeletions (6 to 200 kb) that include the IC or epigenetic changes in the IC occur and are inherited in an autosomal dominant manner. Such defects result in both maternal and paternal copies of genes in the imprinted domain having a maternal imprint and therefore lacking expression of the paternally inherited genes. Finally, about 1% of patients have translocations or other structural abnormality involving chromosome 15, which result in deletion or maternal UPD of the 15q11–q13 region.

Four different genetic mechanisms have been identified in 85% to 90% of AS patients, and all cause loss of *UBE3A* expression.[14] A 4 Mb deletion of the maternal chromosome 15q11–q13 region occurs sporadically in about 65% to 70% of cases due to unequal crossing over, as occurs in PWS. Paternal UPD has been detected in about 3% to 5% of patients and is also due to abnormal segregation of chromosome 15 in meiosis. Approximately 7% to 9% of AS patients have an imprinting defect that results from microdeletions of the IC or epigenetic changes in the IC. Such defects cause lack of expression of the maternally inherited *UBE3A* gene in brain because both maternal and paternal copies have a paternal imprint. Point mutations in the *UBE3A* gene (mostly truncating mutations) are found in 4% to 11% of AS patients.[15] Approximately 10% to 15% of patients with a clinical diagnosis of AS have no identifiable chromosomal or molecular abnormality. It is thought that these patients have some undetected abnormality that affects the *UBE3A* gene or have a mutation in another gene in the ubiquitination pathway.

Clinical Utility of Testing

Diagnostic Testing

Prader-Willi syndrome is a disorder with many manifestations related to hypothalamic insufficiency. The major features include infantile hypotonia, hypogonadism, dysmorphic appearance, small hands and feet, hyperphagia and obesity, developmental delay and MR, and characteristic behavior such as temper outbursts, rigidity, and repetitive thoughts and behavior. In infancy, the differential diagnosis includes neuromuscular disorders associated with hypotonia such as congenital myotonic dystrophy. The differential diagnosis in children and adults includes disorders with MR and obesity such as Bardet-Biedl, Cohen, and fragile X syndromes as well as acquired hypothalmic injury.

AS is characterized by microcephaly, gait ataxia, seizures, severe speech impairment, severe development delay or MR, and characteristic behavior, such as inappropriate

laughter and excitability. The differential diagnosis in infancy includes cerebral palsy, inborn errors of metabolism, mitochondrial encephalopathy, and Rett syndrome. Infants with AS sometimes have been clinically misdiagnosed as PWS because of hypotonia, feeding difficulties, and developmental delay, or because the distinctive features of AS are not apparent until later in development.

Prenatal Testing

The risk of recurrence and the type of prenatal testing vary according to the chromosome or molecular defect found in the proband. For this reason, prenatal diagnosis should be undertaken only after the genetic mechanism in the proband has been determined and the parents have received genetic counseling. A low risk of recurrence is associated with PWS and AS due to a deletion or UPD, if the parents' chromosomes are normal; however, prenatal testing may be offered for reassurance. Since mosaicism has been reported in mothers of AS patients with *UBE3A* mutations, prenatal testing should be offered even if the mother is negative for a mutation. Prenatal testing is also appropriate for families without a child with PWS or AS if a deletion of chromosome 15q11–q13 is suspected on chromosome analysis of CVS or amniotic fluid, if trisomy 15 is noted on CVS but a normal karyotype is found on amniotic fluid, and if a de novo chromosome 15 translocation or supernumerary marker chromosome is found by karyotyping.

Testing Parents and Other Family Members of a Proband

Parents of patients with deletions, specifically fathers of PWS patients and mothers of AS patients, should have chromosome and FISH analysis to determine whether they carry balanced subtle chromosome rearrangements or deletions not expressed as an abnormal phenotype in that parent. Chromosome analysis also is appropriate for parents of patients with UPD combined with a Robertsonian translocation to determine whether the translocation is inherited or de novo. Parents also should be tested for mutations that are identified in the proband such as IC deletions or *UBE3A* mutations. If a parent of a PWS or AS patient is a carrier of a mutation or a chromosomal translocation, then the siblings of that parent should be offered testing.

