جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

الامراض الوراثية والتشخيص الجزيئي

المرحلة الرابعة

الفصل الدراسي الثاني

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Lec 1: Introduction in Genetics Disease

Introduction in Genetics Disease All diseases have a genetic component. However, the extent to which genes contribute to disease varies and much remains to be learned. Advances in understanding the genetic mechanisms behind these diseases enable the development of early diagnostic tests, new treatments, or interventions to prevent disease onset or minimize disease severity. This object provides information about the importance of clinical signs such as family history that may be suggestive of a genetic disease, the different uses of genetic testing, and the different types of genetic diseases. All diseases have a genetic component. Mutations may be inherited or developed in response to environmental stresses such as viruses or toxins. The ultimate goal is to use this information to treat, cure, or if possible, prevent the development of disease.

History and Physical Examination The diagnosis of a genetic disease requires a comprehensive clinical examination composed of three major elements: 1. a physical examination 2. a detailed medical family history 3. clinical and laboratory testing if available. Red Flags for Genetic Disease There are several factors that raise the possibility of a genetic disease in a differential diagnosis. One major factor is the occurrence of a condition among family members that is disclosed when the family history is obtained The occurrence of the same condition in more than one family member (particularly first-degree relatives), multiple miscarriages, stillbirths, and childhood deaths are all suggestive of a genetic disease. Additionally, family history of common adult conditions (heart disease, cancer, dementia) that occur in two or more relatives at relatively young ages may also suggest a genetic predisposition. Genetic Basis of Disease Rare Monogenic • Single mutations of large effect. • Environment less important. (Cystic fibrosis, sickle cell anemia,Duchenne muscular dystrophy

Common Polygenic • Many common genetic variants of small effect • Often strong role of environment (Type 2 diabetes, obesity) Monogenic Disorders Familial Hypercholesterolemia (FH) • Rare disease (1 in 500) • Very high cholesterol levels • Cholesterol deposits • Heart disease by age 60 Familial Hypercholesterolemia

Summary • For rare monogenic diseases, genetics plays a primary role • For common polygenic diseases, genetics and environment are both important • Genes for disease can be found through genetic association studies • Genetic studies have unveiled new biology and drug targets

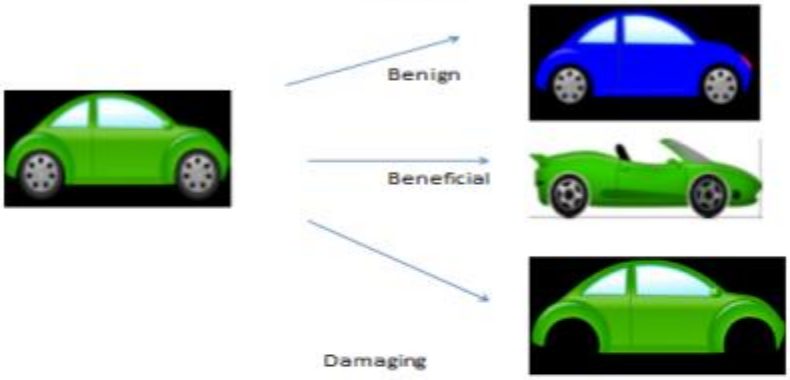
Human Genetic Variation

Humans are 99.9% identical: differ on average 1 in 1000 base pairs.

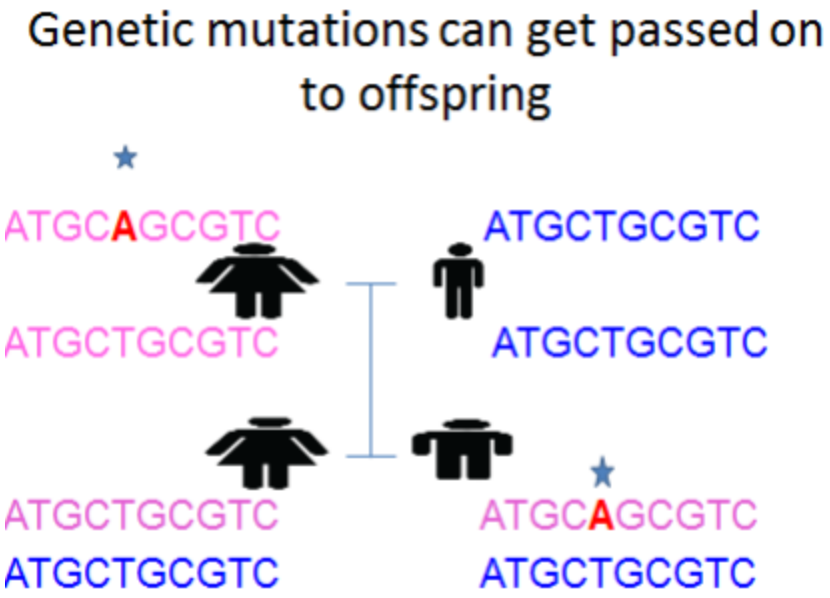
Genetic variants may be common or rare

Reference	A T G **T** G T C G T G C T G C T C
Person 1	A T G **T** G T C G T T C T G C T C
Person 2	A T G **T** G T C G T G C T G C T C
Person 3	A T G **A** G T C G T G C T G C T C
Person 4	A T G **T** G T C G T G C T G C T C
Person 5	A T G **A** G T C G T G C T G C T C
Person 6	A T G **A** G T C G T G C T G C T C
Person 7	A T G **A** G T C G T G C T G C T C
Person 8	A T G **T** G T C G T G C T G C T C
Person 9	A T G **T** G T C G T G C T G C T C
Person 10	A T G **A** G T C G T G C T G C T C

Genetic variants may have a range of effects



Genetic mutations



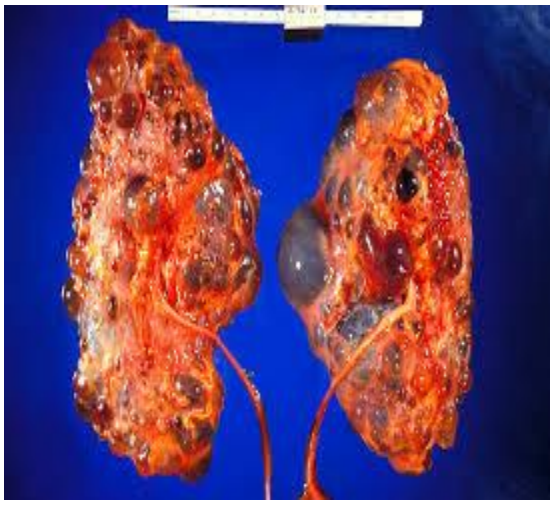
Genetic variant: Any DNA change that is present in the population Mutation: A genetic variant with an effect Conclusions -People have different DNA. -Combination of genes and environment cause disease. - Can find genetic variants that are associated with disease risk. -We can use knowledge of genetics to find new drugs

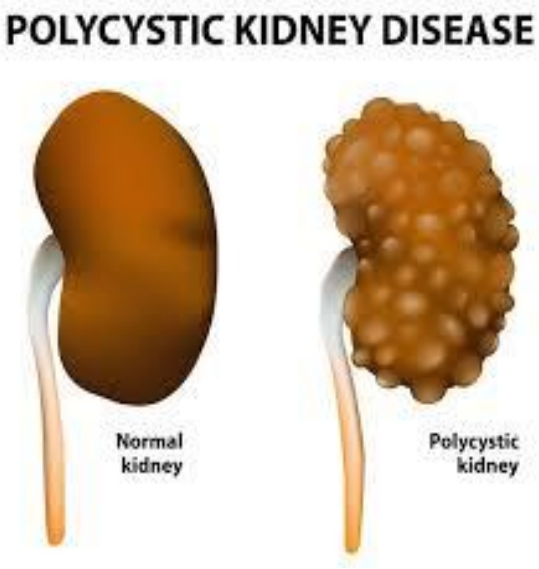
Lec 2

Polycystic kidney disease

Polycystic kidney disease (PKD) is an inherited disorder in which clusters of cysts develop primarily within a kidneys, causing the kidneys to enlarge and lose function over time. Cysts are noncancerous round sacs containing fluid. The cysts vary in size, and they can grow very large. Having many cysts or large cysts can damage the kidneys.Cyst development and growth is gradual, yet despite the massive growth of the kidneys.

Polycystic kidney disease also can cause cysts to develop in the liver and elsewhere in your body. The disease can cause serious complications, including high blood pressure and kidney failure.





Symptoms

Polycystic kidney disease symptoms can include:

- High blood pressure
- Back or side pain
- Headache
- A feeling of fullness in the abdomen
- Increased size of the abdomen due to enlarged kidneys
- Blood in the urine
- Kidney stones
- Kidney failure
- Urinary tract or kidney infections

main types of polycystic kidney disease

- **Autosomal dominant polycystic kidney disease (ADPKD).** Signs and symptoms of ADPKD often develop between the ages of 30 and 40. In the past, this type was called adult polycystic kidney disease.

  Only one parent needs to have the disease for it to pass to the children. If one parent has ADPKD, each child has a 50 percent chance of getting the disease. This form accounts for about 90 percent of cases of polycystic kidney disease.

The most common extrarenal manifestation of ADPKD is the development of hepatic cysts, which usually occur after the development of renal cysts, and are incidental findings in most patients. Other findings in ADPKD include pancreatic, thyroid, subarachnoid, and seminal vesicle cysts. The most lethal extrarenal manifestations of ADPKD are intracranial aneurysms, which has been found to be present in up to 40% of ADPKD patients. These

aneurysms can rupture, causing intracranial hemorrhage and death in 8% to 11% of patients.

- **Autosomal recessive polycystic kidney disease (ARPKD).** This type is far less common than is ADPKD. The signs and symptoms often appear shortly after birth. Sometimes, symptoms don't appear until later in childhood or during adolescence.

Both parents must have abnormal genes to pass on this form of the disease. If both parents carry a gene for this disorder, each child has a 25 percent chance of getting the disease.

In the most severe cases, ARPKD can be detected in utero by the presence of very large echogenic kidneys that occupy much of the abdominal cavity, along with oligohydramnios, due to inadequate renal development.

These patients typically display the characteristic ‘Potter’ phenotype, with findings that include pulmonary hypoplasia, extremity abnormalities, unusual facial appearances, and deformities of the spine, all of which can be attributed to lack of amniotic fluid. These patients often die in the neonatal period due to respiratory complications rather than renal failure, with their renal insufficiency rarely severe enough to be fatal.

Delayed presentations are also possible with ARPKD, with some patients having no clinical or laboratory abnormalities until later in childhood

Inheritance

ADPKD

ADPKD results from mutations in the genes *PKD1* or *PKD2*, which encode the proteins polycystin-1 and polycystin-2, respectively, with *PKD1* being located on the short arm of chromosome 16 (16p13.3 region) and *PKD2* on the long arm of chromosome 4 (4q21.2 region).

ARPKD

ARPKD is a disease primarily of infants and children and is caused by mutations at a single locus, the Polycystic Kidney and Hepatic Disease 1 gene (*PKHD1*), located on chromosome 6p12.

diagnosis

ADPKD

When ADPKD is suspected, patients should be evaluated for a family history of disease, with specific questioning spanning three generations. Although no consensus criteria have been established, with a negative family history of disease, a presumptive diagnosis can be made when there are bilateral renal cysts, and when two of the following criteria are met:bilateral renal enlargement, more than two hepatic cysts, presence of a cerebral aneurysm, or if there is a solitary cyst in the arachnoid, pineal gland, pancreas, or spleen. Given that the number of renal cysts increases with age, it has been proposed that three or more cysts, either unilaterally or bilaterally, is sufficient to make the diagnosis in patients between 15 to 39 years of age.

A gene based diagnosis of ADPKD is also possible, allowing for the detection of specific *PKD1* or *PKD2* mutations. This testing is not commonly performed, however, given the expense of the test and its ability to detect definitive mutations

ARPKD

Autosomal recessive PKD can typically be diagnosed based on clinical findings alone, with liver and kidney biopsies needed only in rare instances diagnosis is suggested by the presence of oligohydramnios,kidney enlargement, and the absence of urine in the fetal bladder, findings typically detectable by US at 18–20 weeks gestation.

DNA analysis by amniocentesis or chorionic villus sampling is currently not part of the routine evaluation of ARPKD patients, with its use typically limited to uncertain cases or for prenatal confirmation

molecular diagnosis

next-generation sequencing (NGS) by multiplexing individually bar-coded long-range PCR libraries and analyzing . The data analysis pipeline has been optimized and automated with Unix shell scripts to accommodate variant calls. analyzed by Sanger sequencing. the NGS method was superior to Sanger sequencing for detecting PKD gene mutations.

Lec 3

Burkitt's lymphoma

Burkitt lymphoma is a cancer of the lymphatic system, particularly B lymphocytes. Burkitt lymphoma is associated with impaired immunity and is rapidly fatal if left untreated. Burkitt lymphoma is named after British surgeon Denis Burkitt, who first identified this unusual disease in 1956.

Burkitt lymphoma is common in young children who also have malaria and Epstein-Barr virus , the virus that causes infectious mononucleosis. One mechanism may be that malaria weakens the immune system's response to Epstein-Barr virus, allowing it to change infected B-cells into cancerous cells. About 98% of African cases are associated with Epstein-Barr infection.

Burkitt's lymphoma

Types of Burkitt Lymphoma

-Endemic (African). Endemic Burkitt lymphomais associated with the Epstein Barr virus (EBV), primarily affects African children ages 4 to 7.

