GENOMICS IN CANCER

AN ADVOCATE'S GUIDE & TRAINING MANUAL

The successful completion of the Human Genome Project marked a major milestone in scientific history. Yet, from the standpoint of improving human health and quality of life, the real achievements still lie ahead. We have just begun to tap into the potential of genomic research to revolutionize the detection, treatment, and ultimately, prevention of common, complex diseases. Cancer is leading this new era.

For the full benefits of the revolution to be realized, it is essential that advocates gain a basic understanding of the human genome and a working knowledge of how genomics may affect biomedical research and healthcare delivery. Given all the demands that advocates have to contend with on a daily basis, that's a tough challenge. However, this guide and training manual — developed by and for the advocacy community — provides an excellent starting point for advocates who want to learn more about this rapidly emerging field.

In a clear, step-by-step fashion, this illustrated guide sets forth the basic principles of genomics and the applications of genomics to cancer and other diseases. Building upon that scientific foundation, the text moves into scenarios often encountered in real life, ranging from evaluating genetic tests to weighing the ethical implications of genomic research. The concluding chapter contains valuable insights on how advocates can apply what they have learned to make a difference.

And making a difference is really what it's all about. As we in the genomic research community strive to move our findings from the lab to the clinic as swiftly as possible, we need you — the advocates — to be at the table with us in every aspect of research planning and execution. This guide represents a modest but significant step towards strengthening that powerful partnership.

Francis S. Collins, MD, PhD
Bethesda, Maryland

Dear Reader,

Welcome to *Genomics in Cancer: An Advocate's Guide & Training Manual* produced by the Research Advocacy Network (RAN). This manual is intended for cancer patient advocates and others interested in having a basic understanding of the world of genomics.

Because genomics is increasingly being integrated into the research and healthcare systems, it is important for advocates and others to understand how it applies to disease and treatment. Understanding the ethical, social and legal issues affecting patients is critical to effective patient advocacy. This is especially true in the area of cancer, where the role of genomic technologies and techniques to develop targeted therapies, tests and assessments is rapidly expanding.

RAN believes this Genomics Manual offers individuals an opportunity to learn what they need and want to know. Some of the material is basic and will not be new to many readers; however, some of the material is advanced and quite technical. We encourage readers to use the Manual in any way that is useful to them. Some will read from cover to cover, while others will refer to this Manual when they need a specific piece of information.

Research Advocacy Network was founded on the concept that advocates are in a unique position to act as liaisons between individuals with disease and the medical and research communities. Advocates bring the patient perspective and the sense of urgency that can help move research forward. Advocate understanding of genomics can bring this perspective into the healthcare and research arena and help advocates communicate more effectively with physicians and researchers.

We could not have completed this project without help from a number of people. We want to give a special thank you to the professionals and advocates that took their valuable time to review the Manual. (See list on next page.) Mary Ann Chapman was able to bring her considerable talent as a medical writer to the project and ensure the Manual's text was clear and easy to read. This manual was made possible by an educational grant and in-kind contributions from Genomic Health, Inc.

We hope *Genomics in Cancer: An Advocate's Guide & Training Manual* will provide advocates with another tool to use in their work.
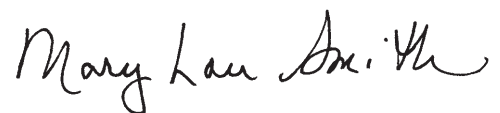
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
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It is the year 2020. We go to our doctor for a routine physical exam. As part of the exam, our doctor draws a tube of blood in order to look at our DNA and our genes to learn about our individual disease risk. The assessment shows that we are at increased risk for breast cancer and high blood pressure. Our doctor recommends that we undergo regular scanning procedures to help detect cancer in the early stages when a cure is likely. Our doctor also recommends that we begin a program of diet and exercise to ward off high blood pressure.

Most experts believe this type of comprehensive gene-based health assessment will be routinely used by physicians in the near future to determine our risk for cancer, diabetes, heart disease, high blood pressure, and many other serious diseases before they actually occur. Today, the risks of a poor outcome from many common diseases can be reduced by changing our behaviors, such as increasing the frequency of screenings or altering our diet and exercise.

Genetic testing can also help physicians decide which treatment is best for us based on our genes. For example, we may have colon cancer that was detected in its early stages. Because of differences in our DNA, we may respond very differently to a given drug, and as a result, we may receive different treatments. Today, a handful of genetic assessments are being developed and some may be widely available within the next few years.

These advances in healthcare are a result of technological developments and an increase in knowledge about human DNA. We've learned that many common diseases involve more than one gene. The subject of this manual is genomics — the study of many, or in some cases all, of our genes, their functions, and their interactions with the environment. Throughout this manual, we've tried to include the most up-to-date information about genomics. However, scientists are learning new things about genomics every day. This new knowledge may challenge old beliefs and lead to changes in our understanding and treatment of disease.

In the first chapter, *Genomics: Studying the Bird Instead of Its Wing*, we further define genomics and consider its impact on health and disease. The second chapter, *What's in a Gene?*, describes the basics of DNA and Chapter 3, *Genes R Us: The Human Genome*, describes the major initiative that has resulted in all of this new knowledge about genomics. The fourth chapter, *DNA Variation: We're Not All Alike*, considers differences in our DNA and the types of genes involved in cancer. The fifth chapter, *Into the Laboratory: Examining Our Genes*, describes the different types of genetic assessments available today and explains some of the general concepts behind testing technologies. Chapter 6, *Evaluating and Regulating Genetic Tests and Assessments* describes how the accuracy of genetic tests is determined and how the tests are regulated. Chapter 7, *Hands Off My Genes!*, addresses some of the ethical, legal, and social implications of genomics research and assessment. Finally, Chapter 8, *How Can Advocates Use This Information?*, considers ways that patient advocates may be able to use information about genomics.

Terms that may be unfamiliar are defined throughout this manual and also in the Glossary after the last chapter. For additional definitions, the *Science* magazine Web site at sciencemag.org features an education section that contains useful glossaries (http://www.sciencemag.org/feature/plus/sfg/education/glossaries.shtml). Important points and summaries are called out in bullet points throughout the text. Links provided in this training manual are current as of February 2006. However, due to

Key Points

Look for these boxed areas throughout for key points found in this manual
for quick and easy reference.

the dynamic nature of the Internet, some of these links will probably move or be deleted over time. Therefore, we have provided the name of the organization that sponsors each Web site and its general Web address, in addition to links to specific Web pages.

Before we get too detailed in our discussion of genomics, it is important to distinguish between genetic assessments that are used for clinical purposes, or for exploring health and disease, and those used for forensic (criminal) or identity purposes. Clinical genetic assessments are used to attempt to answer specific questions related to health or disease such as, "Am I at increased risk for high blood pressure compared with the general population?" or "To which therapy will my lung cancer best respond?" Clinical genetic assessments are not designed to tell anything about our identity, but instead are specific to the health-related question being asked. In contrast, forensic or identity testing is designed to determine whether cells from the blood, mouth, or other tissue that is being examined belong to a certain person in question. Such tests are important in determining whether someone committed or was at the scene of a crime, and in identifying unknown or unrecognizable bodies. Forensic or identity tests are not designed to determine whether the person is at risk for a certain disease or any other health-related information.

Why is it Important for Advocates to Know About Genomics?

As genomics is increasingly integrated into the research and healthcare systems, it is important for us to have a basic understanding of the world of genomics and how it applies to disease, as well as the ethical, social and legal issues that are under discussion today. This is especially true in the area of cancer, where genomic technologies and techniques are permitting the development of targeted therapies and a wide range of tests and assessments.

As advocates, we are in a unique position to act as liaisons between individuals with disease and the medical and research communities. We bring the patient perspective and the sense of urgency that can help move research forward. Our understanding of genomics can help us bring this perspective into the healthcare and research arena and communicate more effectively with physicians and researchers.

NOTES:

GENOMICS: STUDYING THE BIRD INSTEAD OF ITS WING

Within the next decade, scientists will have identified genes that contribute to many different diseases, including diabetes, cancer, Parkinson disease, Alzheimer disease, and high blood pressure. From a quick look at our genome — all of our DNA — physicians will be able to determine which medications may work best for us. In addition, a scan of our genome will reveal our disease predispositions and other things about us that we may or may not want other people to know.

Genetics

A branch of biology that deals with the heredity and variation of organisms. Today, the term genetics is often used to refer to the study of single genes, one at a time, as snapshots.

Genomics

The study of multiple genes, their functions, and interactions.

Genome

All of an organism's DNA.

All of these things are made possible by the study of genomics. But what exactly is genomics?

Genomics is the study of multiple genes working in concert to perform a specific function. In the past, researchers tended to focus on single genes because they did not have the capacity to study many genes and their functions simultaneously. The study of multiple genes at once requires sophisticated computer technology and laboratory methods that have only become available within the past 10 years.

Genomics can be likened to studying the whole bird to understand how it flies instead of just studying its wing. Flying is the result of many different body parts working together, and is more than just a wing flapping up and down. Similarly, the way our body functions is usually the result of sets of genes working together. We can learn a lot by studying a single gene, just as we can learn a lot by studying a bird's wing. However, to understand the whole complex process, we need to study multiple genes working together, just as we need to watch the bird in flight to understand how it can fly.

There are many different areas of study in genomics. Some people who work in the field of comparative genomics study how human genes compare to genes of other living organisms such as mice or bacteria. These comparisons may tell us what genes are important in health and disease and how they function. The comparisons will also help us understand how our body works at a very basic scientific level. Others who work in functional genomics try to figure out what sets of genes actually do in the body. For instance, if one researcher identified a set of 16 genes that was associated with lung cancer, another might ask, "What do these genes do under normal circumstances and how do they increase the risk of cancer?" A third area of study is called pharmacogenomics. Pharmacogenomics focuses on understanding how genes can impact our response to drugs. For example, studying genes related to drug responses may help explain why some of us experience severe side effects from a drug while others may have no reaction at all. These are just a few specialties in the field of genomics.

Key Points About Genomics

- Genomics looks at the functions of genes in health and disease to better understand biology.

- Comparative genomics, functional genomics, and pharmacogenomics are some of the subdivisions within the field of genomics.

Lots and Lots of Terms

A lot of knowledge has been generated in genomics over the past decade. The explosion in the study of genomics has led to a similar explosion in terms used to describe it. Many new sciences that end in the suffix "omics" have emerged, joining the ranks of more familiar fields such as economics. There is even a scientific periodical that is called *OMICS: A Journal of Integrated Biology*.

SOME OF THE NEW "OMICS" SCIENCES	
"Omics" Science	**Description**
Genomics	Study of genes and their function
Oncogenomics	Study of genes in cancer
Comparative genomics	Comparison of all the genes between different living things
Functional genomics	Study of the functions of genes, sets of genes and their interrelationships
Pharmacogenomics	Study of the effect of a person's genes on his or her response to drugs
Epigenomics	Study of control mechanisms (on/off switches) of genes not related to DNA sequence (the basic letter sequence that makes up our genes — described in Chapter 2)
Transcriptomics	Study of gene expression (i.e., genes that are turned on) and the dynamic link between the genome (all of our genes) and the proteome (all of our proteins)
Proteomics	Study of proteins expressed (turned on) in a cell at a given point in time
Metabolomics	Study of metabolites (breakdown products of biochemical reactions) in a cell under given conditions
Cellomics	Study of cell function

The feature that links all of these new fields is that they study many parts working together. Instead of looking at one gene, they look at many. Instead of looking at one protein, they look at many. This is in contrast to earlier studies in genetics and anatomy that took a more isolated approach. The isolated approach has been immensely useful in science and medicine and omics sciences only exist today because of them. For example, we need to understand genetics — the study of single genes and heredity — to understand genomics, the study of how those genes function in relation to one another. However, we now have the tools to look at things in a more comprehensive way, examining the whole instead of the parts. This is leading to a more comprehensive understanding of health and disease than was previously possible.

IMPACT OF GENOMICS ON MEDICINE

Genomics is poised to have an enormous impact in all areas of medicine, and indeed its influence is already evident. This is because nearly all human diseases and disorders have some basis in the genes, and many involve complex sets of genes.

> ### Impact of Genomics on Medicine
>
> • Genomics will significantly impact biomedical research.
>
> • Genomics will significantly impact the delivery of healthcare.
>
> • The influence of genomics in these areas is already being felt today.

Here we consider two areas in which genomics is having a huge impact: medical research and healthcare practice.

GENOMICS AND BIOMEDICAL RESEARCH

1. Understanding disease

Our knowledge of the human genome is making it possible to understand diseases that, in the past, seemed too complex. Many of our most common and deadly diseases, including many types of cancer, high blood pressure, heart disease, and diabetes, are now known to involve more than a single gene. Genomics is making it possible to investigate how sets of genes interact with one another and how they react to our behavior and environment (including diet and exposure to external factors), to cause these diseases.

2. Understanding genetic differences

Advances in genomics are also helping scientists understand the genetic differences between people. This may lead to an understanding of why some people are more susceptible to certain diseases than others. It may also lead to an understanding of why some people respond better than others to different therapies.

3. Development of drugs and biologic therapies

Genomics is having a big impact on the development of drugs and biologic therapies. A drug can be defined as a substance used as a medication. Biologic therapies are medications made by living organisms or living cells. Under these definitions, biological therapies are actually a subclass of drugs. They are often called out as a separate category and are regulated by different governmental agencies. Biologic therapies include such things as antibodies (proteins that attach to specific biochemical targets in our bodies) and vaccines (proteins that stimulate our bodies' immune systems to build antibodies against specific biochemical targets). Although most of us are familiar with vaccines as treatments we receive during childhood to prevent a host of diseases, vaccines are now being actively studied to treat diseases such as cancer. For selective cancers that are caused by viruses, such as cervical cancer (caused by human

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Gene Therapy

Therapies that fix faulty genes directly.

Gene-based Therapy

Therapies that fix problems caused by faulty genes, as opposed to fixing the genes themselves.

papillomavirus), vaccines that might prevent the disease are being studied. Vaccines are also being studied as treatments for some types of cancers not caused by viruses.

Knowing about the genes that cause a disease will permit the development of drugs and biologic therapies that more precisely target the mechanism causing or contributing to it. The ideal treatment for any disease is one that fixes the problem without creating new ones. However, many of our current treatments not only fail to rectify the problem, but also cause unwanted side effects.

For instance, many of our drug therapies treat symptoms instead of correcting the underlying problem. We may take sinus medications to help us breathe better when we have a cold. The sinus medication does not actually help our body get rid of the cold virus. Instead, it treats our stuffy noses — a symptom caused by the virus. We treat symptoms instead of the underlying issue because we don't know exactly what the problem is or we don't have the technology to fix it. As a result, the underlying disease remains until our body manages it (as in the case of colds) or the disease progresses (as in the case of Alzheimer and Parkinson diseases). By understanding the genes that contribute to the development of various diseases and conditions, we can begin targeting treatments at the source of the underlying problem.

Another drawback to many current medicines is that they are not targeted to a specific type of cell or process. Cancer is often treated with chemotherapies designed to kill all cells that grow and multiply rapidly. Therefore, we end up killing some of the healthy cells that happen to grow and multiply rapidly in our body, as well as the cancer cells. In killing these good but fast-growing cells, we deprive our bodies of some important functions, which causes many of the side effects of cancer treatment. For example, chemotherapy often kills healthy bone marrow cells, which can cause low red and white blood cell counts. Low red blood cell counts result in fatigue and low white blood cell counts can result in infections.

Genomics is helping us understand the basis for many diseases so that drugs and biologic therapies can be developed to target the disease more specifically. So, rather than taking a drug that kills both normal and cancerous cells, researchers can design a drug or a biologic therapy that more specifically targets cancerous cells. This will result in more effective treatments that have fewer side effects.

4. Gene therapies

Eventually, we may also be able to fix or replace faulty genes with ones that function properly. This approach is usually referred to as gene therapy. Much research is currently in progress in this area. However, as of early 2006, the United States government has not approved any gene therapies for use as medical treatments. There are many hurdles to this type of therapy and its successful use may be a long way off.

Instead of fixing altered genes, it is often easier to correct the chemical problems that they cause. For instance, lung cancer may be caused by dysfunction in a set of genes that rids the body of harmful chemicals such as those in cigarette smoke. If we know that someone has the faulty gene set, we might give him or her medicines to break down the harmful chemicals instead of trying to fix the genes. This indirect approach to fixing the problems caused by faulty genes is referred to as gene-based therapy.

Targeted Therapy

Therapy that is directed at a specific variable; for instance, targeted cancer therapy may be directed at a specific protein manufactured by an overactive gene, whereas general cancer therapy may be directed at all rapidly dividing cells.
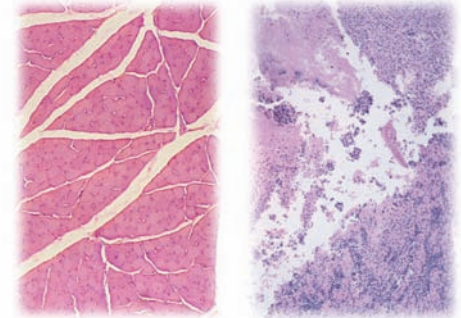
Impact of Genomics on Medical Research

- Genomics will help us understand the science behind many common diseases.

- Genomics will provide the basis for developing new treatments.

GENOMICS AND HEALTHCARE PRACTICE

Genomics is affecting healthcare practice in at least five areas: diagnosis, prognosis, risk reduction, treatment, and surveillance of disease. The influence of genomics in healthcare practice is most readily apparent in genetic assessments designed to detect multiple genes associated with a particular disease.

1. Diagnosis of disease

Genomic technologies are already being used to help diagnose disease and subtypes of disease, and more tests are becoming available every year. Today, certain subtypes of cancers, such as colon and skin, can be diagnosed based on the results of genetic testing. For instance, genetic abnormalities can lead to a type of colon cancer that is called hereditary nonpolyposis colorectal cancer (HNPCC). About 90% of the patients with HNPCC have one

Normal tissue cells   *Skin cancer cells*

of the two most common genetic abnormalities known to cause this syndrome. Individuals with these abnormalities have an 80% lifetime risk for developing colon cancer, and their children are also at significantly higher risk. However, fewer than 3% of colon cancer diagnoses are HNPCC-related. Advances in genomic technologies now allow us to distinguish HNPCC from other forms of colon cancer. Many believe genetic tests will eventually become a primary method of diagnosing cancer and other selected diseases.

2. Prognosis and treatment of disease

Genetic assessments can also be useful in the prognosis of disease, or the expected course of the disease independent of any treatment. For example, doctors can already determine the aggressiveness of prostate cancer based on genetic testing. Additionally, high cholesterol may lead to more or less severe health problems, depending on our genomes. As researchers continue to study large groups of people with slight differences in their genomes, they will be able to relate those differences to disease outcomes. Genetic assessments will then be developed to help predict an individual's course of disease with treatment using a specific drug based on their pattern of genes. Consider two imaginary individuals — Bob and Leah — who both have lung cancer. Genetic assessments may indicate that Bob's disease has a better prognosis (e.g., it will be less aggressive than Leah's). As a result, Bob's disease may be treated differently from Leah's disease. Genetic assessments may predict that Bob's disease will respond to a hypothetical Drug X, which will likely put his cancer in remission for many years. Based on her genetic assessment, Leah is predicted to respond to a different drug, Drug Y.

3. Reducing the risk of disease

The current practice of medicine is mostly reactive: We only go to a doctor when something is wrong and we are in need of treatment. Genomics holds the potential to shift medicine to a more proactive approach and actually prevent us from becoming sick or reduce the risk of becoming sick. Genetic testing can determine whether we are at increased risk of certain diseases before we become ill. By knowing our risks in advance, we can change certain behaviors to help reduce that risk, such as increased

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Prognosis

The expected course of disease, independent of any treatment.

Prediction

The expected course of disease treated with a specific drug.

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cancer screening, preventive drug or other therapy, and lifestyle modifications (e.g., quitting smoking, reducing sun exposure, eating a healthier diet).

4. Treatment of disease

It has long been known that some of us respond better than others to certain medications. The field of study that examines how responses to drugs are influenced by genomics is called pharmacogenomics. In the future, individualized drug therapy based on genomics will become routine.

Genomics will influence treatment of disease by identifying disease subtypes. A specific subtype may contain a feature that can be attacked by a certain drug. However, our neighbor with the same disease may not share the same feature and therefore may not respond to the same treatment. For example, although a number of us may be diagnosed with breast cancer, we won't all have the same type of breast cancer. Some individuals with breast cancer have too many copies of a gene called HER2 (each person normally has two copies). If we have too many copies of HER2, we are much more likely to respond to a drug called trastuzumab (Herceptin®) than are those without the abnormality. The reason for this is that trastuzumab actually targets the extra growth factor receptors that result from the extra copies of HER2. So, before recommending us for trastuzumab treatment, doctors must be sure that we have the abnormality that it is designed to attack. The abnormality can only be detected by genetic assessment.

Another reason why some of us respond better than others to certain medications is that we each process drugs differently (our pharmacogenomics are different). We now know that, in some cases, this is because of our genes. Genetic assessments will help determine which drug and dose we should take based on our ability to break down the drug, which will likely reduce our chances of experiencing serious side effects. For example, we may have a limited ability to break down ibuprofen and would therefore need a lower dosage than most other people to avoid unwanted side effects.

5. Disease surveillance

Genetic assessments may also be useful in the surveillance of diseases such as cancer. For instance, if we possess a gene or genes that put us at increased risk for colorectal cancer, we can schedule more frequent colonoscopies (examinations of the inside of the colon using a flexible fiber optic tube). If polyps, which can develop into cancers, are seen, they can be removed, preventing cancer from developing. If a malignancy develops between colonoscopies, cancers found and removed early are highly curable.

Additionally, diseases can change over time and in response to different treatments. Following a successful treatment regimen in the future, physicians may be able to determine whether cancer-related genes have been turned on or off, which could indicate imminent recurrence of cancer or continued remission. The physician could then decide whether we need additional treatments.

> ## Pharmacogenomics
>
> The field of study that examines how responses to drugs are influenced by the genes or the genome.

Empiric Therapy

This means "try the treatment to see if it works." In practice, this means offering everyone the best available therapy, or the one that benefits the most people, and waiting to see whether it works in each specific individual.

Genomics-based Therapy

Each person's genes are used to guide treatment.

Individualized Medicine

Tailoring treatment to the individual. This applies to the use of genetic tests to classify a person's disease and predict response to treatment. The best treatment for the individual is then selected.

EMPIRIC VS. GENOMICS-BASED THERAPY

Experts agree that we are at a transition point today between empiric therapy and genomics-based therapy. Empiric therapy is a one-size-fits-all approach to treatment that is usually based on scientific evidence that identifies the best therapy for a group of individuals. It is essentially trying the treatment without being sure of its effects. Until recently, this was the best approach to medicine — it provided the best outcomes for the most patients. However, some people have variations of a disease that will not respond well to the most popular drug but will respond better to a lesser-known drug. Still other people have genes that lead them to metabolize or break down drugs differently and they will also fail to benefit from the most popular treatment, or may experience unacceptable side effects.

However, thanks to advances in genomics, treatment is becoming more individualized. Genomics-based therapy is an approach in which each person's genes are assessed prior to treatment to determine which treatment will produce the greatest benefit with the fewest risks for the individual. Individualized medicine is one of the catch-phrases of the genomics era, and is one of the main benefits of genomics that we are likely to see in our lifetimes.

The next important step in moving toward genomic medicine is incorporating genomics into clinical trial designs, which is proceeding rapidly.

Empiric Therapy

1 2 3 4 5

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Drug A

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If Drug A Doesn't Work

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Drug B

Genomics-based Therapy

1 2 3 4 5

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Genetic Testing

1 2 3 4 5

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Drug A **Drug B** **Drug C**
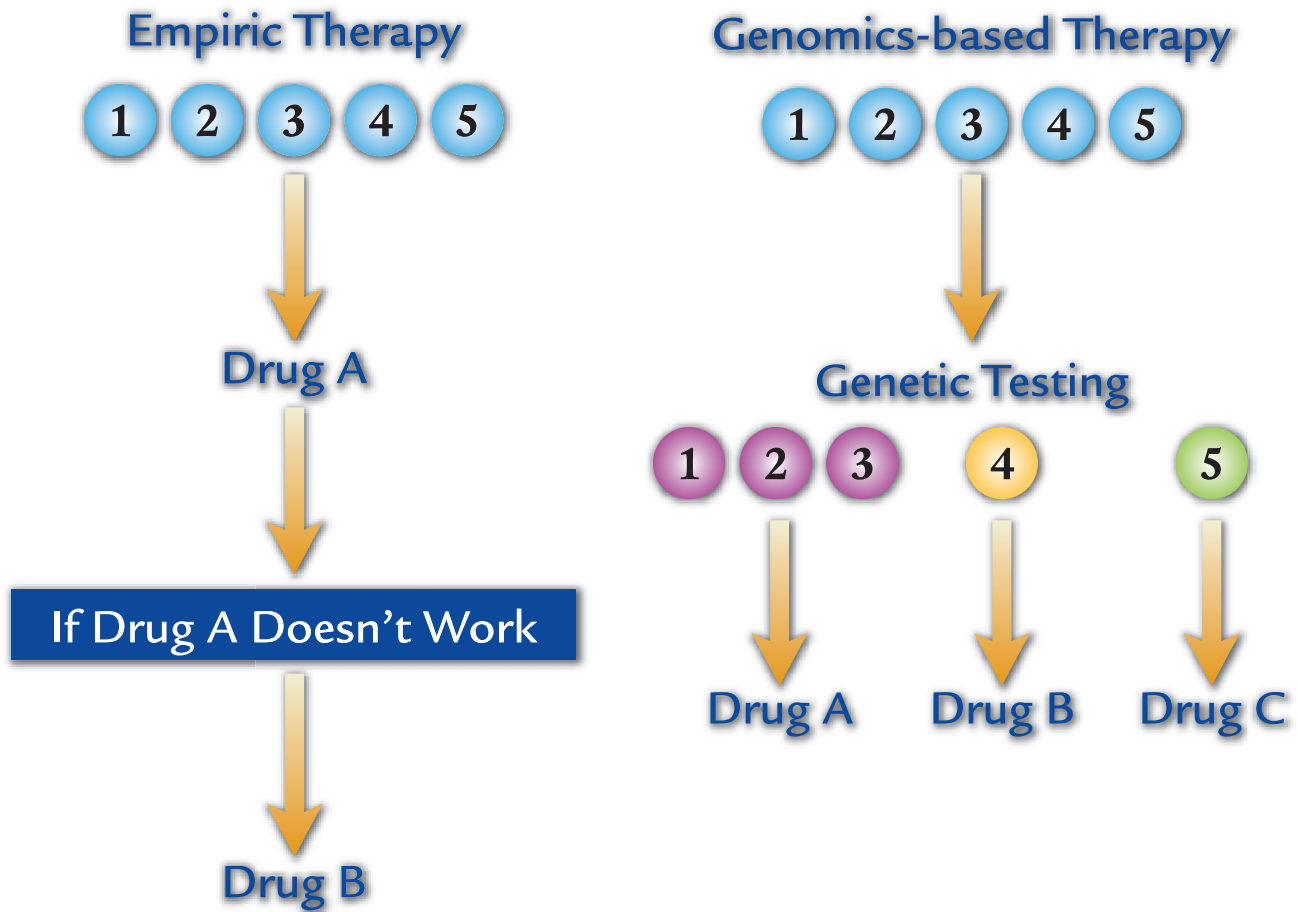
In empiric therapy, each individual (patients #1 through #5) receives the treatment that works the best for the most patients. This is drug A. If drug A doesn't work for a particular individual, drug B is tried.

