NC DNA DAY – Genes and Diseases | Cystic Fibrosis Presenter Notes

Title slide

- Hello! Today we are going to learn about how your physical characteristics are inherited through genetic material and how mutations in this genetic material can sometimes lead to disease.

- We will be focusing on the genetic disease, Cystic fibrosis, which is caused by a mutation in DNA

- We will lead you through an activity that puts you in the shoes of a clinician, where you will determine which patient of four possible patients has a cystic Fibrosis.

- And we will talk about how scientists and doctors are working together to come up with unique and novel treatments for cystic fibrosis patients.

What is DNA Day?

- DNA Day commemorates two major events in the history of our understanding about DNA. In April 1953, James Watson and Francis Crick determined the structure of DNA. 50 years later, in April 2003, the Human Genome Project determined the entire sequence of human DNA. Research scientists are using the knowledge and technology generated by this project to further understand how your DNA sequence can contribute to disease.

What is DNA?

- So What IS DNA? DNA is the most basic molecular building block of you!

- Your DNA dictates your physical appearance but it also plays a role in determining your susceptibility to disease and even your personality!

- As you know, YOU are a unique individual, and part of what makes you unique is your distinct variation of DNA.

Variations in the DNA of different individuals can cause visible changes in individuals

- First of all, lets take a minute to look around us. Just in this room, we can see many examples of genetic variation.

- Some genetic traits, such as the color of your hair or the color of your skin are controlled by multiple genes

- A gene is a segment of DNA that codes for a particular trait

- Others traits, such as the ability to roll your tongue or inability, are controlled by only one gene.

How are Genetic Traits Inherited?

- These traits, the characteristics that help define who we are, can be inherited.
- This is a photo of Amelia's family. (Amelia is one of the creators of this lesson.)
- All of our traits, from the color of our hair, to the color of our eyes, to the shape of our nose and chin are a combination of our parents' traits.

How are Genetic Traits Inherited?

- Our traits are inherited through our genes, remember, a gene is a segment of DNA that codes for a particular trait.
- Genes are carried on chromosomes, which are condensed and highly organized structures made of DNA
- We all have 23 pairs of chromosomes, as shown here.
- We inherit one chromosome from each parent. For every gene, we inherit one copy from our mother, and one from our father.
- This means that every person has two copies of each gene- one of each chromosome in the pair.
- For example on one pair of my 23 chromosomes I have one copy of the gene for tongue rolling from my mother and one copy of the gene for tongue rolling from my father.

Different versions of genes are alleles

- For each gene, you might have 2 different alleles – for example one allele for brown eyes and one for blue.
- OR you might have 2 of the same allele – for example, two alleles for blue eyes.
- Your combinations of alleles determine what traits you will have, such as…

Can you roll your tongue?

- …The ability to roll your tongue.
- Who can roll their tongue? (Raise hand) If you can, you carry a dominant allele, either one copy or two.

- Who cannot? (Raise hand) You carry two copies of a recessive allele (point to blue alleles).

- When we saw an allele is "dominant" this just means that if you inherit both the dominant and recessive allele, the dominant one will be expressed. The only way that a recessive allele can be expressed is if you carry two copies.

- It's important to point out here that if a trait is dominant, it is NOT necessarily more common! For example, the trait of having six fingers on one hand, which is called Polydactyly, is a dominant trait. Does anybody know anyone with 6 fingers? Probably not, it is extremely rare! Dominant does not mean that it is more common.

<u>Genetic Wheel</u>

- Now, to demonstrate how much genetic variation exists between people, we are going to look at the variation that exists in this classroom. To help us with this, I will lead us through an activity called the genetic wheel.

- You should each have a handout for the Genetic Wheel, shown here. As we go through 7 traits that are each controlled by a single gene, we will each color in a genetic wheel to display our individual set of these 7 genetic traits. At the end we will compare your results to those of your classmates.

