BT501-HEALTH BIOTECHNOLOGY

23-Hemophilia

It has been reported that the Arab physician Abu al-Qasim al-Zahrawi, commonly known as Abulcasis, wrote a book on hematology. His book *Al-Tasrif* written during the period 1000 AD provided the first description of hemophilia, a hereditary genetic disorder

Disorder and Genetics

Hemophilia is a hereditary bleeding disorder resulting from a partial or total lack of an essential blood clotting factor. It is a lifelong disorder that produces excessive bleeding, and spontaneous internal bleeding occurs very frequently.

Hemophilia A, the most common form, is caused by a deficiency in clotting factor 8 (factor VIII). Hemophilia B results from a deficiency in clotting factor 9(IX). This disorder is inherited in a sex linked recessive manner.

Prevalence

This disorder occurs more prominently in males than in females. About one-third of new diagnoses occur without a family history. It appears globally and occurs in all racial groups.

Diagnosis and Prognosis

Hemophilia can be diagnosed as type A or type B by blood tests in infants after 9 months of age. Administration of clotting factors helps affected individuals to live with the disease.

Hemophilia, though a serious disease, can be tolerable with proper precautions and therapy, and the prospects for children with hemophilia are excellent. Recent studies have documented a

greatly increased quality of life and life expectancy among hemophiliac patients in developed countries.

However, it is predicted that the number of people with hemophilia in developed countries will increase gradually during the next few decades.

24-Huntington Disease

Disorder and Genetics

Huntington disease is an autosomal dominant genetic disorder; if one parent carriers the defective Huntington disease gene, his or her offspring have a 50-50 chance of inheriting the disease. Huntington disease is a neurodegenerative brain disorder in which afflicted individuals lose their ability to walk, talk, think, and reason. They easily become depressed, lose their short-term memory, and may experience a lack of concentration and focus. Every individual with the gene for the disease eventually develops the disease.

The Huntington disease gene is located on the long arm of human chromosome 4 and encodes a protein called **huntingtin** that is quite variable in its structure. The 5′ end of the Huntington disease gene contains many repeats of the CAG trinucleotide (encodes glutamine). A highly variable number of **CAG trinucleotide repeats** accounts for the Huntington disease gene mutation, which leads to the expression of an abnormally long **polyglutamine tract** at the N terminus of the huntingtin protein beginning at residue 18.

Such polyglutamine tracts increase protein aggregation, which may alter cell function. Thus, like fragile X syndrome, Huntington disease is one of 14 trinucleotide repeat disorders that cause neurological dysfunction in humans. More specifi cally, Huntington disease is classified as a **polyglutamine disorder**.

Healthy unaffected persons have a CAG repeat count of 9 to 35 (Table). However, alleles with more than 36 CAG repeats give rise to Huntington disease (the highest reported repeat length is 250). Incomplete penetrance is found in alleles with 36 to 39 CAG repeats. People with 36 to 40 CAG repeats may or may not develop Huntington disease, while people with more than 40 CAG repeats are rather likely to develop the disorder. Alleles with more than 60 CAG repeats result in a severe form of Huntington disease known as juvenile Huntington disease, and children who get Huntington disease may range from 2 to 20 years of age.

Table- Variation in status of Huntington disease is controlled by number of CAG repeats in the *huntingtin* gene

CAG repeat count	Disease classification	Disease status
<28	Normal	Unaffected
28–35	Intermediate	Unaffected
36–40	Incomplete penetrance	Weakly affected with Huntington disease
>40	Full penetrance	All affected with Huntington disease
>60	Full penetrance	Affected with juvenile Huntington disease

The mass of the huntingtin protein depends mainly on the number of its glutamine residues. Wild-type (normal) huntingtin consists of 3,144 amino acids, contains 6 to 35 glutamines, and has a mass of ~350 kilodaltons (kDa). In Huntington disease patients, huntingtin contains more than 36 glutamines and has an overall higher molecular mass than wild-type huntingtin.

Prevalence

Huntington disease affects males and females equally and crosses all ethnic and racial boundaries. Usually, Huntington disease begins at age 30 to 45, but it may occur as early as the age of 2. Children who develop juvenile Huntington disease rarely live to adulthood. Everyone

who carries the gene develops the disease. In the United States, Canada, and western Europe, Huntington disease affects about 1 in 20,000 people.

Diagnosis and Prognosis

Currently, there is no treatment or cure for Huntington disease. The discovery of the Huntington disease gene in 1993 facilitated the development of specialized testing that may help to confirm the diagnosis of the disease in patients with an affected parent or characteristic symptoms of the disease. Blood samples are taken from patients, and DNA is directly analyzed by PCR for Huntington disease gene mutations to determine the number of CAG repeats in the Huntington disease gene region. Additional blood samples may be obtained from close or first-degree relatives (e.g., the mother or father) with Huntington disease to help confirm the results.

25-Sickle-Cell Anemia

Sickle-cell disease, or sickle-cell anemia or drepanocytosis, is a hereditary blood disorder, characterized by red blood cells that assume an abnormal, rigid, sickle shape. The sickling occurs because of a mutation in the hemoglobin gene.

Disorder

Sickle-cell disease is a blood-related disorder resulting from the substitution of a valine for glutamic acid in the β-globin chain of adult hemoglobin. The mutated sickle hemoglobin undergoes conformational change and polymerization upon deoxygenation, leading to red blood cell hemolysis and deformation (not doughnut shaped) and to pathology due to blockage of

capillaries. Sickled red blood cells cannot migrate through small blood vessels; rather, they cluster and block these vessels, depriving organs and tissues of oxygen-carrying blood. This process leads to periodic episodes of pain and ultimately can damage tissues and vital organs and lead to other serious medical problems. Normal red blood cells live about 120 days in the bloodstream, but sickled red blood cells die much more rapidly, after about 10 to 20 days. Since they cannot be replaced fast enough, the blood is chronically short of red blood cells, leading to the condition of sickle-cell anemia.

Genetics

Hereditary persistence of fetal hemoglobin decreases the severity of sickle-cell disease. Thus, induction of fetal hemoglobin in adults has been a long-standing goal of therapies for sickle-cell disease. Recently, a GWAS for the fetal hemoglobin phenotype revealed a single strong locus, with sequence variants in the intron of a transcription factor, B-cell chronic lymphocytic leukemia/lymphoma 11A (BCL11A). BCL11A represses fetal hemoglobin expression in red blood cells.

Inactivation of BCL11A in a mouse model of sickle-cell disease not only leads to the induction of fetal hemoglobin expression but also corrects the hematologic and pathological defects of the disease. Thus, BCL11A is a potential therapeutic target for sickle-cell disease.

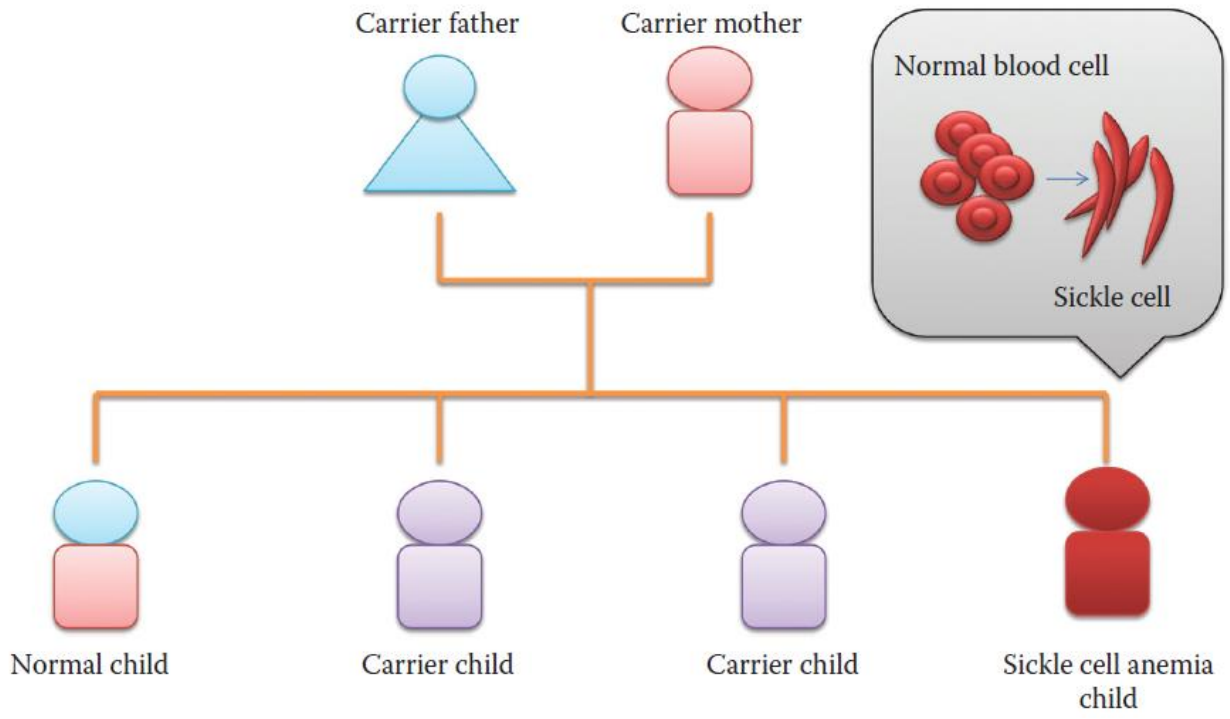


Figure- Single gene inheritance which causes sickle cell anemia

Prevalence

Sickle-cell anemia affects millions of people throughout the world. It is particularly common among people whose ancestors originate from sub-Saharan Africa, South America, Cuba, Central America, Saudi Arabia, India, and Mediterranean countries (e.g., Turkey, Greece, and Italy).

In the United States, it affects around 72,000 people, most of whose ancestors come from Africa. The disease occurs in about 1 in every 500 African-Americans and 1 in every 1,000 to 1,400 Hispanic Americans. About 2 million Americans, or 1 in 12 African-Americans, carry the sickle-cell allele.

Diagnosis and Prognosis

Sickle-cell disease can be diagnosed by a simple blood test. In many cases, sickle-cell anemia is diagnosed when newborns are screened. Vaccines, antibiotics, and folic acid supplements are administered in addition to painkillers. Blood transfusions and surgery are used in severe cases. The only known cure at present is a bone marrow transplant.

26-Tay–Sachs Disease

Disorder and Genetics

Tay–Sachs disease is an autosomal recessive fatal genetic disorder caused by a genetic mutation in the *HEXA* gene on human chromosome 15, which encodes the hexosaminidase A enzyme. This mutation leads to a decrease in function of hexosaminidase A, and as a result, harmful quantities of a sphingoglycolipid, termed ganglioside GM2, accumulate in brain neurons.

HEXA gene mutations are rare and are most frequently detected in genetically isolated populations. Tay–Sachs disease can occur from the inheritance of either two similar or two unrelated mutations in *HEXA* that cause disease. Many *HEXA* mutations have been identified, and these mutations can reach significant frequencies in certain populations (see below).

In the most common form of the disease (infantile Tay–Sachs), abnormal hexosaminidase A enzyme activity and the accompanying harmful accumulation of cell membrane-associated gangliosides in neurons lead to premature neuronal death, paralysis, dementia, blindness, psychoses, and even death of the patient. Although the degeneration of the central nervous

system begins at the fetal stage, the loss of peripheral vision and motor coordination are not evident until ~6 months of age, and death usually results by 4 years of age.

Prevalence

The frequency of Tay–Sachs disease is much higher in Ashkenazi Jews (Jews of eastern European origin) than in others. Approximately 1 in 27 Jews in the United States is a carrier of the Tay–Sachs disease gene. There is also a noticeable incidence of Tay–Sachs disease in non-Jewish French Canadians, known as Acadians, who originated from France and settled in southeastern Quebec.

Interestingly, while the French Canadians and Ashkenazi Jews carry different *HEXA* mutations, the Cajuns (people in southern Louisiana descended from the Acadians) carry the mutation found most frequently in Ashkenazi Jews. The ancestry of carriers from Louisiana families traces back to a single non-Jewish founder couple that lived in France in the 18th century. The Irish are also at increased risk for the Tay–Sachs gene, and among Irish-Americans the carrier rate is currently about 1 in 50. By contrast, the carrier rate in the general non-Jewish population as well as in Jews of Sephardic (Iberian or Middle Eastern) origin in the United States is about 1 in 250.

Diagnosis and Prognosis

Tay–Sachs disease may be diagnosed by a blood test that measures levels of the hexosaminidase A enzyme in serum, lymphocytes, or skin fibroblasts. During the past 25 years, carrier screening and genetic counseling in high-risk populations have greatly reduced the number of children born with Tay–Sachs disease in these groups. Thus, a high percentage of babies born with Tay–

Sachs disease today are born to couples not previously considered to be at high risk. Prenatal tests of hexosaminidase A activity, such as amniocentesis (at 15 to 16 weeks of pregnancy) and chorionic villus sampling (at 10 to 12 weeks of pregnancy), can now diagnose Tay–Sachs disease in the fetus.

27-Thalassemia

Disorder and Genetics

Thalassemia is a blood-related genetic disorder that involves either the absence of or errors in genes responsible for the production of hemoglobin. Each red blood cell contains 240 million to 300 million molecules of hemoglobin. The severity of the disease depends on the gene mutations that arise and the manner in which they influence each other.

Both of the hemoglobin α and β subunits are required to bind oxygen in the lungs and deliver it to other tissues. Genes on human chromosome 16 encode the α subunits, while genes on chromosome 11 encode the β subunits. A lack of expression of a particular subunit determines the type of thalassemia; e.g., a lack of an α subunit results in α-thalassemia. The lack of subunits corresponds to mutations in the genes on the appropriate chromosomes. There can be various grades of the disease depending on the gene and the type of mutations.

Prevalence

The α- and β-thalassemias are the most common inherited single-gene disorders, with the highest prevalence in areas where malaria was or still is endemic. The burden of this disorder in many regions is of such a magnitude that it represents a major public health concern. In Iran, about 8,000 fetuses may be affected by thalassemia each year. In some Mediterranean countries, control programs have achieved 80 to 100% prevention of disease in newborns.

Diagnosis and Prognosis

Diagnosis of thalassemia can be made as early as 10 to 11 weeks in pregnancy using procedures such as amniocentesis and chorionic villus sampling. Individuals can also be tested for thalassemia through routine blood counts. Thalassemic patients may have reduced fertility or even infertility.

Early treatment of thalassemia has been very effective in improving the quality of life of patients. Treatments for thalassemias depend on the type and severity of the disorder. Carriers or individuals who have mild or no symptoms of α- or β-thalassemia need little or no treatment.