Available Assays

Clinical molecular testing for PWS and AS includes the molecular assessment of the parent-specific imprint within the 15q11–q13 region. Methylation, which is involved in the process of genomic imprinting, has been demonstrated for several of the genes in the imprinted domain, including *SNRPN*. Standard molecular techniques for the methylation analysis at the CpG island in exon α of the *SNRPN* gene include (1) double-digest Southern blot analysis using a methylation-sensitive enzyme such as *Not* I along with a methylation-insensitive enzyme such as *Xba* I,[16] (2) PCR following either *Not* I or McrBC digestion with primers for the *SNRPN* promoter, and (3) methylation-specific PCR based on modifying DNA with bisulfite, which converts all unmethylated cytosines to uracils, followed by amplification using primers specific for the unmethylated and methylated alleles.[17] The assessment of *SNRPN* expression by reverse transcription–PCR (RT-PCR) also may be used for the diagnosis of PWS.

For the purpose of genetic counseling, once the diagnosis of PWS or AS is established by abnormal methylation or *SNRPN* expression testing, further tests should be performed to determine the genetic mechanism responsible for the disorder. FISH of metaphase chromosomes using the probes *SNRPN* for PWS and either *SNRPN* or D15S10 for AS will detect the 4 Mb deletion in the majority of patients. For patients without a deletion, DNA marker analysis should be used to detect UPD. Using specimens from both parents and the affected child. Mutations of the IC account for the remaining PWS patients and some of the AS patients, and referral may be made to a research laboratory for further investigation. Patients with an AS phenotype but normal methylation should be assisted to have testing by a clinical laboratory offering mutation analysis of *UBE3A*.

Interpretation of Test Results

If the methylation pattern or methylation-specific amplification is characteristic of only maternal inheritance, then the diagnosis of PWS is confirmed. If the methylation pattern or methylation-specific amplification is characteristic of only paternal inheritance, then the diagnosis of AS is confirmed (Figure 6-3). Methylation assays detect all cases of PWS and AS caused by deletions, UPD, and IC defects; however, such assays will not detect the rare small deletions not involving the *SNRPN* locus or *UBE3A* mutations. While the methylation assays cannot differentiate among the various mechanisms producing PWS and AS, 99% of PWS patients and approximately 78% of AS patients will be detected. Approximately 50% of the remaining patients with an AS phenotype have mutations in *UBE3A*, which are identified by single strand conformation polymorphism (SSCP) analysis and sequencing.

Two different approaches for the laboratory diagnosis of PWS and AS may be used.[18] The first is to start with an analysis of parent-specific methylation of *SNRPN*. A pattern consistent with biparental inheritance rules out PWS and most cases of AS. If the diagnosis of PWS or AS is confirmed by methylation analysis, FISH and DNA marker analysis should be performed to determine whether the cause is a deletion, a UPD, or an IC defect. The second approach takes into account that deletions are the

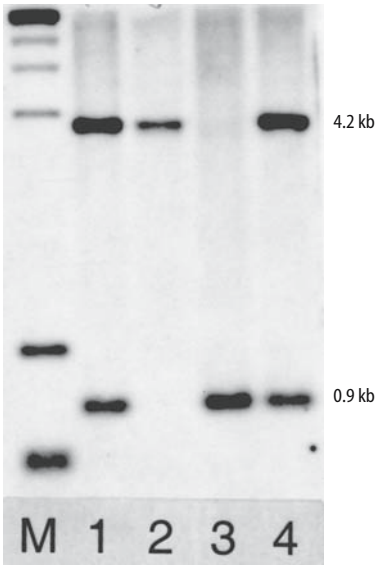

Figure 6-3. Southern blot analysis of PWS and AS. The methylated 4.2 kb (maternal) and the unmethylated 0.9 kb (paternal) fragments generated using *Xba* I and *Not* I are detected by the DNA probe *SNRPN*. Normal individuals exhibit a biparental inheritance pattern (lanes 1 and 4), whereas patients with PWS show a pattern of only maternal inheritance (lane 2) and patients with AS show a pattern of only paternal inheritance (lane 3).

