Control and prevention programmes if effectively implemented can reduce the:
- frequency of homozygous and double heterozygous states
- morbidity
- psychosocial trauma

Successful implementation of control and prevention programmes require awareness amongst:
- professionals
- community
Prevention of Genetic Disease

- Genetic counseling
- Genetic screening and testing
- Carrier Screening
- Premarital counseling
- Neonatal screening
- Prenatal diagnosis and selective abortion
- Pre-implantation genetic diagnosis
- Treatment of genetic disease
- Education
Genetic Testing

**Predictive testing** Tells: a person if she carries a mutation that will cause, or put her at higher risk for, a disease later in life.

**Newborn screening** Detects: common disorders in newborns, where immediate treatment can prevent dangerous symptoms.

**Carrier testing** Tells: a person whether or not he carries a mutation that could be passed on to his offspring. One can be a carrier, but not be at risk for a disease (as in recessive genes).
STAGES OF PREVENTION

Primary Prevention
Secondary Prevention
Tertiary Prevention
Steps towards control and Prevention of Genetic Disorders

Early detection and interaction

- To Prevent birth of an affected child (Primary prevention)
- To Prevent clinical manifestations in affected individuals by appropriate intervention (Secondary Prevention)
- Provision of adequate care and rehabilitation in affected individuals (Tertiary Prevention)

Screening

Counseling
Detection and Intervention of Carrier

Carrier detection

Genetic Counselling

Primary Prevention

(Prevention of birth of a child with homozygous or double heterozygous state)
Population, Premarital and preconception Screening

Objectives

To identify carriers for a particular gene defect
- Genetic counseling for prevention of birth of affected child

To identify individual with a genetic predisposition to a disease
- Genetic counseling to prevent or delay disease development
Autosomal Recessive Disorders Suitable for Population Screening

- α-thal.
- β-thal.
- Hb S.
- G-6-PD deficiency.
- Cystic fibrosis.
- Tay-Sachs disease.
- Multifactorial disorders are included in several population screening programs.
Screening for carriers of recessive genetic diseases

The following criteria must be met

(i) Disease presentation is severe.
(ii) Screening is directed towards high risk population
(iii) Availability of an inexpensive sensitive and specific test.
(iv) Reproduction options are available to couples found to be at risk.
(v) Genetic counselling is available.
Premarital Screening for Beta-Thalassemia in Jordan
Examples of screening to identify individuals at increased risk of having children with genetic diseases

Screening for Hb S or β-thalassaemia

Both partners carriers

Genetic counseling

Prevent the birth of an affected child
Laboratory diagnostic tests for beta-thalassemia

- Carriers are detected by evaluation of red cell indices (MCV)
- Hb electrophoresis
- Molecular analyses
Beta-thalassemia in Jordan

- The carrier prevalence rate of beta thalassemia in Jordan is around 4%.
- The birth incidence for beta thalassemia is about 1 in 2500 livebirths.
- The registered number of beta thalassemia patients in the Kingdom is around 1200.
- It is estimated that without a control program, 80-90 new cases of beta thalassemia will be born annually.
Premarital thalassemia screening program

• The ultimate goal is to reduce the birth incidence of beta-thalassemia in Jordan

• This will provide for better management of present patients blood transfusion and iron-chelating agents
Beta-thalassemia premarital screening program

- Education of the public
- Training of health personnel

Pre-screening Counseling

Screening test

Interpretation of test

- Both or one non-carrier
- Both are carriers

Report that test was done
Beta-thalassemia premarital screening program

Both are carriers

Confirmatory Test

Both are carriers

Both or one non-carrier

Counseling by Specialist

Report that test was done

Confidentiality

Non-stigmatization

Autonomy of decision

Report that test was done
Premarital Screening

Conclusive counseling of identified carriers

- Can influence marriage decision
- Allows informed reproductive decisions
- Marks up individuals for prenatal diagnosis
NEONATAL SCREENING
Types of Genetic Testing

1. Carrier testing: test family members, determine chances of having an affected child

2. Premarital Screening

3. Neonatal testing: New born screening ID individuals for treatment

4. Prenatal diagnosis: determine genotype of fetus

5. Preimplantation diagnosis (PGD): IVF, determine genotype before transfer the fertilized ova

6. Other Technologies
The Cardinal Principles of Screening

Some of the basic criteria for determining which inherited disorders for newborn screening include:

- The disorder has a relatively **high incidence** so that the cost per diagnosed individual is reasonable.