<u>Single-gene traits (dimples and tongue roll)</u>

- Your combinations of alleles determine what traits you will have, such as… laugh dimples.

- Who has laugh dimples? If you do, you carry a dominant allele, either one copy or two.

- Who doesn't? You carry two copies of a recessive allele.

<u>Genetic Wheel (one trait)</u>

- Now we will start to fill in the genetic wheel.

- If you do have laugh dimples, you carry a dominant allele, either one copy or two, and you should color in the box near the center of the wheel with the capital L.

- Your genetic wheel looks like this.

- If you do not have laugh dimples, you have two copies of the recessive allele, and should color in the box near the center of the wheel with two lower case l's.

<u>Single-gene traits (tongue roll)</u>

- Next, who can roll their tongue? If you can, you carry a dominant allele, either one copy or two. Now color in the box with a capital T that connects to or contacts the box you colored in with either a capital "L" or two lower case "l"'s for dimples.

- Those who cannot roll their tongues carry two copies of a recessive allele. You should color in the box with two lower case t's in it.

Genetic Wheel (one trait)

- If you cannot roll your tongue, you should color in the box that connects to the box you just colored in for dimples, with two lower case "t"'s in it. Your genetic wheel looks like this

- The boxes should connect, making a "path".

- By now you should have colored in the box for your tongue rolling trait, either a box with one capital T, if you can roll your tongue, or one with two t's if you cannot.

Single-gene traits (crossing thumbs and pinkies)

- Can everyone clasp their hands together? Now look down and see whether your right or your left thumb is on top. If your left thumb is on top, you carry a dominant allele, and should color in the box with the capital C. If your right thumb is on top, you carry two recessive alleles and color in the box with two lower case c's. You should now have 3 boxes colored in, which are all connected to each other.

- For this next trait, press your two pinkies together side by side. If your pinkies are straight, you carry two recessive alleles. If your pinkies bend away from each other you carry a dominant allele.

- Now you should color in the box with either the capital P or the two lower case p's.

Genetic Wheel (four traits)

- This is an example of a genetic wheel so far. I have colored in a box for each of the four traits we have covered so far.

Single-gene traits (ear lobes and widow's peak)

- For these traits you'll probably need to help your neighbor. First look at your neighbor's ears. Look to see if their ears are attached, as shown in the picture on the left, or free, as shown on the picture on the right. If your ear is attached you carry two copies of the recessive allele, if it's free than you carry a dominant allele.

- For this next trait, we'll look at the hairline. If your hairline is straight across (as shown in the picture on the left) then you carry two recessive alleles. If your hairline points in the middle, as shown in the picture to the right, then you have what is called a widow's peak and carry a dominant allele.

- Continue coloring in the appropriate boxes, one for each trait.

Single-gene traits (hitchhikers thumb)

- For this last trait, I want you to give a "thumbs up". Now look at your thumb and see if it bends backwards (as shown on the left), or if it's straight (as shown on the right). The straight thumb is dominant.

- Now you can fill in the last box, with either one B or two b's.

Completed Genetic Wheel (one person)

- At this point, you have filled in your own genetic wheel. An example is shown here.

- Right next to the last box you colored in (the box with either one B or two b's) is a number. This number represents the specific combination of the traits that help make you "you".

- The number 32 is associated with the example combination of traits.

- Now find the number for your traits.

Completed Genetic Wheel (two people)

- Now look at your neighbor's genetic wheel.
- As you can see in this example, which shows two people's genetic wheels, one in yellow, and one in blue, by looking at just 7 traits we can uncover a large amount of variation between people.
- There are 128 possible combinations from the 7 traits illustrated on the genetic wheel.

- Are you the same as anyone else?

- **If this much genetic variation exists in traits that are visible, imagine how different we all are in ways that we can't see!**

Some diseases are caused by genetic factors that can be inherited

- So far we have looked at the inheritance of traits that do not cause a disease.