most common cause of PWS and AS. It may be preferable to perform FISH analysis first if the patient is an older child or adult with classic features of PWS or AS. Methylation analysis is appropriate if a deletion is not detected. In the case of abnormal methylation, UPD studies using DNA markers should be performed to determine whether UPD is the cause. For either approach, if UPD is not present, then referral to a clinical laboratory for mutation analysis of *UBE3A* should be considered for patients with an AS phenotype. For patients suspected to have PWS, referral to a research laboratory for further molecular investigation of an IC defect should be considered.

Laboratory Issues

If the etiology of developmental delay or MR in a patient is unknown, DNA analysis should be performed as part of a comprehensive genetic evaluation that includes routine cytogenetic analysis (karyotyping). Cytogenetic abnormalities, including unbalanced translocations and deletions, have been identified in individuals referred for testing for PWS or AS. Although it is not necessary for every laboratory to provide all testing methodologies, a smooth progression through the various testing types may be necessary to determine the genetic mechanism causing PWS or AS, and is facilitated if the laboratory is able to perform at least DNA methylation, UPD, and FISH analyses. If sequencing of the *UBE3A* gene is necessary, testing may be referred to a clinical laboratory offering this test. Mutation analysis of the IC is available only on a research basis.

The *SNRPN* probe for Southern blot analysis is available from American Type Culture Collection (http://www.atcc.org). Many laboratories use laboratory-developed testing methods for methylation-sensitive PCR or RT-PCR analysis. Oligonucleotide primers for amplification of marker loci for UPD analysis can be synthesized or obtained commercially. FISH probes are available from two commercial sources (Cytocell, Inc, and Vysis, Inc). Patient control cell lines may be purchased from the Coriell Cell Repositories (http://coriell.umdnj.edu/). The proficiency tests for molecular genetics provided by the CAP include specimens for PWS and AS testing.

RETT SYNDROME

Molecular Basis

Rett syndrome is an X-linked neurodevelopmental disorder that almost exclusively affects females. In the classic form of the disease, affected girls appear to develop normally until the age of 6 to 18 months followed by a characteristic pattern of regression, which includes deceleration of head growth leading to acquired microcephaly, autistic features, loss of speech and purposeful hand use, irregular breathing patterns, stereotypical hand wringing, and seizures.[19] The frequency of classic Rett syndrome is approximately 1 in 15,000 females. Since the gene responsible for Rett syndrome was identified in 1999, however, a broader range of clinical phenotypes have been associated with mutation of this gene. The gene is *MECP2* on chromosome Xq28, which encodes the methyl-CpG-binding protein 2.[20] MECP2 binds preferentially to methylated DNA via its methyl-CpG-binding domain (MBD), and silences transcription by recruiting corepressor complexes through its transcriptional repression domain (TRD). Instead of serving as a global transcriptional repressor, as initially speculated, protein expression studies suggest that MECP2 may have a specialized role in neuronal maturation. The neuropathological findings in Rett syndrome of individuals with brains indicate that arrested neuronal development is an underlying feature of this disorder.[21]

Clinical Utility of Testing

Independent studies have confirmed *MECP2* as the major causative gene for Rett syndrome by the identification of multiple pathogenic mutations in approximately 95% of classic cases. Approximately 85% of classic Rett patients have point mutations and small rearrangements within the coding region; more recently, large gene rearrangements involving *MECP2* were identified in ~10% of classic cases.[22] Mutations also were found in atypical mild variant cases and in severe early-onset variant cases of Rett syndrome. *MECP2* mutations also have been identified in rare affected males with variable phenotypes ranging from neonatal-lethal encephalopathy and a Rett syndrome–like presentation in mosaic or Klinefelter males, to males with uncharacterized MR. In addition, *MECP2* mutations were

documented in patients with an AS-like presentation and in patients with autistic phenotypes. The variability in phenotypic severity observed in individuals carrying an *MECP2* mutation can result from allelic heterogeneity, as well as the X-inactivation pattern in females.[21] Given the spectrum of neurodevelopmental phenotypes associated with *MECP2* mutations, the clinical utility of molecular testing is significant.