- An effective and **not overly expensive** treatment is available.

- A relatively **inexpensive screening test** is available that is suitable for **high volume testing** (preferably automatable).

- The screening test has a very **high sensitivity** (i.e. a very low rate of false negatives) and **high specificity** (i.e. low rate of false positives which require expensive follow-up).

- **Diagnostic Urgency**

- **Government Mandate**
Why do Newborn Testing?

• Reduce mortality and morbidity of inherited disease
• Identify congenital disorders
• Improve patient outcomes through early detection and treatment
  ▪ Minimizing the impact of disease
  ▪ Offering essentially a “normal” life
• Offer a cost benefit to society
Potential Advantage and Disadvantages of Genetics Screening

**Advantages**

- Informed choice
- Improved understanding
- Early treatment when available
- Reduction in births of affected homozygotes

**Disadvantages and hazards**

- Pressure to participate causing mistrust and suspicion
- Stigmatization of carriers (social, insurance and employment)
- Inappropriate anxiety in carriers
- Inappropriate reassurance if test is not 100% sensitive
### Conditions for Which Neonatal Screening Can be Undertaken

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Test/method</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Phenylketonuria assay</td>
<td>Guthrie&quot; or automated fluorometric assay</td>
</tr>
<tr>
<td>• Congenital hypothyroidism hormone</td>
<td>Thyroxine or thyroid stimulating hormone</td>
</tr>
</tbody>
</table>

#### Other inborn errors

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Test/method</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Biotidinase deficiency</td>
<td>Specific enzyme assay</td>
</tr>
<tr>
<td>• Galactosaemia</td>
<td>Modified Guthrie</td>
</tr>
<tr>
<td>• Homocystinuria</td>
<td>Modified Guthrie</td>
</tr>
<tr>
<td>• Maple syrup urine disease</td>
<td>Modified Guthrie</td>
</tr>
<tr>
<td>• Tyrosinaemia</td>
<td>Modified Guthrie</td>
</tr>
</tbody>
</table>

#### Miscellaneous

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Test/method</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Congenital adrenal hyperplasia</td>
<td>17-Hydroxyprogesterone assay</td>
</tr>
<tr>
<td>• Cystic fibrosis analysis</td>
<td>Immunoreactive trypsin and DNA analysis</td>
</tr>
<tr>
<td>• Duchennemuscular Dystrophy</td>
<td>Creatine kinase</td>
</tr>
<tr>
<td>• Sickle-cell disease,</td>
<td>Hemoglobin electrophoresis</td>
</tr>
</tbody>
</table>
Newborn Screening Programs
Types of Genetic Tests

1. Cytogenetic
2. DNA
3. Metabolic
Criteria for Newborn Screening

• Disorder produces irreversible damage before onset of symptoms
• Treatment is effective if begun early
• Natural history of disorder is known
Prevention of Congenital Hypothyroidism

- Neonatal Screening
- Hormone Replacement Therapy

Prevention of congenital hypothyroidism
Prevention Of Neural Tube Defect

Normal baby

Folic Acid supplementation prior to & during Pregnancy
### Time Line for Specimen Collection for PKU Screening

**Treatment:** Milk without Phenylalanine

<table>
<thead>
<tr>
<th>Day of Life</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (hours)</td>
<td>24</td>
<td>48</td>
<td>72</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Specimen Quality</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>
Newborn Screening Program In Jordan

- Thyroid
- Galactosemia
- PKU
PRENATAL SCREENING
Indications for prenatal diagnosis:

• Advanced maternal age
• Previous child with a chromosome abnormality
• Family history of a chromosome abnormality
• Family history of single gene disorder
• Family history of a neural tube defect
• Family history of other congenital structural abnormalities
• Abnormalities identified in pregnancy
• Other high risk factors (consanguinity, poor obst., history, maternal illnesses)
Indications for Prenatal Diagnosis

• High Genetic Risk
• Sever Disorder
• Treatment not available
• Reliable Prenatal Test
• Termination Pregnancy Acceptable
Methods of prenatal diagnosis

**Invasive:**
- Amniocentesis
- Chorionic villus sampling
- Cordocentesis
- Fetoscopy
- Preimplantation genetic diagnosis

**Non-invasive testing:**
- Maternal serum AFP
- Maternal serum screen
- Ultrasonography
- Isolation of fetal cells/DNA from maternal circulation
list of some of the more common genetic diseases that can be detected. Any gene disorder in which the DNA base pairs or code is known, can be detected by PND & PGD.