-Sporadic Burkitt lymphoma occurs worldwide. Globally.

-Immunodeficiency-associated. This variant of Burkitt lymphoma is most common in people with HIV/AIDS.

Genetics

Burkitt lymphoma results from chromosome translocations that involve the *Myc* gene. A chromosome translocation means that a chromosome is broken, which allows it to associate with parts of other chromosomes. The classic chromosome translocation in Burkitt lymophoma involves chromosome 8, the site of the *Myc* gene. This changes the pattern of *Myc*'s expression, thereby disrupting its usual function in controlling cell growth and proliferation.

Inheritance

Burkitt lymphoma (BL) is not an inherited condition. It almost always occurs in people with no family history of BL. To our knowledge, there has been one report (in 1986) describing BL in more than one family member (two sisters). However, this occurrence was thought to be due to an inherited lymphocyte disorder that may have predisposed the sisters to developing BL.

Molecular diagnosis

Gene-expression profiling is an accurate, quantitative method for distinguishing Burkitt's lymphoma.

Tumor-biopsy specimens from patients with aggressive lymphomas were profiled for gene expression and were also classified according to morphology, immunohistochemistry, and detection of the t(8) *myc* translocation.

A classifier based on gene expression correctly identified pathologically verified cases of classic Burkitt's lymphoma. Burkitt's lymphoma was readily distinguished by the high level of expression of *myc* target genes.

Multiple endocrine neoplasia

The term multiple endocrine neoplasia (MEN) include several distinct syndromes featuring tumors of endocrine glands, each with its own characteristic pattern. In some cases, the tumors are malignant, in others, benign.

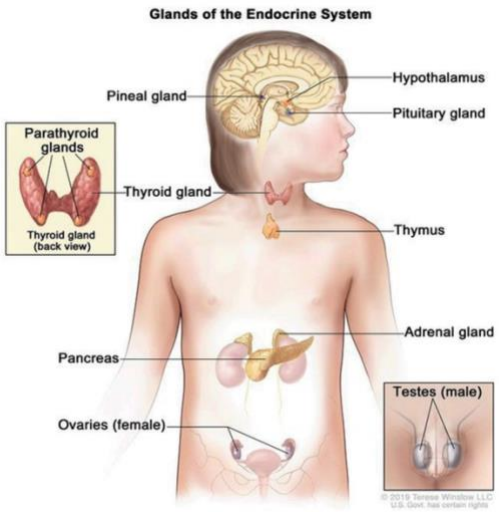
The major forms of multiple endocrine neoplasia are called type 1, type 2, and type 4. These types are distinguished by the genes involved, the types of hormones made, and the characteristic signs and symptoms.

-Type 1 frequently involves tumors of the parathyroid glands, the pituitary gland, and the pancreas. Tumors in these glands can lead to the overproduction of hormones.

- multiple endocrine neoplasia type 2 is a form of thyroid cancer . type 2 is divided into subtypes:

type 2A, type 2B (formerly called type 3).

-Multiple endocrine neoplasia type 4 appears to have signs and symptoms similar to those of type 1, although it is caused by mutations in a different genes.



Genetics

Mutations in the *MEN1*, *RET*, and *CDKN1B* genes can cause multiple endocrine neoplasia.

-Mutations in the *MEN1* gene cause multiple endocrine neoplasia type 1. This gene provides instructions for producing a protein called menin. Menin acts as a tumor suppressor, which means it normally keeps cells from growing and dividing too rapidly or in an uncontrolled way. *MEN1* gene localizes to chromosome 11q13.

-Mutations in the *RET* gene cause multiple endocrine neoplasia type 2. This gene provides instructions for producing a protein that is involved in signaling within cells. The RET protein triggers chemical reactions that instruct cells to respond to their environment, for example by dividing or maturing. Mutations in the *RET* gene overactivate the protein's

signaling function, which can trigger cell growth and division in the absence of signals from outside the cell. chromosome 10q11.

- Mutations in the *CDKN1B* gene cause multiple endocrine neoplasia type 4. This gene provides instructions for making a protein called p27. Like the menin protein, p27 is a tumor suppressor that helps control the growth and division of cells. Mutations in the *CDKN1B* gene reduce the amount of functional p27, which allows cells to grow and divide unchecked. This unregulated cell division can lead to the development of tumors in endocrine glands and other tissues. chromosome 12p13.

Inheritance

Multiple endocrine neoplasia type 1 usually has an autosomal dominant pattern of inheritance. People with this condition are born with one mutated copy of the *MEN1* gene in each cell. In most cases, the altered gene is inherited from an affected parent. The remaining cases are a result of new mutations in the *MEN1* gene and occur in people with no history of the disorder in their family.

Multiple endocrine neoplasia type 2 and type 4 are also inherited in an autosomal dominant pattern. In these cases, one copy of the mutated gene is sufficient to cause the disorder. individuals often inherit an altered *RET* or *CDKN1B* gene from one parent with the condition

Molecular diagnosis

Before MEN can be diagnosed it must be suspected. Suspicion should be raised in any patient with a family history of endocrine tumors of the pancreas, family members with pituitary or parathyroid disease or a family history of endocrinopathy.Identification of a gene mutation in the polymerase chain reaction by nucleic acid sequencing and restriction analyses.

Lec 4

Neurofibromatosis

The neurofibromatoses are a heterogeneous group of hereditary cancer syndromes that lead to tumors of the central and peripheral nervous systems, as well as other organ systems.

The diseases traditionally known as neurofibromatosis have now been formally separated into two types: neurofibromatosis type 1 or NFl (the type described by von Recklinghausen) and neurofibromatosis type 2 or NF2 (a much rarer form).' It is now recognised that although they have overlapping features, including an inherited propensity to neurofibromas and tumours of the central nervous system .

Neurofibromatosis type 1

Neurofibromatosis type 1 (NFI) is the commonest form of neurofibromatosis and has a frequency of about 1 in 3000 Although the gene is almost 100% penetrant, the disease it self has extremely variable expressivity. About 30% of all NFl cases are considered to be new mutations. The high mutation rate may be due in part to the large size of the gene and its transcript, or possibly to the presence of sequences within the gene highly susceptible to mutation.

Typically, NFl is associated with the formation of multiple tumour types in addition to the neurofibromas, including optic gliomas, neurofibrosarcomas, astrocytomas, meningiomas, ependymomas, and phaeochromocytomas.

Neurofibromatosis type 1 is a condition characterized by changes in skin coloring (pigmentation) and the growth of tumors along nerves in the skin, brain, and other parts of the body. The signs and symptoms of this condition vary widely among affected people. Beginning in early childhood, almost all people with neurofibromatosis type 1 have multiple which are flat patches on the skin that are darker than the surrounding area. These spots increase in size and number as the individual grows older. Freckles in the underarms and groin typically develop later in childhood. Most adults with neurofibromatosis type 1 develop neurofibromas, which are noncancerous (benign) tumors that are usually located

on or just under the skin. These tumors may also occur in nerves near the spinal cord or along nerves elsewhere in the body. Some people with neurofibromatosis type 1 develop cancerous tumors that grow along nerves. These tumors, which usually develop in adolescence or adulthood, are called malignant peripheral nerve sheath tumors.

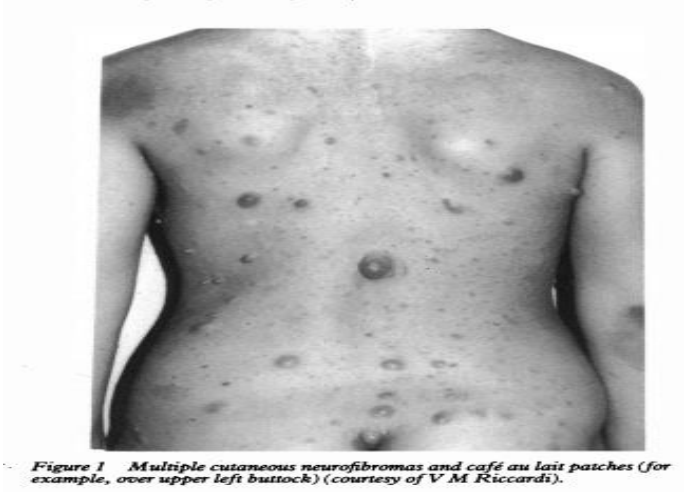


Figure 1 Multiple cutaneous neurofibromas and café au lait patches (for example, over upper left buttock) (courtesy of V M Riccardi).

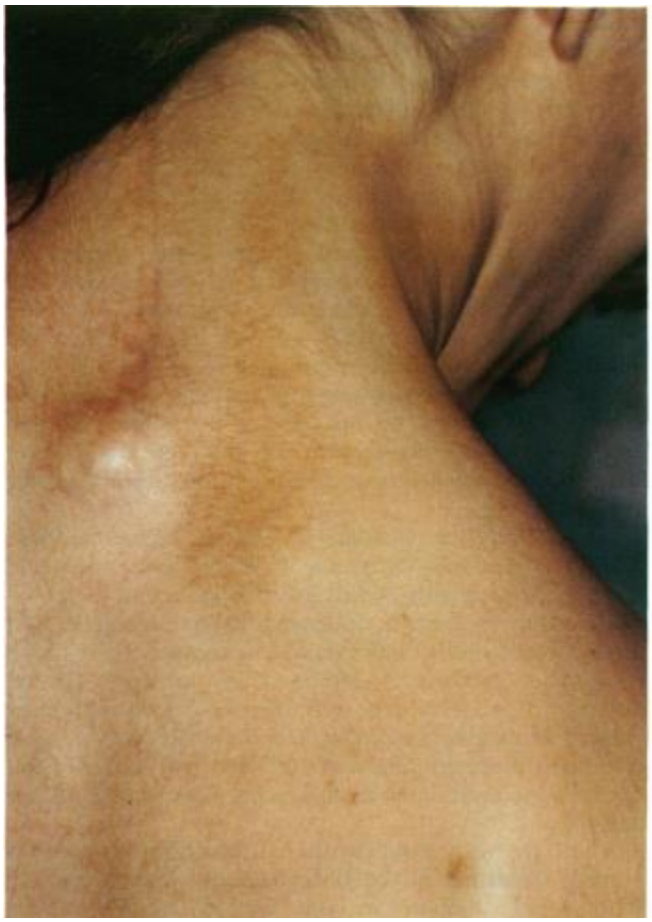
Picture 1 NF1

Neurofibromatosis type 2

neurofibromatosis type 2 (NF2), formerly known as central neurofibromatosis, has been defined as an entity distinct from neurofibromatosis type 1 only in the last decade or so. Clinical""" and genetic evidence" have now confirmed this. Since this division, a clearer, but also continuously evolving, clinical picture 2 of NF2. NF2 is a much rarer disease than NFI, with a population incidence of 1 in 33000-40000.

Neurofibromatosis type 2 is a disorder characterized by the growth of noncancerous tumors in the nervous system. The most common tumors associated with neurofibromatosis type 2 are called vestibular schwannomas or acoustic neuromas. These growths develop along the nerve that carries information from the inner ear to the brain (the auditory nerve). Tumors that occur on other nerves are also commonly found with this condition. The signs

and symptoms of neurofibromatosis type 2 usually appear during adolescence or in a person's early twenties, although they can begin at any age. The most frequent early symptoms of vestibular schwannomas are hearing loss, ringing in the ears (tinnitus), and problems with balance. In most cases, these tumors occur in both ears by age 30. If tumors develop elsewhere in the nervous system, signs and symptoms vary according to their location.



Picture 2 Paraspinal subde area ofhyperpigmentation and subcutaneous schwannoma overlying a spinal tumour in a patient with NF2

Genetics

Mutations in the *NF1* gene cause neurofibromatosis type 1. The *NF1* gene provides instructions for making a protein called neurofibromin. This protein is produced in many cells, including nerve cells and specialized cells surrounding nerves, Neurofibromin acts

as a tumor suppressor, which means that it keeps cells from growing and dividing too rapidly or in an uncontrolled way. Mutations in the *NF1* gene lead to the production of a nonfunctional version of neurofibromin that cannot regulate cell growth and division. As a result, tumors such as neurofibromas can form along nerves throughout the body.

Mutations in the *NF2* gene cause neurofibromatosis type 2. The *NF2* gene provides instructions for making a protein called merlin (also known as schwannomin). This protein is produced in the nervous system, particularly in Schwann cells, which surround and insulate nerve cells (neurons) in the brain and spinal cord. Merlin acts as a tumor suppressor, which means that it keeps cells from growing and dividing too rapidly or in an uncontrolled way.

Inheritance

Neurofibromatosis can either be an inherited disorder or the product of a gene mutation. Both NF1 and NF2 are caused by two separate abnormal genes and may be inherited from parents who have NF or may be the result of a mutation in the sperm or egg cells. NF is considered an autosomal dominant disorder..The gene for NF1 is located on chromosome 17. The gene for NF2 is located on chromosome 22. Children have a 50 percent chance of inheriting the genes that cause NF if the parent has NF. The type of NF the child inherits will be the same as that of the parent. Therefore, if the parent has NF1, there will be a 50 percent chance the child will have NF1. If the parent has NF2, there will be a 50 percent chance the child will have NF2.

The only difference between the child and the parent in these circumstances is the severity of NF and the appearance of symptoms. The presence of only one changed or affected gene can cause the disorder to appear. However, the action of the unaffected gene that is paired with the dominant gene does not prvent the disorder from appearing.

