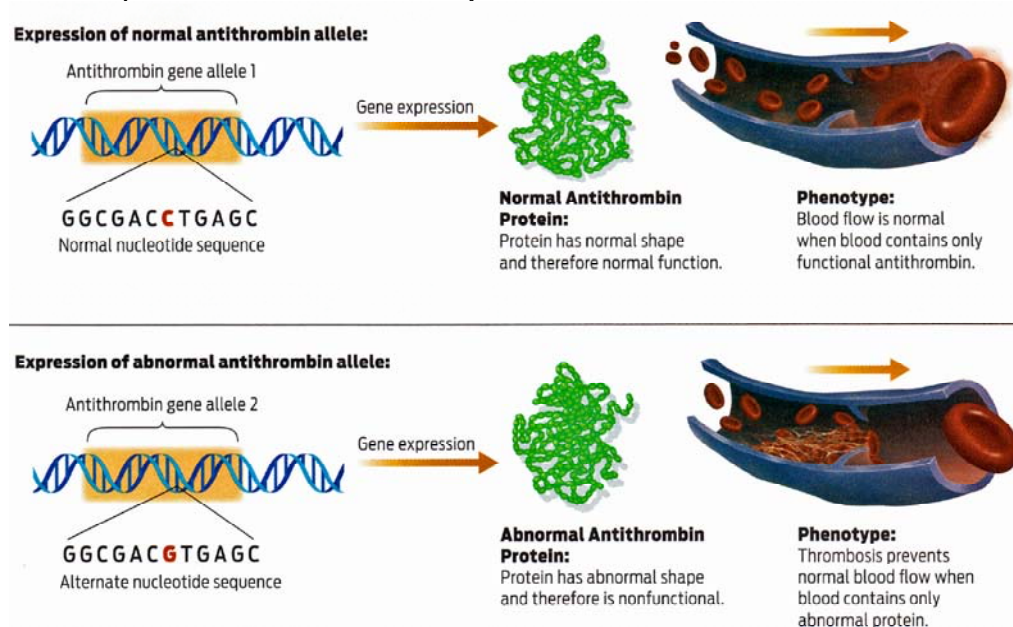


Effects of Mutation on Protein Function and Genetic Diseases

As mentioned in our inheritance section, many human diseases are the result of mutations, often leading to non-functional alleles. The effect of such mutations if the altered allele is expressed is an **altered protein**.



Mutations can result in enzymes that do not function, abnormal protein structure or changes in protein conformation that result in a non-functioning or differently functioning protein.

The effect of mutations is often expressed in the way in which genes and the environment interact, which, under the "right" conditions, may cause disease or make an individual more susceptible to disease. This complex interaction of the genotype and the environment means that much of our "normal" health is genetically **influenced**, including our susceptibility to chronic health diseases such as cardiovascular disease, hypertension, diabetes and cancers. These health issues for humans that may have both genetic and environmental components are multifactorial.

It should not be surprising that many genetic diseases are "caused" by recessive alleles. A mutation in one allele so that it can no longer code for the correct protein can be masked in the phenotype if the unaffected allele on its homologous chromosome can still code for sufficient enzyme or other gene product. It is difficult to remove recessive alleles from the population when individuals who are heterozygous have the altered allele but do not exhibit the problem. In human inheritance, individuals who are heterozygous for a genetic "disorder", but do not exhibit symptoms are called **carriers**. Carriers can pass the allele to the next generation.

Mutations that are always expressed as dominant alleles have greater impacts when harmful to the individual hence are less common in the human population. We will look at some effects of mutation in the context of human genetic diseases.

The Effect of Mutation on Enzyme Function

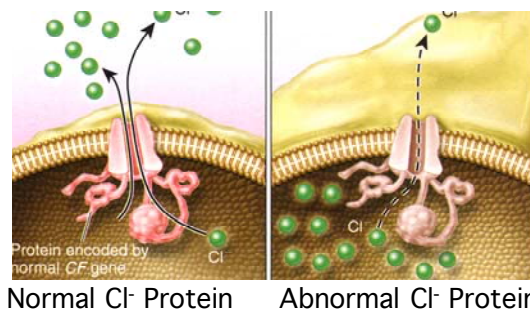
Mutations in DNA that lead to non-functional or dysfunctional **enzymes** are among those now readily detected through genetic screening tests (*see later*). If the enzyme is critical for survival, affected homozygous recessive individuals will die if there is no treatment available. Some human genetic diseases caused by enzyme-coding genes include:

- Galactosemia: the inability to convert galactose to glucose in the liver.
- Phenylketonuria: the inability to process the amino acid, phenylalanine.
- Lactose Intolerance: the inability to digest lactose to glucose and galactose.
- Tay-Sachs Disease: the inability to produce critical brain cell lysosome lipases. Tay-Sachs is fatal in early childhood. About 1 in 28 Ashkenazi Jews in the United States are Tay-Sachs carriers.