Three types of treatments are used for moderate and severe forms of thalassemia. These include blood transfusions, iron chelation therapy (removes excess iron in the blood that increases after transfusions), and folic acid supplements (vitamin B supports red blood cell growth).

Currently, genetic testing and counseling, and prenatal diagnosis, play an increasingly important role in informing individual as well as professional decisions related to the prevention, management, and treatment of this disease.

28-Polygenic disorders- Diabetes

Genetic disorders are **polygenic** if they are causally associated with the effects of many genes. Such disorders may also be **multifactorial**, if they are influenced by several different lifestyles and environmental factors.

Examples of multifactorial disorders include cancer, heart disease, and diabetes. Although such disorders frequently cluster in families, these disorders do not have a simple Mendelian pattern of inheritance. Moreover, as many of the factors that cause these disorders have not yet been identified, it is rather difficult to determine the risk of inheritance or transmission of these disorders.

Some principal features of the pattern of multifactorial inheritance of a polygenic diseases are summarized in Table.

No.	Characteristic
1	A simple pattern of inheritance of a polygenic disease does not exist within a family.
2	A lower incidence of a polygenic disease within a population is associated with a higher risk of disease in first-degree relatives.
3	The relative risk of a polygenic disease is much lower in second-degree and more distantly related family members.
4	The risk of recurrence of a polygenic disease in a family is higher when more than one family member is affected.
5	The more severe the malformation associated with a polygenic disease, the greater is the risk of disease recurrence.
6	If a polygenic disease is more frequent in one sex than the other, the risk is higher for relatives of patients of the less susceptible sex.
7	If an increased risk of recurrence of disease occurs when the parents are consanguineous (e.g., first-degree relatives), many factors with additive effects may be involved.

An individual may not be born with a disease but may be at high risk of acquiring it, a condition referred to as **genetic predisposition** or **genetic susceptibility**. The genetic susceptibility to a particular disease due to the presence of a gene mutation(s) in an allele(s) need not lead to disease.

Diabetes is a disease in which the body does not produce or properly use insulin. Insulin is a hormone that is needed to convert sugar, starches, and other food into energy needed for daily life. The cause of diabetes continues to be a mystery, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles. There are three major classes of diabetes: type 1 diabetes, type 2 diabetes, and gestational diabetes. Only type 1 diabetes, a representative polygenic autoimmune disease.

29-Diabetes mellitus type 1

Disorder

Type 1 diabetes is an autoimmune disease that presents clinically with hyperglycemia resulting from the immune-mediated progressive destruction of insulin-producing pancreatic islet β-cells and associated metabolic dysfunction.

The resulting insulin deficiency requires lifelong exogenous insulin treatment for survival, and long-term complications can cause substantial disability and shorten life span. About 90 to 95% of the β-cell mass is already destroyed when symptoms of type 1 diabetes first appear, demonstrating that prediction and prevention are high priorities.

This disease affects about 1 in 300 individuals in North America. More than 20 million people worldwide (mostly children and young adults) are estimated to have type 1 diabetes.

Genetics

The etiology and pathogenesis of type 1 diabetes are largely accounted for by genetic predisposition. Within a pair of monozygotic twins (originating from one placenta), if one twin has type 1 diabetes, the probability of the other twin becoming a type 1 diabetic is about 40 to 50%. In pairs of dizygotic twins (originating from two placentas) in which one twin is type 1 diabetic, the probability of the other twin becoming a type 1 diabetic is only about 5 to 6%. This lower probability of susceptibility to type 1 diabetes is the same as that for unrelated children in the general population. These findings are consistent with the observed overall global increase in type 1 diabetes incidence during the last 20 to 30 years and strongly support the idea that environmental factors are also important in the control of susceptibility to type 1 diabetes.

The majority of genetic research to date has focused on the heritability that predisposes to islet autoimmunity and type 1 diabetes. The evidence indicates that type 1 diabetes is a polygenic, common, complex disease, with major susceptibility genes located in the *HLA* complex on human chromosome 6 and with other smaller effects found in non-*HLA* loci on other chromosomes. Recent advances in DNA technology, including high-throughput SNP typing and sequencing and GWASs, have advanced our understanding of the immune pathogenesis of type 1 diabetes.

Diagnosis

Type 1 diabetes is checked by analyzing the plasma glucose level and is diagnosed by fasting plasma glucose level at or above 126 mg/dL (considered as prediabetic conditions) and plasma glucose at or above 200 mg/dL (considered as diabetic condition).

To confirm type 1 diabetes, most physicians prefer to measure a fasting glucose level and according to the current definition, two fasting glucose measurements above 126 mg/dL are considered diagnostic for diabetes mellitus. Moreover, patients with fasting glucose levels from 100 to 125 mg/dL are considered to have impaired fasting glucose.

Furthermore, patients with plasma glucose at or above 140 mg/dL but not over 200 mg/dL, 2 h after a 75 g oral glucose load are considered to have impaired glucose tolerance. Interestingly, among these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus as well as cardiovascular disease.

30-Diabetes mellitus type 2

It is known as Non Insulin dependent diabetes (NIDD) and is characterized by reducing response of body against insulin or relatively reduced insulin secretion by the beta cells. Type 2 diabetes is the most common type and starts appearing after the age of 30 years. It is due to the combination of sedentary lifestyle and genetic factors.

Obesity has been found to contribute to approximately 55% cases of the type 2 diabetes. However, because type 2 diabetes develops slowly, some people with high blood sugar experience no symptoms at all.

***Treatment*:** Onset of type 2 diabetes can be delayed or prevented through proper nutrition and regular exercise. If the condition progresses, medications may be needed.

The synthesized insulins are used medically to treat patients with Type 1 diabetes mellitus, whereas patients with Type 2 diabetes mellitus are insulin resistant.

31-Alzheimer-diseases

Alzheimer's disease (AD), also known in the medical literature as Alzheimer disease, is the most common form of dementia. There is no cure for the disease, which worsens as it progresses, and eventually leads to death. It was first described by a German psychiatrist and neuropathologist Alois Alzheimer in 1906 and was named after him. Many different clinical features are associated with Alzheimer disease, including the inability to create new memories, the loss of short-term memory, and the inability to concentrate.

Disorder

Alzheimer disease is a devastating neurodegenerative disorder with a relentless progression. The pathogenesis of Alzheimer disease may be triggered by the accumulation of the amyloid β peptide (Aβ), which is due to overproduction of Aβ and/or the failure of mechanisms to clear the peptide. Aβ self-aggregates into oligomers of various sizes and forms diffuse and neuritic plaques in the parenchyma and blood vessels. Aβ oligomers and plaques are potent mediators of

Aβ toxicity, block proteasome function, inhibit mitochondrial activity, alter intracellular Ca2+ levels, and stimulate inflammatory processes. Loss of normal Aβ function also contributes to neuronal dysfunction.

Aβ interacts with the signaling pathways that regulate the phosphorylation of the microtubule-associated protein tau. Hyperphosphorylation of tau disrupts its normal function in regulating axonal transport and leads to the accumulation of neurofibrillary tangles and toxic species of soluble tau. Degradation of hyperphosphorylated tau by the proteasome is inhibited by the actions of Aβ. These two proteins and their associated signaling pathways therefore represent important therapeutic targets for Alzheimer disease. Abnormalities in Aβ and tau can be measured upon neuropathological examination, in cerebrospinal fluid or by positive emission tomography scans.

Genetics

Susceptibility to Alzheimer disease is genetically controlled, and about 13% of Alzheimer disease is inherited as an autosomal dominant disorder. **Late-onset familial Alzheimer disease** is a complex polygenic disorder controlled by multiple susceptibility genes, and the strongest association is with the *APOE* e4 allele at locus *AD2*.

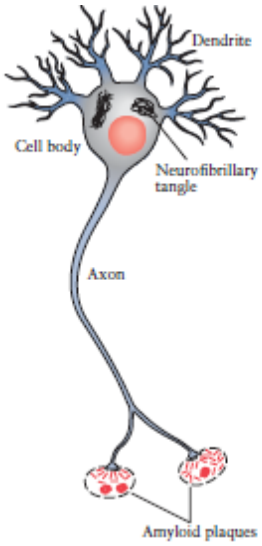
Mutations in three genes—those for **amyloid** precursor protein (*APP*), presenilin-1 (*PSEN1*), and presenilin-2 (*PSEN2*)—account for less than 5% of all cases of Alzheimer disease and often cause early onset of the disease in individuals who are 60 years old or younger. Thus, despite considerable research effort, the root cause of Alzheimer disease in the vast majority of cases is unknown.

Prevalence

Alzheimer disease is the most common cause of dementia in North America and Europe, with an estimate of four million affected individuals in the United States. The prevalence of Alzheimer disease increases with age: about 10% of persons older than 70 have significant memory loss, and more than half of these individuals have Alzheimer disease. An estimated 25 to 45% of persons over age 85 have dementia.

Diagnosis and Prognosis

A proper diagnosis of Alzheimer disease relies on a clinical neuropathological assessment. The formation of Aβ plaques and neurofibrillary tangles is thought to contribute to the degradation of the neurons in the brain and the subsequent symptoms of Alzheimer disease. A hallmark of Alzheimer disease is the accumulation of amyloid plaques between neurons in the brain. Amyloid is a general term for protein fragments that the body produces normally. Aβ is a protein fragment cleaved from a larger amyloid precursor protein. In a healthy brain, these Aβ protein fragments are broken down and eliminated. In Alzheimer disease, the fragments accumulate to form hard, insoluble plaques (deposits). The plaques are composed of a tangle of protein aggregates that have a fiber appearance and are called amyloid fibers. Neurofibrillary tangles consist of these insoluble twisted amyloid fibers found inside neurons. These tangles are formed primarily by a protein called tau, which forms part of a microtubule that helps to transport nutrients and other substances from one part of a neuron to another. In Alzheimer disease, however, the tau protein is abnormal and the microtubule structures collapse. As a result, neurons are deprived of certain nutrients and undergo cell death. In Alzheimer disease, however, the tau protein is abnormal and the microtubule structures collapse. As a result, neurons are deprived of certain nutrients and undergo cell death.



Neurofibrillary tangles and amyloid plaques associated with neurons in the brain are hallmarks of Alzheimer disease.

Three forms of early-onset familial Alzheimer disease caused by mutations in one of three genes—Aβ (A4) precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*)—are recognized. Molecular genetic testing of the three genes is available in clinical laboratories.

Early- onset familial Alzheimer disease A form of Alzheimer disease that arises at a younger age (<65 years) in families. About 1 to 6% of all Alzheimer disease is early onset, and about 60% of early- onset Alzheimer disease runs in families.

Late- onset familial Alzheimer disease A complex polygenic disorder controlled by multiple susceptibility genes. The strongest association is with the *APOE* e4 allele at locus *AD2*.

Senile plaque A dense spheroid amyloid body found outside of neurons and often associated with Alzheimer disease

32-Breast Cancer

Disorder

Cancer generally results from the sequential acquisition of mutations in genes that regulate cell multiplication, cell repair, and the ability of a cell to undergo malignant transformation. This multistep process is not an abrupt transition from normal to malignant but may take 20 years or more.

Genetics

This malignancy is one of the most commonly inherited cancers based on observations that

(i) 20 to 30% of all patients with breast cancer have a family history of the disease

and

(ii) Twin studies show that 25% of breast cancer cases are heritable.

Discovery of the breast cancer type 1 susceptibility (*BRCA1*) and *BRCA2* genes more than 10 years ago has had a high impact on patient care, allowing for early detection and prevention of breast cancer. However, deleterious mutations in the *BRCA1* and *BRCA2* genes cause at most 3 to 8% of all breast cancer cases.

Prevalence

Lung, stomach, liver, colon, and breast cancers cause the most cancer deaths each year. Combined, these cancers are responsible for more than 4.2 million deaths annually.

Current epidemiological evidence predicts that 1 in 8 women will be diagnosed with breast cancer in her lifetime. Deaths from cancer worldwide are predicted to continue rising, with a

projected 13.1 million deaths in 2030. Breast cancer is the second most common malignancy diagnosed in women and the second leading cause of cancer-related deaths.

Diagnosis and Prognosis

The roles that genes play differ greatly, ranging from completely determining the disease state (disease genes) to interacting with other genes and environmental factors in causing cancer (susceptibility genes). The primary determinants of most cancers are lifestyle factors (e.g., smoking, dietary and exercise habits, environmental carcinogens, and infectious agents) rather than inherited genetic factors.

Breast cancer is a heterogeneous disease often characterized by the presence or absence of expression of estrogen receptors and human epidermal growth factor receptor 2 (HER2) on tumor cells. These molecular markers used to classify breast cancer subtypes may also predict typical responses to targeted therapies.

Genetic tests will be useful in determining the best course of treatment for cancers, which are presently classified as a single disease but may ultimately be classified into different types, each best managed by a different therapeutic strategy.

Thus, genetics will continue to play an important role in the control of cancer, including

(i) The identification of individuals at risk for a specific cancer, leading to preventative or screening strategies for an individual or family members, and

(ii) The identification of the subtype of a cancer so that treatment can be tailored to target that specific disease.

Women with a type of breast cancer that overproduces the human epidermal growth factor receptor 2 (HER2) protein on the surface of the tumor may be effectively treated with the humanized anti-HER2 monoclonal antibody trastuzumab (Herceptin).

Trastuzumab: The First Humanized Monoclonal Antibody Approved for the Treatment of Breast Cancer.

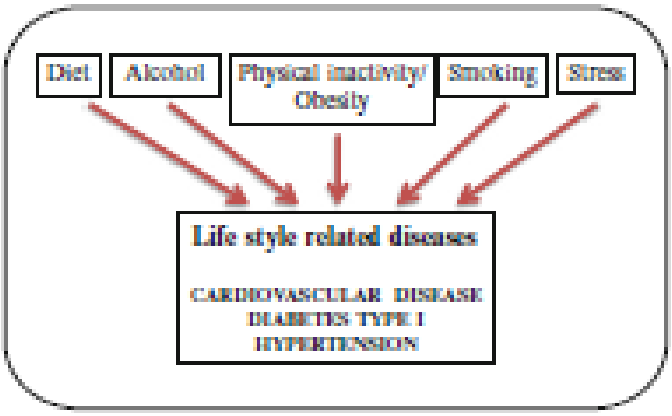
33-Cardiovascular Disease

Disorder

Major cardiovascular diseases include several subtypes, such as coronary heart disease, cerebrovascular disease, heart failure, rheumatic heart disease, and congenital heart disease. Cardiovascular disease is a leading health problem, affecting more than 80 million individuals in the United States. In Canada, 1 out of 3 deaths is attributable to cardiovascular disease.

