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BioEd℠

Complex Traits

Mapping a Mutation

Nancy P. Moreno, Ph.D.

Baylor College of Medicine

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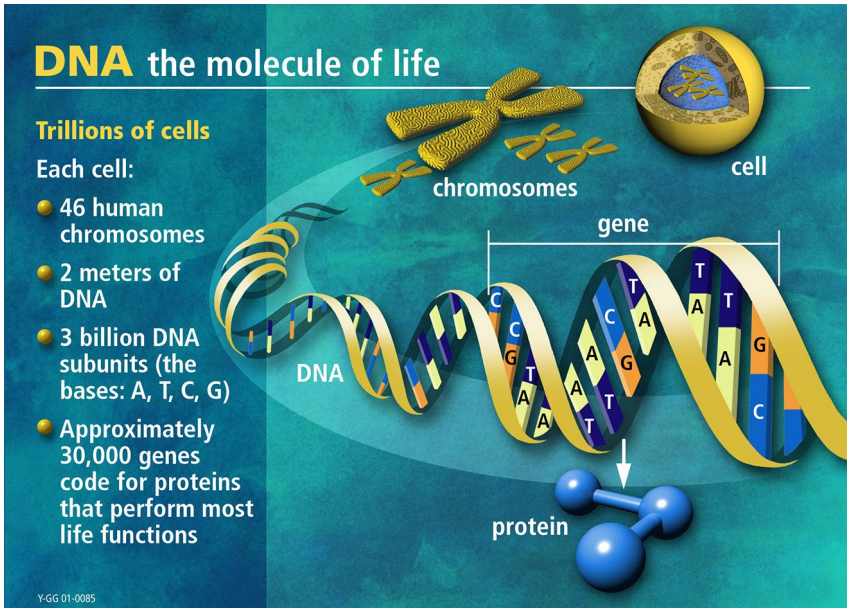
OVERVIEW

Students learn about the genetic code, and how small substitutions within the code can lead to physical changes in individuals. Students use authentic data to uncover the single nucleotide polymorphism (SNP) responsible for long hair in dogs.

Mapping a Mutation

In the activity, "Genotyping a Mixed-breed Dog," students were introduced to the variety of characteristics found across breeds of dogs. They used information produced by modern genetics research to learn which breeds of dogs were most related and how genetics information is used commercially to help people figure the history and possible parentage of mixed breed dogs. These examples represent applications of genomics—a field that offers a new way of thinking about genes and heredity.

Traditionally, genetics researchers have focused on identifying single genes, their variant forms (alleles) and their functions. Genes are hereditary units that occupy a fixed location on a chromosome or DNA molecule. Each gene has a specific influence on the characteristics of an individual (phenotype) and provides the instructions for creating proteins (technically, certain sections of genes code for strings of amino acids or polypeptides). Usually a single gene has various forms, known as alleles. Since every individual has two sets of chromosome, each individual also has two versions or alleles for each gene. The interaction between the two alleles, and of a particular gene with other genes and the environment, determines the phenotype of the individual.



DNA graphic courtesy of the Office of Biological and Environmental Research of the U.S. Department of Energy's Office of Science. science.energy.gov/ber/

Genes are encoded along a pair of twisted DNA (deoxyribonucleic acid) strands, known as a double helix. Each strand is made of a sequence of four much smaller molecules, adenine (A), guanine (G), cytosine (C) and thymine (T). The strands are complementary, so that an A on one strand is matched by a T on the other strand, and a C is matched by a G (see graphic, above). The human genome consists of more than 3 billion of these pairs, called base pairs, packaged into 23 pairs of chromosomes. A small amount of DNA also is found in the mitochondria (where energy is processed inside cells).

Humans have about 20,000–25,000 genes. The average human gene consists of about 3,000 bases. Dogs have 39 pairs of chromosomes, which contain approximately 2.5 billion base pairs, and about 19,000 genes.

The "central dogma" of genetics explains that DNA codes for RNA (ribonucleic acids, another class of long chain molecules), which in turn, encodes and manufactures all of the proteins needed by an organism. The process of creating RNA from DNA is called transcription. Even though DNA has two strands, only one strand is read during transcription of a given sequence (genes can be located on either strand). The other

strand serves as a template to produce a molecule of RNA with the same sequence as the "coding" or "non-template" strand. We now know that 80% of human DNA does not code for proteins. These noncoding regions have other functions, including preserving important gene sequences and regulation of gene functions.

