Phenotypic Expression

1. **Penetrance**
2. **Expressivity**
3. **Variable age of onset**
4. **Pleiotropy**
5. **Genetic heterogeneity**
6. **Sex-limited**
7. **Sex-influenced**

Penetrance

- **Penetrance** refers to the <u>all or none</u> expression (The disease is present or absent "black-or-white", independent of disease severity) of a mutant genotype.

➢ It usually refers to **dominant** traits in *heterozygotes*, and means that even though an individual has inherited the mutant allele, there may be no expression of the phenotype. If a condition is expressed in less than 100 % of persons who have one copy of the mutant allele, it is said to have reduced penetrance.
If a condition/feature is expressed in less than 100% of individuals who carry the responsible allele, then it is said to have reduced penetrance

- The probability of expression of the phenotype given the genotype
- Term used for dominant conditions

Penetrance

- There are some diseases where **NOT** all individuals with the disease-causing variant show the phenotype.

- If 100 individuals carry the same mutation and **all of them** develop the disease, then the disease is ***100% penetrant* (fully penetrant)**.

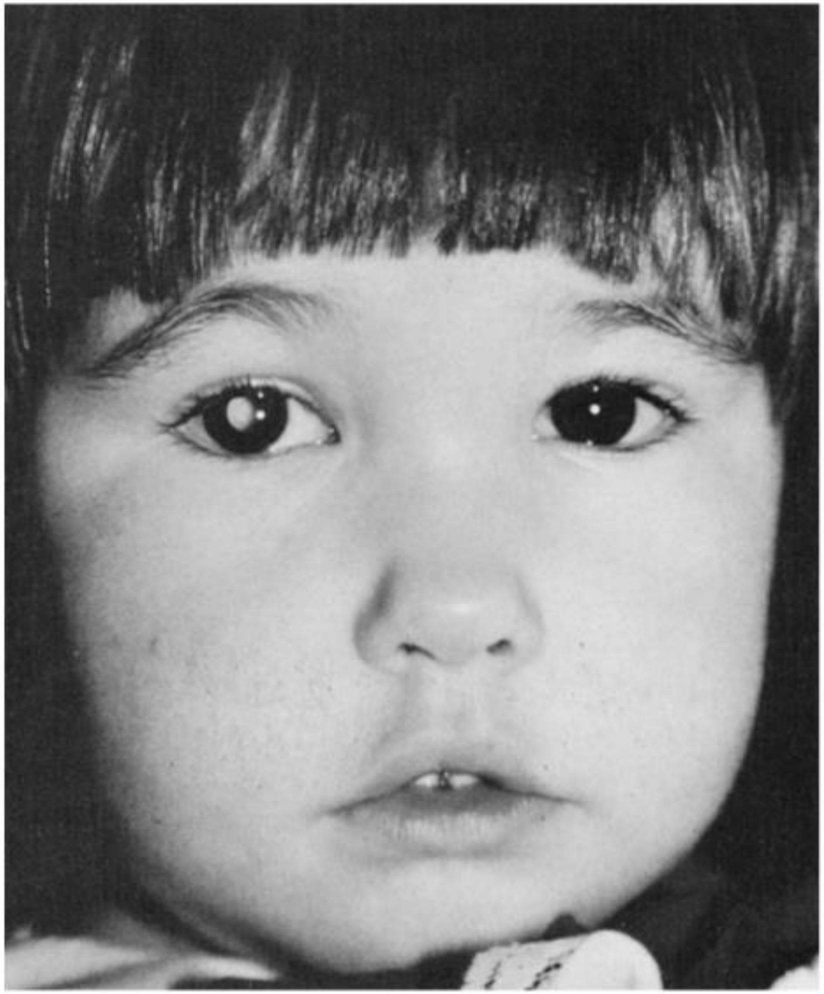
- However, if only **90 out of 100** individuals show clinical manifestations while 10 individuals remain unaffected despite carrying the mutation, then the disease has **reduced (incomplete) penetrance**, in this example ***90% penetrance***.

✓ Therefore, NOT every individual with a disease-causing variant necessarily manifests the disease, even in some **<u>dominant disorders</u>** *where a single mutant allele is sufficient to cause disease.*

Reduced Penetrance

- **Retinoblastoma:** a malignant eye tumor. About 10% of individuals who transmit the mutant allele are **unaffected**. Therefore, the mutant allele is 90% penetrant.

  - Most affected individuals develop the disease by the age of 5 years.



Retinoblastoma

Reduced Penetrance

The mutation exhibits **complete dominance**; however, the phenotype demonstrates reduced penetrance.

- **Waardenburg syndrome:** a congenital sensorineural deafness, heterochromia (each eye has a different color), displacement of the inner canthi, white forelock, and other features. Since only about 20% of people with Waardenburg syndrome are deaf, this shows reduced penetrance of this feature of this syndrome

Deafness in Waardenburg syndrome

- Deafness can be classified as conductive or sensorineural hearing loss. Conductive hearing loss is usually related to problems in the outer ear or middle ear, affecting sound transmission. In contrast, sensorineural hearing loss results from damage to the inner ear structures, particularly the cochlea or the auditory nerve.

- ***Syndromic disease*** involves **multiple** affected organs or tissues, resulting in several clinical manifestations. In contrast, a ***non-syndromic disease*** is characterized by a **single** clinical complication affecting only one system. For example, non-syndromic hearing loss refers to isolated hearing impairment *without* additional clinical features. In syndromic hearing loss, the patient presents with hearing loss **accompanied by other clinical manifestations** affecting different organs, such as vision loss (Blindness) in Usher syndrome.

Variable Expressivity

- It's known as variable severity
- The extent to which a trait is expressed
- If expression ranges from mild to severe then it is said to have variable expressivity
- However, it is **never completely unexpressed**
  - Eg. Neurofibromatosis & myotonic dystrophy (muscle weakness)

- The disease severity or clinical manifestations may vary among different individuals. Even when individuals carry the same gene mutation — or even the exact same variant — the degree of disease severity and the clinical presentation can differ.

- The difference between penetrance and variable expressivity is that in variable expressivity the **disease is present**, but its severity varies among individuals.

✓ Penetrance → Is the disease present or absent?
✓ Variable expressivity → Disease present, but severity differs.

The doctor explained the concepts using real-life examples; watching the lecture is highly recommended.

Variable age of onset & pleiotropy

- **Variable age of onset** refers to the variation in the time to phenotypic expression of mutant gene (s). Example: the onset of ***Huntington disease*** is typically in the 40's, however, age of onset may range from the 20's to 60's.

