
2 Nontraditional Inheritance

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SUMMARY

The “rules” of segregation of alleles originally defined by Gregor Mendel explained much of the phenomena associated with inheritance and have been dogmatically applied in the field of genetics. However, there are situations in which the rules of Mendelian inheritance cannot explain observed phenomena. A variety of molecular mechanisms have been identified that explain certain phenomena that are not easily explained by traditional Mendelian patterns of inheritance. These non-Mendelian mechanisms differ on a molecular basis, but can be described as a group by the term “nontraditional mechanisms of inheritance” or “nontraditional inheritance.” Stated simply, nontraditional inheritance refers to the pattern of inheritance of a trait or phenotype that occurs predictably, recurrently, and in some cases familially, but does not follow the rules of typical Mendelian autosomal or sex chromosome inheritance. Examples discussed in this chapter are the triplet repeat expansion mutations, and genomic disorders including genetic imprinting, mitochondrial inheritance, and multi-allelic inheritance.

Key Words: Angelman syndrome (AS); fragile X; Mendelian; mitochondrial inheritance; multifactorial inheritance; non-Mendelian; Prader–Willi syndrome (PWS).

INTRODUCTION

The “rules” of segregation of alleles originally defined by Gregor Mendel explained much of the phenomena associated with inheritance and have been dogmatically applied in the field of genetics. However, there are situations in which the rules of Mendelian inheritance cannot explain observed phenomena. A variety of concepts have been suggested to explain such phenomena, including the idea that individual genes may function in cooperation with each other and with environmental factors to produce a given phenotype. This concept of multifactorial inheritance is well accepted; however, specific examples for which the various factors can be well defined have been difficult to identify. Other natural phenomena, such as anticipation, in which genetic traits or disorders become more severe or pronounced in successive generations, or genetically determined conditions that appear to depend on the sex of the parent of origin of the involved chromosome, have been difficult to explain, even using concepts of multifactorial inheritance.

A variety of molecular mechanisms have been identified, which explain certain phenomena that are not easily explained by traditional Mendelian patterns of inheritance. These non-Mendelian mechanisms differ on a molecular basis, but can be described as a group by the term “nontraditional mechanisms of inheritance” or “nontraditional inheritance.” Stated simply, nontraditional inheritance refers to the pattern of inheritance of a trait or phenotype that occurs predictably, recurrently, and in some cases familially, but does not follow the rules of typical Mendelian autosomal or sex chromosome inheritance. Examples discussed in this chapter are the triplet repeat expansion mutations and genomic disorders including genetic imprinting, mitochondrial inheritance, and multi-allelic inheritance.

TRIPLET REPEAT EXPANSION

The first disorder identified as resulting from this form of nontraditional inheritance is fragile X syndrome (FRAXA). FRAXA is a well-recognized disorder that causes mental retardation, autistic-like behaviors, and a subtle, but characteristic, external phenotype in all males and many females possessing the mutation. Early studies confirmed that the locus of interest was on the X chromosome, and that in some cases the trait was associated with a cytogenetically visible fragile site on the X chromosome, seen only when cells were grown in a folic acid-deficient medium. The mental retardation syndrome was inherited in a classic pattern of X-linked inheritance, with carrier mothers who might have affected brothers or uncles passing the trait on to half of their male offspring. However, some unusual families caused confusion because of a pedigree pattern demonstrating what came to be known as the Sherman paradox. Specifically, there were families identified in which a male appeared to have passed the trait on to his daughters, but he himself was not affected, even though he might have affected brothers or uncles. This pattern could not be explained by typical X-linked inheritance.

The solution to the Sherman paradox became apparent when the molecular basis of the FRAXA was found to be a unique type of mutation that occurs in a region of repeated nucleotides in the genetic sequence. Specifically, in the FMR1 gene (*Xq27.3*) there is a repeated sequence of CGG nucleotides, a “triplet repeat” (Fig. 2-1). In normal individuals this sequence is repeated 5–44 times, but in an affected individual the sequence is repeated more than 200 times. Even more interesting, the mothers of the affected individuals were found to have triplet repeats with 60–200 copies. The normal allele is stably copied during the process of meiosis,

Table 2-1
Examples of Other Triplet Repeat Expansion Disorders

| <i>Disorder</i> | <i>Inheritance</i> | <i>Triplet sequence</i> | <i>Normal number of repeats</i> | <i>Number of repeats associated with disorder</i> |
|-------------------------------|--------------------|-------------------------|---------------------------------|---|
| Myotonic dystrophy | AD | CTG | 5–27 | >50 to >1000 |
| Huntington disease | AD | CAG | 9–37 | >37 |
| Spinocerebellar ataxia type I | AD | CAG | 19–38 | 40 to >80 |
| Friedreich ataxia | AR | GAA | 7–20 | >200 |
| Fragile X syndrome | XLR | CGG | 6–52 | >200 |
| X-linked spinobulbar atrophy | XLR | CAG | 19–25 | >40 |

AD, autosomal-dominant; AR, autosomal-recessive; XLR, X-linked-recessive.

repeated triplet of bases, and the number of repeats associated with the disease state. The molecular mechanism that causes disease is likely different, because some triplet repeat expansions are in coding regions of the gene (exons), some are in noncoding regions (introns), and others are completely outside of the gene, apparently affecting transcriptional regulation. In some cases, the triplet repeat expansion causes disease because it leads to the loss of function of the normal protein product. The triplet repeat expansion may also cause some new function or interaction, which has been shown to be the case in Huntington disease. Interestingly, the vast majority of disorders known to result from triplet repeat expansion are disorders of the neurological system, especially ataxias and other movement disorders.

