Family Secrets
Genetic Testing PowerPoint Script

*Notes on use:

Each bulleted portion goes with a mouse click advance on the PowerPoint. Sometimes, the mouse click advances a slide, and sometimes the mouse click activates a custom animation.

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- Introduction slide to the Family Secrets Genetic Testing Talk

- Welcome to our Genetics Conference. Today we are going to talk about genetic disease and genetic testing. We will talk about the process by which a patient might go about needing or wanting a genetic test, and then spend some time going over a few different types of genetic tests, and the laboratory techniques used to achieve them. We will also talk about the personal and social implications of genetic testing and address the future of genetic testing.

- Most people are familiar with a situation in which a particular disease or the symptoms of a disease are passed on through many generations, as shown here in this pedigree. Affected individuals are shown in orange.

- This kind of pattern is indicative of an inherited disease.

- Inherited diseases come about because of stable mutations in the DNA. Since the DNA, shown here as 23 sets of maternal and paternal chromosomes, are passed on to an individual's offspring…

- …any mutations in the DNA will also be passed on. Here we see an example of a particular chromosome that has a dominant mutation on it…

- …any family member who inherits this chromosome is affected by the disease.

- Because the mutation is in our genetic material, our DNA, inherited diseases are also called genetic diseases.

- Two reasons a patient may suspect that he has a genetic disease are:

- A pattern of disease in the family - he may know that some family members are affected by a similar disease and may suspect that the disease is caused by a genetic mutation.

- Alternatively, he may come down with symptoms and be seen by a physician, who might inform him that they are common in a particular genetic disease.

- At this point, the patient is faced with a decision – should he decide to find out if he has the genetic disease? If he wishes to find out, he will undergo a form of genetic testing known as predictive testing.

- There are three general classes of genetic testing, one of which is predictive testing. This type of testing is done to determine if an individual carries a mutation that either directly causes a disease, or increases the individual's risk of disease. The other two are newborn screening, and carrier testing. Newborn screening involves testing a newborn infant for an array of mutations, in the hopes of catching the disease at a point where treatment can partially or completely eliminate any adverse symptoms. Carrier testing involves testing an individual who may carry a recessive mutation – that individual will not come down with the disease, but carries a mutation. This individual may want to know if she is a carrier in case her partner carries the same mutation – in this case, any of their children would have a ¼ chance of inheriting both mutations and thus be affected by the associated disease.

- Today, we will talk about predictive testing, as it is probably the most controversial of the three, as we will see during the talk.

- So let's return to our patient.

- His physician has informed him that it is possible he is at risk for a genetic disease. The physician may refer him to a…

- …genetic counselor if she feels that she doesn't have enough knowledge of genetic diseases…

- …or if she is knowledgeable about genetic diseases she may work with a genetic counselor to address the patient's needs. The physician and genetic counselor may suspect a particular genetic disease, and know that it can be diagnosed with a particular genetic test.

- They may wish to test family members who have already exhibited symptoms, if the patient has not, to confirm their suspicions.

- If no such family members are present, they may suggest to the patient that he get tested himself. However, the decision to test is entirely up to the patient.

- There are many issues about genetic testing that must be weighed before one plunges into a genetic test.

- One issue is that of treatment. Certain genetic disease, like Huntington's disease, can not be cured at this point. The knowledge that one is positive for a fatal genetic disease and that there is nothing one can do about it can be psychologically traumatizing.

- Another is the issue of prevention. The presence of certain mutations, such as those associated with an increased risk for breast cancer, does not mean that the patient will absolutely get breast cancer later. Knowing those mutations are present allows the patient to change their lifestyle to help prevent the disease, or it may encourage them to receive regular health check-ups, so that if the disease does manifest itself, it can be treated immediately.

- Another issue involves reproduction. Knowing one has an incurable genetic disease may affect one's desire to have children. Having a dominant mutation such as the one that causes Huntington's disease means that one's children have a 50/50 chance of also having the disease. These questions must be considered by the patient.

- A physician, and/or genetic counselor can help guide the patient through the pros and cons of genetic testing, but in the end, the decision must come from the patient himself.

- If our patient decides he wants to have genetic testing, the type of test that he will receive depends on the suspected mutation. At this point, we will discuss some different techniques involve in detecting mutations.

- As we have discussed, genetic mutations exist in the DNA. However, that mutation cascades down into a mutated RNA being translated into a mutated protein sequence, which may affect the ability of the protein to fold and assume its proper function.

- Genetic testing looks at all of these elements.