The relatively new field of genomics looks at an organism's entire complement of DNA, including all of its genes. The nucleus of every cell (except for red blood cells) contains a copy of the entire genome of the organism. The human genome was sequenced for the first time in 2003. In 2005, a complete dog genome was sequenced using DNA from a female boxer named Tasha.

TASHA

Mutations provide the raw material for genetic variation, and come about through mistakes when DNA is copied or environmental factors, such as ultraviolet radiation from the sun. A mutation is a permanent change in the sequence of DNA. Mutations range in size from a change in a single DNA base (point mutation) to a change in a large segment of a chromosome. When a single nucleotide at the same location differs between individuals, the variation is called a "snip" (short for single nucleotide polymorphism or SNP).

In this activity, students are introduced to genetic variation by looking at recent research results on hair length in dogs. Short hair is the ancestral (original) condition in dogs. Long-haired dog breeds were found to have a mutation in the gene that signals for the termination of hair growth. The mutation causes a loss of function in hair growth regulation, presumably leading to longer hair.

The researchers began by studying a gene that was known to contribute to hair growth in other species (called FGF5, named for the "fibroblast growth factor 5" protein). By comparing sequence data from different dog breeds, they found that a single mutation (from G to T) at one location consistently correlated with the presence of long hair. The mutation causes an amino acid change (cystein to phenylalanine). Only dogs that are homozygous (have two copies) for the mutation have long hair. Students will use some of the original published data to find the location and nature of the mutation.

FAST FACTS

The nucleus of every cell (except for red blood cells) contains DNA, which is the hereditary material in organisms.

A gene is DNA material that occupies a fixed location (section) on a chromosome or strand of DNA.

A DNA strand containing a single gene also contains various forms of that gene, known as alleles.

Each gene has a specific influence on the characteristics an individual or organism (phenotype).

A genome is an organism's complete set of DNA.

MATERIALS

- Complex Traits slide set (slides 16–23), available at http://www.bioedonline.org/slides/classroom-slides1/genetics-and-inheritance/complex-traits/
- Computer and projector, or interactive whiteboard
- Copies of the student page (one per student)

Tasha the boxer courtesy of the National Human Genome Research Institute. Public domain. (https://www.genome.gov/17515860/2005-release-researchers-publish-dog-genome-sequence/) Help Me Understand Genetics. Genetics Home Reference. U.S. National Library of Medicine. https://ghrnlm.nih.gov/

PROCEDURE

1. Introduce the topic of genes and genomes by showing Slide 16. Write on the board or project the following conversation for students to see (Slide 17).

   Person 1: hru?
   Person 2: hv hw
   Person 1: cul8r?
   Person 2: gr8 tty**s**

   Ask students, *When would you write something like this?* [text message on a smart phone] Follow by asking, *Does the message use symbols that everyone can read?* [Only people who know how to send text messages in English can understand the code.] Have one or more students translate the text messages into full sentences.

   Person 1: How are you?
   Person 2: Have homework.
   Person 1: Call you later?
   Person 2: Great. Talk to you soon.

   Ask students to share other examples of text messaging codes. Ask, *What are the advantages of using a code?* [A code conveys information in a compact way, but the receiver has to know how to make sense of the code.]

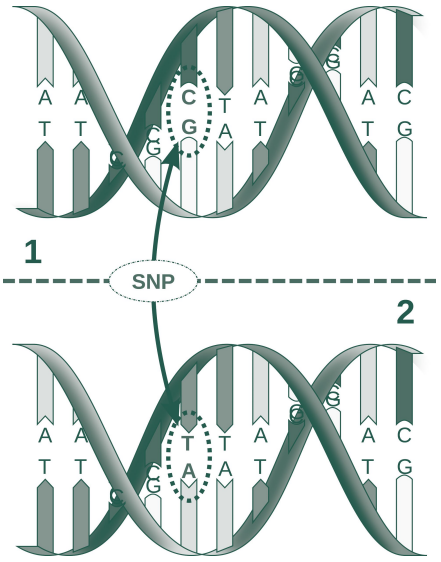
2. Tell students that living cells also use a code, which contains all of the instructions for a living organism. This information, which is contained on long DNA molecules, is packaged inside the nucleus of every cell and are found on chromosomes (Slide 18). Show the following three-minute video created by the BBC (Knowledge Explainer DNA), which provides a general explanation of the genetic code in DNA.