This mechanistic heterogeneity extends also to the meiotic instability of the triplet repeat expansions. Some triplet repeats are more prone to expansion during female meiosis (e.g., fragile X and myotonic dystrophy) whereas others are more likely to expand when inherited from the father (e.g., Huntington disease). The FMR1 gene also has mitotic instability, so that there may be variation in the size of expansion in different cells and different tissues in the same individual. This is not a generalized trait of triplet repeat expansions, though, as it does not occur with the Huntington disease gene, *Huntingtin* (*4p16.3*).

Anticipation refers to an observed phenomenon where a genetic disorder appears to become more severe in successive generations, a condition not easily explained by simple Mendelian inheritance. For many years there was a controversy as to whether this observation was true, or was a result of ascertainment bias because mildly affected parents may only be identified if they have a more severely affected child. It is now known that anticipation does occur, at least in many triplet repeat expansion disorders, because of increasing size of the triplet repeat expansion in successive generations. In Huntington disease, the child of an affected father may present at a significantly earlier age than the father because of further expansion of the abnormal allele during male meiosis. A similar situation occurs with myotonic dystrophy, a disorder characterized by progressive weakness, especially in the distal extremities, associated with myotonia (difficulty relaxing a contracted muscle), cataracts, and frontal hair loss. A mildly affected mother, who may not know she has the disorder, can give birth to an infant with severe hypotonia and weakness causing respiratory compromise and often death in the neonatal period. The mother may have few symptoms, which can be as subtle as difficulty releasing a handshake. A similar pattern of a severely affected infant born to a mildly affected parent has been described with massive triplet repeat expansions occurring in genes associated with some forms of spinocerebellar ataxia.

GENOMIC DISORDERS AND IMPRINTING

Prader–Willi syndrome (PWS) and Angelman syndrome (AS) exemplify several aspects of nontraditional inheritance. In the case of PWS and AS, the parent of origin of chromosome 15 affects the expression of some genes. The discredited hypothesis of Lamarck suggested that the parental factor of inheritance is somehow “imprinted,” and that acquired traits can be passed on to the offspring. Although Lamarck was incorrect, the concept of imprinting has survived, in this case meaning that expression of certain genes is determined by the sex of the parent who passed on that chromosome.

These imprinted genes, which reside on autosomes, exist in two copies, as do all autosomal genes. The inactivation of one copy of these genes resulting from imprinting makes the genes dosage dependent. Stated another way, some autosomal genes are normally expressed only from one member of the gene pair even though both genes in the pair have normal base sequence because one allele has been inactivated. This exposes at least three different, and fascinating, causes of nontraditional inheritance not because of variation in the DNA sequence of the genes involved, but instead because of changes affecting the way the genes are transcribed and expressed. “Genomic disorders” are those disorders resulting from the loss of function of a dosage-dependant gene as a result of loss, duplication, or disruption of the region of the genome in which the genes reside. Often, these events are mediated by deletions or duplications caused by aberrant recombination resulting from closely spaced low copy number repeats flanking the critical region. Points in the DNA that break are more susceptible to breakage because of intrinsic characteristics of the DNA sequence that predispose to abnormal DNA looping at the time of meiosis. The following discussion uses PWS and concerning to demonstrate these mechanisms of nontraditional inheritance.

PWS is characterized by two distinct clinical phases, both of which are seen in essentially all individuals with the disorder. The first phase, marked by profound hypotonia, can be noted prenatally with decreased fetal movement and breech position in the uterus. After birth the infant is profoundly hypotonic, sleeps excessively and has difficulty with feeding and weight gain. There is a global developmental delay, often accompanied by hypoplastic genitalia and cryptorchidism (in boys), strabismus, and evidence of growth hormone deficiency. By the end of 1 yr the feeding difficulty is generally resolved, and behavioral issues are mild.

The second phase typically begins around the age of 2–4 yr. The child is noted to have an apparently insatiable appetite leading to profound obesity if not carefully monitored. There is a typical,

