



## **Advanced Lab. Techniques Theoretical**

Lecture No. 7

# **Molecular Diagnostic Techniques for Genetic Disorders of Hemoglobin**

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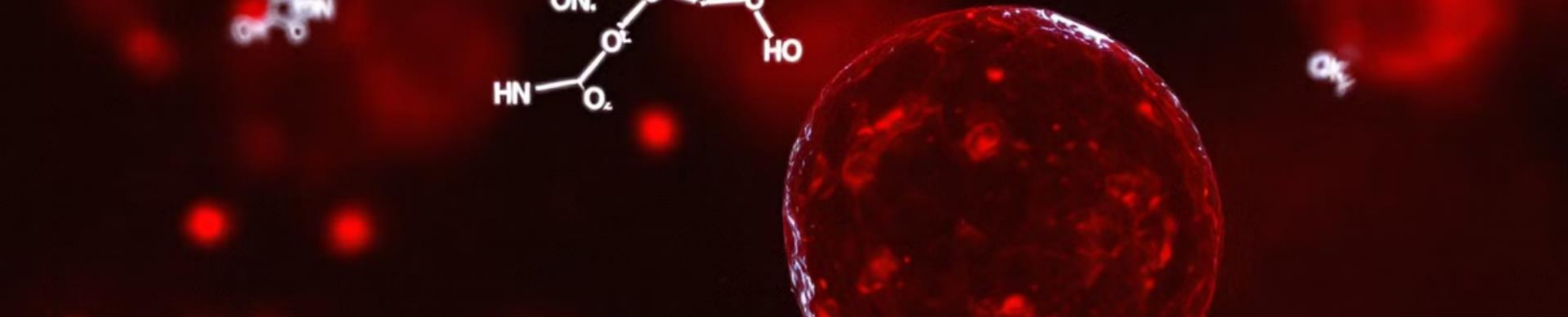
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# **Molecular Diagnostic Techniques for Genetic Disorders of Hemoglobin**

Hemoglobinopathies are a group of inherited blood disorders affecting the structure and function of hemoglobin, the protein responsible for oxygen transport in red blood cells.

Genetic disorders of hemoglobin, such as sickle cell anemia and thalassemias, result from mutations in the genes coding for hemoglobin. These disorders can lead to significant morbidity and mortality if not diagnosed early. Molecular diagnostic techniques play a pivotal role in detecting these mutations, allowing for precise diagnosis, early intervention, and tailored treatment.





# Introduction to Hemoglobin and Genetic Disorders



## Hemoglobin

Hemoglobin is a protein in red blood cells that carries oxygen throughout the body.



## Genetic Disorders

Genetic disorders arise from alterations in genes or chromosomes, affecting protein function.

