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Medical Genetics - Lecture 9

Chapter 13 – Genetic Testing and Gene Therapy

Genetic Screening

What diseases do you think we should screen for in a population, and which patients should we test?

We test for common diseases, population at risk of a certain disease, we usually test for treatable diseases. However, we do screen for uncommon diseases if they were life-threatening severe diseases, because the burden of not screening means that these patients will die, especially if we have a treatment. Phenylketonuria (PKU) is definitely not a common disease, but we do a screening test for PKU.

Therefore, the principles of screening are:

- *The disease should be **common**; however, it doesn't have to be common if it is a severe disease.*
- *The disease should be **treatable**.*

For **genetic screening** we need to do **population screening**. You will be screening newborns and heterozygotes (carriers in a population; people who carry one of two alleles of an autosomal recessive disease; people who inter-marry a lot). If you look at all inborn errors of metabolism and various other diseases that are autosomal recessive, Ashkenazi Jews keep coming up and that is because they inter-marry a lot.

There are two types of **prenatal diagnoses** (fetus versus mother):

- **Invasive diagnosis**: means taking a sample from the fetus. For example, in *amniocentesis*, a sample is taken from the amniotic fluid. Moreover, *chorionic villus sample* (CVS) means taking a sample from the chorionic villi which develop from the chorion (outermost membrane surrounding an embryo which contributes to the formation of the placenta). *Cordocentesis* is the process of taking a blood sample from the umbilical cord.
- **Non-invasive diagnosis**: means taking a sample from the mother. Examples include: ultrasound, maternal blood sample, and MRI.

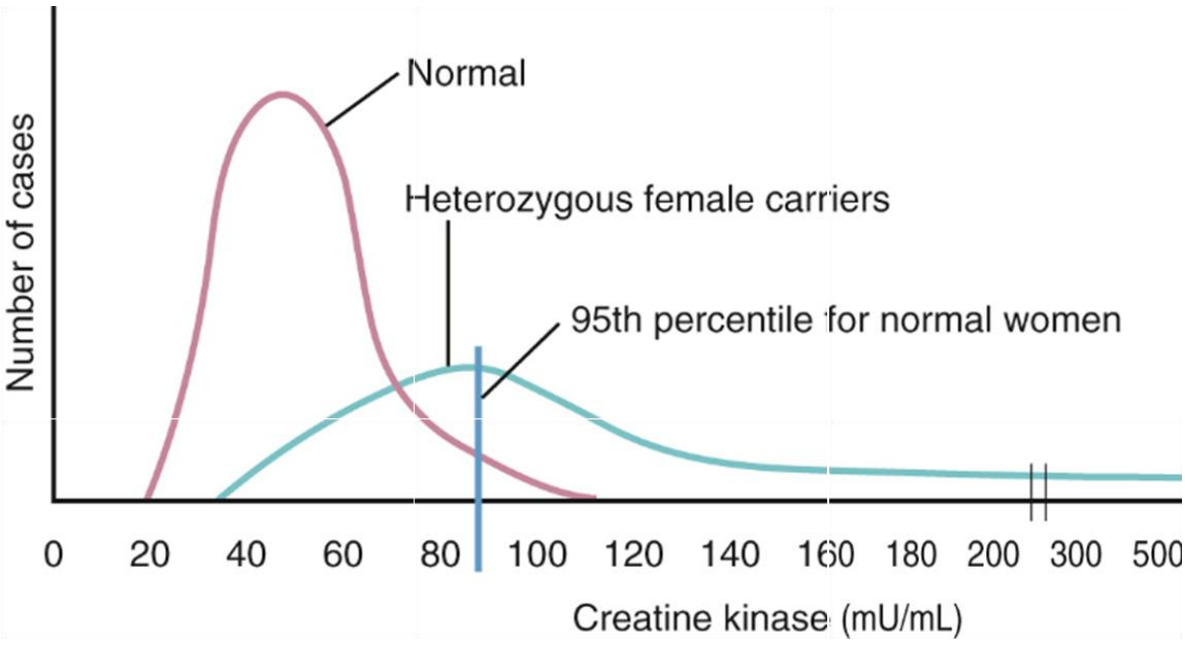
Family screening differs from population screening in that this particular family is at a higher risk to have a mutation than the general population.

Most screening tests are sensitive. Generally, in a screening test, you need to catch as much people as possible, so we want it to be sensitive, even if you catch a false positive. That's why you use a validation test that is more specific. This is called **screening validation**. Especially if the disease in question is a severe fatal disease that means you cannot miss a single patient.