Molecular diagnosis

next-generation sequencing protocol usin identify *NF* mutations for the diagnosis of patients with a prototypic genetic syndrome, neurofibromatosis types. In addition, other

causative genes for classic genetic syndromes were set as the target genes for coverage analysis.

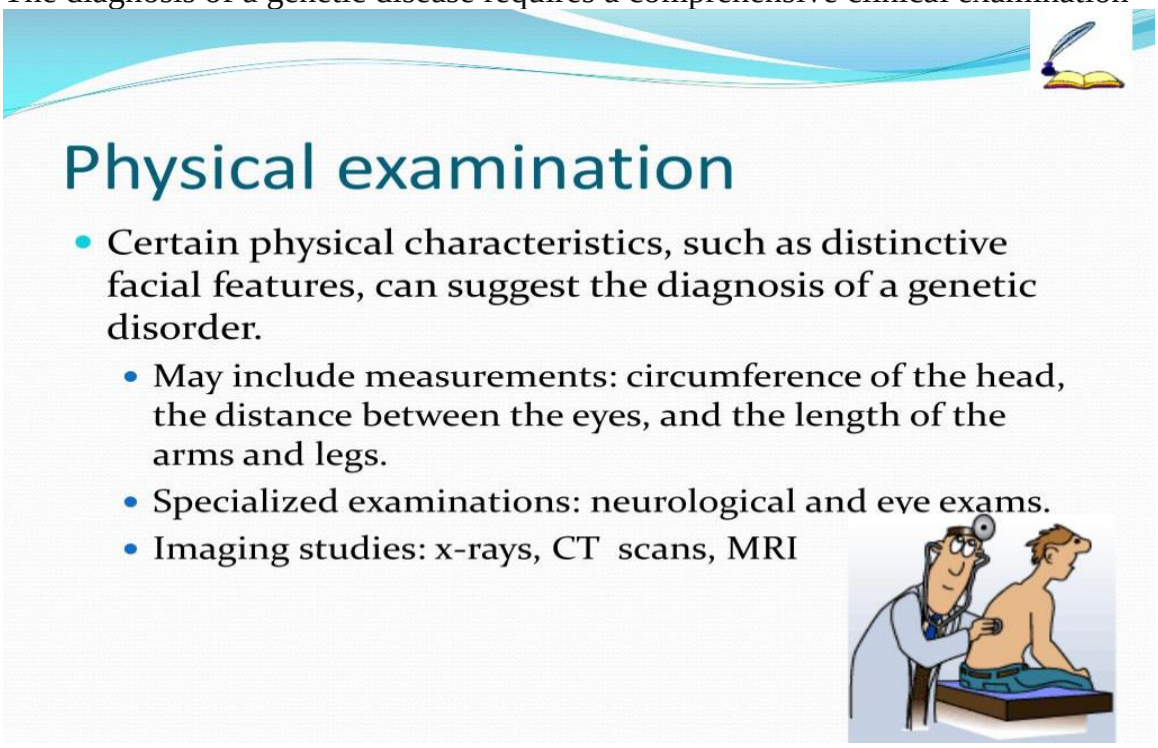
Lec. 5

Diagnosis of a Genetic Disease

a diagnosis of a genetic condition on the basis of a person's physical characteristics and family history, or on the results of a screening test.

1- **History and Physial Examination**

The diagnosis of a genetic disease requires a comprehensive clinical examination



Types of Genetic Testing

Several different methods are currently used in genetic testing laboratories. The type of test will depend on the type of abnormality that is being measured. In general, three major types of genetic testing are available—cytogenetic, biochemical, and molecular

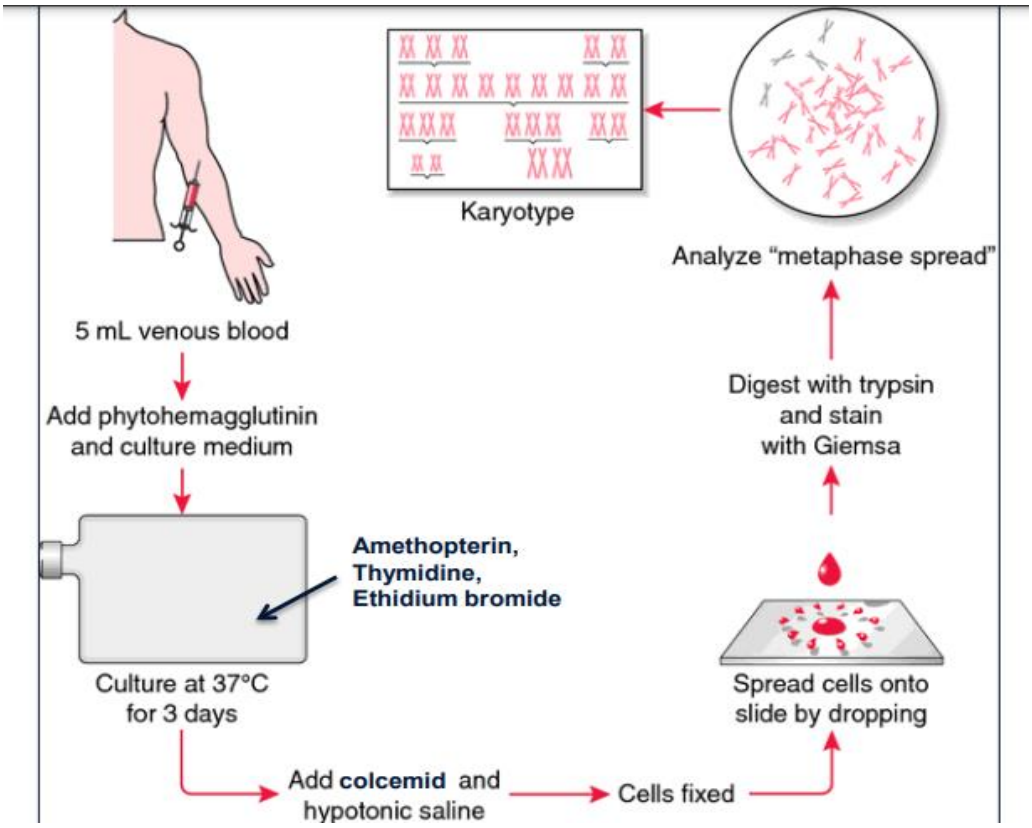
testing to detect abnormalities in chromosome structure, protein function, or DNA sequence, respectively.

1. **Cytogenetic Testing**

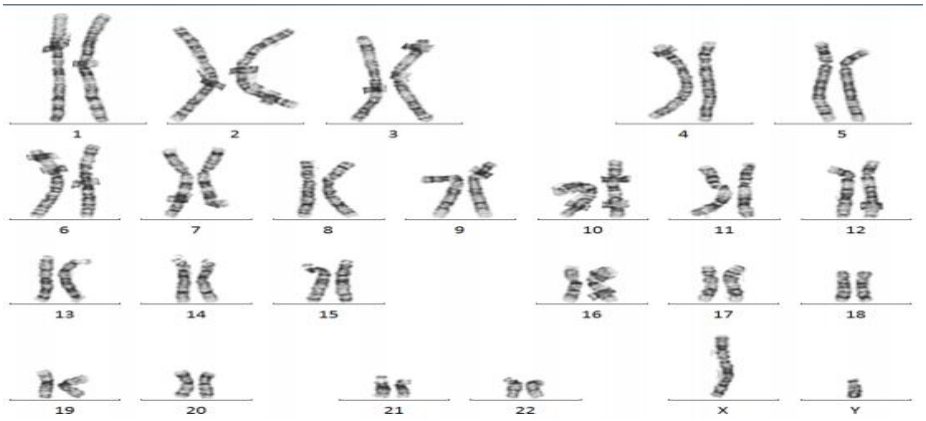
Cytogenetics involves the examination of whole chromosomes for abnormalities. Chromosomes of a dividing human cell can be clearly analyzed under a microscope. White blood cells, specifically T lymphocytes, are the most readily accessible cells for cytogenetic analysis since they are easily collected from blood and are capable of rapid division in cell culture. Cells from other tissues such as bone marrow (for leukemia), amniotic fluid (prenatal diagnosis), and other tissue biopsies can also be cultured for cytogenetic analysis.

Following several days of cell culture, chromosomes are fixed, spread on microscope slides and then stained. The staining methods for routine analysis allow each of the chromosomes to be individually identified. The distinct bands of each chromosome revealed by staining allow for analysis of chromosome structure. Such as

Modified from Preparation of a karyotype



Karyotyping



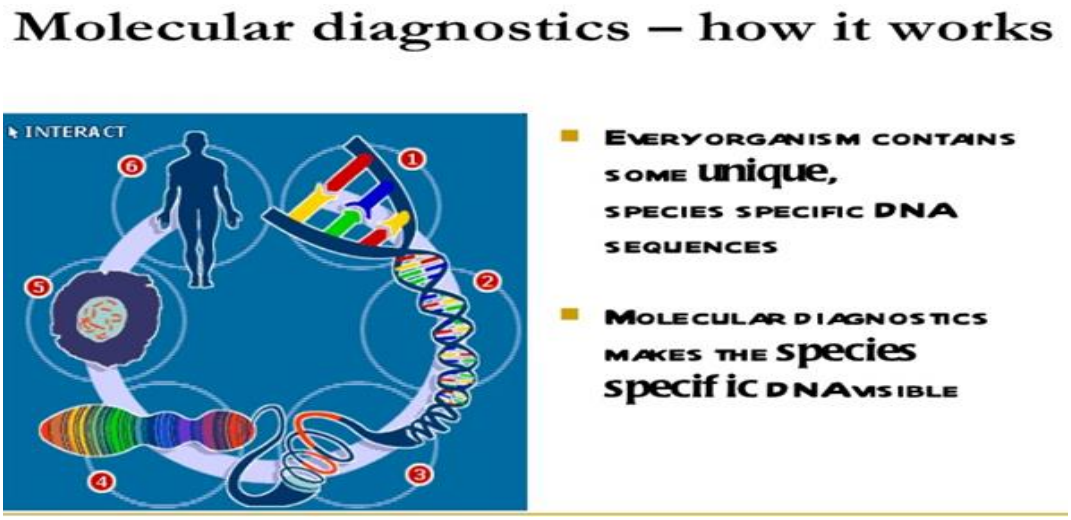
2. Biochemical Testing

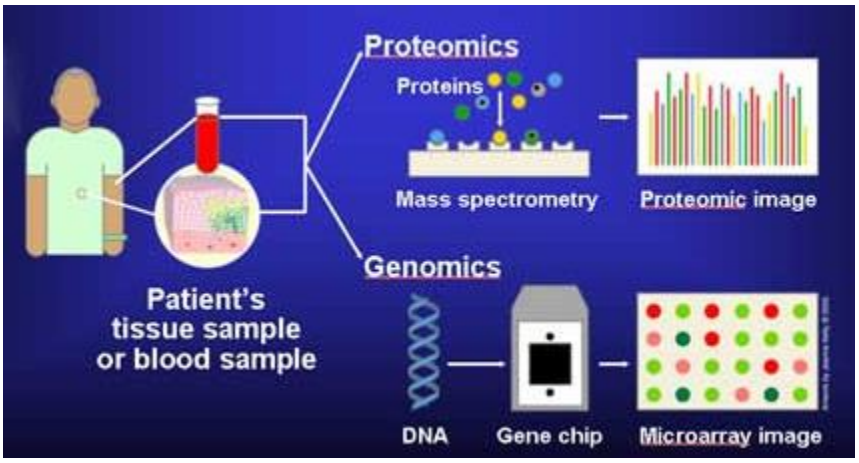
The enormous numbers of biochemical reactions that routinely occur in cells require different types of proteins. Several classes of proteins exist to fulfill the multiple functions, such as enzymes, transporters, structural proteins, regulatory proteins, receptors, and hormones. A mutation in any type of protein can result in disease if the mutation ultimately results in failure of the protein to correctly function.

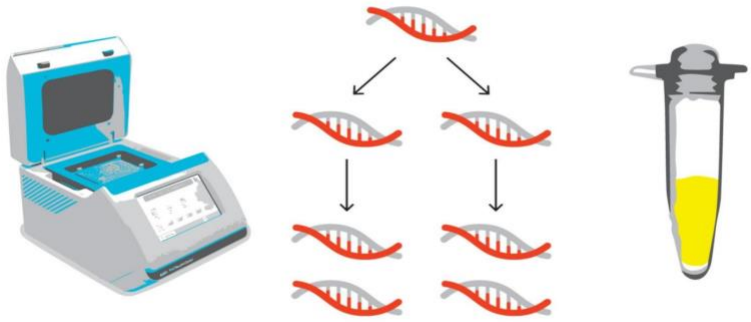
Clinical testing for a biochemical disease utilizes techniques that examine the protein instead of the gene. Depending on the function, tests can be developed to directly measure protein activity (enzymes), level of metabolites (indirect measurement of protein activity), and the size or quantity of protein (structural proteins). These tests require a tissue sample in which the protein is present, typically blood, urine, amniotic fluid, or cerebrospinal fluid. Because proteins are more unstable than DNA and can degrade quickly, the sample must be collected and stored properly and shipped promptly according to the laboratory's specifications.

