

Republic of Iraq
Ministry of Higher Education and
Scientific Research
University of Baghdad
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Genetics disease and molecular diagnosis

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Lecture oneGenetics disease and molecular diagnosis

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

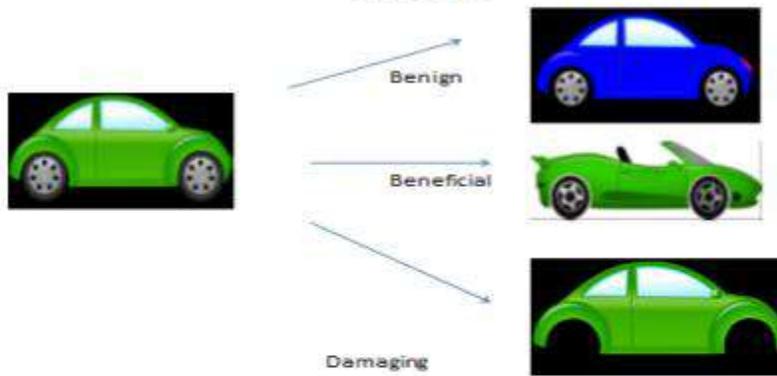
Humans are 99.9% identical: differ on average 1 in 1000 base pairs.



Genetic variants may be common or rare

Reference	ATG TGT CGT GCT GCTC
Person 1	ATG TGT CGT TCT GCTC
Person 2	ATG TGT CGT GCT GCTC
Person 3	ATG A GTC GTG CTG GCTC
Person 4	ATG T GTG CGT GCT GCTC
Person 5	ATG A GTC GTG CTG GCTC
Person 6	ATG A GTC GTG CTG GCTC
Person 7	ATG A GTC GTG CTG GCTC
Person 8	ATG TGT CGT GCT GCTC
Person 9	ATG TGT CGT GCT GCTC
Person 10	ATG A GTC GTG CTG GCTC

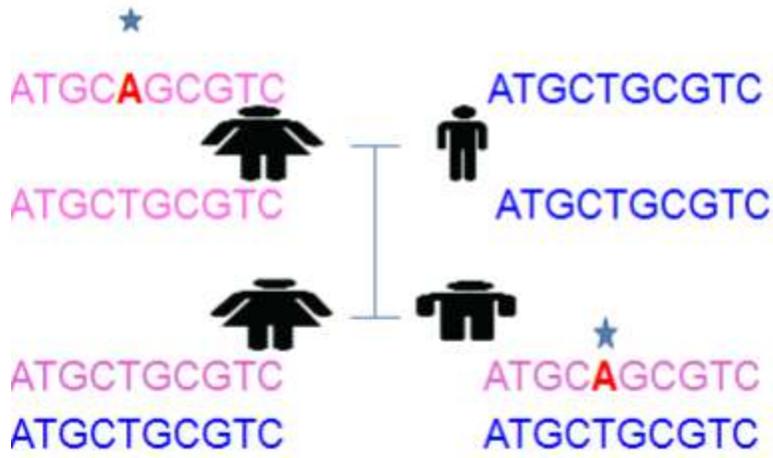
Genetic variants may have a range of effects



Genetic mutations



Genetic mutations can get passed on to offspring



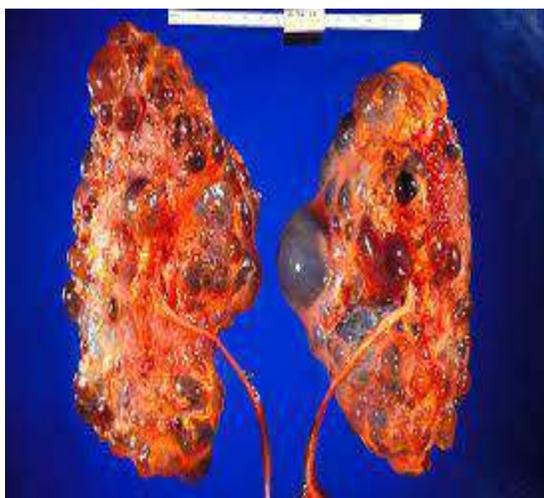
Genetic variant: Any DNA change that is present in the population
Mutation: A genetic variant with an effect
Conclusions -People have different DNA. -
Combination of genes and environment cause disease. - Can find genetic variants that are associated with disease risk. -We can use knowledge of genetics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

Lecture twoGenetics disease and molecular diagnosis

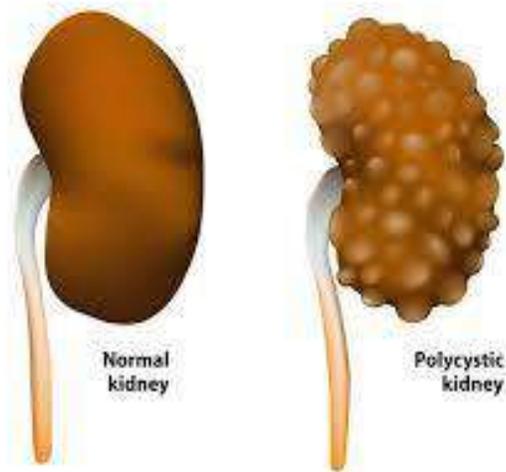
Polycystic kidney disease

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.



POLYCYSTIC KIDNEY DISEASE



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](#).

Lecture threeGenetics disease and molecular diagnosis

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 lymphoma is 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 lymphoma 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.

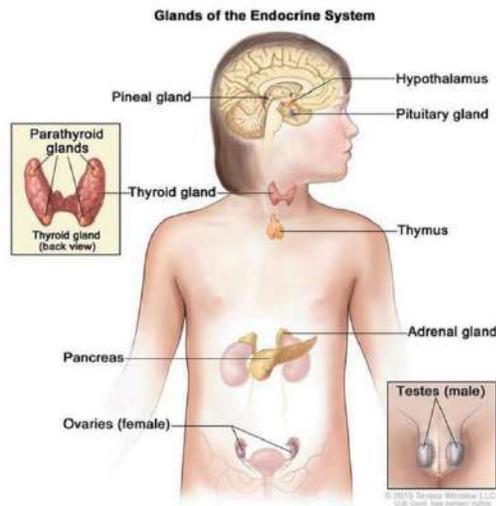
The major forms of multiple endocrine neoplasia are called type 1, type 2, and type 4. These types are distinguished by the genes involved, the types of hormones made, and the characteristic signs and symptoms.

-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.

Lecture fourGenetics disease and molecular diagnosis

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 NF1 (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 (NF1) is the commonest form of neurofibromatosis and has a frequency of about 1 in 3000. Although the gene is almost 100% penetrant, the disease itself has extremely variable expressivity. About 30% of all NF1 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, NF1 is associated with the formation of multiple tumour types in addition to the neurofibromas, including optic gliomas, neurofibrosarcomas, astrocytomas, meningiomas, ependymomas, and pheochromocytomas.

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 café-au-lait spots 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).

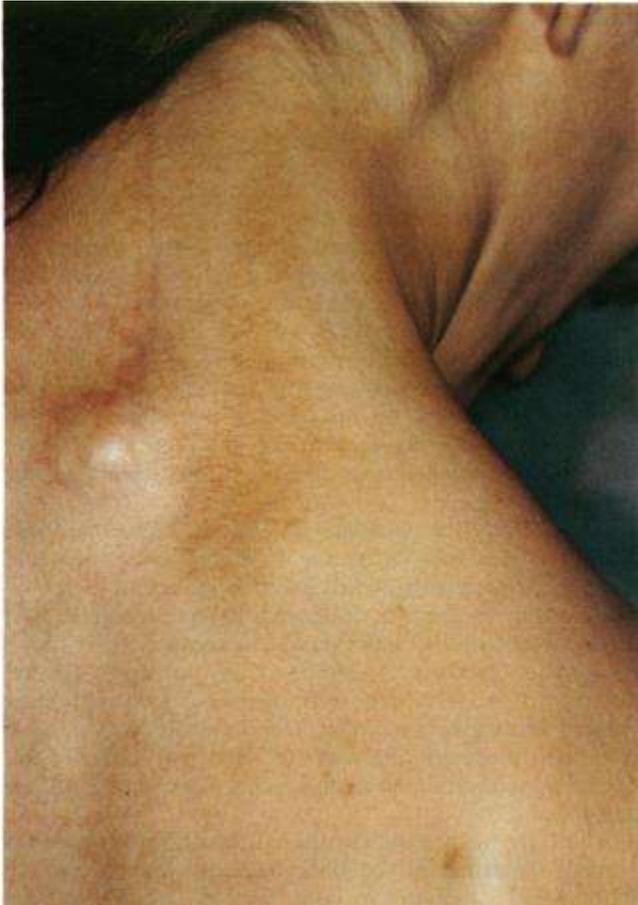
Picture 1 NF1

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 of NF2. NF2 is a much rarer disease than NF1, 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 of hyperpigmentation 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 prevent the disorder from appearing.

Molecular diagnosis

next-generation sequencing protocol used to 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.

Lecture fiveGenetics disease and molecular diagnosis

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 Physical Examination

The diagnosis of a genetic disease requires a comprehensive clinical examination



Physical examination

- Certain physical characteristics, such as distinctive facial features, can suggest the diagnosis of a genetic disorder.
 - May include measurements: circumference of the head, the distance between the eyes, and the length of the arms and legs.
 - Specialized examinations: neurological and eye exams.
 - Imaging studies: x-rays, CT scans, MRI



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.