- Alpha-thalassemia
- Glycogen storage disease
- Beta-thalassemia
- Hemophilia
- Canavan’s disease
- Huntington’s disease
- Cystic fibrosis
- Marfan’s syndrome
- Charcot-Marie-Tooth disease
- Myotonic Dystrophy
- Down’s syndrome
- Neurofibromatosis
- Duchenne muscular dystrophy
- Polycystic Kidney Disease
- Fanconi anemia
- Retinitis pigmentosa
- Fragile X syndrome
- Spinal Muscular Atrophy
- Gaucher disease
- Tay Sachs
Maternal Serum Alpha Fetoprotein (AFP)

• Major protein produced in the fetus
• Elevated levels with open neural tube defect in the fetus
• Second most common fetal malformation
• Maternal serum testing done between 15-22 weeks
Second Trimester Maternal Serum Screening for Aneuploidy

• Performed at 15-20 weeks
• Singleton gestation
• Adjusts age risk based on levels of
  ▪ AFP
  ▪ hCG
  ▪ Unconjugated estriol (uE3)
  ▪ Inhibin-A

“Triple”
“Quad”

• Detection rate in women
  – <35: 60-75% for DS
  – >35: 75% or more
  – >80% for trisomy 18

• Positive screening rate 5%
Serum Screening Test Performance at a fixed 5% False Positive Rate (Dating by Ultrasound)
Combined use of MSAFP and ultrasound approach the accuracy of AFAFP

In many prenatal diagnosis programs, first or second degree relatives of patients with NTDs may have an MSAFP assay at 16 weeks followed by detailed ultrasound at 18 weeks.
<table>
<thead>
<tr>
<th>Disorder</th>
<th>AFP</th>
<th>hGC</th>
<th>hCG/AFP ratio</th>
</tr>
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<tbody>
<tr>
<td>Trisomy 21</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Anencephaly</td>
<td>↑</td>
<td>N</td>
<td>↓↓</td>
</tr>
<tr>
<td>Spina Bifida</td>
<td>↑</td>
<td>N</td>
<td>↓</td>
</tr>
<tr>
<td>Twins</td>
<td>↑</td>
<td>↑</td>
<td>N</td>
</tr>
<tr>
<td>Fetal death</td>
<td>↓</td>
<td>↓</td>
<td>N</td>
</tr>
</tbody>
</table>
Elevated AFP

- Multiple gestation
- Fetal demise, premature delivery, growth retardation
- Abdominal wall defect
- Congenital nephrosis
- Maternal liver disease
Emerging Technologies
Cell & Cell-Free Fetal DNA Sampling

Timeframe: As early as 6-8 weeks post-LMP

- Very small number of fetal cells migrate into the mother’s circulation – 1 out of $10^7$ nucleated cells
- Techniques have been developed to isolate these cells from the maternal blood and tested diagnostic purposes
- At this time, still in developmental stages
- Fetal cells may remain in circulation for years
- In addition, cell-free fetal DNA is found in maternal circulation – this may prove easier to isolate and to test than the fetal cells
Non-Invasive Prenatal Testing

- Detects cell-free fetal DNA in maternal serum
- Uses mass-parallel sequencing to amplify fetal DNA in order to perform genetic testing
- Currently reporting out increases in fetal DNA for chromosomes 13, 18 and 21
  - It is possible that lab will notify us if other numerical chromosome differences are detected
- In the future, it is possible that applications and reporting will expand
Trisomy Detection – Fetal fraction matters in Cell-free DNA

- As cells turnover, chromosomes fragment releasing DNA into the blood
- Cell-free DNA (cfDNA) are short DNA fragments (50-300 base pairs)
- Trisomy detection via cell-free DNA analysis depends on the fetal fraction (proportion of fetal to maternal cell-free DNA within the sample)
- The higher the fetal fraction the easier it becomes to detect aneuploidy

### 10% fetal DNA in circulation

- **Disomic Chr**
  - Total: 100
  - (Maternal: 90)
  - (Fetal: 10)

- **Trisomy 21**
  - Total: 105
  - (Maternal: 90)
  - (Fetal: 15)

