PEDIGREES AND THE INHERITANCE OF LACTOSE INTOLERANCE

OVERVIEW

This activity serves as a supplement to the film *Got Lactase? The Co-evolution of Genes and Culture* (http://www.hhmi.org/biointeractive/making-fittest-got-lactase-co-evolution-genes-and-culture). Students analyze the same Finnish family pedigrees that researchers studied to understand the genetic basis of lactose tolerance/intolerance. Students also analyze portions of actual DNA sequences to identify the specific mutations associated with the trait.

KEY CONCEPTS AND LEARNING OBJECTIVES

- Mutations associated with a particular trait can be identified by comparing the DNA sequences of individuals with the trait to those of individuals without the trait.
- Mutations occur everywhere in the genome. Mutations that have an effect on traits can occur not only in coding regions of genes but also in the regulatory regions that determine when and where genes are turned on.
- Using both pedigree analysis and genetic analysis, scientists have discovered that the lactose-tolerance trait (lactase persistence) is autosomal dominant; individuals need to inherit only one copy of the lactose-tolerance allele to continue producing lactase as adults.

After completing this activity, students should be able to

- study a pedigree to make a claim based on evidence about the mode of inheritance for a trait;
- determine the most likely inheritance pattern of a trait tracked in a pedigree and the genotypes of individuals included in the pedigree;
- analyze variations in DNA to make claims about which variants are associated with specific traits.

CURRICULUM CONNECTIONS

Curriculum	Standards
NGSS (April 2013)	HS.LS3-1, HS.LS2.A, HS.LS1.A, HS.LS3.A
Common Core (2010)	CCSS.ELA-Literacy.RST.9-10.3, CCSS.ELA-Literacy.RST.9-10.5, CCSS.ELA-Literacy.RST.9-10.7, CCSS.ELA-Literacy.RST.9-10.4, CCSS.ELA-Literacy.RST.11-12.4, CCSS.ELA-Literacy.RST.11-12.3,CCSS.ELA-Literacy.RH.9-10.4, CCSS.ELA-Literacy.RH.9-10.7, CCSS.ELA-Literacy.RH.11-12.4
AP Biology (2012-13)	3.A.3, 3.B.1, 3.C.1, 4.A.1, LO 3.14, LO 3.18, LO 3.19
IB Biology (2016)	2.6, 3.1, 3.4

TIME REQUIREMENT

This lesson is designed to be completed in two 50-minute classroom periods with perhaps a small amount of homework. Viewing the film prior to the activity requires an additional 15 minutes.

SUGGESTED AUDIENCE

This lesson is intended for regular and honors high school biology.

PRIOR KNOWLEDGE

Students should have prior knowledge of the relationships between genes, proteins, and traits and know that the letters A, T, C, and G represent the nucleotides of DNA. Students should know how changes in DNA sequences, or mutations, can lead to changes in traits. Students should also have general knowledge of

autosomal, X-linked, recessive, and dominant patterns of trait inheritance and basic genetics vocabulary that includes the terms homozygous, heterozygous, allele, genotype, and phenotype.

MATERIALS

- Student worksheet

TEACHING TIPS

- Students should watch the short film *Got Lactase? The Co-evolution of Genes and Culture* at http://www.hhmi.org/biointeractive/making-fittest-got-lactase-co-evolution-genes-and-culture before doing this activity.
- Before students see the movie, consider asking how many of them know someone who is lactose tolerant. Then ask what data they would collect to answer the question, "What is the mode of inheritance for lactose tolerance/intolerance?" The question should lead to a classroom discussion that engages students to pay more attention to the film.
- This activity lends itself to a flipped classroom approach. Students can watch the film as homework and then perform this activity in class the following day.
- Another option for integrating film and activity is for students to watch the film until about time 5:15 when they see the Finnish pedigrees. Have students examine the pedigree shown in the film and ask them about the inheritance pattern of lactose tolerance/intolerance. (The pedigree shown in the film is Family I in the original paper, which is not included in this activity.) Have students complete this activity and then watch the rest of the film.
- You may wish to have students work in pairs or small groups to perform this activity.
- Part 2 of the activity involves examining short DNA sequence to find the mutation associated with turning of the switch. If students have limited background knowledge about genes at the time this activity is used, you may need to provide more information or omit part 2.
- To learn more about how the expression of the lactase gene is regulated, proceed through the Click and Learn titled "Regulation of the Lactase Gene" at http://www.hhmi.org/biointeractive/regulation-lactase-gene.