3. Molecular Testing

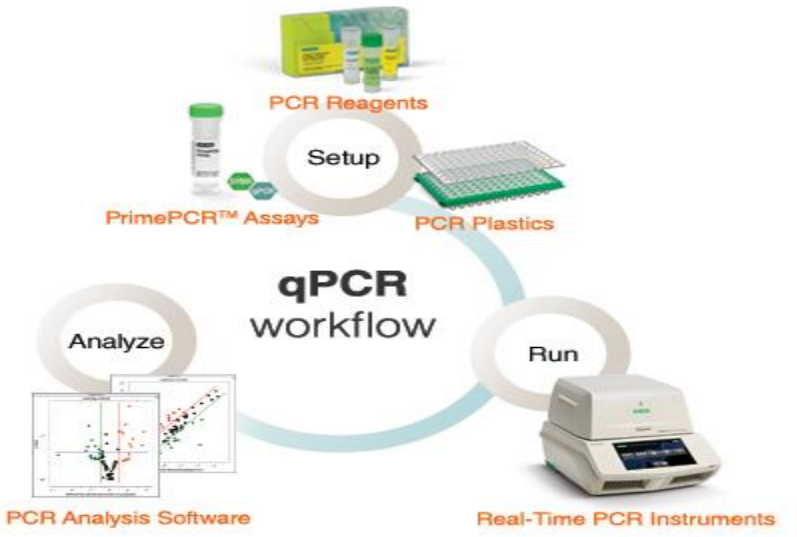
direct DNA testing may be the most effective method, particularly if the function of the protein is not known and a biochemical test cannot be developed. A DNA test can be performed on any tissue sample and require very small amounts of sample. For some genetic diseases, many different mutations can occur in the same gene and result in the disease, making molecular testing challenging.

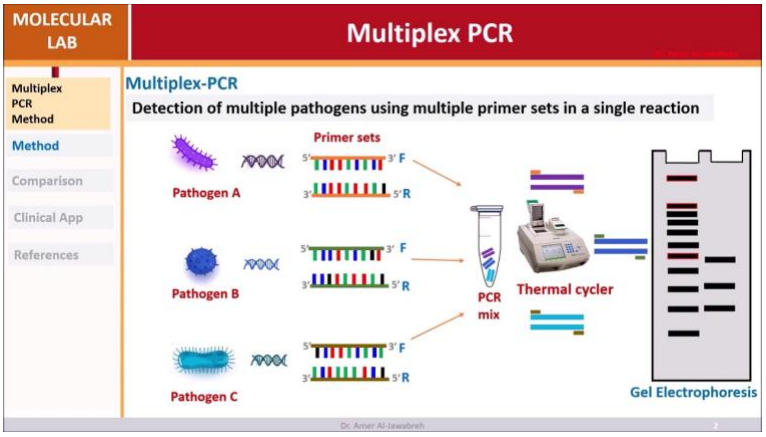






polymerase chain reaction





Uses of Genetic Testing

Genetic tests can be used for many different purposes like

- Newborn Screening
- Carrier Testing
- Prenatal Diagnosis
- Diagnostic/Prognostic
- Predictive/Predispositional

newborn screening : is screened for several genetic diseases. Early detection of these diseases can lead to interventions to prevent the onset of symptoms or minimize disease severity.

Carrier testing :can be used to help couples to learn if they carry—and thus risk passing to their children—a recessive allele for genetic diseases. If both parents are tested, the test can provide information about a couple's risk of having a child with a genetic condition.

Prenatal diagnostic testing: is used to detect changes in a fetus's genes or chromosomes. This type of testing is offered to couples with an increased risk of having a baby with a genetic or chromosomal disorder.

Diagnostic/PrognosticGenetic: tests may be used to confirm a diagnosis in a symptomatic individual or used to monitor prognosis of a disease or response to treatment.

Predictive or predispositional :genetic testing can identify individuals at risk of getting a disease prior to the onset of symptoms . Predictive testing can identify mutations that increase a person's risk of developing disorders with a genetic basis, such as certain types of cancer.

Lecture 6: Newborn screening (NBS)

A term widely used in clinical genetics encompassing the diverse techniques used to identify the molecular basis of genetic disease.

Examples of molecular genetic tests include:

• Genotyping to detect specific pathogenic variants;

• Sequencing of a gene to detect pathogenic variants;

• Amplification or hybridization methods (e.g., qPCR and array CGH)to detect copy number variants involving one or more genes

• Methylation-specific techniques to detect epigenetic changes that influence gene expression

• Exome and Genome sequence.

molecular genetic testing

• Is intended as a public health program to identify infants with treatable conditions before they present clinically. Screening means that disorders can be diagnosed before a baby gets sick. Detected and treated disease early, can lead to significant reduction in disease severity and possibly even prevention of the disease

• Some of the disorders do not show any symptoms at all until the damage has occurred.

• In some of these cases damage is not able to be repaired.

Newborn screening (NBS)

• Early 1990s, DNA from dried blood spots on filter paper was extracted.

• Subsequently, DNA testing was introduced into NBS, allowing the dual use of it in biochemical and molecular tests.

Within 48 hours of a child's birth, a sample of blood is obtained from a "heel stick," and the blood is analyzed for up to 50 diseases.

NSB program in Canada

• More than 95% of all children born in the United States are tested for a panel of diseases. About 3,000 newborns test positive for one of these severe

disorders.

• In Iraq, the Newborn Screening program has been started on April, 2013 as a pilot project taking two provinces: Baghdad and Karbala. for early identification of three assigned disorder which are, phenylketonuria (PKU) ,Galactosemia(GAL) and congenital hypothyroidism(CHT).

Newborn screen test in USA and Iraq

• Second tier molecular tests: Increase sensitivity or specificity of primary assay Cystic Fibrosis (CF)

• Clarify an ambiguous result Hemoglobinopathies

• Supplemental "Just in Time" assay Galactosemia

• Primary molecular test: When no other assay is available –e.g. severe combined immunodeficiency; spinal muscular atrophy

Current Molecular Testing in Newborn Screening Laboratories

• It can improve sensitivity and specificity.

• Increase the speed of diagnosis and treatment.

• Reduce the number of false-positives that can add significant cost to follow-up.

• Molecular testing allows for differentiation between specific disorders, such as sickle cell anaemia and sickle/beta-thalassemia.

In NBS, second-tier molecular testing is performed after a primary test using the same specimen

Lecture 7: Galactosemia

• Galactosemia is the inability to metabolize galactose. This results in toxic levels that cause serious damage to the kidneys and brain among other tissues.

• A rare genetic metabolic disorder that is inherited in an autosomal recessive manner.

• The birth incidence of classic galactosemia is about 1 per 50,000- 60,000 in the Caucasian population

• Classic galactosemia (type1) the most common and severe type, caused bynmutations in the GAL1 gene, and characterized by a complete deficiency of an enzyme called galactose-1-phosphate uridyl transferase(GALT).

• Galactokinase deficiency (type2)- caused by mutations in the GALK1 gene and characterized by a deficiency of the enzyme galactokinase 1.

• Galactose epimerase deficiency (Type3) caused by mutations in the GALKE gene and characterized by a deficiency of the enzyme UDPgalactose-4-epimerase.

Genetics

• The defect in enzymes leads to elevated levels of galactose.

• Galactose is the sugar found mainly in milk, dairy products and produced by the body.

Pathophysiology

Clinical features

1-Galactose (Hill Test): Slight elevations (up to 1. 20 mg/dL) can occur in normal neonates. Galactose metabolites are greatly elevated in infants with galactosemia, if they are receiving a lactose-containing formula or breast milk.

The Hill test is a fluorometric chemical spot test that measures galactose and galactose-1-phosphate.

2- GALT activity: The enzyme test depends upon fluorescence produced by the normal galactose enzyme cascade in red blood cells.

A temporarily abnormal result (absent fluorescent activity) is found in 1:2,000 infants which indicates enzyme activity <50 percent of normal.

Diagnosis

• To increase screening specificity, some programs perform a second-tier DNA test for enzyme or metabolite-positive cases, targeting the most common GALT pathogenic variants.

• False-positive results can occur when blood is put into an (EDTA) tube prior to being spotted onto filter paper deficiency. Transfusion with packed red cells prior to newborn screening can lead to falsely normal GALT enzyme activity.

In these cases it is preferable to proceed with DNA tests if there is a clinical suspicion for galactosemia.

Molecular test

Following an abnormal newborn screen, the diagnosis of classic galactosemia is confirmed by the demonstration of profound deficiency of the GALT enzyme in RBCs and identification of pathogenic variants within the GALT gene by molecular sequencing. States that include total galactose in their screening algorithm can confirm or exclude GALK or GALE deficiency by combining results from the enzyme assay and molecular testing.

Molecular test

• Every 3months in first year provided they are well

• Every 4months in second year

• Every six months till 14 years and

• Annually after 14 years and more frequent in adolescence especially in girls to check pubertal growth.

Review in outpatient (follow up):

Diet Milk and breast milk are not allowed on the modified diet.

• Soy based formula is alternative.

•If there is liver disease, give MCT Casein hydrolyzed protein

• After 1year Soy decrease and need to supplement Ca otherwise its depletion cause decrease in bone density.

• Many medications contain lactose, it should be checked. long-term complications have been noted in older children and adults with classic galactosemia because of endogenous galactose production.

These include :

• speech problems

• poor intellectual function

• neurologic deficits (predominantly extra pyramidal findings with ataxia) ovarian failure in females.

Thus, the need for regular monitoring and evaluation is important

Lecture8: Duchenne muscular dystrophy

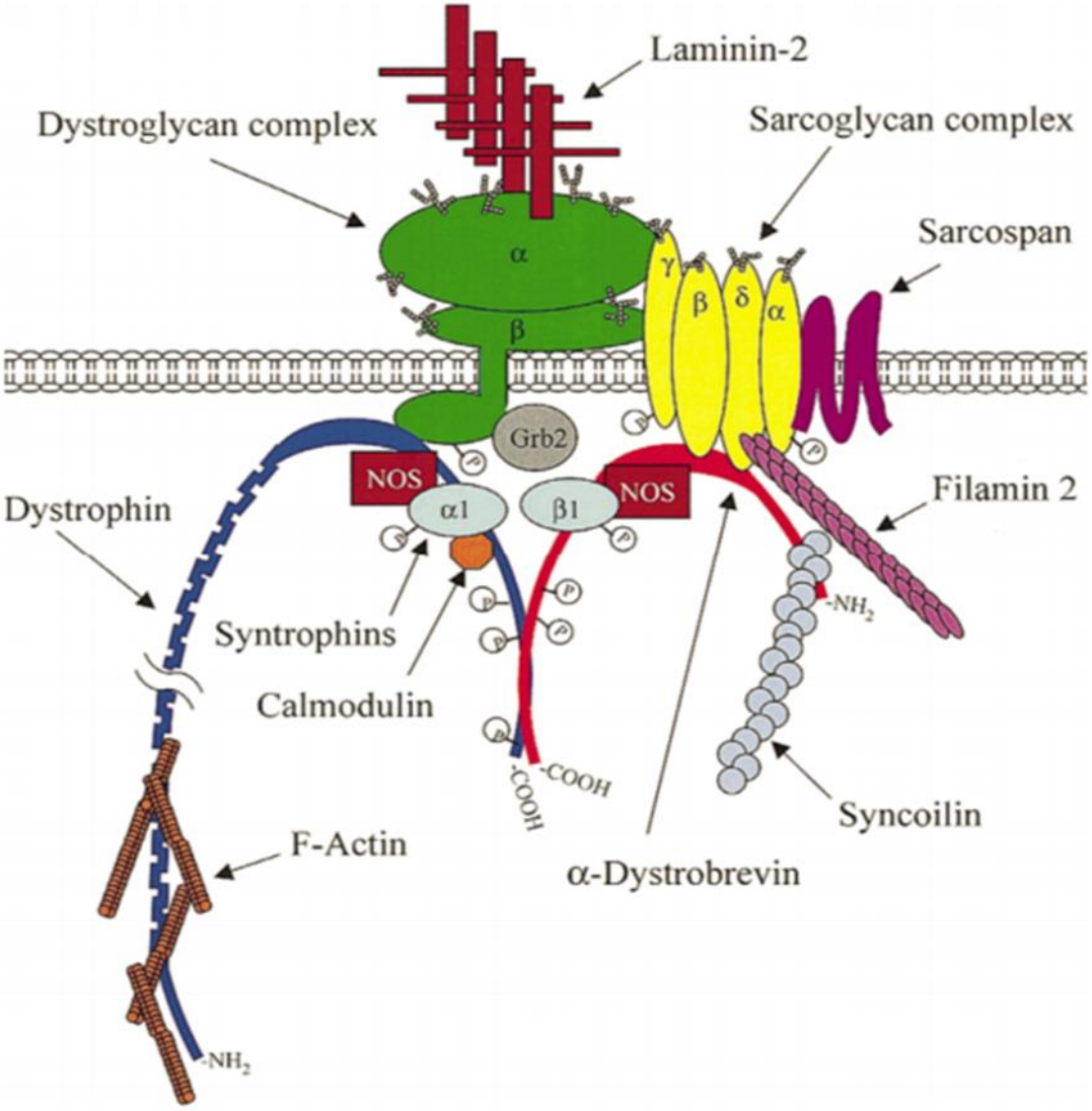
DMD is the most frequent muscular disorder in boys, with an incidence of 1 in 3,500 males The two forms of dystrophin-associated muscular dystrophies, : Duchenne muscular dystrophy DMD(complete absence of DMD protein) Becker muscular dystrophy BMD (truncated protein) caused by genetic defects in the huge DMD gene (Dystrophin)

Dystrophin protein

- Dystrophin is located at the muscle sarcolemma in a membrane-spanning protein complex that connects the cytoskeleton to the basal lamina.
- Dystrophin helps to protect muscle from contraction-induced injury and is

also important as an intracellular scaffold and binding partner, helping to regulate cell signalling *via* its interaction with a sarcolemmal protein complex termed the dystrophin-associated protein complex (DAPC)

- The lack of protein causes membrane destabilization and the activation of multiple pathophysiological processeses.