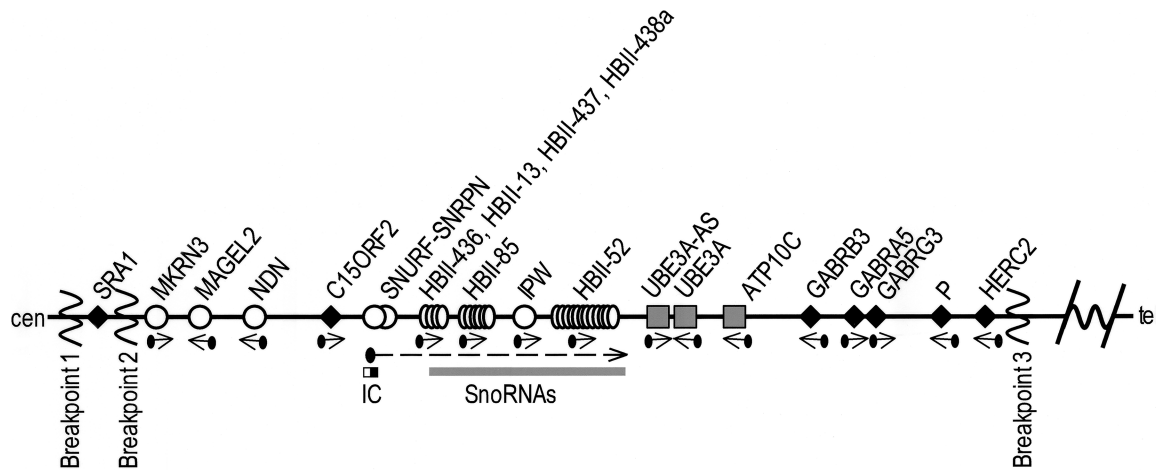


Figure 2-2 Gene map of Prader-Willi syndrome/Angelman syndrome region of chromosome 15. This represents approx 4–5 Mb of chromosome 15 just below the centromere. The common breakpoints of the recurrent deletions are shown. Open circles represent maternally imprinted (expressed only from the paternally inherited chromosome) genes. Gray squares are paternally imprinted genes, and black diamonds represent nonimprinted genes. The open ovals represent clusters of small nucleolar RNAs (SnoRNAs) that have been identified. The function of these RNAs is not known, but they are distributed in intronic regions between the 144 purported exons of SNURF/SNRPN. Arrows show the direction of transcription of genes, with the long, dashed arrow showing the direction and extent of the SNRPN exons. Any of the maternally imprinted genes potentially could contribute to the PWS phenotype, although evidence does not suggest a role for MKRN3 or IPW. The imprinting center is shown as two pieces, with the open rectangle representing the region controlling paternal imprinting (AS), and the filled portion representing the maternal imprint control (PWS) region.

but subtle, facial appearance. There is mild mental retardation or low normal intelligence and a characteristic behavioral profile with temper tantrums, obsessive behaviors, verbal perseveration, skin picking, and a variety of other traits. Management includes avoidance of obesity by careful dietary control and exercise, avoiding exposure to food except at mealtime, use of supplemental growth hormone, and an array of behavioral interventions. Without intervention, the life expectancy is significantly shortened because of complications of obesity such as obstructive sleep apnea, right-sided heart failure, and diabetes.

AS is characterized by more significant mental retardation, severe speech delay or no speech at all, marked gait disturbance with ataxia, and an unusual behavioral profile with a happy demeanor, frequent bursts of laughter for no apparent reason, and rapid escalation of behaviors. There is a characteristic facial appearance, microcephaly, commonly seizures, and there may be a typical EEG pattern.

PWS and AS were delineated clinically in the 1950s and 1960s. In the 1980s, the same recurrent chromosomal deletion was shown to cause both of these disparate clinical syndromes. Further study showed that both result from lack of expression of imprinted genes located near the centromere on the long arm of chromosome 15, but the parental origin of the deletion differed in the two disorders. These two disorders were the first abnormalities resulting from imprinted genes to be recognized in humans. Specifically, at least five different causes of these two disorders have now been delineated, all of which result because genes in the affected region of chromosome 15 are expressed differently when inherited from the mother than when inherited from the father. The known genes in the region are shown in Fig. 2-2, with the sex of the parent in whom the genes are expressed (active) indicated.

Much has been learned about the mechanism by which differential gene expression occurs in imprinted genes. Several genes in the PWS/AS region have an excess of methyl groups attached to

cytosine nucleotides. The methylation appears to block the transcriptional machinery from attaching to or acting on these genes, so that no messenger RNA is made from the highly methylated chromosome. Therefore, the only active copies of the genes in this region are those that are unmethylated. This hypermethylation is found in a parent-of-origin-specific distribution. In Fig. 2-2, genes that are hypermethylated (inactive) when inherited from the mother are shown as white circles, whereas those that appear to be inactive when inherited from the father are shown as gray squares. Any structural change that leads to loss or disruption of the active genes will lead to an absence of the gene product. Several of the genes produce a protein product, although little is known about the function of any of these proteins.

PWS is a result of the loss of genes that are only expressed from the paternally inherited chromosome 15. The most common mechanism leading to PWS is a small interstitial deletion on the chromosome 15 inherited from the father. This recurrent deletion accounts for about 70–75% of all cases of PWS, and occurs with a frequency as high as 1 in 20,000 liveborn infants (the overall incidence of PWS, resulting from all causes, is thought to be 1 in 10,000–15,000). The same deletion accounts for a similar proportion of cases of AS, when it occurs on the chromosome 15 inherited from the mother. The common deletion in both disorders has the same breakpoints, in the vast majority of cases, resulting from small duplicated sections of DNA flanking the region, spanning a distance of about 4 Mb. This type of duplication has been referred to as a “duplicon,” and a number of similar situations appear throughout the genome. Several have already been identified as causing aberrant recombination leading to recurrence of other microdeletions (e.g., deletion 22q11 syndrome).

A second mechanism leading to an individual having no active copies of these imprinted genes occurs when both copies of chromosome 15 are inherited from the same parent, called “uniparental disomy” (UPD). PWS and AS were among the first

disorders caused by UPD to be described. Now, several other conditions have been shown to have a similar mechanism because UPD for chromosomes containing imprinted genes results in absent expression of the imprinted genes. Other examples include some cases of Beckwith-Wiedemann syndrome resulting from UPD for chromosome 11, transient neonatal diabetes resulting from UPD of chromosome 6, some cases of Silver-Russell syndrome resulting from UPD of chromosome 7, and a mental retardation syndrome resulting from UPD for chromosome 14.

UPD most often occurs as a result of a trisomy present at fertilization. PWS occurs when there are two copies of the maternally inherited chromosome 15 present. Most often this results from malsegregation during female meiosis, leading to conception with two copies of chromosome 15 from the ovum and one copy from the sperm. This is nonviable unless there is a second postmitotic event, usually loss of one of the chromosome 15s resulting from malsegregation during mitosis (called “trisomy rescue”). This often occurs because of anaphase lag, where one chromosome fails to move along with the others as the mitotic spindles separate during cell division, and the chromosome is lost into the cytoplasm of one of the daughter cells. It is apparent that there are two possible outcomes from this event. One is the loss of one of the two chromosomes that came from the same parent, resulting in a cell that is now back to the normal and appropriate chromosomal complement, having one chromosome 15 from the father and one from the mother. Alternatively, the chromosome lost in the trisomy rescue process may be from the parent who only contributed one chromosome. This rescues the trisomy, and allows the pregnancy to continue, but if there are imprinted genes on the involved chromosome they will have abnormal expression. Specifically, as seen in PWS, although there are two copies of each gene on chromosome 15, both are from the mother so there will be no gene expression from the imprinted genes.