ADDITIONAL BACKGROUND

The inability of humans to digest lactose was first described by researchers at the University of Manchester in England in the journal *The Lancet* on May 30, 1959. The team studied two siblings, a brother and a sister, both of whom were diagnosed with "failure to thrive in infancy" and had chronic flatulence, diarrhea, and abdominal pain. They determined that neither could digest the sugar lactose, but both could absorb glucose and galactose when consumed together. They provided two hypotheses to explain their observation: 1) neither child was producing the lactase enzyme, or 2) the lactase enzyme was being inhibited by another substance the children were producing. The team further postulated that because both children had the trait, it was likely hereditary, and because the parents of the two children could digest lactose, the trait did not follow a dominant inheritance pattern.

This single report quickly led to other studies, and by the mid-1960s scientists were reporting variations in the distribution of lactose intolerance among people from different ethnic backgrounds. In 1970, Frederick Simoons, a professor of geography at the University of California at Davis, proposed that the addition of goat and sheep milk to human diets starting 10,000 to 6,000 years ago could explain the geographic distribution of lactose intolerance. By the mid-1970s, it had become clear to Western scientists and medical doctors that lactose intolerance was the more prevalent condition and the ability to digest lactose, or lactose tolerance,

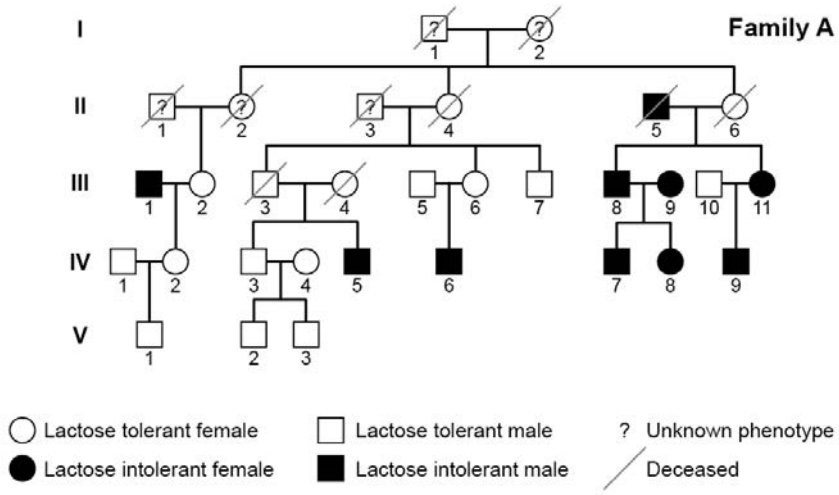
was more rare. By this time, scientists had also concluded that the lactose-intolerance trait was inherited in an autosomal recessive pattern.

You may want to discuss this information with your students. Why did the researchers from the University of Manchester conclude that there was something wrong with the lactase enzyme based on their observations with the two siblings? At first researchers thought lactose intolerance was an "abnormal" or "disease" condition. Ask your students why that was. Think of the countries that many of these researchers were living in.

Students may have questions about the difference between the terms lactase persistence and lactose tolerance. These terms are used interchangeably in the film and this activity, but they don't mean exactly the same thing. Lactase persistence refers to the persistent production of the lactase enzyme into adulthood. Except for some human adults, most adult mammals stop producing the lactase enzyme as adults—in other words they are lactase nonpersistent. Lactose tolerance refers to the ability to digest lactose, the sugar in milk, without any problems, such as bloating, flatulence, or diarrhea. Most lactase persistent adults can digest milk without any symptoms, which means they are lactose tolerant. However some lactase nonpersistent adults may also be able to tolerate small amounts of milk or milk products. In other words, not everyone who is lactase nonpersistent is lactose intolerant—or at least not to the same degree.