   http://vimeo.com/60747882#

3. Help students understand that DNA contains a unique code that directs all of the functions of a living organism (Slide 19). Further, all living organisms use the same code. This code was unlocked about 60 years ago by researchers James Watson and Francis Crick. Show students the video below, in which James Watson describes how they figured out the paired, helical structure of DNA.

   http://www.youtube.com/watch?v=PDeaLx-oL75M

4. Discuss the concepts covered in the video with students: DNA consists of sequences of four molecules or nucleotide bases: adenine (A), guanine (G), cytosine (C) and thymine (T). DNA always has two complementary strands, in which an A on one strand is matched by a T on the other strand, and a G is always matched with a C. The A-T and G-C combinations are called base pairs. Since the strands are complementary, only the sequence on one of the strands is used as a template for the first step in producing a protein molecule.



5. Use Slide 20 to further explain the DNA molecule. Tell students that biologists now can identify the sequence of nucleotides in a section of DNA. Usually, there are many differences in the DNA from one individual organism to another member of the same species. Most differences do not cause any observable changes or diseases. However, sometimes a mutation (change in the DNA sequence) causes some individuals to be

different. By comparing the DNA sequences of two different groups, biologists sometimes can find the genetic change responsible for variation among individuals.

6. Show students Slide 21, which asks, *Is there a genetic explanation for long hair in dogs?* Have students discuss the information. They should conclude that genetics must be involved, because long hair can be inherited by offspring.

DACHSHUNDS (L–R): WIRE HAIR, SHORT HAIR, LONG HAIR

7. Have students work in teams of two or four persons, and give each group a copy of the student sheet (Slide 22), "Understanding Long Hair in Dogs." Refer back to slide Slide 20 to explain SNPs if students have questions.

8. Have students read the instructions carefully, and work to find the location of the change in the genetic code that correlates to long vs. short hair. Students will examine the sequence data to find the location (row) with a pattern of nucleotides that best matches the phenotypes of the individuals (long vs. short hair).

   You may need to guide students with questions, such as, *Which rows do not show any changes from one individual to the next? Can you eliminate these rows from further consideration? Why or why not?* [The rows may be eliminated because they do not help discriminate between the short- and long-haired dogs.]

   Remind students, to think about patterns within each pair of nucleotides, because each individual has two copies at every location. When the two nucleotides are the same within a pair, the condition is called homozygous. When the two nucleotides in a pair are different, the condition is called heterozygous.

9. Have each team present its results and the rationale for the selected location, or write a paragraph describing their findings.

   Students will find that only one row (location 20) completely matches the pattern of long vs. short hair. Dogs with two copies of the T nucleotide at this location exhibit long hair.

10. Discuss students' findings in class. Help them to understand that the single substitution of a "T" for a "C" led to the formation of a defective protein, which in turn, altered the signal for telling individual hairs to stop growing. In other words, the mutation causes a loss of function in hair growth regulation, presumably leading to longer hair. Only dogs with two copies of the mutation have long hair. It is likely that dogs with one copy of the short-hair allele produce enough of the hair termination protein to keep the hair from growing excessively.

11. Remind students of the text messages used to begin the activity. Project Slide 23. Ask, *What would happen if the message were changed in the following way?*

    Person 1: hru?
    Person 2: hv hw
    Person 1: cul8r?
    Person 2: gr8 tty**s** changes to gr8 tty**l**

    "Talk to you soon" becomes "Talk to you later." The change probably would not affect the overall message, although one word was changed. This is similar to the many mutations in DNA that do

not change characteristics (phenotype) of the individual.

12. Next, ask about the following changes.

    Person 2: gr8 ttys changes to gr8 tty**t**

    "Talk to you soon" becomes "Talk to you tomorrow."

    Person 2: gr8 ttys changes to g**2g** tty**t**

    "Great" becomes to "Got to Go"; and "Talk to you soon" becomes "Talk to you tomorrow."

    These changes not only change the words designated by the code, but also alter the meaning of the messages.