Available Assays

The *MECP2* gene is composed of four exons, which give rise to two distinct MeCP2 isoforms.[23] Given the gene structure and mutation profile for *MECP2*, diagnostic testing for Rett syndrome is recommended to begin with analysis of the entire *MECP2* coding region (exons 1 through 4) by mutation scanning or DNA sequencing. One strategy for mutation scanning is denaturing high performance liquid chromatography (DHPLC), wherein sequence variants that give rise to heteroduplex DNA molecules can be detected with a high degree of sensitivity (95% to 100% under optimized conditions). The specificity of DHPLC is low, however, which requires that positive results be confirmed by DNA sequencing to identify the exact nucleotide change. The use of DHPLC coupled with sequencing has identified multiple recurrent and novel *MECP2* mutations of different types (missense, nonsense, splice-site, frameshifting deletions, and insertions).[24] Figure 6-4 shows representative data for DHPLC and DNA sequence analyses, demonstrating a heterozygous nonsense mutation in the *MECP2* gene (880C→T, R294X). This represents one of the more common truncating mutations seen in Rett syndrome patients. Sequencing is largely considered the gold standard for point mutation detection. Approximately 85% of classic Rett syndrome patients have mutations that are detectable by mutation scanning or sequencing of the *MECP2* gene, which is performed by clinical molecular laboratories (http://www.genetests.org/).

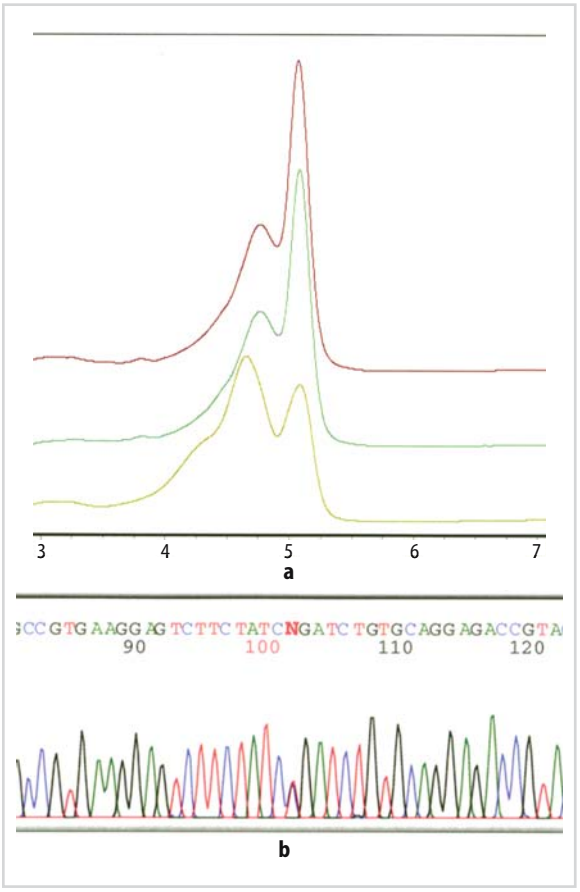

Figure 6-4. (a) Identification of a heterozygous sequence variation in the *MECP2* gene by DHPLC analysis. Elution profiles of *MECP2* exon 4 PCR products corresponding to the wild-type (red and green) and the mutant (yellow) sequences are shown. (b) Identification of the corresponding *MECP2* nonsense mutation by DNA sequencing. A single base change of C to T at nucleotide 880 is identified (N), which predicts an arginine to stop substitution at residue 294 of the MeCP2 protein (880C→T, R294X).