ANSWER KEY

Part 1: Determining the Pattern of Inheritance of Lactose Intolerance



1. *Based on the pedigree above, which of the following terms are appropriate for describing the inheritance of the lactose-intolerance trait (filled-in symbols)? Check all that apply.*

X Recessive ____ Dominant

X Inherited ____ X-linked

2. *Which of the following terms are appropriate for describing the inheritance of the lactose-tolerance trait (empty symbols)? Check all that apply*.

_____ Recessive **X Dominant**

X Inherited _____ X-linked

3. *Use the data in the pedigree and the terms listed in questions 1 and 2 to make a claim about how lactose intolerance is inherited. Provide at least two pieces of evidence in support of your claim*.

The inheritance pattern of lactose intolerance is autosomal recessive. Evidence may include:

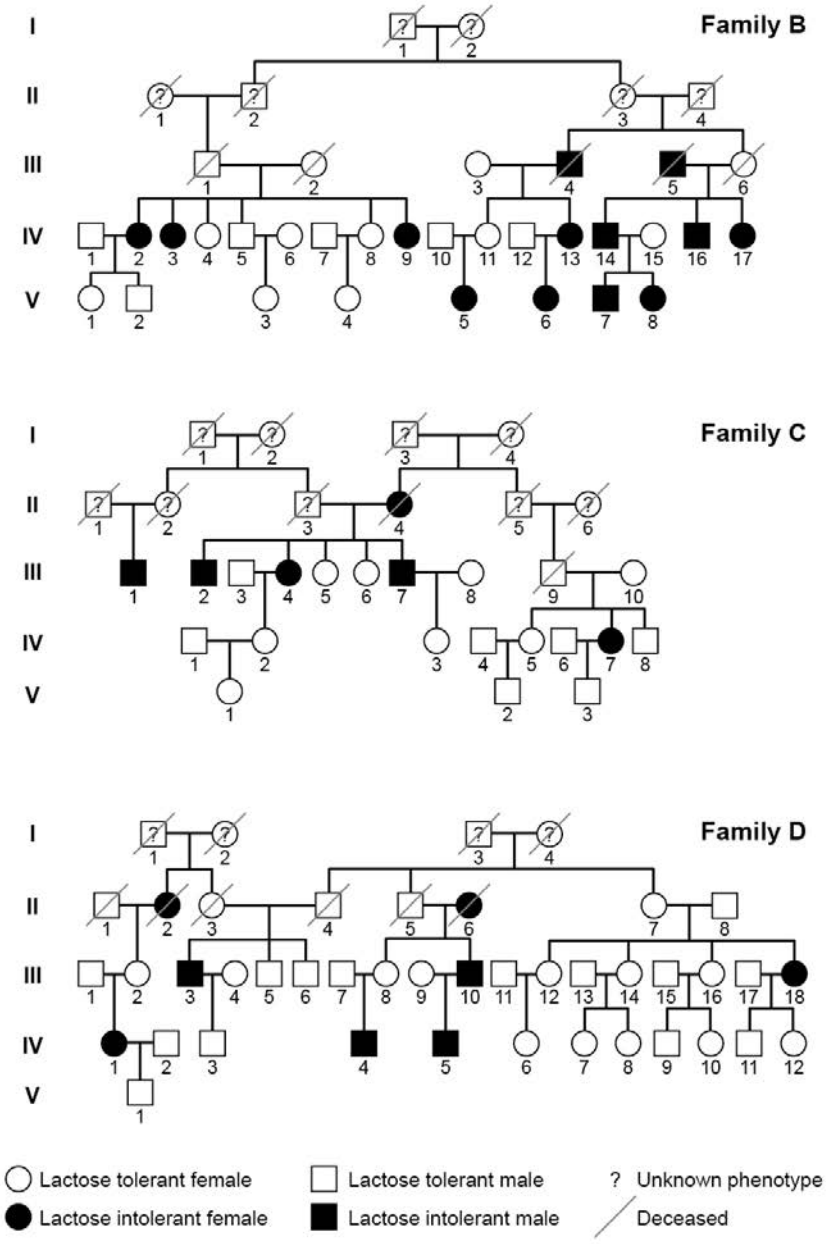
- **The pedigree shows that two parents who are lactose tolerant can have a child (male or female) who is lactose intolerant, which is evidence that the trait is recessive.**
- **The fact that the trait occurs in males and females suggests it is not X-linked**.