- A mutant gene is said to be **pleiotropic** when it produces a wide range of phenotypic effects. Example: ***Marfan syndrome*** (It's a connective tissue disorder. Affected individuals are typically tall with long limbs and joint hypermobility. One of the characteristic features is lens dislocation (ectopia lentis), where the lens is displaced from its normal position in the eye) involves the skeletal, cardiovascular (The most clinically significant complications because connective tissue integrity is impaired, the aortic wall becomes weak. The aorta is exposed to the highest blood pressure in the body, which places patients at increased risk of aortic dilation, dissection, and rupture, which can cause death) , and ocular systems.
    - Another example is ***cystic fibrosis***, in which a variant in the **CFTR gene** affects multiple organ systems, including the respiratory system, digestive system, reproductive system, and sweat glands in the skin.

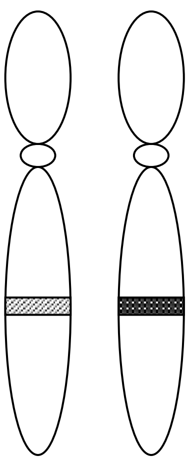
Anticipation: Earlier Age of Onset & Increasing Severity

In the same family

Myotonic dystrophy

Genetic heterogeneity

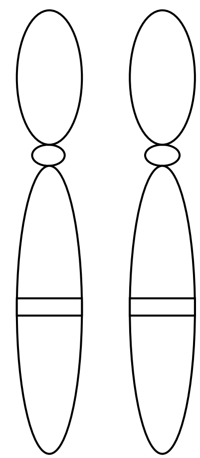
allelic heterogeneity



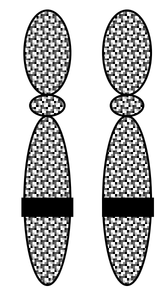
a1 a2

At the CF locus on 7q
a1 = ΔF508 allele
a2 = S549R allele

locus heterogeneity



PAX3 on 2q
Auto dom HL

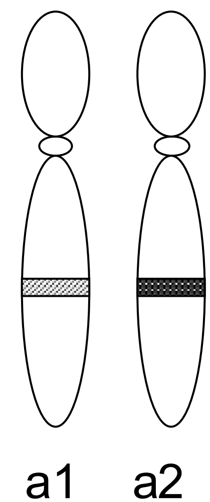


GJB2 on 13q
Auto rec HL

Genetic heterogeneity

- **Allelic heterogeneity** refers to <u>two or more different mutant alleles at the same genetic locus</u> (Example: Duchenne and (the less severe) Becker muscular dystrophy; cystic fibrosis).

- Allelic heterogeneity means that for the same gene, such as the CFTR gene in cystic fibrosis, there are different mutant alleles across different individuals. **The most common is the ΔF508 mutation**, an in-frame deletion of phenylalanine at amino acid position 508. However, there are many other disease-causing variants in the population, such as missense mutations like the substitution of serine to arginine at a specific amino acid position.
- ✓ At the individual level, each person has one or two alleles depending on whether the gene is X-linked (in males) or autosomal, but at the population level there are many different disease-causing alleles for the same gene, which is referred to as ***allelic heterogeneity***.