Mutations that Cause Abnormal Proteins

As discussed earlier, proteins have many functions. Mutations in structural proteins may cause genetic diseases when the mutation results in no protein being synthesized or a conformational change that inhibits the protein from functioning. Some human genetic diseases caused by mutations that code for abnormal proteins include:

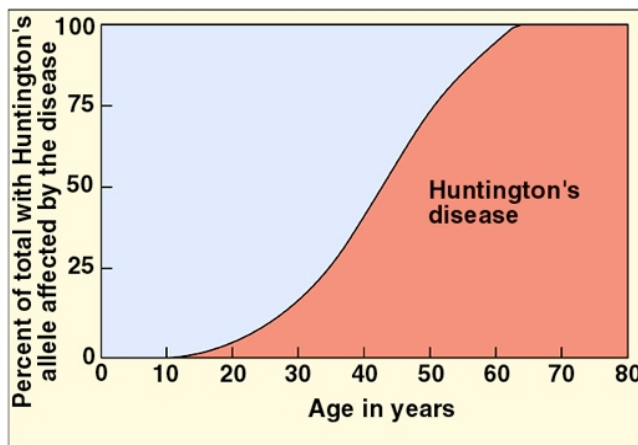
- Duchenne muscular dystrophy: A mutation in the X chromosome that leads to the inability to produce the protein, dystrophin. Dystrophin anchors actin microfilaments to the extracellular matrix of muscle tissue. Without dystrophin, muscle cells lack structural organization and cannot function well.
- Cystic fibrosis: A non-functional chlorine ion channel membrane protein affects water balance and results in thick mucus coatings.



- Hemophilia A: This inability to produce a critical blood-clotting factor is found on the X chromosome.
- Familial Hypercholesterolemia: A non-functional membrane protein receptor results in excess cholesterol. This gene appears to lack dominance; those who are heterozygous are also impacted.

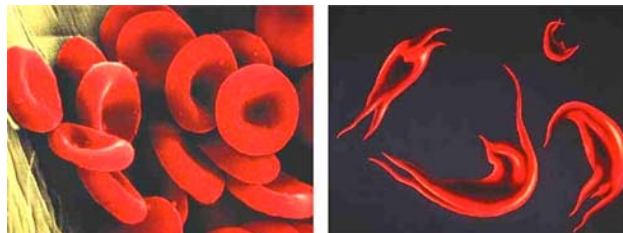
Mutation and Genetic Diseases - 3

- Huntington's disease: An abnormal protein causes the nervous system to progressively deteriorate, resulting in death. Huntington's is caused by a dominant allele, but symptoms usually do not appear until after most childbearing years. Because of work done by Nancy Wexler with a large family in Venezuela in which Huntington's was prevalent, the gene locus for Huntington's was found (tip of chromosome #4) and it is now possible to identify and screen for the Huntington's allele, one of the "trinucleotide repeat" mutations. Those who have the trait in their pedigree may choose to go through the testing procedure (or not). It can be difficult to decide if one wants to **know** that he/she will have the symptoms of this brain disease at "mid-life".



Tracking Huntington's in Venezuela family

- Abnormal Hemoglobin: A variety of mutations in β -globin may result in sickle-cell anemia. The best known is the substitution in amino acid #6, discussed previously. Some β -globin mutations are silent. Such variety in a gene's DNA is known as genetic polymorphism.