In genomics-based therapy, each individual (patients #1 through #5) undergoes genetic assessment before treatment. Test results for patients #1, #2, and #3 show that they are likely to respond to drug A, and this is the treatment they receive. However, patient #4 is deemed unlikely to respond to drug A based on her genes, but is likely to respond to drug B. She is given drug B. The genetic results for patient #5 indicate that she is unlikely to respond to either drug A or B so she is given drug C.

This graphic depicts the ideal situation for both empiric and genomics-based therapies. Because genomics-based therapy is relatively new, there are several obstacles to this paradigm. Genetic testing is not always available for a given disease; there are few conditions for which a patient can be matched to a specific drug; and there may be only one or two drugs to treat a given disease.

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NOTES:
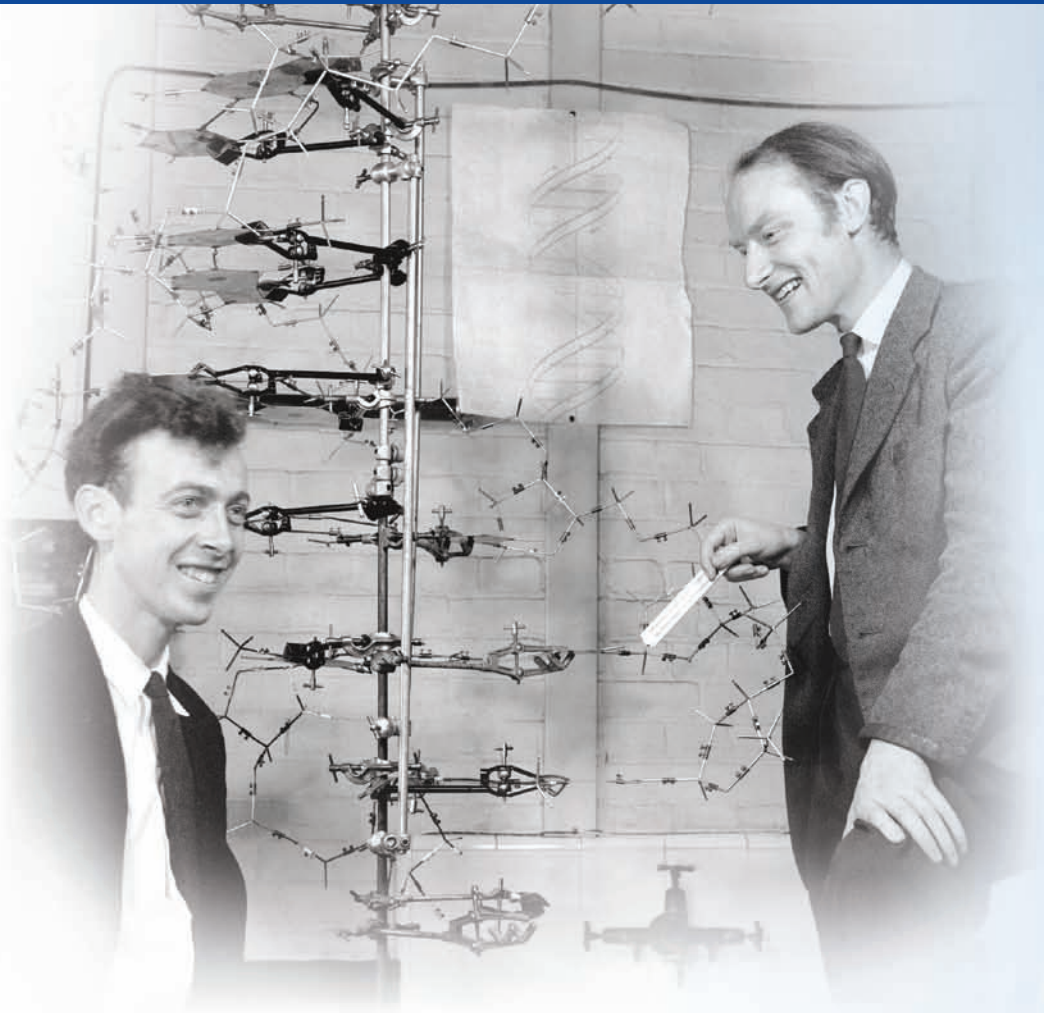
One day in 1953, a scientist named Francis Crick walked
into a British pub with his colleague James Watson and
announced they had found the secret of life. This brash
statement turned out to be true in a technical sense:
Watson and Crick had discovered the structure of DNA,
the material that makes up genes and allows our genetic
information to be transmitted from one generation to the
next. This discovery was important because knowing
the structure allowed scientists to figure out how DNA
could make exact copies of itself and help to form every
single cell in our bodies.

ALL ABOUT DNA

DNA actually stands for deoxyribonucleic acid, a chemical that makes up our genes. Despite its huge job of spelling out all of our genetic instructions, DNA is composed of only four chemical units. These chemical units are known as nucleotide bases called adenine (A), thymine (T), cytosine (C), and guanine (G). The bases are generally referred to as just A, T, C, and G. The exact sequence of chemical letters in our DNA is important and makes up the hereditary information that we transmit to our children. We'll refer to the molecules A, T, C, and G in this training guide as bases.

SO, WHERE IS OUR DNA LOCATED?

DNA can be found in every type of cell in our bodies, except for some red blood cells. When we look at cells under a microscope, we can sometimes see the DNA as long strands called chromosomes.

DNA

Deoxyribonucleic acid, the material that makes up genes. DNA is a series of chemical letters (bases) that spells out the instructions for constructing our bodies and making them work.

Nucleotide Bases

The 4 chemicals in DNA that spell out the instructions for building and running our bodies.
The 4 chemicals are Adenine (A), Thymine (T), Cytosine (C), and Guanine (G).

CHROMOSOMES

Cell

Nucleus

Chromosomes

On the left is an illustration of a cell. The circle in the center of the cell is its nucleus. The nucleus contains chromosomes, the X-like structures shown on the right. DNA strands are found in the chromosomes.

THE DOUBLE HELIX

Inside our cells, DNA exists in two connecting strands. The two strands of DNA twist around into a form called a double helix, which looks like a twisted ladder.

Each strand of the double helix is made up of strings of the bases A, T, C, and G, which form the rungs of the ladder. These rungs are held together by a backbone made up of sugar and phosphate groups (chemical groups containing the element phosphorus). Each of the rungs on the ladder contains a base from one strand paired with a base from the other strand. But nature is very particular about how these strands are paired together. Each of the bases in DNA pairs with only one other base: A only pairs with T and G only pairs with C. This means that if one strand is AAA, the other strand must be TTT in order for them to fit together. The pairs of nucleotide bases on DNA strands are called nucleotide base pairs or base pairs for short.

NUCLEOTIDE BASE PAIRS

A ▭▭ T

G ▭▭ C
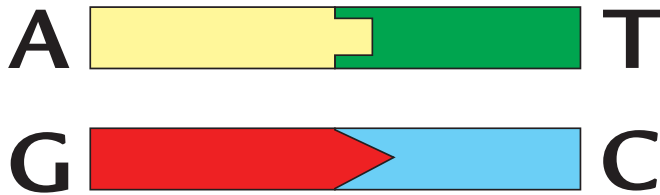
Along the double strands of our DNA, each base pairs with only one other base. If one strand has an A, the other has a T. If one has a G, the other has a C.

Nucleotide Base Pairs

The exclusive pairing of A with T and G with C along the two strands of our DNA. Typically referred to as base pairs.

GENES

Our DNA strands are very long and contain approximately 3 billion base pairs, or 6 billion individual nucleotides. Only parts or sections of our DNA are actually functional or active. Historically, genes have been known as the functional units of DNA, although we now know other parts of DNA may be functional as well. In general, a gene can be defined as a section of the DNA on a chromosome that carries a particular set of instructions to produce a specific chemical product, usually a protein. Each gene is made up of a different order or sequence of the DNA nucleotide bases. According to current estimates, there are about 25,000 genes in a human DNA sequence. However, the exact number of genes has not been unequivocally determined and estimates range from 23,000 to 30,000.

Certain parts of genes are also sometimes called coding regions because they provide the instructions for making proteins. This is where the term *genetic code* comes from. In contrast, non-coding regions are nucleotide base sequences that do not spell out the code for a protein or other specific chemical. These sequences are sometimes involved in regulating genes. Coding regions and non-coding regions are interspersed throughout our DNA.

Today, many people define genes and coding regions the same way. However, this definition of a gene may be too simple and will probably change in the future as scientists learn more. For instance, today scientists know that some stretches of DNA are actually functional but do not produce a protein. These areas are often called DNA non-coding functional elements.

The majority of our DNA — about 97% — is made up of non-coding regions. Most of the non-coding regions on our DNA have yet to be fully explored and understood. Historically, many people have referred to these regions as "junk DNA." However, the term "junk DNA" is somewhat misleading. Some sections of DNA that were once called junk are now known to control and regulate genes, proteins, and our biology. Some of these are called non-coding functional elements. Other non-coding regions on DNA are called promoters, control elements, and regulatory control elements. For our purposes, it is not necessary to memorize the definitions of all of these regions. Instead, it is important to know that some of the non-coding regions play significant roles in our biology. As scientists learn more about genomics, they may find functions for many or most of the non-coding regions. After all, some people argue, why would nature go to all of the trouble of copying the extra DNA into every cell in our bodies if it were useless?

Gene

A section of the DNA on a chromosome. A gene carries a particular set of instructions to produce a specific chemical product, usually a protein.

Coding Regions

Portions of DNA that spell out the instructions for making a protein or other specific chemical.

Non-coding Regions

Portions of DNA that do not spell out the instructions for making a protein or other specific chemical.

Amount of DNA that Comprises Genes

■ Non-coding regions
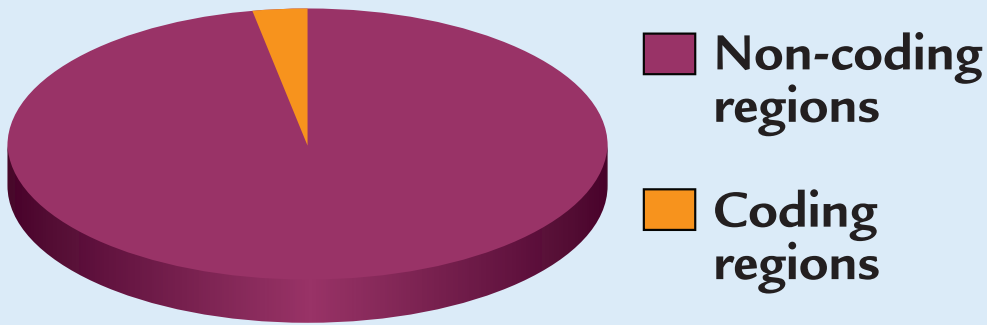
■ Coding regions

About 97% of our DNA does not code for a protein or a specific chemical. Only 3% of our DNA makes up genes. Genes are scattered all along our chromosomes. Some non-coding regions of our DNA are known as promoters, control elements, non-coding functional elements, and regulatory control elements. The vast majority of the non-coding DNA regions have yet to be fully explored and understood. The role of non-coding regions of DNA in cancer is also not understood.

Non-coding Functional Regions

Portions of DNA that do not spell out the instructions for making a protein or other specific chemical but that perform other functions such as turning genes on and off.

Example of the Organization of DNA

GCGCGCGCGCGCGCGCGCGCGCATGCTGCACGACGACACGACGA...
CGCGCGCGCGCGCGCGCGCGCGCGTACGACGTGCTGCTGTGCTGCT...

This long series of letters represents a section of DNA. In this example, the base pairs shown in the box to the left of the arrow are non-gene sequences or non-coding regions. The base pairs in the box to the right of the arrow represent the gene. Chromosomes can contain long stretches of non-coding bases, interspersed with a few genes here and there. At first, the bases in the DNA sequence may seem random. It may seem hard to determine which section of the sequence contains the gene. However, there are often distinct signals in the sequence that indicate where a gene is located. Some of these signals are located in the non-coding regions and some are in the coding regions. One of these signals is the letter sequence ATG, as shown at the beginning of the second box above on the lower line. In almost all human and animal genes, the letter sequence ATG occurs at the beginning of the coding region.

HOW THE BODY USES GENETIC INFORMATION

The sequence of letters in our DNA serves as the recipe for making specific chemical products called proteins. Proteins are known as the workhorses of the cell, doing the work needed to keep the cell healthy. Proteins also help make up the structure of our cells and tissues. Some of the jobs that proteins do are:

- Carry out the chemical reactions of life (as enzymes)
- Allow muscles to contract and expand
- Create the skeletons of cells
- Form key parts of cell membranes
- Make up hair, ligaments and bone
- Function as hormones to send messages between different parts of our body
- Regulate cell growth (as growth factors)
- Transport oxygen to our lungs and blood
- Form antibodies to carry out the work of our immune system

In fact, the structure and function of the many different types of cells in our body are determined by the unique proteins that are present. For instance, a brain cell has different proteins than a muscle cell or heart cell because they perform different functions.

In order to make all of these different proteins correctly, the sequence of the base pairs must be in a precise order. If the base pairs are out of order, the protein may not be made properly and/or may be unable to perform its normal activities in the body.

To understand how the DNA code works, it may be helpful to think about how we make words from letters of the alphabet. When spelling a word, the order of the letters is critical. Ordering the letters a different way will result in a different word. For instance, the letters O, R, D, and O can be used to spell the word ODOR or DOOR. Dropping out a letter O and flipping the order of the letters will result in the word ROD. All of these words have very different meanings. The letters RDOO or DRO do not spell words in the English language.

The same is true for DNA. The bases arranged in one way code for a certain protein. The bases arranged in a different way may code for a different protein. Dropping out bases, adding bases, or rearranging bases may change the gene so it does not do what it is supposed to do. This will be described in more detail later.

Key Points About Genes and Proteins

- Genes are the most common functional units of DNA.
- Genes are recipes for making chemical products, usually proteins.
- Proteins enable all of the structures and functions in the body.

Key Points About Non-coding Regions of DNA

- Most of our DNA is made up of non-coding regions, or portions of DNA that do not spell out a code for proteins or other chemical products.

- These regions may have important functions.
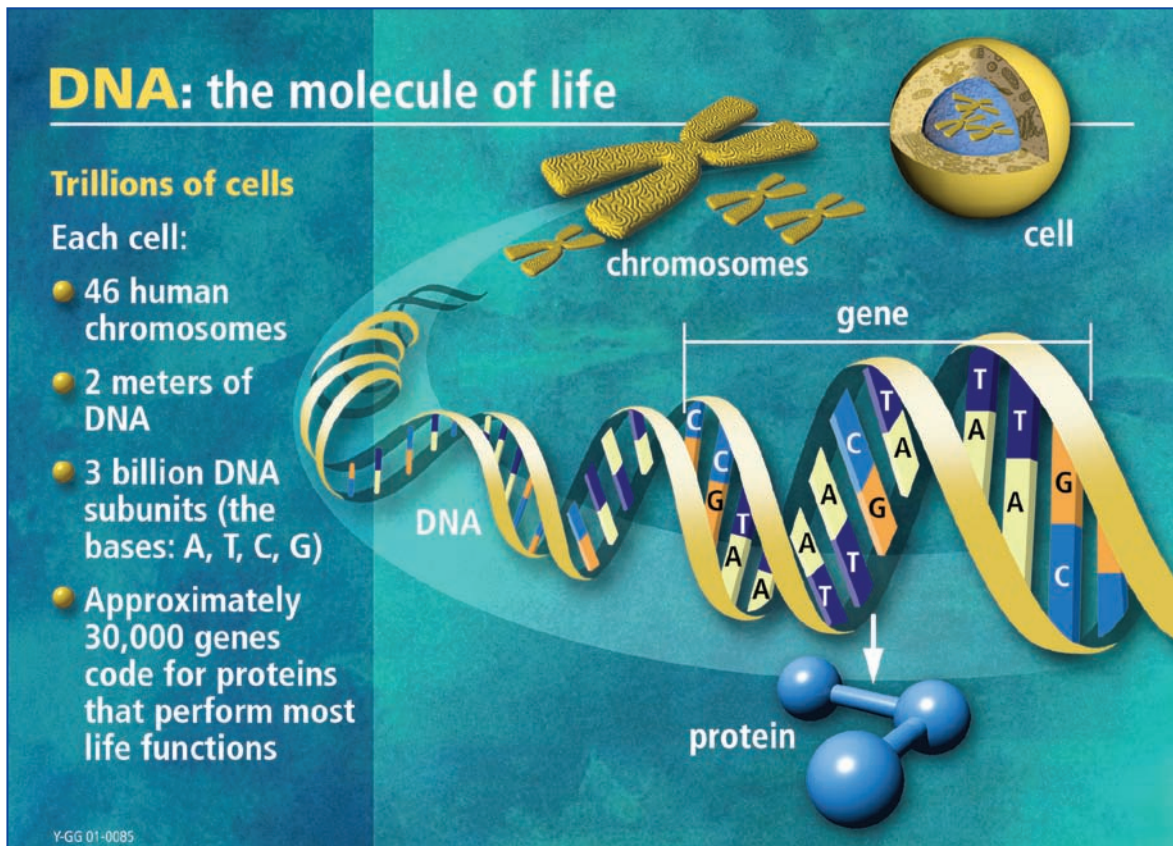
Image courtesy of U.S. Department of Energy Human Genome Program, http://www.ornl.gov/hgmis.

HOW DO WE GET FROM DNA TO PROTEIN?

RNA is related to DNA and is essential to the production of proteins. RNA stands for ribonucleic acid. The structure of RNA is similar to that of DNA but it is single-stranded instead of double-stranded. Like DNA, RNA is made up of 4 nucleotide bases. Three of these bases are the same as those in DNA: adenine (A), cytosine (C), and guanine (G). However, the fourth nucleotide base in RNA is uracil (U) instead of thymine (T). Synthesis of proteins from DNA occurs in 2 steps called transcription and translation. In the first step — transcription — a small portion of DNA unzips from its double-stranded structure to form two single strands. The DNA from one of the strands is used as a template to synthesize messenger RNA (mRNA), which is essentially a complementary copy of the DNA. The mRNA then moves to a part of the cell called the ribosome where its information is translated by another type of RNA called transfer RNA (tRNA). As tRNAs pair with the mRNA, they bring specific amino acids together to form a protein. When making a protein, the tRNA reads or decodes the mRNA in groups of 3 nucleotide bases. Each triplet of nucleotide bases codes for a specific amino acid. Amino acids are the building blocks of protein.

RNA

Ribonucleic acid; a chemical related to DNA. It helps transfer information from DNA into proteins.

Messenger RNA (mRNA)

The form of RNA that is a complementary copy of the genetic information encoded in the DNA.

Transfer RNA (tRNA)

The form of RNA that helps translate the DNA into proteins. tRNA brings amino acids together in the correct order to form proteins. Amino acids are the building blocks of proteins.

HOW DO WE GET FROM DNA TO PROTEIN?
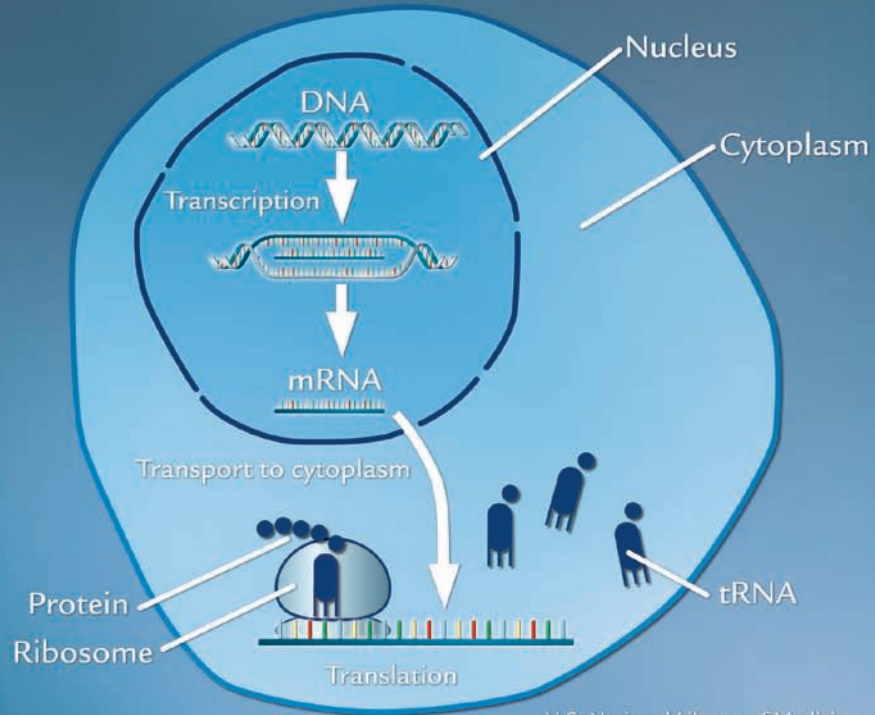
U.S. National Library of Medicine
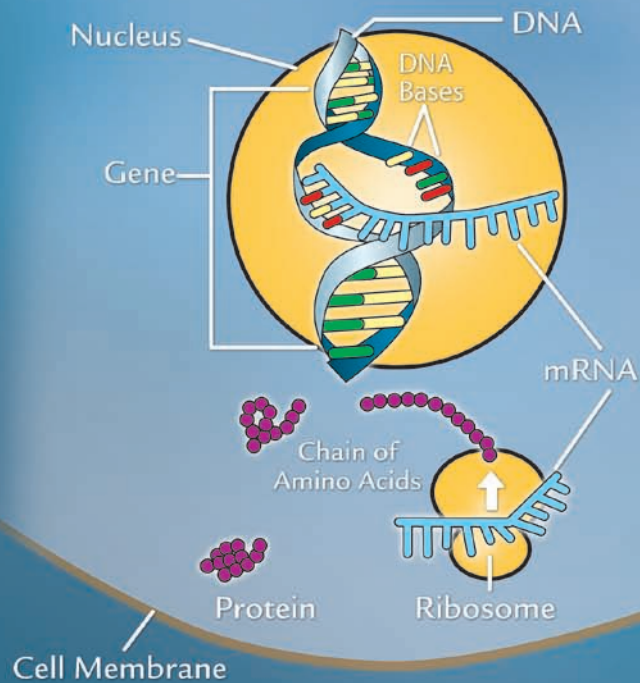
http://www.ghr.nlm.nih.gov/info=basics/show/making_protein
http://rex.nci.nih.gov/behindthenews/ugt/genetestingframe.htm

Putting it All Together
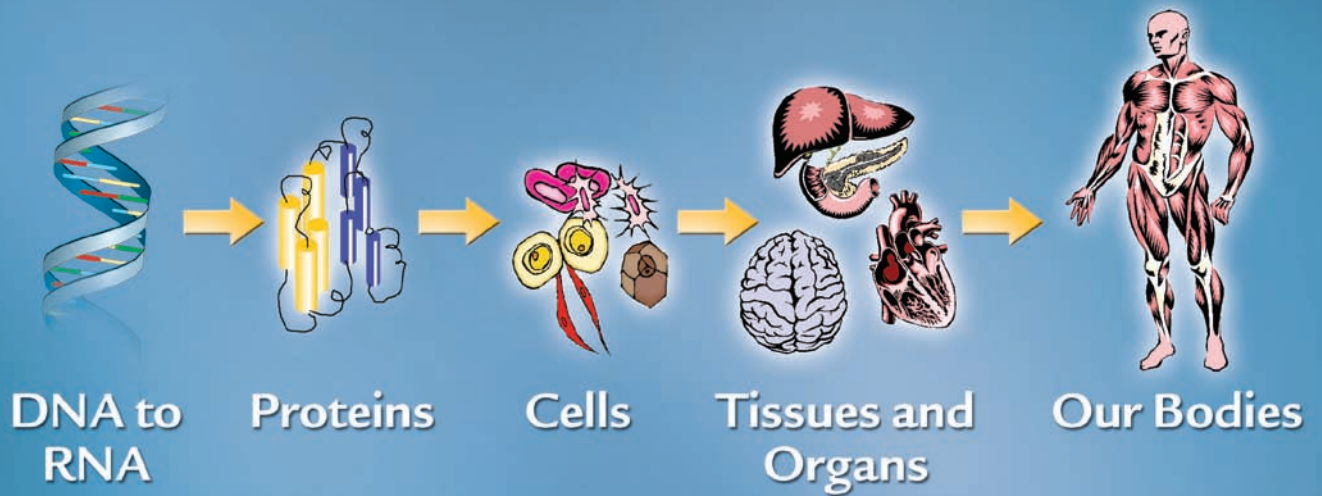
The DNA in our genes provides instructions for making proteins. These instructions are copied to RNA, which helps make proteins. Proteins do many things in our bodies. Proteins help form the structure of our cells and help the different cell types carry out their jobs. Proteins also communicate between cells. Different cell types such as blood cells, skin cells, liver cells, bone cells, brain cells, and muscle cells make up the tissues and organs such as our blood, skin, liver, bones, brains, and hearts. These, in turn, make up our bodies.

For more information on how RNA helps cells make proteins, you may want to visit the Human Genome Project Web site at: www.genome.gov. A page that describes how proteins are made can be found at: http://www.genome.gov/Pages/Education/Kit/main.cfm?pageid=204.

The National Cancer Institute sponsors a useful Web site called the Cancer Genome Anatomy Project or CGAP (http://www.cgap.nci.nih.gov). This Web site contains educational information, including 6 slide sets on basic gene science, and genes and cancer (specific link: http://cgap.nci.nih.gov/Info/concept).

Another resource on the Web is the site sponsored by the Michigan Center for Genomics and Public Health (http://www.sph.umich.edu/genomics/). This site contains information about a variety of genomic topics.

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NOTES:

In 1937, a baby boy who had no eyes was born into a family that had no prior history of such a condition. As the child grew older, other physical abnormalities became evident. The child showed severely delayed mental and physical development. Several decades passed without the condition appearing in any other children. But in the 1950s and 1960s, the disease struck the family again. By then, many individuals in the extended family were terrified of having children. It was impossible to predict where and when the disease would strike next.

Genetic Disease

Pathological condition caused by alterations in genes or chromosomes. Approximately 7,000 hereditary genetic diseases have been identified to date.

For 30 years, researchers tried to solve this genetic mystery. The disease affected boys most severely, which led researchers to zero in on a specific chromosome. Females have two X chromosomes, whereas males have one X chromosome and one Y chromosome. If the genetic abnormalities were located on the X chromosomes, girls might be protected because they had two copies of each gene. However, because boys only have one copy, a genetic alteration on the X chromosome resulted in more severe symptoms.

Even though researchers were able to locate the specific chromosome in this case, they did not know which gene was affected. Without significant knowledge of the human genome, researchers kept running into dead ends with their search. It wasn't until information about the entire human genome was nearly complete that researchers were able to identify the cause. In fact, it only took Dr. Leslie Biesecker and his colleagues at the National Institutes of Health a few months to determine the genetic abnormality given the wealth of information about the human genome that was then available.

As a result of Dr. Biesecker's work, a genetic test has been developed that this and other families can use to determine whether they carry the genetic abnormalities that cause this syndrome, which is called Lenz microphthalmia. Some members of affected families opt to take this test before deciding whether to have children. Unfortunately, there is no treatment or intervention to prevent harm to the infants as a result of this altered gene and protein product. But identifying the genetic abnormality is an important first step toward developing a gene therapy or a gene-based treatment. For this family at least, the mystery was solved and the genetic test can be used to help the family make difficult decisions in the future.

A full description of this story is available on the Human Genome Project Web site (**www.genome.gov**) at this specific link: **http://www.genome.gov/14514560**.