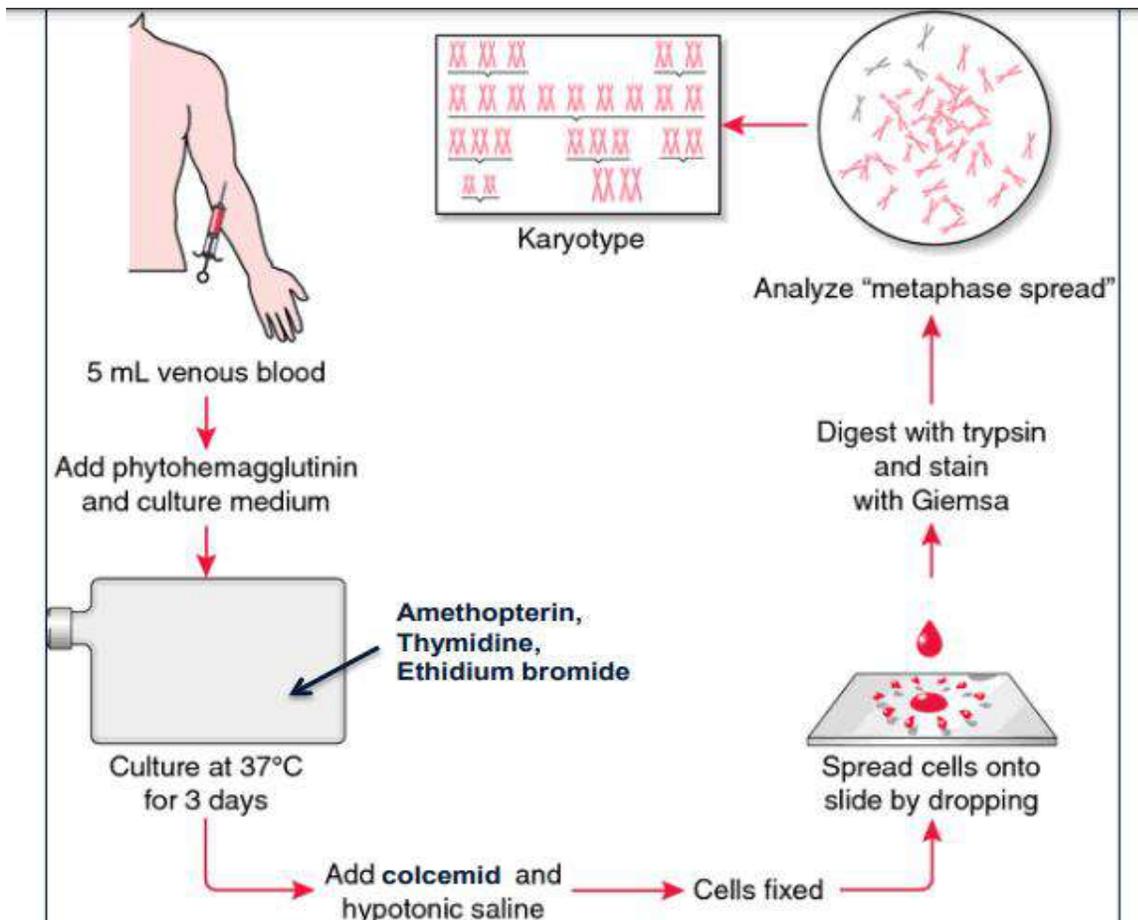
1. Cytogenetic Testing

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

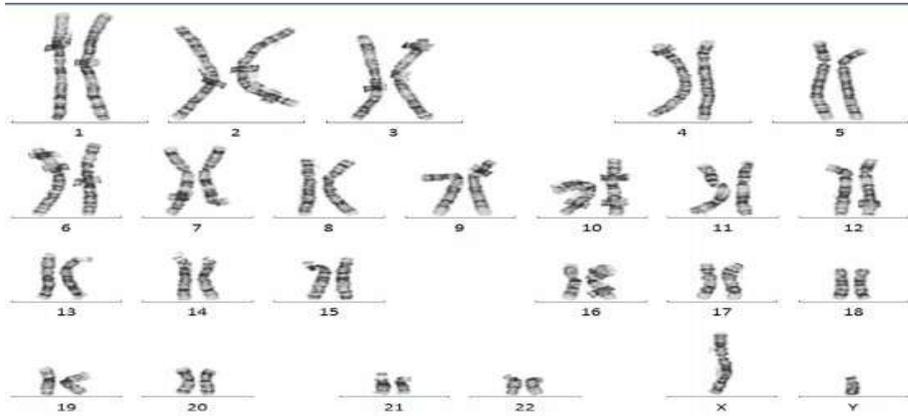
leukemia), amniotic fluid (prenatal diagnosis), and other tissue biopsies can also be cultured for cytogenetic analysis.

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.

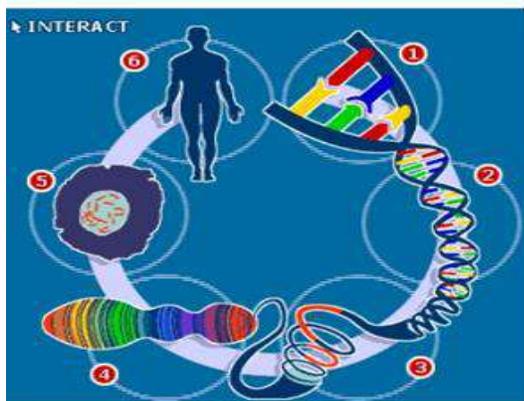
Clinical testing for a biochemical disease utilizes techniques that examine the protein instead of the gene. Depending on the function, tests can be developed to directly measure protein activity (enzymes), level of metabolites (indirect measurement of protein activity), and the size or quantity of protein (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.

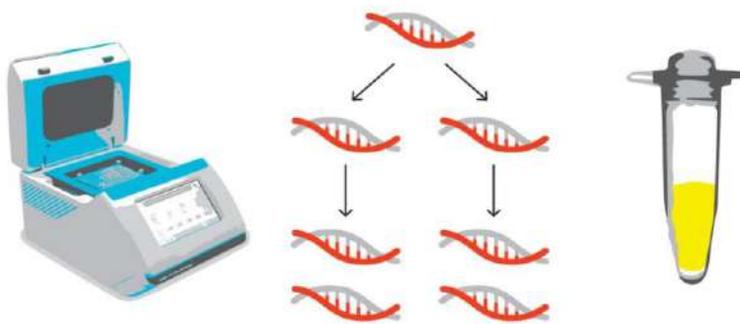
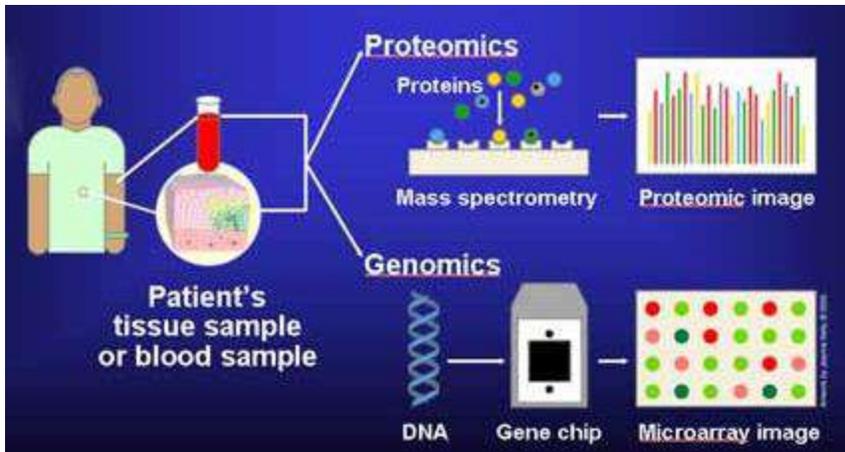
3. Molecular Testing

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.

Molecular diagnostics – how it works

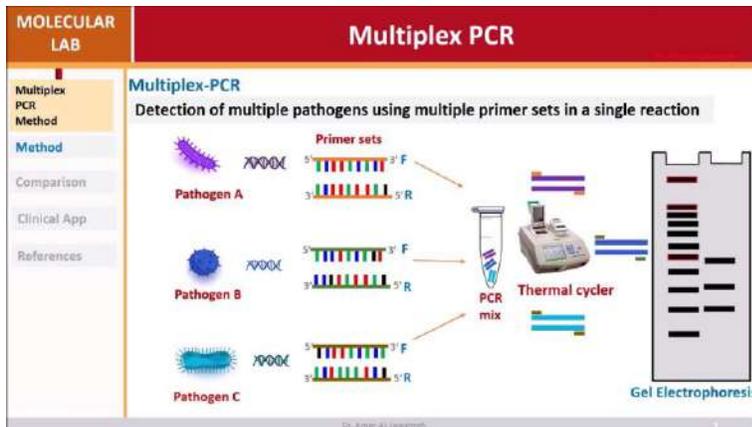


- **EVERY ORGANISM CONTAINS SOME unique, SPECIES SPECIFIC DNA SEQUENCES**
- **MOLECULAR DIAGNOSTICS MAKES THE species SPECIFIC DNA VISIBLE**



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/Prognostic Genetic: 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 sixGenetics disease and molecular diagnosis

Newborn screening (NBS)

molecular genetic testing

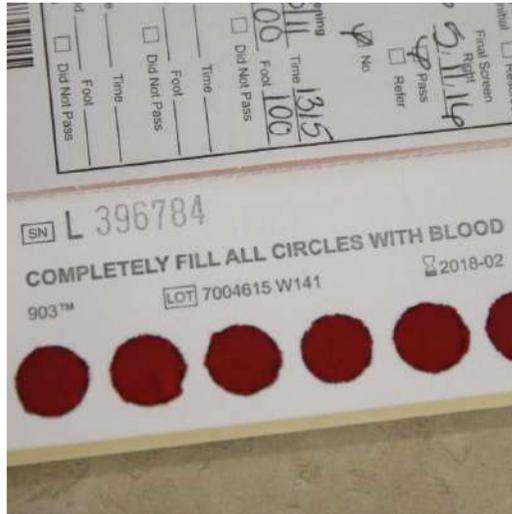
A term widely used in clinical genetics encompassing the diverse techniques used to identify the molecular basis of genetic disease.

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.

Early 1990 s, 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, stick,” and the blood is analyzed for up to 50 diseases.

