GENETICS

Sheet no.13

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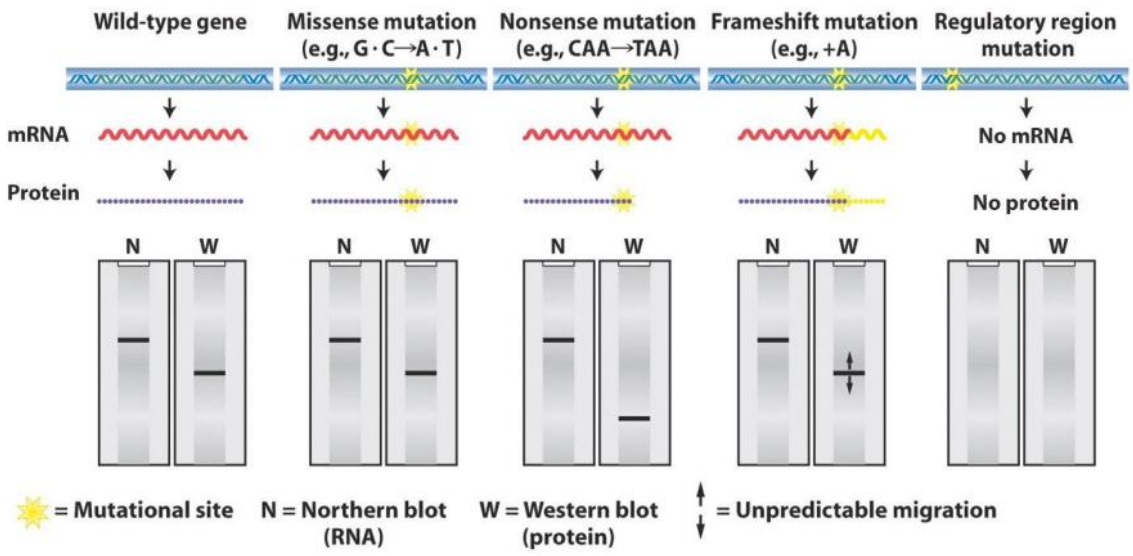
Recap from the previous lec:

-we can use electrophoresis to expect the impact of the mutation at the level of mRNA or protein

- The farther the distance traveled, the smaller the size of the protein or mRNA

-it is called northern blot, if it is mRNA

-and western blot, if it is a protein



-for missense mutation:

*The AA is substituted with another

*the amount of the mRNA and protein isn't affected, the effect is on the structure and the function, so its electrophoresis resembles the wild type.

-nonsense mutation:

*base pair substitution creating stop codon that causes premature termination

*The size is smaller, so the blot travels farther.

-frameshift mutation: (in, out)

*After the position of the mutation, the subsequent AAs will be changed and replaced leading to disruption of the reading frame

*the segment may be smaller so travels faster, if the case is a deletion and premature termination

*or it may be larger so travels slower, if the case is insertion or deletion for the stop codon for ex, causing increase in the amount

-regulatory mutations:

* occur in noncoding regions of genes, such as promoters, introns, and regions coding 5′UTR and 3′UTR segments of mRNA.

*at the level of noncoding regions like splice site mutation, there are introns, where disruption in the splicing pattern may occur either by retention of introns or skipping of exons(smaller proteins)

*mutation in the regulatory region can cause increased, reduced or complete loss of transcription.

*in this electrophoresis example, there is no transcription at all→complete loss→severe mutation→severe nonsense or frameshift →resulting in an abnormal functional protein or defect in mRNA detected by nonsense-mediated decay→no blot

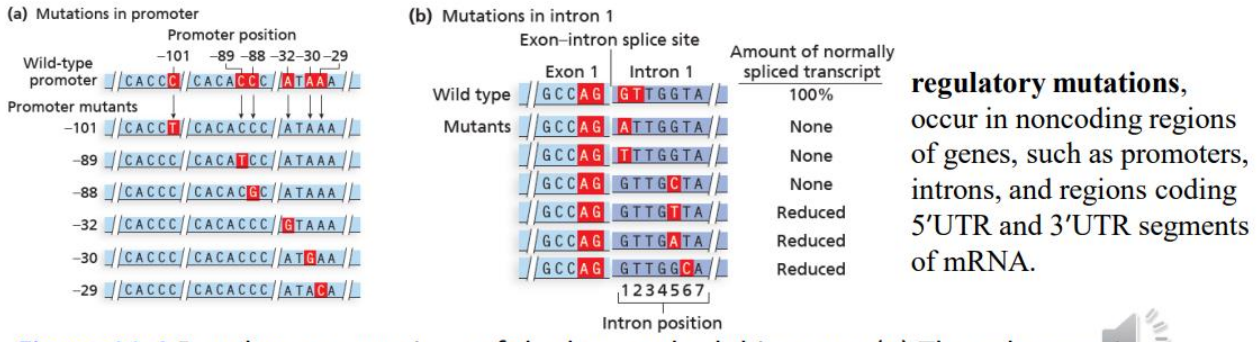


Figure 11.4 Regulatory mutations of the human b-globin gene. (a) These base-pair substitution mutations in the promoter reduce, increase or complete elimination of transcription of the gene. (b) These base-pair substitutions in intron 1 reduce or eliminate normal pre-mRNA splicing.