The disease described here is a hereditary genetic disease. Genetic diseases are caused by alterations in genes or chromosomes. Approximately 7,000 hereditary genetic diseases have been identified to date. Most of these hereditary genetic diseases are rare, meaning they affect fewer than 200,000 people in the U.S. at any given time.

As the story of Lenz microphthalmia syndrome shows, connecting a gene with a hereditary or non-hereditary disease used to be a very slow and frustrating process. Today, the same searches proceed much more rapidly and accurately because much more information is available on the human genome. Despite these advances, however, researchers have thoroughly studied only a small fraction of our genes. In later chapters, we will see how many of our most common diseases, such as cancer, can result from changes in more than one gene or genes.

What is the Human Genome?

The human genome refers to the complete DNA sequence present in humans. Nearly every cell in our bodies contains a copy of the entire human genome.

The human genome is made up of sequences of nucleotide bases (A, T, C, and G) in their correct order. Because each base of the DNA pairs with only one other base (G only pairs with C and A only pairs with T), if you know the sequence of one strand, you automatically know the other. This is why gene sequences are often reported as single lines of letters representing the various bases instead of double lines of bases as they actually occur in DNA.

Aha! It's ATTCGCTTCCGG, just as I thought.

Human Genome

All of the DNA present in humans.

There are about 23,000 classically defined genes in the human genome, which is fewer than many scientists originally expected. Many people thought that because humans are so complex compared with other creatures, we would have at least 120,000 genes in our genome.

An entire single human genome (three billion base pairs) can be stored on a CD like the one held by Senator Tom Harkin in this photo. Nat. Gen. Res. Inst. Photo Credit – Press Photo

THE HUMAN GENOME PROJECT (HGP)

The nucleotide bases of our genes are now available to us all, thanks to a grand-scale international scientific effort known as the Human Genome Project (HGP). The purpose of the HGP was to determine the nucleotide base sequence of the human genome: the sequence of As, Ts, Gs, and Cs that provides the blueprint for life. This major feat was begun in 1990 and completed in 2003. As part of this project, most genes have also been "mapped" or localized to their specific places on the chromosomes.

The HGP is one of science's big success stories. Funded largely by our tax dollars, this unprecedented effort involved many scientists around the world working for several years on a single project. After the sequence of the human genome was complete, other sequencing projects followed to learn about the genomes of "model" organisms that scientists have studied routinely in the laboratory. Model organisms include the mouse, rat, roundworm, plants, and certain bacteria and yeast. By studying the genomes of these model organisms, scientists can compare the workings of our biological systems to theirs. Scientists can also introduce changes into the DNA of these organisms to study the functions of altered genes, as well as test possible genetic treatments. The HGP also developed genomics technologies and transferred that innovation to private companies for commercial use.

Approximately 5% of the annual budget for the HGP National Human Genome Research Institute (the lead agency for the HGP) is devoted to the study of the ethical, legal, and social implications of knowing the location and sequence of all of our genes. This work included the study of insurance concerns and the reaction of families to genetic diagnoses. Researchers and politicians hoped that some of the social and humanistic dilemmas posed by our knowledge about the human genome could be anticipated and addressed even before the final information became available. Of course, this funding did not solve the ethical, legal, and social issues associated with human genome research and these issues are still actively pondered, debated, and legislated today. Some of these controversies are discussed in a later chapter on ethical, legal, and social issues.

An important, though initially controversial, feature of the HGP was the free communication and accessibility of the information produced by the project. Open communication of results is a basic principle of science, and those involved with the HGP were determined to incorporate this as a key feature of the project. As a result, all the information about the sequence of the human genome and the mapping of genes to their specific locations on chromosomes was disclosed immediately and made freely available to the public and private sectors.

The Human Genome Project

A large-scale scientific effort designed to determine the sequence of the human genome: the sequence of As, Ts, Gs, and Cs contained within our DNA. Sequencing and mapping of the entire human genome was completed in 2003.

Genetic Mapping

Localizing a gene to its specific place on a chromosome.

- The DNA sequence of the human genome was completed in April 2003.

- It cost $3 billion to identify all of the nucleotide bases of the human genome.

- To date, millions of single-letter alterations in human DNA have been identified.

Some have asked whose DNA was sequenced for the HGP. It turns out that many people volunteered to have their DNA sequenced for this project and actually donated blood to be analyzed for the project. However, only nine of the samples were used. Even the volunteers themselves do not know whether their DNA was actually among those sequenced. In other words, the human genome project sequenced a composite or representative human genome.

Overall, the HGP has been an amazing success, meeting and exceeding its goals well ahead of schedule. The enormous amount of information gleaned is widely and freely available to citizens, scientists, and private companies around the world. The sequencing and mapping of the human genome is now making it possible to study the functions of genes, proteins, and interactions on a scale never imagined. The implications and possibilities of this new information are still being uncovered and will have a profound impact on healthcare and all biological sciences in the foreseeable future.

Excellent information about the HGP can be found on the National Human Genome Research Institute's Web site at: www. genome.gov. A specific link describing the HGP can be found at: http://www.ornl.gov/sci/techresources/Human_Genome/project/about.shtml.

Another excellent resource about the HGP is the Department of Energy's Web site at: www.doegenomes.org. A specific link that may be helpful is: http://www.ornl.gov/sci/techresources/Human_Genome/publicat/primer2001/index.shtml.

Key Points About the HGP

- As a result of the HGP, we know the nucleotide base sequence of the entire human genome.

- The sequence of nucleotide bases in the human genome is available to everyone.

- The HGP also involved study of the ethical, legal, and social issues brought about by knowing the human genome.

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NOTES:

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DNA VARIATION: WE'RE NOT ALL ALIKE

SIMILARITY IN DNA

99.9% identical DNA between any pair of humans

About 98% identical DNA between humans and chimpanzees

Any discussion about differences in DNA among humans must be considered against the backdrop of our similarity: every person is 99.9% genetically identical to every other person on earth. In other words, only 1 DNA base out of every 1,000 is different for any two people. Depending on how we look at these numbers, we may be ovewhelmed either by how similar we all are or how much difference a mere 0.1% can make. Amazingly, we are about 98% genetically identical to chimpanzees when it comes to the nucleotide base sequence of our DNA. Despite this similarity,

many of the nucleotide base differences between human and chimpanzee DNA could lead to differences in the proteins they produce. Even one difference in a critical part of our DNA could lead to big changes and differences between species.

Because the human genome contains approximately six billion letters (three billion base pairs) in the DNA code, the 0.1% variation among people translates into millions of differences in the nucleotide base sequence between the DNA of any two individuals. Most of these differences occur in the non-coding regions of our DNA. Most of these variations do not result in any measurable differences that we can detect with our current technologies. Some variations in genes are believed to result in relatively minor changes. For instance, variations in the base sequences of multiple genes determine whether we will have blue or brown eyes and whether our noses are long or short. Other variations, though, can cause or predispose us to diseases such as cancer.

TYPES OF VARIATION IN THE HUMAN GENOME

Variation is a general term used to describe differences in our genomes. A variation does not imply good or bad differences. Variations in the human genome come in several types. Nucleotide bases can be changed (polymorphisms), added (insertions) or removed (deletions). Some variations affect only one nucleotide base. Other variations are much longer and more complex and affect tens, hundreds, or even thousands of nucleotide bases. Still other types of variations may result in extra copies of DNA segments, the movement of one part of a chromosome known as a translocation, or even an extra or missing copy of an entire chromosome known as aneuploidy.

Your Sense of Smell Is in Your Genes

Odor is detected by special receptors in our noses that are coded for by about 1,000 genes. More than half of these genes are totally inactive in humans. New research has found a high level of variability in at least 50 of the remaining genes, which are active in some people and inactive in others. As a result, people possess different patterns of active and inactive receptors, leading them to sense smells differently. Because smell is a large component of taste, we probably also taste things differently as a result of this variation. Maybe this is the reason some people crave chocolate more than others.

Testing may soon be available that could examine our olfactory genetics which determine our sense of smell. Companies might then capitalize on this information by marketing perfumes, food, or wine to us based on our olfactory gene pattern.

Adapted from "Why do taste and smell differ among individuals." Wellcome Trust/Weizmann Institute. Available at: http://genome.wellcome.ac.uk/doc%5Fwtd020870.html. Accessed June 22, 2006.

Types of Variations in the Human Genome

- **Polymorphism:** a change in the nucleotide base sequence of our DNA that occurs in at least 1% of the population. It is generally used to mean a change that does not have any clinical significance.

    - **Single Nucleotide Polymorphism (SNP):** a change in only one nucleotide base pair of our DNA sequence that occurs in at least 1% of the population.

- **Mutation:** a change in the nucleotide base sequence of our DNA that occurs in less than 1% of the population. It is generally used to refer to a change that has deleterious effects on the organism.

- **Insertion:** an addition of extra nucleotide bases into our DNA. May be considered a polymorphism or mutation.

- **Deletion:** the loss of nucleotide bases from our DNA sequence. May be considered a polymorphism or mutation.

- **Repeats:** multiple copies of the same nucleotide base sequence.

- **Translocation:** movement of chromosomal material.

- **Aneuploidy:** an irregular number of total chromosomes.

Variation

Differences in our DNA.

SINGLE NUCLEOTIDE POLYMORPHISMS VERSUS MUTATIONS

As scientists studied the DNA of many individuals, they found that there were specific, identifiable alterations that affected 1% or more of the population. These are referred to as single nucleotide polymorphisms (SNPs; pronounced as "snips") and affect only one nucleotide base pair. SNPs account for 90% of all the variation in the human genome. If alterations in the DNA sequence occur in fewer than 1% of individuals, they are typically called mutations. In our everyday language, we usually use the term *mutation* to refer to an undesirable variation in the DNA that causes or predisposes us to disease. However, some mutations can give us an advantage by helping us to better adapt to our environment and still other mutations may have no apparent effect but are carried along because they are near an important gene. Mother Nature experiments with the genome all the time and causes alterations in our genes to help us as organisms adapt and survive.

MORE ABOUT SNPs — THE MOST COMMON TYPE OF VARIATION

Because most of our DNA is made up of non-coding regions, this is where most of the SNPs are found. However, SNPs can also be found within coding regions or genes.

SNPs within a gene are being studied intensely because of their potential to change the function of the protein that the gene encodes. The impact of a SNP within a gene is generally determined by its location within the gene and the type of change (e.g., addition, deletion, or substitution). Some SNPs do not result in any change to the protein structure or function. Other SNPs may result in changes to the protein structure that can then influence its function.

Marker SNP

A SNP located near a gene and usually inherited along with it.

Example of a SNP

Hypothetical DNA sequence of a gene

TCCGAAGA *Sequence of the gene present in most individuals*

TTCGAAGA *SNP: variation in 1 nucleotide base of the DNA*

Some of the SNPs in the non-coding regions of our DNA can serve as "markers" for disease-causing genes. This is because SNPs located near genes are frequently inherited along with them. A marker SNP does not contribute to the disease, but rather can serve as an indicator that the disease-causing gene is present. These marker SNPs can be especially useful if the actual disease-causing gene is not exactly known or is difficult to detect. Genetic tests can frequently be developed to determine whether a disease-causing gene is present by detecting whether the marker SNP is present — sometimes a much easier process than sequencing thousands of base pairs in a gene.

Alleles

Different forms of a single gene.

An example of a SNP located within a suspected disease-causing gene occurs in Alzheimer disease in a gene called apolipoprotein E or ApoE. The ApoE gene contains SNPs at two locations that result in three possible alleles of this gene: E2, E3, and E4. Alleles are different forms of a gene. We get one copy of our ApoE gene from our mother and one from our father. If we inherit at least one copy of the E4 allele, we will have a greater chance of getting Alzheimer disease than the general population.

If we inherit at least one E2 allele, we will have a reduced chance of getting Alzheimer disease compared with the general population. The relationship between these two SNPs and Alzheimer disease is not absolute. We may have two E4 alleles and never get Alzheimer disease. This disease, like many others, is determined by multiple genes and probably environmental factors as well.

The locations of all the different SNPs in the human genome have now been identified and mapped. Our SNP profiles (the pattern of multiple SNPs) may eventually be used to determine our responses to treatment for cancer or other conditions, permitting the individualization of therapy that was described in earlier chapters. These SNPs can also be used to reduce the complexity of searching for genes of interest.

Important Features of SNPs

- SNPs can be located in the coding or non-coding regions of our DNA.

- Some SNPs within coding regions of our DNA are associated with increased risk of disease.

- Some SNPs within non-coding regions of our DNA can serve as markers for genes.

CAUSES AND EFFECTS OF MUTATIONS

Mutations can be caused in a variety of ways. Many mutations are believed to be due to spontaneous errors that develop in the DNA as it copies itself in the process of making new cells. However, other mutations can be caused by our own actions, or by the environment. For example, cigarette smoke can damage the DNA in our lung cells, resulting in lung cancer. Infection by some viruses can lead to cervical cancer and exposure to certain radiation can lead to skin cancer. Chemicals and other elements that can cause cancer are known as carcinogens.

Carcinogens

Chemicals and other substances that can cause cancer.

Mutations may be silent (i.e., cause no effect), harmful, or occasionally, beneficial. An example of a beneficial mutation is one that affects a protein in our blood called hemoglobin, which carries oxygen. People with a certain mutation on one of their chromosomes are more resistant to malaria than those without the mutation. This has been an important survival advantage in certain regions of the world where malaria is common. Like SNPs, some mutations may not result in any change, while other mutations can increase an individual's risk for disease.

What Types of Mutations Can Lead to Cancer?

The term cancer refers to a group of diseases in which cells divide and grow uncontrollably. Cancer may or may not spread to other tissues, although it has the ability to do so. In general, healthy cells maintain very strict control over how and when cells grow. When the cell's controls are altered due to mutations in the genes involved in cell growth, cancer can arise.

All cancer is genetic. Said another way, cancer is a disease of the genome. This does not mean that all cancer is inherited from our parents, but rather that all cancer is caused by changes in our genes. Most of these changes can occur in any type of cell (e.g., lung, colon, liver), at any point in our lifetimes. The changes can give rise to cancer in those particular cells (e.g., lung cancer, colorectal cancer, liver cancer).

Hereditary Mutation or Germline Mutation

A change in the nucleotide base sequence of DNA in the body's reproductive cells (egg or sperm). Germline mutations can be transmitted to the next generation; that is, if a mutation is present in a sperm or egg, then all of the cells derived from that fertilized egg will have the mutation present.
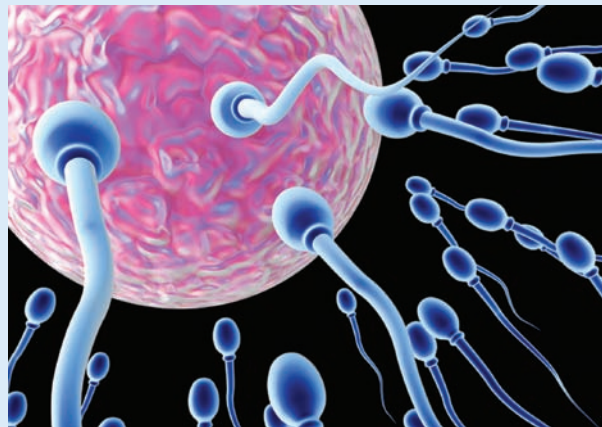
Germ Cells

Reproductive cells (in humans, either egg or sperm cells)

Somatic Cells

Non-reproductive cells (bone cells shown here)

Whether or not a mutation can be passed on to the next generation depends on the type of cell in which it occurs. Mutations can occur in our germ cells or somatic cells. Germ cells are the reproductive cells in our bodies, either egg or sperm cells. In contrast, somatic cells are all of the non-reproductive cells in our bodies such as liver cells, skin cells, and muscle cells.

Because only our reproductive cells can form an embryo, only mutations in these cells can be passed on to the next generation. Mutations that can be passed on to the next generation are called hereditary mutations or germline mutations.

In contrast, mutations in somatic cells such as our liver cells, blood cells, stomach cells, and all other non-reproductive cells cannot be transmitted to the next generation. These are called somatic mutations.

Mutations not inherited from the parents are sometimes referred to as sporadic or spontaneous. Sporadic or spontaneous mutations can be caused by our environment (e.g., sun, radiation, other carcinogens) or random events within our cells. Sporadic or spontaneous mutations can occur in germ cells or somatic cells. If sporadic or spontaneous mutations develop in germ cells, they can be passed on to the next generation.

Somatic mutation refers to the type of cell where the mutation occurs (that is, non-reproductive cells), whereas sporadic or spontaneous refers to how the mutation occurs. It should be noted, however, that even experts disagree on the meanings of somatic, sporadic, and spontaneous mutations (e.g., some experts use the term *sporadic* to refer to non-germline mutations).

Somatic Mutation

A change in the chemical letter sequence of DNA in the body's non-reproductive cells. Somatic mutations cannot be transmitted to the next generation.

Sporadic or Spontaneous Mutation

A change in DNA caused by our environment (e.g., sun, radiation, other carcinogens) or random events within our cells.

MUTATIONS THAT CAN LEAD TO CANCER AFFECT THREE MAJOR TYPES OF GENES

1. Oncogenes

These genes stimulate excessive cell growth and division. They are altered forms of genes called proto-oncogenes that control cell growth and division. Proto-oncogenes act like the accelerator of a car: they tell the cell to divide and grow.

ONCOGENES

Normal cell

Normal genes regulate cell growth

Cancer cell

Oncogenes accelerate cell growth and division

Mutated/damaged oncogene
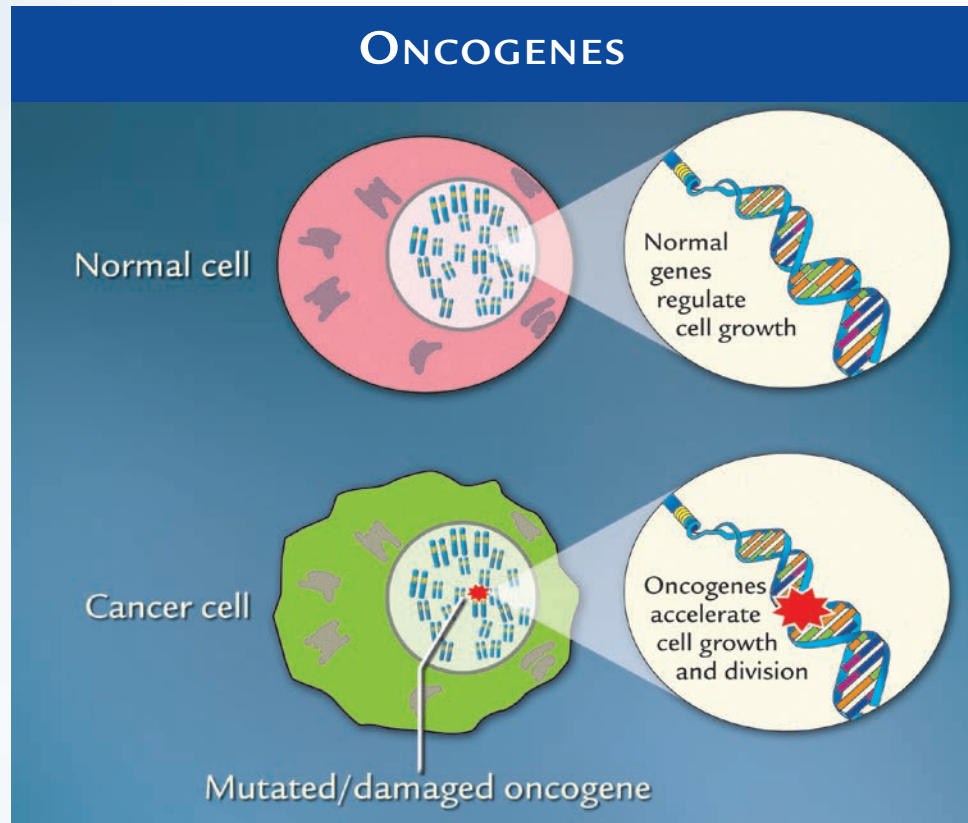
2. Tumor suppressor genes

These genes normally restrain cell growth and division. If they are not functional due to a mutation, the cell may grow and divide repeatedly. Tumor suppressor genes act like the brakes of a car: they tell the cell to stop dividing and growing.

Graphics property of National Cancer Institute. Available at: http://www.cancer.gov/cancertopics/ understandingcancer/cancer/.

TUMOR SUPPRESSOR GENES

Normal cell

Normal genes prevent cancer

Remove or inactivate tumor suppressor genes

Cancer cell

Damage to both genes leads to cancer

Mutated/inactivated tumor suppressor genes

DNA Repair Genes

DNA Repair Genes

Cancer cell

Normal DNA repair

Base pair mismatch

T C G A C
A G C T G
→ No cancer

T C T A C
A G C T G

T C T A C
A G _ T G

T C T A C
A G A T G
→ Cancer

No DNA repair

Graphics property of National Cancer Institute.
Available at: http://www.cancer.gov/cancertopics/understandingcancer/cancer/.

3. DNA repair genes

These genes help repair errors in DNA that occur when the DNA replicates itself or is damaged by exterior forces such as sunlight or chemicals. Without this repair, mutations are more likely to occur in proto-oncogenes and tumor suppressor genes. DNA repair genes function as the mechanic of cells, fixing DNA when errors in the sequence occur.

Although a single mutation in a gene can lead to cancer, uncontrolled cell growth (proliferation) is most often a result of multiple somatic mutations that accumulate over time. This is one of the reasons that cancer is more likely in older individuals.

Accumulation of Mutations May Lead to Cancer

Mutation 1   Mutation 2   Mutation 3   Mutation 4

Cell

Inherited   Environmental or spontaneous
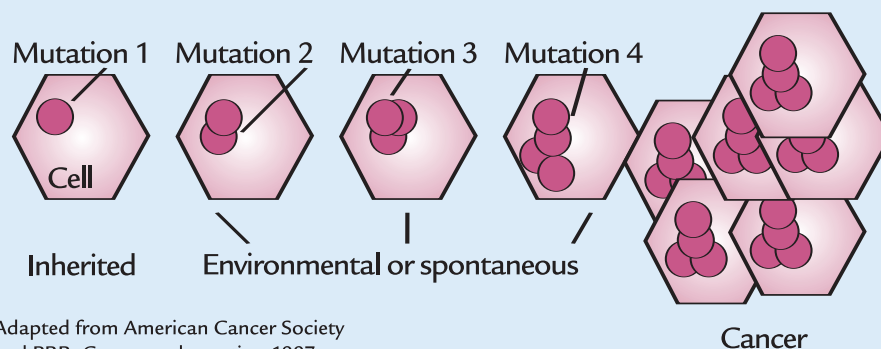
Cancer

Adapted from American Cancer Society and PRR. Cancer and genetics. 1997.

Mutations and Cancer

- Mutations that cause cancer can affect at least three types of genes.
  - Oncogenes
  - Tumor suppressor genes
  - DNA repair genes

- Some mutations are inherited.

- Some mutations are caused by carcinogens in the environment.
  - Radiation
  - Cigarette smoke and certain other chemicals
  - Some viruses and bacteria

- Many mutations are due to spontaneous errors that develop in the DNA as it copies itself in the process of making new cells.

Susceptibility Genes

Genes that increase the likelihood of disease but do not, by themselves, cause disease.

INHERITED SUSCEPTIBILITY GENES

Mutations in some genes may increase our susceptibility to cancer, but do not cause disease by themselves. Mutations in susceptibility genes may be inherited. For instance, women who inherit mutations in at least one of five genes may be at increased risk for breast cancer. In particular, women with certain mutations in the genes called BRCA1 and BRCA2 have a 50% to 80% chance of developing breast cancer and 15% to 45% chance of developing ovarian cancer in their lifetimes. These mutations account for approximately 5% to 10% of all breast and ovarian cancers. The proteins coded for by these genes are involved in DNA repair and recombination.

The fact that the BRCA mutations do not lead to cancer in 100% of individuals underscores their classification as susceptibility genes. Other DNA mutations must be present in order for cells to become cancerous. In many cases, individuals who inherit mutations in susceptibility genes develop cancer at a younger age because fewer mutations need to accumulate to trigger the formation of cancer. If other mutations do not occur, the individual may not develop cancer.

The HapMap Project

A major international project designed to determine the common patterns of DNA sequence variation in the human genome.

THE INTERNATIONAL HAPMAP PROJECT

A major international project called HapMap has determined the common patterns of DNA sequence variation in the human genome. The word Hap stands for haplotype and the word Map refers to a diagram that shows the locations of the sequences. To understand what a haplotype is, we need to back up and consider a few more things about our DNA.

Understanding alleles — different forms of a single gene

As we saw in previous chapters, chromosomes contain our DNA. Healthy individuals have 23 pairs of chromosomes, with one member of each pair from our mother and father. Twenty-two of these chromosome pairs are similar in males and females, but the 23rd pair differs. The 23rd pair is referred to as XX for female and XY for male. A single set of chromosomes is called a haploid set and paired chromosomes are called a diploid set.

The 23 pairs of human chromosomes. Image courtesy of the National Human Genome Research Institute.

Humans have 46 chromosomes, 23 each from our mother and father. Among the chromosome pairs, there are two copies or forms of each gene. The nucleotide sequence of these two may be exactly alike, in which case they would be copies, or they may be somewhat different, in which case they would be different forms of the gene. The different forms of a particular gene, as mentioned earlier in this chapter, are called alleles.

Alleles are responsible for differences in our physical characteristics such as hair color and blood type. Alleles are also responsible for different colored flowers on two plants of the same type. The reason that different alleles may result in different characteristics is because they have somewhat different DNA sequences. For example, if one plant has two alleles of the same type, then it will show the flower color associated with that type. If a plant has two red flower alleles, it will have red flowers. If it has two white flower alleles, it will have white flowers. However, if the plant has two different alleles, such as one red flower allele and one white flower allele, it doesn't make pink. Instead, one dominates the other and determines its physical characteristic. In our example, the red flower allele takes precedence over the white flower allele and is said to be dominant. That is, if the plant has one red flower allele and one white flower allele, the flowers will be red. The allele is said to be dominant if its presence almost always results in a specific physical characteristic such as red petals. The allele is said to be recessive if its presence only results in a specific physical characteristic such as white flowers if two copies are present.

Haploid

Single set of chromosomes.

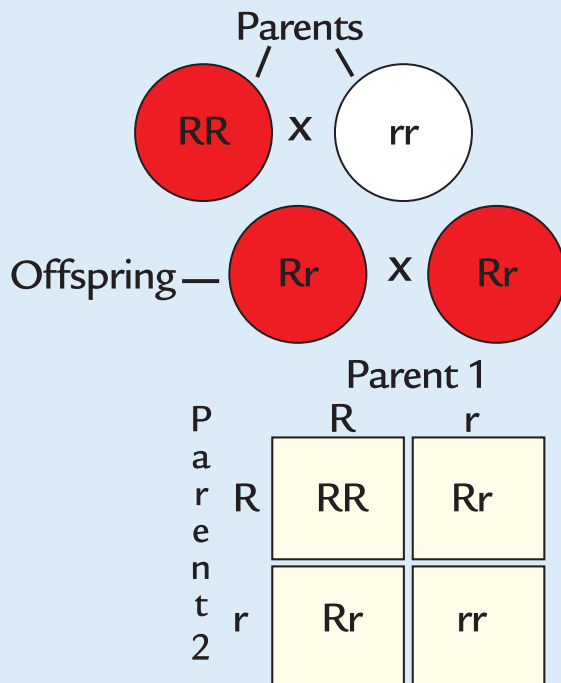### Diploid

Paired set of chromosomes.