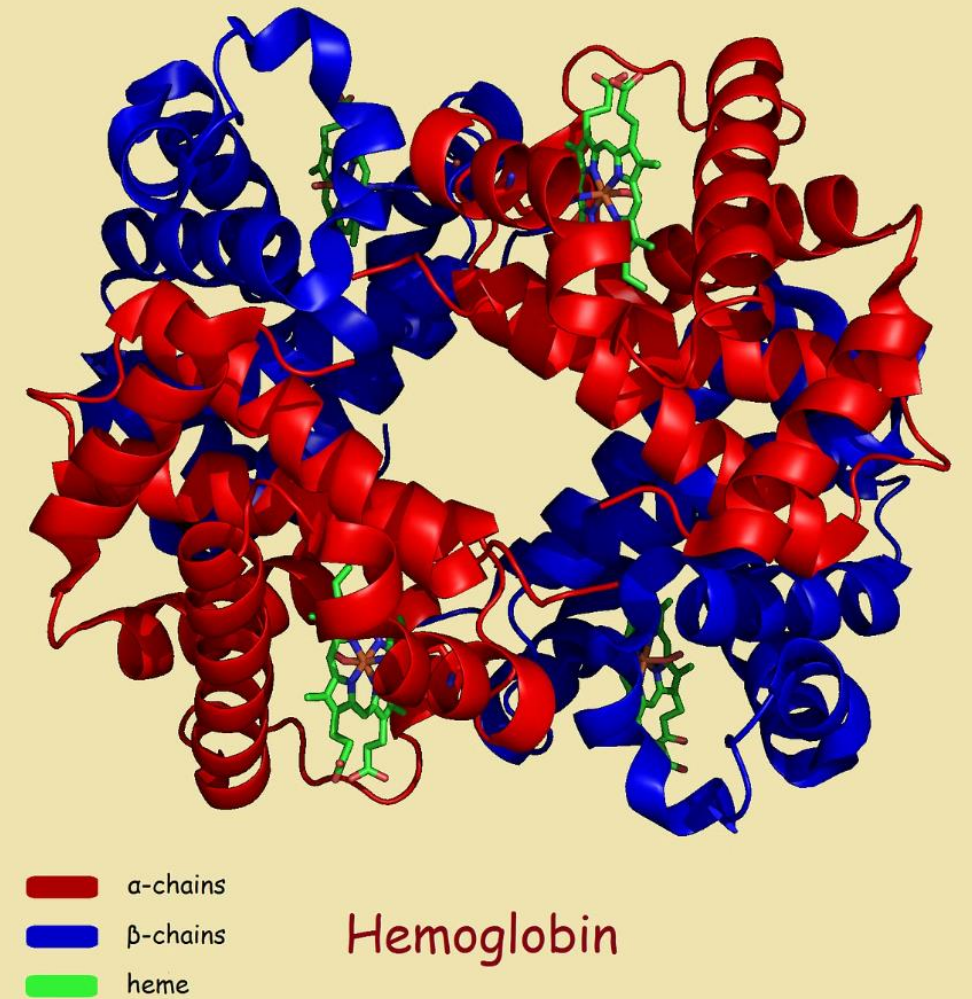


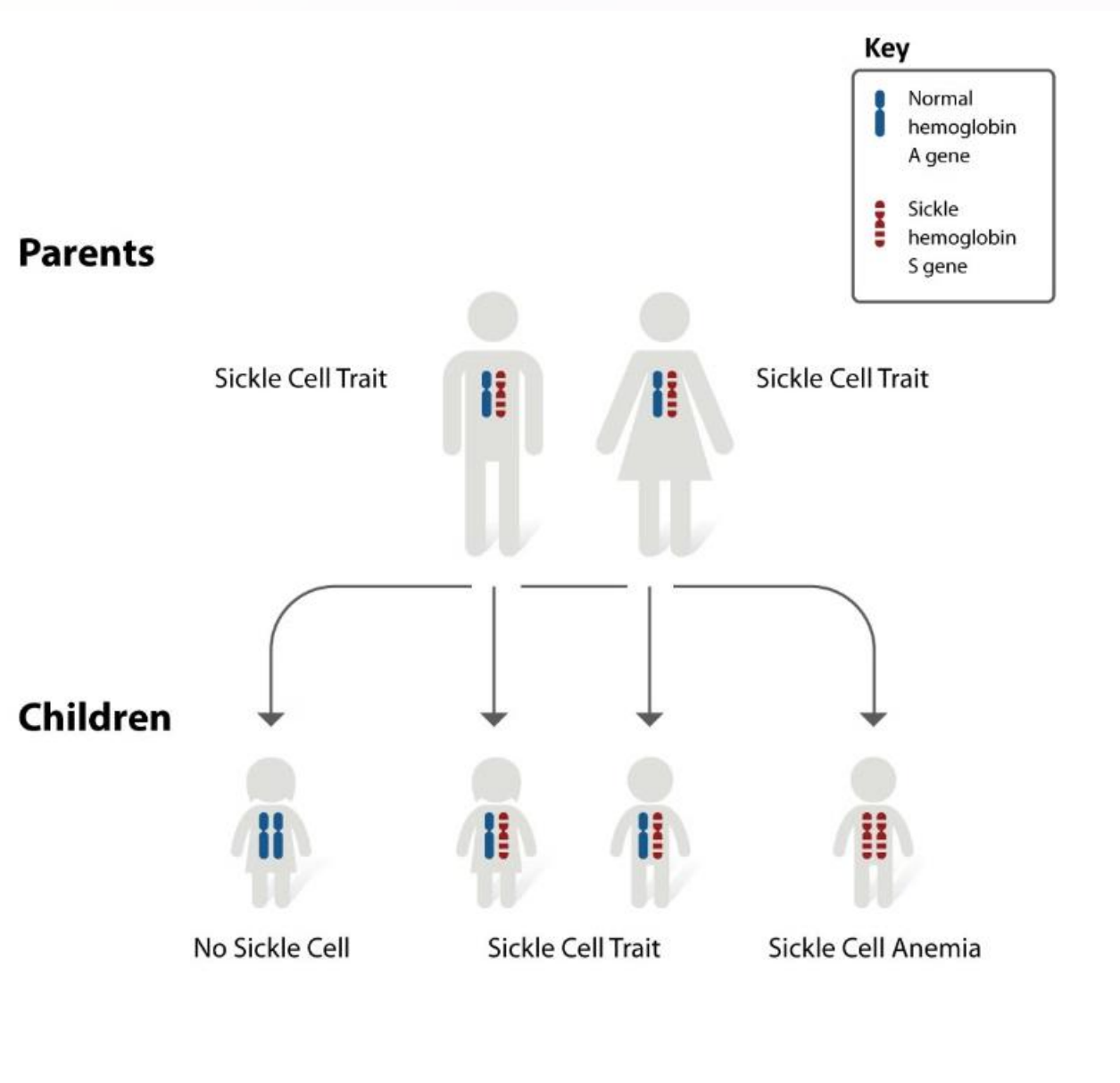
## Hemoglobinopathies

Hemoglobin disorders, or hemoglobinopathies, are genetic conditions that affect hemoglobin structure or production.

## Understanding Hemoglobin Genetics

Hemoglobin is composed of two alpha-globin and two beta-globin chains. Mutations in the genes encoding these chains—**HBA1**, **HBA2** (alpha-globin genes) and **HBB** (beta-globin gene)—lead to various hemoglobinopathies.