### 20% fetal DNA in circulation

- **Disomic Chr**
  - Total: 100
  - (Maternal: 80)
  - (Fetal: 20)

- **Trisomy 21**
  - Total: 110
  - (Maternal: 80)
  - (Fetal: 30)
Phycoll-Hypaque Separation of MNC from Peripheral Blood

Sorting using CD-71 (transferrin receptor to separate nucleated red blood cells.
FISH – for X and Y Signals
Fetal Cells in Maternal Blood

Fetal
Ultrasound

- Noninvasive, uses reflected sound waves converted to an image
- **Transducer** placed on abdomen
- See physical features of fetus, not chromosomes
- May ID some chromosomal abnormalities by physical features
Increased Nuchal Translucency

Normal fluid-filled space between back of the fetal neck and overlying skin, Increased in fetuses with Down Syndrome

- Increased NT > 95th%
- Timing: 11-14 wks EGA

NT > 3 mm is ABNORMAL
Invasive Testing
Chorionic villus sampling (CVS)

Timeframe: 8-10 weeks post-LMP

- Essentially a placental biopsy
- Tissue biopsy from the villous area of the chorion is aspirated transcervically or transabdominally

Cells are cultured and analyzed either for chromosomes or direct DNA mutations or direct assays for biochemical activity
Chorionic villus sampling (CVS)

**Advantages:**
- first trimester diagnosis
- diagnostic results provided
- 99% of the time
- post-CVS fetal loss rate low (1%)
- results usually obtained in 5-7 days

**Disadvantages**
- looks only at extraembryonic material - will not detect a defect arising after embryonic material partitioned off
- confined placental mosaicism may be a problem (2%)
- only gathers cells, not fluid - can’t measure AFP
- Can’t identify NTDs
Review of CVS Procedures
Molecular Testing

Chorionic Villus Material

(b) Chorionic villus sampling (CVS)

Molecular Testing

Karyotyping

Suction tube inserted through cervix

Fetal cells

Several hours

Biochemical tests

Fetus (8–10 weeks)

Placenta

Chorionic villi
Amniocentesis

• An Amniocentesis is a procedure a pregnant woman can have in order to detect some genetics disorders.....such as non-disjunction.
Amniocentesis

**Timeframe:** 15-17 weeks post-LMP
*(Can be done at 10-14 weeks)*

20-30 ml amniotic fluid is collected transabdominally or transcervically with a needle - contains supernatant & fetal cells

Cells cultured & examined for chromosome structure/number and/or direct DNA testing

The amniotic fluid is analyzed for AFP levels
Amniocentesis

• Diagnose > 100 disorders, cells analyzed for chromosomal and biochemical disorders
• Risk of infection and spontaneous abortion
• Normally only used when:
  – Advanced maternal age
  – History of chromosomal disorder
  – Parent with chromosomal abnormality
  – Mother carrier of X-linked disorder
Amniocentesis

Amniotic fluid withdrawn
Amniocentesis

Advantages:
- Can examine AFP levels for spinal defects
- Can be performed by an Ob/Gyn vs. perinatologist
- Fetal loss rate very low (0.5%) - for late Amniocenteses

Disadvantages
- Early amniocentesis has a higher risk of miscarriage (5%)
- Longer wait time for patients than CVS – 1-2 weeks
- Also have some risk of mosaicism
Cordocentesis (PUBS)
Percutaneous Umbilical Blood Sampling

Timeframe: 19-21 weeks post-LMP

Ultrasound-guided puncture of the umbilical cord and withdrawal of fetal blood
Used when rapid diagnosis required (2-3 days) or amnio has failed
also used to diagnose diseases that are analyzed most effectively in blood
Cordocentesis

**Advantages:**

- Rapid diagnosis time, fetal blood cells only need to be cultured for a few days to provide good chromosomes

**Disadvantages:**

- Must be performed by a perinatologist because of difficulty in accessing the umbilical vein
- Higher fetal loss than with CVS or Amnio (2-3%)
Fetoscopy

• Visualisation of foetus by means of endoscope (it has been suppressed by modern US
• It can be undertaken to diagnose a subtle structural abnormalities pointing to a serious diagnosis
• Can also be used to obtain fetal samples for some diagnosis as inherited skin disorders (epidermolysis bullosa) and some metabolic disorders in which enzymes are only in specific organs
Fetoscopy

• A small tube with light source at one end is inserted into womb to inspect fetus for defects of the limbs & face. Blood may be taken.