The major risk factors associated with cardiovascular diseases are cigarette smoking, unhealthy diet, physical inactivity, hypertension, diabetes, and high blood cholesterol. Cardiovascular diseases may also result from a variety of genetic causes, including single-gene mutations, interactions between multiple genes, and gene–environment interactions.

Economic shifts, urbanization, industrialization, and globalization bring about lifestyle changes that promote heart disease. In developing countries, life expectancy is increasing rapidly and is expected to expose people to these environmental risk factors for longer periods.



Genetics

For most cardiovascular disease disorders, the relative risk of disease is attributable to inherited DNA sequence variants. However, the role of inheritance and the magnitude of its effect vary by disease and other factors such as age of disease onset and subtype of cardiovascular disease. Most cardiovascular disease traits, such as myocardial infarction and concentrations of plasma LDL cholesterol, show complex inheritance, suggestive of an interaction between multiple genes and environmental factors.

Disease	Possible causes	Affects	Symptoms	Treatment
Cardiovascular disease	Inherited and acquired, predominant lifestyle disease caused by smoking	Coronary arteries are at risk for narrowing as cholesterol deposits, called plaques, build up inside the artery, affect the heart muscle or the blood vessels of the heart which can lead to congestive heart failure, and may also cause disturbances in normal heartbeat, called arrhythmia; clot in artery due to rupture of plaque can lead to myocardial infarction	Chest pain, shortness of breath, sweating, nausea, and indigestion	Medical intervention with multistep approach, lifestyle modification, regular exercise, cholesterol reduction, quitting smoking and alcohol consumption, and stress-free life
	High blood pressure (hypertension)			
	High cholesterol			
	Diabetes			
	Family history			
	Peripheral artery disease			
	Obesity			

34-Mitochondrial Disorders

Disorders

For cells to function normally, they must generate energy in the form of ATP. In many types of cells, mitochondrial activity is the prime source of ATP. Mitochondria also regulate several other cellular processes, including production of heat in response to changes in temperature and diet, ion homeostasis (maintenance of an internal steady state of ions), innate immune response, production of reactive oxygen species, and programmed cell death (apoptosis).

It follows that if mitochondrial malfunction occurs and leads to deficient energy production and the impairment of other cellular functions, this may result in a mitochondrial disease(s). The knowledge that mitochondrial impairment may be involved in diseases is relatively new; it was first recognized in an adult in the late 1960s and then in children in the late 1980s.

Mitochondrial dysfunction can occur in the cells of many organs and systems in the body; more than 200 inherited diseases of metabolism can affect mitochondria, and more than 40 types of mitochondrial disorders have been reported. The brain, heart, muscles, and lungs are the organs that require the most energy, and when deprived of energy the functions of these organs begin to fail.

The most severe effects of mitochondrial disease occur in the brain and muscles because they are heaviest users of energy. Other commonly affected organs include the liver, nervous system, eyes, ears, and kidneys. Mitochondrial dysfunction is observed in single-gene mitochondrial disorders and may also be associated with the pathogenesis of polygenic diseases such as

Alzheimer disease, Parkinson disease, cancer, cardiac disease, diabetes, epilepsy, Huntington disease, and obesity. In addition, a progressive decline in the expression of mitochondrial genes is a main feature of human aging, raising the possibility that an increased understanding of the aging process may provide more insight into one or more of the above-mentioned polygenic diseases.

Genetics

Mitochondrial dysfunction generally results from a genetic mutation(s). Interestingly, mitochondria have their own DNA for 37 genes, 13 mitochondrial proteins, 2 rRNAs, and 22 tRNAs. Mitochondrial DNA is less complex than nuclear DNA and is not inherited according to Mendelian genetics (inheritance of DNA from both parents). Rather, because egg cells but not sperm contribute mitochondria to a developing embryo, only mothers can transmit mitochondrial traits to their children. Thus, mitochondrial DNA is inherited only maternally.

Mutations in mitochondrial DNA differ from those in nuclear DNA, and many more mutations occur in mitochondrial DNA than nuclear DNA. The mutation rate in mitochondrial DNA is 10 times higher than in nuclear DNA because mitochondrial DNA is subject to damage from reactive oxygen molecules released as a by-product during oxidative phosphorylation in cells. Mitochondrial dysfunction may also be caused by environmental factors (e.g., viral infection or drug treatment) that interfere with mitochondrial activity.

35-Mitochondrial homeostasis

Because neurons require considerable energy to maintain their metabolically active state, they rely heavily on the maintenance of mitochondria in a functional state, a property known as **mitochondrial homeostasis**.

Mitochondria constitute a population of organelles that require a careful balance and integration of many cellular processes, including the regulation of biogenesis, migration throughout the cell, shape remodeling, and autophagy (catabolic degradation of unnecessary cellular components by lysosomes).

These dynamic processes prompt mitochondrial recruitment to critical subcellular compartments, content exchange between mitochondria, mitochondrial shape control, and mitochondrial communication with the cytosol. Mitochondrial homeostasis is the term used to refer to a well maintained balance of these processes. The structure and function of the mitochondrial network are dependent on mitochondrial homeostasis, which is essential for maintaining the signaling, plasticity, and transmitter release of neurons. There are different pathways to maintain mitochondrial homeostasis.

36-Parkinson Disease

After Alzheimer disease, Parkinson disease is the second most common neurodegenerative disease. Parkinson disease affects about 1% of the population that is 60 years of age or older and is characterized by the progressive reduced capacity to initiate voluntary movements arising mainly from the loss of neurons that synthesize the neurotransmitter dopamine.

Among the many factors that mediate the development of Parkinson disease, mitochondrial dysfunction is considered to be a major factor in its etiology and pathogenesis. The dysfunction of mitochondria in Parkinson disease patients may result from one or more of the following conditions:

- Deletion of mitochondrial DNA,
- Accumulation of mitochondrial DNA
- Mutations, increase in oxidative stress from reactive oxygen species
- Deficient expression and function of the mitochondrial respiratory chain
- Abnormal morphology of mitochondria.

Neurodegenerative diseases such as Parkinson disease are often closely associated with an imbalance of mitochondrial homeostasis (Fig.). The levels of many mitochondrial proteins are altered in postmortem samples of brains from persons with Parkinson disease.

Genetic mutations in the *PTEN-induced putative kinase 1* (*PINK1*), *parkin* (*PARK2*), *DJ-1*, *alpha-synuclein* (*SNCA*), and *leucine-rich repeat kinase 2* (*LRRK2*) genes are closely linked to

the recessive Parkinson disease genes. Moreover, these mutations are associated with important functions that maintain mitochondrial homeostasis, which include membrane potential, calcium homeostasis, structure of cristae (internal compartments formed by the inner membrane of mitochondria), respiratory activity, mitochondrial DNA integrity, and clearance of dysfunctional mitochondria from cells.

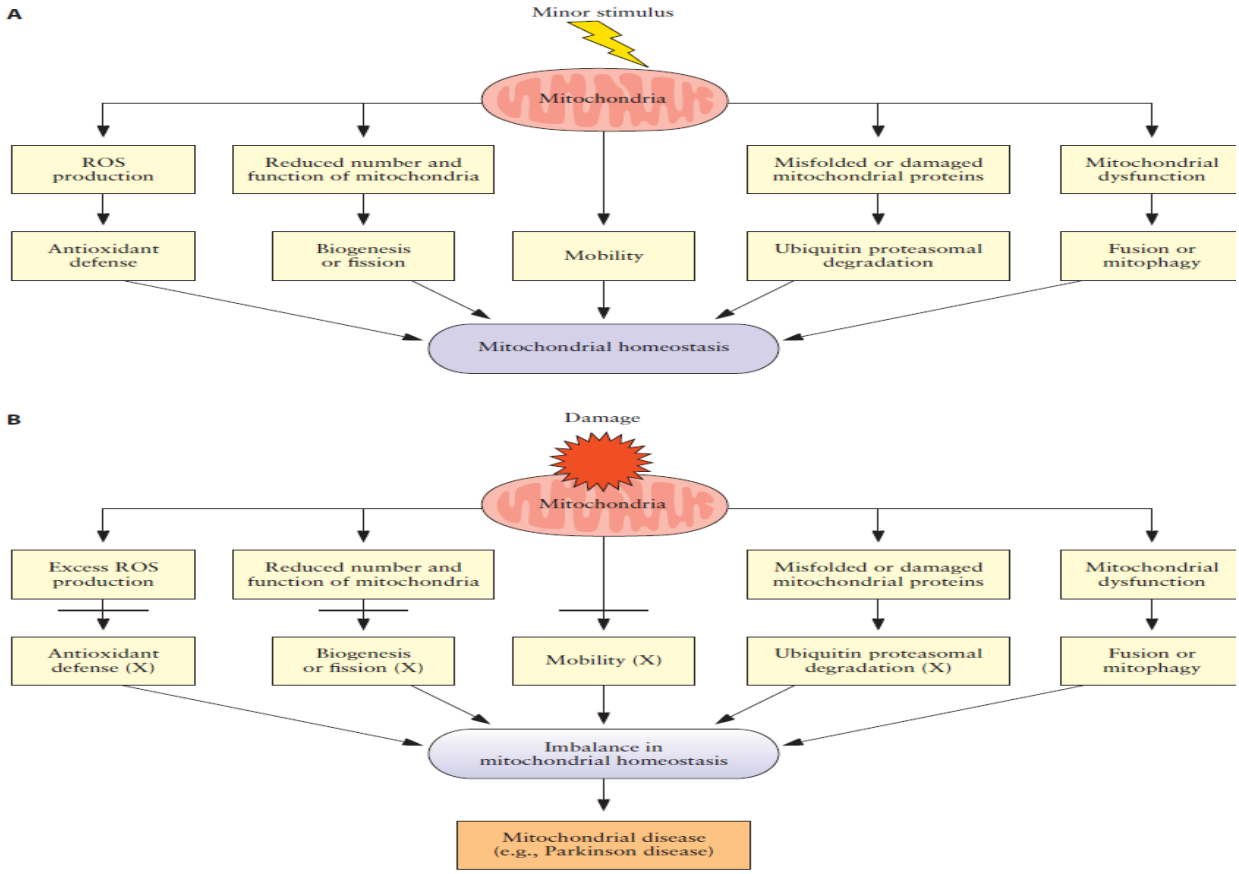


Fig-Parkinson disease is associated with an imbalance in mitochondrial homeostasis. Since mitochondria regulate energy metabolism, they are essential for the control of neuronal function. Mitochondria respond to exogenous and endogenous stimuli and maintain their homeostasis by undergoing continuous fusion, fission, mobility, mitophagy, and biogenesis. **(A)** Normally, upon exposure of mitochondria to a minor stimulus, changes in mitochondrial function and morphology occur to activate different cellular pathways that help to maintain homeostasis. **(B)** After mitochondria incur damage, changes in mitochondria occur that lead to excess reactive oxygen species (ROS) production, reduced number and mass, misfolding

and/or aggregation of damaged proteins, and mitochondrial dysfunction. As a result, an imbalance in mitochondrial homeostasis ensues that may lead to the onset of Parkinson disease. Mitophagy is a catabolic process of self-degradation of mitochondria in a cell. X indicates a mitochondrial dysfunction.

37-Immunological Approaches To Detect Protein Biomarkers of Disease

Proteins play a critical role in all cellular processes, including metabolism, communication, defense, reproduction, transport, and motility. Regulatory proteins maintain tight control over protein production to ensure that cells function normally.

Characteristic changes in proteins have been used extensively to diagnose disease, either by detecting the presence or measuring levels of a specific protein biomarker or by determining protein profiles in polygenic diseases. There are several advantages to using proteins as diagnostic biomarkers of disease. Abnormal levels of gene expression as a consequence of disease-associated mutations are more accurately quantified by measuring the protein directly rather than by measuring mRNA levels, which often do not correlate with protein levels.

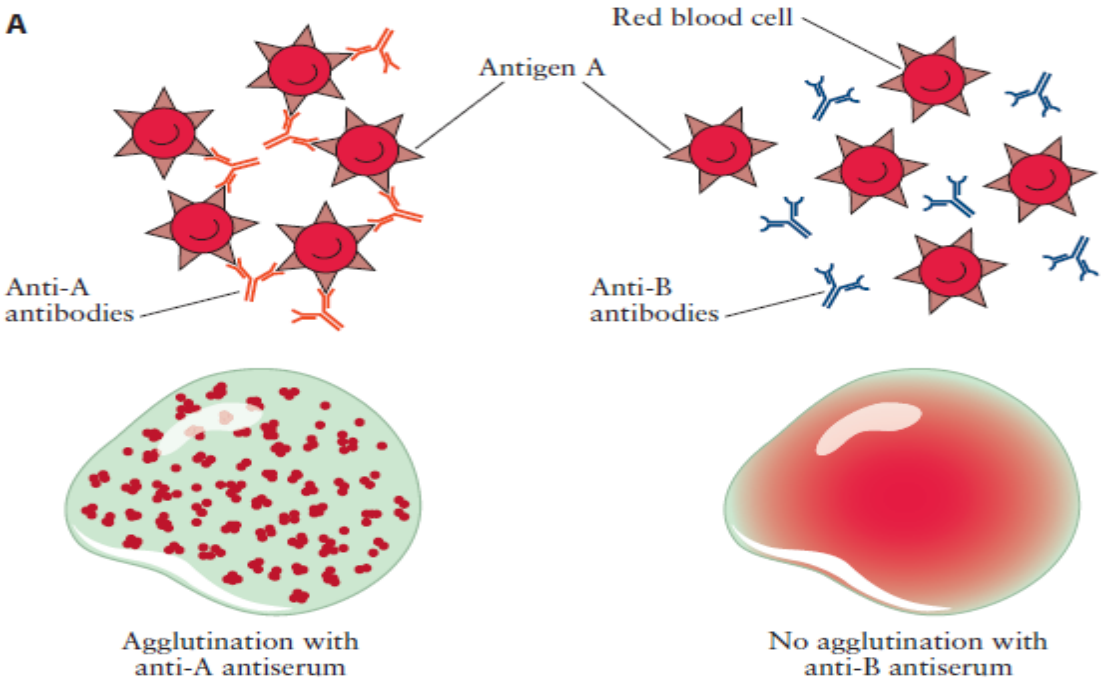
Furthermore, proteins in diseased tissues may exhibit irregularities in posttranslational modification that cannot be detected using nucleic acids. In addition, many diseases often are a consequence of altered protein conformation (e.g., prion diseases and some neurodegenerative diseases) that may not be detected from nucleic acid sequences. On the other hand, the unique shape and often very low cellular levels of a protein are a challenge for development of a diagnostic assay.

Antibodies can be produced that bind to target proteins with high affinity and specificity, and therefore immunological approaches meet the criteria of sensitivity, specificity, and simplicity for diagnostic assays.