Sensitivity: proportion of true positives accurately detected.
Specificity: proportion of true negatives accurately detected.

Threshold of sensitivity changes depending on the degree of severity of the disease. Some diseases could be discovered later on with another test (they are not so severe) so you can give off some sensitivity for specificity.

Simply, suppose you did a test and you have the results. Let's say, for example, that you measured the amount of a certain protein in the serum. The results could range from 20 to 200 and there's an area of overlap between heterozygous and normal patients. You have to set a threshold, or **cut-off point**, where you would say: everyone that has a result that is higher than this threshold is considered a carrier and everyone that has a result lower than this threshold would be considered normal. If I want the test to be highly sensitive I'd set this threshold as 50 for example. In this case I've made sure I've caught all true positives at the expense of catching some false positives too. In contrast, if I want the test to be so specific the threshold should be something near 90 where I've made sure I detected a good proportion of true negatives at the expense of missing some true positives.



Positive predictive value is the fraction of persons with a positive test who truly have the disease**. Negative predictive value** is the fraction of persons with a negative result who truly do not have the disease.

Therefore, you need to take into account all of these factors when you are screening for a disease. Can I catch all affected individuals or is there another test down the line that would catch affected individuals which is not going to be bad and problematic? A physician needs to catch every single one of these patients as early as possible; otherwise they will suffer intellectual disability or worse; death.

What tools do we use?

Biochemical tools, western blotting, microarrays, etc.

Some are more accessible for screening than others, some are more specific and some are better as validation tests.

Limitations of Screening

1. *Test errors* like false positives and false negatives.
2. *Some mutations do not cause a disease*. A lot of tools we use detect mutations; we are not talking about the old biochemical techniques where we are detecting the activity of an enzyme, but we are looking for mutations not necessarily to cause a disease. Even if you find a mutation. It is specifically problematic when you are talking about diseases with reduced penetrance.
3. *Psychological implications* like anxiety, discrimination regarding employment.
   Not so long ago, employment discrimination was a concern. In the states there's an anti-discrimination law which specifically states that genetic tests cannot be used by employers. As we all know, laws are frequently broken and patient's confidentiality is frequently breached as well.
4. Should another genetic mutation be found while doing a screening test for a disease, the physician should inform the patient.
5. *Direct-to-consumer testing*
   The customers send certain companies a cheek swab. When these companies receive the sample, they take it to the lab, make a microarray-based genetic testing and send the results back to the customer. These companies are usually using polymorphisms and known mutations that are currently being considered, not all of them are diagnostic, but they give customers a profile on the common diseases that they may be exposed to like cardiovascular diseases, lung cancer, and other various conditions. The problem is that these customers don't know what a mutation could do and they don't have all treatments yet. The results may be complicated for customers to interpret them since

they've taken out the middle man (the physician). Therefore, customers can become obsessed about these mutations.

6. When you earn your medical degree and go to the US, UK, Germany, or anywhere else, you will find that these patients will go to the clinic after they had genetic tests. These tests can range from few hundred to few thousand dollars, so they are not out of reach of most people. They would present to you saying that the test reveals that I'm at risk of a certain disease. Thus, you have to understand the basics of medical genetics in order to tell them that their DNA encodes certain proteins and some mutations could change these proteins but this change might not be enough to cause a disease.

Prenatal Diagnosis

AFP screening (alpha-fetoprotein)

- Very high levels of AFP indicate that there are neural tube defects.
- Very low levels of AFP indicate that there the fetus has Down syndrome.

You would either take a sample the maternal blood or the amniotic fluid. However, there's a lot of overlap with normal children when talking about high or low levels, so you might find lots of false negatives. For neural tube defects, we can separate the true positives from the false positives simply by ultrasound. *That's why most screening tests need a validation test.* Neural tube defects are severe, so you might do a second test to make sure that your diagnosis is correct.

In addition to AFP screening, you can directly assay fetal DNA from the mom's blood. Some cells from the fetus pass through the placenta and circulate in the mother's blood. You also can find **cell free DNA** (**cfDNA**) that is also circulating the maternal blood. Isolating the cells or the fetal DNA is not an easy task. However, it will overcome the problems with sensitivity in biochemical tests, and is non-invasive. Most researchers are currently trying to figure out non-invasive prenatal screening tests.

Amniocentesis

It is typically needed when the pregnant woman is over 35 of age.