To increase overall mutation detection for the *MECP2* gene, additional testing is available for large *MECP2* gene rearrangements, present in approximately 10% of classic Rett patients. Deletions, insertions, or duplications involving all or part of the *MECP2* gene have been identified by dosage-sensitive DNA testing methods. The classic Southern analysis method has been used to detect copy number differences corresponding to deletions or duplications of specific *MECP2* exons, with detection of associated junction fragments in some cases. Alternative methods for dosage-sensitive analysis of the *MECP2* gene include multiplex ligation-dependent probe amplification (MLPA; MCR Holland, the Netherlands) or quantitative real-time PCR analysis.

Laboratory Issues

One of the issues encountered in sequence-based clinical testing is the interpretation of novel sequence variations, particularly alleles of uncertain pathogenic significance. A valuable resource for laboratories and referring clinicians is provided by the American College of Medical Genetics (ACMG), which has issued recommended standards for interpretation of sequence variations (ACMG Laboratory Practice Committee Working Group 2000; policy statement available at http://www.acmg.net/).[25] Another issue pertaining to Rett syndrome DNA testing at the current time is the value of two-tier testing (e.g., sequencing followed by dosage analysis) to provide comprehensive mutation analysis of the *MECP2* gene. Identification of the mutation in the proband facilitates prenatal testing in subsequent pregnancies. The majority of *MECP2* point mutations are new mutations of paternal origin, with low recurrence risk. Prenatal testing is recommended for fetuses of mothers who are identified to carry a point mutation or large rearrangement in the *MECP2* gene. In cases where the mother is not a carrier, prenatal testing may be sought for parental reassurance due to rare reports of gonadal mosaicism. The current focus on point mutation analysis

within the coding region by sequence analysis does not rule out potential mutations in regulatory elements or other important noncoding regions of the *MECP2* gene. Ideally, barriers to increased uptake of *MECP2* clinical testing may be addressed by improvements in genotype-phenotype correlations, assay design, and clinical services. With the accumulation of additional genetic information and the development of improved DNA technologies, appropriate enhancements can be incorporated into clinical testing of the *MECP2* gene for Rett syndrome.

WILLIAMS SYNDROME

Molecular Basis of Disease

Williams syndrome (WS), also referred to as Williams-Beuren syndrome, is an autosomal dominant disorder that occurs in approximately 1 in 10,000 to 20,000 live births (Online Mendelian Inheritance in Man [OMIM; database online] #194050). Clinical diagnosis is based on the presence of characteristic dysmorphic facial features, growth delay, abnormalities of connective tissue, cardiovascular disease, developmental delay, an outgoing personality type, and a unique cognitive profile of specific mental strengths and weaknesses. A less-frequent finding is hypercalcemia in the first year of life.[26]

Approximately 95% to 99% of individuals with WS have complete deletion of one copy of the elastin gene (*ELN*) located in chromosome region 7q11.23.[26] The most common form of deletion in WS extends well beyond *ELN* in both the 3′ and 5′ directions and results in hemizygosity for genes contained in approximately 1.5 Mb of DNA. The common deletion segment is flanked by repetitive DNA sequences, or "duplicons," that serve as agents for chromosomal rearrangements. This is achieved by creating an opportunity for misalignment of the chromosome 7 homologs during meiotic pairing. Unequal recombination then may yield deletion and duplication products, and in this case, deletion of the 1.5 Mb region between the duplicons results in WS. Deletion also may occur as a result of intrachromosomal rearrangements, and the deletion may involve either the maternal or paternal chromosome 7. These repeats also mediate an inversion of this region, which may result in an increased risk for the WS-causing deletion.[27]