- Here we will discuss the levels at which genetic tests can occur. We have left out RNA, since the majority of tests at this time deal with DNA, protein and protein function, although RNA expression can be tested. DNA can be examined by microscopic analysis of whole chromosomes. Mutations that involve large deletions or insertions, or the presence of extra chromosomes can be detected by this method. DNA can also be examined by analyzing its sequence. All genetic diseases will have mutated DNA sequence, so analyzing sequence allows one to look at all types of mutations. We will talk more about sequence analysis later in the talk. Proteins can be examined by analyzing protein shape, usually by protein gel electrophoresis – a mis-folded protein may migrate through a gel differently than a properly folded protein. Protein function can be analyzed if the protein normally makes a product, whose presence or absence can be detected.

- As I explained before, DNA sequence is always affected in a genetic disease, so we are going to concentrate on methods of detecting mutations in the DNA.

- First let's talk about what kinds of mutations exist.

- For comparison, here is a section of DNA that is "normal" or mutation free.

- One kind of mutation is a single base pair mutation. One base, in this case a "G" has been changed to an "A." If this single mutation changes the amino acid sequence, it can often affect the ability of the protein to fold. Sickle cell anemia is caused by a single base pair mutation.

- Another mutation involves the deletion of DNA sequence. The deletion can be small, as in the case of cystic fibrosis…

- …or large, as in the case of Duchenne muscular dystrophy

- Another mutation involves the insertion of DNA sequence. This occurs in Huntington's disease.

- Some diseases are caused by multiple mutations. Diabetes and breast cancer are examples of disease that can be caused by many different mutations, individually, or together.

- So how can we find out whether these mutations exist in a patient? Essentially, how can we look at the DNA sequence? There are number of different techniques. Today, we will talk about sequencing DNA, RFLP analysis, and the use of probes in dot blots, and microarrays.

- To talk about these techniques, we first have to talk about two techniques that are used in nearly all of the genetic testing procedures we will talk about. One is gel electrophoresis and the other is PCR.

- First we will talk about gel electrophoresis

- Gel electrophoresis does two things – it separates DNA fragments on the basis of size, and once those DNA fragments are separated, the DNA can be visualized on the gel by using a dye that stains the DNA. DNA is negatively charged at neutral pH, and when an electrical current is applied to DNA fragments, they will migrate towards the positive charge. If the DNA is forced to migrate through a matrix of polymers, which can be a gel of agarose or acrylamide, large fragments will be separated from small fragments, since the large fragments will get caught up in the polymer matrix. The process of gel electrophoresis is often called "running a gel."

- The result of electrophoresis of different sized DNA fragments would be a band of fragments of the same size that are in different positions on the gel depending on their size. Small fragments will be found farther away from the well, and larger fragments will be present close to the gel. Once the DNA is present as a band in the gel, it can be visualized by washing the gel in buffer containing a fluorescent or colored dye that binds to DNA.

- Now let's talk about PCR…

- PCR stands for polymerase chain reaction. PCR involves taking a DNA sample and passing it through multiples cycles in which the genomic DNA, contained in all 23 sets of chromosomes, acts as template from which copies of a small region are made. Here we start with a segment of genomic DNA.

- The first cycle is a melting step. The temperature is raised to 94 degrees, at which the double stranded bonds are broken, and the DNA is separated into two single strands.

- The second cycle lowers the temperature to a point where primers can anneal, or pair, to their complementary sequences.

- The third step raises the temperature to 72 degrees, which allows a polymerase, an enzyme that copies DNA, to start from the primers and copy in a 5' to 3' direction.

- These cycles are repeated over and over again…

- Melting

- Annealing

- Elongation. At this point, you may notice that future copies will only contain DNA sequence between the two primers. This is another useful aspect of PCR, which is that you can define what sequences you want copied by making your primers flank that region. This is important if you are starting with genomic DNA, which contains a lot of DNA that you may not be concerned about.

- Here, you can see how many copies of DNA can be made from one double stranded piece of DNA. After just 10 cycles of PCR, there will be 1,024 copies for every one double stranded DNA template. One cell contains two copies of a particular DNA sequence, so 10 cycles of PCR on a single cell gives you 2,048 copies.

- Although PCR is used as a tool to generate many copies of a region of DNA that may be used in other forms of genetic testing, PCR can also be used as genetic test unto itself. For example, if the primers flank a region of DNA that is known to carry a deletion that causes a disease, the PCR process will produce a copy that is smaller than a copy that was made from a normal sequence.

- To be able to see the different sized copies, they are run out on a gel.

- Now we can talk about sequencing DNA.

- One may start with knowledge of what a normal gene's DNA sequence would look like, thanks to sequencing projects such as the Human Genome Project.