-splice site mutation→acceptor, donor, cryptic=skipping of exons or retention of introns

Genetic Variation II

• Describe the functional effect of mutations

• Give examples of loss of function, gain of function,

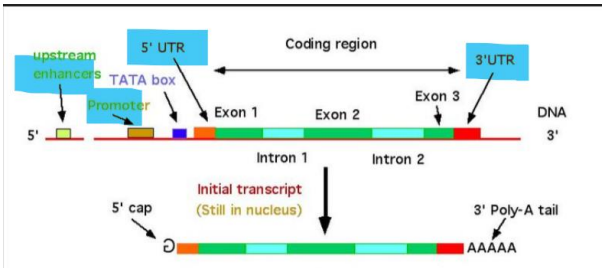
haploinsufficiency, dominant negative effects,

• Genotype-phenotype correlations

Different mutation classes

• A gene cannot be restricted to a coding sequence because it also contains sequences necessary for its expression, i.e. the promoter, the 5 and 3 untranslated regions (5UTR and 3UTR),The polyadenylation signal, and intronic sequences that have to be very precisely excised to reconstitute the exact coding sequence of the messenger RNA (or several coding sequences if the gene is alternatively spliced).

• Therefore, a mutation in a gene will have different effects depending on its site and its nature.(+,-,complete loss)



-5UTR, 3UTR

-Promotor

-enhancer, suppressor

-transcription factors: promotes the binding to the sequences to regulate transcription

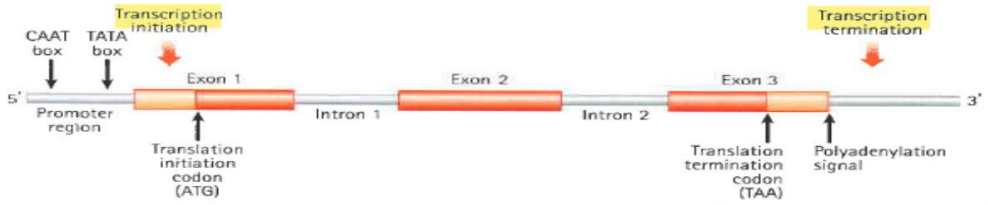
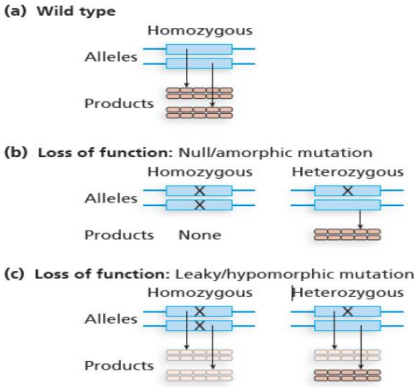


Fig. 2.6
Representation of a typical human structural gene.

• The loss of function mutations causes

– A decrease or a loss of the gene product (quantitative mutations): amount OR

– A decrease or a loss of the activity of the gene product (qualitative mutations);fx



The expression of the products of wild-type alleles produces wild-type phenotype

Null alleles produce no functional product. Homozygous null organisms have mutant (amorphic) phenotype due to absence of the gene product (e.g. nonsense or stop-gain mutations, frameshift and splice-altering mutations).

Leaky mutant alleles produce a small amount of wild-type gene product. Homozygous organisms have a mutant (hypomorphic) phenotype. (e.g. nonsynonymous or missense mutations).

b) null, amorphic mutation:

homo: 2 mutant alleles,, hetero: 50% production

-mutation either prevents the synthesis of the protein or promotes the synthesis of protein incapable of carrying out any fx(activity or product)

c) leaky, hypomorphic:

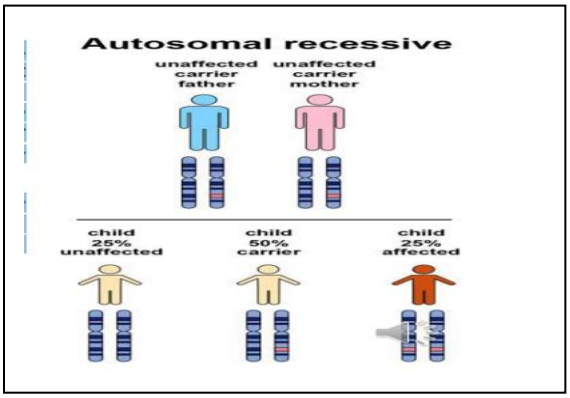
produces a mutant protein that functions less effectively or less in the amount compared to the wild-type, but it is more than 50% production(more than hetero null)

-loss of fx mutation is usually recessive, 2 alleles should be mutated to get the phenotype

• Loss of function mutations frequently have a recessive effect compared with the functional gene effect. In heterozygous: the wild-type allele having an activity

sufficient to compensate for the loss of the mutated gene.