## Common Genetic Disorders of Hemoglobin

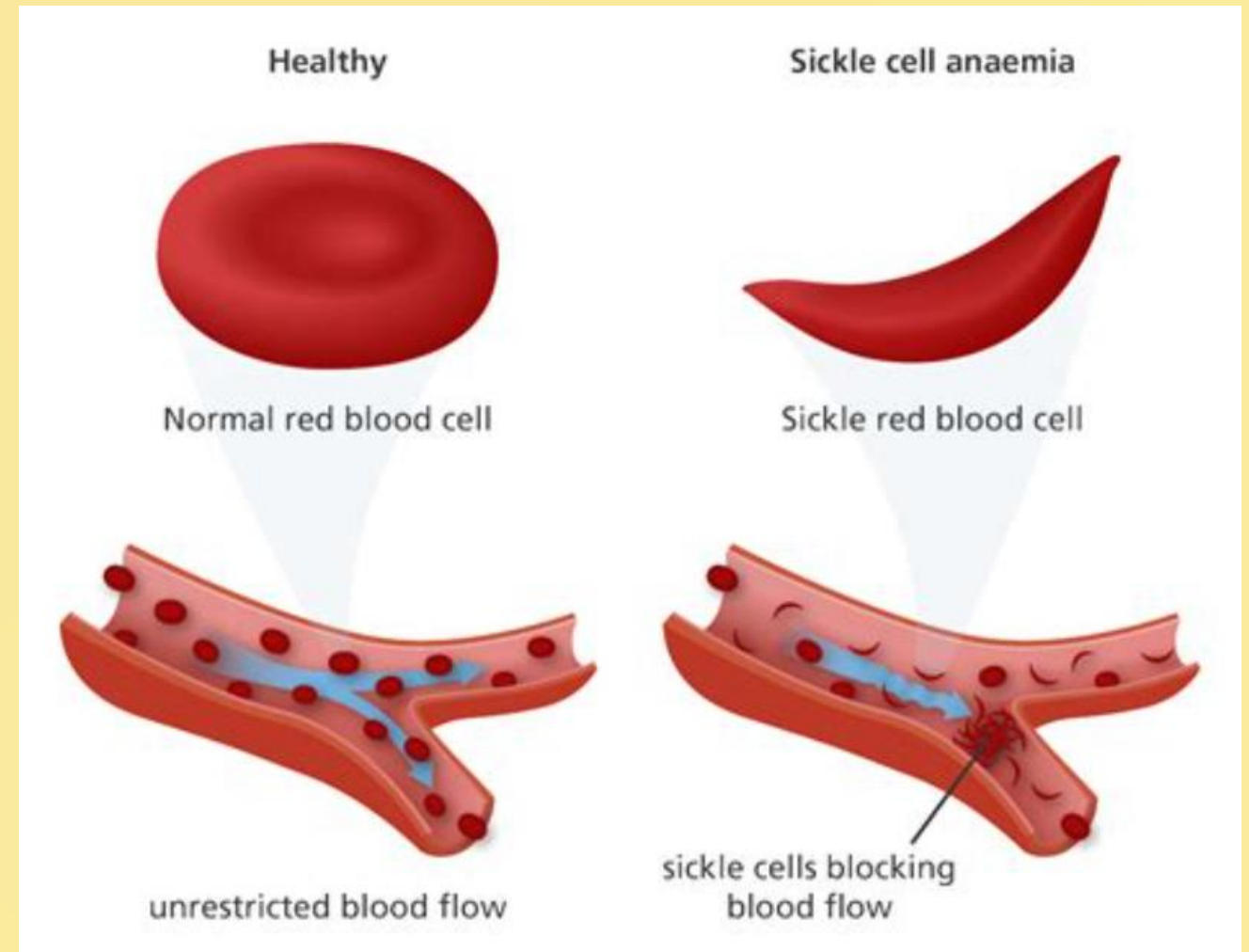
1. **Sickle Cell Anemia:** A point mutation (GAG→GTG) in the HBB gene causing the substitution of glutamic acid with valine at the 6th position.
2. **Alpha-Thalassemia:** Deletions in one or both alpha-globin genes.
3. **Beta-Thalassemia:** Point mutations or deletions in the beta-globin gene.