Agglutination is a simple, inexpensive, rapid, and highly specific immunological test that is widely performed in diagnostic laboratories. For example, it is often used for human blood typing based on the presence of specific antigens on the surface of red blood cells, which vary among individuals.

Antiserum containing antibodies against either A or B surface antigen is mixed with red blood cells. Clumping (agglutination) indicates the presence of the antigen (Fig. A). Some individuals produce only the A antigen (blood type A), which agglutinates with anti-A antiserum but not with anti-B antiserum (Fig. A).

Blood type B individuals produce only the B antigen, which reacts with anti-B antiserum and not with anti-A antiserum (Fig. B). Others carry both antigens (blood type AB) or neither (blood type O). Blood samples from individuals with blood type O, the most common blood type, do not produce a positive agglutination result with either antiserum. This test, referred to as hemagglutination, is important for determining which blood to use in transfusions, as antibodies naturally produced against nonself red blood cell antigens would destroy the introduced blood. In some agglutination tests, the antigen or antibody employed to detect either a specific antibody or antigen, respectively, in patient samples is used to coat the surface of small latex beads.



B

Blood type	Agglutination with serum	
	Anti-A	Anti-B
A	+	–
B	–	+
AB	+	+
O	–	–

Fig-Hemagglutination test to determine blood type. **(A)** Antiserum containing antibodies against A or B surface antigens is mixed with red blood cells carrying A surface antigens. Clumping (agglutination) in the presence of anti-A antiserum but not with anti-B antiserum indicates blood type A. **(B)** Agglutination responses for different blood types.

38-Enzyme-Linked Immunosorbent Assays

Enzyme-linked immunosorbent assays (ELISAs) are widely used for diagnosing human diseases, including various cancers, autoimmune diseases, allergies, and infectious diseases. An ELISA measures antigens or antibodies produced against an antigen in a clinical sample from blood, urine, or tissues. It is based on the specific and high-affinity interaction between an antibody and an antigen and is a sensitive assay that can be used for rapid detection on a large scale (i.e., high-throughput assays). Detection relies on the activity of an enzyme that is covalently bound to an antibody employed in the assay.

An **indirect ELISA** can detect the presence of specific antibodies in patient serum that indicates an immune response to the presence of a particular protein or pathogen. A **sandwich ELISA** detects the presence of a specific antigen in a patient's sample and hence is sometimes referred to as an antigen capture assay.

In an indirect ELISA used for diagnostic purposes, a standardized antigen is bound to a solid support, usually the surface of a well in a microtiter plate (Fig. A). A patient's serum sample is applied to the well, and specific **primary antibodies** in the serum bind to the immobilized antigens. A **secondary antibody** that binds specifically to the primary antibody is applied. Because the primary antibody is present in human serum, the secondary antibody is an anti-human immunoglobulin antibody that was raised in another animal, for example, a goat, by injecting the animal with human immunoglobulin. The secondary antibody is covalently bound (conjugated) to an enzyme such as alkaline phosphatase or horseradish peroxidase that catalyzes

the conversion of a colorless substrate into a colored product. A colorless substrate is applied, and the formation of a colored product by the enzyme indicates the presence of the secondary antibody and, hence, the primary antibody–antigen complex.

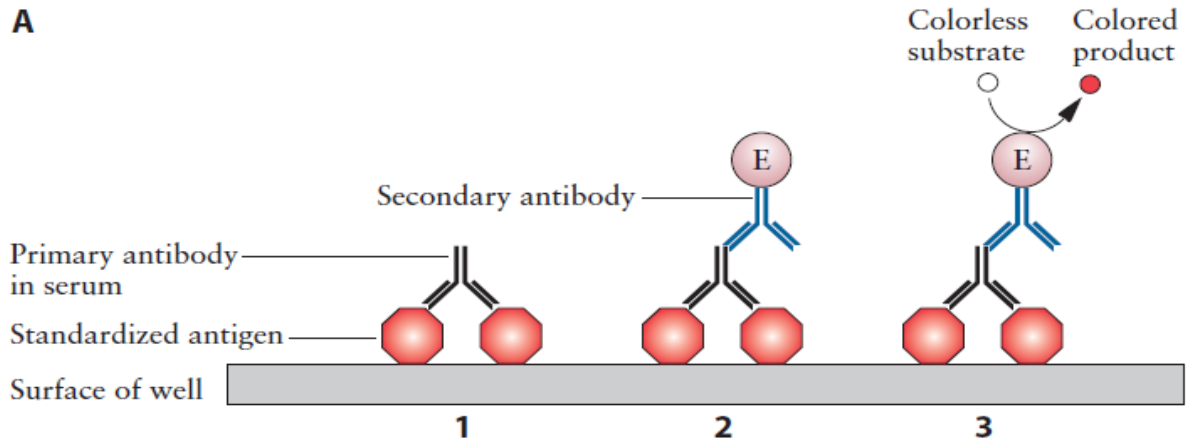


Fig.**(A)** Indirect ELISA. A specific antigen is immobilized on the surface of the wells in a microtiter plate. (1) Patient serum is applied, and primary antibodies present in the serum bind to the antigen. Unbound antibodies are removed by a washing step. (2) A secondary antibody that binds specifically to the primary antibody is applied. (3) The secondary antibody is conjugated to an enzyme (E) that catalyzes the conversion of a colorless substrate into a colored product. The formation of a colored product indicates that the serum sample contains antibodies directed against the antigen.

In contrast to an indirect ELISA, a sandwich ELISA directly detects a particular antigen in a complex clinical sample. To capture the antigen, a **monoclonal antibody** that is specific for the target antigen is first bound to the surface of a microtiter plate (Fig. B). The patient's sample is then added to a well; if the specific antigen is present in the sample, it binds to the immobilized antibody. A labeled primary antibody is added to detect the presence of bound antigen. The labeled antibody may detect a different **epitope** on the same antigen.

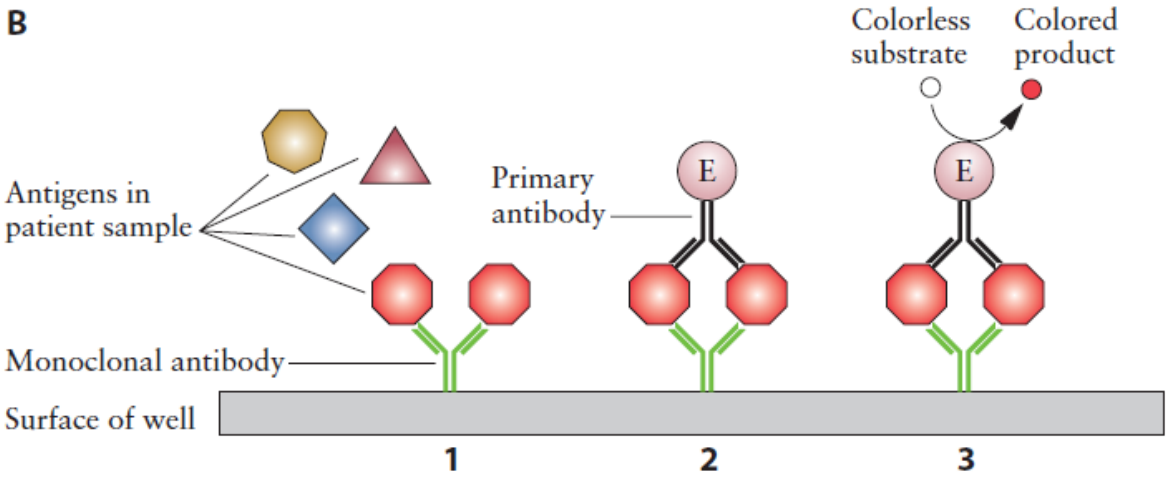


Fig. **(B)** Sandwich ELISA. Monoclonal antibodies specific for a target antigen are bound to the surface of a well in a microtiter plate. (1) A patient's sample is added to the wells, and specific antigens present in the sample bind to the immobilized antibody. A wash step removes any unbound molecules. (2) A primary antibody is applied to detect the presence of bound antigen. (3) The primary antibody is conjugated to an enzyme that catalyzes the formation of a colored product that indicates the presence of the target antigen.

39-Measuring Disease-Associated Proteins by Sandwich ELISA

Ovarian cancer is a devastating disease that kills over 15,000 women a year in the United States. More than 22,000 new cases are diagnosed each year, most at an advanced stage when the survival rate is less than 20%.

An immunoassay widely used to monitor progression and recurrence of ovarian carcinoma measures levels of the protein CA125 in serum. CA125 is a high-molecular-weight glycoprotein that is present at higher levels in 50% of women with ovarian cancer compared to healthy women.

Levels may also be elevated in women with lung, pancreatic, breast, cervical, and colorectal cancers and with noncancerous disorders such as pelvic inflammatory disease, hepatic disorders, and nonmalignant ovarian cysts. Because CA125 may indicate other disorders and is not elevated in all patients with ovarian cancer, especially at early stages of the disease when tumors are small and therefore secrete only low levels of the protein, it is not recommended as a screening test. Rather, it is commonly used to monitor a patient's response to treatment. Researchers have therefore sought to identify other biomarkers that are more specific for ovarian cancer.

A useful biomarker for ovarian cancer is a protein that is secreted specifically by ovarian tumors, and not by normal tissue or other tumor types, and that can be detected at low levels in the blood or urine, enabling early, and noninvasive, detection. Using cDNA microarrays and quantitative polymerase chain reaction (PCR), human epididymis protein 4 (HE4) was identified as a promising new biomarker that was expressed in ovarian carcinomas but not normal tissue or benign ovarian tumors. Mouse monoclonal antibodies were generated against two different HE4 epitopes and used to develop an ELISA. In an initial blinded study (researchers were not informed of the source of the patient samples), HE4 was found to be elevated in sera from patients with early and late-stage ovarian cancer compared to sera from healthy women and from women with benign ovarian tumors.

The U.S. Food and Drug Administration (FDA) has approved the use of a HE4 sandwich ELISA to monitor the recurrence or progression of ovarian cancer in women who are being treated for the disease.

Patient samples are incubated with biotinylated monoclonal antibodies generated against one HE4 epitope in streptavidin-coated wells (Fig.). A second antibody, conjugated to the enzyme horseradish peroxidase, that binds to a different HE4 epitope is added, and after generation of a colored product by the horseradish peroxidase, HE4 is quantified spectrophotometrically. Using this assay, HE4 was found to be present at elevated levels in more than 75% of patients with ovarian cancer, compared to 5% of healthy women and 13% of individuals with other nonmalignant conditions. An increase in HE4 levels of more than 25% is considered significant to suggest recurrence or disease progression, while a decrease of this magnitude suggests a positive response to treatment.

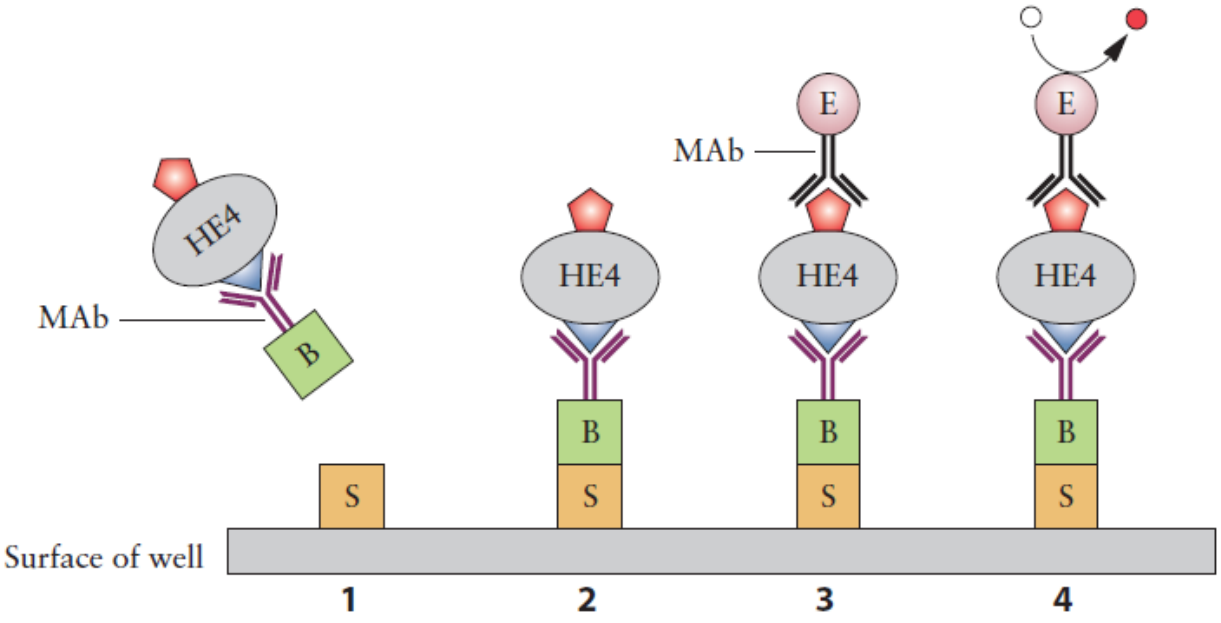


Fig- Sandwich ELISA to monitor progression or recurrence of ovarian cancer. **(1)** Patient samples are incubated with biotinylated (B) monoclonal antibodies (purple MAb) generated against one HE4 epitope (blue). **(2)** Biotin, together with the MAb–HE4 complex, binds with high affinity to streptavidin (S) that coats the wells of a microtiter plate. **(3)** A

second antibody (black MAb) that binds to a different HE4 epitope (red) is added, and after generation of a colored product by horseradish peroxidase (E) conjugated to the antibody **(4)**, HE4 is quantified spectrophotometrically.

40-Diagnosing Autoimmune Diseases by an Indirect ELISA

Autoimmune diseases occur when the body's immune system does not recognize normal cellular molecules and structures as "self" but rather produces antibodies (autoantibodies) that destroy those targets . Several autoimmune diseases have been identified, including celiac disease, type 1 diabetes, lupus erythematosus, and rheumatoid arthritis.

Indirect ELISAs have been developed to diagnose some autoimmune diseases. In these assays, autoantibodies produced against a self-protein are detected in patient blood samples. Rheumatoid arthritis is an autoimmune disease that results in chronic, systemic inflammation of the joints, mainly the synovial (flexible) joints. The synovial membrane that lines these joints secretes a fluid that lubricates the articulating bones. Initially, an inflammatory response occurs in the synovial membranes in the small joints of the hands and feet; it then occurs in larger joints, causing accumulation of excess fluid and damage to the joints. Systemic inflammation may damage organs such as the heart and lungs. The disease affects about 1% of the population of the United States.