MONTANA DP&HS NEWBORN SCREENING
Public Health Laboratory
200 Box 4380, Helena, MT 59604-4369

SN **238818**

Do Not Write in This Space

RACE OF BABY (Check all that apply)
 White Black Native Amer. Asian Other Unk

ETHNICITY OF BABY (Check only one)
 Non-Hispanic Hispanic Unknown

BIRTH DATE AND TIME: ____/____/____ :__ :__ AM PM

SPECIMEN COLLECTION DATE AND TIME: ____/____/____ :__ :__ AM PM

SPECIMEN: 1st Repeat

SINGLE BIRTH IF MULTIPLE A B C

BIRTH WEIGHT (grams): _____

HAS THE BABY RECEIVED A RBC TRANSFUSION? Y N

DATE OF TRANSFUSION: ____/____/____

MISCELLANEOUS INFORMATION

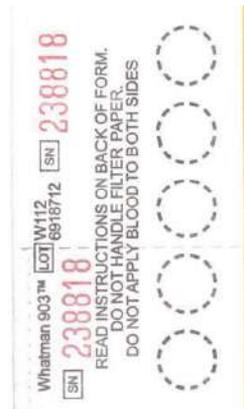
FEEDING METHOD
 TPN Breast Formula, Soy Formula, Lactose

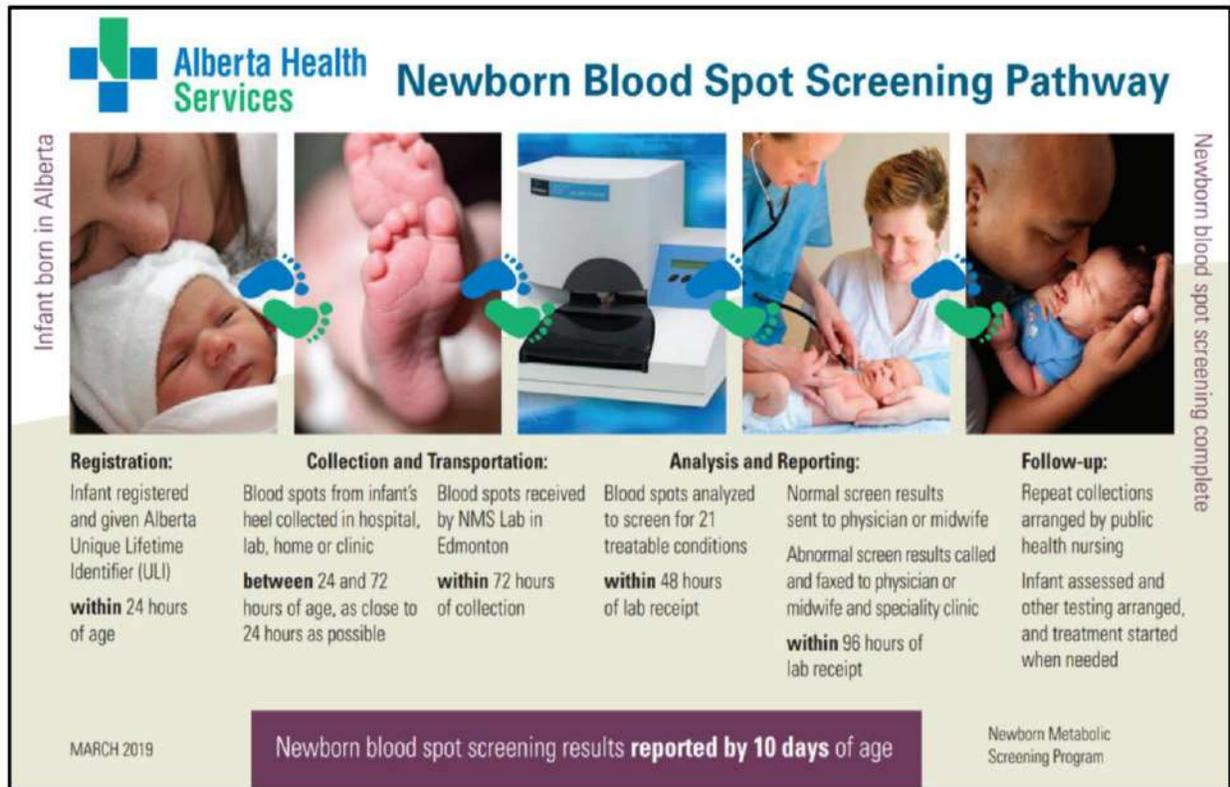
IS MOTHER OR INFANT ON STEROIDS? Y N

IS MOTHER OR INFANT ON ANTIBIOTICS? Y N

OTHER CONSIDERATIONS
 NICU Adopted _____

Submitting Facility: _____





Newborn screen test in USA and Iraq

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 (Galactosemia (and congenital hypothyroidism(CHT)

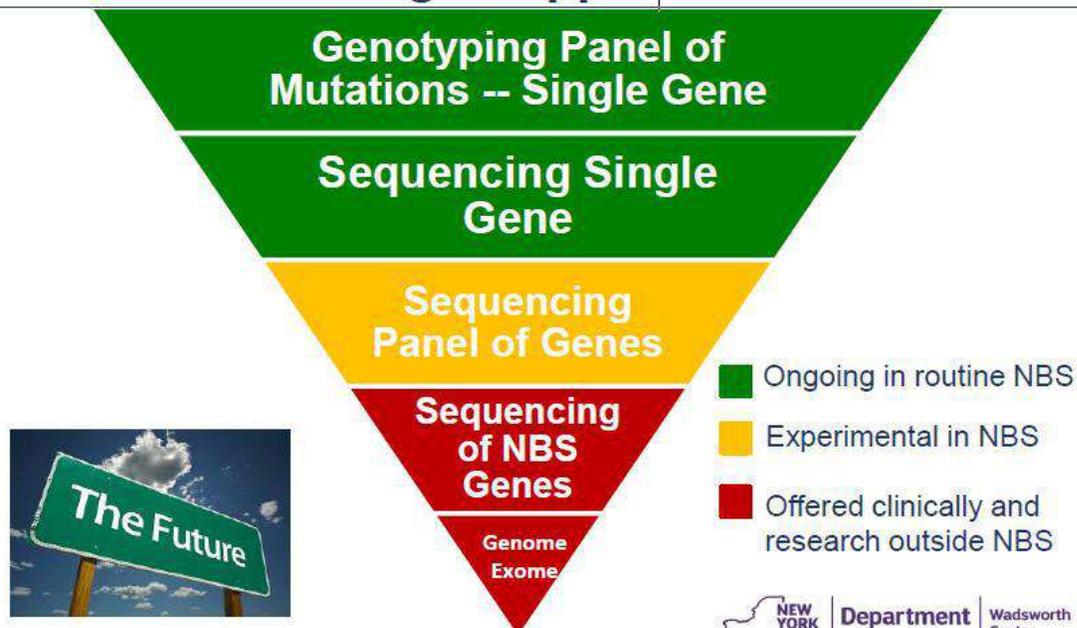
Current Molecular Testing in Newborn Screening Laboratories

- 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

- 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

Molecular Analysis in Newborn Screening A Staged Approach

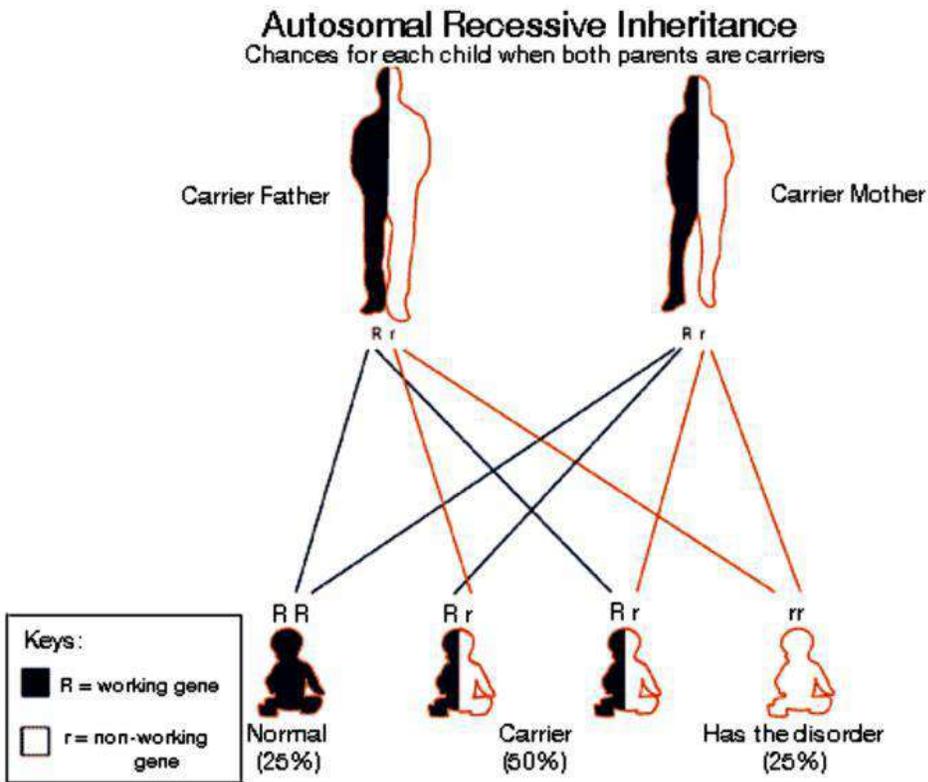


Galactosemia

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



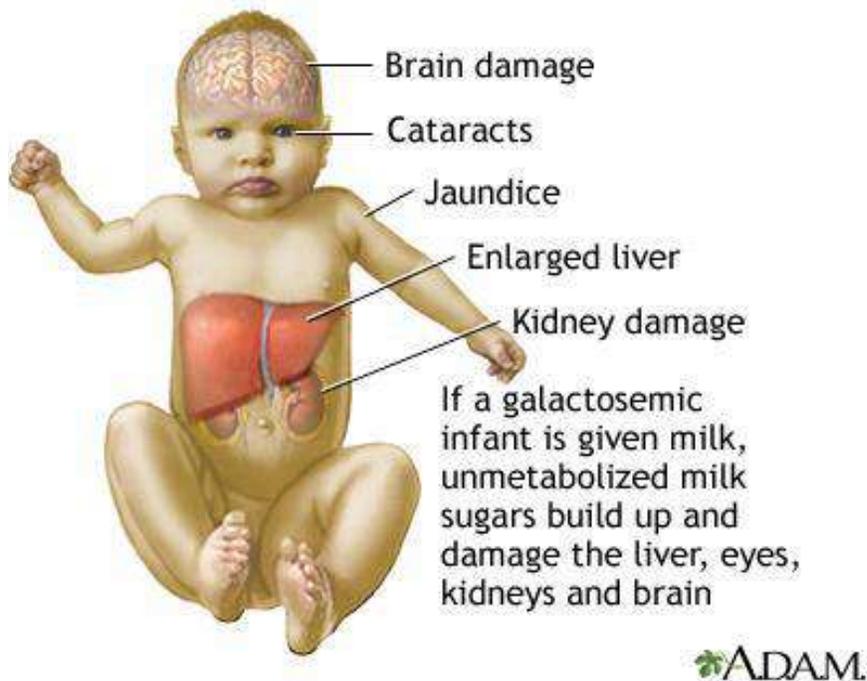
- Classic galactosemia (type 1 the most common and severe type, caused by mutations in the GAL 1 gene and characterized by a complete deficiency of an enzyme called galactose 1 phosphate uridyl transferase(GALT)
- Galactokinase deficiency (type 2 caused by mutations in the GALK 1 gene and characterized by a deficiency of the enzyme galactokinase 1
- Galactose epimerase deficiency (Type 3 caused by mutations in the GALKE gene and characterized by a deficiency of the enzyme UDP galactose 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

- 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.