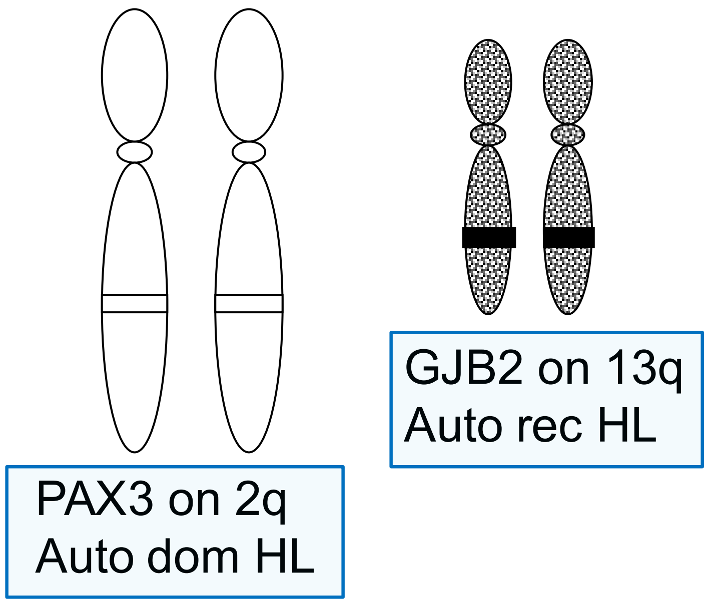


At the CF locus on 7q
a1 = ΔF508 allele
a2 = S549R allele

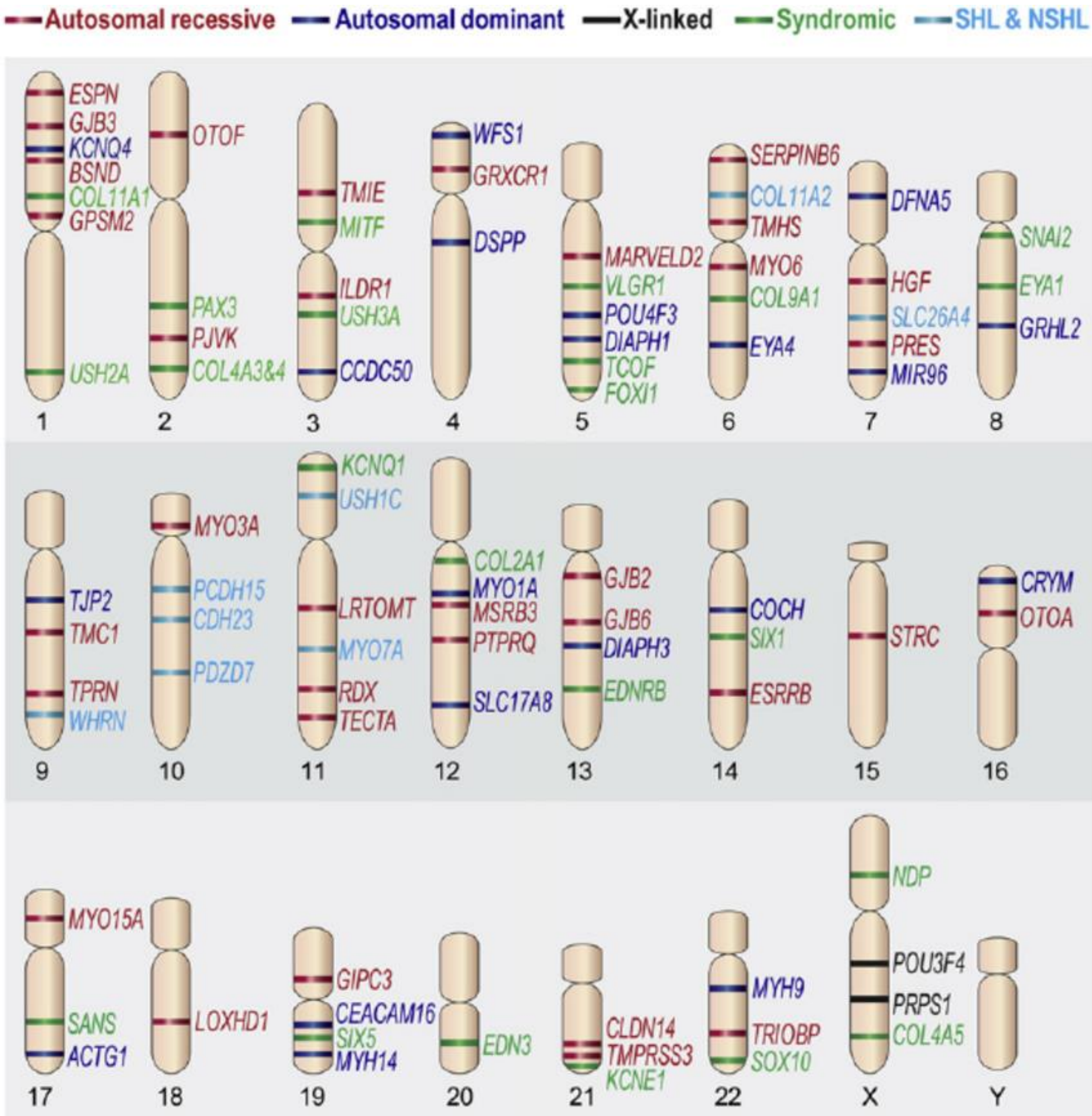
Genetic heterogeneity

- **Locus heterogeneity** is when mutations at two different genetic loci (regions on a chromosome) result in similar phenotypes (Example: congenital deafness). In some cases, the mode of inheritance of the disorders can vary

  - Locus heterogeneity means different genes at different chromosomal locations cause the same disease.
  - ✓ A prime example is **congenital hearing loss**. If a patient has congenital bilateral sensorineural hearing loss (early onset or congenital), we can think of many genes (close to 100 genes), and mutations in any of these genes can lead to the same clinical manifestation. Therefore, ***locus heterogeneity*** occurs because different genes at different loci can cause the same clinical feature.



HL: hearing loss


Autosomal recessive
Autosomal dominant
X-linked
Syndromic
SHL & NSHL
ESPN
GJB3
KCNQ4
BSND
COL11A1
GPSM2
USH2A
1
OTOF
PAX3
PJVK
COL4A3&4
2
TMIE
MITF
ILDR1
USH3A
CCDC50
3
WFS1
GRXCR1
DSPP
4
MARVELD2
VLGR1
POU4F3
DIAPH1
TCOF
FOXI1
5
SERPINB6
COL11A2
TMHS
MYO6
COL9A1
EYA4
6
DFNA5
HGF
SLC26A4
PRES
MIR96
7
SNAI2
EYA1
GRHL2
8
TJP2
TMC1
TPRN
WHRN
9
MYO3A
PCDH15
CDH23
PDZD7
10
KCNQ1
USH1C
LRTOMT
MYO7A
RDX
TECTA
11
COL2A1
MYO1A
MSRB3
PTPRQ
SLC17A8
12
GJB2
GJB6
DIAPH3
EDNRB
13
COCH
SIX1
ESRRB
14
STRC
15
CRYM
OTOA
16
MYO15A
SANS
ACTG1
17
LOXHD1
18
GIPC3
CEACAM16
SIX5
MYH14
19
EDN3
20
CLDN14
TMPRSS3
KCNE1
21
MYH9
TRIOBP
SOX10
22
NDP
POU3F4
PRPS1
COL4A5
X
Y

Sex-limited & Sex-influenced

- **Sex-linked** means that the gene is physically located on a sex chromosome. For example, ***hemophilia*** is caused by a gene located on the X chromosome; therefore, it is an X-linked (sex-linked) disorder.

- **Sex-limited** refers to a phenotype that is autosomally transmitted but <u>expressed only in one sex </u>"gender" (but the gene itself is NOT necessarily to be carried on a sex chromosome). Example: ***Autosomal dominant MALE precocious puberty.*** when a female has the disease-causing variant, she will not have any clinical features.

- **Sex-influenced** (Disease severity may vary depending on the sex of the individual) refers to autosomally inherited traits that are expressed differently, in either degree or frequency, in males and females. Example: ***hemochromatosis*** "iron overload" (autosomal recessive disorder of increased absorption of dietary iron) is more commonly found in **MALES** due to lower dietary intake and menstruation in **FEMALES**.

- Some disorders do not follow Mendelian patterns of inheritance.
- These disorders are clearly genetic (inherited) and their inheritance is classified as non-Mendelian.
- We now understand why some of these disorders do not follow Mendelian patterns and examples include: **mitochondrial inheritance**, **unstable trinucleotide repeats**, and **imprinting**.

Trinucleotide Repeats

- There are diseases such as myotonic dystrophy and Fragile X syndrome (FMR1) in which the mutations are considered dynamic mutations, as they involve repeat expansions.

Some disorders were observed to increase in severity from one generation to another,

and/or the age of onset of symptoms became earlier in successive generations.

This was termed **anticipation** and the mechanism was a mystery since mutations were presumed to be inherited in a stable manner from one generation to another.

Furthermore, in some disorders the sex of the parent who passed on the disorder seemed to influence the severity or age of onset of symptoms.

This too was a puzzle because in Mendelian traits maternal and paternal DNA was assumed to be equivalent.

Anticipation and **parent of origin** effects are now known to be due to a novel type of **dynamic mutation** known as unstable trinucleotide repeats.

Trinucleotide Repeats

Tandemly repeated trinucleotides (i.e. CGG, CTG) within or adjacent to a gene that may increase or decrease in number during formation of egg or sperm cells and thus disrupt the functioning of the gene and lead to disease

Examples:

- Fragile X Mental Retardation syndrome
- Huntington disease
- myotonic dystrophy
- spinocerebellar ataxia
- Kennedy disease
- Joseph disease
- Friedreich Ataxia

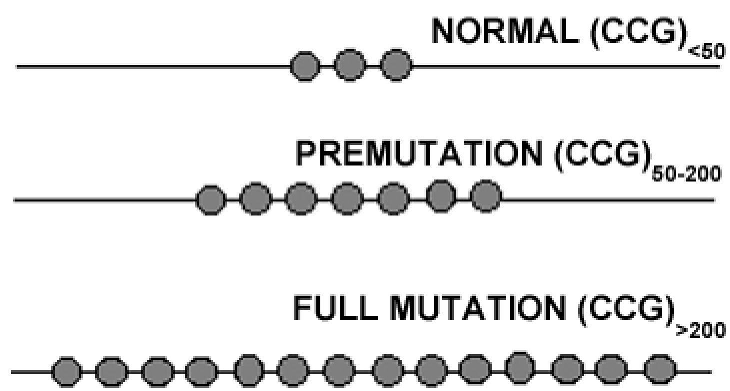
✓ All are examples of diseases in which expansion of trinucleotide repeats causes the disease.