4. *How comfortable are you with drawing a firm claim about the inheritance of lactose intolerance? What might you do next to increase your confidence in making this claim?*

Answers will vary. Some students may indicate that they are uncomfortable making a claim about the inheritance pattern of a trait based on a single pedigree and that they would want to analyze more families.

5. *Study Individuals 5 and 6 of Generation III in Family A and their child. The two parents are lactose tolerant, yet their son is lactose intolerant. Are these data consistent or inconsistent with the claim you made in question 3? Explain your answer*.

The two parents are lactose tolerant, meaning they can be homozygous or heterozygous for the dominant allele. The only way to explain how their son could be lactose intolerant is if both parents are heterozygous and each passed the recessive allele to their son.



6. *Do the data presented in these additional pedigrees support your claim in question 3 above? Explain your answer.*

Students should point out that the data from the additional pedigrees are consistent with lactose intolerance being inherited as an autosomal recessive trait. There are a few examples of lactose-tolerant parents with lactose-intolerant offspring.

7. *Based on your claim, which symbols would you use to represent the genotype of an individual who is lactose tolerant?* **LL or Ll** *Lactose intolerant?* **ll**

Students' answers will vary depending on how they choose to designate the allele. In the example above, we used uppercase "L" for lactose tolerance and lowercase "l" for lactose intolerance.

8. *Based on your claim, what is the genotype of Individual 3, Generation IV in Family C? Explain your answer.*

Individual 3, Generation IV in Family C is lactose tolerant; she must be either heterozygous (Ll) or homozygous (LL) for the lactose-tolerance allele. Her father is lactose intolerant, which means that he has two recessive alleles for lactose intolerance (ll). Thus Individual 3 must have inherited one of those two alleles. That means that she is heterozygous, having one lactose-tolerance allele and one lactose-intolerance allele.

9. *Based on the same claim, what is the genotype of the father of Individual 4, Generation IV in Family D? Explain your answer.*

The father of Individual 4 in Family D is heterozygous; the genotype is Ll. We know this because Individual 4 is lactose intolerant and must therefore be homozygous recessive (ll). Individual 4 received one recessive allele from each parent, who must both be heterozygous because neither of them is lactose intolerant.

10. *Individuals 8 and 9, Generation IV in Family B are sisters. Based on your inheritance claims, what is/are the possible genotype(s) of*:

Individual 9? Homozygous recessive (ll)

Individual 8? Could be heterozygous (Ll) or homozygous dominant (LL)

The parents of Individuals 8 and 9? **Both are heterozygous (Ll)**

11. *Individual 4, Generation IV in Family B is the sister of Individuals 8 and 9 in the question above. What is the probability that Individual 4 is homozygous?* **1/3** *Heterozygous?* **2/3**

Given that both parents are heterozygous (Ll), their children have a 1/5 chance of being LL, 1/2 chance of being Ll and a 1/4 chance of being ll. However, in this individual's case, we know she isn't homozygous recessive (ll) because she is lactose tolerant. Given the 3 remaining states, she has a 1/3 probability of being homozygous (Ll), and a 2/3 probability of being heterozygous (LL).

Part 2. Finding the Responsible Mutation

1. *Study the two tables below. Fill in the phenotype (e.g., lactose tolerant or lactose intolerant) of each individual in the second column of the table. (You will need to look at the pedigrees in Part 1 to complete this step.)*

2. *Identify and circle all the nucleotides that differ between at least two sequences in each table.*