• This is the case for most of the recessive diseases (β–thalassemia,cystic fibrosis, haemophilia,Duchenne/Becker myopathy)



-however, there are some dominant functional loss mutations:

Dominant-negative variants

-resulted in a mutated protein that interferes with the normal fx of the wild-type protein

-the mutant protein will dominate the wild-type protein, preventing it from functioning correctly

- A dominant-negative variant, a mutated protein disrupts the function of the normal protein. This often happens when proteins work in groups or complexes.
- Dominant-negative variants can complicate genetic disorders, as the presence of one mutant allele can significantly disrupt normal protein function even if the other

allele is normal. This contrasts with simple recessive mutations, where one normal allele is often sufficient to maintain normal function.

-example: the tumor suppressor gene P53, a mutation in it, will change it to tumor producing gene

-the dominant negative variant happens in multimeric proteins(multi-subunits):

Different subunits are encoded by different genes, and a mutation in one of these subunits will cause a toxic effect on the remaining normal subunits interfering with their act.

Dominance and recessivity are explained by molecular pathology

Example: Fibrillin-1 Gene (FBN1):

Normal Function: Fibrillin-1 is a protein that is essential for the formation of elastic fibers found in connective tissue.

Dominant-Negative Variant: In Marfan syndrome, mutations in the FBN1 gene can lead to the production of defective fibrillin-1, which disrupts the integrity of connective tissues, resulting in symptoms like long limbs, flexible joints, and cardiovascular problems.

Mechanisms of dominant disease

haploinsufficiency, dominant negative, or gain-of-function effects

• Haplo-insufficiency: Loss-of-function mutations in the heterozygous state in which half normal levels of the gene product result in phenotypic effects.

-haploinsufficiency: the wild type allele is insufficient to do normal fx, since it is dominant, 1 allele is sufficient to cause the defect

-it differs than neg dominant, it does not result from interfering, it is an abnormal protein per se.

-it can be homozygous or heterozygous, homo is more severe

-the most severe mutations are frameshift and nonsense→pathogenic variant→loss of fx of the alleles due to premature termination

-The mutant allele has novel function that produces a mutant phenotype in homozygous and heterozygous organisms and may be more severe in homozygous organisms.

Figure 9.30 Haploinsufficiency: Some loss-of-function mutant alleles are dominant to wild-type alleles. The *GLI3* gene is haploinsufficient.
heterozygotes *GLI3* exhibit polydactyly (extra fingers and toes).

Polydactyly in both the grandpa and grandchild due to GLI3 mutation at heterozygote level

At the homozygote level, polydactyly varies at the level of toes and fingers(expressivity)

To sum up:

Loss of fx mutation has different types: reduction in the gene production or the activity/ blocking transcription/ deletion for part or whole gene leads to complete loss of fx

-loss of fx can be qualitative(activity) or quantitave(amount)→ if lost completely→it is known as null(zero) or amorphic(without form)

-it may be also a partial loss to the activity and fx→known as leaky mutation or hypomorphic → reduced form but still there is activity

-the phenotype depends on the percentage of the activity or amount of gene product,

The lower the percentage than the normal, the worse the phenotype, and vise versa