# Overview of Sickle Cell Disease and Thalassemias

Sickle cell disease and thalassemias are inherited blood conditions that affect how red blood cells work.

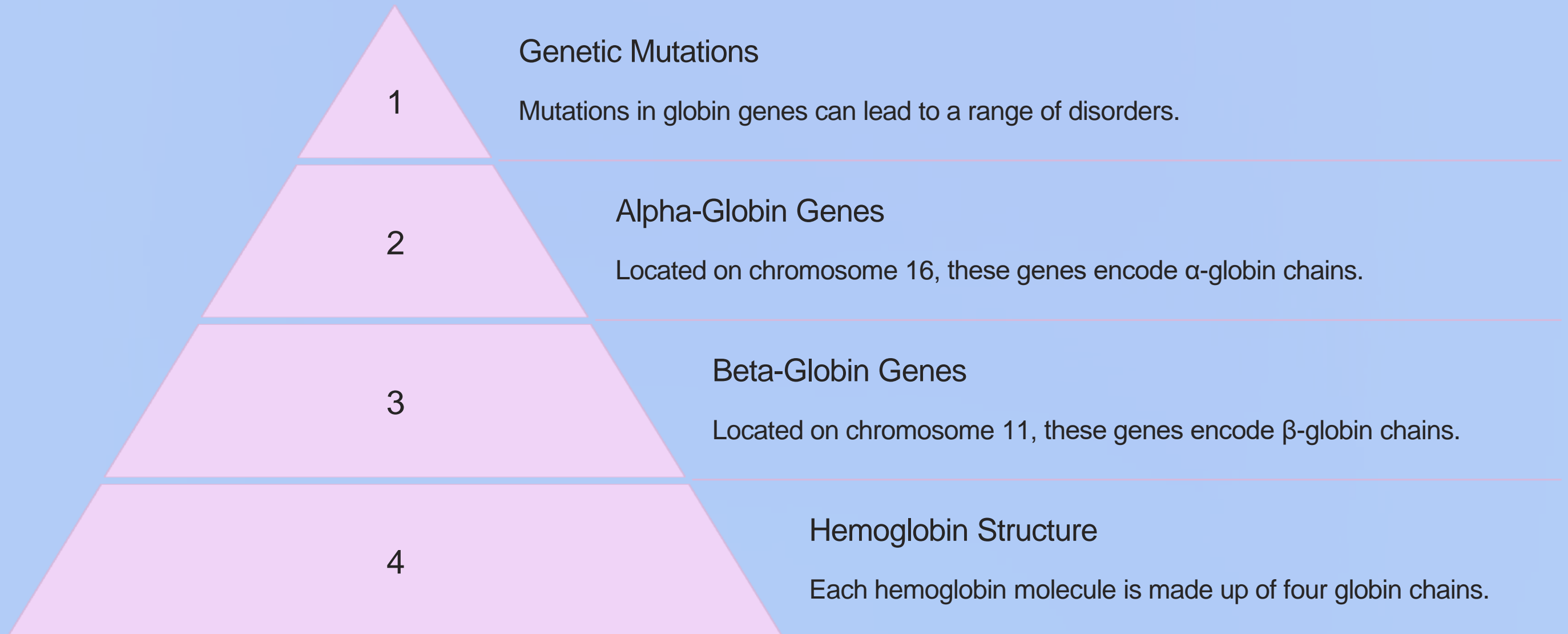
In sickle cell disease, a gene change causes red blood cells to become crescent-shaped. These unusual cells are weak and can block blood vessels, leading to pain and health problems.