- Now, we will shift gears and talk about how the inheritance of some genetic factors can lead to disease.

- In order to understand genetic diseases however, I first want to review how information from DNA is translated into the proteins that make up our bodies and how this process can sometimes go wrong which in some cases can lead to disease.

Genes contain instructions to make proteins.

- So far we have learned about genes. On a molecular level, a gene is a segment of DNA. That piece of DNA tells your cells how to make a certain protein or series of proteins.

- In order to make that protein, the information on the DNA, shown here in dark blue, must first be copied into RNA shown here in light blue. This process is called **transcription**.

- That RNA copy then can be used by machinery in your cells to make proteins, shown in green. This process is called **translation**.

- Proteins are long intricately folded chains of amino acids. Amino acids are the most basic molecular building blocks of proteins.

- Proteins work together to form the functional machinery that makes up a cell.

How does an Altered Gene Result in an Altered Protein?

- Let's look more closely at how a change in DNA can result in the wrong protein.

- We'll use this sentence - SAM AND TOM ATE THE HAM - as an example of a DNA message.

- Much like the alphabet, Information in DNA is stored in as a series of 4 distinct molecules called bases. Each base has been assigned a letter, A C T or G.

- This series DNA bases is "read" in three-letter groups. Each three-letter group stands for a specific amino acid, similar to one word in a sentence. As we learned, these amino acids are strung together to make a protein, which is a complete functional unit, much like a completed sentence.

- What happens If we change a single letter in our sentence SAM AND TOM ATE THE HAM, For example – the "A" in HAM to an "I"

- This substitution changes the meaning of the sentence. In this case the sentence no longer makes sense.

- Similarly, if we change a letter in the DNA sequence – the central A to a C – one of the amino acids in the protein will be different. This new protein may not work properly.

- A change in the DNA is called a mutation.

- This is just one example of a mutation - there are many other ways that an altered gene can result in an altered protein. There are many types of mutations.

- Variations in the DNA of different individuals can cause physical changes in those individuals or even cause disease. An example of a disease caused by a genetic mutation is Cystic Fibrosis.

Cystic Fibrosis

- 1 in 30,000 Americans have it and there are 2,500 new cases per year
- Can lead to organ inflammation
- Infertility
- Most notably the inability to clear the thick mucus in the airway
- Diseases are mostly defined by the symptoms a patient can experience.
- Initially, antibiotics became the common treatment for CF, but because this didn't lead to people being cured, scientists began thinking about how CF is a genetic disease. To know the cause of CF, we first need to see what healthy lungs look like.

<u>The lungs are lined with specialized cells</u>

- Here is a depiction of what the lungs look like and here is a close up view. At first glance, some people might think that the lungs are just big hollow bags. In reality they are more like sponges, which increases the area inside the lungs that is available for blood to meet with the air.

- Your lungs need to be clean in order to function properly. The air you breathe is not always clean. For this reason, your lungs have a cleaning system. Dust, dirt, and germs are trapped in the **mucus** that lines your nose, trachea and lungs. The glands that make the mucus are called **mucus glands**. The dirty mucus is then pushed out of your lungs and into your throat by tiny hairs called **cilia**. This system is called the **mucus escalator**. Without it, we would have no way to get rid of germs from our lungs and we would be sick all the time!!

<u>These special cells clear dust and excess mucus</u>

- Think of this ball as mucus and the guy inside is dirt. Without the hands in the crowd, that ball would fall and not move. The inability to move the dirt or other particulates causes breathing problems.

- So what helps the hands move the dirt?.....Right, it's the mucus layer.

<u>Cystic Fibrosis Transmembrane Regulator (CFTR) Protein helps clear the mucus</u>

- Let's remember how proteins are made, you have a gene in the DNA that is… (have them answer transcribed) and then RNA is …(have them answer translated) into protein. Remember, these genes in our chromosomes are inherited. Now that we have our protein, let's see how it is incorporated into the cell membrane.