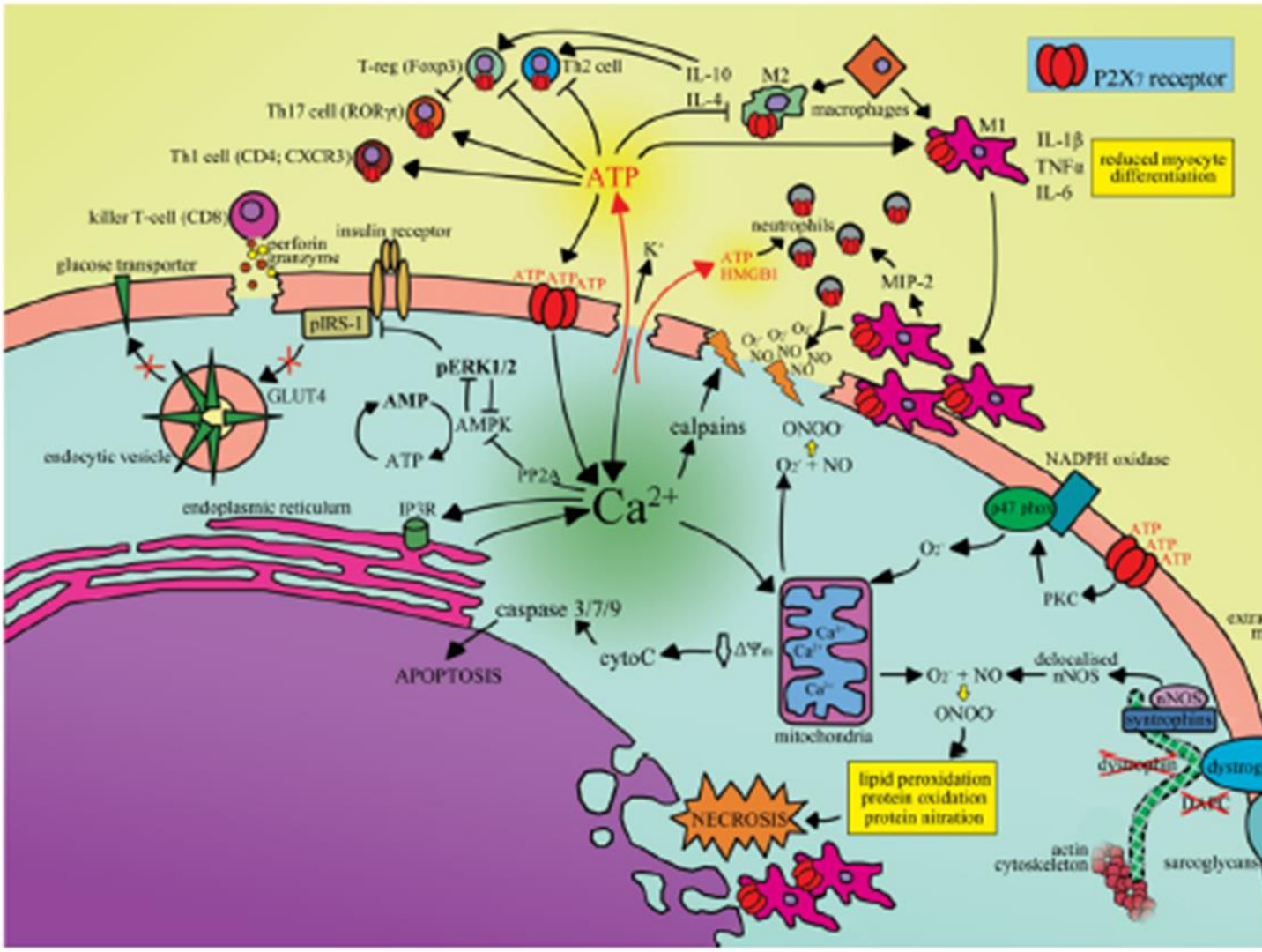

Laminin-2
Dystroglycan complex
Sarcoglycan complex
Sarcospan
α
β
γ
β
δ
α
Grb2
NOS
α1
β1
NOS
Dystrophin
Filamin 2
Syntrophins
Calmodulin
-NH2
-COOH
-COOH
Syncoilin
F-Actin
α-Dystrobrevin
-NH2

Mutation of DMD gene

The large size and complex structure of the dystrophin gene makes it vulnerable to mutations and therefore this gene has a high mutation frequency of 1 x 10–4 genes per generation.

- Two-third of the DMD cases is caused by deletion or duplication in one or more exons.

  - ❑ Nearly 65% of patients, deletions or duplications of one or more exons, with hot spots in the 5′ part (exons 3–20) and the central part (exons 44–55) of the gene
  - one-third of DMD patients is caused by point mutations



DNA Diagnosis in BMD/DMD Patients

- **Method**

multiplex PCR deletion analysis

60% of the patients have a deletion of one or more exons.

- **Diagnosis**

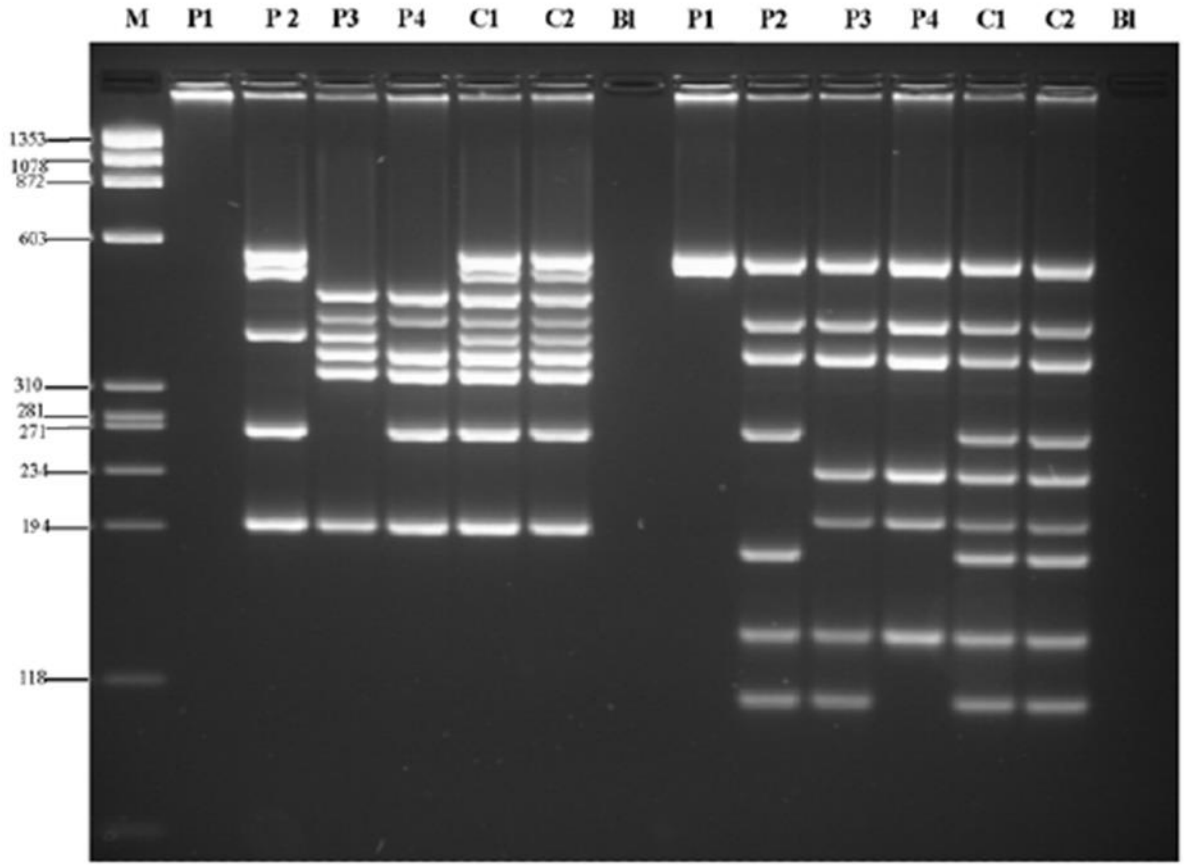
To identify a deletion in the patient's DNA, **two multiplex PCRs** should be preformed in which 9 exons can be amplified simultaneously, and 98% of all deletions could be identified After PCR and agarose gel electrophoresis

❖ **Result**

the absence of amplicons in the gel can simply be scored as a deletion of the corresponding exon

Preparation in Batches of Multiplex PCR Kits

1. Prepare the master mix for each kit. Final concentrations of the mix for the deletion detection kits are 1X multiplex PCR buffer, 10% DMSO, 1.5 m*M* dNTPs, 170 ng/µL BSA,
and 0.5 µ*M* of each primer.
2. To test the master mix, perform a PCR with **DNA of patients** with different deletions (positive controls), **normal controls**, and a no-**DNA control** .
3.Place the PCR tray in a preheated thermal cycler, set at 95°C (hot start). Perform an initial denaturation of 5 min at 95°C followed by 25 cycles of 94°C for 30 s, 53°C for 30 s, and 65°C for 4 min, with a final extension period of 5 min at 65°C. Cool down to 4°C after the PCR has finished.
4.Place the gel in an electrophoresis tank containing 1X TBE running buffer with 0.2 µg/mL ethidium bromide.
5. Load 25 µL of the PCR reaction on gel, including the no-DNA template reaction. Use a clean tip for each loading.
6. Electrophorese the gel for 2–3 h at 100–150 V. The bromophenol blue dye must migrate at least 10 cm into the gel for a good separation.
7. Prepare an image of the gel using an imaging system. Check for absence of product formation in the no-DNA control.



Gel Image of the two multiplex PCR assays for deletion detection in patients.
For Patient 1, a deletion of all DMD exons with the exception of exon 1 is detected (amplicon Pm is present). Patient 2 shows a minimal deletion of exons 8 to 19. In Patient 3, the exact deletion of exons 44 to 50 is characterized using this multiplex because exons 43 and 51 are present. Patient 4 has a minimal deletion of exons 45 to 52, the deletion may extend further at the 3′ end (exons 53 and beyond). Note that for all four patients, the observed deletion confirms the results of both multiplex PCRs.

Lecture 9: Cystic fibrosis (CF)

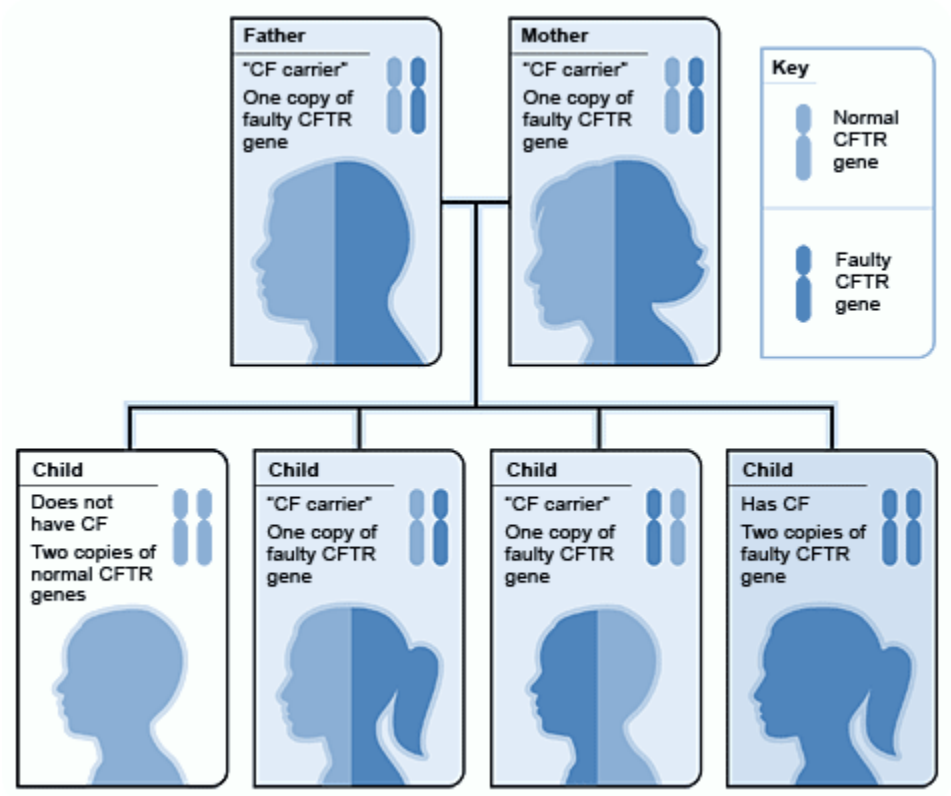
Cystic fibrosis (CF) is an inherited disorder that causes severe damage to the lungs, digestive system and other organs in the body.