The point in meiosis where nondisjunction occurs is another important factor concerning UPD. Meiosis I nondisjunction is a failure of separation of the homologous chromosomes, but the sister chromatids divide normally at meiosis II (Fig. 2-3). This results in two gametes that carry one copy of each original parental chromosome (heterodisomy) and two gametes with no copies of the parental chromosome involved. The alternative is that the nondisjunction occurs in meiosis II, after the homologues have successfully separated. Meiosis II defects result in one gamete with two identical copies of the same chromosome (isodisomy), one gamete with no copy of the chromosome and two normal gametes (Fig. 2-3D). Both types of meiotic errors can lead to a trisomic fertilization that is then rescued by loss of one of the chromosomes. Isodisomy can also occur when a gamete that is missing a chromosome (nullisomic) is involved in fertilization. That fertilization results in a monosomic pregnancy, most of which are not viable, unless a mitotic segregation defect occurs that leads to a duplication of the chromosome in question. This mechanism always leads to isodisomy. In AS, the majority of UPD cases have paternal isodisomy, suggesting that the mitotic mechanism is more common. In PWS, both heterodisomy and isodisomy have been seen, making it difficult to determine the mechanism.

Heterodisomy and isodisomy cause a phenotype if there are imprinted genes in the region. Even when the two copies of the chromosome are different (heterodisomy), both carry the identical imprint (i.e., maternally derived or paternally derived) so that the imprinted genes are not expressed. Isodisomy, on the other hand, can also cause a phenotype because nonimprinted recessive disease-causing genes on the duplicated chromosome will be present

on both copies of the chromosome. The very first documented case of UPD was in a child with cystic fibrosis (CF) who was homozygous for the common $\Delta F508$ mutation, a mutation that was only found in one of her parents. She also had short stature. Additional studies demonstrated that both copies of her chromosome 7 were inherited from the parent who carried the CF mutation. This situation has been described for other autosomal-recessive conditions. Furthermore, maternal UPD for chromosome 7 has been shown to be associated with poor growth of prenatal onset, and appears to cause some cases of the primordial dwarfing condition Silver-Russell syndrome.

Several possible evolutionary advantages of imprinting have been put forward. One hypothesis suggests a relative survival advantage to males of being physically large (the idea of the “strapping” boy) weighed against the survival advantage to the female, in this case the mother, of surviving the delivery to reproduce again by having a relatively smaller baby. Supportive evidence for this comes from the fact that pregnancies resulting from duplication of the male genome form a mass of trophoblastic tissue (placentation) with little or no recognizable embryonic tissue, the so-called “hydatidiform mole.” Likewise, the parental contribution of the extra set of chromosomes in triploid pregnancies correlates with the clinical findings. Triploidy with the paternal genome duplicated is usually associated with a very small fetus and large placenta, whereas maternal genomic duplication is associated with a small placenta and an early spontaneous miscarriage. Many of the earliest imprinted genes identified were found to be associated with growth, although that generalization has not held up entirely as more imprinted genes have been found. Other hypotheses purporting an evolutionary advantage for imprinted genes suggest a role either in protection from inappropriate timing of expression of certain genes, or a role in protecting mammalian females from malignant trophoblastic disease because of parthenogenic reproduction, so that paternally contributed genes are required for normal placentation. It has been suggested that there are only 100–200 imprinted genes out of the total estimated less than 30,000 genes in the human genome.

The third mechanism for development of PWS and AS, accounting for less than 5% of cases of each, also results from an imprinting abnormality. During the normal process of gamete production, an individual must change the imprinting pattern of chromosomes inherited from their own opposite sex parent. For example, the chromosome 15 that a man inherits from his mother will be maternally imprinted, and the imprinted genes will not be expressed during that man’s embryonic development. When he passes on that chromosome 15 to his children he must be able to switch the imprint and turn those genes back on. If this does not happen, his offspring will inherit a normal maternally imprinted chromosome 15 from their mother, and an abnormal maternally imprinted chromosome 15 from their father. In this case, there is biparental inheritance of chromosome 15, but both copies are maternally imprinted. When molecular testing is performed there will be no deletion of chromosome 15, nor will there be molecular evidence, usually identified by microsatellite-polymorphism analysis, of UPD. Specific analysis of the methylation pattern in the PWS/AS region of chromosome 15 will be abnormal, though. This “methylation assay” relies on the use of a methylation-sensitive method of evaluating the region, either by use of a methylation-sensitive restriction enzyme, or by use of a specialized methylation-sensitive polymerase chain reaction protocol. In either test, the result will be production of different-sized DNA fragments from