At least 17 genes have been identified in the 1.5 Mb deletion region;[27] however, there is clear evidence for only one of the genes in the pathogenesis of WS. Supravalvular aortic stenosis (SVAS) and peripheral pulmonary artery stenosis are the most common forms of cardiovascular disease in WS. Elastin contributes to the elasticity of tissues such as skin and arteries, and is a major component of the aorta. There is strong evidence that abnormal elastin production is responsible for the arterial disease of WS.[26] Point mutations and intragenic deletions of the elastin gene have been associated with an autosomal dominant form of SVAS. Humans and mouse models hemizygous for *ELN* show that reduced elastin production produces a compensatory increase in lamellar units in response to increased wall stress during development. This results in the obstructive vascular disease of SVAS.[28] Hemizygosity for *ELN* also may contribute to the other connective tissue abnormalities of WS, such as hernias, hoarse voice, joint laxity, and premature aging of the skin.[26]

Evidence for a role of other genes in the clinical aspects of WS is less clear. *LIMKI,* a tyrosine kinase that is expressed in the brain during embryogenesis, may be involved in the cognitive portion of the WS phenotype.[26,29]

Clinical Utility of Testing

Demonstration of deletion of the elastin gene is used to confirm a clinical diagnosis of WS. This deletion is not visible by routine or high-resolution chromosome analysis. Most cases occur sporadically; however, there have been rare cases of multiple affected siblings. Affected individuals have a 50% chance of passing the deletion on to their offspring, and the deletion can be detected prenatally.

Available Assays

FISH is the most commonly used testing method for diagnosis of WS. The analysis is performed with a DNA probe containing a full-length copy of the *ELN* gene. Metaphase chromosome preparations from cultured peripheral blood lymphocytes are hybridized with the probe, and the 7q11.23 region of both chromosomes 7 are analyzed for the presence or absence of a hybridization signal. The assay generally includes a second probe, either for the centromere or a more distal locus on chromosome 7, to serve as an internal hybridization control.

Quantitative PCR can be used to assay the *ELN* copy number; however, there is no significant clinical or cost benefit to PCR over FISH. Whenever available, FISH is the preferred method for assessing genomic copy number because of the value gained by assessing individual cells. FISH testing for WS is usually ordered in conjunction with karyotyping, as other chromosome abnormalities may be part of the differential diagnosis. Addition of FISH testing requires no additional specimen and can be performed on fixed cell pellets after karyotyping is completed. In addition, although it is extremely rare, there have been reports of visible chromosome rearrangements that may result in disruption of the WS region, that would not be detected by PCR.

Interpretation of Test Results

Approximately 95% to 99% of individuals with a WS phenotype have complete deletion of the *ELN* gene, and a FISH assay with the *ELN* gene probe will detect all of these cases.

FISH analysis will not detect the *ELN* mutations that are commonly found in nonsyndromic SVAS; thus, the specificity for the test with respect to WS is very high. Nevertheless, it is possible that a rare *ELN* deletion in an individual with SVAS but without WS may be detected by FISH. As in all genetic disorders, the laboratory result must be interpreted in the context of the individual's complete clinical presentation. Conversely, a negative result should suggest reevaluation of the clinical diagnosis of WS.

Laboratory Issues

The FISH test is performed by cytogenetics laboratories, which routinely process cultured cells. Probes for WS testing are commercially available (sources include Cytocell, Inc, and Vysis, Inc). These probe sets are classified by the US Food and Drug Administration (FDA) as analyte specific reagents. Recommendations for test validation methods have been established by the ACMG (http://www.acmg.net/). Proficiency testing for FISH analysis of microdeletion disorders, such as WS, is available through the CAP.

SUBTELOMERIC DISORDERS

Molecular Basis of Disease

Genetic imbalance caused by a chromosome abnormality frequently results in some form of developmental delay and one or more of the following: abnormal growth, dysmorphic facial features, and congenital abnormalities. Duplications or deletions of very small chromosomal segments are often difficult or impossible to detect by routine or high-resolution chromosome analysis. Though small relative to larger and thus more readily visible rearrangements, these imbalances nonetheless frequently result in an abnormal phenotype due to the resulting imbalance of multiple genes within the affected segment(s).