• Performed between 15 & 18 weeks post conception
Prenatal Diagnosis

What technique do you use?

 Depends upon what you are looking for

- Chromosomal abnormalities - need to look at chromosomes - need live fetal cells obtained from amniocentesis or chorionic villus sampling
- Hormone or enzyme levels - need cells or fluid
- Direct mutation analysis - need DNA (fetal cells)

Tests: Karyotyping, FISH, CGH, Molecular, Biochemical
Overview of Preimplantation Genetic Diagnosis (PGD)
Pre-implantation Genetic Diagnosis (PGD)

What is it?

Genetic analysis of a single cell from an eight-cell embryo done in conjunction with in vitro fertilization (IVF) to improve the chances of a “normal” pregnancy.
Why consider PGD in addition to IVF?

1. recurrent miscarriages
2. one child already affected with a genetic disease
3. family history of inherited disease
4. maternal age older than 38
5. prior failure with IVF
6. family “balancing” for sex
PGD – Clinical Indications

- Single gene defects
- Balanced translocations
- Advanced maternal age (aneuploidy)
- Repetitive IVF failure
- Recurrent pregnancy loss
- Embryo selection
Preimplantation Genetic Diagnosis (PGD)

- PGD is a state-of-the-art procedure used in conjunction with In Vitro Fertilization (IVF) in which the embryo is tested for certain conditions prior to being placed in the womb of the woman.
- PGD was first reported in 1990.
- PGD combines the recent advances in molecular genetics and in assisted reproductive technology.
Indications for PGD

1. Chromosomal Disorders
   - Numerical
     - Chromosomal aneuploidy
   - Structural
     - Inversions
     - Translocations
     - Deletions and duplications

2. Gender determination for severe X-linked diseases with unknown gene

3. Severe monogenic diseases (cystic fibrosis, ß thalassaemia, sickle cell anemia, fragile X syndrome, myopathies)

4. PGD for HLA-typing (to allow selection of embryos that are histocompatible with live siblings)
Preimplantation Genetic Diagnosis (PGD)

1) Eggs are removed from the ovary, fertilized, and grown to the eight-cell stage.

2) Single cells are identified as either male or female.

3) Embryos of the desired sex are selected.

4) The selected embryos are transferred to the uterus for development.
Preimplantation Genetic Diagnosis (PGD)

- Can also be performed on polar bodies
- Polar body is the product of the first meiotic division in oogenesis where the paternal and maternal chromosomes are separated
- If the polar body contains the mutant alleles, the secondary oocyte would have the normal complement and would be used for IVF
The Methods of Preimplantation Genetic Diagnosis

1. Remove a single cell (blastomere) from the 6-8-cell embryo

2. Two types of assessment techniques are common:
   a. chromosome “painting” (or FISH)
   b. genetic testing for specific disease loci (PCR or gene chips)

Limitations of PCR-based tests:

- Both alleles may not amplify equally, leading to misdiagnosis or inconclusive results
- PCR-based tests only detect disorders at target loci; other mutations may exist elsewhere
- Prenatal amniocentesis or CVS is usually recommended
Prohibited Uses of PGD

• Social reasons including sex selection
• S. 11 HART Act 2004
• No selection of embryo on the basis of the sex of the embryo
HLA Tissue Typing
Saviour Siblings

Molly and Adam Nash
Fanconi Anaemia

Zain Hashmi
Beta thalassaemia

Charlie Whitaker
Diamond Blackfan Anaemia
Preimplantation Genetic Diagnosis (PGD)

**Advantages:**
- Very early diagnosis
- Only transfer unaffected (or carrier) embryos

**Disadvantages**
- Cost is extremely high
- “Success”/implantation rate low
- Discard affected or unused embryos, which has raised ethical concerns
International Comparisons

• **Sweden**
  – Severe and progressive hereditary diseases leading to premature death

• **France**
  – Severe genetic disorder known to be incurable at the time of diagnosis

• **UK**
  – Degree of suffering, speed of degeneration, extent of impairment

• **Australia**
  – Victoria: significantly adversely affect health

• **USA**
  – No regulation
Causes of Misdiagnosis

• Human Error
  ▪ Unprotected sex
  ▪ mislabeling, misidentification, misinterpretation
  ▪ wrong embryo transfer
  ▪ incorrect probes or primers