Diagnosis

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

Atemporarily abnormal result absent fluorescent activity) is found in 1 2 000 infants which indicates enzyme activity 50 percent of normal

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 (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

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

Review in outpatient (follow up):

- Every 3 months in first year provided they are well
- Every 4 months 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.

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.

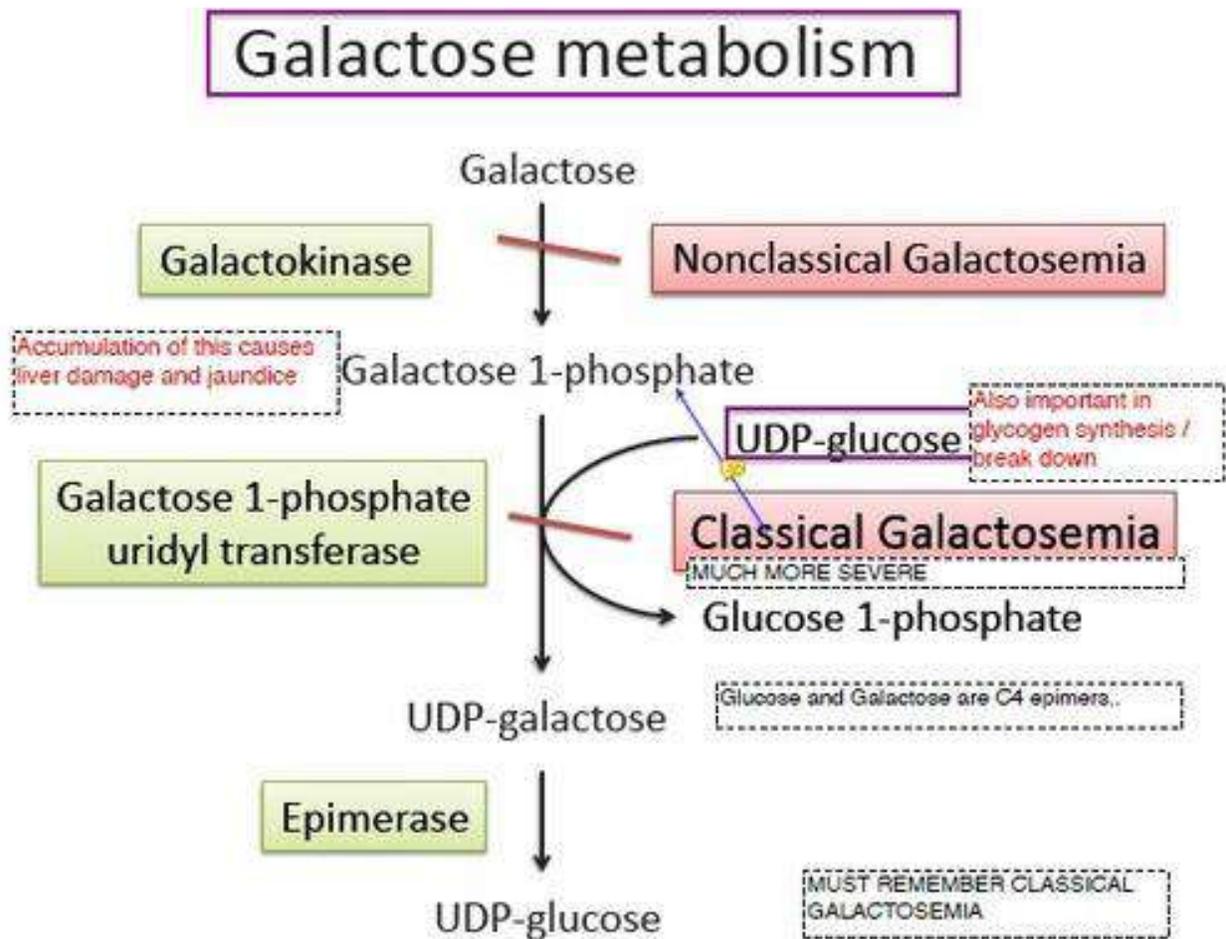
Thus, the need for regular monitoring and evaluation is important

Prognosis

Lecture sevenGenetics disease and molecular diagnosis

Diagnosis of **Galactosemia**

- Classic galactosemia is an autosomal recessive disorder caused by the deficiency of galactose 1-phosphate uridytransferase, GALT
- It presents with vomiting and diarrhea in neonates within a few days of milk intake. Most patients develop jaundice and hepatic failure. If untreated, it is potentially lethal.
- Newborn screening for galactosemia is routine in all states in the USA and in many other countries.
- Elimination of dietary galactose is the main treatment. However, this does not prevent secondary complications such as growth retardation, mental retardation, dyspraxia, cataracts, ataxia, and ovarian failure later in life



GALT enzyme activity assay

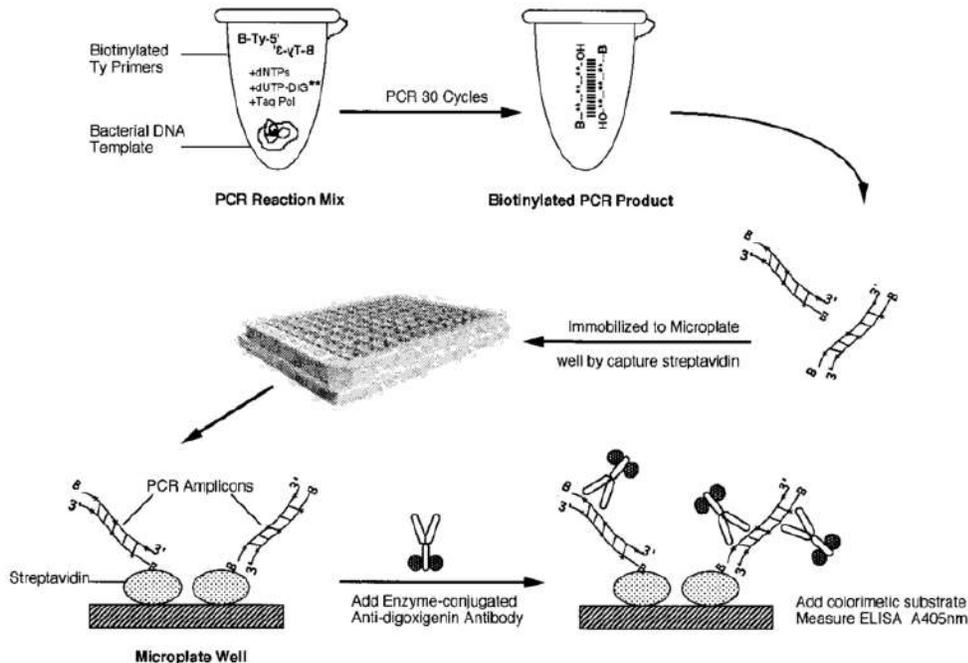
GALT enzyme activity in red blood cells (RBC's) is assayed by measuring the conversion of [14C]-galactose-1-phosphate ([14C]-Gal-1-P) and UDP-glucose (UDPG) to glucose-1-phosphate (Glu-1-P) and 14C-UDP-galactose at 37°C for 1 hour by erythrocyte hemolysate normalized to hemoglobin content.

Result

- The proband presented in the neonatal period with classical galactosemia confirmed by high levels of Gal-1-P [25.1 mg/dL (normal 1.0 mg%)]
- Absent activity of erythrocyte GALT [normal 36.3 1.7 moles/gHbg/(Hr)]

PCR-ELISA test

- PCR-ELISA is containing of PCR and ELISA techniques that allows to detection of nucleic acid instead of protein.
- With this assay, we can determine quantity the PCR product directly after immobilization with biotinylated DNA on a microplate.



- **PCR-ELISA**

- Principle: is based on interaction of between DIG-labeled DNA sequencing and anti-DIG antibody.
- By using of probe in this technique, if target DNA is wrong copied,probe cannot connect to it and the answer will be negative

- **Method:**

- 1- A specific part of the gene is selected, and primers designed for it.
- 2-Amplification was carried out by:
 - digoxigenin-11-dUTP (DIG-dUTP) nucleotide.
 - Probe is labelled with biotin at its 5'end (Preferred probe complementary in the middle of the gene because it can increase the specificity).
- 3- streptavidin which has high affinity to biotin is coated in microplate.
- 4- Double strand DNA is singled by heat shock and is added to microplate. Complementary sequence is connected to probe and extra material is washed with PBST.
- 5- Anti-DIG antibody is added, and optimal density is measured by spectrophotometry.
- This manner is quick, easy and can be getting the exact result at maximum 4 hours, without needed to advance laboratory and professional person. This method is safe and its color is non- mutagenic.

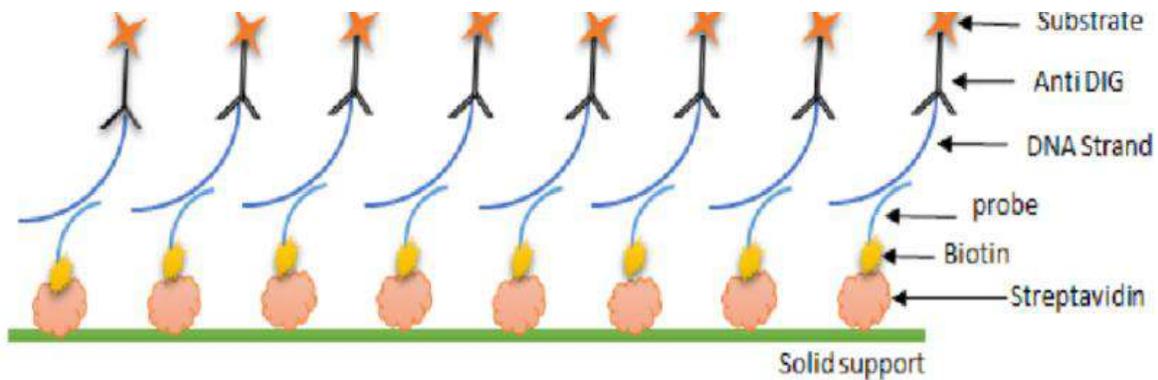


Figure 1. Detection of biotinylated DNA using an anti-DIG-peroxidase conjugate with substrate ABTS to form a blue-green color reaction that is both visible and measured using a spectrophotometer

Advantage of PCR-ELISA :

- This method is more sensitive than agarose gel electrophoresis analysis. Because in this way products are analyzed by colorimetric method,
- The risk of toxicity of color materials and DNA pollution is reduced.
- The specificity of the assay is too high because the detection uses gene-specific probes
- Another advantage of this method is large-scale screening with standard laboratory
- Compared with conventional PCR methods, the analytical time of PCR-ELISA is shorter
- Due to its low cost, if a study does not require very high sensitivity, PCR-ELISA can be a good option instead of quantitative PCR (qPCR)

Primer used in this test

- The GALT alleles can amplify using three sets of primers:
- IVS2-F, S135L-R,
- GALT 6-5, GSINT7R,
- GALT 9-5, and INTJR.