Rheumatoid factor is an autoantibody that targets the Fc region of immunoglobulin G (IgG) antibodies and contributes to rheumatoid arthritis. It is commonly used as a diagnostic biomarker to differentiate rheumatoid arthritis from other forms of arthritis and other inflammatory

conditions. An indirect ELISA has been developed to detect the presence of rheumatoid factor in patient blood samples. In this test, IgG molecules, usually from a rabbit, are the standardized antigen bound to the surface of a multiwell plate, and diluted patient sera are applied to the wells (Fig.).

After incubation to allow binding of rheumatoid factor in the serum to the rabbit IgG molecules and washing to remove unbound molecules, an anti-human IgG antibody is added. The anti-human IgG antibody specifically targets rheumatoid factor (and not rabbit IgG) and is conjugated to an enzyme such as horseradish peroxidase for colorimetric detection. Other ELISAs have been developed that target rheumatoid factor IgM and IgA that can assist in accurate diagnosis of rheumatoid arthritis.

Early diagnosis is important to prevent irreversible joint damage, as rheumatoid arthritis may be managed in the early stages by administering antirheumatic drugs.

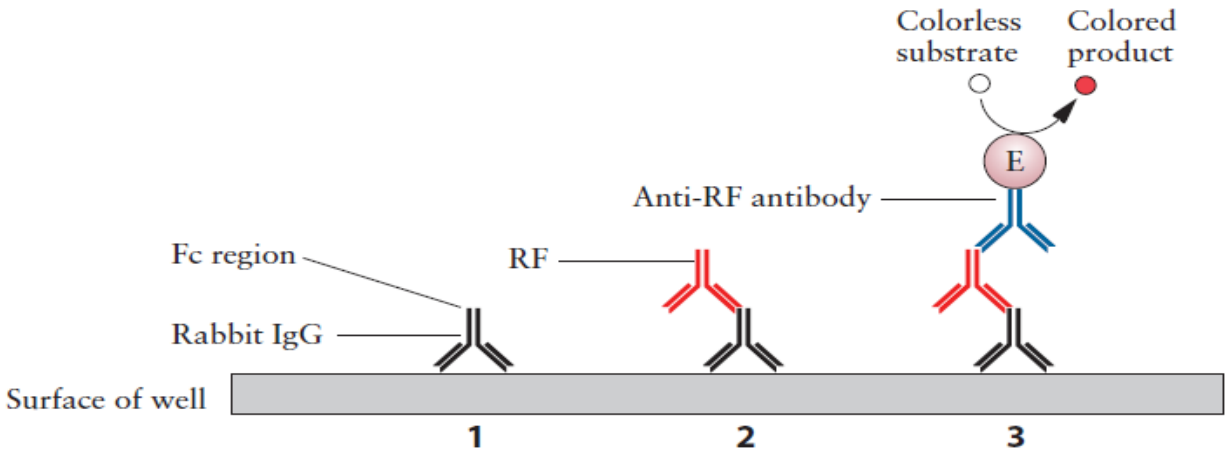


Fig- Indirect ELISA for diagnosis of the autoimmune disease rheumatoid arthritis. **(1)** Rabbit IgG molecules are bound to the surface of a microtiter plate, and diluted patient serum is applied. **(2)** If rheumatoid factor (RF) is present in the serum, it binds to the Fc region of the rabbit IgG molecules. **(3)** After a washing step to remove unbound molecules, an anti-RF antibody conjugated to an enzyme (E) is applied for colorimetric detection.

41-Immunoassay for infectious diseases

Clinical laboratories often identify pathogenic microbes in patient samples based on their physiological or biochemical characteristics. For example, a pathogenic bacterium may ferment specific carbohydrates or produce specific enzymes, and detection of the products of these reactions is the basis for some diagnostic assays. While these methods are effective, they require growth and isolation of the pathogen and therefore are slower than immunological and other molecular methods, typically requiring more than 48 h.

Immunological detection methods such as agglutination assays and ELISAs eliminate the need to grow the pathogen in culture and can be used to detect specific viral, bacterial, fungal, or protozoan pathogens in body fluids and tissues. Because antibody-based approaches detect a target antigen with high specificity and sensitivity, they are well suited to distinguish a specific pathogen from the other hundreds of microbes that are normally present in some human tissues.
Immunological assays for infectious disease may target proteins produced by a pathogen or may detect the presence of antibodies produced against the pathogen. The latter, however, does not differentiate between current and past infections.

To prevent transmission of infectious disease, donated blood is screened for several pathogens, including human immunodeficiency virus and hepatitis viruses. Although five hepatitis viruses can infect the liver and cause liver inflammation, cell death, and even liver failure, most cases of chronic viral hepatitis are caused by hepatitis B and C viruses. Both viruses may be transmitted via blood transfusion.

ELISAs have been developed for routine blood screening for hepatitis B surface antigen, a viral envelope lipoprotein, and for antibodies produced against both hepatitis B and C viruses. Viral surface antigens are the first markers to be detected in serum after infection, while antibodies appear up to several months later. Detection of the viral surface antigen with a sandwich ELISA utilizes specific monoclonal antibodies to capture the hepatitis B surface antigens present in blood in wells of an assay plate (Fig. A). The monoclonal antibodies were generated using recombinant hepatitis B surface protein expressed in yeast. An anti-hepatitis B surface antigen detection antibody with a conjugated enzyme and the enzyme substrate are added to measure virus titers.

An indirect ELISA that detects the presence of anti-hepatitis B virus antibodies in the blood sample is often used to confirm a positive result (Fig. B); however, blood from individuals immunized with a hepatitis B vaccine also produces a positive result with this test. Blood may additionally be screened for hepatitis B DNA and C virus RNA.

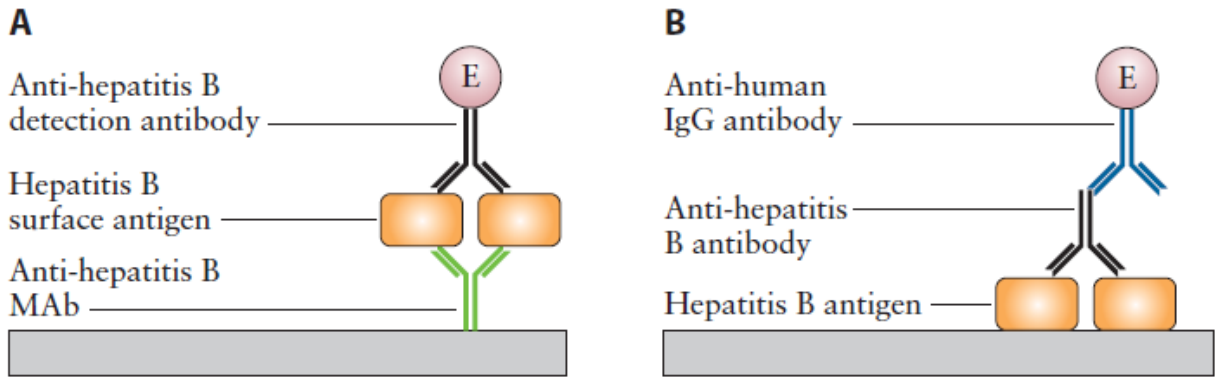


Fig- Diagnosis of hepatitis B virus infection by ELISA. **(A)** A sandwich ELISA detects the presence of hepatitis B surface antigen in blood. **(B)** An indirect ELISA detects the presence of anti-hepatitis B virus antibodies in blood.

42-Protein Array to Detect Polygenic Disease

An ELISA typically measures a single target protein; however, for some diagnoses, it may be more informative to measure multiple target proteins in a single assay. Analysis of **proteomes** is commonly used in research to identify and quantify protein changes in diseased tissue versus normal tissue and is useful for diagnosis of **polygenic diseases** (resulting from mutations in more than one gene) such as breast cancer, Alzheimer disease, type 1 diabetes, and cardiovascular disease.

Protein microarrays are multiplex immunoassays that can detect multiple biomarkers in a clinical sample. Biomarkers may be subsets of proteins in complex clinical samples such as biopsied tumor tissue. Currently, commercially available protein microarrays are most commonly used to detect antibodies in serum from patients suffering from allergies, autoimmune diseases, or infections.

Approximately 25% of the population in industrialized countries suffers from type I allergies, which are IgE-mediated immediate hypersensitivity reactions, such as asthma, hay fever, and eczema. In susceptible individuals, the first exposure to an allergen elicits the production of high levels of IgE antibodies that bind to mast cells. In this way, the individual becomes sensitized to the allergen. On subsequent exposures, the allergen reacts with mast cell-bound IgE and causes bridging between adjacent IgE molecules, thereby stimulating the release of inflammatory

mediators such as histamine, leukotrienes, prostaglandins, and tumor necrosis factor. These biochemicals stimulate smooth muscle contraction and dilation of capillaries that cause edema (swelling), itching, and development of a rash.

Diagnostic allergen microarrays detect IgEs produced against common allergens, for example, pollen, food proteins, and molds. Purified allergens are arrayed in triplicate on a solid supportand probed with sera from allergic patients (Fig.). Specific IgEs present in the serum bind to an immobilized allergen and are retained on the surface of the array. Bound IgEs are detected using an anti-human IgE antibody, often labeled with a fluorescent dye. The array format enables detection of hundreds of allergens in a single assay.

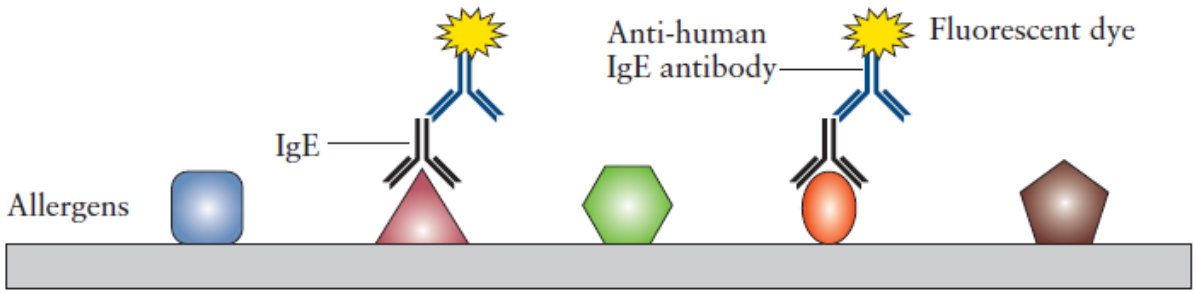


Fig- Diagnostic allergen microarray. Purified allergens are arrayed on a solid support and probed with serum from an allergic patient. Specific IgE present in the serum binds to an immobilized allergen and is detected using an anti human IgE antibody conjugated to a fluorescent dye

Another type of protein microarray measures levels of fifty different protein biomarkers of heart disease in a patient serum sample. Increased or decreased levels of these proteins have been associated with increased risk of heart disease. A liquid bead array has been developed to

quantify levels of the different protein biomarkers are detected in a single well of a multiwell plate (Fig.), which enables rapid analysis of many patient samples simultaneously.

Primary antibodies directed against each protein biomarker are attached to tiny capture beads that are distinguished by either size, color, or shape. A serum sample is applied to a well, and specific proteins in the sample bind to their cognate antibody (Fig. A). For detection of the bound proteins, secondary antibodies conjugated to a fluorescent molecule are added to the well (Fig. B). Each bead, and any captured proteins, is analyzed by passing by a laser that measures fluorescence emitted from bound secondary antibodies and distinguishes the different capture beads by their size, color, or shape.

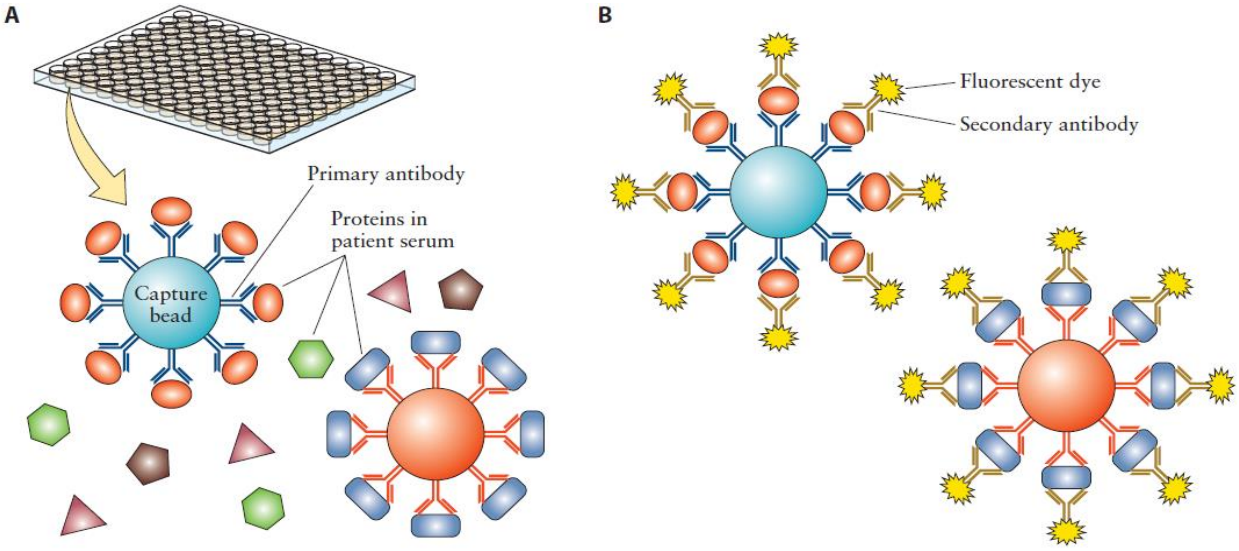


Fig- Liquid bead assay to detect protein biomarkers associated with increased risk of cardiovascular disease. **(A)** Patient serum is applied to a well of a microtiter plate containing capture beads bound to primary antibodies directed against all of the protein biomarkers. Capture beads of different colors distinguish primary antibodies that bind to different protein biomarkers. Target proteins in the sample bind to their cognate antibodies. **(B)** For detection of the bound proteins, secondary antibodies conjugated to a fluorescent molecule are added to the well. Each bead, and any captured proteins, is analyzed by passing by a laser that measures fluorescence. The presence of specific protein biomarkers is determined by the color of the capture bead to which they are bound.

43-Immunoassay for Protein Conformation-specific Disorder

Several human neurological disorders arise as a consequence of protein misfolding that leads to protein aggregation and cell death. Parkinson disease, Alzheimer disease, and prion diseases are examples of protein conformational disorders.

Alzheimer disease is a degenerative brain disorder that is characterized by the progressive loss of abstract thinking and memory, personality change, language disturbances, and a slowing of physical capabilities. Clinical diagnosis of Alzheimer disease is poor, although 1% of the population between 60 and 65 years old and 30% of the population over 80 years old may develop it.