Thalassemias happen when the body doesn't make enough healthy red blood cells. This can cause low energy, weakness, and other health challenges.



# Molecular Genetics of Hemoglobin Disorders

Hemoglobin disorders arise from genetic mutations affecting the globin genes, which encode the protein subunits of hemoglobin.



# DNA-Based Diagnostic Techniques

## DNA-Based Diagnostics

DNA-based diagnostic techniques are highly accurate and reliable for identifying mutations in genes associated with hemoglobin disorders.

They can be used to confirm a diagnosis, predict the severity of the disease, and guide treatment strategies.

## Advantages

DNA-based methods offer several advantages, including sensitivity, specificity, and the ability to detect mutations that are not readily detectable by other methods.

These techniques are also suitable for prenatal diagnosis, allowing for early detection and management of genetic disorders.





# Molecular Diagnostic Techniques

## A. Polymerase Chain Reaction (PCR)

Purpose: Amplify DNA fragments containing mutations for analysis.

1

### DNA Extraction

First, DNA is extracted from a blood sample. The sample is treated with chemicals to remove unwanted proteins and lipids.

2

Design primers flanking the regions of interest (e.g., HBB gene).

Perform PCR cycles: denaturation, annealing, and extension.

3

### Mutation Detection

After amplification, DNA is analyzed for mutations using techniques like gel electrophoresis. This allows for the identification of specific changes in the gene sequence.

4

### Interpretation and Diagnosis

The results are analyzed to determine the presence or absence of known mutations. If a mutation is detected, the diagnosis of a hemoglobin disorder is confirmed.



# Restriction Fragment Length Polymorphism (RFLP) Analysis

1

## DNA Digestion

Restriction enzymes cut DNA at specific sequences.

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2

## Electrophoresis

DNA fragments are separated by size.

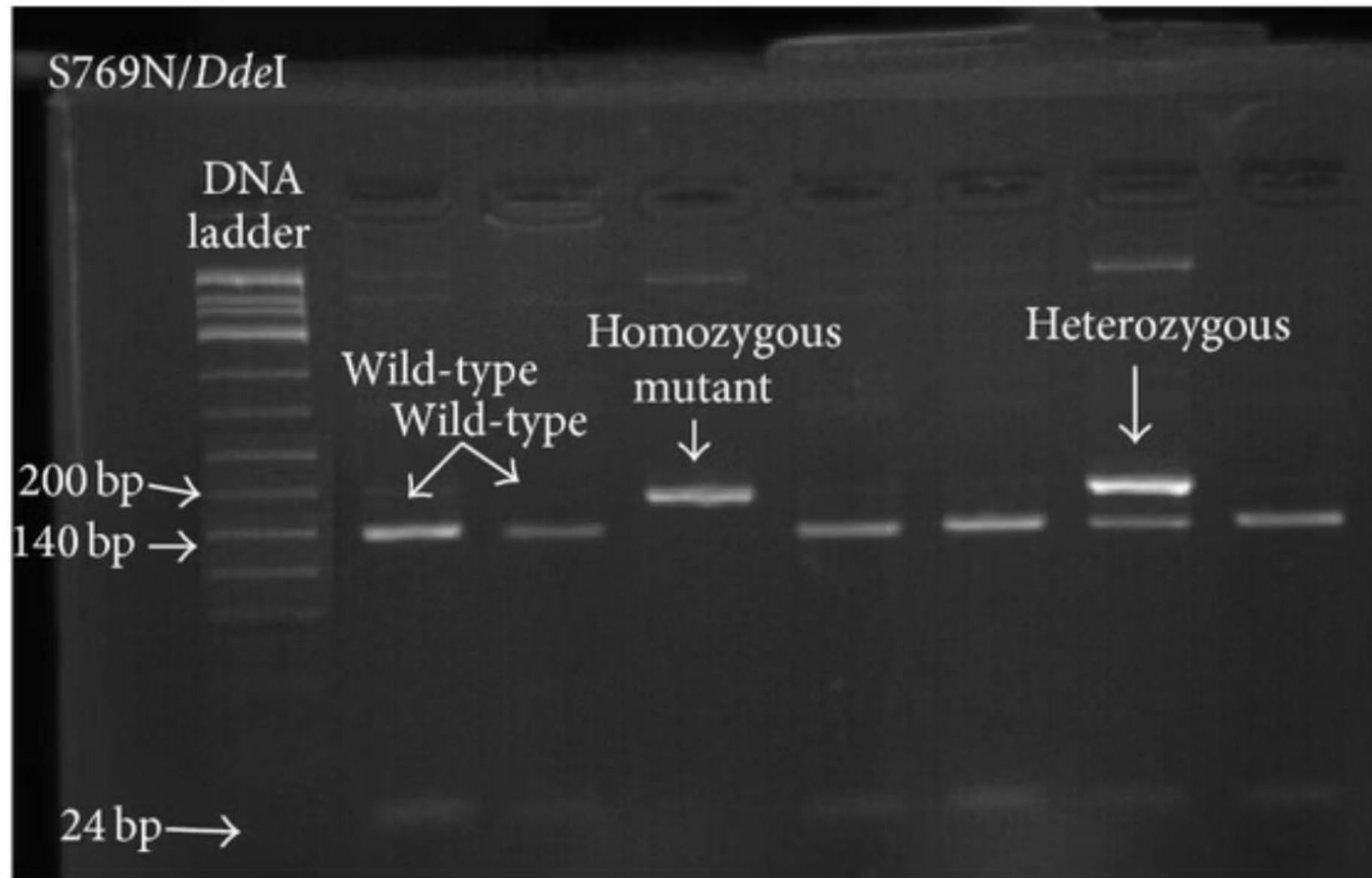
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## Fragment Analysis