Table 1

sed to amplify the GALT gene for specific mutation ana

Primer sequence (5' ≥ 3')

GGGTGGGCCTTCCCTACTCC

GGACCGACATGAGTGGCAGCGTTA

AGGAGGGAGTTGACTTGGTGT

GGGGACACAGGGCTTGGCTCTCTC

GGTCAGCATCTGGACCCCAGG

GCCTGCACATACTGCATGTGA

CACATACTGCATGTGAGAGTC

- **Result**
- Hybridized products developed a green color after incubation with peroxidase substrate.

- This method enabled us to detect the presence of the seven most common mutations of the GALT gene—Q188R, N314D, S135L, L195P, Y209C, IVS2 and K285N.^{2,4}

Interpretation of result

- The GALT genotypes should be compared among mother, father and proband following multiplex PCR of their GALT DNA (Fig. 1).
- Note that from this initial PCR-based analysis, the proband has mutation in the K285N,
- The mother has mutation for the N314D
- Father has mutation for both the K285N and the N314D.

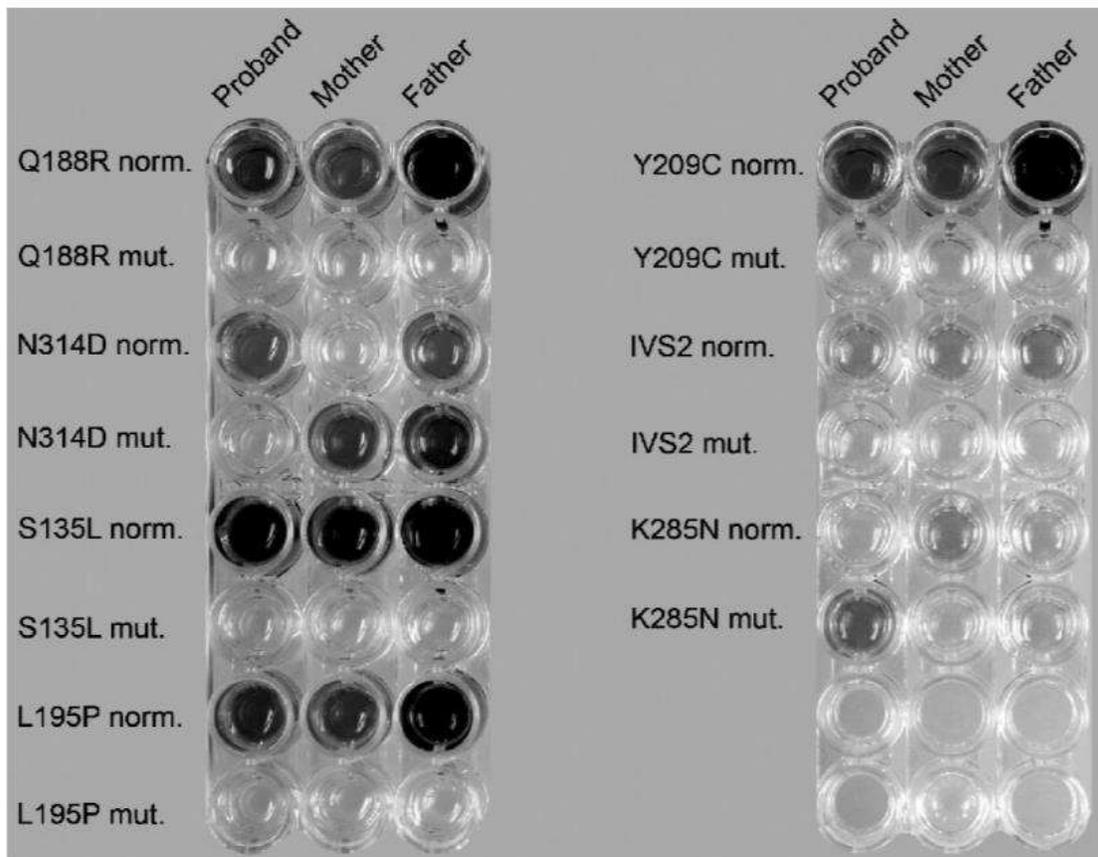


Fig. 1. Allele-specific oligonucleotide (ASO) hybridization for seven most common mutations of GALT gene. PCR amplified DNA fragments from proband, her mother and father were hybridized with biotinylated oligonucleotides corresponding to either normal (norm.) or mutated (mut.) sequence of each mutation.

Lecture eightGenetics disease and molecular diagnosis

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)

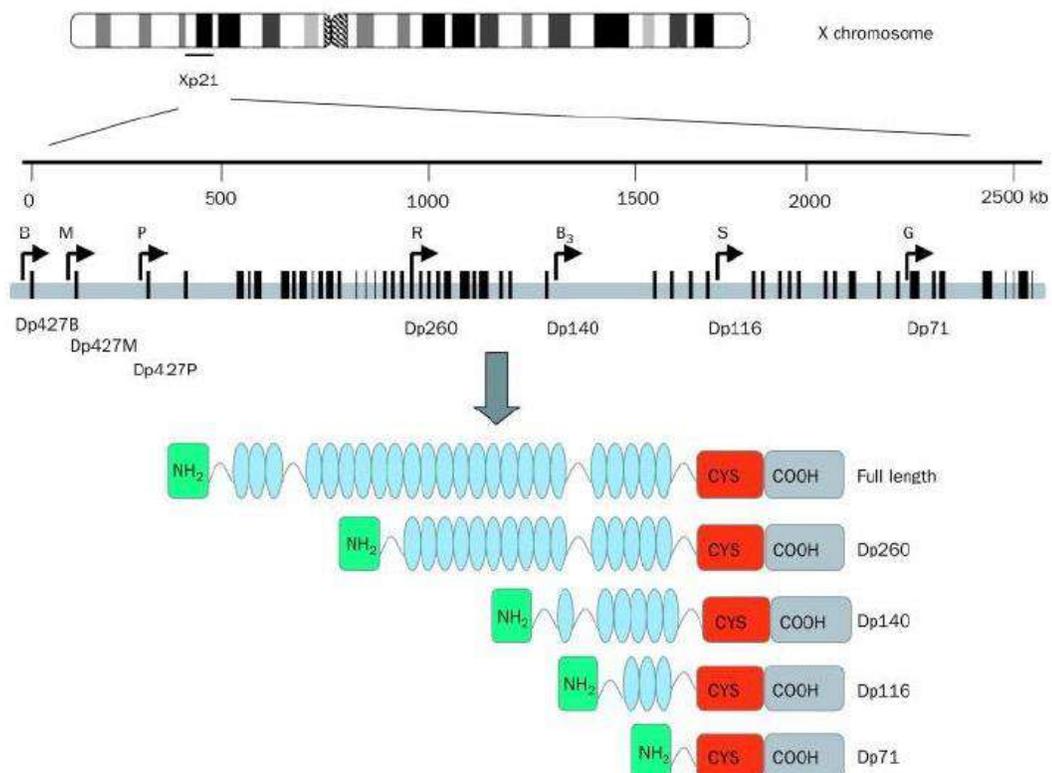
Characteristics of the severe DMD

- DMD patients are clinically normal at birth, although serum levels of the muscle isoform of creatine kinase are elevated.
- between the ages of 2 and 5 years, the child presenting with difficulty in climbing stairs. There is often a delay in walking and difficulty in running.
- Subsequently, the onset of pseudohypertrophy of the calf muscles, proximal limb muscle weakness, and Gowers' sign suggest DMD. Decreased lower-limb muscle strength and joint contractures result in wheelchair dependence, usually by the age of 12.
- Most patients die in their early twenties as a result of respiratory complications. Death can also be the result of cardiac dysfunction.

Dystrophin gene

- Located on the short arm of chromosome X, in the p21 locus
- DMD gene is the largest known gene, with approximately 2.4 mega bases, which represents about 0.1% of the entire human genome.

- The gene consists of 79 exons (1% of the gene) transcribed into a 14 kb full length dystrophin mRNA
- The remaining 99% of the dystrophin gene is composed of non-coding intronic regions.
- The main transcript translates to a cytoskeletal protein named dystrophin, which has a molecular weight of 427kDa and consists of 3685 amino acid residues