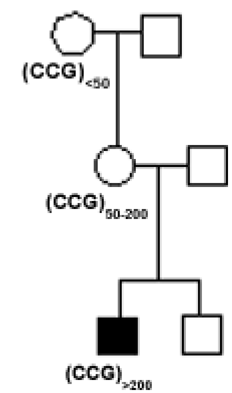
Trinucleotide Repeat Expansion

Fragile X MR Syndrome

- FMR1 is a gene located on the X chromosome, and it stands for Fragile X Mental Retardation gene; however, the term "intellectual disability" is now preferred instead of "mental retardation." Historically, the earlier term was used at the time of its discovery. This gene contains a CGG trinucleotide repeat sequence (CGG rather than CCG, although the complementary strand is CCG). In normal individuals, this CGG repeat is present in tandem repeats fewer than 50 times and is not associated with clinical features.

- Back in the day, in the past, they used to test for the number of repeats by doing a karyotype, and when you do a karyotype for an individual with higher than normal CGG repeats, during the testing process the X chromosome appears fragile or may break. And that is why it is called Fragile X. However, nowadays the standard testing for repeat expansions in Fragile X is PCR-based.





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Let's discuss dynamic mutation:

- When the number of repeats expands to **more than 200**, this is considered a **full mutation**, and an individual with more than 200 CGG repeats will be clinically affected. That is why it is called *dynamic*.
- ✓ Dynamic means that the number of repeats changes across generations. It is not a gain, loss, or substitution mutation; rather, it is an **expansion** or increase in the number of repeats over generations.
- If an individual has a **premutation**, meaning the repeat number is above the normal range (above ~50 repeats) but below 200 repeats, this individual is considered a premutation carrier. This person is **not clinically affected** with Fragile X syndrome.
- However, because the repeat number is already expanded beyond the normal range, during **gametogenesis** these repeats are likely to expand further. Therefore, although the parent is unaffected, the repeats may expand to a full mutation (>200 repeats) in the gametes, resulting in an affected offspring.
- This phenomenon is called **anticipation**, where disease severity increases or appears earlier in successive generations due to repeat expansion.
- ✓ Anticipation is a known mechanism in **dynamic mutations**, and the **parent of origin** is important. Parent of origin refers to whether the premutation is inherited from the mother or the father.
- In **Fragile X syndrome**, anticipation shows a **maternal origin effect**. When the mother carries the premutation, the repeats can expand to a full mutation during **oogenesis** (egg formation). In contrast, if the father carries the premutation, it is usually transmitted as a premutation during **spermatogenesis** and does NOT expand to a full mutation.
- ✓ Therefore, parent-of-origin effects are closely related to dynamic mutations involving repeat expansions.

FX MR Clinical Features

1. High Incidence of about 1 in 5000 males; presumed incidence in females (have 2 X chromosomes) is about one-half that of males.
2. Most common cause of inherited **mental** retardation in males.

According to the American Academy of Pediatrics (AAP) guidelines, testing for FMR1 mutations is specifically recommended in patients presenting with intellectual disability or developmental delay.

3. Phenotype in males includes moderate mental retardation, large head, long face, prominent forehead and chin, protruding and larger ears, large testes after puberty, speech delay, and loose joints. Behavior abnormalities include hyperactivity, hand flapping, hand biting, temper tantrums and sometimes autism spectrum disorder. نوبات غضب
4. Approximately 50% of female carriers of a full mutation have mental retardation that is usually **less severe** than in affected males. Keep in mind the concept of random X inactivation.
5. About 30% “This percentage is impacted; higher repeat number in the premutation range increases the risk of expansion (e.g., 150 > 60) “of males who carry a premutation will develop Fragile X-associated tremor/ataxia syndrome (FXTAS) which is characterized by late-onset, progressive cerebellar ataxia and intention tremor.
6. About 20% of females who carry a premutation will develop premature ovarian failure (POF). (At an earlier age)

Genetic Features

A. Atypical X-linked inheritance showing parent of origin effect.

B. In affected males associated with a fragile site at Xq27.3 in 10-40% of metaphase spreads, however, this cytogenetic testing is no longer used for diagnostic testing.

C. Amplified 'CGG' trinucleotide repeat as well as abnormal methylation (hypermethylation) of the FMR-1 gene. The normal protein product, FMRP, is an RNA-binding protein that seems to function as a nucleocytoplasmic shuttling protein and it binds several mRNAs including its own. It also seems to affect cytoskeletal structure, synaptic transmission and neuronal maturation. The FMR-1 gene mutation results in gene silencing and the loss of function results in suppression of translation of proteins from its RNA targets.

- Those repeats are located on either the coding or non-coding region of the gene. So for example, in ***Fragile X***, those repeats are in the **non-coding region**, specifically in the UTR (untranslated region). It is non-coding, but it turns out that when the CGG repeat number is high, this induces **methylation** of the gene, and the gene becomes **hypermethylated**. When the gene is hypermethylated, it is not expressed. An example of a different mechanism is ***Huntington disease***, where the repeats are located in the **coding region**. In Huntington disease, the repeat leads to **expansion of amino acids (glutamine)**, resulting in an abnormal protein structure.

Genetic Features

D. Allele sizes (these categories are not absolute):
   - Normal alleles: 5-54 repeats
   - Premutation alleles: **55-200 repeats** (not associated with MR but there is risk for FXTAS and POF; may expand to full mutation in female carrier)
   - Full mutation alleles: > 200 repeats (affected individuals)

E. Existence of transmitting males who are of normal intelligence but can transmit the Fragile X chromosome to their daughters. These daughters are of normal intelligence, however, their children are at risk for mental retardation.

F. The change from phenotypically normal to affected state (i.e. expansion of the trinucleotide repeats into the full mutation range) has only been observed following oogenesis.

Huntington's Disease: A Late-Onset Lethal Disease

- **Huntington's disease** is a degenerative (progressive deterioration in the NS) disease of the nervous system
- The disease destroys cells in the **basal ganglia**, the part of the brain that controls movement, emotion, and cognitive ability
- The disease has no obvious phenotypic effects until the individual is about 35 to 40 years of age (Late onset)
- Once the deterioration of the nervous system begins the condition is irreversible and fatal

○ Patients with Huntington disease feel that something is wrong with their movements and start to struggle with voluntary motor control.