- However, if a patient were to approach us, we would have no idea what his sequence is. To find out, we can sequence his DNA.

- Sequencing is a lot like PCR.

- It uses a primer, which is close to the region of DNA you suspect may contain the gene that is mutated.

- The PCR process is used to copy the patient's DNA, but included in the reaction are special nucleotides called dideoxy-nucleotides. These dideoxy-nucleotides, or ddNTP's, differ from the nucleotides found in DNA strands, which are deoxynucleotides. ddNTP's stop the elongation process.

- During the course of a sequencing reaction, ddNTP's will be incorporated during elongation at random, making copies of DNA that are of different lengths.

- The ddNTP's used in modern sequencing reactions are colored with different fluorescent labels, the purpose of which you'll see in a moment.

- The goal of sequencing is to have fragments of all different sizes. For example, a 10 base pair sequence that you want to sequence will have fragments that are 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 bases long, each with a colored ddNTP at the end.

- These fragments are run out on a gel…

- The gel continues to run until the bands come out of the bottom of the gel.

- As they come out of the gel, they are detected by a computer, which reads the color of the ddNTP at the end of each fragment. This is translated by the computer into the DNA sequence, as shown here.

- The sequence read by the computer is complementary to the patient's sequence, so…

- … we can now fill in the patient's sequence and compare it to the known sequence.

- A careful look at the sequence tells us that there is a mutation in the patient's sequence

- Some issues to consider about sequencing are that although it gives a lot of information about sequence, it is expensive and time consuming to perform.

- Another way to look at DNA sequence is by RLFP analysis.

- RFLP stands for Restriction Fragment Length Polymorphism. RFLP analysis relies on the use of restriction enzymes. These enzymes recognize specific DNA sequences (usually palindromes) and cut them.

- Restriction Fragment Length Polymorphism means that there are polymorphisms, or differences between people in the number of restriction sites, and therefore the length of the cut fragments. This person has 4 fragments after restriction digest. This person has a mutation that eliminates one of the sites that the restriction enzyme cuts at. Therefore, this person has 3 fragments, one of them being much larger than the rest. If this mutation was associated with a disease, a restriction digest would show that a carrier of the mutation had 3 fragments instead of 4.

- You may have noticed that we are only addressing a small portion of the DNA.

- Genomic DNA contains much more DNA than this small piece, and cutting the genomic DNA with a restriction enzyme would result in an enormous number of different bands. Here is where it is advantageous that PCR only copies a small portion of the DNA.

- Before RFLP analysis, these small fragments would be copied in a PCR reaction, so that the number of small fragments made by PCR vastly outnumbers the amount of genomic DNA, so that when we run the fragments out on a gel to see the different sizes, we only see the fragments from this piece of DNA, and not from the genomic DNA.

- The fragments are run out on a gel to detect the different sizes.

- In this case, we can now see how the four fragments in the normal sequence would appear on a gel, and we can see the three fragments in the mutated sequence, where one of the fragments is now much larger than the others.

- Some issues regarding RFLP analysis are, that in order for RFLP analysis to work, the mutation has to eliminate, or sometimes create, a restriction enzyme cut site. This is not always the case.

- Now we will talk about some different techniques that utilize probes. First, let's discuss what a probe is.

- To understand how probes work, one must understand the concept of base pairing. DNA is a double stranded molecule, and the bases pair with their complementary bases. A pairs with T, G pairs with C.

- A probe is a short piece of DNA that is complementary to a region on the genomic DNA. If the DNA is made single stranded by denaturing the DNA, by applying high heat – just as in the melting step of PCR, and then the temperature is lowered, the small probe will bind to its complementary sequence faster than the long DNA strands. This is the same concept that we talked about in the annealing step of PCR.

- The ability of a probe to bind depends on its complementarity to the DNA strand. A single base pair difference can affect binding, depending on the stringency of the conditions during which it is trying to bind to the DNA. Stringency is a term used to describe conditions that will affect whether a probe must absolutely bind to its exact complementary sequence, or whether small differences are acceptable. This depends on factors such as temperature and what buffers are used during probe binding. At low stringency conditions, a probe with a single base change can still bind. At high stringency conditions, this probe can not bind.

- Now lets apply this concept to a dot blot.

- This procedure can use either genomic DNA, or DNA copies made by PCR that are only of a region of interest to the genetic test. A patient's DNA is denatured and applied to a membrane. Application of DNA in this manner is also called "spotting."

- The membrane is washed in a solution that contains the probe, which is usually radioactively labeled. The probe binds to any complementary sequences on the membrane.

- Excess probe is removed by washing the membrane.

- Now the membrane is exposed to autoradiography film.