In our example, two flowers may have the same physical characteristics or phenotype such as red flowers, but different alleles. The set of alleles on both chromosomes is called the genotype. In our example, it is also possible for offspring to have a different phenotype than their parents. For instance, if two red flowers are mated, the offspring may have white flowers. If this happens, the parent flowers must have each had two different alleles. That is, both parent flowers must have had a white flower allele and a red flower allele. Moreover, each must have passed the white flower allele to the offspring in order for it to have white flowers.

In humans, the same sort of dominant and recessive processes are at work. For instance, the genes for human blood types are A, B, and O. The A and B genes are co-dominant over the O gene. This means that if a person has one A gene and one O gene, his or her blood type will be A. Similarly, if a person has one B gene and one O gene, his or her blood type will be B. With blood type, it is possible that a mother with type A blood and a father with type B blood will have a child with type O blood. This could happen if the parents had genotypes A/O and B/O. In this way, it is possible for a characteristic (e.g., type O blood) to show up in offspring but not in parents.

Most human characteristics, including eye color, are controlled by more than one gene. This makes them a bit more complex to study.

Usually we talk about genotype in reference to a specific gene. For instance, in our previous example of Alzheimer disease, we discussed three possible alleles for the ApoE gene — a gene that may be involved in Alzheimer disease. The three alleles of the ApoE gene are E2, E3, and E4. Because each person has two alleles (one on each copy of the chromosome), the following combinations are possible: E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, E4/E4. Each of these combinations is a genotype.

Dominant

An allele that produces a trait or disease if only one copy is present.

Recessive

An allele that produces a trait or disease only if two copies are present.

An Example of Genotypes, Phenotypes, and Alleles in Crossing Red and White Flowers

Parents

RR X rr

Offspring — Rr X Rr

	Parent 1	
	R	r
R	RR	Rr
r	Rr	rr

(Parent 2)

This figure shows the mating of parents with different phenotypes and genotypes, often referred to in genomics as "crossing." The capital letter R stands for the dominant red flower allele and the lowercase r stands for the recessive white flower allele. Crossing a red parent flower with an RR genotype with a white parent flower with an rr genotype will result in offspring with red flowers that have the Rr genotype. If two red flowers with the Rr phenotype are crossed, three possible genotypes result: the RR genotype (red flowers), the Rr genotype (red flowers), and the rr genotype (white flowers). This situation occurs because the red flower gene (R) is dominant and the white flower gene (r) is recessive. A plant must have two copies of the white flower allele r in order to have white flowers.

Understanding haplotypes — sets of alleles that are inherited together

An important topic in genomics that is related to alleles is haplotype. Haplotype refers to a set of alleles on a single chromosome that tend to be inherited together.

The following example may help clarify the difference between alleles, genotype, and haplotype.

In this example, there are three genes of interest on chromosome 4. Each person has two copies or forms of each gene — one on each chromosome (designated A and B). The genes can either be straight or dashed, which represent different sequences of DNA. For gene #1, Sam's genotype is straight/dashed, whereas Joan's genotype is straight/straight. For gene #2, both have the straight/straight genotype, meaning that both have a pair of straight alleles. For gene #3, Sam's genotype is dashed/dashed (two dashed alleles) and Joan's is straight/dashed (one straight allele and one dashed). Research has found that the alleles for these three genes tend to be inherited together. Sam's haplotype for the three genes on chromosome 4 copy A is straight, straight, dashed and on copy B is dashed, straight, dashed. Joan's haplotype for the 3 genes on chromosome 4 copy A is straight, straight, straight, and on copy B is straight, straight, dashed.

Research has found that many people share common haplotypes. If our haplotypes can be identified, it may make it unnecessary to determine the nucleotide base sequence of long stretches of our DNA to find out important diagnostic, prognostic, and treatment-response information related to our genes. For example, research may show that one of the haplotypes on chromosome 4 is associated with increased risk of Parkinson disease and that this haplotype dominates over other haplotypes. This may mean that if we have that specific haplotype, we will show an increased risk of Parkinson disease, regardless of our other set of alleles. If we want to know whether we have this increased risk, we can assess our haplotype instead of taking a test to determine all of the chemical letters on chromosome 4. Haplotype assessments are generally technically much easier to determine than some other assessment methods because fewer nucleotide bases need to be sequenced. Eventually, our haplotype may allow prediction of our drug response, predisposition to disease, and other factors. Knowing our haplotypes may speed up the sort of individualized medicine that we discussed in the preface and first chapter of this manual. This will require knowledge about the association of various haplotypes with diseases, conditions, treatment response, or other health-related variables.

> ### Haplotype
> A set of closely linked alleles (genes or DNA polymorphisms) on a single chromosome that tend to be inherited as a unit. A contraction of the phrase "haploid genotype."

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NOTES:

INTO THE LABORATORY: EXAMINING OUR GENES

Did you know that nearly every infant born in the United States today receives at least one genetic test? The test screens for a disease called phenylketonuria or PKU, a disorder in which the body can't appropriately use a chemical called phenylalanine that is fairly common in our diets. In infants with alterations in the gene responsible for helping the body appropriately use the chemical phenylalanine, the build-up of phenylalanine can be lethal to the nervous system and cause severe mental retardation. By avoiding phenylalanine in the child's diet, the dreaded effects of this disorder are preventable. The genetic test determines whether the newborn has this disorder and tells parents whether an intervention is necessary.

WHAT IS A GENETIC TEST?

A genetic test or assessment is one that examines variations in our genes, either directly or indirectly. In this manual, we use the word *test* to refer to those procedures that examine DNA or RNA directly. In contrast, we use the word *assessment* to refer to measurements that are more indirect. For instance, genomics — the study of all of our genes — involves looking at patterns of genes and systems instead of just a single gene. These procedures involve assumptions and interpretations that are not required in direct tests of a single gene. In order to distinguish direct tests from those that require more assumptions and interpretations, we have included the term *assessments*. Most of the sources cited in this manual do not distinguish between tests and assessments.

Genetic tests can be designed to look for a deleterious variant of a gene or genes or simply a pattern of genetic variations. The general types of genetic tests are as follows:

Types of Genetic Tests/Assessments

- **Direct DNA or RNA testing:** looks directly at the nucleotide base sequence of our genes.

- **Cytogenetic assessment:** examines our chromosomes.

- **Linkage assessment:** assesses DNA markers inherited along with a genetic variant known to cause disease.

- **Biochemical assessment:** assesses proteins or metabolites (by-products of chemical reactions) that signal a certain genetic variant.

Genetic Variant

An alteration in the normal sequence of a gene.

Marker

Nucleotide base sequence located near a gene and usually inherited along with it.

Genetic Test or Assessment

A procedure that examines our genes, either directly (test) or indirectly (assessment). The test can be designed to detect a deleterious genetic variant or variants or simply a pattern of genetic variations. Genetic testing in today's world increasingly means "genomics testing," although we still usually refer to these as genetic tests. The phrase "gene test" is often used the same way.

The types of genetic tests and assessments just described can be designed to detect a variety of different things.

- Direct DNA or RNA tests can be used to determine whether the genes in question have a nucleotide base sequence known to cause disease. These tests can also be used to examine whether our DNA contains a pattern of genetic variants that makes us susceptible to certain diseases.

- Cytogenetic assessments can be used to determine whether an individual has an extra copy of a chromosome, is missing part or all of a chromosome, or has experienced the translocation of a chromosome.

- Different types of tests can be used to measure gene expression — whether a gene has been turned on (activated) or over-activated, whether it has been silenced (i.e., inactivated), or whether it is being under- or over-expressed, including having more than the expected number of copies of the gene.

- Over- and under-expression of certain genes can be examined with direct DNA/RNA tests or with biochemical assessments that measure the quantities of protein product of the genes.

- Tumor marker assessments detect substances in blood, urine, saliva, or other tissues that occur at abnormal levels when some cancers are present in the body. These are usually classified as biochemical assessments.

The definition of genetic tests and assessments that we've just provided — a test that directly or indirectly examines our genes — is a basic and general definition. It does not capture all of the nuances of genetic tests. It also does not define the specific types of tests that qualify as genetic. The National Institutes of Health have developed a very specific definition of genetic tests that specifies exactly what they consider these tests to include. This definition is summarized here for our reference, but is not meant to be memorized. This definition has been edited in several places, indicated by brackets, in order to be more understandable. Let's consider several specific features of this definition.

WHAT ARE SOME OF THE USES OF GENETIC TESTS?

According to this definition, a genetic test is one that is used for clinical purposes as opposed to research purposes. The clinical purposes include predicting risk for disease, identifying individuals who have a specific DNA sequence associated with disease but who do not actually have the disease (i.e., pre-symptomatic or at-risk individuals), and establishing prenatal and clinical diagnosis or prognosis. We will consider each of these clinical uses in turn.

Specific Definition of a Genetic Test from the National Institutes of Health

The analysis of human DNA, RNA, chromosomes, proteins, and certain metabolites in order to detect heritable [i.e., things that are inherited] gene-related profiles associated with disease for clinical purposes. Such purposes include predicting risk of disease, identifying carriers [individuals who possess a certain genetic abnormality but do not actually have the disease], establishing prenatal and clinical diagnosis or prognosis. Prenatal, newborn and carrier screening, as well as testing in high-risk families, are included. Tests for metabolites are covered only when they are undertaken with high probability that an excess of deficiency of the metabolite indicates the presence of heritable [alterations] in single genes. Tests conducted purely for research are excluded from the definition, as are tests for somatic (as opposed to heritable) mutations, and testing for forensic purposes [from the Task Force on Genetic Testing Definition, Final Report, 9/97 (NIH-DOE-ELSI report)].

Preimplantation testing/assessment

A technique called *in vitro* fertilization is sometimes used to help couples conceive a child. Once a woman's eggs have been fertilized in a test tube, the embryos (fertilized eggs) begin to divide. Once the cells begin dividing, one or a few cells can be tested for a variety of genetic diseases before being implanted into the woman. Some couples with a family history of a rare genetic disease will opt to have only an embryo that they know does not have the condition implanted into the woman.

Prenatal testing/assessment

Some genetic tests are used to look for gene-related abnormalities in developing fetuses. An example of this is a test for Down Syndrome, a condition in which the developing fetus has an extra chromosome. The extra chromosome leads to mental retardation and other symptoms. For instance, some tests for Down Syndrome determine whether the fetus is at increased risk of the disease, whereas another test is diagnostic (i.e., tells us with reasonable certainty that the disease is present).

Newborn screening

Genetic tests may be performed on newborns to detect certain genetic diseases for which early treatment is available. These are often performed as part of a public health program. One example of a newborn screening test that we discussed previously is for phenylketonuria (PKU).

Carrier testing/assessment

Carrier tests are usually performed on adults to look for the presence of altered genes that are known to cause specific diseases. A carrier is someone who has one copy of the altered gene, when two copies of the altered gene are needed to cause the disease. Thus, a carrier does not have the disease. However, a carrier may pass the gene on to his or her offspring. If the offspring has two copies of the gene, he or she is likely to show the disease. For this reason, carrier tests are often requested by individuals with a family history of certain genetic diseases before they have children. Some examples of carrier tests are those for cystic fibrosis, sickle-cell anemia, and Tay-Sachs disease (a lethal disorder of metabolism).

Predictive testing/assessment

Predictive tests examine the likelihood that a healthy individual may develop a certain genetic disease at some point in the future. For instance, predictive tests are available for some types of cancers in which a specific genetic alteration puts a person at much higher risk of disease than those without the mutation. An example of this is testing for BRCA1 and BRCA2 mutations where individuals who have mutations in these genes have a 50% to 80% chance of developing breast cancer later in life. Predictive tests can also indicate the presence of genetic alterations that will definitely lead to disease, such as Huntington disease — a progressive condition of uncontrollable movements and problems in thinking.

Diagnostic testing/assessment

Diagnostic testing is used to identify or confirm the presence of a disease. Diagnostic tests or assessments may be used for the following purposes:

- Diagnose primary disease — test for and identify the disease the first time it occurs.
- Identify cancer subtypes — some cancers are divided into subtypes that are more or less aggressive.

A diagnosis and/or identification of disease subtype may influence disease prognosis; that is, test results may indicate expected severity and symptom progression without treatment based on outcomes of other individuals with similar genetic profiles. It may also direct treatment. For instance, cancer is many different diseases, all of which respond differently to various treatments. A diagnosis that accurately identifies the type and/or characteristics of cancer will also help to identify the type of treatment that maximizes the chance of a cure.

Other uses

- Evaluate response to treatment — some tests show whether the cancer is responding to treatment.

- Detect minimal residual disease — some tests check for cancer cells that remain after treatment is completed.

- Monitor remission or progression — if a cancer is in remission, frequent tests may help detect the cancer if it returns and/or determine whether it is progressing.

In the immediate future, genetic testing is likely to help individualize treatment and indeed some tests are already available. This means that if we have a serious condition, it can be identified properly and we can be offered aggressive and innovative therapies that may prolong our lives. Conversely, if we have a mild condition, it too can be identified correctly and we can be spared unnecessary treatments. Perhaps the greatest promise of future genetic testing and assessment is their potential for preventing diseases from occurring in the first place.

Uses of Genetic Tests and Assessments

- Preimplantation analysis to detect presence of disease-causing gene(s) in embryos

- Prenatal genetic analysis

- Newborn screening for treatable diseases

- Carrier testing, primarily for family planning

- Predictive testing to assess risk of disease

- Disease diagnosis
   - *Primary diagnosis*
   - *Disease subtype*

- Other uses
   - *Evaluate treatment response*
   - *Detect residual disease*
   - *Monitor disease regression or progression*

EXAMPLES OF GENETIC TESTS/ASSESSMENTS

An increase in the number of genetic tests and assessments available is one of the most visible products of the genomics era. According to a government-sponsored database, more than 900 clinical tests are currently available and another 296 are available for research. The tests provide information that can often be valuable in making healthcare decisions or behavioral modifications. On the other hand, some available tests look for genetic variants that lead to diseases for which there is no cure. Some examples include current tests for Alzheimer disease and Huntington disease, both of which affect the brain and lead to progressive disability. Both of these diseases are incurable today.

The table on the following page lists some of the diseases for which genetic tests and assessments are currently available. The table also lists an example of the type of genetic test/assessment, what it's designed to detect, its clinical use, and what the test result tells us. As can be seen from the table, some tests and assessments can determine whether we are at increased risk for the disease but not whether we will definitely get the disease. In other cases, such as Down Syndrome, the tests can determine definitively if we have the disease. For many of these diseases, more than one type of genetic test is available. This table is meant to give some examples, but does not include each possible type of genetic test or assessment that is available for each disease. Some of the tests examine constitutional DNA, or DNA that makes up a person's genome. Constitutional DNA can be contrasted with DNA that is obtained from tumor cells from a specific tissue. An example of tumor cell testing is the testing of HER-2 (human epidermal growth factor receptor-2) in breast cancer. For this test, a sample of a tumor in breast tissue is obtained and the cancerous cells examined.

Constitutional DNA

DNA that makes up an individual's genome. This is in contrast to DNA obtained from tumor cells in a specific tissue.

SOME EXAMPLES OF GENETIC TESTS AND ASSESSMENTS* AVAILABLE TODAY AND THEIR CLINICAL USES

Disease	Description/ Symptoms	Example of Type of Test/Assessment	What Does the Test or Assessment Detect?	Clinical Uses
Cystic fibrosis	Thick mucous secretions clog the lungs and lead to deadly lung infections	Direct DNA	Alterations in the DNA sequence that may lead to the disease (2 altered copies must be present to have cystic fibrosis)	Carrier testing or after *in vitro* fertilization prior to implantation, prenatal, diagnosis
Down syndrome	Physical and mental retardation due to an extra chromosome	Cytogenetic	Abnormalities in chromosome number	Diagnosis, prenatal
Leukemias	Cancers involving the white blood cells (leukocytes)	Cytogenetic	Abnormalities in chromosomal structure	Prognosis, response to chemotherapies
Pulmonary hypertension	High blood pressure of the pulmonary arteries	Linkage	Variations in DNA sequence that are near or within the gene for bone morphogenetic protein receptor type IIa	Adult screening to determine risk of disease in families where no specific mutations in the gene of interest have yet been determined
Chronic myelogenous leukemia (CML)	Cancer involving the white blood cells; usually due to a mutation called BCR-ABL that produces an abnormal protein	Direct RNA	Levels of RNA encoding the BCR-ABL mutation	Determining treatment response to Gleevec®, a drug that can inhibit the abnormal protein in CML
Breast cancer	HER2 (human epidermal growth factor receptor 2) positive subtype: too many copies of the HER2 gene in tumor cells	Direct DNA/RNA

Biochemical | Overexpression of the HER2 gene

Analysis of amount of HER2 receptor proteins | Diagnosis of disease subtype, predicts response to Herceptin®, a drug that can inhibit tumor growth by binding to HER2 receptor proteins |
| Breast cancer | Mutations in BRCA1 and BRCA2 genes associated with an increased risk of breast cancer | Direct DNA | Mutations in BRCA1 and/or BRCA2 genes | Risk assessment; presence of these genes may suggest various risk reduction options |

*Many diseases can be detected by more than one type of genetic test; the different tests may have different clinical uses.
For all of the examples shown here except HER2 overexpression, only one type of test is described.

Genetic testing/assessment raises some significant concerns, including the accuracy of the test or assessment, the ability of the method to predict an important clinical outcome, privacy of the results, psychological impact of the results, implications for family members, and cost. In Chapter 7, we discuss some of the ethical issues related to genetic testing and assessment. For more information on genetic testing and further discussion of these issues, the Web sites in the following box may be useful.

Genetic Testing References on the Web

- The National Cancer Institute sponsors a Web site (www.cancer.gov) that provides a good overview of gene testing and also reviews the basic concepts of DNA. A link that may be helpful is: http://rex.nci.nih.gov/behindthenews/ugt/genetestingframe.htm.

- A similar Web site is sponsored by the U.S. Department of Health and Human Resources (www.accessexcellence.org). A link that may be helpful is: http://www.accessexcellence.org/AE/AEPC/NIH/.

- The National Institutes of Health sponsor a Web site called Gene Tests (www.genetests.org). This site provides reviews of diseases, a directory of genetic testing laboratories, an international directory of genetics and prenatal diagnosis clinics, as well as educational materials.

- The U.S. National Library of Medicine sponsors a Web site called Genetics Home Reference (www.ghr.nlm.nih.gov/). This site contains a handbook with information on genetic testing and information about a number of specific genetic tests.

- The Centers for Disease Control and Prevention also have a Web site that discusses genetic testing from a public health perspective (www.cdc.gov). A specific link that may be helpful is: http://www.cdc.gov/genomics/gtesting/resources.htm. On this page, the sections entitled Genetic Testing Public Health Perspectives and Fact Sheets may be of interest.

INTO THE LABORATORY

We've just had a blood, tissue, or cheek swab sample taken for genetic testing. Now what? How do scientists figure out which genetic variants we have, which genes work and don't work, and which genes are turned on or turned off?

In this section, we explore some key techniques used in the genomics laboratory. The development of these techniques, combined with computer technology, has enabled scientists to design studies to look at many genes simultaneously. By comparing the genes in individuals affected with cancers and other diseases to healthy individuals, scientists can identify which sets of genes are associated with disease. Once the target genes have been identified, tests can be developed and validated that may eventually be used in clinical practice.

Before we get our genetic test, a healthcare professional or a genetic counselor should first discuss the risks and benefits of testing with us. If we agree to have the testing performed, we then provide a sample of body fluid or tissue, usually blood. The blood or other tissue sample is sent to the testing laboratory where technicians isolate the DNA, RNA, or protein. Some genetic abnormalities are large, such as an additional chromosome in the case of Down Syndrome. In this case, cytogenetic testing may be performed. However, the change can be as small as a single chemical letter in the DNA code. There may be an abnormal number of copies of a gene or a gene may be too active, not active enough, or absent altogether.

Some Types of Genetic Abnormalities

- Too many or too few chromosomes

- Chromosomes switched, translocated, or rearranged

- Change in the nucleotide base sequence of DNA in a gene

- Too many copies of a gene

These and other types of abnormalities may cause a gene to be too active, not active enough, or absent.

An Example of Genetic Testing

A blood sample
is taken → The sample
contains cells → The cells
contain DNA → The test looks
for misspellings
in the DNA


```
TTAACCGGGA
GGAACCTCAC
TACAGGCCAT
GTACCTTACT
CCTT    ATA
GTACTACGAG
TAGCTACTTT
```

```
TTTTCCGGGA
GGAACCTCAC
TACAGGCCAT
GTACCTTACT
CCTT    ATA
GTACTACGAG
TAGCTACTAA
```
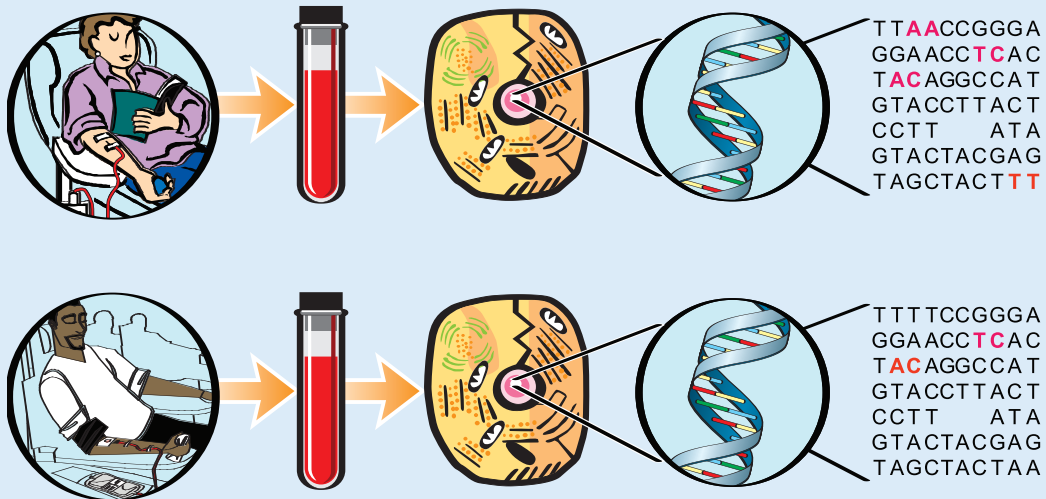
Many genetic tests are conducted on the DNA contained in our blood.
The genetic tests then look at the nucleotide base sequence of certain genes.
In this example, a woman and a man are providing blood samples for
the same genetic test. The test is looking for specific alterations in the
nucleotide base sequence of the DNA ("misspellings") or nucleotide
base deletions in the 7 lines of DNA. The woman in the graphic has
misspellings in lines 1, 2, 3, and 7 that are shown in bold red letters.
The woman also has a deletion of letters in line 5, shown as a blank spot.
The man in the graphic has the same misspellings in lines 2 and 3,
as well as the deletion in line 5. However, his genes in lines 1 and 7
are normal. The test results for these two individuals then need to be
interpreted. Based on the results of the laboratory's analyses, we may learn
the woman in our example may be at high risk for disease and the man
at slightly increased risk of disease, relative to the general population.

Genetic tests may be performed on blood or other tissues and fluids, such as samples of a tumor, urine, or cheek swabs. In the example shown here, the genetic test assesses the risk of disease. However, as we saw earlier in this chapter, other genetic tests may assess our response to a particular drug treatment, help predict the aggressiveness of the disease, or help predict our treatment outcome. Still other genetic tests may be used in the diagnosis of disease.
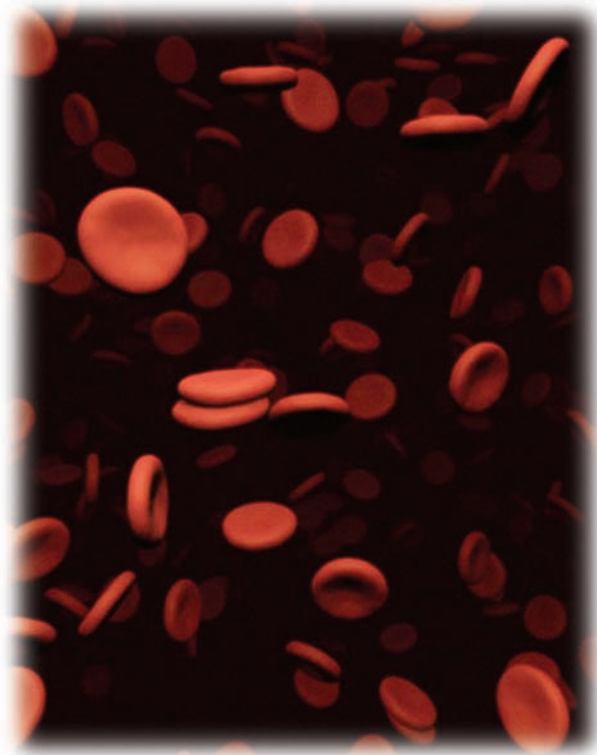
IMPORTANT TECHNIQUES OF THE GENOMICS ERA

Many important techniques have been developed over the past 10 years that have helped us learn about genomics. New techniques are being developed regularly, and the ones that are popular today may be replaced by different techniques in the future. Some genomics methods examine DNA, some RNA, and some protein. In many cases, methods are combined so that an RNA technology is used in one part of the test and a DNA technology is used in another part of the test. However, for our purposes, we will lump the tests into these three categories based on what is being examined in the sample. More complete information about selected genomics techniques is provided in the Appendix.

What Do Genetic Tests/Assessments Examine?

DNA or RNA or Protein

Tests that examine DNA

- *Type of information provided:* These tests examine our DNA directly, either by assessing our chromosomes, the nucleotide base sequence of our DNA, or both.

- *Pros:* DNA is quite stable, even after undergoing the processing steps necessary for conducting tests.

- *Cons*: Sequencing a long strand of our DNA can be a laborious and time-consuming process, as can examining individual chromosomes. These tests can also be very expensive. Furthermore, we don't always know whether alterations in the DNA get expressed as RNA and eventually as proteins that don't work properly. The association between mistakes in the DNA and problems with the proteins that they encode is not always straightforward.

Tests that examine RNA

- *Type of information provided:* These tests examine our RNA directly, which tells us whether the gene or genes in question are expressed and the level of that expression.

- *Pros:* Gene expression can be quantified with some techniques; we can tell that the gene or genes are being expressed.

- *Cons:* The amount of RNA does not always predict the amount of protein created. Additionally, RNA is readily lost because it is broken down by a protein that is commonplace in our environment. Long strands of RNA are unstable.

Assessments that examine proteins

- *Type of information provided:* Assessments that examine proteins allow us to examine the end products of genes. We can tell whether there is too much or too little of a protein and how the protein levels change over the course of time. We can also tell how well a protein functions and whether it does what it is supposed to do.

- *Pros:* These methods can be sensitive to the modifications that proteins undergo after they are produced and the dynamic changes in protein expression and amount.

- *Cons:* Tissue processing methods can affect the results of these tests more than for DNA or RNA. Some techniques require substantial expertise; other techniques are promising but have yet to be validated. Additionally, the tests that measure protein in tissues are not always standardized, so that results may vary widely from laboratory to laboratory and may not always reflect what is being measured.

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NOTES:

Genetic tests and assessments can be extremely useful, but only if we can get them, afford them, and have confidence in them. In this chapter, we describe how genetic tests and assessments are regulated and distributed and the factors that influence whether and how we can get them. We also consider the quality of these tests because this determines how confident we can be in the results and whether the results will be useful.

AVAILABILITY OF GENETIC TESTS AND ASSESSMENTS

Genetic tests and assessments are available to us basically one of two ways: through a physician or as a commercial product. Tests that are available commercially do not need to involve a healthcare intermediary. That is, physicians or other healthcare practitioners do not need to order the test for us. Instead, we can purchase the test or assessment from a store or directly from the company that conducts the analysis. In this case, we send off our sample (such as a swab from inside our cheek that contains cells) and the results are mailed back to us. Examples of tests that are currently available to the general public without a physician visit or prescription are several that claim to provide personalized diet and lifestyle recommendations. The tests supposedly examine how our diets influence the way our genes work. Most experts believe that we do not yet have enough information for these tests to be useful.