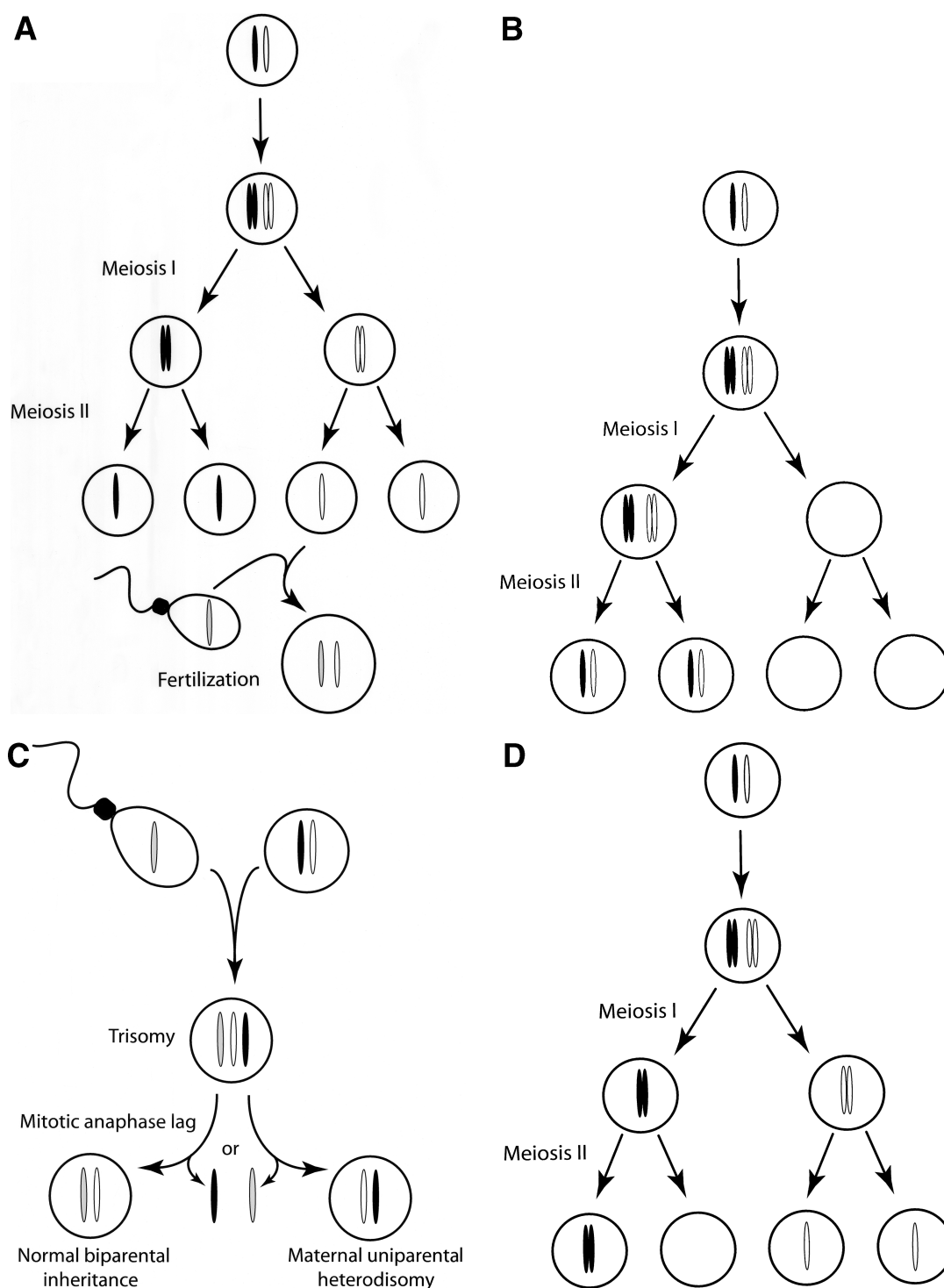


Figure 2-3 (Continued)

maternally and paternally imprinted DNA, demonstrating the presence (or absence) of maternally and paternally imprinted genes. This test will identify all cases of PWS and AS resulting from deletion, UPD, or imprinting defects.

The specific mechanism by which the parent of origin imprint is switched has not been elucidated. The region of chromosome 15 involved, the “imprinting center,” has been defined through examination of a series of chromosome rearrangements and progressively smaller deletions. There appear to be distinct, slightly

separated, regions responsible for initiating the imprint for the maternally silenced genes and the paternally silenced genes. Unlike deletions and UPD, which occur sporadically, some imprinting defects result from imprinting mutations (mostly very small deletions of sequence around the imprinting center) that may be familial. Imprinting mutations cause a unique situation in which the first individual in a family to acquire the defect will be normal, but half of the chromosomes that they pass on will be abnormal because the imprint will not be properly switched on