Differences in fragment lengths indicate mutations.

RFLP is a classic technique used to detect mutations in hemoglobin genes. It relies on restriction enzymes, which cleave DNA at specific sequences. The resulting fragments are then separated by electrophoresis, and differences in fragment lengths, or RFLPs, can indicate the presence of mutations.



Gel electrophoresis results showing normal, carrier, and affected patterns.

# Homozygous

- **Meaning:** An organism is **homozygous** for a gene when it has two identical alleles for that gene, one inherited from each parent.
- **Example:**
  - **Homozygous Dominant:** Both alleles are the dominant version (e.g., AA).
  - **Homozygous Recessive:** Both alleles are the recessive version (e.g., aa).
- **Application in Hemoglobin Disorders:**
  - In **sickle cell anemia**, a person with two copies of the mutated HBB gene (HbS/HbS) is homozygous.

# Heterozygous

- **Meaning:** An organism is **heterozygous** for a gene when it has two different alleles for that gene, one dominant and one recessive (e.g., Aa).
- **Example:**
  - A person carrying one normal allele and one mutated allele for the HBB gene (e.g., HbA/HbS in sickle cell carriers).
- **Significance:**
  - In hemoglobin disorders, heterozygous individuals are often carriers and may not show disease symptoms but can pass the mutation to offspring.

# Wild Type

- **Meaning:** The **wild type** refers to the most common or "normal" version of a gene in a population. It represents the standard sequence without mutations.
- **Example:**
  - For the HBB gene, the wild-type allele produces normal hemoglobin (HbA).
- **Application in Diagnosis:**
  - Comparison with the wild type is essential to identify mutations.

## illustration Example

- **Wild Type:** Normal hemoglobin gene (HbA/HbA).
- **Heterozygous:** One normal allele, one mutated allele (HbA/HbS).
- **Homozygous:** Two mutated alleles (HbS/HbS for sickle cell anemia).

# DNA Sequencing for Hemoglobin Mutations

1

## Direct Sequencing

This method directly analyzes the DNA sequence of the beta-globin gene. It identifies mutations by comparing the patient's sequence to a reference sequence.