This is done 15 to 17 weeks after the patient's last menstrual period (LMP). You will obtain a sample of amniotic fluid from which you can assay AFP. Additionally, you will get some fetal cells, but you will not get a lot of fetal cells so you need to grow them in a cell culture for nearly a week. After that week has passed, you can do the various genetic tests we talked about in all the previous lectures. Typically we are talking about a turnaround period of 10 -12 days. Psychologically that is very difficult for the parents. Occasionally, you can do fluorescence in situ hybridization on a few cells early on, but again those tests are specific which means you are suspecting a certain disease rather than screening for everything. If there's a family history of a disease; a certain mutation or deletion, we use FISH to see if the child has the problem or not. You can do this if you there's a previous child that had a certain problem, family history, paternal history and especially if there was an abnormal screening test.

Amniocentesis is *not a screening test.*

The risks are: leakage, infection and a small percentage risk of fetal loss (0.3% higher than the general population). This sounds like a lot if you're talking about patients ending up losing the fetus because of this test, that's why it is not a screening test. However, fetal losses are rare.

Chorionic Villus Sampling

It could be done earlier; 10-12 weeks after LMP. It is never performed before 10 weeks after LMP because this massively increases the risk of fetal loss.
Post-CVS fetal loss rate is 1% above the background rate vs. 0.3% for amniocentesis. However we still do it if there's higher suspicion and you need to detect earlier. The sooner you detect the better, so you can induce abortion upon the parents' request.

Therefore, it is generally safe but there's a higher risk than amniocentesis. There are one or two studies that indicated increased risk of limb deficiency because of a potential of introducing vascular insult and causing this limb to be hypoxic.

You cannot measure amniotic fluid AFP while taking CVS. Additionally, you don't have to culture the cells so the turnaround period is shorter- psychologically better, especially for parents who would consider an abortion.

Cordocentesis

It is also known as **percutaneous umbilical blood sampling.** It is quicker; 2-3 days (vs. 10-12 days in amniocentesis). You can detect all the genetic diseases you can't detect in amniocentesis. Remember that some babies maybe mosaic so any cell you can get from the chorionic villus or from the amnio that may be different from the other cells in the baby. To take from blood you will overcome the problem of mosaicism.

We do not take blood unless you had found an abnormality by ultrasound. Blood would also help you diagnose new problems like hematological and immune diseases.

Ultrasound and MRI

They are non-invasive tests. You can detect limb problems, fetal growth, cardiac defects, diaphragmatic defects and other abnormalities mentioned in Box 13-6 (p.279). See figure 13-6 at the book or go back to the slides. There you can find a photograph of an ultrasound of a normal spine and another with neural tube defects where there was accumulation of fluids just next to the spinal column.

Preimplantation Genetic Diagnosis

It is usually done for patients who go for **in vitro fertilization (IVF)**. Therefore, if you fertilize an ovum and they reach that stage of 6 to 8 cells, you can take one or two of these cells, grow them in a petri dish, extract the genetic material and do the genetic testing to make sure that this baby is fine, especially if you are expecting certain disease. You implant few of the zygotes that proved to be unaffected and you have a healthy baby.

Furthermore, it is considered unethical to manipulate the baby's genetic material. It is much easier to find the healthy zygote and plant it than trying to correct the abnormal zygote. If it is a normal zygote and you change the genetic material to enhance the child, you got to the whole "designer baby" issue.

How to fix it?

The easier answer is: treat the symptoms. If the patient has hyperlipidemia, treat their hyperlipidemia. Typical enzymatic problems can be dealt with by drugs. However, some problems cannot be solved by drugs and some could be treated more effectively if we alter somatic genetic material.

Gene Therapy

Somatic Cell Therapy

Basically you take the cells from the patient, alter their genetic material by adding a certain gene for example, a healthy copy of a mutated gene, and then you reintroduce these cells back to the patient. To introduce genetic material into the cell, a virus that has the required genetic material is used.

The **transduction** (adding genetic material to a cell through a virus) rate is typically less than 1%. However, they'd still be effective because they proliferate; they are the healthier cells so by natural selection they will surpass the other cells.

In severe combined immunodeficiency that can be caused by multiple gene abnormalities, one of them is deficient adenosine deaminase. This gene has been introduced by a retrovirus to lymphocytes taken from a patient. However, lymphocytes have a short life span which means that we have to come up with an alternative. Bone marrow stem cells are a good alternative. They are taken from the bone marrow, modified genetically and put back in the patient. These cells proliferate and the patient no longer has severe combined immunodeficiency.

However, retroviruses are known for integrating preferentially near promoters and a few patients have had viral integration right next to a proto-oncogene, so they are no longer immunodeficient but they end up with a leukemia-like disease which is not exactly untreatable but a lot more treatable than severe immunodeficiency.