<u>The CFTR in lung cells</u>

- [Point everything out] These are lung epithelia cells (can anyone tell me what epithelial means, cells that line surfaces in the body). These are the Cilia (green), and here are the cell's nuclei (blue). Here is CFTR present on the surface of the cells below the cilia.
  Slide definition:
  Epithelial Cell – Cells that form the covering or lining of all internal and external body surfaces

Mutations in the CFTR gene blocks CFTR protein function

- In a common mutation in the DNA of the CFTR gene, the amino acid sequence is changed in the protein and the protein does not fold properly. This mutated protein gets degraded before it ever reaches the plasma membrane.
- As you saw before the normal mucus layer is thin and can move well to move dirt out. The CF mucus is thick and a lot harder to move. This makes it difficult to breathe. Now what is the relationship between the mucus consistency and CFTR?

Cystic Fibrosis airways are dehydrated and cannot clear mucus

- In Cystic Fibrosis, the outside of the lung cells (airway) is dehydrated.
- Instead of being able to move freely like in the healthy airway, the cilia in a CF airway are trapped in thick mucus (think GAK!) and cannot move.
- The thick mucus gets stuck in the lungs and becomes a breeding ground for all sorts of infectious bacteria.
- How does water move in and out of cells?
- Answer: osmosis/water follows concentration gradient of solute
- Turns out, the CF airway mucus has a lack of water above the cilia. It is the CFTR protein that helps regulate the balance of water in the mucosal layer.

Cystic Fibrosis airways accumulate bacteria and inflammatory cells

- So what happens with this thick mucus layer? Turns out dirt is just the beginning of your problems, you also have to worry about bacteria such as PA. PA is an opportunistic pathogen, which means it infects people who are immunocompromised or already sick. Although the genetic disease is bad, it is the PA infection which is the ultimate demise of the CF patients. You also get buildup immune cells in the lung.
  Slide definitions:
  Opportunistic pathogen – An infection that does not cause disease in a host with a healthy immune system, but does in a host who's immune system is compromised. Another way to think of it is: A pathogen that causes disease when there's an "opportunity".
  Immunocompromised – A deficient immune system

Cystic Fibrosis Activity

- So now let's do an activity in which you will be the investigator. Your challenge is finding the person who has CF. **SEE HANDOUT FOR CF ACTIVITY 1.** Pass out investigator sheets to the class.

Investigate the patient samples

- [read slide]

Who do you think has cystic fibrosis?

- Remember patient A is a 1 year old girl, patient B is a two year old boy, patient C is a 3 month old male, and patient D is a new born girl.

- Let's take a look at their symptoms.
- Patient A has 1 of the 3 symptoms. It is doubtful they have CF.
- Patient B has 3 of the 3 symptoms. It is very possible this person has CF
- Patient C has 0 of the 3 symptoms. It is very doubtful they have CF
- Patient D has 2 of the 3 symptoms. It is possible this person has CF
- Let's take a closer look at patients B and D. To discover how to confirm our diagnosis, let's learn how we can use what we know about the genetics of CF to diagnose the disease.

Sequence DNA and look for mutations in the *cftr* gene

- So now that we think that patients B and D possibly have cystic fibrosis, what could we do next to confirm this diagnosis?

- We could sequence the DNA of the *cftr* gene and look for mutations that are known to cause cystic fibrosis

- There are about 900 mutations in the *cftr* gene that lead to cystic fibrosis, so we have lots of mutations to look for!

- We have sent samples from patients B and D to the lab and have had their DNA sequenced. You can see the DNA sequences on the handout. **HANDOUT 2.**

How will we determine if patients B and D have mutations in CFTR?

- In order to determine if mutations in the *cftr* gene for patients B and D result in mutations in the CFTR protein, we need to remember how proteins are made. DNA is transcribed to RNA which is translated to protein. If there is a mutation in the DNA, there could possibly be a mutation in the protein.