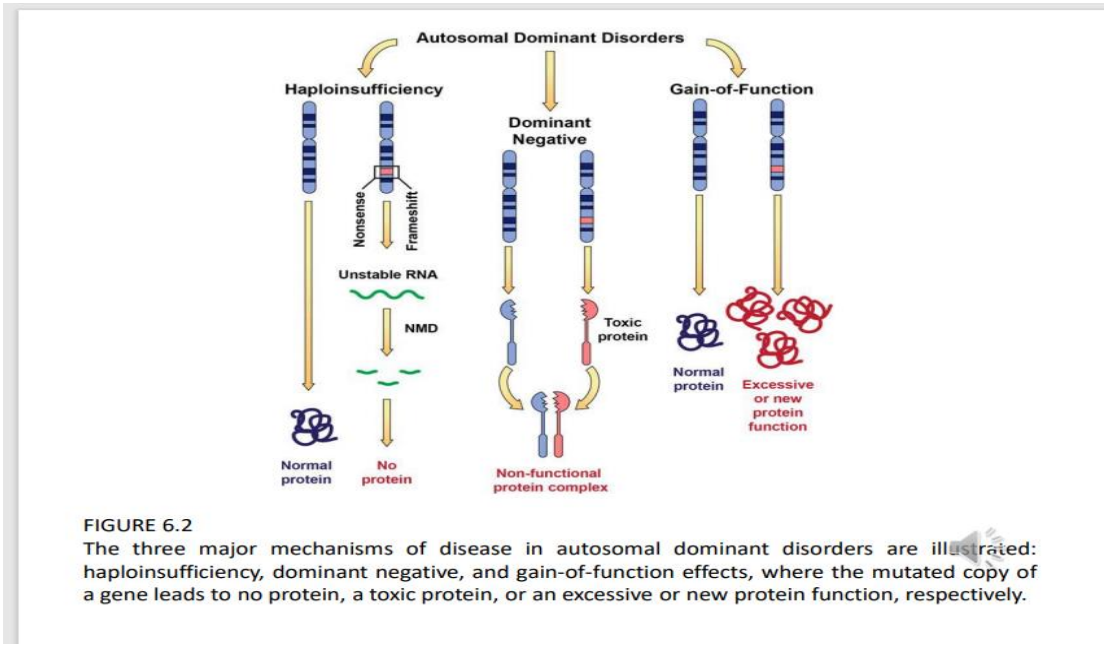


FIGURE 6.2
The three major mechanisms of disease in autosomal dominant disorders are illustrated: haploinsufficiency, dominant negative, and gain-of-function effects, where the mutated copy of a gene leads to no protein, a toxic protein, or an excessive or new protein function, respectively.

Although the majority of loss of fx mutations are recessive, there are AD disorders of loss of fx mutations though:

1-haploinsufficiency (-)

2-dominant negative: in multimeric proteins, subunits make complex to make functional proteins requiring all genes to be normal, a mutation in one subunit(polypeptide) will have toxic interfering and spoiling effect on the normal polypeptides, ex: p53 and collagen

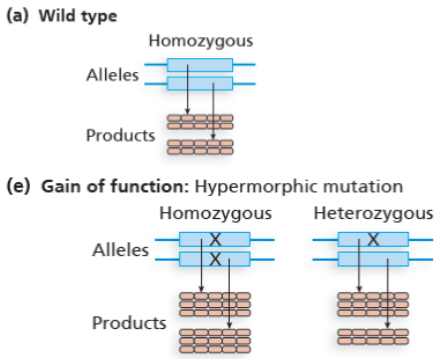
-collage is trimeric protein(3 polypeptides): 2 subunits of COL1A1, 1 subunit of COL1A2, a hetero mutation in COL1A1 or homo in COL1A2 will cause OI(osteogenesis imperficta)

3-gain of fx(+):

Gain of function mutations: Hypermorphic

• The gain of function mutations causes an increase in the amount of gene product (quantitative mutation) or an increase in its activity

– Sometimes creates a new property, leading to a toxic product responsible for a pathological effect (enhanced activity or activation at the wrong time and place)



Excessive expression of the gene product leads to excessive gene action. The mutant phenotype may be more severe or lethal in the homozygous genotype than in the heterozygous genotype.

• Hypermorphic allele where the protein activity cannot be switched off.

• e.g. Receptor usually is only activated if bond by ligands. A receptor that transduce signals without ligands is Constitutively Active

-Cell signaling often starts with an extracellular molecule (called ligand, peptides or chemicals) binding to its specific receptor protein. The conformational change of the receptor leads to the activation of the enzymatic activity of the receptor or its downstream binding proteins

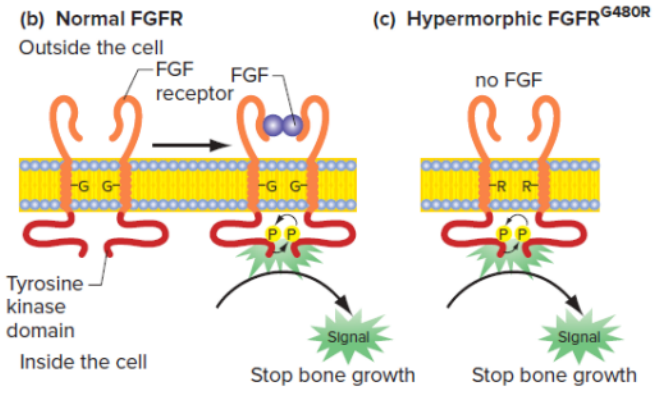


Figure 9.31: **(b)** FGFR3 encodes a dimeric transmembrane receptor that is normally activated only when it is bound to the hormone FGF. The tyrosine kinase domain of one activated FGFR3 subunit adds phosphate groups (P in yellow circles) to the other subunit and vice versa. These phosphorylations initiate a signal that ultimately stops bone growth. **(c)** Mutant FGFR3 (p.G480R) protein is always activated, whether FGF is present or not, leading to improper bone development.