Two hallmarks of Alzheimer disease found in the brain are (i) neurofibrillary tangles of the cytoskeletal protein tau that accumulate within nerve cell bodies and (ii) dense extracellular aggregates of insoluble proteins called amyloid plaques that develop at the ends of inflamed nerves (Fig.).

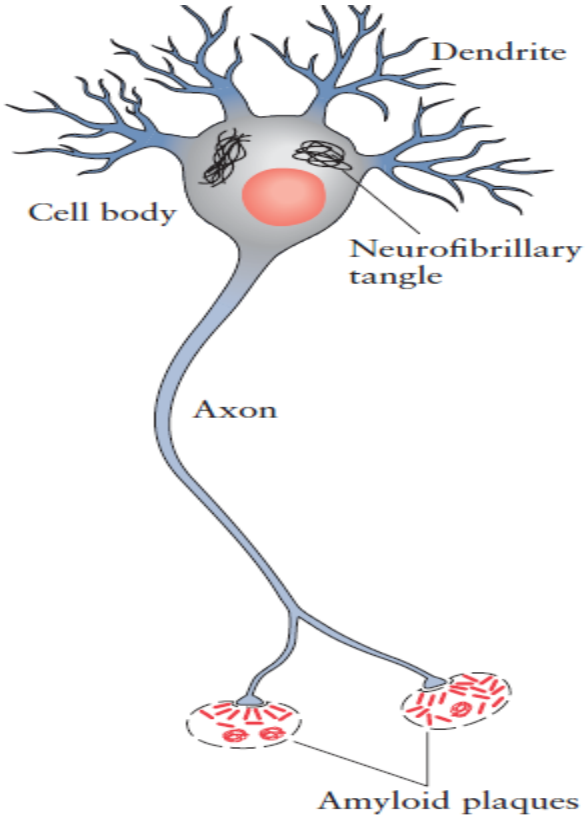


Fig- Neurofibrillary tangles and amyloid plaques associated with neurons in the brain are hallmarks of Alzheimer disease.

The principal protein of an amyloid plaque is a small protein called Aβ (β-amyloid protein). The Aβ protein ranges in length from 39 to 42 amino acid residues; the Aβ40 and Aβ42 forms are the main variants. All Aβ proteins are derived from the β-amyloid precursor protein (APP) by proteolytic cleavage. Abnormal cleavage of APP results in production of Aβ40 and Aβ42 and alters protein folding, causing exposed regions of the protein to self-interact; hence, the proteins aggregate in amyloid plaques.

Diagnosis of Alzheimer disease using immunological methods may exploit the development of antibodies that differentiate between the conformations of the disease-associated Aβ proteins and

normal APP. To develop conformation-specific antibodies, researchers used an approach that mimics protein aggregation during the disease process, that is, the self-interaction of the Aβ proteins. They grafted short motifs (10-mers) from the Aβ42 protein into a complementarity-determining region (CDR3, VH domain) of an antibody to generate antibodies that would interact with aggregated Aβ42 via the grafted motif (Fig. A).

Out of twelve antibodies, carrying different but overlapping Aβ42 sequences, three bound specifically to insoluble Aβ42 aggregates but not Aβ42 monomers (Fig. 8.11B). A motif of four amino acids, common to all three positive antibodies, was found to be the minimal sequence required for binding. To determine whether binding of the grafted antibodies is mediated by interactions between the Aβ motif on the antibody and the same motif in the aggregated Aβ proteins, each grafted antibody was bound to immobilized Aβ42 aggregates and then a second antibody was applied. Binding of the second antibody was reduced only when it recognized a motif that overlapped with that grafted on the first antibody. This relatively simple approach may be used to generate conformation-specific antibodies against other proteins that not only aid in accurate disease diagnosis but also may be used in therapeutic strategies to target these proteins.

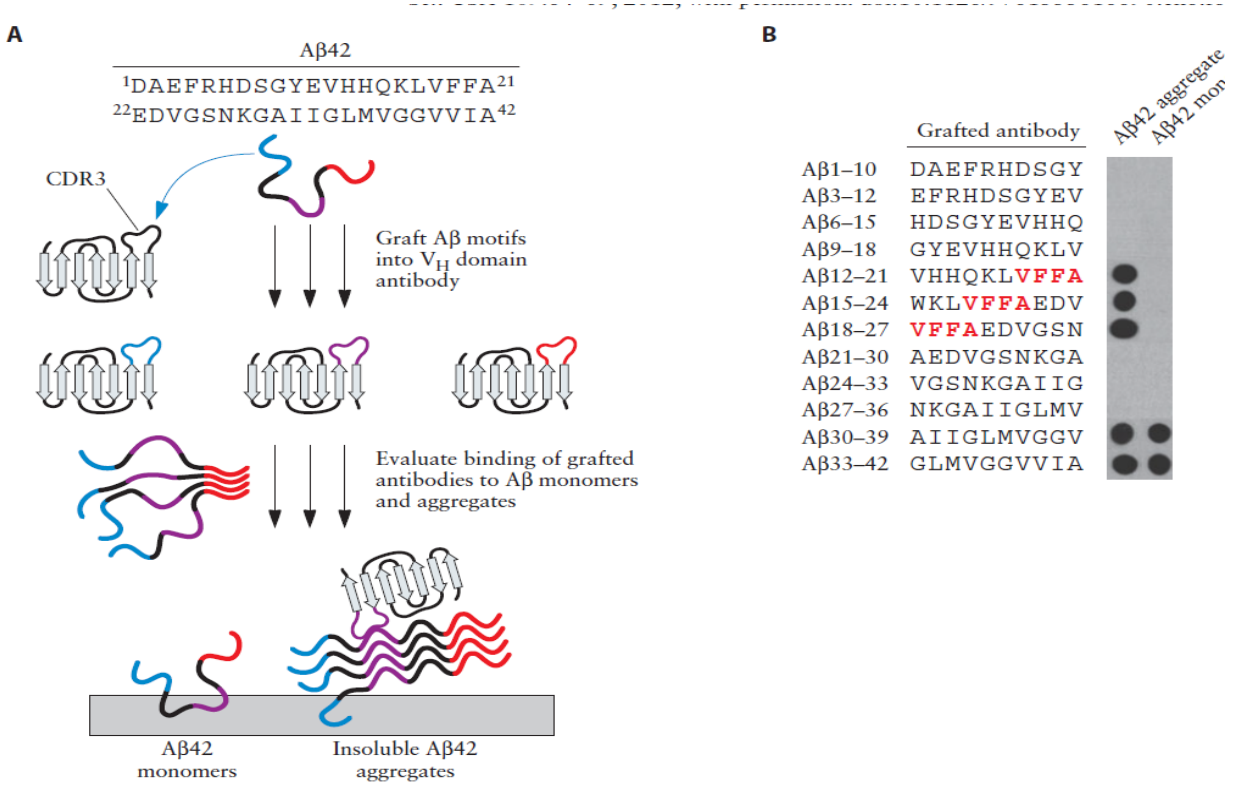


Fig- Development of conformation-specific antibodies to detect aggregated Aβ proteins indicative of Alzheimer disease. **(A)** Short (10-amino-acid) overlapping Aβ42 peptide segments were grafted into a complementarity-determining region (CDR3, V_H domain) to generate antibodies that bind specifically to aggregated, insoluble Aβ42 proteins. **(B)** Out of 12 grafted antibodies, three (Aβ12-21, Aβ15-24, and Aβ18-27) bound to aggregated Aβ42 proteins, and not Aβ42 monomers, that were deposited on a membrane. A sequence of 4 amino acids (highlighted in red) was found to be the minimal requirement for binding.

44-DNA based approaches to diagnosis diseases

DNA-based diagnostic tests determine the existence of specific nucleotide sequences, including human genetic mutations and sequences present in human pathogens. They are highly sensitive and specific and can detect single nucleotide mutations or copy number variations. The ability to

diagnose diseases in humans at the genetic level makes it possible to determine the cause of an illness and to predict whether individuals or their offspring are predisposed to the disease.

Because a DNA-based test does not require expression of a gene, in contrast to diagnostic detection of proteins, DNA analysis can be used for the identification of asymptomatic carriers of hereditary disorders, for prenatal diagnosis of serious genetic conditions, and for early diagnosis before the onset of symptoms.

DNA sequence-specific diagnostic approaches include hybridization of a unique DNA probe to a complementary target sequence, target sequence amplification by PCR, microarray analysis to detect multiple sequences in a single sample, and mass spectrometry to identify single-nucleotide polymorphisms (SNPs).

Hybridization Probes:

Hybridization is the formation of hydrogen bonds between two complementary strands of nucleic acids. A diagnostic test involving DNA hybridization utilizes a DNA probe to detect a complementary target DNA sequence that is characteristic of the disease. The probe is labeled with a reporter molecule that indicates hybridization between the target and probe DNA.

Hybridization probes are often employed to detect the presence of microbial pathogens. Malaria, caused by the parasite *Plasmodium falciparum*, is one of the most common infectious diseases and is especially fatal in young children.

A DNA diagnostic test for active infections that measures the presence of the pathogen was developed by using highly repetitive DNA sequences (present in many copies) from *P. falciparum*. The DNA sequence that was chosen as a specific probe hybridized with *P. falciparum* but not with *Plasmodium vivax*, *Plasmodium cynomolgi*, or human DNA, despite the fact that *P. vivax* causes a less severe form of malaria. This probe can detect as little as 10 picograms (pg) of purified *P. falciparum* DNA or 1 nanogram (ng) of *P. falciparum* DNA in blood.

45-Allele specific Hybridization

In addition to infectious diseases, hybridization probes are widely used to detect specific disease-associated alleles. Monogenic diseases are caused by mutations in a single gene; however, any one of several alterations to the normal nucleotide sequence of a gene may be responsible.

This is exemplified by cystic fibrosis, a common lethal autosomal recessive disorder that affects approximately 1 in every 2,500 live births. Mutations in a single gene, the cystic fibrosis transmembrane conductance regulator (CFTR) gene, result in defects in chloride ion transport.

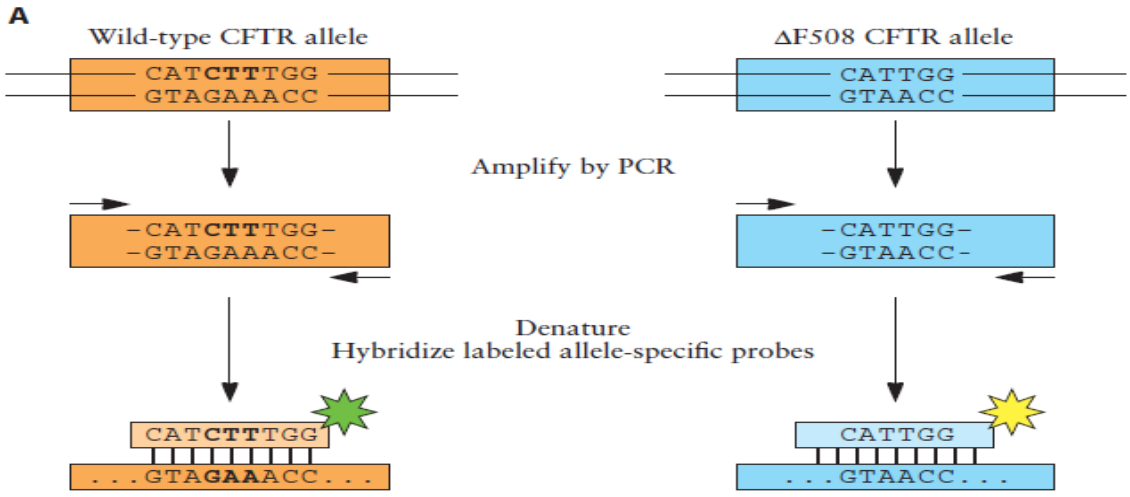
As a consequence, the mucus of lung and other mucosal tissues is thick and viscous, obstructing the respiratory, digestive, and reproduction system functions. Every state in the United States now routinely screens newborns for cystic fibrosis. An ELISA is used to detect higher-than-normal levels of immunoreactive trypsinogen in blood. This protein is produced by the pancreas and is linked to cystic fibrosis. Premature babies and babies from stressful births also may have

elevated levels of immunoreactive trypsinogen, and in these cases, genetic tests are employed for confirmation.

About 1,900 different mutations are reported to occur in the CFTR genes of patients with cystic fibrosis. Screening individuals who may be at risk for cystic fibrosis for such a large number of possible mutations is a daunting task. However, some of the mutations that cause cystic fibrosis are much more common than others. The most common mutation is an in-frame deletion of three nucleotides in exon 10 of the CFTR gene that leads to loss of the amino acid phenylalanine at codon 508 (ΔF508).

Over 90% of cystic fi brosis patients carry at least one ΔF508 allele, and nearly 50% of cystic fibrosis patients are individuals who are homozygous for ΔF508. It is estimated that about 160 different mutations account for 96 to 97% of cystic fi brosis alleles.

Allele-specifi c hybridization is commonly used to screen for cystic fibrosis. With this technique, an individual's CFTR gene is amplified by PCR and then hybridized to labeled oligonucleotide probes for the mutant (e.g., ΔF508) and wild-type genes, separately (Fig. A). In this way, it is possible to distinguish between healthy individuals with two wild-type alleles, cystic fibrosis carriers with one mutant and one wild-type allele, and cystic fibrosis-affected individuals with two mutant alleles (Fig. B).



B

Source of blood sample	Hybridization test results
Normal individual	
Cystic fibrosis carrier	
Cystic fibrosis-affected individual	

(A) The CFTR gene is amplified by PCR, and then the PCR products are denatured and incubated with oligonucleotide probes that specifically hybridize to wild-type or mutant (usually ΔF508) alleles (note that only part of the sequence of the CFTR gene and probe are shown). After a washing step to remove unbound probe, hybridization is detected by measuring fluorescence emitted by the fluorophore attached to the probe.

(B) Healthy individuals are homozygous for the wild-type allele, while those affected by the disease carry two mutant alleles. Cystic fibrosis carriers are heterozygous for the CFTR alleles.

Diagnostic kits are commercially available that test patient blood samples for the presence of a panel of common CFTR mutations as recommended by the American College of Medical Genetics.

46-Oligonucleotide ligation assay

The oligonucleotide ligation assay (OLA) is also commonly used to detect SNPs known to be associated with human diseases with a high degree of accuracy. In this diagnostic assay, two short oligonucleotide probes (~50 nucleotides) are designed to anneal to adjacent sequences within a gene that encompass the polymorphic nucleotide (Fig.).

Importantly, one of the probes (allele-specific probe) has as its last base at the 3′ end the nucleotide that is complementary to the polymorphic nucleotide. The second probe (common probe) is complementary to the sequence immediately downstream of the polymorphic nucleotide.