The way that the test or assessment is packaged and sold is one factor that determines whether it is available through physicians or, increasingly, directly to consumers. The test or assessment may be packaged and sold as a service or a kit. If a sample must be sent to a company's laboratory to be analyzed using their genetic test, it is considered a service. If the physical materials to conduct the test are provided to physicians so that they can perform the tests in their offices or affiliated labs, it is considered a kit or diagnostic device.

The two categories of tests or assessments, those sold as kits and those sold as services, are regulated somewhat differently.

Availability of Genetic Testing/Assessment

- Genetic tests and assessments are available through physicians or as a commercial product without a healthcare intermediary.

- Whether a test is available through a physician or commercially depends, in part, on how the test is packaged and sold.

  - *If the sample must be sent to a company's laboratory to be analyzed using their analysis, it is considered a service.*
  - *If the physical materials to conduct the test are provided to physicians so that they can perform the tests in their offices or affiliated labs, it is considered a kit or diagnostic device.*

Regulation of tests/assessments sold as kits

Approval by the Food and Drug Administration (FDA) is required for all tests — not just genetic tests or assessments packaged and sold as kits or those considered as diagnostic devices that have a major impact on the health of the patient. The extent of FDA oversight depends on the test's intended use and risk. The risk for harm from these tests does not refer to physical danger from taking the test itself. Instead, it is the potential for harm based on the test results. Examples include misuse of the results by insurance agencies or employers (e.g., discrimination) and psychological damage to the individual from knowing the results (e.g., increased risk of disease). Based on the test's intended use and risk, it is classified as Level I, II, or III. Higher-level tests require more oversight and data on accuracy, interpretation, and utility than lower-level tests. Level III is the strictest classification and requires the most data and oversight. Below is a brief summary of the different levels.

- **Level I:** These tests are associated with minimal harm to the consumer. An example is a pregnancy test. Most Level I tests do not have to undergo formal approval by the FDA before being sold.

- **Level II:** These tests pose an intermediate risk to consumers. Examples include tests for drug abuse or amounts of various substances in our blood, such as proteins and blood cells.

- **Level III:** These tests have the most potential for harm. Examples include cancer diagnostic tests.

Regulation of tests/assessments sold as services

Tests or assessments, including genetic, sold as services are regulated under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). The CLIA regulations are conditions that all laboratories must meet to be certified to perform testing on human specimens. They were devised to ensure quality laboratory testing. Genetic tests or assessments offered as laboratory services do not require FDA approval, and these account for most genetic tests available today. Currently, components of the tests sold as services, though not the tests themselves, may be regulated by the FDA. The FDA only allows the essential ingredients (reagents) to be sold to organizations that qualify as one of the following: (1) diagnostic device manufacturers; (2) clinical laboratories that are qualified to perform highly complex tests; or (3) organizations that use the reagents to make tests for non-medical uses (such as academic research or forensics).

> **Clinical Laboratory Improvement Amendments (CLIA)**
>
> Law that governs the use of laboratory tests provided as services.

> For more information about how genetic tests get to market and the levels of FDA classification, the following Web site, Lab Tests Online, may be useful (http://labtestsonline.org/ index.html).

Key Points on Government Regulation of Tests, Specifically Genetic Tests

- Government regulation of genetic tests is maturing and adapting as science evolves and the commercial marketplace grows.

- Some genetic tests are sold as kits (which are products) but most are sold as laboratory services (i.e., send in a sample to be analyzed by the company or institution).

- The government regulations are different for products and services.

> ## FDA Regulations Pertaining to Tests, Specifically Genetic Tests, Sold as Services
>
> • FDA regulates the key ingredients (reagents) used in genetic tests sold as services.
>
> • The key ingredients in genetic tests can only be purchased by the following:
>
> > - *Diagnostic device manufacturers*
> > - *Clinical laboratories that are qualified to perform highly complex tests*
> > - *Organizations that use the reagents to make tests for non-medical uses*

Government regulation of genetic tests and assessments is maturing and adapting as science evolves and the commercial marketplace grows. It seems likely that regulations will change over time.

ACCESS TO GENETIC TESTS AND ASSESSMENTS

The way a genetic test or assessment is regulated plays a role in how we gain access to it. We can gain access to tests sold as kits through our healthcare provider. In this way, physicians are the gatekeepers of the tests or assessments. In other words, physicians may or may not decide to use the test. Just because a test is approved by the FDA and shown to be effective does not mean that physicians will use it in their practices or prescribe it for us. The physician's decision to recommend a test depends on a variety of factors. First, he or she must be aware that the test is available. Second, he or she must be convinced that the test is right for us. This may include the physician's belief that the test is accurate, reliable, and useful. It may also include a judgment on the part of the physician that the test can identify factors of clinical usefulness and that we can understand the test results. Many other potential factors may influence a physician's decision, too, including marketing or advertising of the test, experience with the test, reimbursement of the test, time associated with the test, and availability of genetic counseling.

Most genetic tests or assessments are not available commercially. However, some of these that are sold as services may be available to us via mail order, although an increasing number are becoming available at drug stores, as previously noted. In order for us to have access to these tests, we must know about them and be able to afford them. Thus, access depends on our ability to find out about the test, as

well as our socioeconomic status. Some companies may use more aggressive marketing strategies than others and may engage in campaigns to create an awareness and market for their tests that reach a broad range of people. Other companies may have tests that most of us will never know about, although our physicians will. As a result of these differences, access to tests sold as services is variable.

COST OF GENETIC TESTS AND ASSESSMENTS

The cost of genetic tests and assessments is influenced by a number of factors, including the patent rights to the gene being examined. If a company owns the patent on the gene, it is possible that only that company can develop and market a test and determine the cost of the test. Our cost is influenced by whether we have health insurance and, if so, by the types of tests covered by that insurance. Reimbursement of tests by insurance companies is evolving in much the same way as government regulation. It is likely that the reimbursement landscape for these tests and assessments will change over the coming years.

Reimbursement of genetic tests and assessments depends heavily on education. Consumers, healthcare providers, and reimbursement professionals must know about genetic tests and assessments and be convinced of their utility. Many health insurance companies follow industry standards, which often means that companies will cover tests that are covered by Medicare. Reimbursement is also influenced by the quality of the test, which we discuss in the next section. As people learn more about genetic tests and as the government becomes increasingly convinced of their utility, it is likely that the numbers and types of tests reimbursed by healthcare companies will increase substantially over the coming decade.

TESTING THE TESTS: QUALITY ASSURANCE OF GENETIC TESTS AND ASSESSMENTS

We are used to hearing about clinical studies or trials to determine whether a treatment is effective and safe for a given condition. However, we may think less about testing the tests that are used in medical practice, including genetic tests and assessments. With such tests increasingly used to make healthcare decisions, the importance of testing the tests is becoming clear. Just as with medical treatments, medical tests must meet high quality standards. After all, if a test is being used to determine whether we should undergo conservative or aggressive treatment for cancer, we want it to be accurate, reproducible, and of the highest quality.

So, how do we test genetic tests, or any test for that matter? In the following sections, we describe the ideal case or what sort of evidence tests should ideally have for us to be confident in their results. In reality, not all tests are thoroughly "tested" for their accuracy and usefulness.

The evaluation of any test involves the concepts of validity and reliability. Validity refers to how well the test measures what it is supposed to measure. Reliability refers to the consistency of the measure or its ability to get the same result each time.

> ### Test Validity
>
> How well a test measures what it is supposed to measure.

> ### Test Reliability
>
> The test's ability to get the same result each time, even when performed by different people.

For example, we use a thermometer to determine whether a roast we've put in the oven is done cooking. It is important that the thermometer actually measures the temperature of the roast and not of the surrounding air or metal oven rack. In other words, it must be valid. It is also important that the thermometer reads the same temperature each time, within an acceptable range. For example, if it reads 160 degrees on the first try and 165 on the second try, its reliability is probably acceptable. If it reads 85 degrees on the second try, our thermometer is not reliable.

HOW TO DETERMINE WHETHER A TEST IS VALID?

With our roast example, it's easy to determine whether the thermometer is valid: We cut the roast, observe its color, and taste it if need be. Our assessment of the roast is an unequivocal test of the roast's doneness.

When determining the validity of tests used in clinical medicine, two types of validity are usually considered: analytical validity and clinical validity. Each type of validity has two components: sensitivity and specificity.

Analytical validity

Analytical validity is a measure of how well the test measures what it is supposed to measure. An analytically valid test should be able to accurately detect an abnormality when it is present in the test sample (known as sensitivity) and not detect it when it is not present (known as specificity). Analytical validity, like all of the other aspects of test validity described here, applies to tests in general, not just genetic tests. For instance, we may provide a blood sample to see if we have West Nile virus. The test may detect antibodies in our blood. We want to be sure the test will give us a positive result if we really do have the antibodies in our blood (sensitivity). On the other hand, we don't want the test to give us a positive result if we do not have the antibodies in our blood (specificity). For instance, a test for West Nile virus should not give a positive result if we have a different virus. A sensitive test will give a positive result when the thing we are looking for is present. A specific test will give a negative result when the thing we are looking for is not present. If a test is unable to perform these functions well, we will have a hard time believing the test result.

The analytical validity of a newly developed test is a measure of its sensitivity and specificity and is often judged by comparing the results to those obtained from the best available test, sometimes referred to as the "gold standard."

Clinical validity

The other important feature of test validity is clinical validity. In our example of West Nile virus, not only do we want to know whether we have the antibodies, but also that the antibodies measured by the test actually tell us whether or not we have the disease. We want to be sure that only people who have the disease have a positive result on the test (clinical sensitivity) and that people who do not have the disease will have a negative result on the test (clinical specificity). That is, we want to be sure that the antibody test gives us clinically relevant information. The clinical validity of a test refers to its ability to provide clinically relevant information.

In the world of genomics, tests are often used to classify us into different categories. It is important that the different categories are related to some important clinical outcome such as treatment response. That is, the different results on the genetic test must correspond to different responses to drugs, different outcomes, or some other clinical categorization that is relevant to our health. Clinical validity is assessed in clinical trials.

Analytical Validity

A measure of how well a test measures what it is supposed to measure.

Sensitivity

The ability of a test to detect something (e.g., a faulty gene) when it is actually present.

Specificity

The ability of a test to give a negative result when the thing we are looking for is not present.

Clinical Validity

The ability of a test to provide clinically relevant information.

Clinical utility

Yet another important test-related concept is clinical utility. Clinical utility refers to the benefits versus drawbacks associated with use of the test in routine clinical practice. Clinical tests should provide some sort of benefit such as diagnosing a disease, ruling out a disease, suggesting strategies that minimize risk of disease, or identifying subgroups that may respond to certain treatments. If the test does not provide any benefit to us, it does not have clinical utility.

The drawbacks of the test must also be considered. If the test provides benefit but is associated with a high risk, it may not have clinical utility. Risks from genetic tests and assessments are typically related to the potential for harm that comes from knowing the results (e.g., misuse by employers or insurance companies, psychological trauma from learning about personal risk of disease). Financial and practical drawbacks must also be considered. Specific examples of situations in which tests may lack clinical utility include:

- If the blood test for West Nile virus could only be performed in another country and we had to send all of our blood samples there, the test may have low clinical utility for Americans.

- If the test required special equipment that could only be purchased by the richest hospitals, the test may have low clinical utility in routine care.

- If taking a blood test for West Nile virus had a 50% chance of exposing us to a more serious virus via needle contamination, it clearly would not be worth the risk and the test would not be clinically useful.

- If there were two tests that provided nearly identical information about West Nile virus but one was half the cost, the other test may not be considered clinically useful.

- If a genetic test could detect every single alteration in our genome but we didn't have any idea of what those alterations meant, the test would not be clinically useful.

It is important to note that the concepts of reliability, validity, and clinical utility do not apply only to genetic tests and assessments, but to all health-related tests. In fact, reliability and validity are important concepts for any test, such as the tests for college admission and assessment of mental health.

Clinical Utility

The benefits versus drawbacks of a test in the context of clinical use.

PARAMETERS OF TEST EVALUATION

Test Parameter	Definition	Example: We are trying to detect a specific DNA sequence in someone's blood sample
Analytical validity	How well the test detects the target compared to a gold standard test	The test detects the sequence just as well as our best available test (For some new genetic tests, there is no gold standard)
Clinical validity	How well the test can accurately predict a clinically important outcome	Does a positive result for the sequence relate to disease progression, response to drugs, or other clinically important outcome?
Sensitivity (part of validity)	Likelihood that the test will detect the target if it is actually present	The likelihood that the test will detect the sequence if we actually have it
Specificity (part of validity)	Likelihood that a test will give a negative result when the target is not present	The likelihood that the test will say that the sequence is not present if we do not actually have it
Reliability	Repeatability of results	The likelihood of obtaining a positive result for the sequence each time we test a sample in which it is present; the likelihood of obtaining a negative result for the sequence each time we test a sample in which it is not present
Clinical utility	The value of a test in diagnosing/ruling out a disease, in suggesting treatment or risk reduction versus its drawbacks	Does detection of the sequence help us diagnose or rule out a disease with a minimum of risk? Is it cost effective? Does it provide new information that is not duplicated by another test? Does it help us select a treatment? Does it suggest risk-reduction strategies? Is it practical in the clinic?

A CLOSER LOOK AT CLINICAL VALIDATION

Genomics tests must be validated in clinical trials to ensure their relevance to patients. A test might be very useful in the laboratory for detecting the presence of a group of genes (analytically valid) but if that test does not provide unique information about patient diagnosis, prognosis, treatment response, or outcomes, it will not be useful in healthcare practice.

IMPORTANT QUESTIONS TO ASK ABOUT THE CLINICAL VALIDITY AND UTILITY OF GENETIC AND GENOMIC TESTS

1. What clinical parameter is this test designed to detect or predict?

Is this a diagnostic test? Does it purport to detect recurrence? Is it providing a prognosis? Does it purport to classify individuals into groups and, if so, of what utility are the groups?

2. What clinical studies have been conducted to validate this test?

Peer-reviewed, published clinical studies must document the relationship of the test with some important outcome. For instance, the test may predict risk of recurrence or response to therapy. If the test claims to predict risk of recurrence, has this been tested in a clinical study and documented? How good is the test at predicting recurrence? Have the results been independently reproduced or have multiple positive trials been conducted? It is also important to consider whether the studies were well designed. This will tell us how confident we can be in the results. For instance, we can be more confident if the study included 200 people instead of 10. We can be more confident if a study included some sort of control group than if it did not. (For more discussion of clinical trials as they relate to genomics, please see the next section.)

3. Does this test provide unique information?

Is it highly correlated with existing tests? Is it more/less accurate than existing tests?

4. Does this test support actionable clinical decision making?

Will patients and healthcare providers be able to make better clinical decisions because of this test?

TEST VALIDITY AND TEST REGULATION

As described previously, tests that are marketed to physicians as kits must be approved by the FDA. As part of this approval process, the test must be shown to have both analytical and clinical validity. Laboratories that provide tests marketed as services come under CLIA regulations, which require analytical but not clinical validity. Various efforts are underway to revise the current regulations to include demonstration of clinical validity of all tests.

In the current regulatory arena, some types of genetic tests are required to meet more rigorous standards than others. Some tests have been validated in the clinic but many have not. That is why it is important for us to ask about a genetic test's validity before agreeing to have the test performed. Experts generally agree that additional government oversight of genetic tests is important. This additional oversight will probably happen in the coming years as the number of genetic tests increases and their use becomes more prevalent in healthcare.

CLINICAL TRIALS AND GENOMICS

We've just noted how important clinical trials are in determining whether a genetic test is clinically valid. The growing field of genomics is certain to result in many new genetic tests that will all need to be validated in clinical trials. This raises questions about the number of clinical trials that will need to be run and where the dollars will come from.

A clinical trial is usually defined as a prospective study involving human participants that examines the effects of an intervention on some aspect of health to determine whether it is effective and/or safe. The intervention can be a drug, test, procedure, device, diet, counseling, or behavioral strategy. A clinical trial often involves a fairly large group of participants (e.g., 100 or more), although most definitions do not specify an exact number. Clinical trials have well-defined outcome measures. The outcome measure depends on what is being studied. In cancer trials, outcome measures often include disease progression. Most clinical trials include safety as an outcome measure, which is measured by adverse events, toxicities, and/or laboratory tests.

The term *clinical study* is often used interchangeably with *clinical trial*. However, the distinction between these terms is evolving. It seems likely that, in the future, a formal definition for clinical study will be available that will distinguish it from clinical trial. In everyday language, the term clinical study is broader than clinical trial and encompasses research that meets the formal definition of a clinical trial provided in the previous paragraph, as well as research that does not meet that definition.

Clinical Trial

Usually defined as a prospective study designed to determine whether an intervention is effective and/or safe; it has well-defined outcome measures and usually involves a minimum number of patients.

Clinical Study

Sometimes used interchangeably with *clinical trial*. However, in everyday usage, the term *clinical study* is broader than *clinical trial*.

General Description of Phase 1 through 4 Trials/Studies

The descriptions of study phases that follow were developed by the U.S. Food and Drug Administration and are required for all drugs and some other healthcare products.

Phase 1: These are conducted when a product (i.e., drug or other healthcare product that is required to undergo these studies) is initially introduced for use in humans. They are usually conducted in healthy volunteer subjects or in patients, many of whom have few treatment choices. They are designed to determine the metabolism (breakdown) and pharmacology of a drug and side effects with increasing doses. These generally involve 20 to 80 participants.

Phase 2: These are early, controlled clinical studies designed to provide preliminary evidence of the effectiveness of a product in individuals with a given condition or disease. These studies are also designed to examine the common short-term side effects and risks. These generally involve several hundred participants.

Source: U.S. Food and Drug Administration Center for Drug Evaluation and Research. CDER handbook. Available at: http://www.fda.gov/cder/handbook/. Accessed February 26, 2006.

Randomized, controlled trials

In medicine, the Phase III prospective, randomized, controlled trial is considered the optimal type of trial. This type of trial provides the highest possible scientific proof that whatever we are testing actually works. It is designed to eliminate as much bias as possible. By eliminating bias, we can be sure that any differences in the things we are measuring (e.g., disease progression, relapse) are due to our intervention (e.g., the test, drug, or treatment).

The word *prospective* means the study is designed first and then the participants undergo the treatment or test. A retrospective study is designed to study participants after they have already undergone the treatment or test. For instance, if we wanted to determine whether eating bananas during the winter could reduce our risk of getting the flu, we could do one of two things. We could ask one group of people to eat a banana per day and another group to refrain from bananas. We would then monitor whether the numbers of people who got the flu differed between the groups. This is a prospective study. Alternatively, we could look at the supermarket records of how many times people purchased bananas over the winter and see if those people who bought more bananas had fewer bouts of the flu. This is a retrospective study.

The word *randomized* means that everyone participating in a study has an equal chance of getting the treatment or test in question. For instance, in our banana study, we want to make sure that each person has an equal chance of being in the banana group and the no-banana group. If we put the 10 males who contacted us into the banana group and the 10 females into the no-banana group, our assignment of individuals to groups would not be random. It may be that females are more susceptible to the flu than males. In other words, we cannot be sure that a reduction in flu is due to the bananas. In reality it is not possible to predict all of the ways that groups could be different. This is why researchers resort to randomization, or procedures designed so that everyone who comes in the door has an equal chance of being in the

group that receives the treatment in question. In large clinical trials, randomization is determined by computer. Sometimes procedures are used to ensure that the groups will be approximately the same on some important variable such as age. This is called stratification and the stratification factors are determined before the study begins.

The word *controlled* in randomized, controlled trial refers to a comparison group. In our banana example, the control group is the one that did not get bananas. In some clinical trials, the control group receives the accepted standard of care for the condition being studied.

Prospective, Randomized, Controlled Trial:

A type of clinical study that provides the best possible test of whether an intervention works.

- **Prospective:** the study is designed first and then we undergo the intervention (e.g., treatment or test).

- **Randomized:** every person has an equal chance of being in each group.

- **Controlled:** the intervention group is compared to a group that does not get the intervention.

Designing clinical trials to validate genetic tests

Rigorous clinical trials are an important part of medicine. However, prospective, randomized, controlled trials cost a lot of money, take a lot of work, and take a lot of time. In many cases, it is very important that we conduct randomized, controlled trials, despite their drawbacks. For instance, before we replace one medicine with another, we want to be as sure as possible that the new one is at least as effective as the old one and is at least as safe.

However, in the case of genetic tests, it may not be necessary to always conduct prospective, randomized, controlled trials. For instance, if a test is relatively low risk and has a high likelihood of benefit, we may elect to compromise on prospective, randomized, controlled trials. A rigorously-designed retrospective study may be able to get a useful test into clinical practice more quickly where it can benefit us. For instance, if we are diagnosed with a serious disease today, we may prefer to have the retrospectively-validated genetic test today rather than wait for two years for the prospective trial to be completed. In genomics, many mathematical models are used that provide rigorous and convincing evidence for the utility of a test without doing the prospective trial. Thus, genetic testing may be an area in which our society chooses to make compromises about the types of studies used to validate them. This is not to say that the trials need not be rigorous or well designed; it is only to say that the urgent need for an assessment may call for a study design that brings it into practice sooner than a prospective trial would.

- Prospective, randomized, controlled trials are the gold standard for determining whether a treatment is effective.
    - *These trials provide the strongest possible scientific evidence.*
    - *These trials can be expensive, labor intensive, and long.*

- Retrospective study designs can provide highly useful information and can be conducted more quickly and economically than prospective, randomized, controlled trials.

- Retrospective studies may be particularly useful for examining genetic/genomics tests that have a high potential to help us in the immediate future.

With these considerations in mind, we now turn to a few examples of clinical validation studies.

EXAMPLE: VALIDATION OF ONCO*TYPE* DX™

Onco*type* DX is a diagnostic test that predicts the likelihood of breast cancer recurrence in women with newly diagnosed, stage I or II, node-negative, estrogen receptor-positive breast cancer that will be treated with tamoxifen. The test analyzes the expression of 21 genes.

This particular test has undergone clinical validation testing and can serve as an example of the types of questions asked and the relevance of outcomes.

Synopsis of an Example Clinical Validation Study

- **Question asked:** Can Onco*type* DX accurately quantify the likelihood of breast cancer recurrence in the defined population?

- **Procedure:** Surgical tissue samples from 668 women (node-negative, estrogen receptor-positive, tamoxifen-treated) were tested for the 21-gene panel using an RNA test. Each sample was assigned a Recurrence Score™ from 0 to 100 based on the results of the RNA test. Outcomes of the women who provided the samples had been tracked over many years.

- **Results:**
    - *Recurrence Score accurately grouped patients into low risk and high risk of recurrence groups based on actual outcomes.*
    - *Recurrence Score was a better predictor of patient outcome than age, tumor size, and tumor grade (current standard predictors of outcome).*
    - *Recurrence Score was predictive of overall survival and could be used to predict distant recurrence in individual patients.*

- **Conclusion:** The Recurrence Score is valid for quantifying the likelihood of distant recurrence in this patient population.

Source: Paik S, Shak S, Tang G, et al. N Eng J Med. 2004;351(27):2817-26.

EXAMPLE: VALIDATION COMPARISON FOR TESTS DESIGNED TO DIAGNOSE ASTHMA

Asthma is a chronic lung disease in which inflammation causes the airways to become smaller and filled with mucus, making it more difficult to breathe. A number of effective medications are available for asthma. Accurate diagnosis is important so that asthma can be treated if it is found to be the cause of someone's breathing difficulties. The following box describes a study designed to compare the clinical validity of several common tests used to assess the presence of asthma. As previously noted, the concept of validity does not just apply to genetic tests. The following example describes a test for asthma that is not a gene-based test.

Synopsis of an Example Clinical Validation Study

- **Question asked:** Which test is the most useful for diagnosis of asthma in patients with difficulty breathing?

- **Procedure:** The medical and test histories of 195 patients with difficulty breathing (141 of whom had asthma) and 18 control patients were examined to determine the diagnostic accuracy of 5 different tests.

- **Results:**
    - *Tests for hyper-responsiveness (i.e., extra-sensitive responses) of the large airway passages called the bronchii showed a diagnostic accuracy of 93%.*
    - *Skin-prick allergy tests showed a diagnostic accuracy of 62%.*
    - *Other tests showed a diagnostic accuracy of less than 50%.*

- **Conclusion:** Testing for bronchial hyper-responsiveness is the most accurate method for diagnosing asthma in individuals with difficulty breathing.

Source: Popovic-Grle S, Mehulic M, Pavicic F, et al. Coll Antropol. 2002;26 Suppl:119-27.

Examples of Specific Gene-based Tests

The following section describes several gene-based tests that are used to screen for different types of cancers. In some cases, these tests are used to diagnose disease and in other cases, they are used to predict treatment response.

Example: PreGen-Plus™ DNA screen for colorectal cancer

PreGen-Plus is a screening test that looks in the stool (bowel movements) for cells that contain DNA alterations that are associated with colorectal cancer. Cells containing the altered DNA are shed into the stool by cancerous and pre-cancerous areas of the colon and rectum. PreGen-Plus is intended for use in individuals 50 years of age and older who do not have symptoms of colorectal cancer and are at average risk for developing the disease. In several studies, this test has been found to correctly identify 65% (sensitivity) of people known to have colorectal cancer. It has also been found to correctly identify 95% (specificity) of the people known not to have colorectal cancer.

Example: DNAwithPap™ for cervical cancer

DNAwithPap is a screening test for cervical cancer that combines a pap smear with DNA testing for detection of the human papillomavirus (HPV) — the primary cause of cervical cancer. This combined test has been found to correctly identify 72% (sensitivity) of women who actually do get cervical cancer within several years of the test. The tests have also been found to correctly identify 99% (specificity) of the women who do not get cervical cancer within a few years of the test.

Examples of General Gene-based Tests

The next few examples of gene-based tests describe general types of tests as opposed to specific products as described in the previous two case studies. Therefore, specificity and sensitivity data are not included because these would be different for each test.

Hereditary nonpolyposis colorectal cancer test

Hereditary nonpolyposis colorectal cancer is a type of inherited cancer of the digestive tract, particularly the colon (large intestine) and rectum. Inherited abnormalities in any one of four genes increase the risk of this cancer. The 4 genetic abnormalities are referred to as MLH1, MSH2, MSH6, and PMS2. These genes normally help to correct errors in DNA that can occur when cells divide. When the gene cannot perform this function due to the inherited abnormalities, cancer can result. Not all people who inherit these genetic abnormalities will develop cancer.

A number of different tests are available to determine whether someone has these abnormalities. The type of genetic alteration we have can tell us something about disease prognosis. For instance, if we have the alteration in the MSH2 gene, we have a higher risk of developing cancers outside the colon, such as endometrial cancer in women. Families that have these genetic alterations are recommended for more frequent colonoscopies and testing for endometrial, ovarian, and urinary tract cancer. Prophylactic (preventive) surgeries (i.e., those that remove parts of the colon) are more aggressive than those indicated for non-inherited colorectal cancers.

Chronic myeloid leukemia BCR-ABL testing

Chronic myeloid leukemia is a cancer of the bone marrow in which blood cells fail to develop as they should. It is usually diagnosed by finding the Philadelphia chromosome. The Philadelphia chromosome is a result of an exchange of genetic material between chromosomes 9 and 22. As a result of this exchange, two genes come together that are not supposed to. These genes are called the BCR (breakpoint cluster region) from chromosome 22 and the ABL (Ableson leukemia virus) gene on chromosome 9. These genes contain the nucleotide base sequence that codes for a hybrid protein that leads to uncontrolled growth of some blood cells, leading to leukemia. Gene-based tests can determine whether we have the BCR-ABL abnormality, which then can be used to guide treatment.