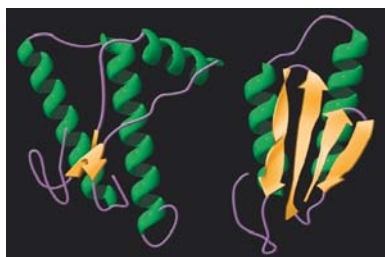
Normal and Sickle-cell RBCs

Prions - A Protein Conformation Disease Agent

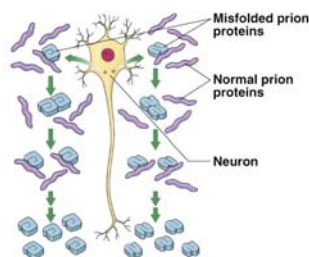
Although we have been discussing the effect of mutation on protein expression in genetic diseases, there is at least one well-known exception to the association of gene mutation to malformed proteins: the **prion**. Prions are **infectious protein particles**, notorious for being the proteins that are responsible for "mad cow" disease or scrapie, (both properly called transmissible spongiform encephalopathy), which is found in a number of mammals besides cows, including deer, sheep, goats and in human Creutzfeldt-Jakob disease. All are degenerative brain diseases. An early symptom is shaking and staggering, as well as rubbing fur or skin off. Prions, which are **glycoproteins**, can be transmitted in food consumed from animals that have the prion disease. Prions accumulate very slowly in brain tissue. The incubation period before symptoms appear is more than ten years for Creutzfeldt-Jakob disease. Prions, to date, have been indestructible.

Stanley Prusiner won the Nobel Prize for his work on how prions probably work. A prion is a variant of a normal brain protein (Prp^c) that has a different tertiary structure (PrP^{sc}). When the prion comes in contact with a normal form of the protein, it converts the normal protein's shape into a prion shape, increasing the number of prions in a chain reaction, until enough are accumulated to cause death.

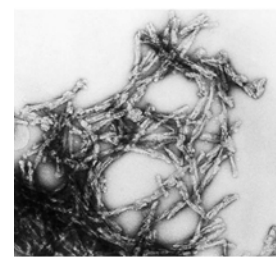
There is no treatment to stop prion formation once the prions are introduced into an individual. Prions are an exception to the central dogma of $\text{DNA} \rightarrow \text{RNA} \rightarrow \text{Protein}$ because no genetic molecules are needed for the protein transformation. No one knows how the original malformed prions originated, or whether prion "mutations" can be spontaneous as well as transmitted by ingestion of food that contains prions. However, scientists from the Scripps Research Institute have shown that prions may be capable of evolving. Prion populations from brain cells were transferred to other cells in culture and observed how they changed. The two populations were then mixed again with both populations out-competing each other in their respective adapted environments, indicating that prions can change, or evolve, to suit their environment. The normal form of the prion protein (Prp^c) inhibits the enzyme that catalyzes the formation of B-amyloid, a plaque-forming protein associated with Alzheimer's disease. People with early-onset Alzheimer's disease have less Prp^c in their brains than people who age "normally". However, there is no association of prions and Alzheimer's disease.



Normal PrP^c and Prion PrP^{sc}



Prion Formation



Prions

Disease Identification and Genetic Screening

Genetic screening for early detection of genetic disorders, accompanied by counseling about the potential effects of identified gene abnormalities and possible treatment, is available for many genetic diseases. Screening can test for both phenotype and genotype, and increasingly, uses DNA analysis to confirm the presence of specific DNA alterations associated with the condition. *DNA analysis tools are discussed in our genetic technology section.*

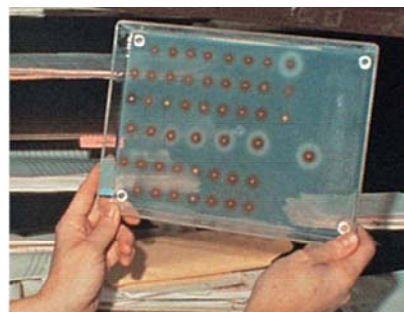
- Some genetic diseases can be identified by their distinctive phenotypic effects.
- Some chromosome abnormalities are visible on karyotypes.
- Pedigree analysis can predict probabilities of one's children having certain genetic diseases.
- Some genetic diseases can be identified by biochemical tests that identify the altered gene's phenotypic expression.
- Some genetic diseases can be diagnosed by direct DNA analysis.

Many screening and diagnosis techniques are in use today, and we will mention a few below. However, it helps to know where a gene is in the genome and its DNA sequence for screening, diagnosing and treating genetic diseases. We shall also examine how DNA analysis using some of the tools discussed earlier in this section is used to identify abnormal genes most accurately.