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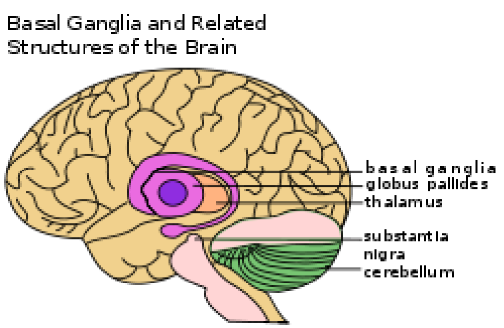


Genomic Imprinting

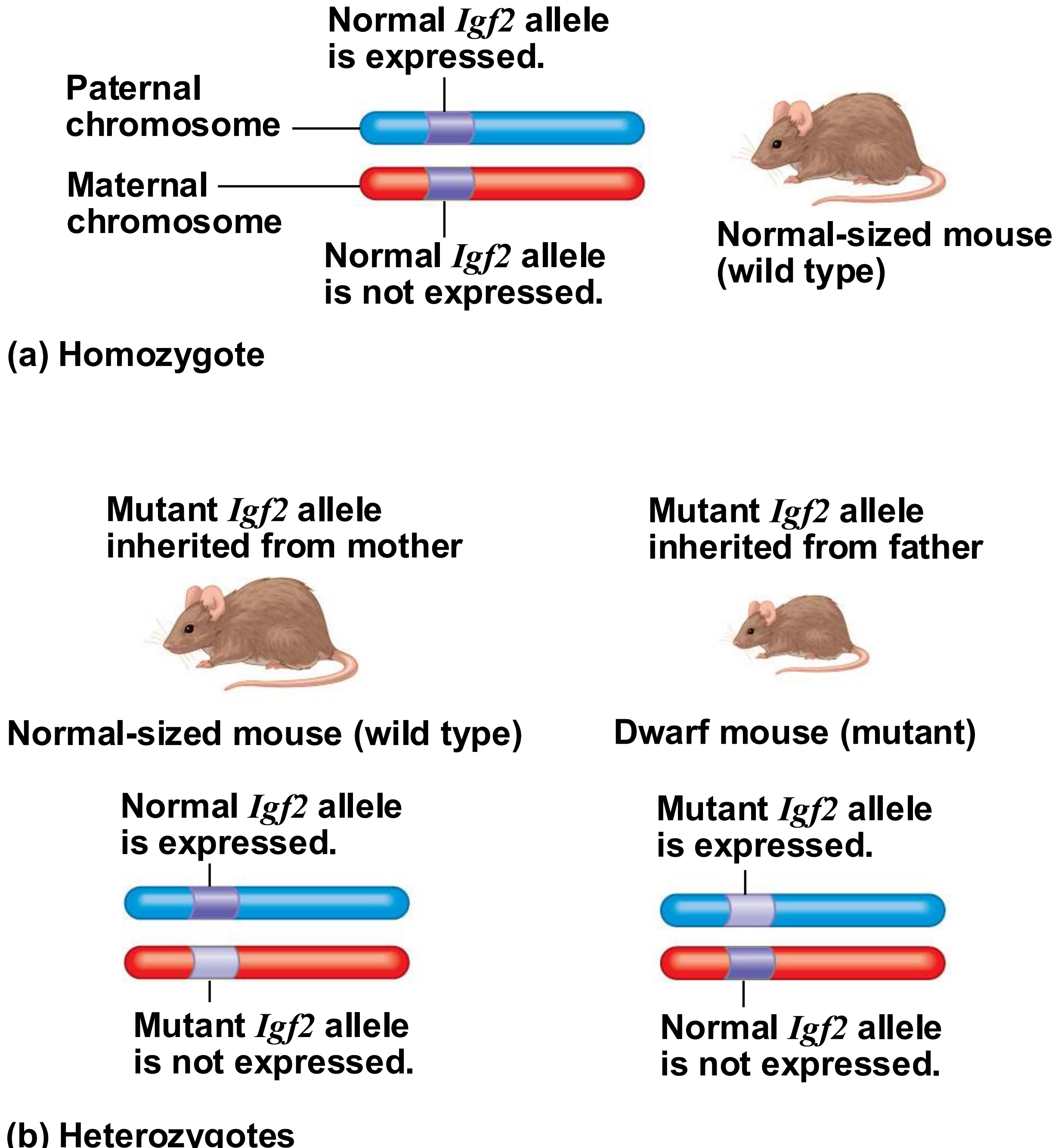
- ***Genomic imprinting*** refers to a phenomenon in which the DNA sequence remains the same, but differences in **methylation patterns** depending on the parent of origin influence gene expression and clinical features.

- For a few mammalian traits, the phenotype depends on which parent passed along the alleles for those traits
- Such variation in phenotype is called **genomic imprinting**
- Genomic imprinting involves the silencing of certain genes that are "stamped" with an imprint during gamete production

Genomic Imprinting

- Humans were initially thought to have around 100,000 protein-coding genes, assuming that each gene produces one protein. However, it is now known that humans have approximately 20,000 protein-coding genes, yet they can produce more than 100,000 different proteins. This diversity is mainly explained by mechanisms such as **alternative splicing**, where different exons are included or skipped to generate multiple mRNA transcripts from a single gene. Introns are always removed from mature mRNA, whereas exons may be variably expressed depending on the splice variant.

- In addition to protein-coding genes, humans also possess more than 20,000 **non-coding genes**, which are transcribed into RNA but not translated into proteins. Furthermore, a subset of several hundred genes undergo **differential methylation** during gametogenesis. During oogenesis and spermatogenesis, specific genes are methylated and silenced in a parent-of-origin–dependent manner, meaning that some genes are silenced in the egg while others are silenced in the sperm, forming the basis of genomic imprinting.



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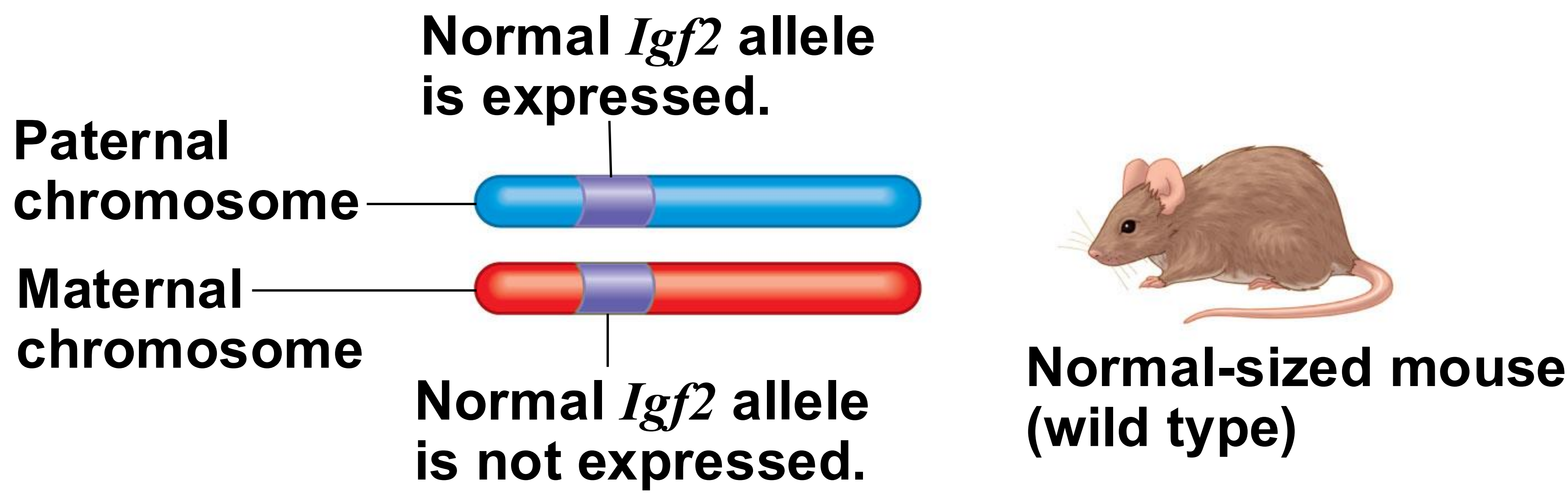
- In this simple example, we start with a normal fertilized egg that contains both a paternal and a maternal chromosome, meaning two alleles of the same gene.