Role of cytogenetics in acute myeloid leukemia testing

Acute myeloid leukemia is another cancer of the bone marrow in which blood cells fail to mature as they should. In most cases, the cause of acute myeloid leukemia is unknown. Many different chromosomal abnormalities have been found in acute myeloid leukemia and these can be detected using cytogenetic techniques or techniques that look directly at the chromosomes. Different types of chromosomal abnormalities are associated with different levels of responsiveness to treatment. One type of chromosomal abnormality is an indication for a different type of treatment altogether. Therefore, cytogenetic testing is used to guide treatment of acute myeloid leukemia.

In the next few examples, we describe several different tumor markers. Tumor markers are usually proteins that are produced by tumor cells or by the body in response to tumors. As the numbers of tumor cells increase, tissue gets damaged and these proteins leak into the blood. Blood tests are performed to determine levels of the tumor marker, which can be useful for monitoring various types of cancers. Tumor markers are not usually used by themselves to diagnose cancers.

Carcinoembryonic antigen (CEA) in different cancers

Carcinoembryonic antigen is a protein that is found in the blood of the developing fetus, but not usually in healthy adults. However, levels of CEA are increased in the blood when we have certain cancers — especially cancers of the colon and rectum. For this reason, it is often used as a tumor marker. That is, high levels of this protein in the blood may mean that we have cancer. However, many other conditions can also increase levels of this protein, including benign tumors, smoking, inflammatory bowel disease, infections, pancreatitis, and cirrhosis of the liver. Also, early tumors do not usually increase CEA and neither do some advanced tumors. A high CEA level can lead to the use of more specific tests for colon cancer.

With a diagnosis of widespread cancer, changes of CEA in the blood can be used to monitor treatment. Levels may decrease with successful treatment. Blood levels of CEA may also be monitored to determine whether cancer has returned. These represent the clinical utility of tests that are reliable and valid indicators of CEA.

Prostate-specific antigen (PSA)

Prostate-specific antigen (PSA) is a protein produced by the cells of the prostate gland. Men normally have low levels of this protein in the blood, but levels can be increased in men with prostate cancer. Increased levels of PSA in the blood are also associated with some non-cancerous conditions.

Prostate-specific antigen is sometimes used as a tumor marker. It is used along with a digital rectal exam to help diagnose prostate cancer. It can also be used to help determine whether the cancer has returned.

More about tumor markers can be found on the National Institutes of Health's Web site (www.cancer.gov). A specific link that may be useful is: http://www.cancer.gov/cancertopics/factsheet/Detection/tumor-markers.

The following table summarizes some of the gene-based tests that have been used in cancer diagnosis and treatment. The table is not comprehensive, but instead provides examples. In some case, the protein tests are used to provide information about several different types of cancer.

EXAMPLES OF SOME GENE-BASED TESTS		
Gene or Marker Name	Type of Cancer	Relationship of Gene or Marker to Cancer
BRCA1 and BRCA2	Breast	Changes in these genes increase the risk of cancer
BCR-ABL	Leukemia	Genetic material is exchanged between two chromosomes, causing two genes to come together that are not supposed to. These genes are called the BCR and ABL genes. These genes spell out the chemical letter code for a hybrid protein that leads to uncontrolled growth of some blood cells, leading to leukemia
MLH1, MSH2, MSH6, and PMS2	Hereditary nonpolyposis colorectal cancer	These genes are part of the system that corrects errors in DNA. When they do not have the correct chemical letters, errors in DNA may go unchecked and lead to cancer
HER2/neu (human epidermal growth factor receptor 2)	Breast	A gene that helps control how cells grow, divide, and repair. Cancers with too many copies of the HER2 gene have too many HER2 receptors and tend to grow fast
Estrogen and progesterone receptors (ER/PR)	Breast	Cancers that are ER-positive are more likely to respond to anti-estrogen therapies
CA-125 (cancer antigen – a protein)	Ovarian and digestive organs (but other conditions can elevate this protein)	Function of this protein is unknown, blood levels used to monitor response to treatment and recurrence
CA 15-3	Breast	Blood protein that is used to monitor treatment response and recurrence
CA 19-9	Pancreatic	Blood protein that is used to aid diagnosis and monitor disease
Beta-HCG (Beta-subunit human chorionic gonadotropin)	Testicular	Blood protein that is used to aid diagnosis and monitor disease
CA 27.29	Breast	Blood protein that is used to monitor treatment response and recurrence

American Association for Clinical Chemistry. Lab tests online. How clinical laboratory tests get to market. Available at: http://www.labtestsonline.org/understanding/features/market.html. Accessed September 1, 2005.

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Ross JE. Molecular diagnostic techniques in breast cancer. In: Ross JS, Hortobagyi GN (Eds). *Molecular Oncology of Breast Cancer.* Sudbury, Mass.: Jones and Bartlett Publishers, Inc. 2005:12-33.

Terry S. Comments to the Secretary's Advisory Committee on Genetics, Health, and Society: "Examining the issues surrounding the coverage and reimbursement of Genetic Tests and Services." Available at: http://www.geneticalliance.org/ws_display.asp?filter=tests_coverage. Accessed September 15, 2005.

Notes:

With the sequence of the human genome now in hand, our society is increasingly faced with new challenges posed by this wealth of information. Ethical, legal, and social issues, including who should have access to what information, who owns the genetic information stored in each individual's DNA, and what limits should be placed on genetic assessments, are also becoming paramount.

Although many of the ethical, legal, and social issues associated with genomics are complex and controversial, others are not. For instance, the use of validated genomics tests (an extension of the diagnostic tools already available) to assist in the diagnosis and prognosis of disease is not a controversial topic. Many gene-based tests, such as those for phenylketonuria (PKU) for newborns, have been used for years and most people support testing in the context of a preventable or manageable disease. To the extent that there is controversy in this area, it concerns whether testing should be mandated as is sometimes the case with newborn screening, and when and how to integrate new tests into clinical practice. For example, there may be controversy as to whether evidence of clinical utility or cost-effectiveness should be required before a test is routinely offered to patients or paid for by insurers.
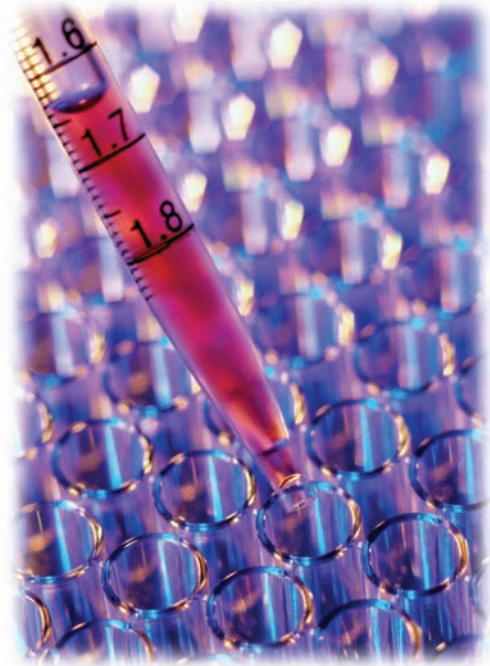
EXAMPLE OF REGULATORY ACTION

Another area in which there is not much controversy is the use of predictive genetic information, to discriminate against individuals for purposes of employment or health insurance. Most people oppose this use: in a U.S. government poll, 85% of people said they wouldn't want insurance companies to have their genetic information. Indeed, a bill entitled the Genetic Information Nondiscrimination Act of 2005 has passed the Senate unanimously (98-0) and may be signed into law by the President in 2006. Although not all advocates for nondiscrimination are satisfied with the enforcement sections of this particular bill, all agree that some protection is needed. According to Dr. Francis Collins, the Director of the Human Genome Project:

"This bill could just as well be known as the bill to protect people with DNA. That would be all of us! Since all of us have dozens of genetic glitches that put us at risk for disease, we all have a reason to be concerned about the possible misuse of genetic information."

The bill prohibits discrimination in both health insurance and employment based on a person's genetic information, unless that genetic information concerns a disease or disorder that is already manifested in that person. This bill would also prevent insurers and employers from requesting or requiring genetic information (or the taking of a genetic test) except in very limited circumstances. As of February 2006, this bill still faces real challenges in the House.

Some controversy also exists over the extension of anti-discrimination protections to other types of insurance such as long-term care and life insurance, which are not currently part of the bill. Additionally, some commentators think we would be better off with generic laws that prevent insurers and employers from gaining access to any predictive health-related information.

The following example describes DNA analysis for forensic rather than medical purposes, but it speaks to a generally accepted use of genetic testing and assessment.

DNA evidence is also routinely used to solve crimes. When DNA evidence is collected from the scene of the crime and then matched to DNA that police already possess, few people protest. However, this issue does become controversial when individuals who match a general profile are asked to provide DNA samples "voluntarily." This situation occurred in November 2004 in Charlottesville, Virginia when police, attempting to find a serial rapist, asked 197 African-American men who resembled a composite drawing to provide DNA samples. A total of 187 men provided samples in this so-called DNA sweep. Many view this type of sweep as an invasion of civil liberties, and thus this practice is controversial. Another controversial issue is the practice in some states of keeping the DNA of suspects on file, instead of just the DNA of those who are convicted.

ETHICAL, LEGAL, AND SOCIAL IMPLICATIONS (ELSI) PROGRAM

Approximately 5% of the annual budget for the Human Genome Project was used for the analysis of ethical, legal, and social issues, which proceeded in parallel with the sequencing efforts. Of course, these issues have not been solved as a result of the spending of this money, but the decision to set aside funds for these studies showed recognition on the part of scientists and the government that these issues are important and must be addressed in concert with scientific progress. The program that developed as part of the Human Genome Project was called the Ethical, Legal, and Social Implications (ELSI) Program. This program continues today as the largest supporter of research into the ethical, legal, and social implications of genetics and genomics.

The ELSI Program has identified 4 high-priority topics:

1. Use and interpretation of genetic information

2. Clinical integration of genetic technologies

3. Issues surrounding genetics research

4. Public and professional education and training about these issues

BASIC PRINCIPLES OF MEDICAL ETHICS

When analyzing the ethics of genomics practices, it may be useful to have a framework that can be applied to each new dilemma. Some members of the bioethics community have identified basic principles that may be used as part of such a framework. The principles include beneficence, nonmaleficence, individual rights (sometimes described using the terms "autonomy" or "self-determination"), privacy, and justice/equity.

PRINCIPLE	DEFINITION	EXPLANATION
Beneficence	Duty to do more good than harm	Who will benefit from this technology and in what ways?
Non-malfeasance	Duty not to cause harm	Who might be harmed by this technology? How might this technology be misused?
Individual rights	Respect for an individual's right to be his/her own person and choose course of action	Are rights of all individuals considered and respected?
Privacy	Control over one's body and personal information, and freedom from interference with personal choices	Protecting confidentiality is an important aspect of protecting privacy, but are there any limits? How does this principle stand up when such information may be necessary to save another's life?
Justice/equity	Fair, equitable treatment for all	Are the interests of all in the community considered and potential discrimination prevented? Are resources allocated fairly?

A PRACTICAL APPROACH TO THE ETHICS OF GENOMICS

An editorial that appeared in the scientific journal *Nature Genetics* in 2001 considered the advantages of a practical approach to the ethics of genomics, in contrast to the traditional, more abstract pursuit. In this vision, a practical approach would have as its goal real-world solutions to real-world problems and would involve maximizing benefits and minimizing risks. It is essential for citizens, patients, and commercial industry (such as genomics-testing companies), as well as researchers and policy makers, to discuss problems and develop solutions together.

A second component of this practical approach is the inclusion of a global perspective. This philosophy urges that the needs and circumstances of all people be taken into account and warns that it is unethical to allow genomics to develop in a way that ignores the disadvantaged.

Practical Ethical Concerns for Advocates

Many ethical questions and dilemmas, such as those in the following section, are identified by governmental, academic, and legal institutions. In our day-to-day work as advocates, we are often concerned with more practical and immediate ethical concerns such as those involving tissue donations. Tissue donation refers to the provision of blood, part of a tumor, or other biological specimen from our bodies for research purposes.

With tissue donation, a number of ethical concerns related to privacy and use become important. For instance, we want to ensure that our tissue will be used for research purposes only after all of the necessary medical tests have been done that pertain to our healthcare. Secondly, we want to know who will have access to our tissue and the data derived from it. We usually want to be sure that our tissue and data will be used to benefit research and not just for the benefit of a commercial company trying to sell a product. Furthermore, we want to know whether the tissue sample will be linked to any of our personal information. We want to be assured of privacy so that our insurers or employers cannot misuse our genetic information. We want to know who has control of our tissue and how to contact them. These immediate issues are important to us today and represent a practical approach to the ethics of genomics.

Federal and state guidelines have been developed to protect individuals who donate tissue or participate in other forms of research. For instance, privacy and security regulations have been issued under the Health Insurance Portability and Accountability Act (HIPAA), the National Institutes of Health, and voluntary standards.

A number of ethical controversies surround the use of tissue donated to research. One such controversy concerns the use of tissue that was donated years ago. That is, should researchers be required to obtain re-consent for use of this tissue even though the tissue may not be linked to current contact information? If individuals did not originally agree to have their tissue used for the kind of research being planned, it may be argued that re-consent is necessary. However, tracking down all of the individuals for each proposed project could be extremely expensive and, if some individuals agree to use and others do not, it could bias the research. Another ethical concern is the ownership of the tissue. In most states, the legal ownership status of donated tissue has not yet been resolved.

Ethical Questions Related to Genomics

The ELSI program and a number of other organizations have identified research questions that illustrate some of the prominent ethical issues emerging as a result of genomics. We have combined these with some specific ethical dilemmas into several categories on the next page. The categories are not mutually exclusive. That is, a given issue may fit into several categories. The following list was based primarily on possible issues that the ELSI program has identified as ones that they may address over the next five years. The list is not exhaustive, and is not meant to represent the relative importance of these questions over others. Instead, the examples are provided to give an idea of some pertinent ethical issues related to genomics and to provoke thought about them.

Privacy and discrimination issues

- What strategies should be used to balance the needs for privacy and safety of individuals and groups with the scientific goals of creating resources for DNA sequencing and human variation research?

- What are appropriate and inappropriate uses of genetic testing in the employment setting? For instance, are there conditions under which it might be ethical and/or legal to use genetic testing to identify those employees who may have a susceptibility to workplace hazards?

- What issues emerge from the collection, storage, and use of blood and other tissue samples, including collections by the military, civil and criminal justice systems, commercial entities, and federal and state public health agencies?

  - Should the government be allowed to retain samples of individuals' DNA in the same way it retains fingerprints?

- What are the implications of potential commercial applications resulting from the availability of genetic information about individuals and groups? For instance, should commercial companies have access to personal genetic data for targeted product marketing?

- How is the impact of genetic testing in clinical and non-clinical settings affected by concepts of race and ethnicity and other social or economic factors? For instance, will particular communities and groups be more vulnerable to employment discrimination based on genotype?

- Should we be required to disclose the results of genetic tests to our family members? If yes, under what circumstances? Should we only be required to disclose if the genes are for a serious disease that can be prevented or treated?

- What should be the policies regarding genetic screening of adopted children? Adopted children may benefit from knowing their biological parents' genetic make-up. What policies should govern their right to know versus the parents' (both biological and adopted) rights to privacy?

Societal and cultural issues

- What are the clinical and societal implications of identifying common polymorphisms that predict disease susceptibility or resistance? For instance, will genetic testing promote risky behavior in persons found to be genetically resistant to particular pathogens, such as HIV, or environmental hazards, such as cigarette smoke?

- What are the implications of obtaining genetic information for use in adoption proceedings and establishing child custody and child support?

- What are the implications of behavioral genetics for traditional notions of personal, social and legal responsibility? For instance, what role will the discovery of assumed genetic predispositions to violent behavior play in criminal prosecutions?

- How are individual views about the value of genetic research, the importance of access to genetic services, and the meaning and relevance of genetic information affected by concepts of race and ethnicity and by socioeconomic factors?

- In what ways are access to, and use of, genetic information and services affected by ethnicity, race or socioeconomic status?

- What are the most effective strategies to ensure that genetic counseling and other genetic services are culturally sensitive and relevant?

- Will the discovery of DNA variation influence current concepts of race and ethnicity?
- What new concerns are being raised by the commercialization and patenting of DNA sequence information in the public, academic, and private sectors?
  - What should be our policy regarding the patenting of genes? The following U.S. government (Human Genome Project Information) Web site provides pros and cons of patenting genes: **http://www.ornl.gov/sci/techresources/Human_Genome/elsi/patents.shtml**.
- What regulations should be placed on genetic testing? Should genetic tests be marketed directly to the public?

Educational issues

- What are the most effective strategies for educating healthcare professionals, policy makers, the media, students, and the public regarding the interpretation and use of information about genetic variation?
- What are the best strategies for educating healthcare providers, patients, and the general public about the use of genetic information and technologies?
- What are the potential uses and abuses of genetic information in educational settings? (e.g., Is placement of students on the basis of genetic data any more or less beneficial or harmful than tracking on the basis of traditional categories or classifications?)

Healthcare issues

- What are the potential risks and benefits of integrating genetic testing for complex diseases, behaviors, and other traits into healthcare?
  - For instance, what would happen if we took a genetic test when we were 50 years old and found that we had a 90% chance of developing Alzheimer disease by age 75? Today, there is no cure for Alzheimer disease. Should such tests be available when no cure for the disease is known? Do we have the right to decide if we want to know our risk of disease regardless of whether there are measures that can reduce our risk?
- Will the availability of genetic information influence provider practice, change patient behavior, reduce morbidity and mortality, and/or reduce healthcare costs?
- What should be the goal(s) of genetic counseling? Should counseling be mandatory for all types of tests? For some types of tests?
- Should there be rules or laws regarding a physician's duty to inform us about the availability of a genetic test? Will there be inequities in who receives genetic tests? This is sometimes referred to as the "failure-to-test" issue.
- What if we have received successful treatment for cancer and a genetic test identifies us as "likely to recur"? Should the physician be required to give us this information even if there is nothing we can do to prevent it?
- What if a genetic test identifies us as a non-responder to current standard of care? Should we be recommended for an experimental treatment on the basis of that test? Should the standard-of-care treatment be withheld on the basis of the genetic test? Should an insurer be able to refuse coverage of standard-of-care treatment on the basis of the genetic test?

Genetic enhancement issues

- What are the implications of genetic enhancement technologies for conceptions of humanity?

    - Will scientists be able to clone humans? Today, this is not possible. But what if it becomes possible? Should this be allowed? What if our child or grandchild were killed in a car wreck — should this be allowed then?

    - What are the implications of passing on genes to our offspring that have been altered for disease resistance?

- Should parents be allowed to use genetic techniques to select traits in their children? Consider parents who are carriers for a serious genetic disease. Should these parents be allowed to select an embryo (fertilized egg) for implantation that does not have the disease? Should parents be able to select the sex of their children?

THE ECONOMICS OF GENOMICS

The advances in genomics are not only accompanied by ethical implications, but also economic ones. In fact, some prominent authors (e.g., Juan Enriquez, Director of the Harvard Business School Life Sciences Project) argue that the influence of genomics on our economy will be so profound that it will determine which countries and companies end up being dominant over the next 50 years. On both the private and public level, the United States and many other countries are making huge financial investments in genomics. Large portions of these investments are directed toward health-related genomics research, including the development of new assessments and technologies.

However, economic issues in genomics go beyond the research and development of tests and treatments. Economic concerns also relate to the delivery of and access to gene-related services, health insurance, and issues of gene discovery (such as licensing genes).

In this section, we will consider a few of the economic aspects of genomics.

Publicly funded genomics research

In addition to the Human Genome Project and the HapMap Project that we've already discussed, the U.S. government funds many smaller health-related genomics projects. These include developing research resources, identifying genes that may be important in disease, developing genomics technologies building on basic research (such as new tests and therapies), and sponsoring clinical trials. The government also funds research and study into the ethical, social, legal, and economic aspects of genomics.

Genomics-based companies and nonprofit institutions

Many private companies are based on genomics platforms. These include pharmaceutical companies that focus on genomics research to develop their drugs, as well as biotechnology companies that develop genomics technologies (e.g., microarrays). Other companies develop genetic tests using genomics methods. Nonprofit institutions such as the J. Craig Venter Institute also raise and spend millions of dollars studying genomics.

Insurance and other issues in genetic testing

As more genetic tests become available, health insurance companies are increasingly faced with decisions as to whether, and under what circumstances, they should pay for such tests. Some tests are likely to be very expensive. Insurance companies will be concerned about balancing benefits and costs. Concern about possible inequity in access to genetic tests has been voiced by many groups. They argue, among other things, that the Human Genome Project is for all of us and we should all be able to reap its benefits. The goal is to make genetic tests widely available and this is an area of active discussion and debate.

A few additional economic issues are listed below:

- Licensing, royalty charges, and other issues associated with gene patenting
- Funding the development and commercialization of genomics-based tests
- Allocation of national/international resources to genomics-based research
- Costs of large-scale clinical trials to assess and validate genomics technologies
- Genomics information and health insurance

  The following Web sites provide more information about the ethical, legal, and social issues associated with genomics.

- National Human Genome Research Institute, ELSI Research Program:
  General Web site: **http://www.genome.gov**
  Specific link: **http://genome.gov/PolicyEthics**

- U.S. Department of Energy, Human Genome Project Information, Ethical, Legal, and Social Issues:
  General Web site: **http://www.ornl.gov**
  Specific link:
  http://www.ornl.gov/sci/techresources/Human_Genome/elsi/elsi.shtml

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HOW CAN ADVOCATES USE THIS INFORMATION?

WHY IS IT IMPORTANT FOR ADVOCATES TO KNOW ABOUT GENOMICS?

In the preceding chapters, we have described the basics of genomics and the application of genomics to cancer. This information can be valuable to us as advocates in understanding the language used by the physicians and researchers with whom we interact. Because we now know that cancer is essentially a genomics problem, this language is heard more and more frequently in all areas of cancer treatment and research. Knowledge of genomics will help us interact more confidently with these individuals. As a result, we gain credibility for our viewpoint. It may be helpful to remember that most physicians were not formally trained in genomics. However, most physicians have the basic knowledge of genomics that is presented in this training manual.

In addition to increasing our ability to "talk the talk," knowledge of genomics can help us ask more informed and pointed questions as we participate in research advocacy, policymaking, and other activities. Such knowledge may also help us suggest improvements to the current research system. As advocates, we are in a unique position to understand what genomics research means to individuals with cancer and other diseases. We have critical insider knowledge that makes us uniquely qualified to assess at least four important issues pertaining to genomics. These issues are summarized in the following figure.

SOME IMPORTANT ISSUES SURROUNDING GENOMICS THAT ADVOCATES ARE UNIQUELY QUALIFIED TO ADDRESS

COMPROMISES
What sorts of compromises are reasonable for individuals with disease?
(e.g., In donating tissue, how much loss of privacy are individuals willing to risk in order to benefit future patients?)

OUTCOMES
What outcomes are most important for individuals with disease?
(e.g., Can individuals easily obtain access to their tissue sample if a new biomarker is found that could help them or their families?)

PRACTICALITY
Are the proposed genomics tests feasible for individuals with disease?
(e.g., financially, timewise)

SAFEGUARDS
Are individuals who take these tests protected from harm, within reason?
(e.g., Are protections in place to guard medical information attached to tissue samples? Are specimens totally de-identified? Are they coded? Can the data and tissue donor be re-identified?)

A number of training modules and programs exist to help prepare advocates for roles in research and policy.

- The Research Advocacy Network's Advocate Institute (**www.researchadvocacy.org**) provides information about donating tissue, communicating with members of the research community, and sets of SkillBuilders and ScienceBuilders. The Web site also contains information about other advocate organizations and how to get involved in advocacy, including contact information for groups that you may be interested in joining.

- The National Breast Cancer Coalition (**www.natlbcc.org**) sponsors a program called Project LEAD, an intensive 4-day program designed to provide basic scientific knowledge to strengthen and empower breast cancer activists. The following link may be useful: **http://www.natlbcc.org/bin/index.asp?Strid=483&btnid=4&depid=7**.

- The American Association for Cancer Research (**www.aacr.org**) Scientist/Survivor Program, associated with their annual meeting, is designed to promote communication and unity among the scientific and cancer survivor/patient advocacy communities. Advocates are matched with scientist members with the goal of promoting partnerships, enhancing communication, and increasing mutual understanding. Advocates attend tailored lectures, participate in small group meetings, and have one-on-one meetings with scientific mentors. An expanded curriculum is available in a 4-day workshop. More information is available at the following Web site: **http://www.aacr.org/page3782.aspx**.

- The Coalition of National Cancer Cooperative Groups sponsors a booklet and interactive curriculum entitled "A Guide to Cancer Clinical Trials" (**www.cancertrialshelp.org**) that explains cooperative groups, cancer clinical trials, drug development, and other topics. This is available at the following Web site: **http://www.cancertrialshelp.org/selfStudyGuide_public.jsp**.

- The National Cancer Institute's Education Series is designed to educate people about cancer clinical trials. Materials available on the Web site include a basic workbook, an in-depth training guide, an advocacy guide, and a trainer's guide. Information is available from the National Cancer Institute's homepage (**www.cancer.gov**) or the following link: **http://www.cancer.gov/clinicaltrials/learning/clinical-trials-education-series**.

- The American Society for Clinical Oncology (**www.asco.org**) sponsors a Communications and Patient Information Department that is responsible for ASCO's patient advocate relations and programs. The Annual Meeting Patient Advocates Program components include: discounted registration rate, ASCO-sponsored patient advocacy booth, individual patient advocacy booths, an advocate reception, a mentor program, and a patient advocates lounge. ASCO also has a Patient Advocate Program in place for the Society's Small Meetings, which includes: reduced registration rate and a discounted rate for patient advocacy organizations exhibits. ASCO cancer advances are summaries of recent findings presented at the annual ASCO meeting. These summaries are written in lay language and are reviewed by medical experts. The summaries can be found on the following Web site: **http://www.asco.org/ac/1,1003,_12-002138,00.asp** searching for "cancer advances patient information."

FINDING OUT ABOUT THE LATEST DISCOVERIES

In order to be as effective as we can be, it may be beneficial for us to keep up on the most recent developments in cancer research.

- National Comprehensive Cancer Network (NCCN; **www.nccn.org**) Guidelines are a set of recommendations developed by the NCCN that are based on scientific evidence and are reviewed and revised annually. These well-respected guidelines relate to screening, treatment, and symptom management. The NCCN Web site contains a section called "Treatment Guidelines for Patients" that can be accessed online or ordered as a booklet.

- The Wellness Community (**www.thewellnesscommunity.org**) sponsors a series of materials that describe new discoveries in cancer entitled "Frankly Speaking About New Discoveries in Cancer." These slide sets can be viewed on the following Web site: **http://www.thewellnesscommunity.org/programs/ frankly/newdiscoveries/newdiscoveries_home.htm**.

HOW CAN ADVOCATES MAKE A DIFFERENCE?

Advocates can and do make a difference in the practice, research, and politics of medicine. As a result of breast cancer advocacy groups, federal spending on breast cancer research has increased enormously. For instance, in 2005 the National Cancer Institute spent $605 million on breast cancer research — more money than was spent on any other cancer. Advocates have played a big role in bringing research funding for breast cancer to the forefront. Advocates have also influenced the nature of cancer research, bringing the patient perspective to the table and a sense of urgency to research. Following are some examples of advocate accomplishments:

- *1995:* Advocates became voting members on the Department of Defense's cancer research program review panels.