Biochemical Screening for the Gene Product – Protein Testing

Biochemical tests that can measure low enzyme production or the absence of an enzyme (by measuring levels of a substrate or alternative product that results from the absence of an enzyme) are effective at detecting carriers of some genetic diseases and for the early detection of diseases, particularly in infants. Because some genetic diseases can be adequately treated when detected in newborns, newborn screening is mandatory in some countries, including the United States. At least 25 genetic diseases can be detected through newborn screening. Genetic diseases that can be detected by biochemical screening include:

- Tay-Sachs
- PKU (carriers and newborns)
- Sickle-cell disease
- Common form of cystic fibrosis
- Congenital hypothyroidism

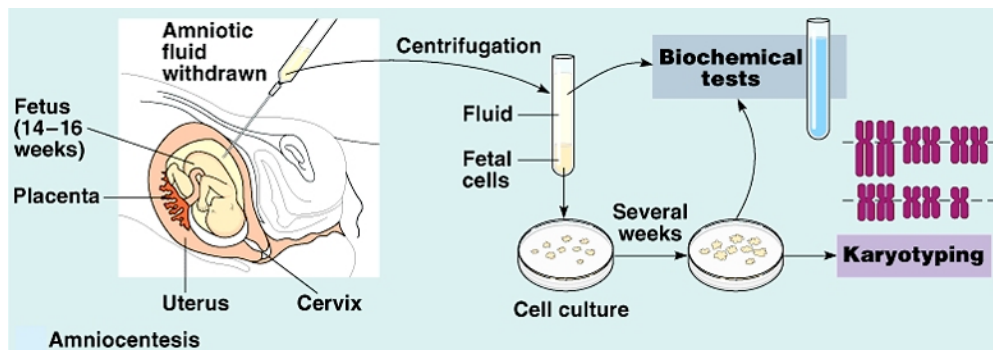


Testing for Phenylketonuria in Newborn Blood Samples

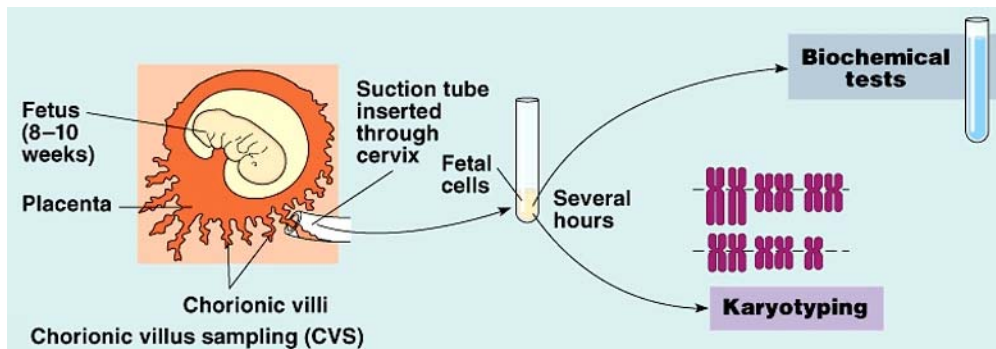
Prenatal Screening

Prenatal screening can help potential parents determine the risks of genetic disorders in the developing embryo or fetus, and perhaps apply genetic technology to correct gene defects in the embryo or newborn. Prenatal screening is often recommended for older mothers and those who have carriers of particular diseases within their families. Prenatal screening methods include:

- **Amniocentesis**, which obtains fetal cells found in the amniotic fluid. Amniocentesis is done in the 14th to 17th week of pregnancy and may require several weeks to culture and analyze the sample.



- **Chorionic villus sampling** takes fetal tissue from the placenta, which can provide more cells than from amniocentesis. Chorionic villus sampling can be done earlier in pregnancy and results take just a few days.



- **Ultrasound** of the developing fetus is a no-risk option for major anatomical abnormal development
- **Fetoscopy** uses a thin viewing scope and fiber optic light source inserted through the uterus and has results similar to ultrasound
- **Pre-implantation analysis** is used with in vitro fertilization. One or a few cells from very early embryo development can be examined for certain chromosomal abnormalities, such as Huntington's, muscular dystrophy and cystic fibrosis. With direct DNA testing, discussed below, even more genetic abnormalities can be detected. If no abnormality is detected, the remaining mass of cells can be implanted into the female for development.