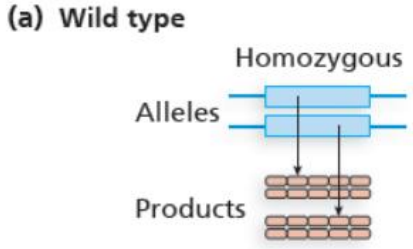
Example: Achondroplasia: FGFR3: p.G480R

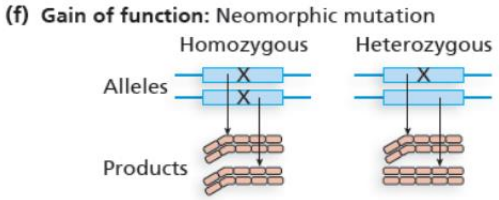
-fibroblast growth factor receptor 3 (FGFR3) works normally when binds to the FGF ligand leading to growth inhibition if a heterozygous substation mutation happens from Gly to Arg at position 480, the receptor will be overactivated even in the absence of the ligand, meaning inhibition of growth → leads to achondroplasia.

Another example: myeloproliferative neoplasms (MPNs), particularly essential thrombocythemia: JAK2: p.V617F, substitution mutation from Val to Phe at position 617 causes overgrowth of bone marrow cells and increases platelets.

Gain of function mutations: Neomorphic

• Gain-of-function mutations resulting from neomorphic ("new form") mutations acquire novel gene activities not found in the wild type and are usually dominant.





Genetic Analysis An Integrated Approach by John L. Bowman, Mark L. Sanders (z-lib.org)

• Some neomorphic alleles produce mutant proteins with new functions or properties - notice the pictures-.

• Function of the gene is completely changed → new product is maybe being produced or the gene is being expressed in a new location/new time (normal protein but in the wrong time or place).

• It can be homozygotes or heterozygotes however, Homozygotes for a neomorphic allele may exhibit a more severely affected phenotype than do heterozygotes.

• Cancers are one of the best examples on gain of function mutations.-the doctor said you can READ these two examples;)-

• Gain of Novel Function: Unlike loss-of-function mutations that reduce or eliminate the normal activity of a gene, neomorphic mutations lead to a new function

- Example: The BCR-ABL fusion protein in chronic myeloid leukemia (CML) gains abnormal tyrosine kinase activity.

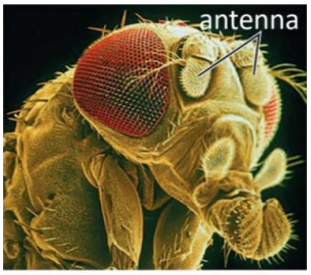
• Altered Protein Activity: The mutated gene product (protein) may have a completely new activity not present in the wild-type protein.

- Example: IDH1 and IDH2 Mutations in Gliomas
- Normal Function: Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are enzymes involved in the citric acid cycle, converting isocitrate to αketoglutarate (α-KG).
- Neomorphic Mutation: Mutations in IDH1 (e.g., R132H) and IDH2 (e.g., R172K) lead to a new enzymatic activity that converts α-KG to 2-Hydroxyglutarate (2-HG), an oncometabolite.
- Impact: 2-HG accumulation disrupts cellular metabolism contributing to oncogenesis. This leads to altered gene expression and promotes the growth of gliomas and other cancers.

• Ectopic Expression: The gene may be expressed in different tissues or at different times compared to its normal expression pattern, potentially leading to abnormal effects.

• The gene is expressed in tissues or developmental stages where it is normally inactive but this type of mutation switches on the gene in the wrong place or time.

• Example: Antennapedia mutation, where a gene that normally specifies leg development is aberrantly expressed in the head where normally there are antennae, leading to the development of legs instead of antennae.

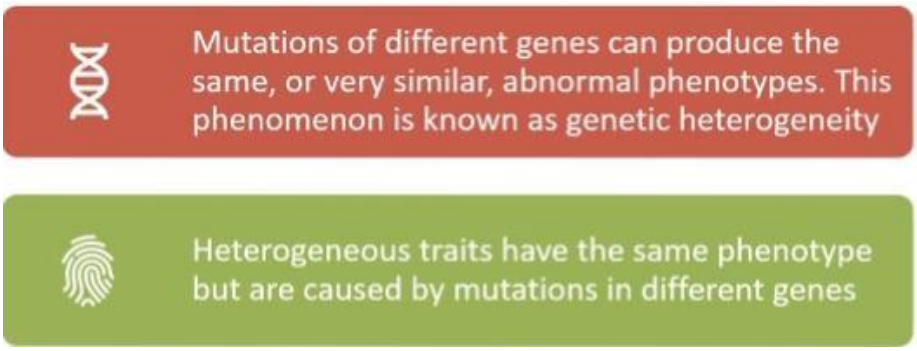


Normal fly

Mutant fly

Genetic heterogeneity can be explained by molecular pathology

• People can have the same phenotype but if we did genetic analysis we will find different mutations in different genes.