- If the probe has bound to the DNA, the spot will appear as a dark spot on the film.

- Here is an example of how dot blots are used to detect a mutation that causes cystic fibrosis. DNA from a patient is spotted out twice, one spot will be used with a probe complementary to the normal sequence, the other will be used with a probe that is complementary to a mutated sequence. Here we are looking at dot blots for three patients. One is homozygous for the normal sequence, one is homozygous for the mutation, one is a heterozygote for a mutation. The normal patient's DNA will bind to the normal probe, but not the mutated probe. The homozygous patient's DNA will only bind to the mutated probe. The heterozygous patient's DNA will bind to both probes. Since a heterozygote has one copy of the normal gene and one copy of the abnormal gene, both probes can bind, but only half as much binds, making the dot lighter.

- The advantages of dot blots are that a patient's DNA can be exposed to many different probes, making it easier than sequencing to see if a patient has multiple mutations. However, if the mutation is a single base pair mutation, it can be difficult to find conditions that are stringent enough to prevent the probe from binding.

- The last technique we will talk about involves the use of microarrays. This is relatively new technology that requires the use of specialized machinery and computers.

- A microarray is essentially a dot blot in reverse. Microarrays start with a "chip" on which is spotted many different probes for different mutations. Each dot represents a different probe. If this were a dot blot, it would be called a "reverse dot blot" because the PROBE is spotted, rather than the DNA. The advantage to microarrays as opposed to reverse dot blots is that the process is automated, and many more probes can be tested in a single experiment.

- The microarray chip is washed with a patient's DNA, most likely genomic DNA that has been cut with restriction enzymes to make them into small pieces, denatured, and labeled with a fluorescent dye.

- If a patient's DNA binds to spotted probes, a computer detects this by the measuring the intensity of the fluorescence emanating from that spot. A homozygote for a cystic fibrosis mutation will have a more intense spot than a heterozygote for the same mutation. In this hypothetical example, a patient's DNA binds to probes for mutations in cystic fibrosis, colon cancer and breast cancer.

- The big issue with microarrays is expense. The equipment needed to process a microarray chip is very expensive, and it is also time consuming to spot a chip for every patient that needs one. However, as this technology develops, microarrays may become cheaper and easier to use.

- Right now, there are predictive tests available for the following diseases, and others.

- So we've just talked about some of the different techniques that might be used to test our patient's DNA. Now let's return to our patient. He knows the potential benefits of genetic testing. If he's negative, he may feel relief, and undergo fewer health check ups that would be associated with being in a family that tends to have a higher risk of a particular genetic disease. If he's positive, he may be able to make informed lifestyle decisions, and may find that it's possible to reduce the risk of coming down with severe symptoms.

- He also knows the limitations of genetic testing. That the presence of mutations does not always lead to disease, that existing tests only look for common mutations, making less common mutations undetectable. He knows that there is a small chance of error in the testing procedure, and very importantly, he knows that testing is not always matched by treatment for a particular disease.

- From societal viewpoint, one of the biggest limitations of genetic testing is that testing is not always matched with treatment. While knowing one is positive for an untreatable disease may allow an individual to make personal choices regarding reproduction and lifestyle, confidentiality of a positive test result is an important issue.

- Who has the right to know if a person is positive for a genetic disease? Health insurance companies may want to know – they may want to refuse coverage for a person with a debilitating disease that will cost them thousands of dollars in care later in life. While this seems cruel, one viewpoint is that the cost for one person's care is matched by an increase in insurance rates for other people covered by the same insurance company. Another issue is whether an employer has the right to know that they are hiring a person who may be adversely affected by a genetic disease. Would you feel safe being a passenger on a plane that is being flown by a person who is positive for a neurodegenerative disease? Another issue is whether a family has a right to know a patient's test results. If our patient was positive for a degenerative disease, would that affect potential relationships if his partner knew about the disease, and would existing children, who may not want to be tested for their own reasons, have to know that they are also at risk? These ethical, legal and social issues must be addressed as our ability to test for different genetic diseases increases, and our ability to treat them does not follow suit.

- The future of genetic testing involves resolution of ethical, legal and social issues, through discussion that includes experts in the fields of genetic testing, ethics, law, and policy makers, to name a few. It also involves advancing the field of genetic testing to develop cheap, effective methods of testing so that all people can have access to this technology, not just those that can afford it. Importantly, it requires we advance scientific research in the treatment and prevention of the symptoms of genetic diseases. Examples of current lines of research are gene therapy and drug design. Genetic testing offers us great knowledge that we can use to improve our health. With it comes great responsibility to use this knowledge for the benefit of all.