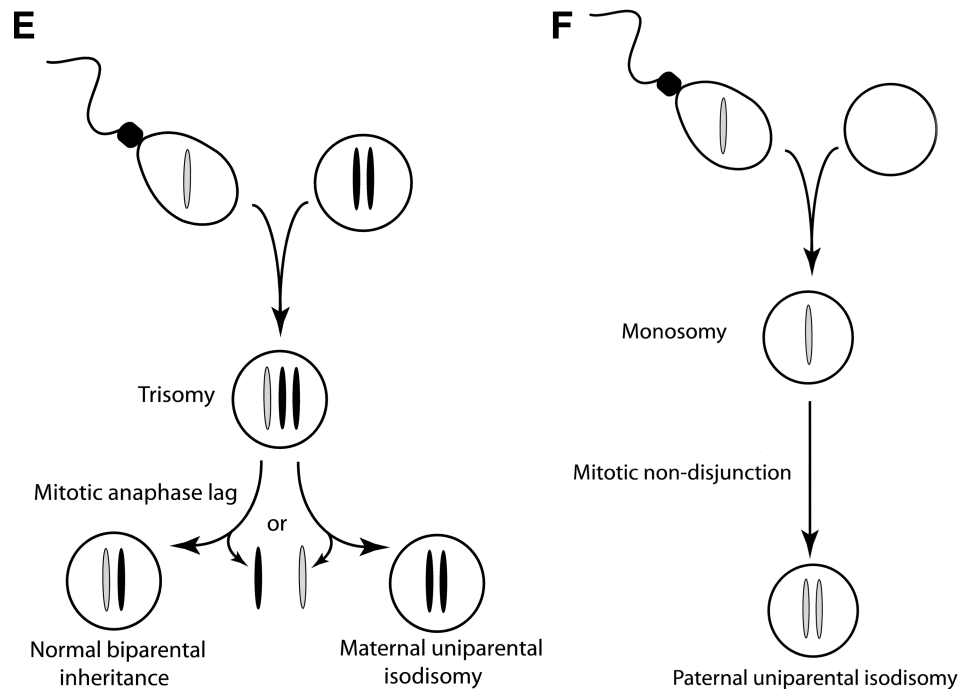


Figure 2-3 Uniparental disomy. This figure follows a single pair of chromosomes through a variety of meiotic and mitotic outcomes to demonstrate how UPD occurs. The chromosomes are shaded differently so that parent of origin for individual chromosomes can be followed easily. All of the meioses are shown in ova, but the process is similar in spermatogenesis. **(A)** Normal female meiotic gametogenesis. After duplication, the homologues separate during the first meiotic division so that each cell contains two identical chromosomes (sister chromatids). During the second meiotic division the sister chromatids separate so that each gamete contains one copy of each chromosome. **(B)** In meiosis I, errors the homologues fail to separate, but the sister chromatids do separate normally during the second meiotic division. Thus there are two potential types of gametes, those that contain one copy of each of the parental chromosomes, and those that contain no copy of the chromosome. The first produces a trisomic fertilization **(C)** that is not viable unless a second error occurs. Mitotic anaphase lag rescues the pregnancy by loss of one of the chromosomes. Depending on which chromosome is lost the result may be either normal biparental inheritance (left side) or uniparental heterodisomy (right side). **(D)** Meiosis II errors occur after the normal separation of the homologues, but with failure of separation of the sister chromatids during the second meiotic division. In this case, there are three potential chromosomal complements for the gametes, isodisomy, nullisomy, and normal. Fertilization of the disomic gamete **(E)** leads to a trisomy that can be rescued by loss of one chromosome. This results either in normal biparental inheritance (left side) or in uniparental isodisomy (right side). Note that in meiosis II errors two of the gametes are normal, whereas in meiosis I defects all of the gametes are abnormal. **(F)** Fertilization of the nullisomic gamete resulting from either type of meiotic error leads to monosomy, which can then be rescued by a mitotic nondisjunction event leading to duplication of the single chromosome. This always results in uniparental isodisomy.

one of their chromosomes. For example, if a woman acquires a new imprinting mutation she will be normal. If that mutation arose on the chromosome 15 that she inherited from her mother, there will be no problem in her offspring because the imprinting pattern does not need to switch. Likewise, each of her daughters who inherit this mutated chromosome will also be fine, as will all of their offspring. However, the son of a mother with a maternally inherited imprinting mutation will be unable to switch the imprint when he passes on that chromosome 15, so that half of his offspring will inherit a maternally imprinted chromosome 15 from their father and will have PWS. This fact makes it important that every child with PWS have the cause thoroughly investigated to rule out this 50% recurrence risk for offspring of the father who carries an imprinting mutation. When analyzing the pedigree of this family it will appear that the trait may skip generations, leading to what has been called a “grandmatrilinal” inheritance pattern for PWS. Similarly, if the original imprinting mutation arises in a male, the eventual result will be females with a 50% risk of having children with AS.

Both PWS and AS have been caused by apparently balanced translocations that either disrupt specific genes, or, more likely,

interfere with imprinting in the region. AS can also be caused by mutations in a single gene in the region, UBE3A, possibly accounting for 10% of cases. This gene is unusual in that it appears to be expressed from both alleles in most tissues, but only from the maternal allele in certain regions of the brain. This tissue-specific imprinting pattern is not typical of the genes in the region and the mechanism is poorly understood. It does not appear to be resulting from hypermethylation of CpG islands at the 5' end of the gene, the most common silencing mechanism for imprinted genes, but may be a result of expression of an antisense transcript of UBE3A. This antisense transcript is in the area of the imprinting region, upstream of UBE3A. It is speculated that on the paternal chromosome the imprinting center allows transcription of the paternally active genes as well as allowing transcription of the paternal copies of the UBE3A antisense region. This antisense transcript, through unknown mechanisms, may interfere with expression of the UBE3A from the same chromosome. The result is that when the imprinting center is “off,” the paternally expressed genes are transcribed, as is an antisense transcript that stops expression of UBE3A from that chromosome. Alternatively, when the imprinting center is “on,” the maternally expressed genes are

silenced, and the UBE3A antisense transcript is not expressed, thus allowing normal expression of UBE3A from that chromosome.

No mutation in a single gene has been shown to cause PWS, and the abnormal methylation pattern is almost always seen in individuals with typical PWS. This supports the idea that PWS is a true contiguous gene syndrome, meaning that the full phenotype is the result of a combination of effects from several genes that are not properly expressed. There is also support for this idea from the various mouse models of PWS as well, none of which fully recapitulate the complete PWS phenotype.