- *1995:* Advocates became members of the U.S. Food and Drug Administration's cancer drug advisory committee; became voting members in 1998.

- *1998:* The National Cancer Institute established the Director's Consumer Liaison Group to provide the advocate's perspective to the Director.

- *2000:* The number of advocates on review panels of the Susan G. Komen Breast Cancer Research Foundation increased from 7 to 24; advocate scores began to be included in the ratings of grant applications.

Source: National Cancer Institute. Cancer advocates in research: the movement evolves. Available at: NCI: http://www.cancer.gov/clinicaltrials/developments/advocates0101. Accessed February 26, 2006.

Advocates can make a difference in many ways and using many styles, as a group or as individuals. For instance, any individual can join a grassroots campaign that ultimately exerts a powerful influence through collective action. The National Breast Cancer Coalition (NBCC; www.natlbcc.org) organizes many grassroots campaigns. For instance, the NBCC sponsors a campaign entitled "Personal Stories, Public Action" in which breast cancer survivors share their healthcare experiences to help others who have been diagnosed. For more information, contact NBCC at (800) 622-2838 or visit the following Web site: http://www.natlbcc.org. By developing these stories and additional communication aids, advocates can help others navigate the healthcare system. Thus, the healthcare system itself represents a place for advocates to exert influence.

> Advocates can make a difference in many ways and using many styles, as a group or as individuals. The important thing is to get involved today.

Many additional opportunities exist for advocates who are willing to play higher-profile, individual roles such as participating on committees. Some of us may prefer to begin our journey as advocates in a collective role and later switch to a role in which we have more individual responsibility. Some of these individual roles follow.

WAYS TO GET INVOLVED IN ADVOCACY TODAY

Today, one of the most important ways that we can influence cancer treatment is by participating in the development and oversight of research. It is through research advocacy that we can truly influence the future of cancer care. Genomics technologies are developing rapidly and will benefit greatly from advocate involvement. For instance, today, only about 5% of individuals diagnosed with cancer participate in clinical trials. These numbers must increase drastically to ensure that beneficial new tests and treatments become part of healthcare practice within the next decade.

Advocates can be critically influential in increasing awareness of, and enrollment in, clinical trials. Advocate roles span many different sectors of research, from federal policy to local committees in developing and approving research protocols. Following are some of the research-related activities in which advocates are needed today. To find out more about advocate participation in these organizations, contact local research organizations directly or contact the Research Advocacy Network via e-mail at info@researchadvocacy.org.

Institutional review boards (IRB)

Institutional review boards are independent committees made up of individuals with diverse medical and non-medical backgrounds that review and approve all documents related to all studies that involve human participants at a research institution. Every major institution that conducts research with human participants and has federal funding of their research must have an IRB. The primary purpose of the IRB review is to protect and assure the safety, rights, and welfare of study participants. An IRB approves a variety of different materials, including study protocols, informed consent forms, and participant recruitment materials. The IRB can approve or disapprove research or can require modifications that it feels are in the best interest of the participants. IRBs typically consist of researchers, physicians, students, community members (non-affiliated members, non-scientific members) who are not research experts, ethics experts, and/or lawyers. Because IRBs provide local protections, they are an ideal place for patient advocates.

Protocol review committees

Protocol review committees evaluate, approve or reject, monitor, and re-review human research protocols. A protocol is a description of the way a study is performed, including the number and characteristics of individuals to be enrolled, the procedures that participants undergo, and how the data are collected and analyzed. Protocol review committees may also examine whether the research study is designed to answer an important question, whether the study is adequate to answer the question, and whether the study can actually be completed as designed. Protocol review committees are distinguished from IRBs because they do almost all their work before the protocol reaches the IRB and they are focused on the research design, methods, and scientific merit, whereas IRBs are focused on the protection of participants. Protocol review committees have a similar member composition as IRBs; that is, they include research experts and may include advocates.

Data and safety monitoring boards (DSMBs)

Data and safety monitoring boards oversee the conduct of clinical trials to ensure the safety of participants and the validity and integrity of the data. These boards monitor data that are generated to make sure that the treatment isn't unexpectedly harming subjects and to assess whether the treatment is so valuable that the trial should be stopped and everyone given the new treatment. DSMBs also close trials because of slow accrual. Data safety and monitoring boards are required by the National Institutes of Health (NIH) for all of the Phase III studies they support financially.

Study sections (National Cancer Institute)

Study sections are part of all divisions, including the National Cancer Institute. Study sections are groups of individuals organized around scientific areas, which review applications for federal research grants within each area. The money for these grants comes from our tax dollars. The study sections are made up of scientific experts who are typically peers (i.e., they are researchers themselves) and, more recently, advocates and others such as lawyers. Study sections make recommendations as to the priority of the research, by way of a score, which is then taken into account by the National Cancer Institute in deciding which research to fund.

Patient representative or advocacy committees/working groups in cooperative groups

These committees have been established by various oncology cooperative groups such as the Eastern Cooperative Oncology Group (ECOG) to incorporate the patient perspective into their organization and operation. Nearly all Phase III trials currently being designed in the Cooperative Groups incorporate a correlative science component in addition to the clinical component. The scientific component typically involves the study of participant tissue for clues about cancer — what causes it, which treatments might work, etc. Advocates are key to improving researchers' understanding of patient concerns about donating their tissue for cancer research.

Specialized Programs of Research Excellence (SPOREs) sponsored by the National Cancer Institute

These programs were established in 1992 to promote interdisciplinary research and facilitate exchange between basic and clinical science. The goal of these programs is to incorporate novel ideas for reducing cancer incidence and mortality found in the laboratory into clinical research that will improve survival and quality of life for cancer patients.

Advocates can participate in SPORE's Patient Advocate Research Team (PART) Program. The PART Program helps SPOREs build effective collaborations with cancer patient advocates. Most SPORE patient advocates are willing to make a personal commitment to work directly with cancer researchers in a specific SPORE program.

Currently, patient advocates work with approximately 50 of the 58 SPOREs programs throughout the U.S. While most SPOREs include patient advocates at an advisory level, at least 25 SPOREs are involving patient advocates at an operational level. SPORE patient advocates can be involved in a wide range of activities, including:

- Input on strategic direction
- Grant reviews
- Tissue issues: consent forms, collection processes, usage, patient follow-up procedures
- Clinical trials
- Development: input on design, informed consents, IRB representation
- Accrual: letters on clinical trials, presentations to patient groups
- Adherence: support and guidance for patients, input for amendments
- Education: forums and "training days" for patient advocate community; presentations in professional training courses (to scientific staff), newsletter

More information can be obtained from the homepage (**www.nci.nih.gov**) or at the following link: **http://spores.nci.nih.gov/**.

Consumer Advocates in Research and Related Activities (CARRA)

The National Cancer Institute (NCI) of the National Institutes of Health has established a novel program designed to integrate the views of the cancer community in directing Institute activities and research. Consumer Advocates in Research and Related Activities (CARRA) includes roughly 200 cancer survivors and consumer advocates from across the country. The members of CARRA are diverse in terms of the types of cancer with which they are familiar, their age, and ethnic origin. CARRA members not only serve as an integral part of NCI activities, but also as liaisons linking back to the cancer community networks. CARRA members serve 3-year terms and are called upon as needed for input into particular activities, including peer review of grant applications and evaluation of information geared toward the cancer community. CARRA was developed to make the input of advocates more systematic and routine and to facilitate two-way communication between the Institute and the community. More information can be obtained from the homepage (http://carra.cancer.gov).

Patient Representative Program at the U.S. Food and Drug Administration

In this capacity, advocates provide the FDA and the advisory committee, patient and family perspective on issues, problems, and questions related to the viewpoint of patients and family members living with a specific serious or life-threatening disease. Advocates may be voting or non-voting members of the advisory committee. More information can be obtained from the FDA's homepage (www.fda.gov) or at the following link: http://www.fda.gov/oashi/patrep/patientrep.html.

ETHNICALLY- OR CULTURALLY-BASED INITIATIVES

A number of ethnically- or culturally-based initiatives have been undertaken to provide support, information, and programs for specific groups of people who are united by a common heritage. Such initiatives are particularly important in genomics, as certain genetic variations may be more common in some subgroups of individuals than others. Following is a non-comprehensive list of some ethnically- or culturally-based initiatives.

Genetic Education for Native Americans (GENA)

Genetic Education for Native Americans is a project designed to provide culturally competent education about genetics and genetic research to Native American college and university students. The secondary goal is to increase the number of Native people who have access to scientific mentoring experiences in genetic counseling, education, research, and other opportunities or careers.

The GENA project is comparing two variations of a newly designed culturally relevant genetic education program and provides mentoring opportunities to Native American students who are interested in genetic education, research and medicine. More information is available at the following Web site: http://members.aol.com/natamcan/gena.htm.

Native American Cancer Survivors' Support Network

Native American Cancer Survivors' Support Network offers social and emotional support and culturally specific educational materials to cancer patients and survivors. It conducts one-on-one interviews with survivors to learn more about how they are dealing with their cancer and how their care can be improved, and it connects members with other Native survivors who have had similar experiences. An important activity is the support of a confidential database to monitor members and track the types of cancer and cancer care. More information can be found at the homepage www.natamcancer.org or at the following link: http://natamcancer.org/community.html.

Redes En Acción: The National Hispanic/Latino Cancer Network

Redes En Acción is an initiative designed to combat cancer among Latinos. This National Cancer Institute-funded initiative focuses on cancer prevention and control by building a nationwide network of community-based organizations, research institutions, government health agencies, and the public. Core activities include promoting training and research opportunities for Latino students and researchers, generating research projects on key Latino cancer issues, and supporting cancer awareness activities within the Latino community. More information is available at the following Web site: http://www.redesenaccion.org/.

Sisters Network, Inc.

Sisters Network, Inc. is a national African-American breast cancer survivorship organization with 39 affiliate chapters around the nation. It provides support, breast education programs, resources, information, and research through its affiliated chapters. In 1999, Sisters hosted the nation's first national African-American Breast Cancer conference to specifically address the impact of breast cancer among black women, which is now in its sixth year. Community outreach programs include "The Gift for Life Block Walk™", The Pink Ribbon Awareness Campaign, STOP THE SILENCE: Changing the Face of Early Breast Health Intervention, and The S.P.I.R.I.T. program, a national partnership with the UT M.D. Anderson Cancer Center. More information is available at the following Web site: www.sistersnetworkinc.org.

Intercultural Cancer Council

The Intercultural Cancer Council (ICC) promotes policies, programs, partnerships, and research to eliminate the unequal burden of cancer among racial and ethnic minorities and medically underserved populations in the United States and its associated territories. The ICC works to facilitate the access of minorities and medically underserved populations to the healthcare system. It also strives to include survivors, minorities and culturally diverse individuals in the development of health policies and programs intended for their communities. More information is available at the following Web site: www.iccnetwork.org.

Research advocacy possible scenarios

Next we consider a few possible scenarios to show how research advocates may be involved with various groups.

- Elaine is a 15-year survivor of cancer and a community member of the Protocol Review Committee at the University of Michigan. Elaine has just reviewed a research study that an investigator at the university wants to conduct. This study examines the effects of a new treatment called X therapy that would be given to participants in addition to their standard chemotherapy. Although preliminary research has found that X therapy may help keep the cancer from returning, it is also associated with the side effect of muscle weakness. The researcher proposes to record whether or not participants experience muscle weakness. However, Elaine is concerned about the effects that muscle weakness may have on the participants' daily lives. Elaine not only wants to know whether muscle weakness occurred, but how disruptive it is for the participants. In the meeting, Elaine brings up this point and suggests that the researcher add a way to measure this, possibly by incorporating a questionnaire about daily activities into the research protocol. The researcher agrees that this is a good idea and an easy thing to add.

- Sarah has just joined the Eastern Cooperative Oncology Group as an advocate on a Patient Representative Committee. Sarah and her colleagues are discussing a study that is planned to determine the validity of a genetic test. The genetic test is supposed to determine whether an individual's cancer will respond to a new treatment Y. As with all studies done in people, informed consent must be obtained. That is, the study must be thoroughly explained to each potential participant in a language that they can understand. They must be able to understand both the risks and benefits of the test. Sarah has just completed the genomics training manual and now has a basic understanding of genetic tests. She offers to lead the group in developing a patient education brochure that will be used in the consent process.

- Leo has just been asked to serve as a representative patient advocate on the National Cancer Institute's study section that evaluates research grant proposals on genomics screening tests. For the meeting, Leo is asked to review 4 proposed research studies examining various types of tests. One of the studies proposes the use of a tissue preparation method for the genetic test that is costly and complex for routine medical use. Specialized equipment must be provided to the research investigators and they must undergo a lengthy training session in order to learn how to prepare the tissue. Leo knows that many other genetic tests do not require this type of extensive tissue preparation. In the meeting, Leo reviews the grant proposal. During his review, he expresses concern that the complex tissue preparation technique is unlikely to be useful in clinical practice. Several members who are researchers disagree that this should be an issue when judging the grant proposal. Leo reiterates the patient perspective that the ultimate goal of this research is for the test to be used in clinical practice. The two researchers continue to disagree with Leo and, in the end, the grant is recommended for funding.

Thoughts for the Future

Advocates are poised to have an increasing presence in and influence on medical research in the coming years. Some of the future issues that advocates may be involved in include the following:

- How can research results best be accepted into clinical practice? Are there ways to make this happen faster?

- What are the clinical outcomes that are most important to individuals with disease? Are these being incorporated into clinical trials?

- What sort of validation should be required of genomics tests?

- How can individuals with disease be encouraged to participate in clinical trials that may benefit them?

- How can we maximize the information gained from tissue samples while still protecting the individual with disease?

- How can we expand the adoption of genomics into clinical trials? (see table on next page)

- How can we network with a diverse group of medical professionals and organizations such as pathologists, biospecimen repositories, IRBs, and others?

HOW CAN ADVOCATES INFLUENCE THE ADOPTION OF GENOMICS INTO CLINICAL TRIALS?

Raise genomics issues during trial design phase	Are researchers thinking about this? Advocates are.
Help weigh cost-benefit considerations	Including genomic analyses in clinical trials will increase costs but potential benefits to patients are significant. For example, findings may reduce treatment costs in the long run by making it possible to target treatments to patients who are likely to respond; savings also in terms of reducing patient adverse events; improving patient satisfaction with decisions about therapy. An example of how costs can be reduced by targeting therapies to a subgroup with a certain genomic profile occurred with the drug Herceptin®. By dividing groups into those who were HER2/neu-positive and those who were HER2/neu-negative, Genentech saved an estimated $35 million in clinical trial costs, and earned an estimated $2.5 billion from the acceleration of its product. An estimated 120,000 patients were able to receive the drug as a result of the positive effects reported in the HER2/neu-positive subgroup (from Press MF, Seelig SA. Lesson Learned From the Development of a Diagnostic to Predict Response to Herceptin, 2004 Targeted Medicine 2004 Conference Presentation). Cited in Frueh F. PGx and biomarkers: Current role in drug development. Available at: http://www.accel-eratingworkshop.org/workshop/Workshop_Materials/. Accessed September 22, 2005
Help weigh ethical considerations of genomic testing	If advocates don't represent the patient perspective, it will be left to ethics experts. Advocates can help keep the discussions reality-based and goal oriented
Help protect patient confidentiality	Advocates can evaluate the proposed protections and identify other methods for maintaining confidentiality, and help streamline institutional policies to make it easier for researchers to adhere to
Help word consent forms so that patients understand genomic testing	Advocates can help ensure that the language used is understandable to study participants and all scientific terms are defined
Convey a sense of urgency to including genomics	The presence of advocates reminds researchers of the urgency of including new technologies such as genomic screening; human lives are at stake
Keep the underserved populations in the picture	Advocates can represent or remind researchers of the different cultural views of donating tissue and how to respect those views

Evidence-based Advocacy

In a 2003 article published in the *Journal of Clinical Oncology*, Musa Mayer describes evidence-based advocacy and contrasts it with access-based advocacy. Evidence-based advocacy is a strategy in which advocates base their efforts on available medical evidence, and, in the absence of the evidence, urge physicians and researchers to conduct more rigorous studies. In contrast, the philosophy of access-based advocacy is that the rapid approval of new drugs is crucial. The focus is on obtaining early access to investigational drugs for patients with advanced cancer who have run out of approved treatment options.

In her article, Ms. Mayer argues that new treatments are not always better than current treatments and may be associated with an increased risk of severe or even lethal side effects. She argues that emotion needs to be set aside in favor of decision making based on medical evidence. Although this approach may actually delay access to a particular treatment, she suggests that it may be the best way of assuring the development and approval of treatments that are at least as safe and effective as the current standard treatment; in her words, "evidence should not be sacrificed for speed."

Breadth of Advocacy Opportunities

Opportunities for advocate participation are growing, with an increasing number and type of organizations seeking the advocate's perspective. Listed below is a wide variety of organizations that have expressed interest in engaging advocates. These do not necessarily represent immediate opportunities for advocate participation but serve to illustrate the breadth of interest in including advocates in their activities. More information about these organizations can be gleaned from typing their names into a Web browser and reading about their goals and needs.

International health organizations

- WHO: World Health Organization, Office of Genomics and Disease Prevention
- United Nations Health Office
- Cochrane Collaboration

Governmental organizations

- FDA: Food and Drug Administration, Patient Consultant Program and Office of Special Health Issues
- National Cancer Institute
- U.S. Department of Health & Human Services
- OHRP: Office for Human Research Protections
- CDC: Centers for Disease Control and Prevention, Office of Genomics and Disease Prevention
- AHRQ: Agency for Healthcare Research and Quality
- NAS: National Academy of Sciences
- CMS: Centers for Medicare and Medicaid Services

Industry organizations

- BIO: Biotechnology Industry Organization
- PhRMA: Pharmaceutical Research and Manufacturers of America
- AdvaMed Medical Technology Association

Professional organizations

- ACLA: American Clinical Laboratory Association
- American Association for the Advancement of Science
- IOM: Institute of Medicine
- ASCO: American Society of Clinical Oncology
- ONS: Oncology Nursing Society

Insurance/managed care organizations

- Blue Cross/Blue Shield Association
- American Association of Health Plans

Community organizations

- Association of Community Cancer Centers

Non-profit research and policy development organizations

- The Commonwealth Fund
- The RAND Foundation
- IHPS: Institute for Health Policy Solutions
- The National Quality Forum

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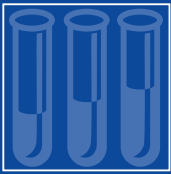
APPENDIX

GENETIC ASSESSMENT TECHNIQUES

This appendix covers some of the genetic assessment techniques in more detail and explains how some of them work. Genetic tests can be grouped into at least 3 categories based on what molecule they are designed to detect, as shown in the following table: DNA tests, RNA tests, and protein tests.

SOME TYPES OF GENETIC TESTS		
DNA TESTS	RNA TESTS	PROTEIN TESTS
• Routine cytogenetics • Chromogenic *in situ* hybridization (CISH) • Fluorescence *in situ* hybridization (FISH) • Comparative genomic hybridization (CGH) • DNA microarrays • Oligonucleotide arrays • Classic gene-sequencing methods • Gel and flow cytometry • Southern blots • Microsatellite instability tests • Polymerase chain reaction (PCR) sequencing	• Northern blot • Reverse-transcriptase PCR (RT-PCR) • *In situ* hybridization (including FISH and CISH)	• Two-dimensional gel electrophoresis • Matrix-assisted laser desorption ionization time of flight (MALDI-TOF) • Surface-enhanced laser desorption and ionization time of flight (SELDI-TOF) mass spectroscopy • Immunohistochemistry (IHC)

Adapted from Ross JE. Molecular diagnostic techniques in breast cancer. In: Ross JS, Hortobagyi GN (Eds). Molecular Oncology of Breast Cancer. Sudbury, Mass.: Jones and Bartlett Publishers, Inc. 2005:12-33.

METHODS USED TO DETECT DNA

DNA probes

DNA probes are one of the most common tools used to investigate the sequence of specific genes. The probes work on the basic principle of DNA nucleotide base pairing — namely, that each letter in the DNA pairs with only one partner: A with T and C with G. The probes themselves consist of short strings of DNA whose letter sequences will stick to or pair with the letter sequences in a gene. So, to detect a faulty gene that you know is spelled CCCCCCC, you could send in a probe that is spelled GGGGGGG. The Gs will stick to the Cs but not Ts or As.

The probes, which are attached to fluorescent markers, are mixed with DNA sequences obtained from blood or tissue samples and then washed off. If the probe located a matching DNA sequence, it will have stuck to it and won't wash off. If the visible marker is seen in the sample after washing, then the faulty sequence is found in the sample. If the probe did not find a matched DNA sequence, it will wash off and there will be no visible markers.

> ### DNA Probe
>
> Short strings of DNA whose chemical sequences will stick (hybridize) to or pair with the matching letter sequences in a gene (i.e., G with C and A with T).

An Example of Genetic Testing

Probe   Gene

A — T
T — A
G — C
       C
       A
       A
       G
       G
       T
       A
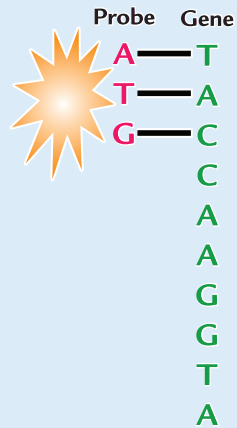
If you want to detect the mutation TAC, you could send out a probe containing ATG. The probe will bind to the sequence TAC. Attached to the probe is a chemical that makes it visible under certain conditions. If your DNA "lights up" after being exposed to the probe, it indicates the presence of the TAC mutation.

In reality, the probes are much longer than just 3 base pairs.

DNA microarrays

DNA microarrays were developed to allow the detection of thousands of genes at once, which makes them useful in genomics. Sequences of DNA from sections of genes (DNA probes) are "arrayed" or spotted on a very small surface (usually glass), about the size of a typical microscope slide or smaller. The DNA microarray actually looks like thousands of tiny dots arranged in precise rows and columns. Each dot contains a single DNA probe or a string of DNA with a nucleotide base sequence that is designed to pair with the one being detected. Because there are many spots for probes, many different DNA sequences can be detected at once. This allows so-called "high throughput" or the analysis of many genes in parallel. These assessments are known by a number of different names, including genome chip, GeneChip® (actually a trade name of a specific product), and gene array. Arrays can be placed on other

surfaces besides glass. Bead arrays, capillary arrays, and well arrays all work the same way. That is, strands of DNA whose nucleotide sequence is known are attached to the array surface, allowing thousands of genes or even the whole genome to be examined in a single experiment.

Once all of the DNA probes are placed in the microarray, a sample of DNA is spread over all of the spots at once. The DNA in the sample is then allowed to pair or bind with the DNA probes. Another name for this binding is hybridization. If the gene is not present in the DNA sample, no binding will occur in that spot. If the gene is present, binding will occur in that given spot. Bound and unbound DNA is then detected by fluorescence (or light emission) following laser excitation. If the gene is present, it will "light up" on the specific spot. The computer knows which spot corresponds to which gene and can identify the presence of each one in the DNA sample. Different technologies use one color, two colors, or four colors in the detection scheme.

The first microarray was approved for clinical use in December 2004 to detect specific gene sequences in two genes associated with response to certain drugs. A lot of controversy still surrounds microarray technology. One major point of debate concerns the length of the DNA strands that are stuck to the array; some argue in favor of longer strands and some shorter. Scientists also debate the number of duplicate experiments that should be performed in order to confirm results. Microarray technology is also relatively new and lacks standardization and reproducibility. The issues surrounding the use of microarray testing in the clinic are likely to be a focus of regulatory scrutiny for the foreseeable future given the potential importance of the information these tests can generate.

DNA Microarray

A technology and method used to detect many genes at once. The microarray consists of thousands of dots arranged in orderly rows and columns. Each dot contains a DNA probe or a known sequence of DNA and will stick to the genes we are trying to detect in our sample.

Image of a DNA Microarray (AKA: An Array "Heat Map")

This graphic shows a DNA microarray. It consists of tiny dots that contain DNA probes. Some of these dots are lit up, indicating that the sample contained the spelling of chemical letters that the test was trying to detect. The computer knows which DNA probes each dot contains. The different colors of the dots correspond to different levels of the DNA detected. Sometimes different colored dots are used to detect different genes being studied.

Image courtesy of U.S. Department of Energy Genomics: GTL Program, http://doegenomestolife.org

DNA Microarrays and Gene Expression Profiles in Breast Cancer

DNA microarrays are making the biological classification of human breast cancers possible. Differences in the active genes have been found between estrogen receptor-positive and estrogen receptor-negative tumors and between subtypes within these groups. The subgroups have been associated with different clinical survival rates and drug resistance profiles. As yet, the microarray analysis of breast cancer has not been incorporated into routine clinical use. Before this can happen, quality control and assurance must be established. Differences between microarray techniques, measurements, controls, and several other features must be resolved and the tests standardized.

Cytogenetic testing

Cytogenetic testing involves examining the number and structure of chromosomes. Cytogenetic testing is often divided into several types based on the phase of cell division in which a test is performed. Conventional or routine cytogenetic testing (also called karyotyping) detects structural or numerical chromosome abnormalities in cells during the phase of cell division called metaphase. Metaphase is a term used to refer to a specific stage of cell division during which the chromosomes are aligned.

Conventional cytogenetic tests involve taking cells from a certain area of the body (such as bone marrow — the spongy material inside our bones) and growing the cells in a test tube for at least one day. The cells that are dividing are then stopped or essentially "frozen" in the process of division. It is essential that the cells are dividing because only in this stage can the chromosomes be seen using a regular microscope. The dividing cells are then placed onto a microscope slide. Each chromosome is evaluated in multiple cells (usually at least 20 cells) by looking under a regular microscope. This type of test is often used to detect Down Syndrome, in which the affected individual has an extra copy of a chromosome. This genetic disease can be detected simply by counting the chromosomes.

Cytogenetics is also used for the typing of blood cancers such as leukemia. One example of this is a chromosomal abnormality called the Philadelphia chromosome, which is associated with leukemia in some children. This chromosome is named after the Children's Hospital of Philadelphia, where the tiny chromosome was discovered in bone marrow samples of children with leukemia. The presence of the Philadelphia chromosome is an indication for aggressive treatment. The Philadelphia chromosome (actually chromosome #22) is a result of chromosome rearrangement — an exchange of genetic material — between chromosome #22 and chromosome #9. This disrupts some of the genes on these chromosomes. Chromosomes for this test, as in other conventional cytogenetic tests, are examined during the phase of cell division called metaphase.

Cytogenetic Testing

Examining the number and structure of chromosomes.

Conventional Cytogenetic Tests

Assessments of structural or numerical chromosome abnormalities in cells during the phase of cell division called metaphase.

Fluorescence *in situ* hybridization (FISH)

FISH, or molecular cytogenetic testing, is a way to visualize and map (document the location of) genetic material, including specific genes or portions of genes. FISH is used to look for the presence, absence, relative positioning, and/or number of specific DNA segments under a special type of microscope called a fluorescence microscope. Unlike conventional cytogenetic techniques, FISH does not have to be performed on cells that are actively dividing. This is one of its advantages.

FISH uses DNA probes, which we described in the previous section. The probes are attached to fluorescent dye. The probes are allowed to bind with the chromosomes and are then viewed under a fluorescent microscope. When a probe binds to a chromosome, its fluorescent tag provides a way for researchers to see its location.