DNA Analysis for Genetic Diseases

As mentioned, DNA analysis provides us with accurate information about gene and allele sequences. Once we know the normal DNA sequence for a gene, we have a number of techniques and tools available for identifying mutant alleles that cause genetic disease. With the ability to amplify a DNA sample using the polymerase chain reaction (PCR), only one or two cells are needed. Direct DNA testing can be a more accurate and timely way of screening for genetic diseases than some traditional techniques.

Locating Disease-Causing Genes

Molecular geneticists find mutation differences in DNA and then trace the gene to the intended protein product. Techniques for finding genes use **genetic markers**. All DNA has unique sequences that can be used to locate specific mutated regions of DNA that cause diseases. These small pieces of DNA (*which we will be discussing with our genetic technology unit*) have allele polymorphisms within the human population. These differences using techniques such as DNA hybridization and DNA probes find and match, or not match, with test subjects' DNA, hence identifying individuals that have the unique DNA sequence or mutation.

Identification of disease-causing alleles is corroborated by analysis of DNA from people who have the disease and people who do not in order to find DNA polymorphisms that will lead to the disease-causing allele. mRNA analysis is often used as an adjunct to determine the protein for which the DNA codes and relating the protein involved with normal or abnormal phenotypic expression. Once disease-causing allele sequences are confirmed, tests are developed for diagnosis in individuals.

Genetic analysis has confirmed a multitude of point mutations and chromosomal abnormalities, including learning that abnormal gene products can result from an assortment of point mutations in the gene. Over 500 different point mutations have been identified in the gene that codes for phenylalanine hydroxylase. Several of these result in failure to code for the enzyme, which results in phenylketonuria. Many of these point mutations are single nucleotide polymorphisms (SNPs), which abound in DNA. Mutations involving short tandem repeats (STRs), repeating short DNA sequences, are also common, as in the trinucleotide repeat mutations mentioned previously. (*STRs and SNPs will be discussed in our Genetic Technology and DNA Analysis techniques section.*)

DNA screening is now used to identify mutations in the β -globin gene that result in sickle cell disease, and is also available for cystic fibrosis and phenylketonuria (PKU). Some of these techniques will be discussed in our genetic technology section.

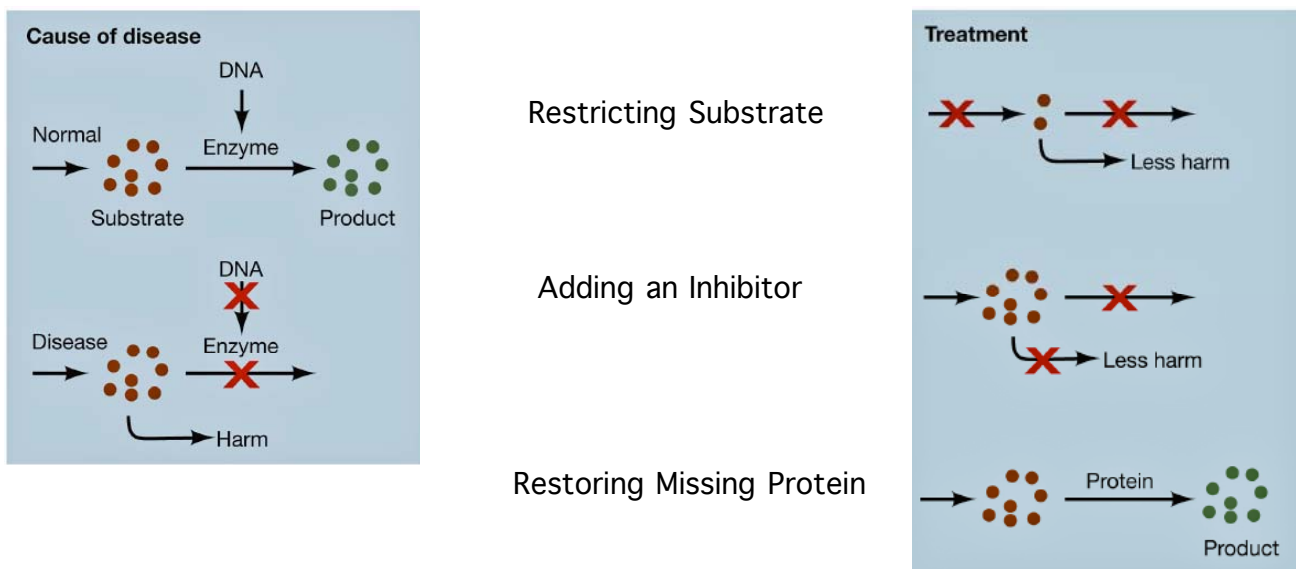
Treating Genetic Problems

To treat genetic diseases, we have to understand how the disease works at the cellular and molecular level and also intervene with treatment before it's too late for treatment to be of benefit.