The subtelomeric region of each chromosome arm is located between the telomere, the functional chromosome cap, and more proximal chromosome-specific sequences.[30] Subtelomeric regions and their adjoining chromosome-specific areas are of particular clinical interest for several reasons. They are particularly gene-dense regions, and shared homology within these segments may facilitate relatively frequent recombination events that may result in loss or gain of genetic material.[31] This suggests that genetic imbalances in these regions may have considerable clinical consequences. Additionally, the majority of all chromosome abnormalities involve imbalance for, or movement of, a segment that contains a subtelomeric locus. Thus, testing panels have been designed to assess the genomic copy number of arm-specific loci within or near the subtelomeric regions of each chromosome for identification of imbalances that are not readily detected by standard chromosome analysis methods.

Clinical Utility of Testing

Because developmental delay is a frequent finding in chromosomal imbalance, the main focus for these assays has been in studies of individuals with idiopathic mental retardation (IMR). An average of 6% of individuals with IMR have an imbalance that involves one or more distal chromosomal segments.[32] Based on these data, subtelomeric testing is the next logical step following a normal routine or (ideally) high-resolution karyotyping study, for individuals whose phenotype suggests an underlying chromosomal etiology. Subtelomeric panel testing is not routinely used for prenatal diagnosis at this time due to constraints of the test methodologies and to potential limitations in test interpretation in the absence of a well-defined phenotype (see Interpretation of Test Results section below).

Available Assays

Telomere FISH tests of metaphase chromosome preparations are currently in use in clinical laboratories. The test is also referred to as a "telomere panel" or "telomere testing," as it is the telomeric or distal end of the chromosome that is assessed. The test utilizes a complete set of chromosome arm-specific probes for loci that are within several hundred kilobases of their respective telomeres.[33] The short arms of the five acrocentric chromosomes are not included since they contain only repetitive sequences, and imbalance of these regions has no clinical consequence. Regions of homology exist for the terminal ends of Xp and Yp, and also for Xq and Yq; therefore, one set of probes hybridizes to both sex chromosomes. This leaves a minimum number of 41 probes needed for complete analysis. The test uses standard FISH methodology in which two or more probes are grouped together into one reaction mixture by using different probe colors, and multiple hybridizations are carried out with each group of probes for one complete test (Figure 6-5).

Research surveys have used PCR analysis of genetic markers, such as VNTRs or STRs, on DNA samples. A disadvantage to this method is that it requires parental DNA samples, which are not always available. STR analysis is more amenable to automation than the FISH assay, and could ultimately be less costly; however, it is more likely to yield false-positive results because of the presence of sequence polymorphisms.[32] The FISH method is more labor-intensive and requires actively dividing cells, but can also determine the position of each signal. This has particular benefit in detecting reciprocal translocations, because a de novo balanced translocation could generate an abnormal phenotype through gene disruption.

A recently introduced testing method uses comparative genomic hybridization (CGH) with subtelomeric FISH probes in a microarray format. The microarray contains bound subtelomeric probes that are hybridized with patient