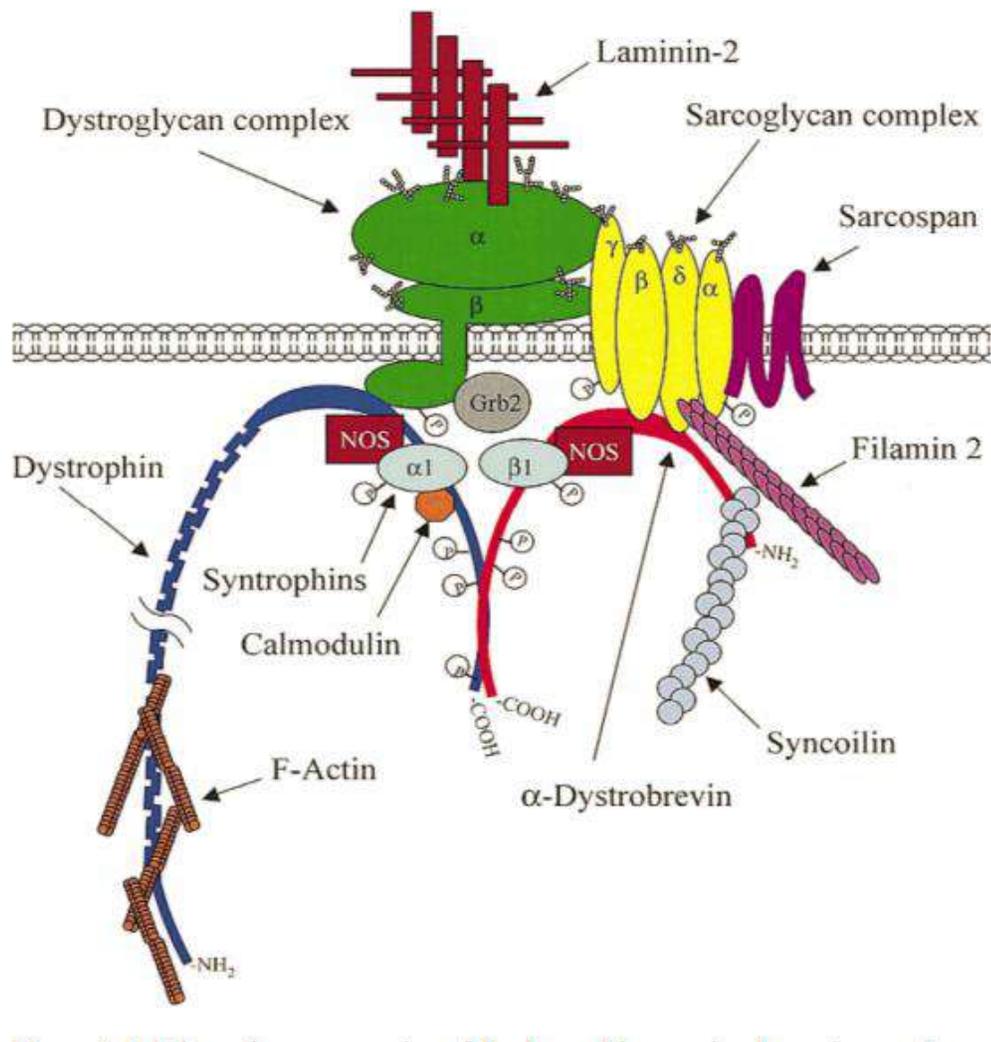


Dystrophin protein

- 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 processes.



Mutation of DMD gene

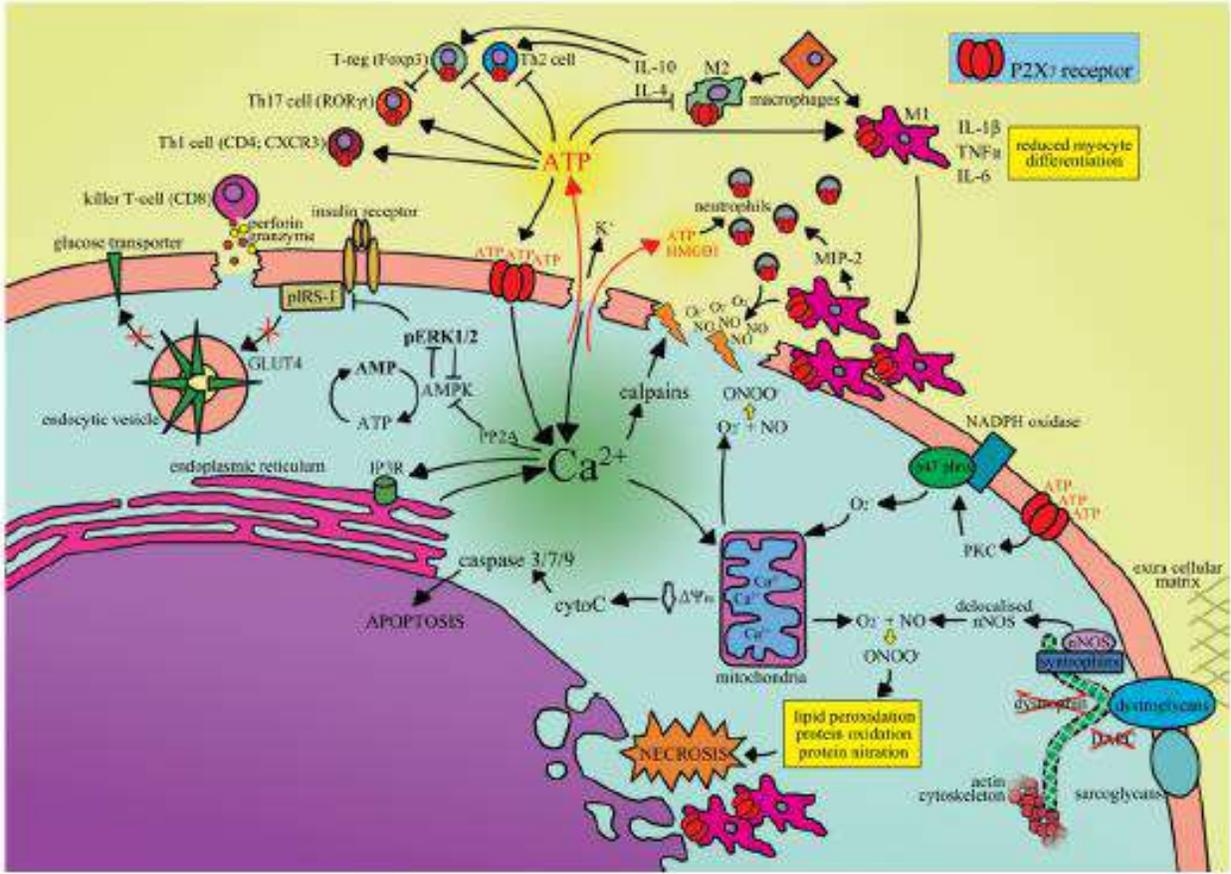
The large size and complex structure of the dystrophin gene makes it vulnerable to mutations and therefore this gene has a high mutation frequency of 1×10^{-4} genes per generation.

- 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

Downstream effects of the absence of dystrophin

Major secondary systemic and cellular level abnormalities that are faithfully replicated in animal models of this disease include:

- Chronic inflammation of dystrophic muscles.
- Chronically elevated intracellular Ca^{2+} levels in skeletal muscle fibres.
 - A regeneration defect, such that damaged muscle is increasingly less able to be repaired over time.
- Cell signalling dysregulation.
- A metabolic crisis, such that muscle becomes less efficient at utilising and managing nutrients.
- Elevated levels of oxidative stress mediators

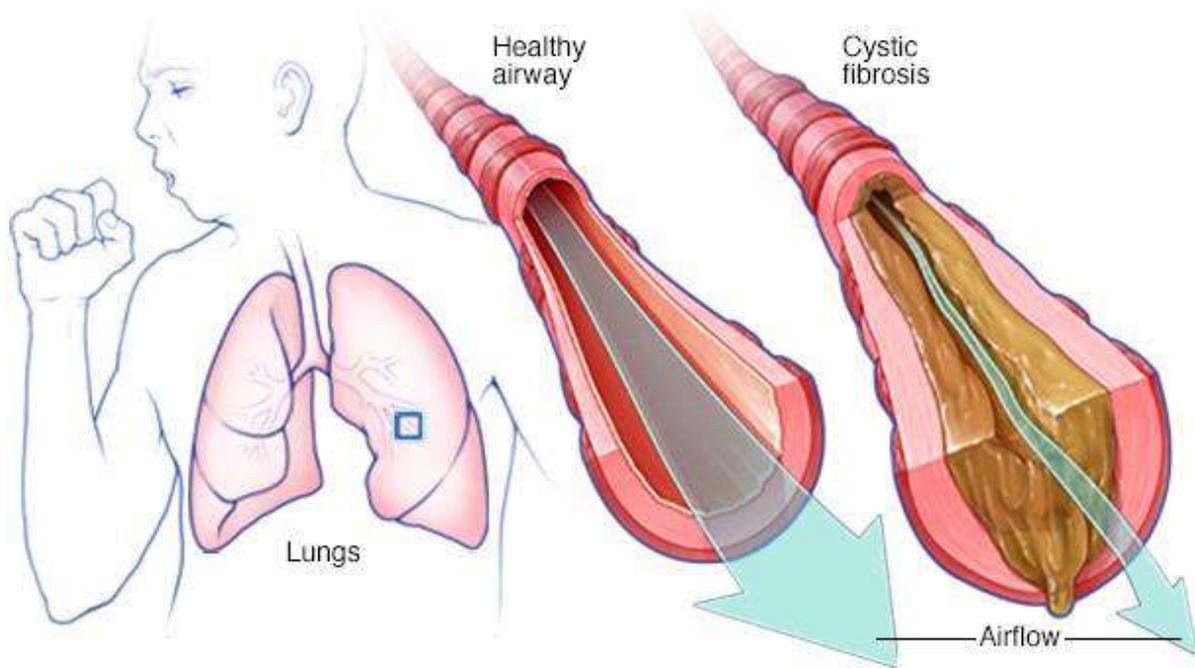


Lecture nineGenetics disease and molecular diagnosis

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.

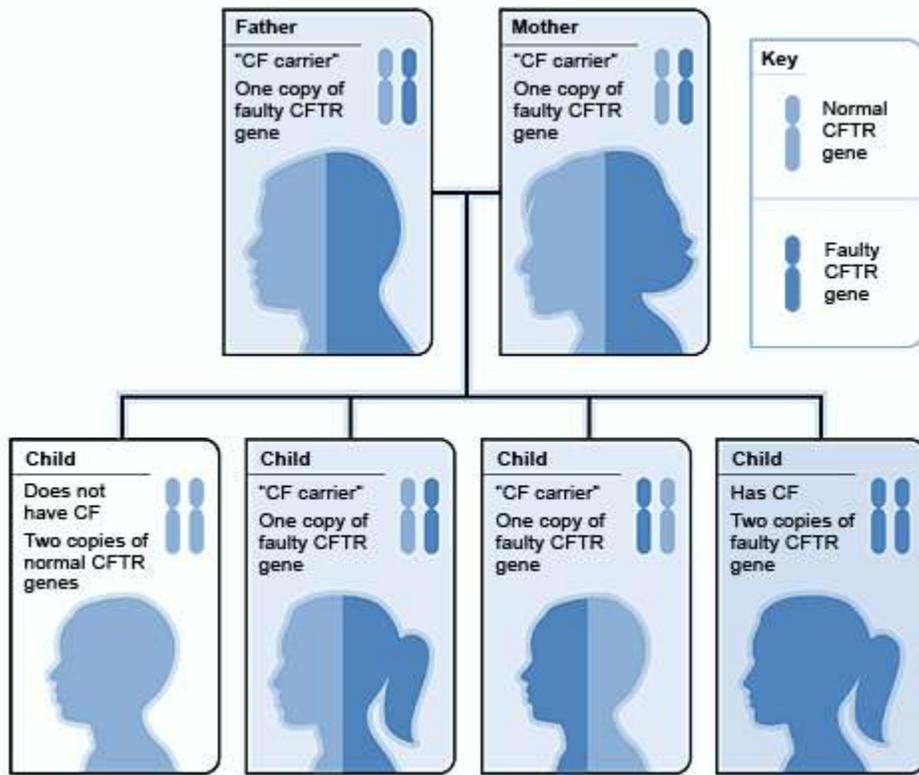
- Cystic fibrosis affects the cells that produce mucus, sweat and digestive juices. These secreted fluids are normally thin and slippery. But in people with CF, a defective gene causes the secretions to become sticky and thick. Instead of acting as lubricants, the secretions plug up tubes, ducts and 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.