Although correcting the mutant allele within the cells and tissues of the affected individual is a long-term goal, the immediate goal for most genetic diseases is treating the phenotypic symptoms of the disease, particularly when the disease is the result of a single gene mutation.

Treatments for Single Gene Mutations Involving Enzymes and Needed Proteins – Treating the Phenotype

When the effect of the genetic disease is known, particularly when the mutation affects enzyme production, treatments include restricting the substrate so its negative impacts are avoided, adding a metabolic inhibitor to minimize the negative impacts of accumulated substrate (or alternative harmful product) or providing the missing enzyme in the cellular environment.



Removing the Missing Enzyme's Substrate from the Environment

If the genetic disorder is one that fails to produce a critical enzyme for the breakdown of some food or nutrient (substrate) so that the nutrient accumulates in toxic quantities, the individual can be treated by providing a diet that minimizes consumption of or does not include the substrate. This is also known as **Diet Therapy**. Although it can be challenging to achieve, those who successfully do so live productive "normal" lives. Genetic conditions that can be treated by substrate restriction include:

- Lactose intolerance
- Phenylketonuria (phenylalanine intolerance)
- Galactosemia (galactose intolerance)

Metabolic Inhibition of the Substrate or Product – Molecular Medicine

Drugs or medications that can block synthesis or inhibit cell activity that produces symptoms of a genetic disease can be used to treat some genetic diseases.

- People with hypercholesterolemia can take medications that block synthesis of cholesterol, limiting the amount of cholesterol in circulation. In combination with diets that restrict intake of cholesterol and saturated fats, they have good success.
- Chemotherapy, often used to destroy cancer cells, can also sometimes inhibit gene activity when the gene produces some harmful substance in genetic diseases other than cancers.
- Chelation agents can also be used with some chemicals that can build to toxic levels

Supplying the Missing Protein

For some genetic diseases, the treatment is to provide the protein. Generally, this cannot be done through diet since proteins are denatured during digestion, but in some cases, injection of the missing protein works.

- Clotting factor can be provided to those with hemophilia A.
- People who have sickle cell disease can receive periodic blood transfusions to supply them with sufficient normal red blood cells to alleviate their symptoms.
- Children born who cannot produce sufficient growth hormone can be supplied with growth hormone to achieve normal growth.
- Diabetics can be provided insulin, which works in conjunction with a conscientious diet and blood glucose level monitoring.

Environment Adjustment

A few genetic conditions can be ameliorated by avoiding the environment in which the condition is aggravated. For example:

- People with sickle-cell disease try to avoid excessive oxygen demand situations, or, in some cases, transfusions with normal blood cells provide the person with short-term better oxygen delivery.
- Myopic people wear spectacles.
- Humans that are albino conscientiously avoid sun exposure.

Corrective Surgery

If the gene mutation results in developmental structural effects, sometimes surgery can correct the "defect". It may be that repeated surgeries are necessary. The degree to which surgery is effective is highly variable.

Gene Therapy

The potential for Gene Therapy, an active area of genetic research that inserts the correct gene into an individual's cells will be discussed in our genetic technology section.

Even with these methods, few of our genetic disorders are treatable today, and many are still unidentified.

Another issue in genetic counseling is how one uses the information obtained by genetic screening. For some, the decision to have or not have children may be based on the results of genetic screening. For some, knowledge of a genetic abnormality in a fetus or newborn can guide how one promotes the best life possible, or to make a decision not to continue a pregnancy. In some countries political legislation restricts decision options for families when faced with having children with serious genetic diseases. For some, economic situations affect access to treatments that are often prohibitively expensive.

Genetic research can be limited by legislation rather than by our science knowledge. Some think that genetic screening and allele alterations may be a way for parents to select traits they think their children should have – the so-called designer babies. The concern that research findings can be "abused" is one way that science research can be limited.

No matter how one views the potential uses of our increasing knowledge of genes and inheritance, and the applications of this knowledge, it's critical for each of us to learn as much as possible about this science in order to make educationally informed decisions. *We shall revisit this topic at the end of our discussion on genetic technology.*