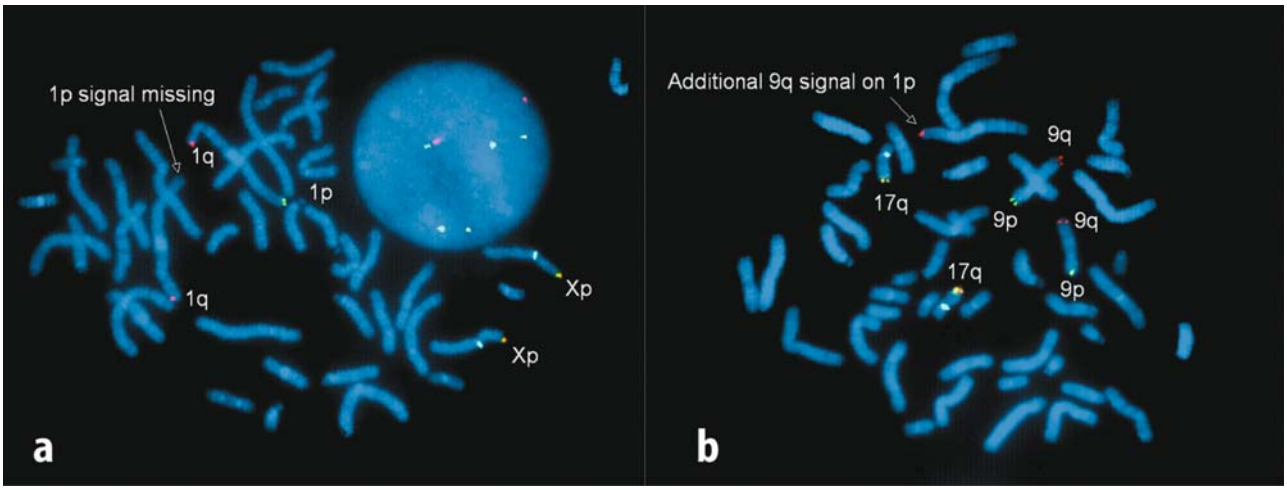

Figure 6-5. Subtelomeric FISH detection of abnormal chromosome 1, which is missing material from the distal short arm (1p) and contains additional material from distal 9q. (a) Hybridization mixture with probes for subtelomeric regions of 1p, 1q, Xp/Yp, and X centromere (control probe; aqua signals). Note absence of green signal on one 1p terminus. (b) Cell from the same individual after hybridization with probes for 9p, 9q, 17q, and 17 centromere (control probe; aqua signals). Note additional 9q signal on the end of 1p. The mother carries a reciprocal translocation between chromosomes 1 and 9. (Courtesy of Emory Genetics Laboratory, Atlanta, GA)

DNA labeled in one color, and a control DNA labeled in a second color. Significant deviation from a 1 : 1 hybridization ratio indicates an area of imbalance in the patient's DNA. All probes can be assessed on a single chip from one DNA specimen, without the need for cultured cells or parental samples, and with the potential for automation.

Interpretation of Test Results

FISH tests have had the widest usage in clinical laboratories. The individual probe sensitivities and specificities are generally very high. One caveat is that the exact origin of an imbalance that involves an additional Xp/Yp or Xq/Yq subtelomeric signal cannot be determined without additional analysis because of the shared homology within these regions. In general, the FISH test is able to detect any imbalance of a segment that contains a probe locus. Because this test has been in clinical use for only a few years, its level of specificity is less certain.

An abnormal result in a proband should ideally be confirmed by FISH analysis with a second probe for the same region. Follow-up performance of a targeted FISH analysis on parental chromosomes to determine whether the imbalance in the proband is due to an inherited rearrangement is very important for genetic counseling. A significant proportion of abnormalities detected by subtelomeric assays are familial in nature, and these cases may have significant risk for recurrence. Reports of families in which identical abnormal signal patterns have been found in a clinically abnormal proband and a clinically normal parent provide a note of caution for interpretation of results. Such findings may indicate locus polymorphisms, imbalances for which there are no obvious clinical significances, or imprinted areas in which the parent of origin determines the clinical consequence of the imbalance.

Some abnormalities detected by this assay fall into categories of well-described deletion or duplication syndromes. Other cases may represent unique situations in which the abnormality consists of a derivative chromosome containing imbalances from two different chromosomal segments. The clinical literature regarding the phenotypes associated with small subtelomeric imbalances is rapidly expanding; however, it must be remembered that this assay does not determine the size of the imbalance. Two individuals with identical abnormal hybridization patterns may have very different phenotypes due to differences in the extent of genetic imbalance.

Laboratory Issues

Two commercial sources for FISH probes are currently available (suppliers are Vysis, Inc, and Cytocell, Inc). A CGH-based microarray test system also is commercially available (Vysis, Inc). The FISH probe sets are classified by the FDA as analyte-specific reagents. Recommendations for FISH test validation methods have been established by the ACMG (http://www.acmg.net/). Proficiency testing is not available for subtelomeric FISH testing; however, the CAP does provide proficiency testing for other types of FISH assays.

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