• Technical
  ▪ Probe or primer failure
  ▪ contamination (maternal, paternal, operator, carry-over)

• Intrinsic (embryo)
  ▪ Mosaicism
  ▪ Allele drop out
  ▪ Uniparental Disomy
ESHRE-Misdiagnosis

- **Single Gene Disorders**
  - 14 cases (0.3%), 86% PCR
  - 8 babies born, 78% Prenatal Diagnosis
- **Translocations (FISH)**
  - 3 cases, (0.08%)
  - No live births
- **PGS (FISH)**
  - 10 cases, (0.08%)
  - One baby born with T-21
- **SS**
  - One case (0.2%), 46 XX, pregnancy terminated
Causes of Misdiagnosis

• Human Error
  ▪ Unprotected sex
  ▪ mislabeling, misidentification, misinterpretation
  ▪ wrong embryo transfer
  ▪ incorrect probes or primers

• Technical
  ▪ Probe or primer failure
  ▪ contamination (maternal, paternal, operator, carry-over)

• Intrinsic (embryo)
  ▪ Mosaicism
  ▪ Allele drop out
  ▪ Uniparental Disomy
The Basics of Counselling

- Pre-test counselling
- Gather information
- Risk assessment
- Patient education
- Identify options – engage in dialogue
- Promote autonomous decision-making
- Psychological assessment
- Non-directive
“genetic counselling”

- **What is a GC:** A communication process which deals with the human problems associated with the occurrence, or risk of occurrence, of a genetic disorder in a family….

- Comprehend the medical facts about a disorder
- Appreciate the way in which heredity contributes to the disorder and to the risk of recurrence
- Understand the options for dealing with the risk of recurrence
- Choose the course of action which seems most appropriate to them
- Make the best possible adjustment to the disorder in an affected family member
Patient Education

• Available options
• Difference between screening test and diagnostic test
• Benefits and limitations of screening and diagnostic testing (if appropriate)
• Option of no testing
Benefits of Genetic Screening

- Diagnosis
- Reduce testing
- Appropriate intervention (prevention, management, treatment)
- Informed decisions
- Reproductive choices
Risks of Genetic Screening

- Risk of miscarriage
- Psychological impact
- Family relations
- Insurance issues/concerns
- Privacy
Control and Prevention Programmes For Genetic Diseases

Genetic Diseases (Control and Prevention)

- Increase awareness
- Genetic Screening
- Appropriate management and consultation programmes

High risk | Premarital | Prenatal | Neonatal | General populations

Genetic Counselling

- Patients, families and community
- Clinical staff and premedical personnel
- Health policy makers and administration
GENE THERAPY

- Replacement Therapy
- Gene transfer
- Gene manipulation
- Cloning
- Stem cell
Gene Therapy Strategies

- Interference with gene products
- Replacement of a missing or defective gene
- Introduction of gene(s) to influence cellular process
Gene therapy could be very different for different diseases

- **Gene transplantation**
  (to patient with gene deletion)

- **Gene correction**
  (To revert specific mutation in the gene of interest)

- **Gene augmentation**
  (to enhance expression of gene of interest)
Replacement strategy

- Applies to diseases caused by single gene defects
- Transfer of a functional copy of the defective or missing gene
- Examples: enzyme deficiencies
Replacement strategy

- To apply this strategy, three requirements must be met:
  1. The specific gene defect must be known
  2. A functional copy of the gene must be available
  3. Target cells must be available and amenable to transfection methods resulting in long-term expression
## Replacement strategy

<table>
<thead>
<tr>
<th>Gene with defect</th>
<th>Disease/Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenosine deaminase (ADA)</td>
<td>SCID</td>
</tr>
<tr>
<td>( \alpha )-1-antitrypsin</td>
<td>Emphysema</td>
</tr>
<tr>
<td>CF transmembrane regulator</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>Clotting factor VIII</td>
<td>Hemophilia A</td>
</tr>
<tr>
<td>Clotting factor IX</td>
<td>Hemophilia B</td>
</tr>
<tr>
<td>( \beta )-chain of hemoglobin</td>
<td>Sickle cell anemia</td>
</tr>
</tbody>
</table>
Gene Transfer: Types of Vectors

- **RNA viruses (Retroviruses)**
  1. Murine leukemia virus (MuLV)
  2. Human immunodeficiency viruses (HIV)
  3. Human T-cell lymphotropic viruses (HTLV)

- **DNA viruses**
  1. Adenoviruses
  2. Adeno-associated viruses (AAV)
  3. Herpes simplex virus (HSV)
  4. Pox viruses

- **Non-viral vectors**
  1. Liposomes
  2. Naked DNA
  3. Liposome-polycation complexes
  4. Peptide delivery systems
Viral Vectors

- Gene therapy typically uses inactivated viruses to deliver genes throughout the body.
Liposomes

Next level idea – why naked DNA?