2

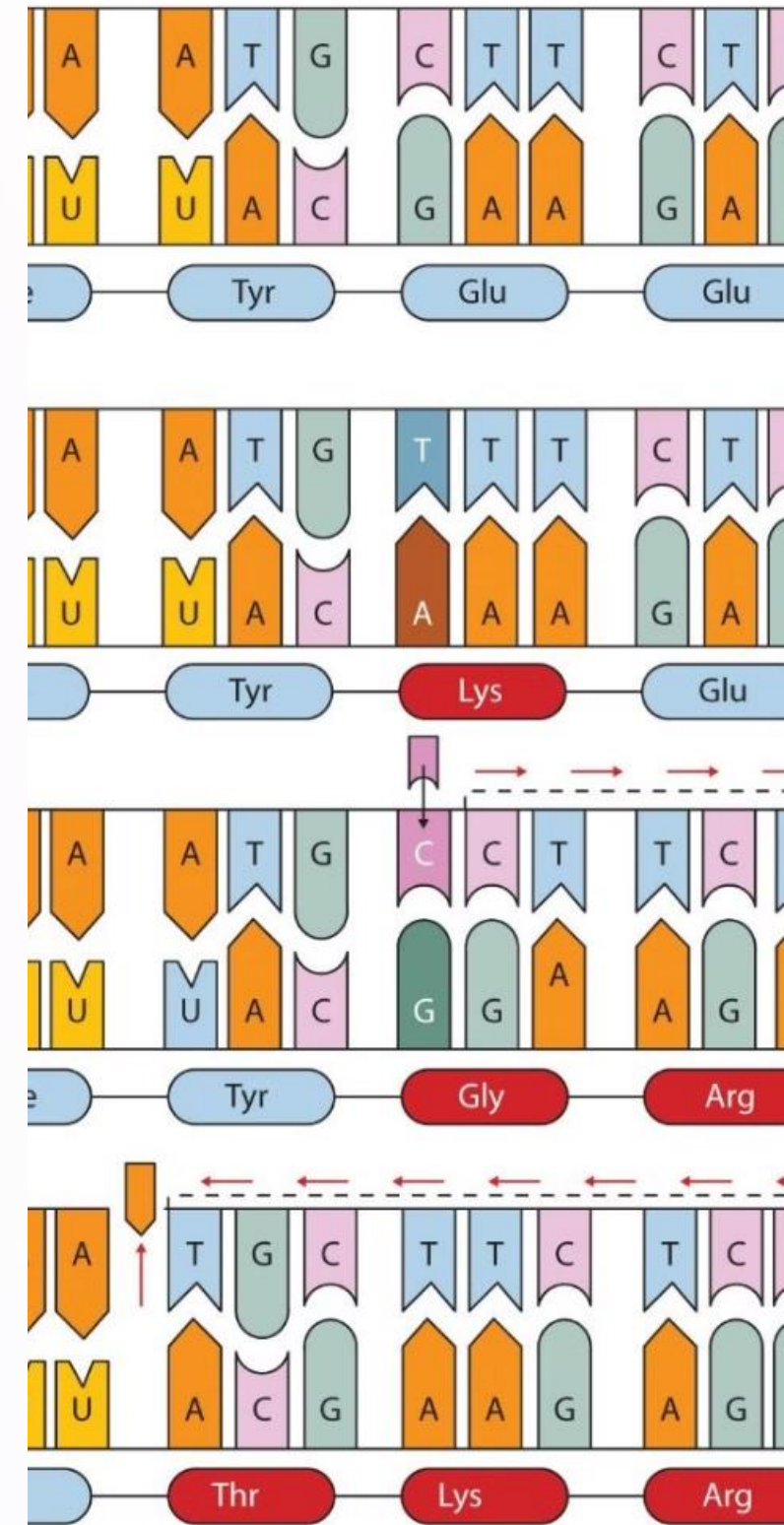
## Next-Generation Sequencing (NGS)

NGS allows for simultaneous sequencing of multiple genes, including the alpha and beta globin genes. This approach is particularly useful for identifying rare or complex mutations.

3

## Whole Genome Sequencing (WGS)

WGS provides comprehensive genetic information, including the entire beta-globin gene sequence. It can identify mutations and other genetic variations that might influence the diagnosis and management of hemoglobin disorders.



# Prenatal Diagnostic Testing



## Prenatal Diagnosis of Hemoglobin Disorders

Prenatal diagnosis enables parents to make informed decisions about their pregnancy based on the genetic health of their unborn child.



## Ultrasound Screening

Ultrasound can reveal certain structural abnormalities in the fetus, potentially associated with hemoglobin disorders.



## Amniocentesis

Amniocentesis involves extracting amniotic fluid from the pregnant woman's uterus for genetic analysis.



## Chorionic Villus Sampling (CVS)

CVS involves extracting chorionic villi from the placenta for genetic testing, providing early prenatal diagnosis.



# Newborn Screening for Hemoglobin Disorders

## Early Detection and Intervention

Newborn screening programs identify infants with hemoglobin disorders, allowing for early treatment and management of these conditions.

## Blood Sample Collection

A simple heel prick blood sample is collected from newborns, allowing for rapid and non-invasive testing.

## Screening Techniques

Various methods are used, including DNA-based tests, to detect specific genetic mutations associated with hemoglobin disorders.

## Public Health Impact

Newborn screening programs have significantly improved outcomes for individuals with hemoglobin disorders, reducing morbidity and mortality.