The recurrent nature of the common deletion in PWS and AS is a result of the genomic structure around the region, which is flanked by highly homologous stretches of DNA that predispose to aberrant recombination and deletion or duplication. Such areas can be found throughout the genome, and are thought to explain several recurrent deletion syndromes. It is important to note that one or more of the genes included in the deleted region must be dosage sensitive, so that the loss of a single copy can lead to disease. Developmental abnormalities resulting from this phenomenon of genomic architecture leading directly to a mechanism of disease that does not involve a traditional type of mutation, or traditional inheritance, have been called “genomic disorders.” There are other examples of genomic disorders. Smith–Magenis syndrome, a recognizable mental retardation syndrome, is caused by a recurrent microdeletion on chromosome 17p11.2. Charcot-Marie-Tooth disease type 1A results from a recurrent duplication nearby on 17p11.2, involving the peripheral myelin protein 22 gene, which, when deleted instead of duplicated, causes a different disorder, a hereditary neuropathy with liability to pressure palsy. These genomic rearrangements, once they occur, segregate following Mendelian principles, as can be seen with Charcot-Marie-Tooth, long known to be inherited in an autosomal-dominant fashion. There is some evidence that this particular aspect of genomic architecture, whereas in some instances predisposing to genomic disorders, is actually part of the process of primate evolution, as some of these regions appear to be associated with new genes developing as part of gene families resulting from genomic duplication.

MITOCHONDRIAL INHERITANCE

The idea of nontraditional inheritance developed in response to contradictions between Mendel’s laws and observed biological facts and was initially used to describe imprinting defects. The concept, though, can be further extended to include a variety of other interesting phenomena that lead to situations in which inheritance is not easily explained by Mendel’s laws. A well-recognized example of this is the condition of mitochondrial inheritance, which appears in a matrilineal pattern. This means that the disorder can be seen in males or females, but can only be transmitted from an affected female to her children. Affected males do not transmit the disorder (although this, like most biological “rules” has not been shown to be 100% true). The cause of this inheritance pattern is now well understood, because the mitochondria contain their own small genome. Mitochondrial DNA (mtDNA) is a small, circular DNA containing only 16,569 bp, encoding 13 proteins, each of which is a part of one of the subunits of the mitochondrial electron transport chain. The mitochondrial genome also encodes a unique set of transfer RNAs (tRNAs), as well as two ribosomal RNAs. Mutations throughout the intronless genes on the mtDNA can cause disease, all of which are manifest by disturbances in

energy metabolism, as would be expected by the roles of the known proteins.

During the process of gametogenesis the ovum accumulates a large number of mitochondria, each of which contains multiple copies of the mitochondrial genome. The nucleotide sequence of these mitochondrial genomes is not identical, so that in any particular ovum there may be a variety of mutations, none of which are present in every copy of the mtDNA. The sperm compartmentalizes its mitochondria to the motor unit of the tail, so that none of the mitochondria are delivered into the fertilized egg. Therefore, the mother, explaining the matrilineal inheritance pattern, supplies all of the mitochondria in the fertilized egg.

Another hallmark of mtDNA diseases is that there can be tremendous clinical variation. Different mutations may predispose to different phenotypes, but even with the same mutation the phenotype may vary. One of the reasons for this becomes clear from the fact that multiple different copies of the mtDNA exist in each egg. After fertilization, mitochondria, and their mtDNA component, replicate and segregate during cell division. In this way, different developing tissues may acquire different complements of mtDNA mutations, and, depending on the effect of the mutation and the energy requirements of the tissue in question, there may be selection for one mtDNA genome over another, leading to accumulation or loss of a particular mutation in a particular tissue type. This variation of mitochondrial complement in different tissues is referred to as heteroplasmy.

MELAS, or mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, is a recurrent mtDNA phenotype most often resulting from a point mutation in the mitochondrial leucine tRNA (nucleotide 3243). There is often an accumulation of mutant mtDNA in successive generations, leading to increased severity with earlier onset in the younger generations. Affected individuals may present with poor growth, lactic acidosis, seizures and ataxia, severe headaches, recurrent strokes or stroke-like episodes, cortical blindness, or muscle weakness. The symptoms tend to progress, with death resulting from respiratory complications, infections, or bowel obstruction. Affected individuals may be identified across many generations and branches of the family, always inherited through females.

Kearn-Sayre syndrome is a progressive disorder consisting of peripheral weakness, pigmentary retinopathy, progressive external ophthalmoplegia (because of weakness of the extraocular muscles), heart block or cardiomyopathy, and, occasionally, diabetes mellitus. Most cases of Kearn-Sayre are associated with large deletions of the mitochondrial genome, but some cases have been reported with point mutations in the same leucine tRNA associated with MELAS. The fact that two distinct phenotypes may result from defects in the same tRNA may be a result of tissue heteroplasmy, but it also points to the difficulty in predicting phenotype from genotype in mitochondrial diseases.

Many other phenotypes have been described with mtDNA mutations, some more predictable than others, but it is important for the clinician to remember that defects of the oxidative phosphorylation process may produce almost any symptom in almost any tissue at almost any time of life. Also, although this section discusses defects resulting from mtDNA changes, many more nuclear-encoded genes are involved in the production of electron transport protein subunits and the formation and maintenance of the mitochondrial membranes, transporters and oxidative phosphorylation complexes, defects of which are most often inherited as traditional autosomal-recessive traits.