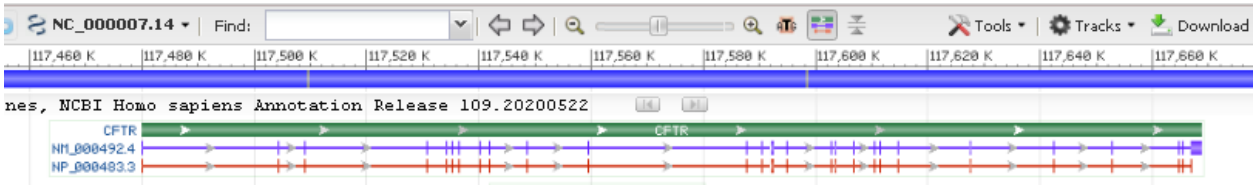
- Cystic fibrosis affects the cells that produce mucus, sweat and digestive juices. These secreted fluids are normally thin and slippery. But in people with CF, a defective gene causes the secretions to become sticky and thick. Instead of acting as lubricants, the secretions plug up tubes, ducts and passageways, especially in the lungs and pancreas.
- Although cystic fibrosis is progressive and requires daily care, people with CF are usually able to attend school and work. They often have a better quality of life than people with CF had in previous decades. Improvements in screening and treatments mean that people with CF now may live into their mid- to late 30s or 40s, and some are living into their 50s.

Inheritance Pattern for Cystic Fibrosis

- CF is a common autosomal recessive genetic disorder with a prevalence of ~1 in 2500 live births (two abnormal genes in order for the disease to develop)
- results from the presence of mutations in the CF transmembrane conductance regulator (*CFTR*) gene on chromosome 7q31
- A person inherits one abnormal copy of the *CFTR* gene (a carrier).

More than 1,500 *CFTR* sequence changes have been reported to the CF mutation database (http://www.genet.sickkids.on.ca/cftr/), including point mutations, small deletions and insertions, frameshifts, splice-site mutations, and exon deletions and duplications


Father
"CF carrier"
One copy of faulty CFTR gene
Mother
"CF carrier"
One copy of faulty CFTR gene
Key
Normal CFTR gene
Faulty CFTR gene
Child
Does not have CF
Two copies of normal CFTR genes
Child
"CF carrier"
One copy of faulty CFTR gene
Child
"CF carrier"
One copy of faulty CFTR gene
Child
Has CF
Two copies of faulty CFTR gene


NC_000007.14
Find:
Tools
Tracks
Download
117,460 K
117,480 K
117,500 K
117,520 K
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117,560 K
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nes, NCBI Homo sapiens Annotation Release 109.20200522
CFTR
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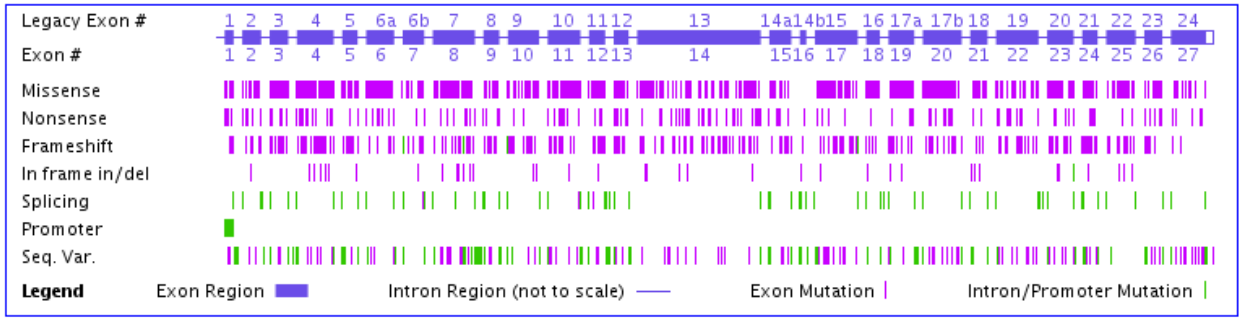
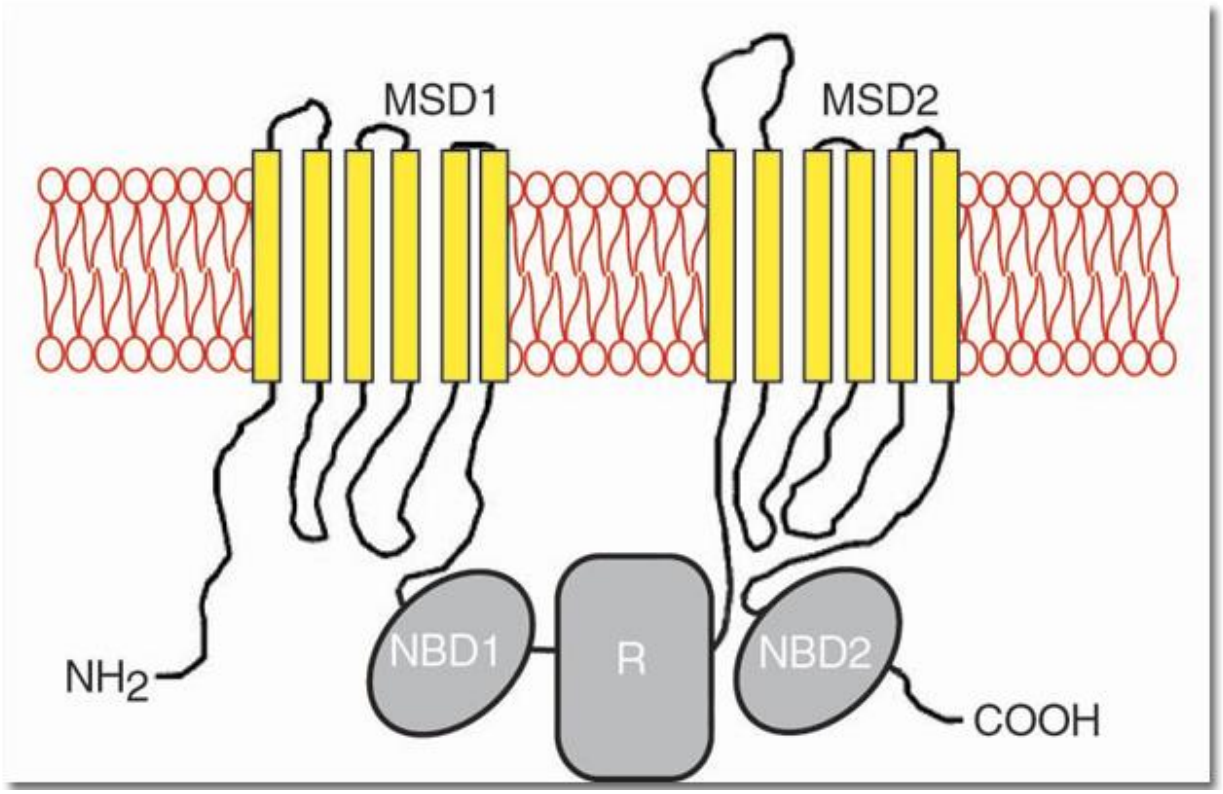
NP_000483.3


Legacy Exon #
1 2 3 4 5 6a 6b 7 8 9 10 11 12 13 14a 14b 15 16 17a 17b 18 19 20 21 22 23 24
Exon #
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Missense
Nonsense
Frameshift
In frame in/del
Splicing
Promoter
Seq. Var.
Legend
Exon Region
Intron Region (not to scale)
Exon Mutation
Intron/Promoter Mutation

The most common mutation, called **delta F508**, is a deletion of one amino acid at position 508 in the CFTR protein. The resulting abnormal channel breaks down shortly after it is made, so it never reaches the cell membrane to transport chloride ions.

Mechanism of disease

The *CFTR* gene provides instructions for making a protein called the cystic fibrosis transmembrane conductance regulator. This protein functions as a channel across the membrane of cells that produce mucus, sweat, saliva, tears, and digestive enzymes. The channel transports negatively charged particles called chloride ions into and out of cells. The transport of chloride ions helps control the movement of water in tissues, which is necessary for the production of thin, freely flowing mucus. Mucus is a slippery substance that lubricates and protects the lining of the airways, digestive system, reproductive system, and other organs and tissues. The CFTR protein also regulates the function of other channels, such as those that transport positively charged particles called sodium ions across cell membranes. These channels are necessary for the normal function of organs such as the lungs and reas.



The protein is comprised of two, six span membrane bound regions each connected to a nuclear binding factor which binds ATP. Between these two units is an R-domain which is comprised of many charged amino acids. The R-domain is a unique feature of CFTR within the ABC superfamily.

Respiratory signs and symptoms

The thick and sticky mucus associated with cystic fibrosis clogs the tubes that carry air in and out of your lungs. This can cause signs and symptoms such as:

- A persistent cough that produces thick mucus (sputum)
- Wheezing
- Exercise intolerance
- Repeated lung infections

- Inflamed nasal passages or a stuffy nose
- Recurrent sinusitis

Digestive signs and symptoms

The thick mucus can also block tubes that carry digestive enzymes from your pancreas to your small intestine. Without these digestive enzymes, your intestines aren't able to completely absorb the nutrients in the food you eat. The result is often:

Foul-smelling, greasy stools

Poor weight gain and growth

Intestinal blockage, particularly in newborns (meconium ileus)

Chronic or severe constipation, which may include frequent straining while trying to pass stool, eventually causing part of the rectum to protrude outside the anus (rectal prolapse)

Laboratory Diagnosis

Q-PCR

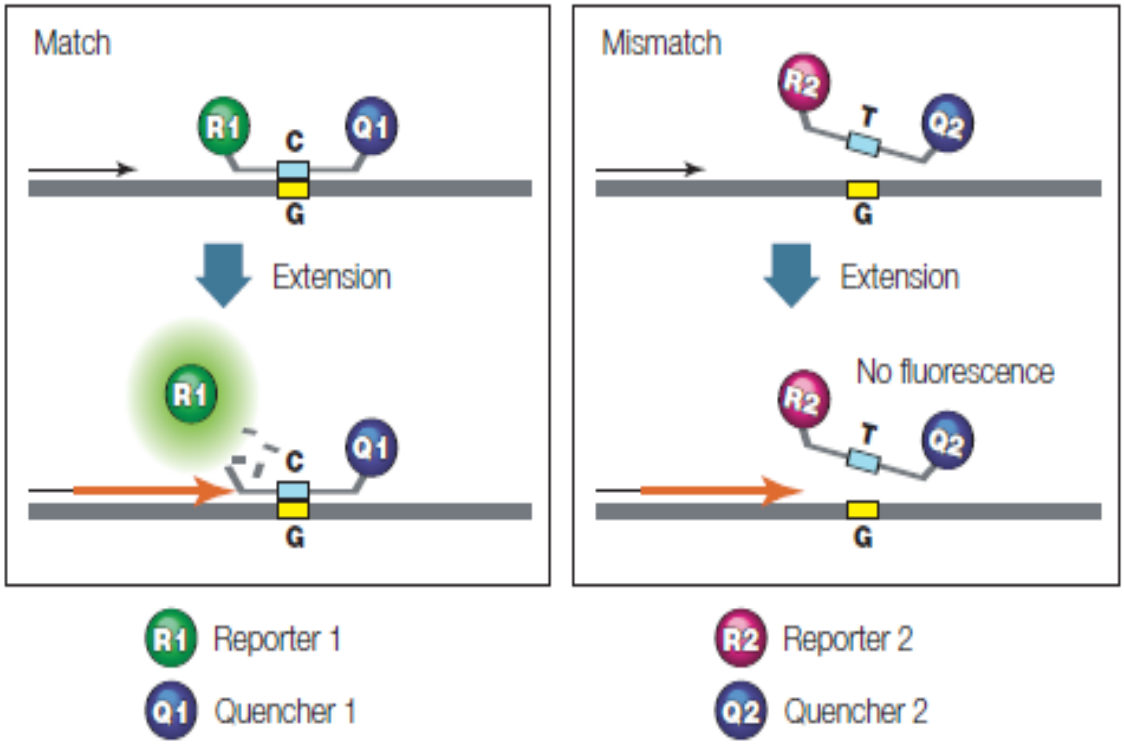
- Q-PCR combined with melting curve analysis allows for faster detection
- Carry-over contamination can be prevented in the closed-tube system.
- The quality control of the synthesized PCR products is assured in the determinations by performing the melting curve analysis.
- The whole diagnostic procedure takes 1 h for 32 samples.
- Multiplexing can be assessed by using several primer-probe detection systems to screen for larger numbers of mutations in the same capillary tube at the same time
-

How are TaqMan probes used to discriminate between allelic variants

The TaqMan probes used for allelic discrimination are differentially labeled fluorescent

probes that are specific for each allele. One probe is specific for the wild-type (WT) allele and another probe is specific for an allelic variant.

The probes are differentially labeled with a 5' fluorescent reporter dye.