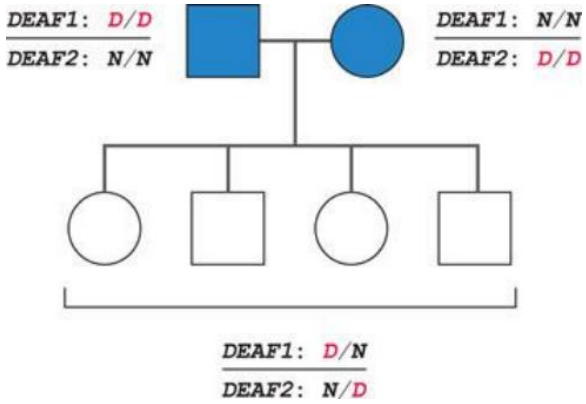
Locus heterogeneity: one disease/several genes

• The simplest way to consider a Mendelian disease is as a monogenic disease, meaning a disease for which every patient is affected in the same gene (one disease/one gene).

- E.g. phenylketonuria, cystic fibrosis Beta thalassemia and familial mediterranean fever.
- The disease is monogenic

• Locus heterogeneity: Different genes within the genome can cause the same clinical condition. This can complicate genetic diagnosis and research because identifying a single causative gene might not explain the genetic basis for a condition in all patients.

• Locus heterogeneity explains how parents who are both affected with the same common recessive disorder produce multiple unaffected children.



•**Deafness** in humans can be caused by mutations in ~ more than 60 different genes

• In this pedigree, you can notice that both parents are deaf, but none of their children is affected. This is because the mutant gene that caused deafness in the father is different from the one that caused it in the mother. So, the father has homozygous or compound heterozygous mutations that are different from the ones that are found in the mother and vice versa. The children, on the other hand, are heterozygotes for the mutations, which is not enough to cause the disease.

• **Hypertrophic Cardiomyopathy:** Over 20 genes involved.

• **Retinitis pigmentosa**

- A common cause of visual impairment due to photoreceptor degeneration associated with abnormal pigment distribution in retina.
- known to occur in AD, AR, and X-linked forms

Allelic heterogeneity: one gene/several diseases

• Allelic heterogeneity occurs when different mutations arise within the same gene locus result in same phenotype but with variability -variable Phenotypes-.

• These mutations can involve various types of genetic alterations, including point mutations, insertions, deletions, and structural rearrangements.

Allelic Heterogeneity in Cystic Fibrosis (CF):

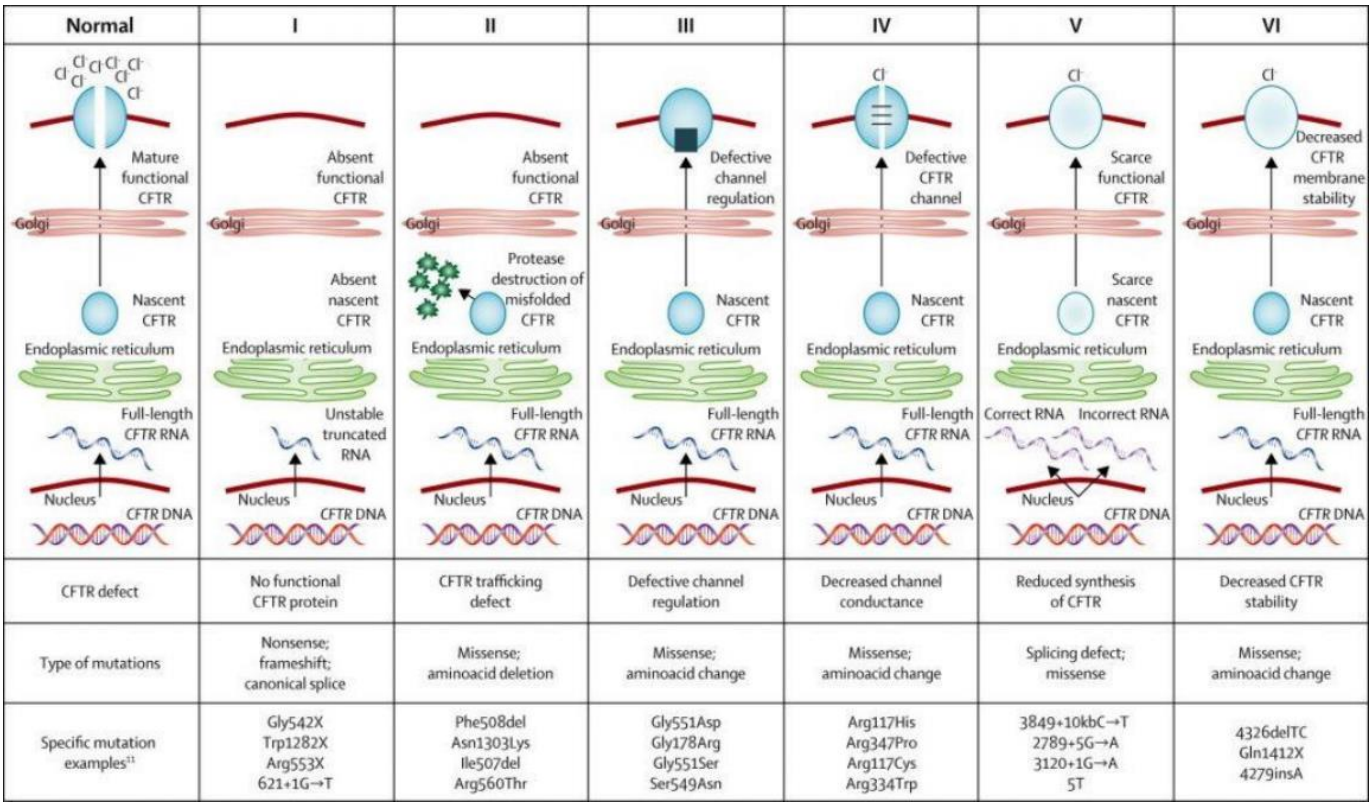
• CF is primarily caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene located on chromosome 7.