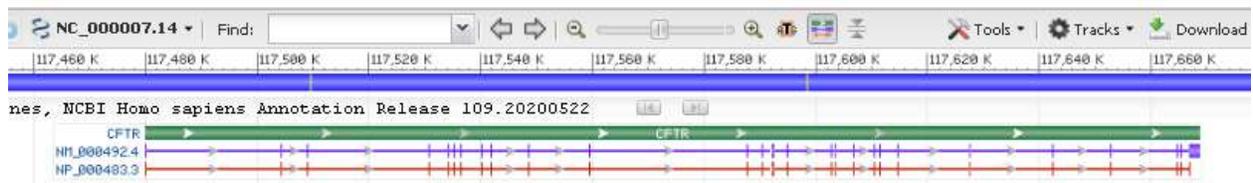
Inheritance Pattern for Cystic Fibrosis

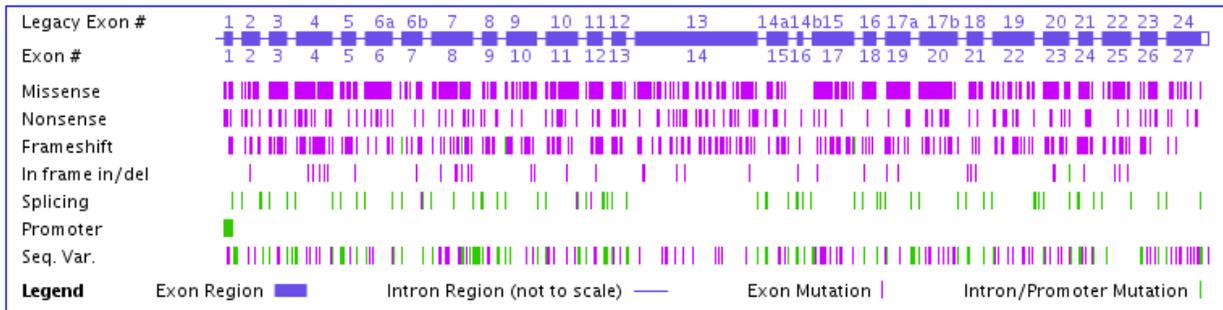
- CF is a common autosomal recessive genetic disorder with a prevalence of ~1 in 2500 live births (two abnormal genes in order for the disease to develop)
- results from the presence of mutations in the CF transmembrane conductance regulator (*CFTR*) gene on chromosome 7q31

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

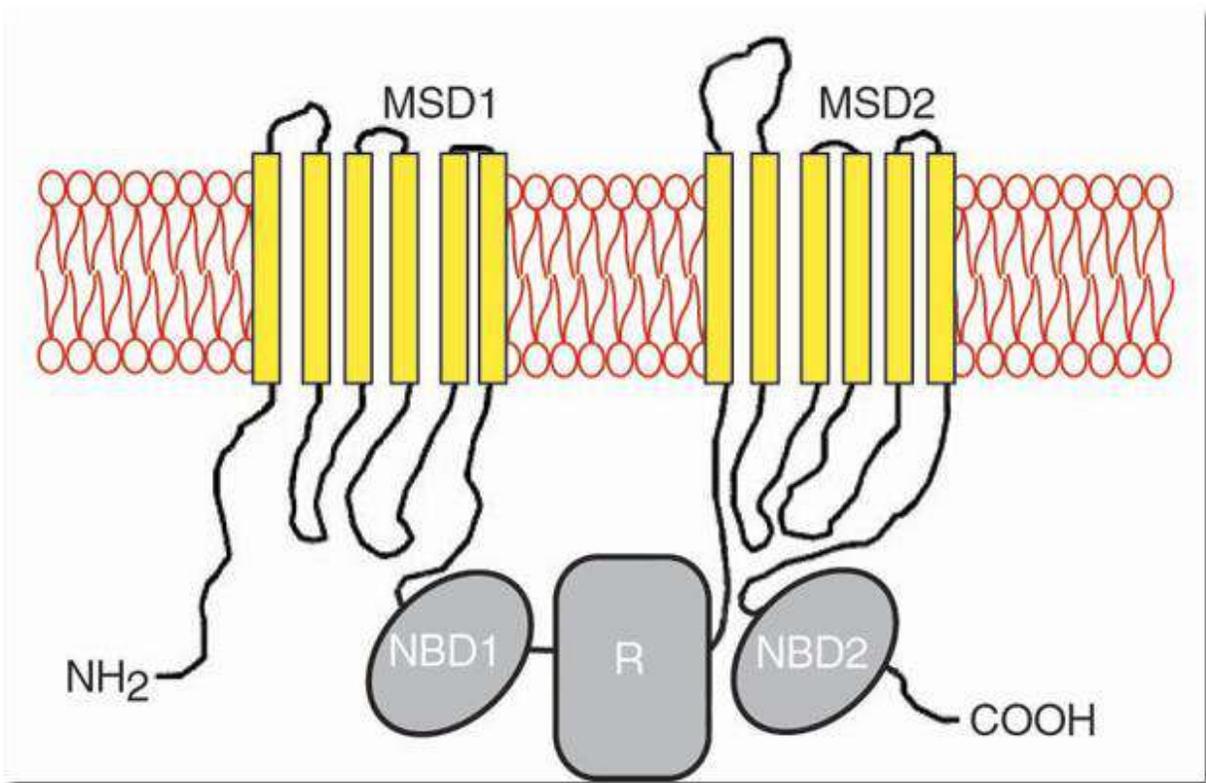




The most common mutation, called **delta F508**, is a deletion of one amino acid at position 508 in the CFTR protein. The resulting abnormal channel breaks down shortly after it is made, so it never reaches the cell membrane to transport chloride ions.

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 pancreas.



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:

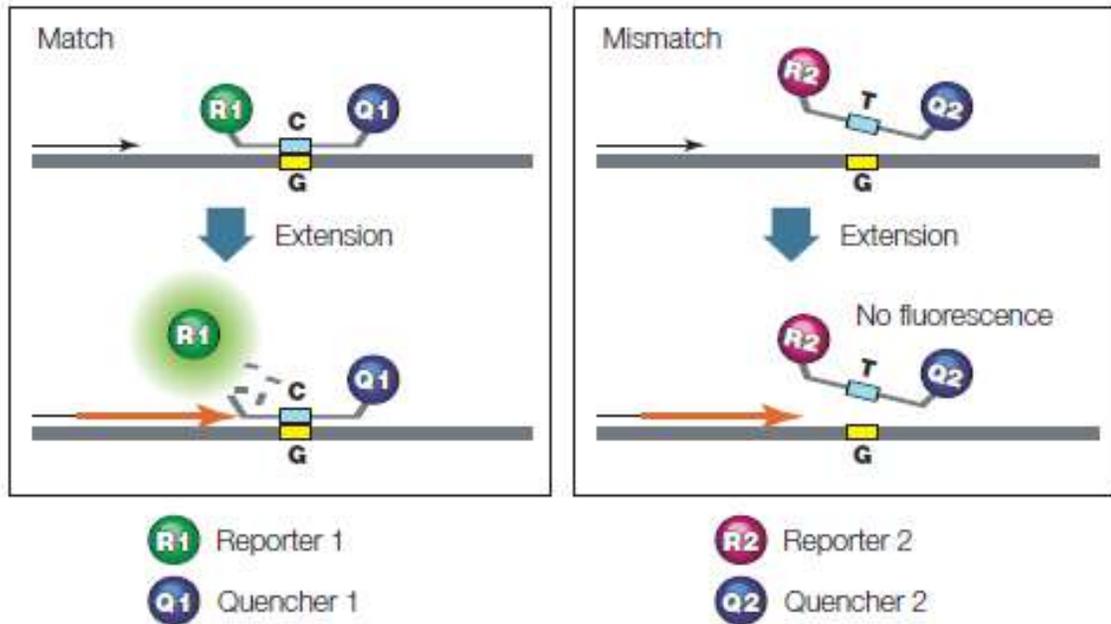
- Foul-smelling, greasy stools
- Poor weight gain and growth
- Intestinal blockage, particularly in newborns (meconium ileus)
- Chronic or severe constipation, which may include frequent straining while trying to pass stool, eventually causing part of the rectum to protrude outside the anus (rectal prolapse)

Lecture tenGenetics disease and molecular diagnosis

Diagnosis of cystic fibrosis

- 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

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/ μ l	
Final volume	20 μ l	

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

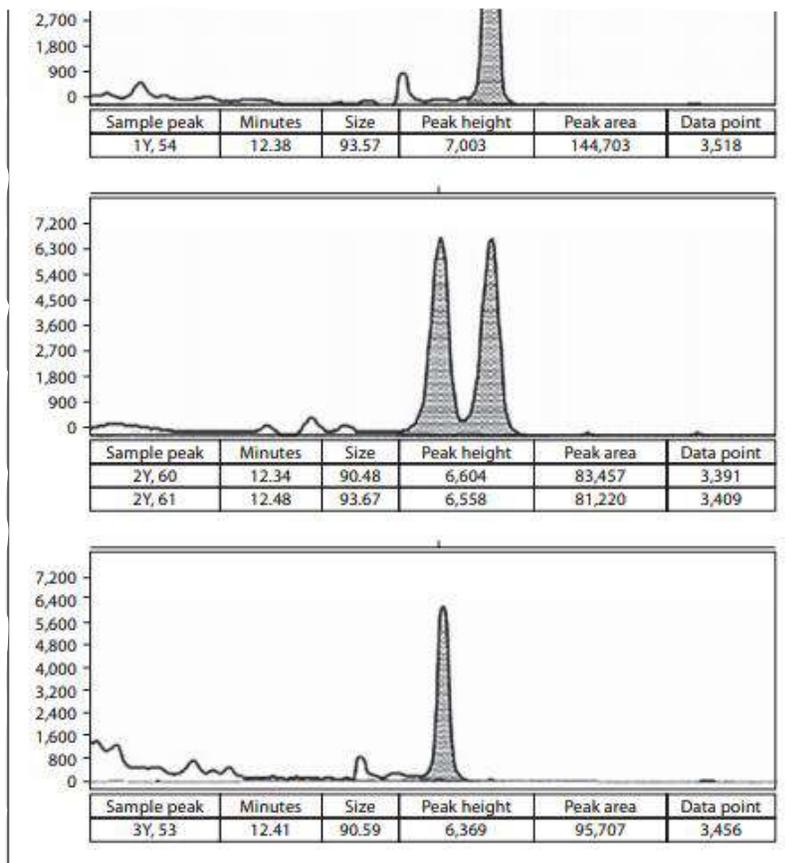
P2 LCRed640-GAA-AAT-ATC-AT-CTT-TGG-TGT-TTC-C-P