Quantitative Real-Time PCR and Melting Curve Analysis

material	1n	xn
Master mix	10	
Fv primer	1	
Rv primer	1	

Probe 1	1	
probe2	1	
H2O	?	
DNA	40 ng/µl	
Final volume	20µl	

Quantitative real-time PCR

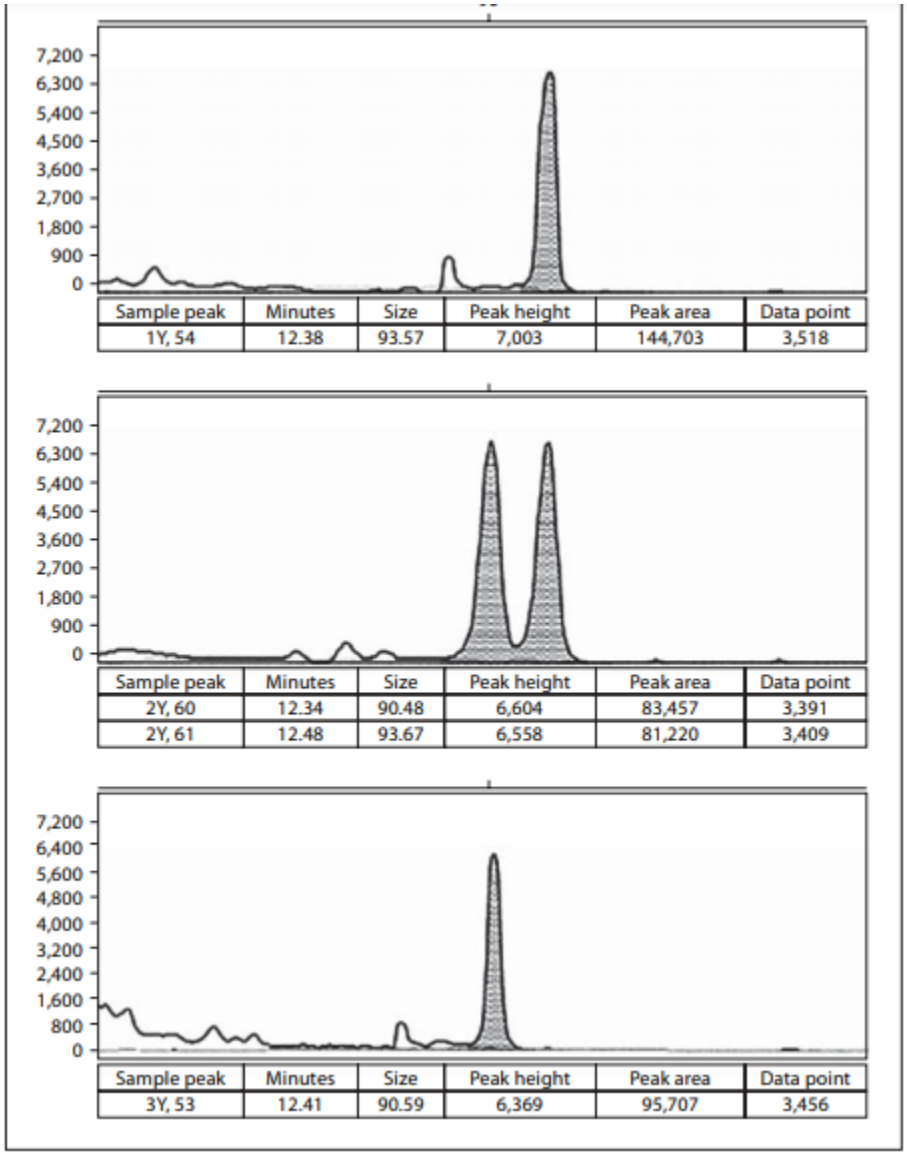
Q-CF forward	5′-GGA-GGC-AAG-TGA-ATC-CTG-AG-3′
Q-CF reverse	5′-CCT-CTT-CTA-GTT-GGC-ATG-CT-3′
P1	5′-TTT-TCC-TGG-ATT-ATG-CCT-GGC-ACC-ATT-AA-F
P2	LCRed640-GAA-AAT-ATC-AT-CTT-TGG-TGT-TTC-C-P

- **The samples should be tested in duplicates** and in each run there was also a **normal healthy sample**, a **heterozygous sample** and a **D.W** as controls.
- The initial 10 min denaturation at 95 ° C was followed by 35 cycles, at denaturation (95 ° C; 0 s), annealing (63 ° C; 25 s) and extension (72 ° C; 5 s).
- Melting curve analysis was performed following the PCR and Tm were determined.

Result

1.Detection of F508del using fluorescent PCR and DNA fragment analysis. The electrophoretograms show a healthy (wild type) sample with a PCR fragment of 93 bp at

the top, a heterozygous sample with 90 and 93 bp fragments in the middle, and a homozygous F508del with a 90 bp fragments at the bottom



Sample peak	Minutes	Size	Peak height	Peak area	Data point
1Y, 54	12.38	93.57	7,003	144,703	3,518

Sample peak	Minutes	Size	Peak height	Peak area	Data point
2Y, 60	12.34	90.48	6,604	83,457	3,391
2Y, 61	12.48	93.67	6,558	81,220	3,409

Sample peak	Minutes	Size	Peak height	Peak area	Data point
3Y, 53	12.41	90.59	6,369	95,707	3,456

Figure 2 shows the melting curves of Q-PCR. The Tm of the F508del PCR product is 49 ° C, while the wild type PCR product has a Tm of 60 ° C.

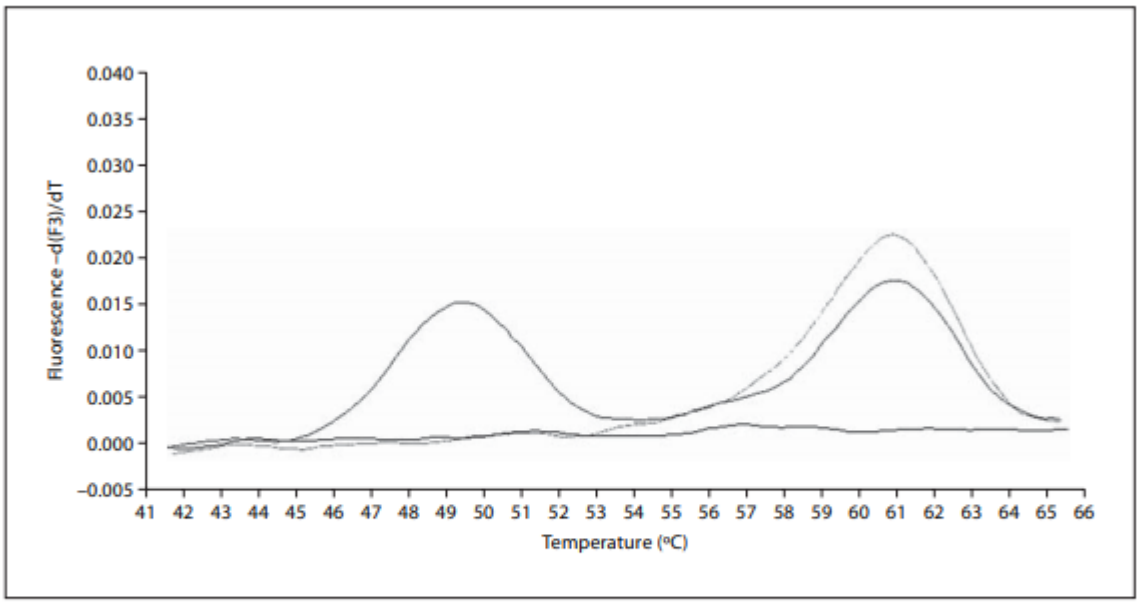
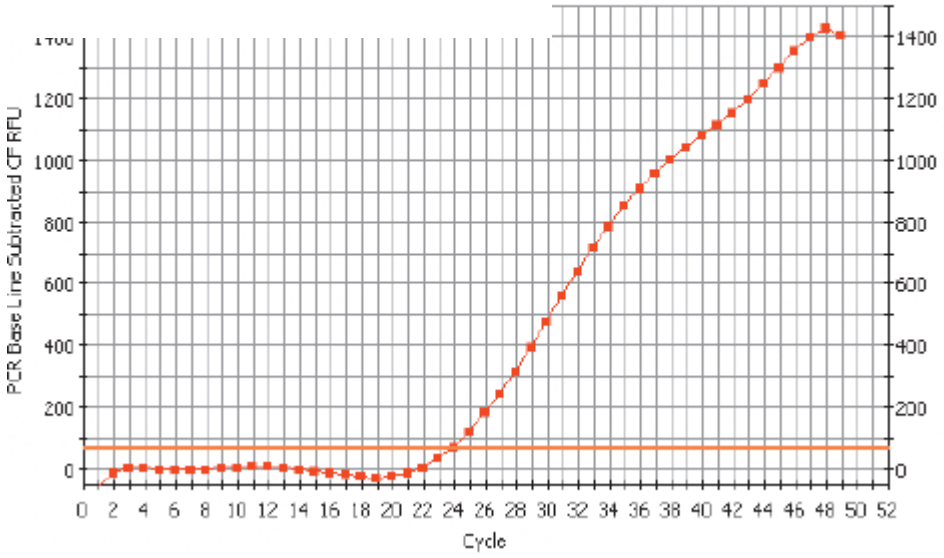
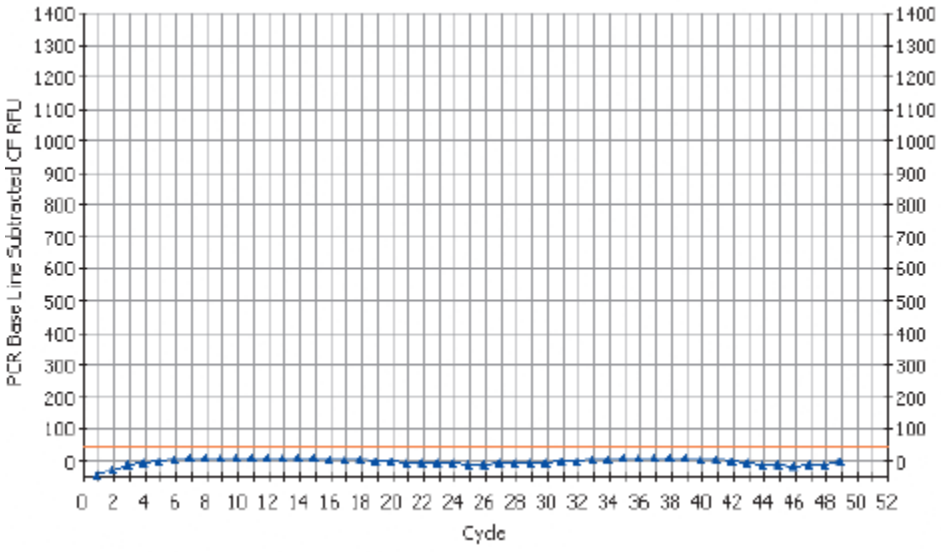


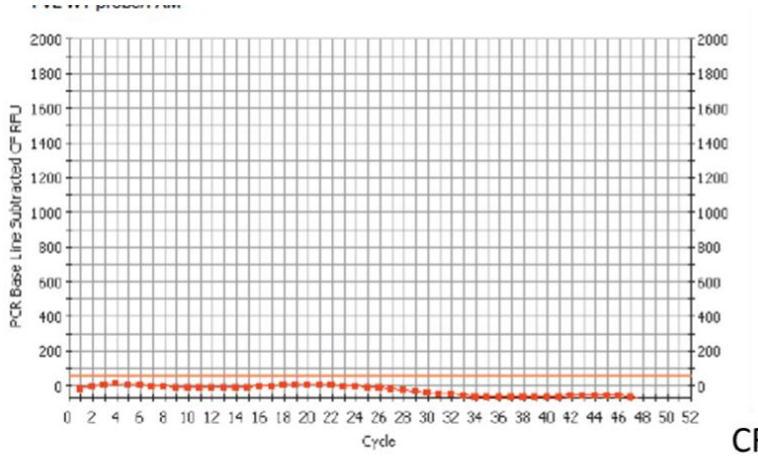
Fig. 2. Detection of *ΔF508del* using Q-RT-PCR and melting curve analysis. The figure shows the melting curve analysis curve of a heterozygous (double peaks), a healthy wild sample (one peak) and a d.water (negative control; no peak). The PCR product of *ΔF508del* has a T_m of 49°C and the wild type 60°C.