To avoid this issue, adenoviruses are used.

Adenoviruses do not integrate. The problem with adenoviruses is that because they do not integrate DNA, their effect is *transient* (they don't last). Therefore, you have to continuously give patients their treatment to reach therapeutic levels. Another problem with adenoviruses is that they are highly immunogenic, so with every treatment they will have a worse immune reaction.

Adeno-associated viruses (parvoviruses) can replicate at the presence of adenoviruses. The problem with this virus is, again, transient transduction - the transcription is transient. However, you do not get the immune reaction you get with adenoviruses.

Lentiviruses (a famous example is HIV) are retroviruses, but unlike other retroviruses, they do not integrate in areas of coding genetic material or promoters, so you get around this problem of inducing a mutation. HIV retroviruses are used as vectors.

All these viruses must have most of their genetic material stripped out (to avoid an immunological response). Alternative genetic material is added, so that they are replication deficient (to stay in the host).

We are limited to kilo bases, some are 36 kb, but some of our genes, like the dystrophin gene is half a million kb so you can't package that into a virus!

In order to be able to package larger genes, non-viral vectors are used. **Liposomes** are vesicles consisting of lipid. They do not contain any protein content so they don't induce an immunologic response. They allow genetic material to pass through the cell membrane and they are big enough to take several million bases. The genetic material will reach the cytoplasm, the efficiency of delivering the genetic material is less than viruses, and a lot of the genetic material tends to be degraded in the cytoplasm. Nevertheless, it works. You can even inject the DNA directly, and you can have some of this DNA entering the cell.

You can also construct **human artificial chromosomes**. A chromosome that has functional telomeres, centromeres and you can introduce a giant genetic material and deliver them into a cell and this will replicate along with the chromosomes of the cell. All of these are experimental.

The problem with viral vectors and liposomal vectors is that *we do not really have much control over how much protein is to be produced*. In combined immunodeficiency, excess adenosine deaminiase is not a problem, but in certain thalassemia it is important for the number of alpha and beta chains to be closely balanced, so it is not the ideal way to fix the response.

All of the treatments we discussed so far are trying to replace a missing enzyme, or a defective protein. What if you had over activity of a protein? You would block it (similar to miRNA discussed previously in other courses). **Gene blocking** is potentially giving a copy of the complementary strand (the other strand where you produced the mRNA from) that binds the mRNA and now you have a double stranded RNA. Your cells don't like double stranded RNA, they think it is a virus; they start to chop it up by the same enzymes used to make miRNA (dicer and the rest). Therefore, you reduce the amount of the mRNA and the amount of the protein produced.

Ribozyme therapy means using certain RNA molecules that work and function like an enzyme. They bind to these particular DNA molecules and degrade them. Therefore, not all enzymes are proteins, some can be RNA.

RNAi is, again, based on the mechanism of miRNA. You produce a double stranded piece of RNA that has piece of a particular region of the mRNA. When the double-stranded RNA molecules (which are more stable than single stranded RNA and act as indicators of viral invasion) enter the cell, the cell immunity machinery will think that dsRNA is a virus and chop it up into 20 base-pair bits. Thus, the dicer will chop them into small complementary RNAs that would bind to mRNA and potentially it can also bind to DNA and shut down the transcription all together, reducing the amount of protein produced.

Gene Therapy for Non-inherited Diseases

This is significant for multifactorial diseases. In addition, cancer could be targeted by gene therapy. As you've already noticed RNAi could target *KRAS* in colon cancer, *HER2* in breast cancer, and *BCR-ABL* in chronic myelogenous leukemia.

Germline therapy

This type of therapy includes all of the techniques we talked about but instead of altering somatic cells they work on germline cells. There's serious ethical consideration and most of the scientific community discourages germline alteration, because while you may be adding a genetic factor that enhances one thing, you might screw with another thing because of the principle of pleiotropy (one gene has multiple effects; mentioned in p.74). We don't understand the whole genome, so we shouldn't don't screw with it. Even though we cloned many animals, humans should not be cloned. In all cloning experiments you hear about the success: Dolly the sheep. An ovum is taken, the genetic material is taken out and the nucleus from a somatic cell is introduced where you have manipulated its epigenetics to reopen all of the chromosomes and make sure you can transcribe all the factors you need for embryogenesis.

A lot of the initial trial ended up with massive malformations, and even Dolly the sheep became more ill as she grew older. Therefore, cloning humans is a big NO, even though there have been one or two reports where it have been done.

Next lecture: Genetics in clinical practice

Good Luck!

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