When these two probes are hybridized with the target gene (which has been amplified by PCR), base-pairing occurs between the allele-specific probe and the target sequence, including the 3′ nucleotide if the complementary polymorphic nucleotide is present, and the common probe binds immediately downstream. DNA ligase, added to the reaction, covalently joins the two probes.

However, if the nucleotide at the 3′ end of the allele-specific probe is mismatched, it will not base-pair with the polymorphic nucleotide in the target DNA sequence, although the common probe will be perfectly aligned. As a consequence of the single-nucleotide misalignment, DNA ligase cannot join the two probes.

In short, OLA is designed to distinguish between two possibilities: ligation if the probes are perfectly matched and no ligation if the allele-specific probe carries a mismatched nucleotide. Other oligonucleotide probes may be designed to detect different SNPs in the same gene.

To determine whether ligation has occurred, one of the two probes is labeled with a reporter molecule. For example, the allele-specific probe may be labeled at the 5′ end with a fluorescent dye that can be detected by laser excitation and fluorescence emission. Probes may be labeled with different fluorescent molecules corresponding to different SNPs. If an individual is homozygous for a particular allele, either two copies of a normal gene or two copies of a disease-associated SNP, a positive signal will be detected only from one of the fluorescent molecules. Heterozygous individuals will yield positive signals from two different probes. Overall, the OLA system is rapid, sensitive, highly specific, and amenable to automation.

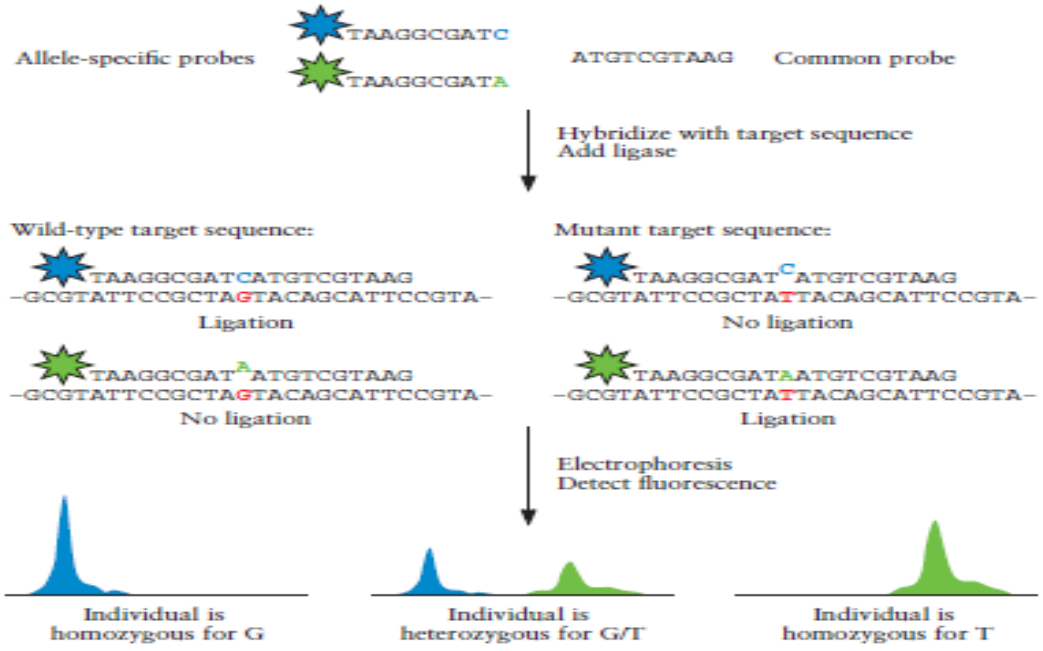


Fig-OLA. Ligation of the allele-specific and common probes occurs only when the allele-specific probe is perfectly complementary to the target sequence. Longer ligated probes may be separated from shorter unligated probes by electrophoresis, and then probe fluorescence can be detected to distinguish homozygous wild-type, homozygous mutant, and heterozygous individuals.

47-Padlock Probes

Diagnostic assays for SNPs that use padlock probes are very similar to those that use OLA probes, except that the former utilizes only one probe rather than two as used in the OLA procedure.

A padlock probe is an oligonucleotide that is complementary to a target sequence at its 5′ and 3′ ends but not in its middle region (Fig.). When a padlock probe hybridizes to its target sequence, the 5′ and 3′ ends of the probe come into close proximity to each other and the middle portion loops out. If the ends of the probe are exactly complementary to the target sequence, after hybridization they can be joined together by DNA ligase. If there is a mismatch between the target and probe, ligation does not occur.

The requirement for both ends of the probe to bind perfectly to the target sequence for ligation to occur ensures a high specificity of detection and therefore the ability to easily detect SNPs. Following the ligation reaction, the probe–target hybrid can be detected by the activity of reporter molecules attached to the middle (linker) portion of the probe. Padlock probes typically have sequences approximately 15 to 20 nucleotides in length at each end that are complementary to the target sequence and a middle region of approximately 50 nucleotides.

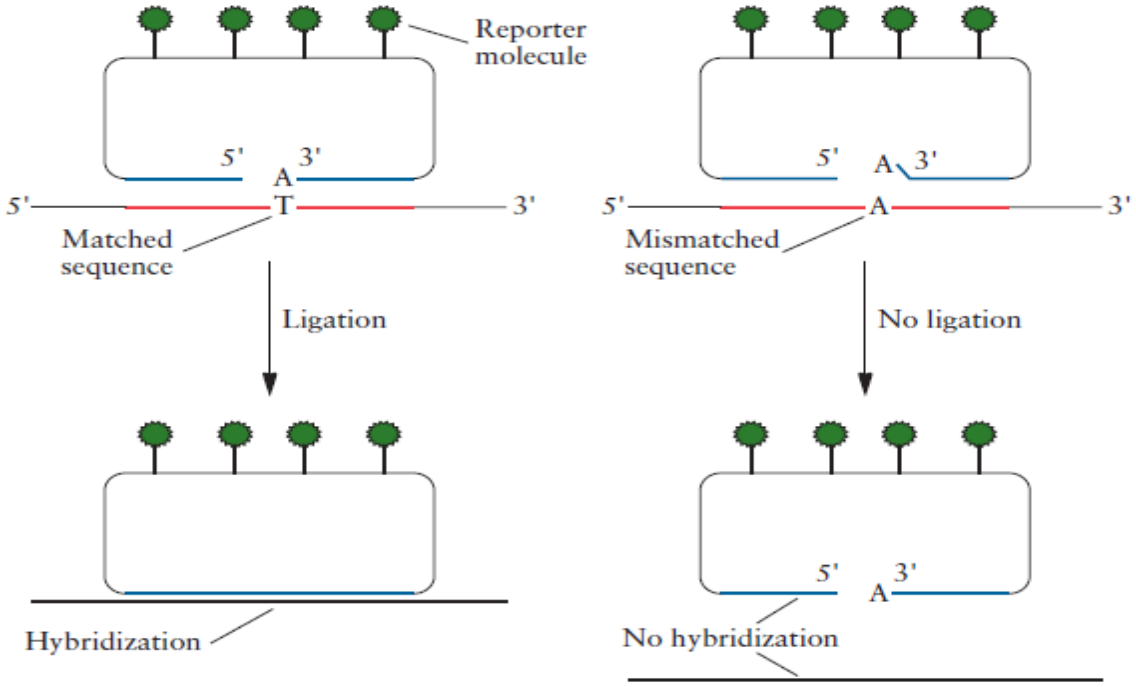


Fig-A padlock probe. Ligation (circularization) of the padlock probe occurs only when the 5′ and 3′ ends of the probe are perfectly complementary to the target sequence. When there is a single-base mismatch at the 3′ end of the probe, ligation does not occur and the probe assumes a conformation that does not allow hybridization. Under stringent conditions, the ligated probe remains bound to the target DNA, while a nonligated probe is removed in a washing step. Hybridized probe can be detected by the activity of the reporter molecule, for example, by fluorescence emitted from a fluorescent dye.

48-Allele-Specific PCR

Many nucleic acid diagnostic tests are based on PCR. Advantages of PCR-based tests include

(i) specificity that enables detection of a particular nucleotide sequence in complex samples,

(ii) sensitivity that enables detection of low-abundance targets,

(iii) an amplification step that generates substantial amounts of a target sequence for additional analyses such as hybridization or sequencing,

(iv) rapid analysis (usually completed in 1 to 2 h or less),

(v) multiplexing that enables identification of multiple targets in a single sample, and

(vi) low cost. Conventional PCR has been used extensively in diagnostic laboratories over the last 15 years; however, more recently, it has become possible not only to detect but also to quantify the pathogen in a clinical sample using quantitative real-time PCR.

Allele-specific PCR (also referred to as PCR amplification refractory mutation system) screens for known SNPs. Different forward PCR primers are used to distinguish among alleles that differ by a single nucleotide. One primer is exactly complementary to the normal DNA sequence, and another primer anneals to a variant sequence containing the disease-associated SNP.

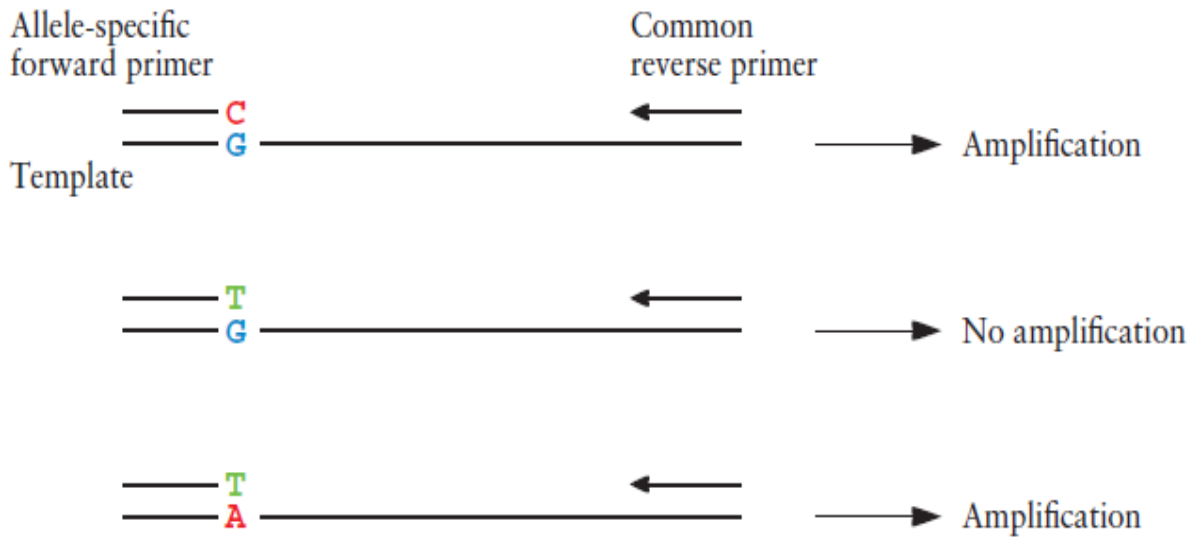


Fig- Allele-specific PCR to detect SNPs. Amplification occurs only when the forward and reverse PCR primers perfectly match the target sequence. Different allele-specific primers that carry an SNP-specific nucleotide at the 3′ end are employed to distinguish among alleles that differ by a single nucleotide.

The primers are usually designed to place the polymorphic nucleotide at the 3′-terminal end of the primer because most polymerases used for PCR do not extend 3′ mismatched primers efficiently. A third reverse primer is complementary to the opposite strand and is common to all

reactions. Each reaction contains only one of the allele-specific forward primers and the common reverse primer, together with the patient DNA sample, a thermostable DNA polymerase, and all four deoxyribonucleotides.

PCR amplification occurs only when a forward primer is present that is exactly complementary to the target sequence in the patient sample; mismatches between primer and template DNA prevent primer annealing and therefore primer extension during DNA synthesis. An advantage of this method, compared to allele-specific hybridization described above, is that the amplification and diagnostic steps are combined.

One variation of allele-specific PCR is known as competitive oligopriming, in which two different SNP-specific forward primers are included in a single reaction. To discriminate between the PCR products, each forward primer is labeled with a different fluorescent dye. For example, one forward primer that is exactly complementary to a normal allele may be labeled at its 5′ end with rhodamine (which fluoresces red), while another forward primer that is complementary to the disease-associated allele may be labeled at its 5′ end with fluorescein (which fluoresces green)

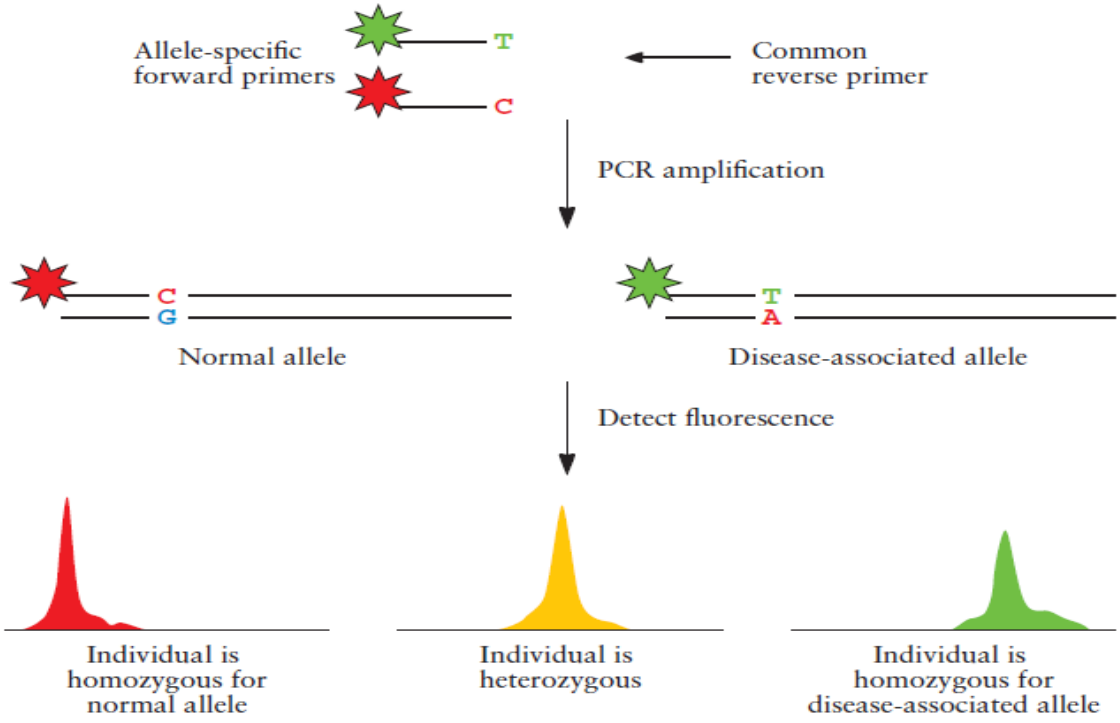


Fig- Competitive oligopriming to detect SNPs. Two allele-specific forward primers that differ in the nucleotide at the 3′ end are included in a single reaction together with a reverse primer that anneals to a sequence that is common among variant alleles. PCR amplification occurs only when a forward primer is perfectly complementary to the target DNA. To discriminate between PCR products corresponding to normal and disease-associated alleles, each forward primer is labeled with a different fluorescent dye. In the example shown here, PCR products from individuals who are homozygous for the normal allele fluoresce red, those from individuals who are homozygous for the disease associated allele fluoresce green, and those from individuals who are heterozygous fluoresce yellow.