- Under normal physiological conditions, only the **paternal allele of the *Igf2* gene** is expressed because it is **not methylated**, whereas the **maternal allele is methylated and silenced** during oogenesis. Therefore, normally only the paternal allele is expressed and produces mRNA and protein, while the maternal allele remains inactive.

- If a mutation occurs in the **maternally inherited *Igf2* allele**, the phenotype is NOT affected because this allele is already silenced and not expressed. However, if the same mutation occurs in the **paternal allele**, the phenotype becomes evident, and the mouse develops growth deficiency or dwarfism. This happens because ONLY the paternal allele is functionally expressed.

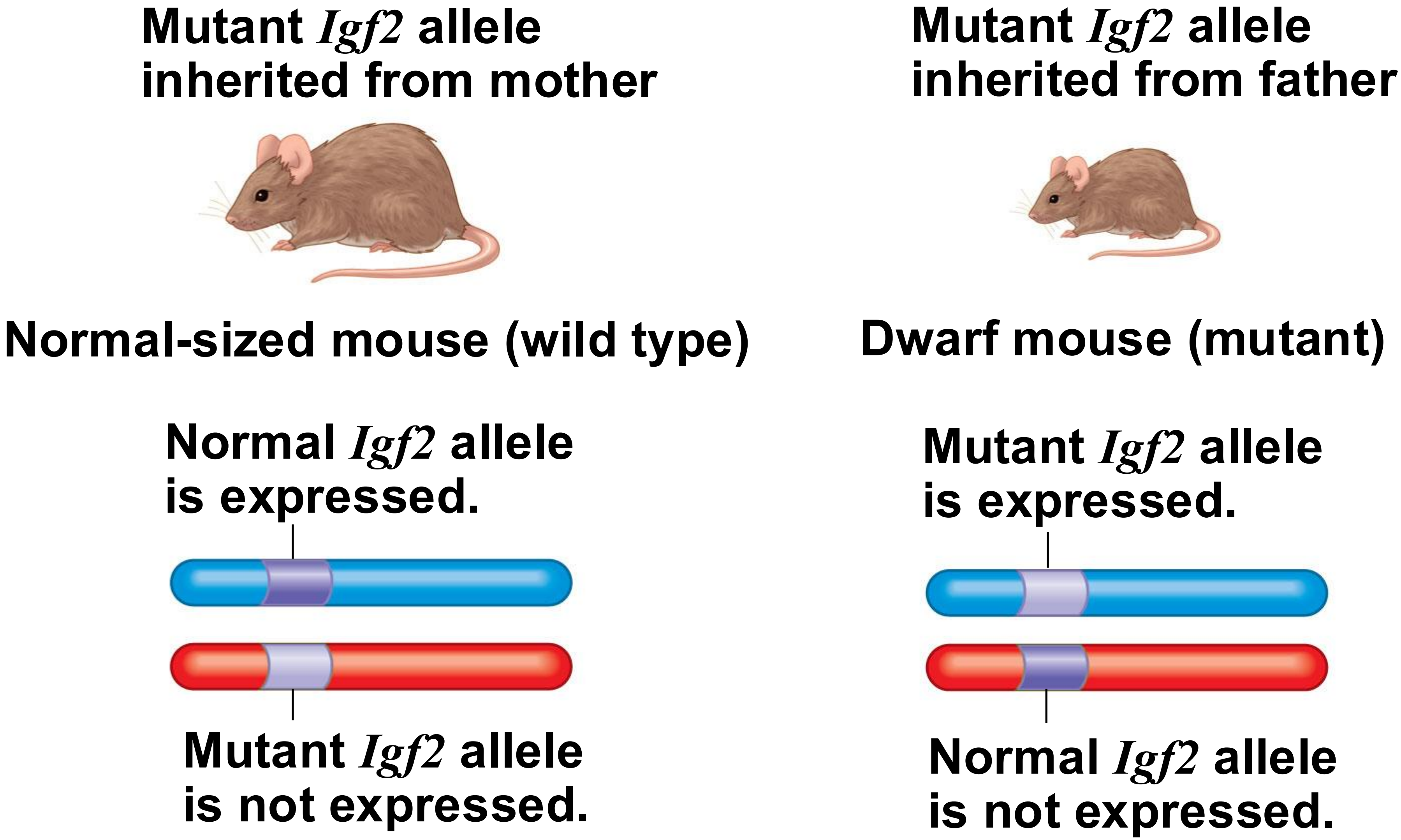
- Phenotypically, mutations in the maternal allele have no effect since the allele is inactive from the beginning. However, when this male later undergoes **spermatogenesis**, imprinting marks are reset. Both alleles can be transmitted in sperm cells, and each sperm carries one allele with a paternal imprint. Consequently, 50% of the offspring may inherit the mutated expressed allele and develop the disease.