- 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 T_m 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



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

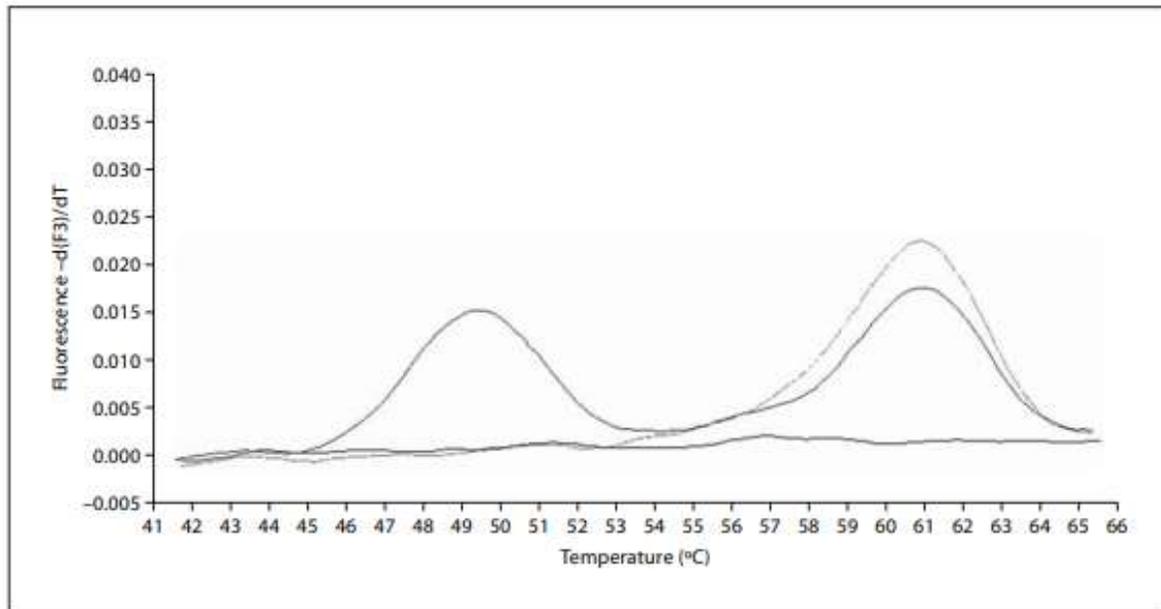
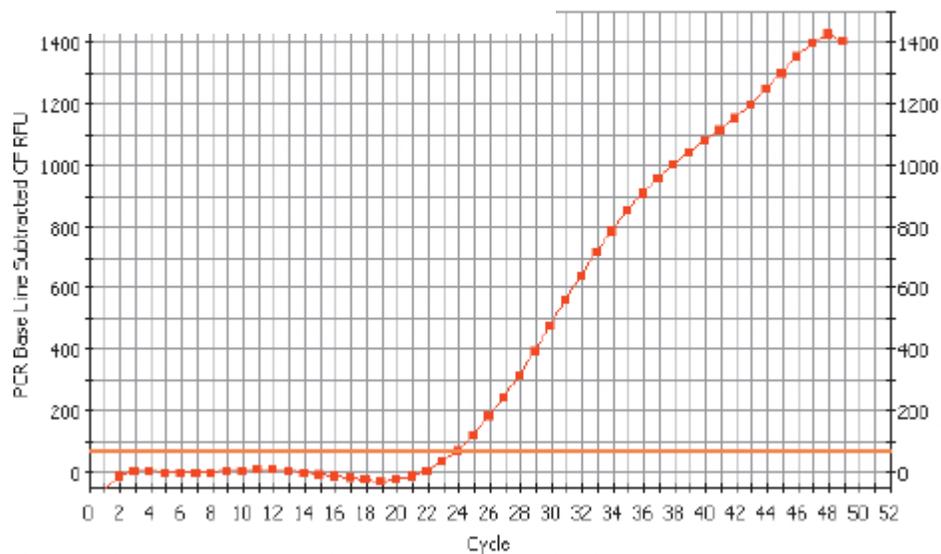
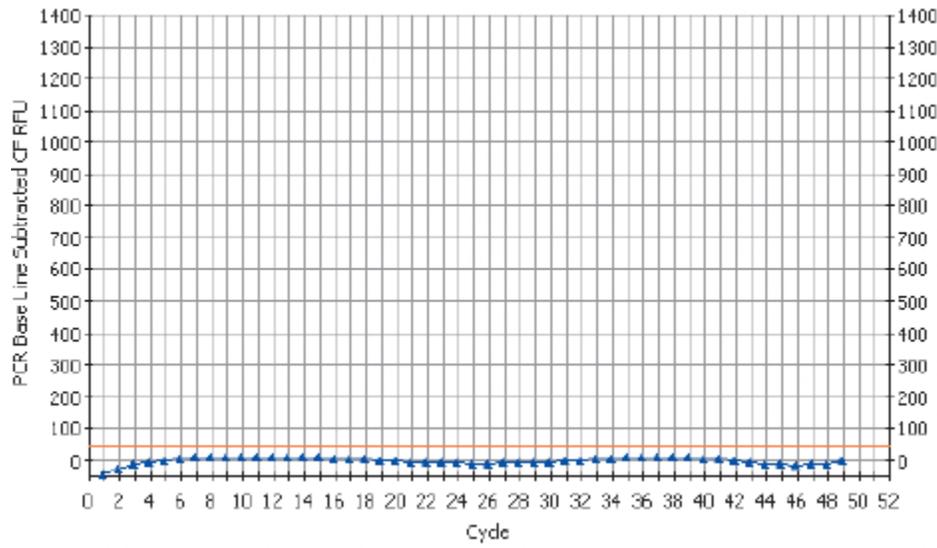


Fig. 2. Detection of $\Delta F508del$ using Q-RT-PCR and melting curve analysis. The figure shows the melting curve analysis curve of a heterozygous (double peaks), a healthy wild sample (one peak) and a d.water (negative control; no peak). The PCR product of $\Delta F508del$ has a T_m of 49°C and the wild type 60°C .

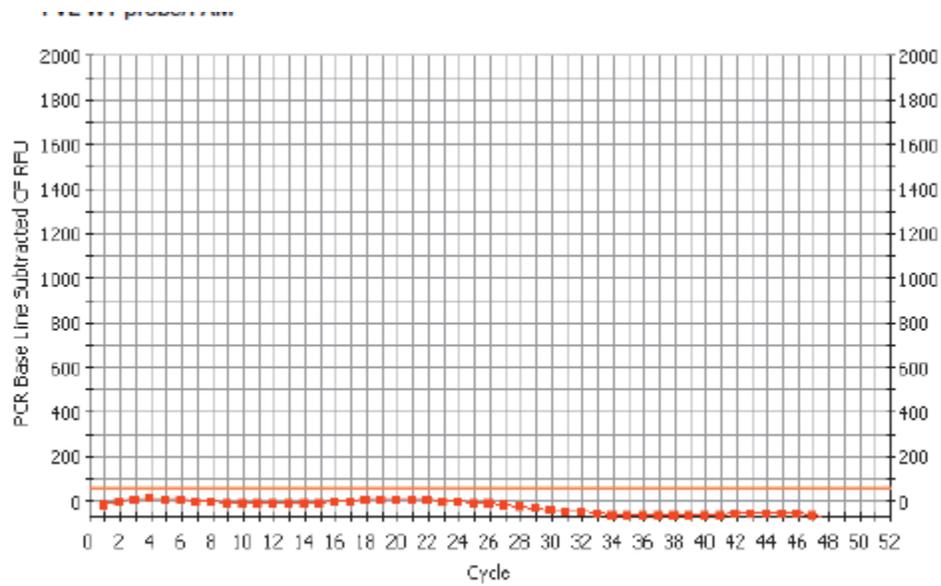
WT

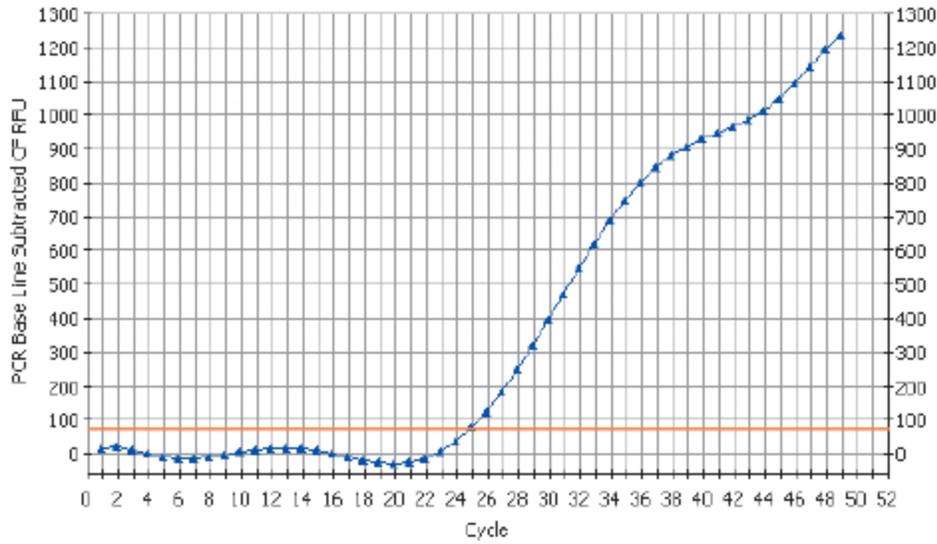
CFTR wt probe/FAM





mut





Heterozygote