In both cases, amplification requires a third, unlabeled primer that is complementary to the opposite strand. PCR amplification occurs only when a forward primer is exactly complementary to the target DNA; therefore, the presence of these two forward primers in the same reaction mixture will result in the amplification of either the normal or disease-associated DNA sequence or both, depending on which alleles are present. If an individual is homozygous for the normal

allele, after PCR and removal of unincorporated primers, the reaction mixture will fluoresce red; if he or she is homozygous for the disease-associated allele, the reaction mixture will fluoresce green; and if he or she is heterozygous, the reaction mixture will fluoresce yellow. This assay can be automated and adapted for any single-nucleotide target site of any gene that has been sequenced.

49-TaqMan PCR

The TaqMan PCR protocol is used to screen individuals for the presence of SNPs that are indicative of any of a variety of genetic diseases. Made popular by one particular company, it is based on the 5′ nuclease activity of *Taq* polymerase, which is commonly used to amplify DNA in PCR applications.

To simultaneously detect normal and disease-associated alleles, two TaqMan probes are utilized. Each probe is exactly complementary to either the normal or the disease-associated DNA sequence, and each probe has a different fluorescent dye attached to its 5′ end (Fig.). Intact probes, whether unbound or bound to template DNA, do not fluoresce because of the presence of a quencher molecule at the 3′ end of the probe.

PCR primers anneal to sequences that flank the probe hybridization site, and as PCR amplification proceeds, the TaqMan probe is displaced by the growing DNA strand. The 5′ nuclease activity of the *Taq* polymerase degrades the 5′ end of the TaqMan probe, thereby releasing the fluorescent dye and removing it from the proximity of the quencher molecule. Thus, only TaqMan probes that were previously bound to target DNA are degraded and subsequently fluoresce. Any mismatched probes that are not complementary to the SNP in the

region where the TaqMan probe binds will be displaced but not cleaved, so they will not fluoresce.

By monitoring the fluorescence at two different wavelengths (one for each TaqMan probe), it is possible to distinguish individuals that are homozygous for each SNP (either normal or disease associated) and heterozygous (carrying one normal and one disease-associated allele). This technique may be used to detect more than two different SNPs at the same time as long as the fluorescent dyes attached to each SNP-specifi c probe have well-separated, nonoverlapping fluorescence maxima.

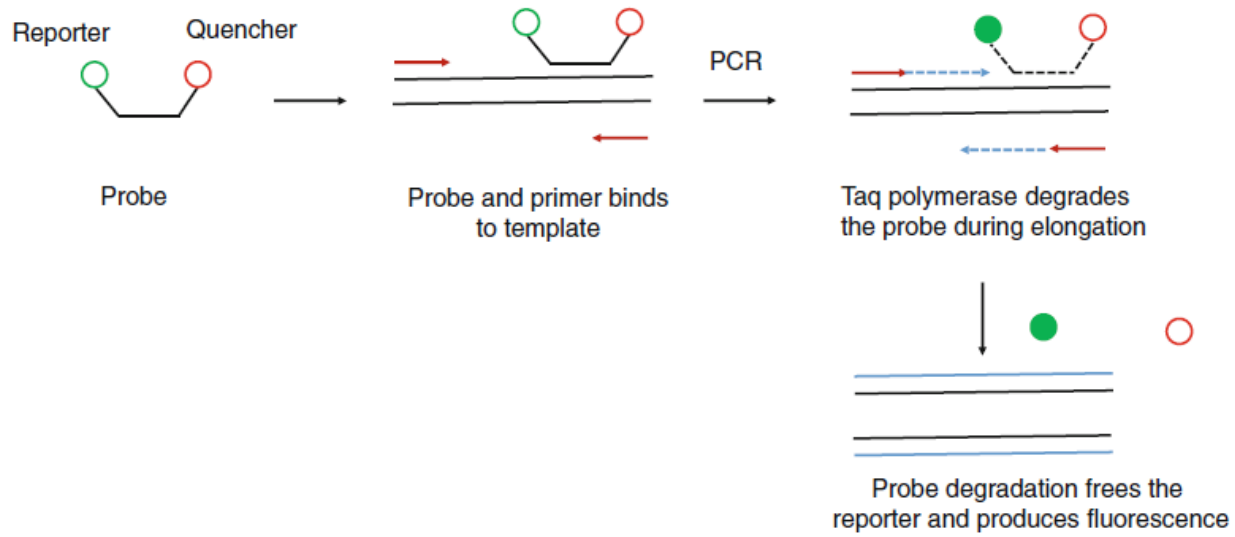


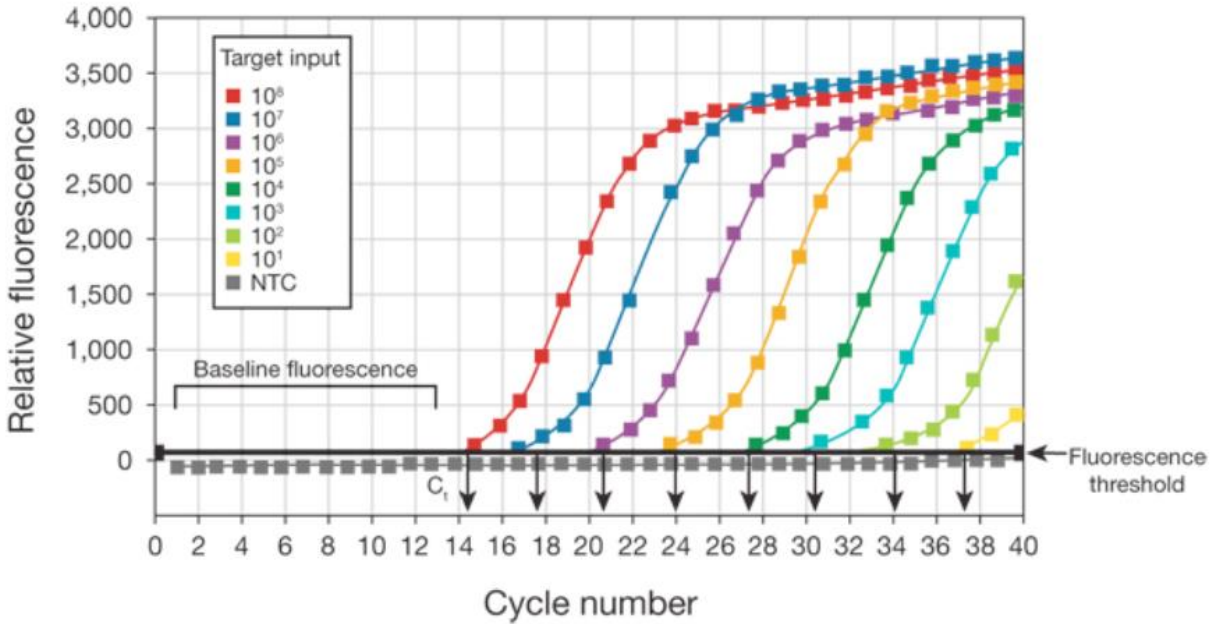
Fig-TaqMan q-PCR principle. A gene-specific probe with a reporter and quencher binds along with the primer. Since the reporter is close to the quencher, it does not produce fluorescence. During PCR reaction, Taq DNA polymerase degrades the probe due to 5′ nuclease activity. The released reporter produces fl uorescence signal that is captured after each cycle

50-Real-Time PCR To Detect Infectious Disease

Nucleic acid detection and accurate quantitation has become integral in all areas of scientific research.It has a wide range of applications in basic science, biotechnology, medicine, diagnostics, and forensic science. q-PCR is a valuable technique for detection and quantification of DNA and RNA. Unlike endpoint PCR where the amplicon is quantitated and detected at the end of the reaction, in real-time PCR , the amount of DNA is measured after each cycle based on increasing yield of fluorescent signal produced by the accumulation of fluorescent dye.

The signal is directly proportion to the number of molecules generated. Data collected in the exponential phase of the reaction yield quantitative information on the starting quantity of the target. The change in fluorescence is measured by a thermal cycler that has fluorescent dye scanning capability.

By plotting fluorescence against the cycle number, it generates an amplification plot that represents the accumulation of product over the duration of the entire PCR reaction.



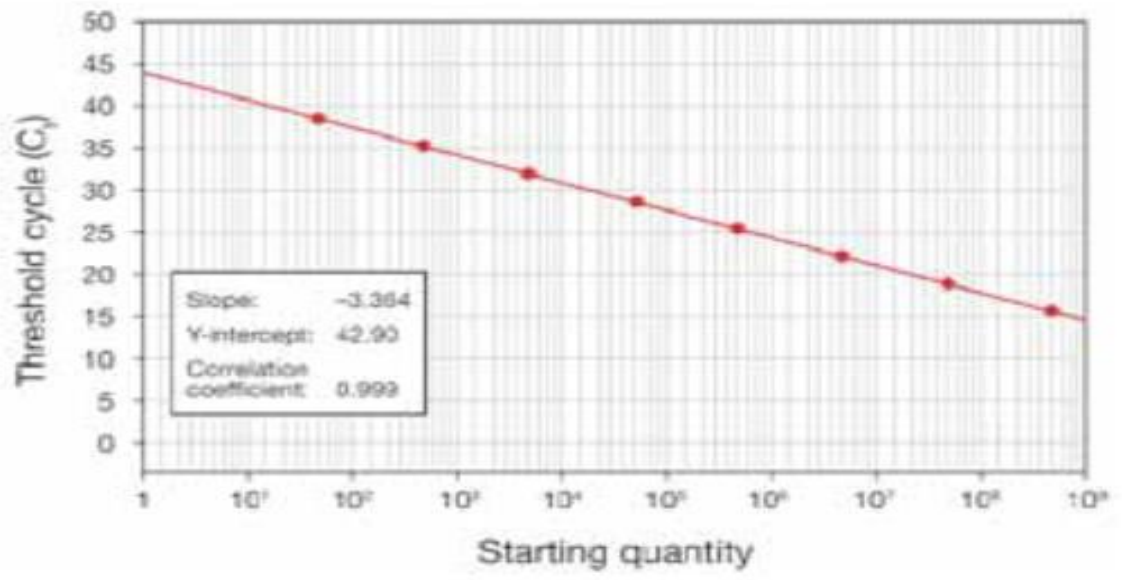
The steps in q-PCR are similar to that of conventional PCR . It includes denaturation of the DNA template, followed by primer annealing and extension. In case of RNA quantitation, a reverse transcription step is done before PCR where the RNA is reverse transcribed to complementary DNA (cDNA). The cDNA is used as a template for q-PCR. After the completion of every cycle, the fluorescence signal is measured and plotted against the cycle number to provide an amplifycation curve (Fig.).

Amplification curve has two phases: exponential and non-exponential phase. In exponential phase, the PCR product doubles after every cycle. Hence the fluorescence signal increases exponentially. The cycle number at which the fluorescence signal reaches detectable limit is called as threshold cycle or C T . Since C T is calculated at exponential phase when the reagents are not limiting, it can be used to calculate the initial amount of template accurately and reliably.

C T value is inversely related to the starting quantity of the template. The more the amount of the template, the earlier it will reach the C T . q-PCR can be used for exact quantitation or relative quantitation of a target. For exact quantitation, it is important to create a standard curve with known amount of the target. This can be done by performing q-PCR reaction with serial dilution of the purified target. Log of known concentration of the target template is plotted against the corresponding C T value to prepare the standard curve. This is used for determining the exact quantity of the target template in the experimental sample and also to calculate the efficiency of the reaction. Most of the time, relative quantitation is done where the expression of the target template is calculated as fold changes with regard to an internal control that could either be a single- copy gene or a housekeeping gene that is expressed steadily in all tissue in every condition.

Standard curve

A dilution series of known template concentrations can be used to establish a standard curve for determining the initial starting amount of the target template in experimental samples or for assessing the reaction efficiency (Fig.) The log of each known concentration in the dilution series (x-axis) is plotted against the Ct value for that concentration (y-axis). From this standard curve, information about the performance of the reaction as well as various reaction parameters (including slope, y-intercept, and correlation coefficient) can be derived.



Correlation coefficient (R2)

The correlation coefficient is a measure of how well the data fit the standard curve. The R2 value reflects the linearity of the standard curve. Ideally, R2 = 1, although 0.999 is generally the maximum value.

Y-intercept

The y-intercept corresponds to the theoretical limit of detection of the reaction, or the Ct value expected if the lowest copy number of target molecules denoted on the x-axis gave rise to statistically significant amplification. Though PCR is theoretically capable of detecting a single copy of a target, a copy number of 2–10 is commonly specified as the lowest target level that can be reliably quantified in real-time PCR applications. This limits the usefulness of the y-intercept value as a direct measure of sensitivity. However, the y-intercept value may be useful for comparing different amplification systems and targets.

Slope

The slope of the log-linear phase of the amplification reaction is a measure of reaction efficiency. To obtain accurate and reproducible results, reactions should have an efficiency as close to 100% as possible, equivalent to a slope of -3.32

There are two types of PCR chemistry that is widely used for q-PCR: SYBR ® Green dye-based assay and 5′ nuclease assay or TaqMan ® Assay.

Most nucleic acid diagnostic tests for etiological agents of infectious disease are based on PCR. As for other molecular approaches, PCR circumvents the requirement to grow the pathogens, which is time-consuming for viruses and slow-growing or fastidious bacteria. Not only does this technique enable identification of a viral, bacterial, or fungal pathogen, but also it can reveal specific characteristics of that pathogen, for example, the presence of antibiotic resistance genes that can be used to determine the best course of treatment.

In a real-time PCR, the double-stranded DNA product is bound by a fluorescent dye and the fluorescence is measured after each amplification cycle to quantify the amount of product in real time. Post-PCR processing is not required, and therefore, time to pathogen identification is shorter than for conventional PCR.