Figure 15.17a



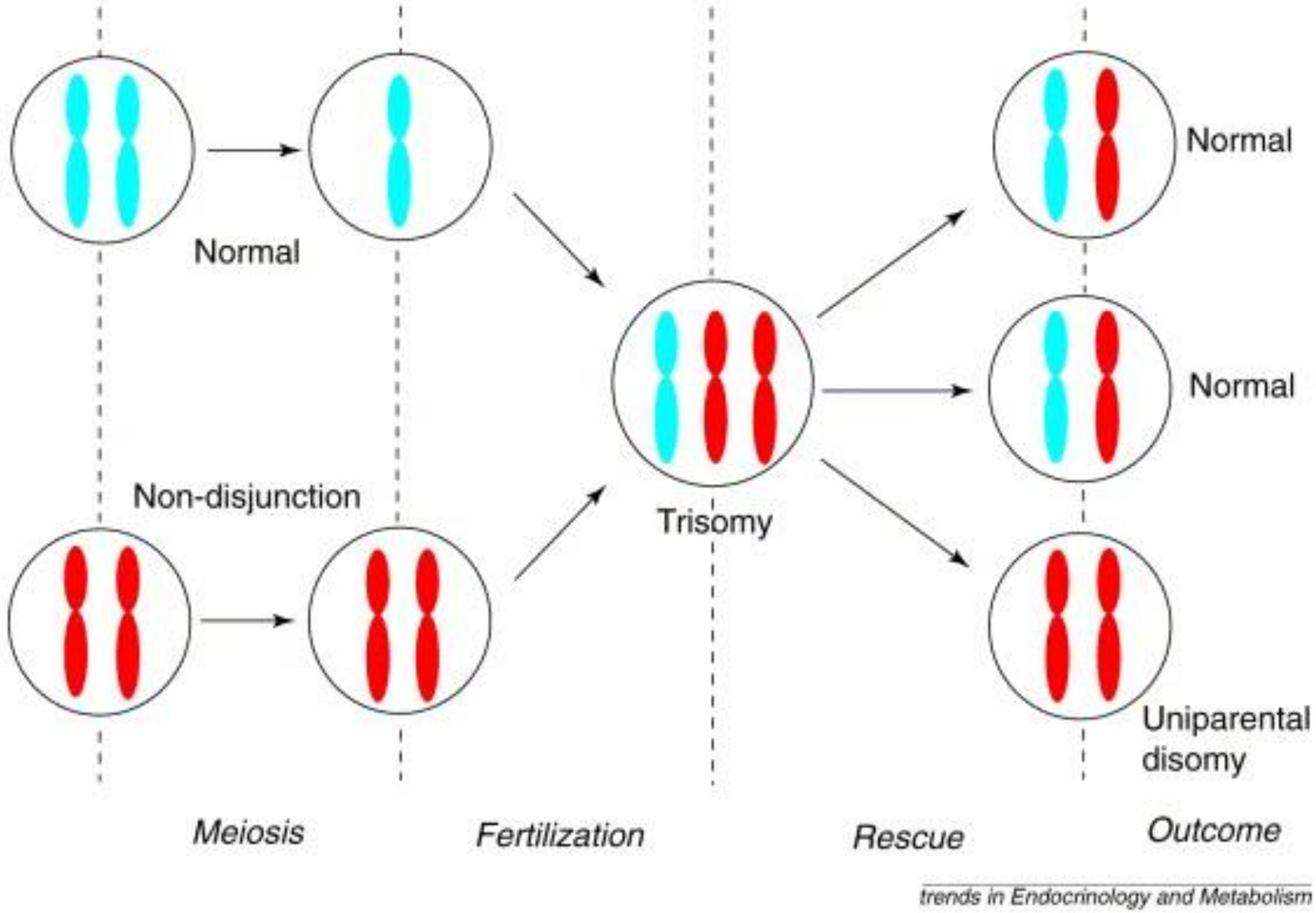
(a) Homozygote

Figure 15.17b



(b) Heterozygotes

- It appears that imprinting is the result of the methylation (addition of $–CH_3$) of cysteine nucleotides
- Genomic imprinting is thought to affect only a small fraction of mammalian genes
- Most imprinted genes are critical for embryonic development



- Trisomy is the presence of an extra chromosome, which may be of maternal origin. In a trisomy, the individual has three copies of a chromosome. In some cases, a process called **trisomy rescue** occurs, where the fertilized egg tries to eliminate the extra chromosome. If the chromosome that is lost is the only chromosome from one parent, and the two remaining chromosomes are from the same parent, this results in **uniparental disomy** (UPD), meaning **both chromosomes come from one parent**, either maternal or paternal.

Imprinting

Prader-Willi syndrome **Angelman syndrome**

- ✓ Those are two different clinical diseases, but they originate from the same genetic region.

Imprinting

I.Definition: the differential expression of a gene depending on the sex of the parent from which it is inherited (i.e., the parental origin of the gene).

Implications:

A.Implies that there is a critical or sensitive period during development (i.e. during or before gametogenesis) during which the genetic information is marked or imprinted in order to permit differential expression based on parental origin.

B.The imprint must persist stably through DNA replication and cell division in the body cells.

C.The imprint must be capable of affecting gene expression (i.e. turning genes on or off).

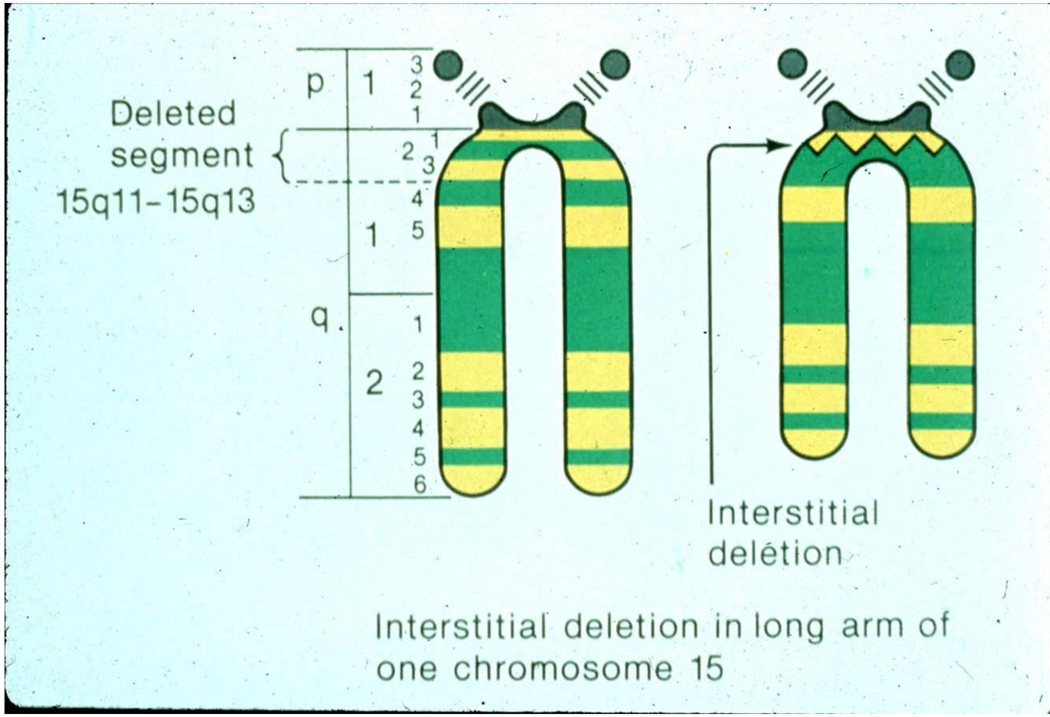
D.Imprinting is not a permanent alteration since it must be erased in the germ cell line of every individual so that new imprinting may be introduced.