13. Help students understand that mutations in DNA act in similar ways. Most mutations lead to no detectable changes in phenotype. Other mutations cause variations that are helpful or harmful, or have neutral effects. Variations at a single location along a DNA strand are called "single nucleotide polymorphisms."

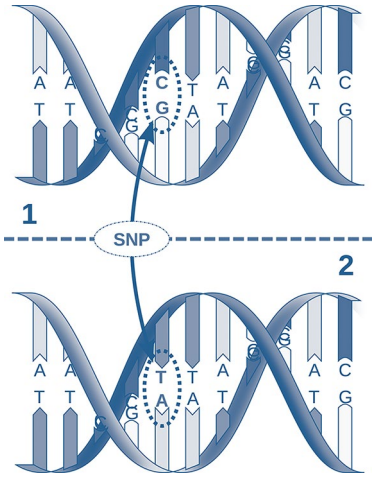
    Note: The mutation for long hair causes a loss of function in dogs that receive two copies of the allele.

Understanding Long Hair in Dogs

The table to the right presents sequence data from a gene that controls hair growth in dogs. The gene is found on dog chromosome number 32, and contains about 70,000 nucleotides (or "letters").

Certain sections of the gene appeared to be more variable, and those sections were sequenced in 10 individual dogs of different breeds. The investigators found a number of locations where individual dogs had different nucleotides at the same spot along the DNA strand. These locations are given in the table.

The columns in the table represent the 10 sampled dogs. Each row corresponds to a single location where the nucleotides (letters) were different. Biologists refer to a mutation at a single nucleotide position as a SNP, pronounced "snip" (which stands for single nucleotide polymorphism). Many times, these tiny mutations do not cause any differences between individuals. Remember that each dog has two versions of the nucleotide (one on each chromosome).



When a single nucleotide at the same location differs between individuals, the variation is called a "snip" (SNP).

Your challenge is to find the SNP location that explains the differences between the dogs that were sequenced. In other words, which SNP location best matches the pattern of long vs. short hair?

LOCATION	LONG HAIR				SHORT HAIR					
	1	2	3	4	5	6	7	8	9	10
	Havanese	American Eskimo	Standard Poodle	Dachshund (long hair	Entlebucher Mountain Dog	Dachshund (short hair)	Dachshund (short hair)	Collie	Border Terrier	Pug
1	CC	CC	TC	CC	CC	CC	CC	CC	CC	CC
2	GG	GG	GG	GT	GG	GT	GG	GG	TT	TT
3	AA	AA	AA	AG	AA	AG	AA	AA	GG	GG
4	CC	CC	CC	CT	CC	CT	CC	CC	TT	TT
5	GG	GG	GG	GG	GT	GG	GG	GG	GG	GG
6	TT	TT	TT	CT	TT	CT	TT	TT	CC	CC
7	AA	AA	AA	AT	AA	AT	AA	AA	TT	TT
8	AA	AA	AA	GA	GA	GA	AA	AA	GG	GG
9	TT	TT	TT	GT	TT	GT	TT	TT	GG	GG
10	CC	CC	CT	CT	CT	CT	CC	CC	GG	GG
11	TT	CT	CT	CT	TT	TT	TT	TT	TT	TT
12	GG	GA	GA	GA	GA	GG	GG	GG	GG	GG
13	GG	GA	GA	GA	GA	GG	GG	GG	GG	GG
14	CC	CT	CT	CT	CC	CC	CC	CC	CC	CC
15	CC	CC	CT	CC	CC	TT	TT	TT	TT	TT
16	GG	AA	AA	AA	AA	GG	GG	GG	GG	GG
17	AA	AA	AA	AA	GG	AA	AA	AA	AA	AA
18	CC	CC	CC	CT	TT	CC	CC	CC	CC	CC
19	GG	GG	GG	GG	GG	AA	AA	AA	AA	AA
20	TT	TT	TT	TT	GG	GG	GG	GG	GG	GG
21	GG	GG	GT	GT	TT	TT	TT	TT	GG	GG
22	TT	TT	CT	CT	CT	CC	CC	CC	TT	TT
23	AA	AA	AA	AA	AA	CC	CC	CC	AA	AA
24	GG	GG	GG	GG	GG	GG	GG	GG	GG	GG
25	CC	CC	CT	CT	CC	TT	TT	TT	CC	CC

Graphic courtesy of NIH/David Hall.