MULTI-ALLELIC INHERITANCE

In the later 19th and early 20th century, as Mendel's ideas about the independent segregation of traits were being re-evaluated, it was understood that some traits were clearly not the result of single genes. Sir Francis Galton established that height was a trait that could not be explained by Mendelian arguments, and Ronald A. Fisher, a statistician, later showed how multiple genes, each contributing more or less to the final outcome, could explain Galton's findings on height and other quantitative traits. The concept of polygenic inheritance followed, and along with that the idea that genetic factors may interact with environmental factors to produce a trait in a multifactorial way. Multifactorial inheritance, then, can be invoked to mathematically model empirically observed incidences of a variety of traits, and the concept is now fully accepted in genetic thinking and counseling. This multifactorial model requires several assumptions, including that the genes involved all contribute something to the phenotype, without being dominant or recessive, and that they act together in an additive fashion. The number of genes and environmental factors involved in a multifactorial trait is not infinite, and may vary from just a few to a great many (as has been suggested for the development of hypertension).

Findings now point to a form of nontraditional inheritance that is neither fully Mendelian nor fully multifactorial. Three specific examples are digenic inheritance, synergistic heterozygosity, and triallelic inheritance. None of these are completely unique and independent concepts, but all serve to illustrate the complexity of genetic and biological interactions. Retinitis pigmentosa (RP) is a genetically heterogeneous condition of progressive vision loss because of degeneration of the retina associated with increased retinal pigment deposition. It can be isolated or associated with a variety of genetic syndromes, and at least 26 loci have been described in the genome that cause isolated RP, some as X-linked, others as autosomal-dominant and -recessive traits. All of them, though, result from mutations in a single gene. A unique inheritance pattern was identified when a form of RP was found in several different families because of the combination of heterozygosity for mutations in two different genes, *ROM1* and *RDS*. Homozygosity for mutations in the *RDS* gene can also cause RP, but heterozygosity for a mutation in either, by itself, does not. This finding, that heterozygosity at two different unlinked loci is a requirement for the development of the phenotype, represents a newly recognized form of inheritance that is neither Mendelian nor multifactorial, but is instead digenic. Specifically, it cannot be said to be multifactorial or polygenic inheritance because it is not an additive effect of the two genes, but a synergistic effect. Similar findings have now been shown for several conditions, including one form of hereditary deafness, some cases of Hirschsprung disease, and severe insulin resistance. It is interesting to note, though, that pedigree analysis of affected families might be suggestive of autosomal-recessive inheritance, because the recurrence risk for the parents of an affected child would be 25% with each pregnancy.

In some cases digenic inheritance could result from mutations in two genes that interact in a developmental pathway, or that both contribute to the same developmental pathway although they may not physically interact with each other. An analogous situation has been described in several individuals presenting with metabolic myopathies. Investigation into the usual causes of disruption in fatty acid oxidation or electron transport pathways led to the observation that in some cases, heterozygosity for mutations in

two different genes involved in cellular energy metabolism may cause myopathy. Specific genes involved include those for carnitine palmitoyl transferase II, very long chain acyl-CoA dehydrogenase gene, and some of the nuclear-encoded subunits of the electron transport chain. Each, by itself, will be considered a recessive allele, not expected to cause disease if the other partner of the gene pair were normal. It appears that in the heterozygous state the reduction in flux through a particular pathway may be tolerated, but if there is a mild (heterozygous) defect in a different part of the same pathway, the sum of the reductions in flux through the pathway may lead to insufficiency of energy production during periods of metabolic stress. This condition has been referred to as synergistic heterozygosity, but the parallel to digenic inheritance is obvious.

Although both of these forms of inheritance would lead to Mendelian (recessive) proportions of affected individuals on pedigree analysis, triallelic inheritance would not. This fascinating example of nontraditional inheritance has been described in an isolated population with a high rate of an unusual disorder called Bardet-Biedl syndrome (BBS). Individuals with BBS present with pigmentary retinal dystrophy, polydactyly, obesity, reduced cognitive function, and renal abnormalities. There is genetic heterogeneity for the disorder, with eight loci having been identified. In studying several families it was found that some affected individuals were homozygous for mutations in a previously identified BBS gene, whereas there were unaffected family members that were also homozygous for the same mutations. Further investigation revealed that the difference between the affected and the unaffected individuals was that those affected also had heterozygous mutations of another gene known to be associated with BBS. Therefore, in this population, homozygosity for mutation in the first locus was not sufficient to cause the phenotype, but required a third abnormal allele in a different gene, thus triallelic inheritance. There are different ways to interpret these findings, and debate continues whether these may really represent modifier effects; nonetheless, the complexity of inheritance is much greater than was previously imagined.

Similar arguments could be made for some complex traits. A good example is in the risk of blood clotting resulting from inherited thrombophilia. This is one area in which genetic dissection of a complex, multifactorial trait has led to the recognition of a variety of more or less common genetic factors predisposing to thrombosis. The identification of certain of these factors, including the Leiden mutation in the gene for clotting factor V and the prothrombin 20210G > A mutation, along with certain environmental factors, such as cigaret use and oral contraceptive use, allows, at least partially, for the determination of broad categories of risk of abnormal thrombosis and of the relative contribution of individual factors to that risk.

New technologies for exploring the human genome, the Human Genome Project, and the dedicated work of thousands of researchers are beginning to unravel the complexities of human inheritance in ways that could not have been imagined by Mendel, Galton, or other pioneers of genetics. This chapter has reviewed some of the complexities of non-Mendelian, nontraditional inheritance and discussed new ways of understanding old paradoxes. It is likely that more complexities will be discovered, giving new insight into genetic disorders both rare and common, and informing new therapeutic approaches. At the least, it seems likely that these new genetic findings will lead to more personalized medical

information and risk assessments. The immediate impact, unfortunately, is to make the job of the physician much more complicated. Patient demands for genetic information will make it impossible to ignore these advances, so physicians need to find tools and resources to keep up to date, and to find approaches to share this information with patients in ways that will be beneficial without raising inappropriate expectations or fears.

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