Example of Imprinting in Humans

Prader-Willi syndrome (PWS) and Angelman syndrome (AS)

1. Both map to and may involve deletions of 15q11-13 but they have distinct phenotypes.
2. PWS is characterized by obesity, voracious appetite, and mental retardation, whereas, Angelman is characterized by gait ataxia, smiling facies and happy demeanor (happy puppet) , and mental retardation.
3. Deletions are found in about 50-60% of cases of PWS and AS.
4. If the deletion is paternally derived (only maternal 15q11-13 present) then PWS.
5. If the deletion is maternally derived (only paternal 15q11-13 present) then AS.
6. Some cases of PWS (about 30%) have been attributed to maternal uniparental disomy and some cases of AS (about 5%) have been attributed to paternal uniparental disomy. About 10-15% of cases of AS are caused by a single gene mutation in the UBE3A gene. Other causes of PWS and AS include defects in the imprinting center, chromosomal translocation within the PWS/AS critical region, and unknown cause.

PWS & AS both involve chromo 15q11-13



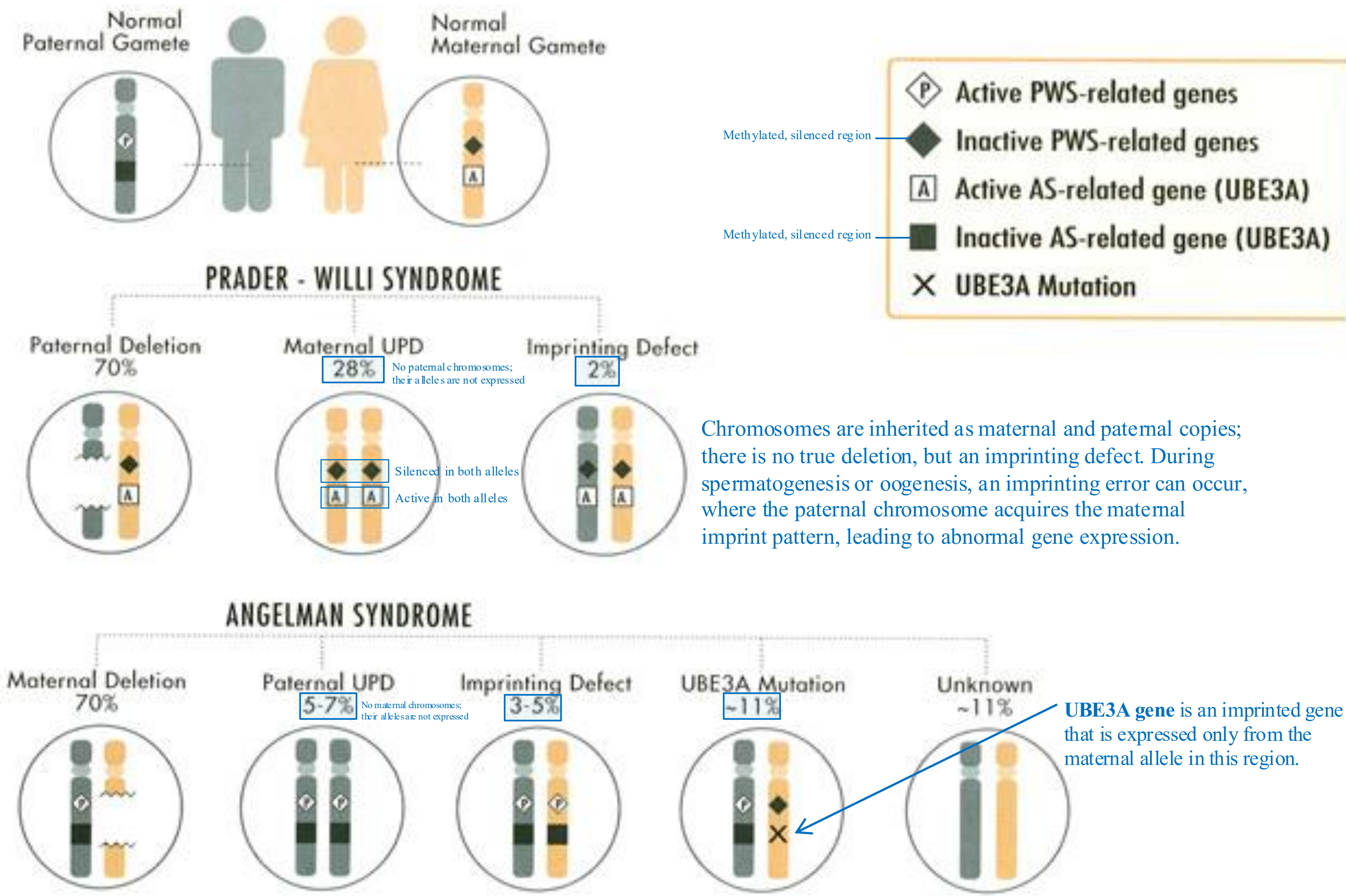
Acrocentric chromosome

Keep in mind the concept of chromosomal regions and subregions that we covered in the midterm

Deletions account for ~ 70% cases of PWS & AS

- If paternal deletion of 15q11-13 → PWS
- If maternal deletion of 15q11-13 → AS

Causes of PWS and AS



Chromosomes are inherited as maternal and paternal copies; there is no true deletion, but an imprinting defect. During spermatogenesis or oogenesis, an imprinting error can occur, where the paternal chromosome acquires the maternal imprint pattern, leading to abnormal gene expression.

UBE3A gene is an imprinted gene that is expressed only from the maternal allele in this region.

In AS, if testing for chromosomal deletions and uniparental disomy is negative, about 11% of cases are due to UBE3A mutations.

Adapted from Journal of the American Academy of Child and Adolescent Psychiatry, 2000;39:388

Inheritance of Organelle Genes

- Extranuclear genes (or cytoplasmic genes) are found in **Mitochondria** (16kb circular DNA)
- Extranuclear genes are inherited maternally because the zygote's cytoplasm comes from the egg

- Mitochondrial diseases are maternally inherited because mitochondria are transmitted through the oocyte, while sperm contributes only nuclear DNA. Therefore, affected males do not transmit mitochondrial DNA mutations to their offspring.

- Mitochondrial genes encode proteins required for mitochondrial function (mainly energy production). Some defects in mitochondrial genes prevent cells from making enough ATP and result in diseases that affect the muscular and nervous systems
  - For example, mitochondrial myopathy (**myopathy** is a muscular disease)
  - Leber's hereditary optic neuropathy (damage to nerves)

✓ Optic nerve atrophy leading to blindness.