- On your handout, you can see the partial DNA sequences for patients B and D. We have to do two things to check if there are mutations in the patients' CFTR proteins. First, we have to transcribe the DNA to RNA. Second, we have to translate the RNA sequences to protein sequences. Using the instructions on your worksheet, determine the protein sequences for each patient's CFTR protein.

- PAUSE FOR STUDENTS TO COMPLETE THE WORKSHEET

Which patient has the defective CFTR protein?

- Here are the results from the worksheet. Yours should look like this.

- You now have the partial CFTR protein sequence for patients B and D. Comparing the protein sequence to the normal CFTR protein sequence, which patient has the defective CFTR protein?

- Based on DNA sequencing, we have found that patient D has two mutations in the *cftr* gene. One mutation does not cause the amino acid to change. This is called a silent mutation. The other mutation caused an amino acid to change and this is called a point mutation. This leads to a defective CFTR protein. Patient D therefore has cystic fibrosis.
- While patient B had symptoms of cystic fibrosis, the patient does not have a mutation and does not have cystic fibrosis. Perhaps patient B has bronchitis or a sinus infection that could lead to these symptoms.

How do you treat CFTR?

- We just found out that patient D has the defective CFTR protein and has cystic fibrosis. How do you treat cystic fibrosis?

Current cystic fibrosis treatments

- There are currently three therapies that are used together to treat cystic fibrosis

- 1. Bronchodilators → these are oral, inhaled, or injectable drugs that open up (dilate) the airways of the lungs

- 2. Airway clearance techniques → once the airways are opened up, you need to clear the mucus out since your lungs are not doing this on their own. By simple coughing, you can clear some mucus. There are also assist devices, such as those shown here. These devices contain small metal balls inside of them. When you blow into them, the metal ball vibrates, which causes vibrations through your mouth, trachea, and into your lungs. These vibrations help to dislodge mucus, which you can then cough out.

- 3. Antibiotic treatment → since cystic fibrosis patients have lots of mucus in their lungs, this is a breeding ground for bacterial infections. By using oral, inhaled, or injected antibiotics, the harmful bacteria in the lungs can be killed to prevent infections

But what if…

- These current methods treat the symptoms of cystic fibrosis, but what if we could treat the cause of the disease?

- How might we be able to do that?

- What if we could change the DNA, to fix the CFTR mutation which would make a normal CFTR protein and thus cure cystic fibrosis?

Gene therapy

- This type of treatment is being developed by scientists and doctors and is called gene therapy.

- The goal of gene therapy is to treat the cause of the disease, not just the symptoms.

- In gene therapy, the idea is to add "normal" DNA that does not have the mutation to cells using a special virus, which injects the normal DNA into cells. The normal DNA will be transcribed and then translated to normal protein. In the case of cystic fibrosis, by adding normal DNA that does not have a mutation in the CFTR protein, normal CFTR protein can get made which will then go to the cell membrane and help to effectively clear mucus, thus curing cystic fibrosis.

- Currently, gene therapy is not approved in the USA, but trials are being conducted to prove its safety and effectiveness.

    - Problems
    - How best to deliver genes to cells?
    - Will cells accept genes?
    - Will normal protein be made?

Conclusions

- Our physical characteristics, from our appearance to our susceptibility to disease, can be inherited genetically from our parents.

- Cystic fibrosis is caused by a mutation in a single gene.

- Gene therapy can be used to treat the cause of many genetic diseases, including cystic fibrosis.

Vocabulary List

- Silent mutation – a change in DNA that does not result in a change in amino acid

- Point mutation – a change in DNA that results in a change in amino acid

- Bronchodilator – an oral, injectable, or inhalable drug used to open up (dilate) airways

- Gene therapy – method to treat the cause of genetic diseases by adding normal DNA to cells (usually via a viral vector) to allow expression of normal, functional protein