• The CFTR gene encodes a protein involved in the regulation of Chloride and Sodium ion transport across cell membranes.

•Example: >2000 different mutations in CFTR in cystic fibrosis.

- Some mutations cause: Only the abnormality of the male reproductive tract, Not the normal cystic fibrosis.
- Some mutations cause: Pancreatic insufficiency, severe progressive lung disease and congenital absence of vas deferens in males. (classic form)
- Some mutations cause: lung disease but normal pancreatic function.

- As you can see the phenotypes are highly variable



CF classification - Study the figure carefully -

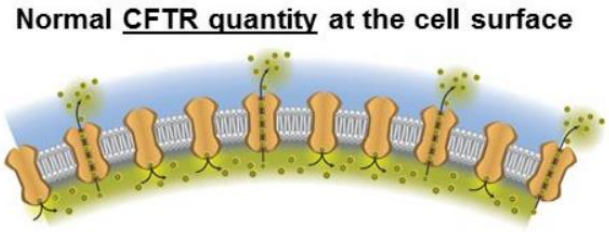
- CF is an autosomal recessive disease, there are 6 classes of this disease depending on the mutation and its impact on the function of the protein.
- In healthy individuals, the CFTR is formed inside the cell then moves to the cell membrane -ponder the figure, it starts by transcription → translation → folding → etc. -, then it will act as a channel that will allow Chloride to flow out the cell.
- Mutations can reduce CFTR or alter its function.
- Disruption of Chloride flowing out will increase sodium absorption into epithelial cells, which will cause blockage and thick mucus production in multiple organs such as the lungs, pancreas, etc.
- Class I: the synthesis of CFTR is defected. Note that there is transcription with no translation, so no CFTR is produced. Mutations that cause this class are mostly nonsense, frameshift or splice site mutations, which are severe.
- Class II: the defect is in the processing and trafficking, caused by defects in post-translational modifications, folding, or transporting the protein to the cell surface. Mutations are usually missense, frameshift or deletion.
- Class III: the defect is in the CFTR channel gate, normal production but the channel's open probability is reduced or blocked. Mutations are missense, which are less intense than nonsense mutations.

- Class IV: there is a defect in the CFTR channel conductance, the protein is at the surface but the ions movement is impaired.
- Class V: there is reduction in the synthesis of CFTR proteins, the mutations are usually splice site mutations.
- Class VI: there is a reduction in the stability of the CFTR proteins, their turnover will be accelerated.
- In conclusion, not all CF patients share the same phenotype, but they have some similarities.
- -the patient may come just infertile, without the usual phenotype of mucus thickening

• CFTR activity can be defined as the total ions transported by the CFTR channels in the cell surface, which can be determined by the quantity – number of CFTR channels on the cell surface -or function – the functional ability of each channel to open and transfer the ions- of CFTR.