Let’s wrap it in something safe to increase transfection rate

Lipids – are an obvious idea!

Therapeutic drugs
Barriers to successful gene therapy:

1. Vector development
2. Corrective gene construct
3. Proliferation and maintenance of target cells
4. Efficient transfection and transport of DNA to nucleus for integration into genome
5. Expansion of engineered cells and implantation into patient
Ex vivo gene therapy

- Cells from diseased person are removed
- Then, they are treated in lab (using techniques similar to bacterial transformation)
- Finally, they are reintroduced to the patient
- More effective than *in vivo*
- **Transfection** is the introduction of DNA into animal or plant cells
In vivo gene therapy

- Introducing genes directly into tissues or organs \textbf{without} removing body cells
- Challenge is \textit{delivery} only to intended tissues
- Viruses act as \textbf{vectors} for gene delivery, but some injected directly into tissue
Creation of recombinant DNA molecules \textit{in vitro}

plasmid cloning vector
Protein Production in Transgenic Sheep

1. Holding pipette
2. Implant into foster mother
3. Transgenic progeny are identified by PCR
4. Expression of YFG is restricted to mammary tissue
5. Obtain milk from transgenic animals
6. Fractionate milk proteins
7. YFG product is secreted into milk
8. Pure YFG product
Introduction of drug-sensitivity genes

- Suicide gene therapy
- Gene that converts non-toxic prodrug into a toxic metabolite
- Bystander effect
- Gancyclovir triphosphate
- Problem: it can transfec normal cells too
Drug Activation Gene Therapy for Cancer

The diagram illustrates the process of drug activation through gene therapy for cancer. It involves the transfer of a gene construct that includes an erb-B2 promoter and an enzyme gene. The gene construct is delivered through a plasmid or viral construct.

- In normal cells, the gene construct is transcribed, but no toxic prodrug is produced due to the absence of the enzyme.
- In tumor cells, the enzyme is expressed, converting the non-toxic prodrug into a toxic drug, leading to cell death.
Interference nucleic acids

- DNA
  - Antisense oligodeoxynucleotide (ODN)
  - DNAzyme

- RNA
  - Antisense RNA
  - Ribozyme
  - Small interfering RNA (siRNA)
  - Short hairpin RNA (shRNA)
  - microRNA (miRNA)
Spectrum of Gene expression

• Cancer Gene Therapy
  1. Oncogene inactivation
  2. Augmentation of tu. supressor genes
  3. Cell targeted suicide – prodrug to toxic metabolite by transfer of converting enzyme gene into tumor cells
  4. Chemoprotection – transfer of MDR gene into bone marrow cells to decrease effect of cytotoxic agents
Areas of investigation

Introduction of gene(s) to influence cellular process

- Enhancement of anti-tumor response
- Introduction of drug-resistance genes
- Introduction of drug-sensitivity genes
- Replacement of tumor suppressor genes
STEM CELLS
Major Types of Stem Cells

**Embryonic Stem Cells**
- Totipotent: cell can develop into all cell types
- "Immortal": can self-renew indefinitely
- Plentiful

**Adult Stem Cells**
- Multipotent: cell can develop into a few cell types but not all
- Located in few organs or may be unidentified
- Hard to find
Embryonic Stem Cells

Examples:
- Circulatory System
- Nervous System
- Immune System
The Promise of Stem Cell Research

- Identify drug targets and test potential therapeutics
- Culture Pluripotent Stem Cells
- Study cell differentiation
- Understanding prevention & treatment of birth defects
- Toxicity Testing
- Tissues/Cells for Transplantation

- Bone marrow for leukemia & chemotherapy
- Nerve cells for Parkinson's & Alzheimer's disease
- Heart muscle cells for heart disease
- Pancreatic islet cells for diabetes

Courtesy of the National Institutes of Health
Human Embryonic Stem Cell (hESC) Based Therapy