Fluorescence *In Situ* Hybridization (FISH)
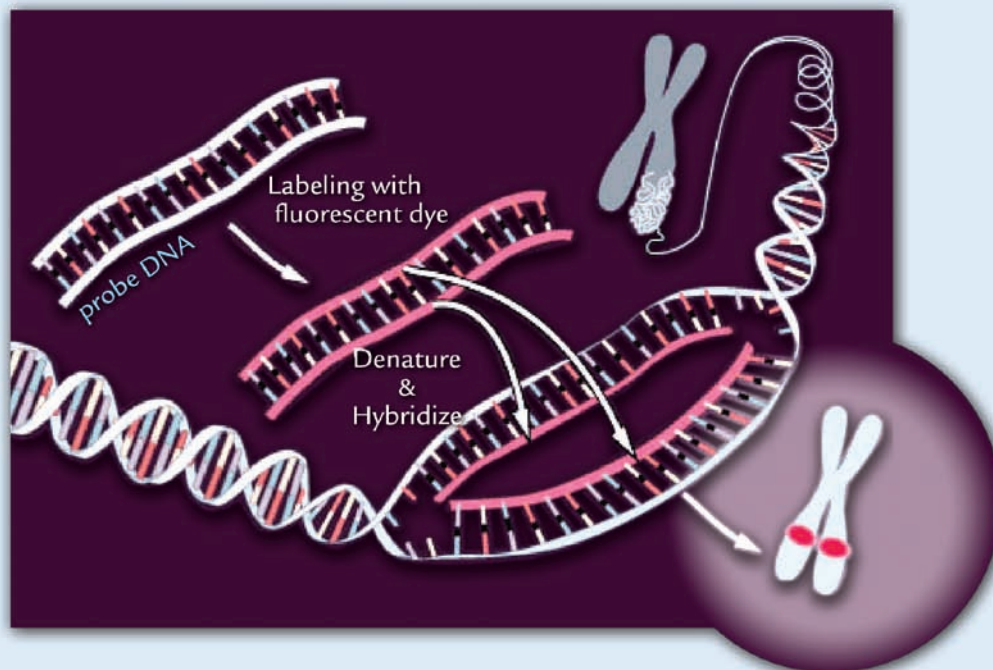
In FISH, a DNA probe is labeled with a fluorescent dye. The DNA is then denatured (i.e., the two strands are separated) so that the probe can bind to DNA on the chromosomes. Chromosomes are often represented as capital Xs, as shown here. If a DNA sequence of the probe matches a DNA sequence on the chromosome, it will bind. This binding can be seen as a fluorescent color under a special type of microscope. The red ovals on the chromosome in this graphic indicate the probe has paired with a specific sequence located on the lower portion of the chromosome.

Image courtesy of National Human Genome Research Institute (NHGRI).
Available at: http://www.genome.gov/10000206. By artist Darryl Leja.

Primers

A primer is a sequence of DNA nucleotide bases that serves as a starting point for DNA copying. The proteins that promote the replication of DNA, called DNA polymerases, cannot begin synthesizing a new DNA strand from scratch. They can only add to an existing strand of nucleotides. This is where primers come in: they begin the process and DNA polymerase then completes it.
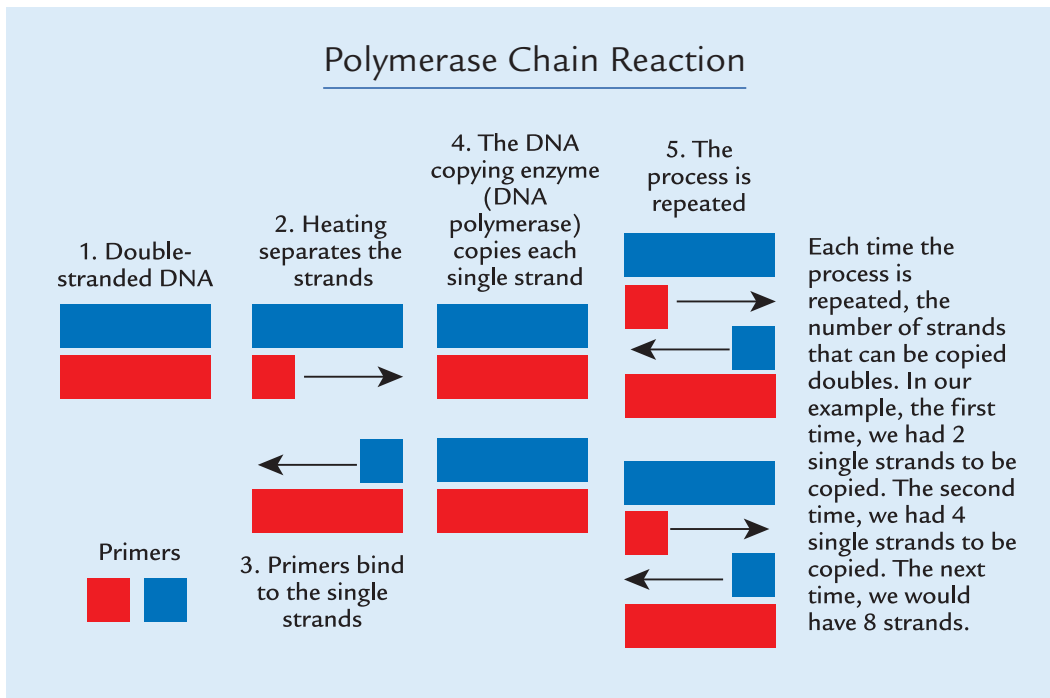
Polymerase chain reaction (PCR)

PCR is a laboratory method that uses primers and is often combined with other techniques. It has been referred to as "xeroxing DNA." In PCR, four components are placed together in a test tube. These ingredients are: 1) the sample DNA (e.g., the DNA from a tumor), 2) lots of nucleotides (As, Cs, Gs, and Ts), 3) an enzyme that is responsible for copying DNA, and 4) primers, short sequence of nucleotides which lie on either side of the DNA fragment of interest and signal the start of DNA copying.

The tube is then heated to separate the paired strands of the DNA double helix into two single strands. The test tube is then cooled, which permits the primers to pair with the single-stranded DNA. The tube is heated once again to a temperature at which the copying enzyme is most active. The copying enzyme, called DNA polymerase, then copies each strand of DNA. This process is repeated 20 to 30 times. By repeating this process about 30 times, 1 million copies can be made in about three hours.

With PCR, relatively small sequences of known DNA can be copied millions of times fairly rapidly. This is useful because it makes the DNA easier to detect. PCR is used with microarrays to copy the bits of DNA to be placed on the array. PCR is also useful for detecting minimal residual disease, in which a small amount of disease left after treatment may lead to recurrence but is typically not detectable with other techniques.

PCR is used to examine the DNA from humans or animals that have died — in many cases, long ago. DNA is remarkably stable. Often, small amounts of DNA can be obtained from tissues that nature has preserved in some way. In these cases, PCR can be used to make enough copies of the DNA so that it can be analyzed. For instance, PCR was used to amplify or copy the DNA found in the tissues of humans who lived 8,000 years ago in what is now Florida. This allowed scientists to determine that the humans were genetically different from today's Native Americans. Another example pertains to former vice president Hubert H. Humphrey. In the 1960s, Mr. Humphrey's tests for bladder cancer were negative, although he eventually died of the disease. In 1994, researchers analyzed a small amount of DNA obtained from a 27-year-old urine sample with the help of PCR. This analysis showed that Hubert Humphrey had an alteration in one of the genes that is now known to suppress tumors.

Primer

A sequence of DNA nucleotide bases that serves as a starting point for DNA copying.

DNA Polymerase

A protein that assembles new DNA by copying an existing strand.

Polymerase Chain Reaction

1. Double-stranded DNA

Primers

2. Heating separates the strands

3. Primers bind to the single strands

4. The DNA copying enzyme (DNA polymerase) copies each single strand

5. The process is repeated

Each time the process is repeated, the number of strands that can be copied doubles. In our example, the first time, we had 2 single strands to be copied. The second time, we had 4 single strands to be copied. The next time, we would have 8 strands.

> **Polymerase Chain Reaction**
>
> A method for making millions of copies of specific DNA.

METHODS THAT EXAMINE RNA

Reverse transcription-PCR

Reverse transcription (RT)-PCR is a technique that can be used to detect the degree to which genes are activated or expressed by making millions of copies of DNA from an RNA sequence. Regular PCR can be used to detect genes that are present but does not necessarily tell us whether they are expressed. This advantage of RT-PCR has made it a very useful addition to the techniques available for research and medicine.

RT-PCR detects RNA as opposed to DNA. RNA is the intermediate chemical that mediates the translation of a DNA sequence into proteins. RNA is built like DNA — a series of four chemical letter bases — except that one of its bases is different: RNA uses a base called uracil (U) instead of the thymine (T) in DNA. We really do not know why this substitution takes place, but the A in DNA pairs exclusively with the U in RNA, just as it does with the T in DNA.

RT-PCR uses the same steps as PCR, but instead of DNA, the sample (e.g., from a tumor, blood, urine, etc.) contains a type of RNA. RT-PCR is finding many uses in cancer research and treatment. Because RT-PCR can detect even low levels of genes that are activated, it is being used to determine whether cancers have spread to distant regions. RT-PCR is also used to detect the presence and activity of certain viruses such as human immunodeficiency virus (HIV).

> **Reverse Transcription-PCR**
>
> A method for making millions of copies of DNA from an RNA sequence, which tells us which genes are activated or expressed.

METHODS USED TO DETECT PROTEIN

Immunohistochemistry

Immunohistochemistry is a technique for identifying proteins. This technique takes advantage of the method our immune system uses to rid the body of foreign proteins — that is, it uses antibodies. In immunohistochemistry, a sample (e.g., blood, tumor, etc.) in which we are trying to find a specific protein is placed together with antibodies that are known to bind to that protein. The antibodies are labeled beforehand with some sort of marker, often a fluorescent one that can be seen under a fluorescence microscope. The antibodies are mixed with the sample in a test tube and given time to bind or pair. If the protein is present in the sample, the antibodies will bind and a colored label will be seen under the microscope.

This technique is often used to detect various protein markers that occur on some cancer cells (e.g., colon cancer and Hodgkin's disease). Immunohistochemistry is also used to determine subtypes of breast cancer. For instance, immunohistochemistry is used to detect estrogen receptor and progesterone receptor status. Receptors are parts of cells that can attach to certain substances such as hormones that circulate in the blood. Estrogen and progesterone are hormones that play important roles in the growth and treatment of breast cancer. Women with breast cancer that is estrogen and progesterone receptor-positive tend to have a better prognosis and respond better to hormone therapy than women whose cancers lack these receptors.

Serum proteomics

Serum proteomics is the study of proteins that are present in the liquid portion of our blood (also called serum). The types of proteins in our serum change depending on a variety of factors. One of these factors is disease. If we have a certain disease such as ovarian cancer, our bodies may produce proteins that they would not normally produce. If these could be detected in the blood, they could be used to assist diagnosis.

Early diagnosis of ovarian cancer is important because the prognosis is much better than if the disease is diagnosed in its later stages. Some researchers at the National Institutes of Health are working to find ways of detecting ovarian cancer in its early stages. One of the ways they are doing this is by looking for proteins in the serum. This research has led to the identification of so-called biomarkers (in this case, serum proteins) that may be able to determine whether women have ovarian cancer, even when the disease is in its earliest stages.

SELDI

The technique that the researchers used to find this proteomic pattern is called surface-enhanced laser desorption and ionization (SELDI). SELDI is a technique that separates proteins according to their mass (size) and electrical charges. SELDI gives a series of visible peaks on a computer. The larger the peak, the more protein in the sample. With SELDI, it is possible to examine 15,000 or more proteins at one time.

Immunohisto-chemistry

A technique that uses the antibody-protein reaction to identify proteins.

Serum Proteomics

The study of proteins present in the liquid portion of our blood.

QUANTITATIVE TECHNIQUES

Most of the techniques we have been talking about up to now are generally used to tell us whether the thing we are looking for (e.g., a gene, a chromosome, a genetic abnormality) is present in the sample. For instance, the techniques can answer questions like, "Does this sample contain the Philadelphia chromosome?" or "Is this sample estrogen receptor positive?"

Quantitative methods, in contrast, allow investigators to answer the question "how much?" This is important because we are often interested in how active a gene is or how much protein is present. In some cases, the difference between disease and health is determined by the amount of a substance instead of just its presence. This may lead investigators to ask questions like, "How many times more active is this gene than the normal gene?" Quantitative methods allow investigators to attach numbers to these types of questions. Studies can then be conducted to determine whether a certain amount of the substance (say, 70% higher than normal) is associated with a different prognosis, predicts response to treatment, or other clinical outcomes.

An example of a case in which quantitative techniques are useful is in the detection of prostate-specific antigen. Prostate-specific antigen is a protein produced by the cells of the prostate gland. Many men normally have low levels of prostate-specific antigen in their blood. However, high levels can indicate prostate cancer. The higher the level, the more likely that prostate cancer is present, although there are other reasons for elevated levels of this protein. In this case, a quantitative test is clearly needed. It is not enough to know whether the prostate-specific antigen is present in the blood. Instead, it is important to know how much of this protein is present. For this reason, the tests for prostate-specific antigen used in healthcare practices are quantitative.

There are many different quantitative methods. Some of these can be combined with the techniques we've already discussed. For instance, FISH or immunohistochemistry may use a fluorescent marker to determine whether the substance is present. The amount of fluorescent marker can be calculated using a computerized program. One of the most common tests for prostate-specific antigen uses an antibody that has been labeled with radioactivity. If the antibody binds to the prostate-specific antigen protein, the amount of radioactivity can be counted using special equipment.

Reverse-transcription PCR is also frequently used along with a quantitative method. This technique allows investigators to determine levels of specific RNA sequences in tissue samples. To make RT-PCR quantitative, special fluorescent markers are added in that stick to the newly made PCR product. The fluorescence can then be quantified.

> **Quantitative Techniques**
>
> Techniques that determine how much of something is present.

TISSUE DISSECTION

When a tissue sample is obtained from the body, it contains many different types of cells in complex interactions. For instance, a sample of cancerous cells from breast tissue contains not only the tumor cells but also other nearby cells, connective tissue, blood vessels, glands, fat cells, and immune cells. The cancerous cells must be separated out prior to analyzing the DNA so it is not contaminated with DNA from non-cancerous cells. This is where tissue dissection and microdissection methods come in. Dissection refers to the separation of tissue and microdissection refers to the separation of tissue under a microscope. There are a variety of different types of methods for separating tissue.

Web Sites of Interest

More information about techniques is available from the National Cancer Institute's Cancer Genome Anatomy Project (CGAP), available at http://cgap.nci.nih.gov/.

Specific links include
1. Microdissection: http://cgap-mf.nih.gov/Protocols/Microdissection/Overview.html

2. Overview of probes, microarrays, other techniques: http://www.cancer.gov/cancertopics/understandingcancer/CGAP/allpages

The Cold Spring Harbor Laboratory sponsors a Web site that contains animated graphics of some techniques we have described: http://www.dnalc.org/home.html.

For information about PCR and DNA arrays, the following link may be useful: http://www.dnalc.org/ddnalc/resources/animations.html.

NOTES:

Glossary

- **Alleles:** Different forms of a single gene.
- **Analytical Validity:** How well a test compares to the best available test.
- **Aneuploidy:** An irregular number of total chromosomes.
- **Base Pairs:** See nucleotide base pairs.
- **Bases:** See nucleotide bases.
- **Biochemical Testing:** Assesses proteins or metabolites (by-products of chemical reactions) that signal a mistake in a gene.
- **Carcinogens:** Chemicals or other substances that can cause cancer.
- **Chromosomes:** Compacted structures of long strands of DNA located inside almost all of our cells.
- **Clinical Laboratory Improvement Amendments (CLIA):** A law that governs the use of laboratory tests sold as services.
- **Clinical Study:** Sometimes used interchangeably with *clinical trial*. However, in everyday usage, the term *clinical study* is broader than *clinical trial*.
- **Clinical Trial:** Usually defined as a prospective study designed to determine whether an intervention is effective and/or safe; it has well-defined outcome measures and usually involves a minimum number of patients.
- **Clinical Utility:** The benefits versus drawbacks of a test in the context of clinical use.
- **Clinical Validity:** The ability of a test to provide clinically relevant information.
- **Coding Regions:** Portions of DNA that spell out the instructions for making a protein or other specific chemical.
- **Comparative Genomics:** Comparison of all the genes in different living things.
- **Constitutional DNA:** DNA that makes up an individual's genome. This is in contrast to DNA obtained from tumor cells in a specific tissue.
- **Conventional Cytogenetic Tests:** Assessments of structural or numerical chromosome abnormalities in cells during the phase of cell division called metaphase.
- **Cytogenetic Testing:** A type of genetic test that examines the number and structure of chromosomes.
- **Deletion:** The loss of nucleotide bases from our DNA sequence. May be considered a polymorphism or mutation.
- **Diploid:** Paired set of chromosomes.
- **Direct DNA or RNA Testing:** Looks directly at the chemical letter sequence of our genes.

- **DNA:** Deoxyribonucleic acid, the material that makes up genes. DNA is a series of chemical letters (base pairs) that spells out the instructions for constructing our bodies and making them work.

- **DNA Microarray:** A technology and method used to detect many genes at once. The microarray consists of thousands of dots arranged in orderly rows and columns. Each dot contains a DNA probe or a known sequence of DNA that will stick to the faulty genes we are trying to detect in the sample.

- **DNA Polymerase:** A protein that assembles new DNA by copying an existing strand.

- **DNA Probe:** Short strings of DNA whose letter sequences will stick to or pair with the letter sequences in the faulty gene.

- **DNA Repair Genes:** The proteins made from these genes help repair errors in DNA that occur when the cell is duplicating itself. Without this repair, mutations can be more likely to occur in proto-oncogenes and tumor suppressor genes.

- **Dominant:** An allele that produces a trait or disease if only one copy is present.

- **Empiric Therapy:** This means "try the treatment to see if it works." In practice, this means offering everyone the best available therapy, or the one that benefits the most people, and waiting to see whether it works in each specific individual.

- **Empiric-based Therapy:** An approach to therapy that involves trying the treatment to see if it works.

- **Enabling Techniques:** Techniques that pave the way for easier or more accurate use of genetic tests.

- **Functional Genomics:** A field of study that looks at the biological functions of multiple genes and their products.

- **Gene:** A section of the DNA on a chromosome. A gene carries a particular set of instructions to produce a specific chemical product, usually a protein.

- **Gene Therapy:** Therapies that fix faulty genes directly.

- **Gene-based Therapy:** Therapies that fix problems caused by faulty genes, as opposed to fixing the genes themselves.

- **Genetic Disease:** Pathological condition caused by absent or defective genes or by abnormalities in the chromosomes.

- **Genetic Mapping:** Localizing a gene to its specific place on a chromosome.

- **Genetic Test:** A test that examines our genes, either directly or indirectly.

- **Genetic Variant:** An alteration in the normal sequence of a gene.

- **Genetics:** A branch of biology that deals with the heredity and variation of organisms. Today, the term genetics is often used to refer to the study of single genes.

- **Genome:** All of an organism's DNA.

- **Genomics:** The study of multiple genes, their functions, and their interactions or all of our genes acting together.

- **Genomics-based Therapy:** An approach to therapy in which each person's genes are used to guide therapy.

- **Genotype:** A set of alleles, as opposed to physical appearance.

- **Germ Cells:** Reproductive cells in our bodies (either egg or sperm cells).

- **Haploid:** Single set of chromosomes.

- **Haplotype:** A set of closely linked alleles (genes or DNA polymorphisms) inherited as a unit.

- **The HapMap Project:** A major international project designed to determine the common patterns of DNA sequence variation in the human genome.

- **Hereditary Mutation (also called germline mutation):** A change in the chemical letter sequence of DNA in the body's reproductive cells.

- **Human Genome:** All of the DNA present in humans.

- **Human Genome Project (HGP):** A large-scale scientific effort designed to determine the nucleotide base sequence of human genes: the sequence of As, Ts, Gs, and Cs contained within our DNA. Sequencing and mapping of human genes was completed in 2003.

- **Immunohistochemistry:** A technique that uses the antibody-protein reaction to identify proteins.

- **Individualized Medicine:** Tailoring treatment to the individual. This applies to the use of genetic tests to classify a person's disease and predict response to treatment. The best treatment for the individual is then selected.

- **Inherited Mutation:** A change in DNA transmitted to us by our parents.

- **Insertion:** An addition of extra nucleotide bases into our DNA. May be considered a polymorphism or mutation.

- **Linkage Testing:** Assesses markers inherited along with a gene known to cause disease.

- **Marker:** Nucleotide base sequence located near a gene and usually inherited along with it.

- **Marker SNP:** A SNP located near a gene and usually inherited along with it.

- **Messenger RNA (mRNA):** The form of RNA that is a complementary copy of the genetic information encoded in the DNA.

- **Metabolomics:** All metabolites breakdown products in a cell under given conditions.

- **Molecular Cytogenetic Tests:** Assessment of specific genes or portions of genes on chromosomes.

- **Mutation:** A change in the nucleotide base sequence of our DNA that occurs in less than 1% of the population. It is generally used to refer to a change that has deleterious effects on the organism.

- **Non-coding Functional Regions:** Portions of DNA that do not spell out the instructions for making a protein or other specific chemical but that perform other functions such as turning genes on and off.

- **Non-coding Regions:** Portions of DNA that do not spell out the instructions for making a protein or other specific chemical.

- **Nucleotide Base Pairs:** The exclusive pairing of A with T and C with G along the two strands of our DNA. Typically called base pairs for short.

- **Nucleotide Bases:** The 4 chemical letters in DNA that spell out the instructions for building and running our bodies. The 4 letters are A (adenine), T (thymine), C (cytosine), and G (guanine). Often called bases for short.

- **"Omics" Sciences:** Fields of study that focus on multiple parts working together or the whole instead of the individual parts.

- **Oncogenes:** Genes that stimulate excessive cell growth and division. They are altered forms of normal genes called proto-oncogenes that control cell growth and division.

- **Pharmacogenomics:** The field of study that examines how responses to drugs are influenced by genes or the genome.

- **Phenotype:** Observable traits or characteristics.

- **Polymerase Chain Reaction (PCR):** A method for making millions of copies of specific DNA.

- **Polymorphism:** A change in the nucleotide base sequence of our DNA that occurs in at least 1% of the population. It is generally used to mean a change that does not have any clinical significance.

- **Prediction:** The expected course of disease treated with a specific drug.

- **Primer:** A sequence of DNA nucleotide bases that serves as a starting point for DNA copying.

- **Prognosis:** The expected course of disease, independent of any treatment.

- **Proteomics:** The study of the entire protein expression of a living thing at a given point in time.

- **Proto-oncogenes:** Genes that control cell growth and division. They act like the accelerator of a car, telling the cell to divide and grow.

- **Quantitative Techniques:** Techniques that determine how much of something is present.

- **Recessive:** An allele that produces a trait or disease only if two copies are present.

- **Repeats:** Multiple copies of the same nucleotide base sequence.

- **Reverse Transcription Polymerase Chain Reaction (RT-PCR):** A method for copying RNA into DNA, which tells us which genes are activated or expressed.

- **RNA (Ribonucleic Acid):** A chemical that is related to DNA; helps transfer information from DNA into proteins.

- **Sensitivity:** Likelihood that a test will give a positive result when the thing you are trying to detect is present.

- **Serum Proteomics:** The study of proteins present in the liquid portion of our blood.

- **Single Nucleotide Polymorphism (SNPs or "snips" for short):** A change in only one nucleotide base pair of the DNA sequence that occurs in at least 1% of the population.

- **Somatic Cells:** Non-reproductive cells in our bodies.

- **Somatic Mutation:** A change in the chemical letter sequence of DNA in the body's non-reproductive cells.

- **Specificity:** Likelihood that a test will give a negative result when the thing you are trying to detect is not present.

- **Sporadic or Spontaneous Mutation:** A change in DNA caused by our environment (e.g., sun, radiation, other carcinogens) or random events within our cells.

- **Susceptibility Genes:** Genes that increase the likelihood of disease but do not, by themselves, cause disease.

- **Targeted Therapy:** Therapy that is directed at a specific variable; for instance, targeted cancer therapy may be directed at a specific protein manufactured by an overactive gene, whereas general cancer therapy may be directed at all rapidly dividing cells.

- **Test Reliability:** The test's ability to get the same result each time.

- **Test Validity:** How well a test measures what it is supposed to measure.

- **Transfer RNA (tRNA):** The form of RNA that helps translate the DNA into proteins. tRNA brings amino acids together in the correct order to form proteins. Amino acids are the building blocks of proteins.

- **Translocation:** Section of DNA from one chromosome that switches places with a section on another chromosome.

- **Translocations:** Movement of chromosomal material, such as a section of DNA from one chromosome that switches places with a section on another chromosome.

- **Tumor Suppressor Genes:** Genes that normally restrain cell growth and division. If they are not functional due to a mutation, the cell may grow and divide repeatedly. Tumor suppressor genes act like the brakes of a car: they tell the cell to stop dividing and growing.

- **Variation:** Differences in our DNA.

The Research Advocacy Network (RAN) was formed in 2003 to bring together participants in the research process with

Research Advocacy Network

Advancing Patient-Focused Research

the focus on educating, supporting, and connecting patient advocates with the medical research community. While there are many organizations addressing the needs of patients with specific diseases, political advocacy, cancer education and fundraising, no organization has focused on advancing research through advocacy. Research Advocacy Network is committed to improving patient care through research. Our goals are to get results of research studies (new treatments) to patients more quickly, to give those touched by the disease an opportunity to give back and to help the medical community improve the design of its research to be more attractive to potential participants.

RAN represents a new approach to advocacy. Because research holds the hope for improvements in treatment, diagnostics and prevention, we are dedicated to patient-focused research. We believe dissemination of research results to the medical community and patients can have a major impact on clinical practice. We have produced a number of "What It Means for Me" Fact Sheets — short, easy-to-read recaps of studies that will change clinical practice available at **www.researchadvocacy.org**. These Fact Sheets can be used by sites to inform their patients about new research findings.

Research Advocacy Network collaborates with all stakeholders in the medical research community to enhance the effectiveness of advocates and advocate organizations. We have collaborated with Siteman Cancer Center and the St. Louis Komen Affiliate to develop a program to increase awareness of and participation in research advocacy. RAN directs the Advocate Core at the Indiana University Department of Defense Breast Cancer Center of Excellence and is developing a network of advocates and advocate organizations to support the Center's research. RAN has developed a model for advocate programs at cancer centers modeled after best practices in performance evaluation used in Fortune 500 companies.

To provide those touched by the disease an opportunity to give back, RAN created the Advocate Institute™. This virtual learning center provides advocates with multiple methods of learning to improve their effectiveness in interactions with the research "world." The Institute uses an innovative curriculum, on-site presentations and online learning opportunities. RAN has used the latest technology to reach a larger audience of advocates through Focus on Research™. This is a system of preparatory conference calls, virtual classrooms (webinars), learning materials and mentoring to prepare advocates to attend research-oriented meetings. Focus on Research prepared advocates to attend the American Society of Clinical Oncology's Annual meeting in 2005 and 2006.

RAN applies best practices from the world of market research to inform research design. Using the models of focus groups and structured interviews, RAN was able to inform the design of the PACCT-1 (now renamed as TAILORx) clinical trial.

Patient advocacy in research has many opportunities for all kinds of people to make a contribution. RAN has training and educational programs, publications and tools for advocates on our Web site, and experience in effectively working with researchers in cancer centers. RAN works with advocates and organizations to effectively integrate advocates into research activities. Please learn more about us at our Web site at **www.researchadvocacy.org** or contact us about our work by e-mailing us at **info@researchadvocacy.org** or by phone or FAX at 877-276-2187. We look forward to hearing from you!