# Applications in Personalized Medicine

## 1 Targeted Therapies

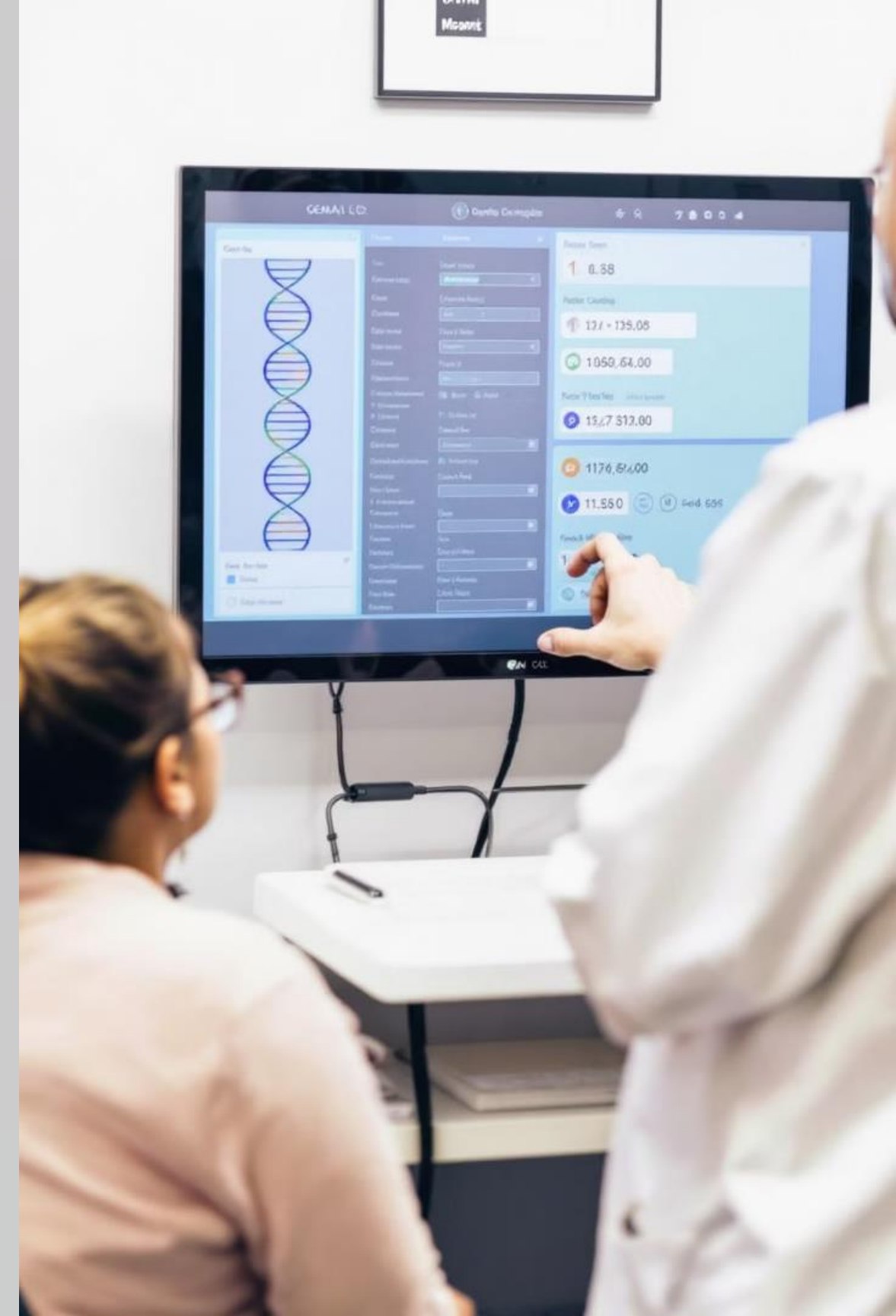
Molecular diagnostics can guide the selection of treatments based on specific genetic mutations.

## 2 Risk Prediction

Identifying individuals at higher risk for developing hemoglobin disorders can allow for early intervention and preventive measures.

## 3 Carrier Screening

Premarital and prenatal carrier screening can help couples understand their risk of having children with hemoglobin disorders.



# Conclusion and Future Directions

Molecular diagnostics for hemoglobin disorders have revolutionized clinical practice. These techniques offer accurate and rapid diagnosis, enabling timely intervention.

Advances in next-generation sequencing and CRISPR-Cas9 technology hold promise for personalized medicine and gene therapy.



**Thanks**