CFTR wt probe/FAM



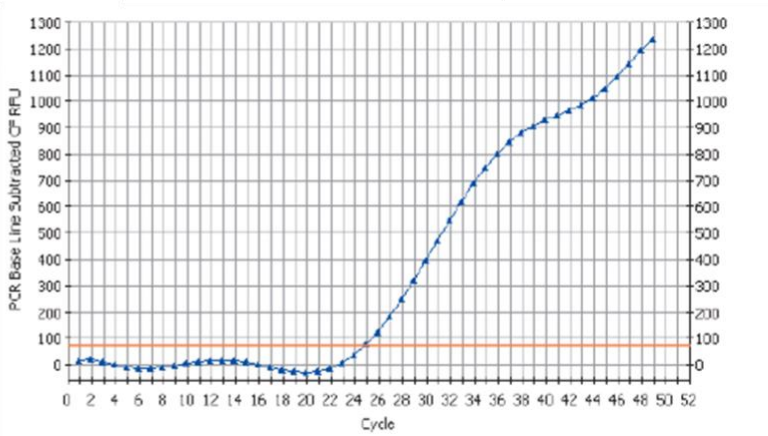
CFTR mut probe/VIC

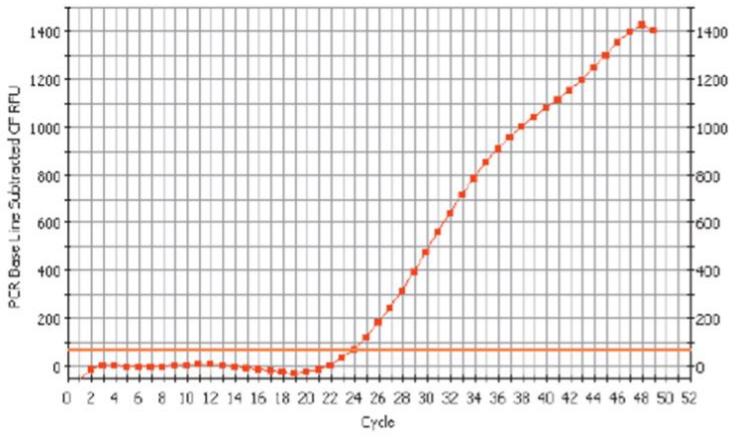




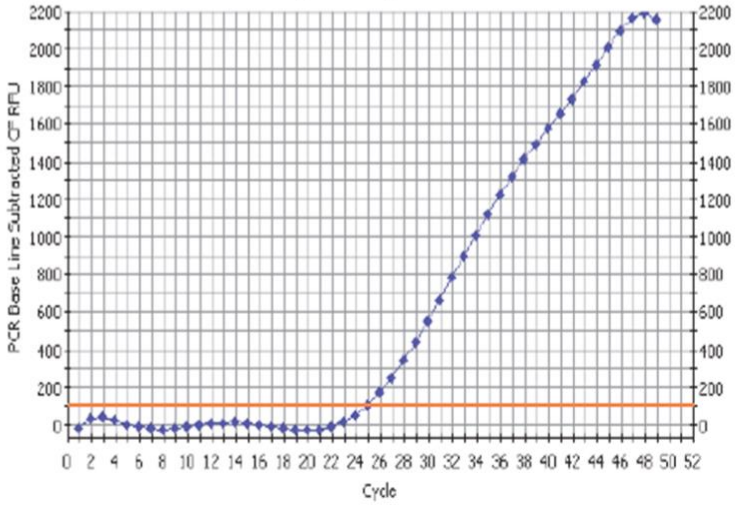
CFTR mut probe/VIC

mut





CFTR mut probe/VIC



Heterozygote

Prevention

- If you or your partner has close relatives with cystic fibrosis, you both may choose to have genetic testing before having children. The test, which is performed in a lab on a sample of blood, can help determine your risk of having a child with CF.
- If you're already pregnant and the genetic test shows that your baby may be at risk of cystic fibrosis, your doctor can conduct additional tests on your developing child.

Genetic testing isn't for everyone. Before you decide to be tested, you should talk to a genetic counselor about the psychological impact the test results might carry.

Lecture 10: Sickle Cell Anemia

Sickle Cell Anemia:

Sickle cell anemia (SCA) is one of a group of inherited disorders known as Sickle Cell Diseases (SCD). SCD are the most important hemoglobinopathy worldwide in terms of frequency and social impact, recently recognized as a global public health problem by the World Health Organization. Sickle cell anemia affects people all over the world, but it is especially common in families whose ancestors come from Africa, South or Central America, India, Saudi Arabia, the Caribbean islands, and Mediterranean countries such as Turkey, Greece, and Italy.

The molecular basis of sickle cell anemia

Hemoglobin is a blood protein which is tetrameric molecule formed from two α-chains and two β-chains each chain is associated with a heme group as shown in (Figure 1). Hemoglobin in the red blood cells picks up oxygen and transports it to all the muscles, tissues, bones, and organs in the body. Once the hemoglobin has released the oxygen into other cells, it takes away carbon dioxide, a by-product of cell respiration to lungs.

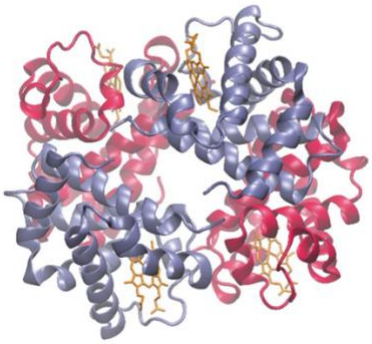


Figure 1: Human Hb A, represented by two α-chains (in ice blue), two β-chains (in red) and four heme groups (in orange).

There are several types of hemoglobin. Hemoglobin A (abbreviated as HbA) is the most common and abundant type in adults. The gene *HbA* codes for the normal β hemoglobin chain, which consists of 146 amino acids. A mutant allele of this gene, *HbS*, causes the β chain to have in the sixth position the amino acid valine instead of glutamic acid (Figure 2).

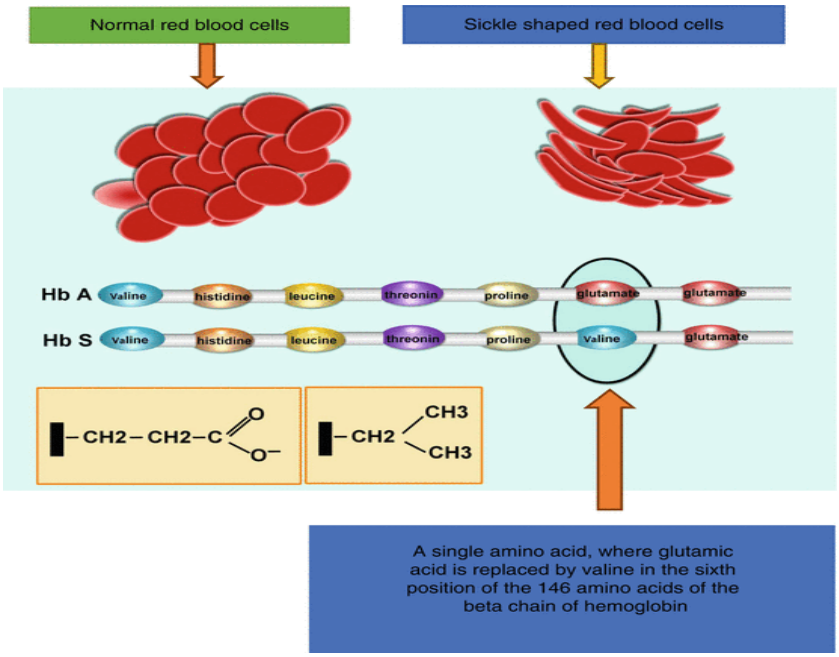


Figure 2: Single amino acid substitution at the sixth residue of the beta (β)-globin subunit (p.Glu6Val), which results in the production of the characteristic Hemoglobin S (HbS)

This apparently minor substitution modifies the properties of hemoglobin. Red blood cells with normal hemoglobin are smooth, disk-shaped, and flexible. They can move through the blood vessels easily. Cells with sickle cell hemoglobin are stiff and sticky. When they lose their oxygen, they form into the shape of a sickle or crescent, like the letter C blocking small blood vessels causing painful and damaging complications as shown in Figure 3.

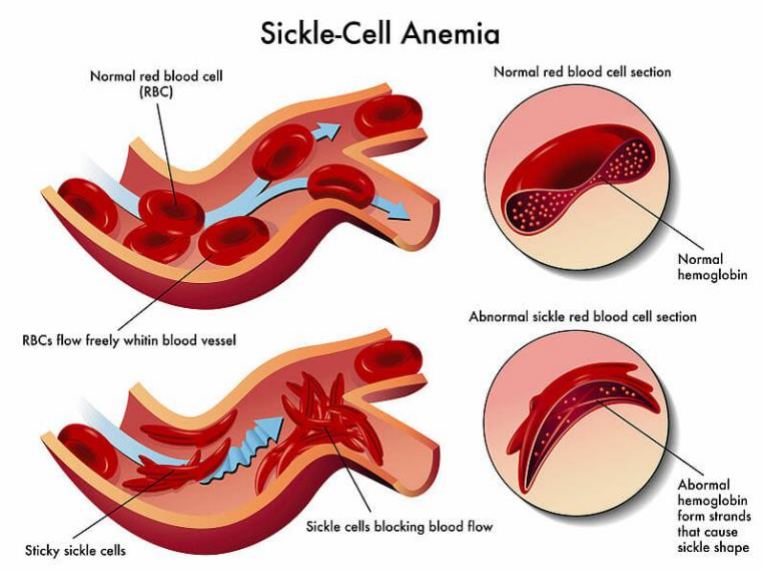
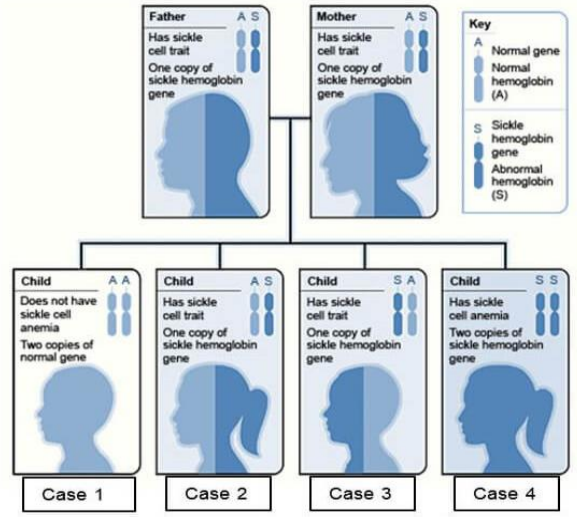


Figure 3: Normal and abnormal sickle red blood cells

Sickle Cell Anemia inheritance pattern:

- Sickle cell anemia is the most common type of a group of Sickle Cell Diseases. It accounts for 70% of SCD.
- Sickle Cell Anemia is an autosomal-recessive genetic disorder. A person carrying just one abnormal sickle cell gene is said to have sickle cell trait (*HbAHbS* carrier)
- If both parents are carriers, then 25% of their childı will be homozygotes with the mutant allele, (*HbSH* suffer from a severe form of anemia that in most cases lead to death before the age of reproduction.



Symptoms:

Signs and symptoms of sickle cell anemia usually appear around 6 months of age. They vary from person to person and may change over time. Signs and symptoms can include:

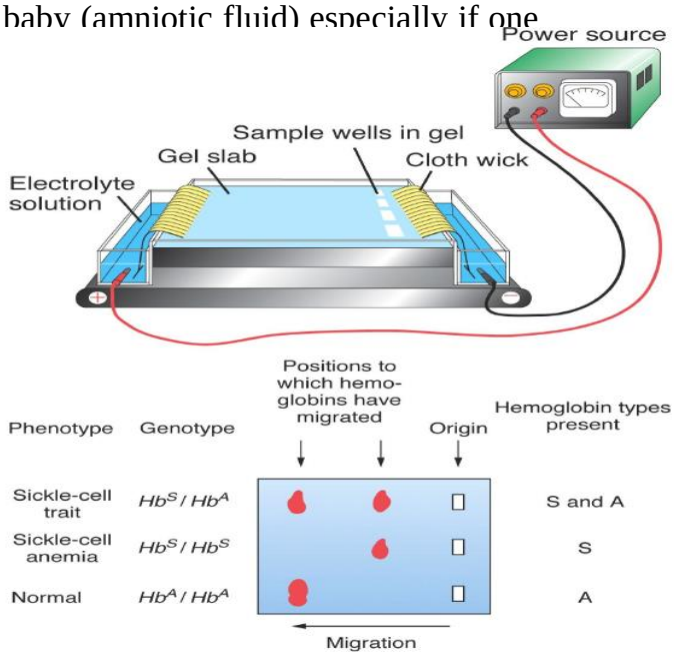
- **Anemia.** Sickle cells break apart easily and die. Red blood cells usually live for about 120 days before they need to be replaced. But sickle cells typically die in 10 to 20 days, leaving a shortage of red blood cells (anemia). Without enough red blood cells, the body can't get enough oxygen and this causes fatigue.
- **Episodes of pain.** Periodic episodes of extreme pain, called pain crises, are a major symptom of sickle cell anemia. Pain develops when sickle-shaped red blood cells block blood flow through tiny blood vessels to your chest, abdomen and joints.
- **Swelling of hands and feet.** The swelling is caused by sickle-shaped red blood cells blocking blood circulation in the hands and feet.
- **Frequent infections.** Sickle cells can damage the spleen, increasing vulnerability to infections. Infants and children with sickle cell anemia commonly receive vaccinations and antibiotics to prevent potentially life-threatening infections, such as pneumonia.
- **Delayed growth or puberty.** Red blood cells provide the body with the oxygen and nutrients needed for growth. A shortage of healthy red blood cells can slow growth in infants and children and delay puberty in teenagers.
- **Vision problems.** Tiny blood vessels that supply the eyes can become plugged with sickle cells. This can damage the retina — the portion of the eye that processes visual images — and lead to vision problems.

Complications:

Sickle cell anemia can lead to a host of complications, including: Stroke, Acute chest syndrome, Organ damage such as kidneys, liver and spleen, Leg ulcers, Gallstones and pregnancy complications.

Molecular diagnosis:

- Hemoglobin electrophoresis is a test that measures the different types of hemoglobin in the blood. It also looks for abnormal types of hemoglobin.
- Newborns test as early as 24-48 hours after birth are screened for sickle cell status as part of the newborn screening program.
- During pregnancy It's best to have the test before the 10^{th} week of pregnancy by sampling some of the fluid surrounding the baby (amniotic fluid) especially if one of the parents have the trait (carrier) of



Treatment:

Management of sickle cell anemia is usually aimed at avoiding pain episodes, relieving symptoms and preventing complications. Treatments might include medications (such as Hydroxyurea, Crizanlizumab, Voxeloto) and blood transfusions. For some children and teenagers, a stem cell transplant might cure the disease.