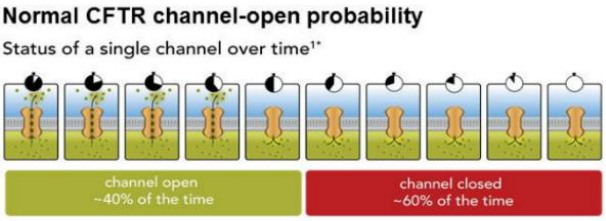
• CFTR quantity is determined by:

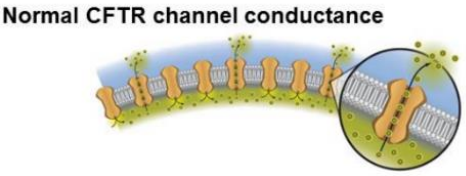
- CFTR synthesis: normal transcription, proper splicing and normal translation.
- CFTR processing and trafficking: maturation the CFTR and its delivery to the cell surface.
- CFTR surface stability: the amount of time of CFTR at the cell surface before it is removed.



• Function of CFTR is determined by:

- Channel-open probability: the fraction of time that a single CFTR protein channel is open and transporting ions. In the normal state, it is 40% open and 60% closed.
- Channel conductance: rate at which ions move through open channels





Clinical and genetic heterogeneity

• Many diseases present a clinical heterogeneity (phenotypic heterogeneity) characterized by the severity of the symptoms, by the severity or the way the disorder evolves.

• One part of the clinical heterogeneity can be explained by allelic heterogeneity, by the fact that different mutations can have variable phenotypic effects, either in their type or in their strength.

• Another part of the clinical heterogeneity, notably between affected individuals within the same family, can be explained by the effect of modifying genes – different than the original genes- that can increase or decrease the effect of pathological mutations in the gene principally involved, without being responsible for the appearance of the pathology.

• Finally, the effect of the environment on the appearance of the disease and on its clinical variability should not be neglected. Can be a positive or negative effect. Could affect the age of onset.

اللهم صلّ على سيدنا محمد وعلى اله وصحبه وسلّم

A 34-year-old woman comes to the office due to dysuria. The patient has a history of recurrent urinary tract infections. A urine sample is collected and sent for culture. Gram-negative bacteria isolated from the urine are found to form pink colonies on lactose-containing MacConkey agar. Several days later, bacterial isolates from a second urine sample are found to form white colonies when plated on the same type of medium. Genetic analysis shows that the more recent isolates have a single nucleotide deletion within the *lac* operon DNA sequence. This genomic change is most consistent with which of the following?

- A. Conservative mutation
- B. Frameshift mutation
- C. Missense mutation
- D. Nonsense mutation
- E. Silent mutation

An 8-year-old boy of Ashkenazi Jewish ancestry is brought to the office after developing reduced sensitivity to pain, impaired tear formation, and orthostatic hypotension. Familial dysautonomia is suspected due to the patient's symptoms and heritage. This disorder is caused by loss of function of the IKAP protein, which is essential for development and survival of sensory and autonomic neurons. *IKAP* gene sequencing reveals a single nucleotide substitution that causes a guanine residue to be replaced by adenine at the highlighted position in the normal gene sequence shown below. Exon sequences are represented by capital letters and introns by lowercase letters.

5'-GTACAAACATTGCTGCTGGGAAAGCCGCCGCCGCCACCATG
GGCTATGGGAGTGGTGGTAGCACTGGAGTTAACACCGAAATTG
GCAAGATCCGGGATGAAATGGTGG AACAGAACAGGAGAGAAC
ACCCCTTCAGCAAAAACTAGATGAATTTAAAGTCATCTCCCTTA
TTTGCATTGCAGGCTGGATCATAAATATTGGGCACTTCAATGAC
CCGGTTGATGGAGGGTCCTGGATCAGAGGTGCTATTTACTACT
TAAAATTGCAGTGGCCCTGGCTGTAGCAGGTGATTCCATTCCT
AAGgtctgcctgcagtcatcaccacctgcctggctctggaactcgcagaagaaaaatgcc
cattggaagcctcccgtctgtggaaaccttggttgtacttctgttatctgctcagacaagactgg
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AAGCTATGGGAGTGGTGGTAGCAACTGGAGTTAAACCGAAATT
GGCAAGATCCGGGATGAAATGGTGGCAACAGAACAGGAAGAA
CACCCCTTCAGCAAAAACTAGATGAATTTGGGGAACAGCTTTCC
AAAGTCATCTCCCTTATTTGCATTGCAGTCTGGATCATAAATTTG
GGACTTCAATGACCCGGTTCATGGAGgtcmgatcagaggtgctatttacta
ctttaaaattgcagtggccctggctgtagcagccattcctgaaggtctgcctgcagtcatcacac
ctgccGggctctGggaactcgcagaatggcaaagaaaaatgccattgttcgaagcctcag

Which of the following is the most likely effect of this mutation?

- A. Decreased mRNA export to the cytosol
- B. Impaired ribosomal attachment to mRNA
- C. Incorrect splicing of pre-mRNA
- D. Increased degradation of mRNA by 5' exonucleases
- E. Translation of the 3'-untranslated region of mRNA

B C