Table 1

Individual	Phenotype	Sequence 1*
A IV-3	Lactose tolerant	*Copy 1, Chromosome 2:* TAAGATAATGTAGTCCTGG *Copy 2, Chromosome 2:* TAAGATAATGTAGTCCTGG
B IV-4	Lactose tolerant	*Copy 1, Chromosome 2:* TAAGATAATGTAGTCCTGG *Copy 2, Chromosome 2:* TAAGATAATGTAGTCCTGG
B IV-8	Lactose tolerant	*Copy 1, Chromosome 2:* TAAGATAATGTAGTCCTGG *Copy 2, Chromosome 2:* TAAGATAATGTAGCCCTGG
B IV-9	Lactose intolerant	*Copy 1, Chromosome 2:* TAAGATAATGTAGCCCTGG *Copy 2, Chromosome 2:* TAAGATAATGTAGCCCTGG
C IV-3	Lactose tolerant	*Copy 1, Chromosome 2:* TAAGATAATGTAGTCCTGG *Copy 2, Chromosome 2:* TAAGATAATGTAGCCCTGG
D IV-4	Lactose intolerant	*Copy 1, Chromosome 2:* TAAGATAATGTAGCCCTGG *Copy 2, Chromosome 2:* TAAGATAATGTAGCCCTGG

*Sequence 1 is a nucleotide sequence corresponding to nucleotides 19923-13902 upstream from the start of the lactase gene.

Table 2

Individual	Phenotype	Sequence 2*
A IV-3	Lactose tolerant	*Copy 1, Chromosome 2:* ATAAAGGACATCTTGACAA *Copy 2, Chromosome 2:* ATAAAGGACATCTTGACAA
B IV-4	Lactose tolerant	*Copy 1, Chromosome 2:* ATAAAGGACATCTTGACAA *Copy 2, Chromosome 2:* ATAAAGGACATCTTGACAA
B IV-8	Lactose tolerant	*Copy 1, Chromosome 2:* ATAAAGGACATCTTGACAA *Copy 2, Chromosome 2:* ATAAAGGACGCTTGACAA
B IV-9	Lactose intolerant	*Copy 1, Chromosome 2:* ATAAAGGACGCTTGACAA *Copy 2, Chromosome 2:* ATAAAGGACGCTTGACAA
C IV-3	Lactose tolerant	*Copy 1, Chromosome 2:* ATAAAGGACATCTTGACAA *Copy 2, Chromosome 2:* ATAAAGGACATCTTGACAA
D IV-4	Lactose intolerant	*Copy 1, Chromosome 2:* ATAAAGGACATCTTGACAA *Copy 2, Chromosome 2:* ATAAAGGACATCTTGACAA

*Sequence 2 is a nucleotide sequence corresponding to nucleotides 30192-30173 upstream of the start of the lactase gene.

3. *Pretend that you are the researcher who discovered the variations and you are writing to a colleague describing what you found.*

a. *How would you describe the variation(s) you found in Sequence 1?*

An example of a student answer is: I found that nucleotide 14 in Sequence 1 can be either a thymine or a cytosine.

b. *How would you describe the variation(s) you found in Sequence 2?*

An example of a student answer is: I found that nucleotide 10 in Sequence 2 can be either an adenine or a guanine.

4. *Based on the sequence data you analyzed, which variation is associated with lactose tolerance (lactase persistence)? Support your claim by providing at least three pieces of evidence.*

The presence of a thymine in place of a cytosine at position 14 in Sequence 1 is associated with lactose tolerance. Evidence includes:

- **All lactose-tolerant individuals have at least one copy of the sequence with the T instead of a C at position 14.**
- **None of the lactose-intolerant individuals have a T at position 14 of Sequence 1.**
- **Individuals only need one copy of the T variant to be lactose tolerant, which is consistent with an autosomal dominant trait.**
- **Neither version of the variation identified in Sequence 2 is consistently found in lactose-tolerant or -intolerant individuals.**

5. *Is the variation associated with lactose tolerance (lactase persistence) found on one chromosome or both chromosomes in individuals with the trait? Explain your answer.*
One chromosome, which is consistent with lactose tolerance being a dominant trait.

6. *From the pedigrees and the DNA sequencing data, what do you know about how the variation associated with lactose tolerance was inherited for Individuals B IV-4 and B IV-9?*

We know that Individual B IV-4 must have inherited two alleles associated with lactose tolerance—in other words, she is homozygous for the lactose-tolerance alleles. Another way of saying this is that she inherited one chromosome with a T at position 14 of Sequence 1 from her mom and one from her dad. On the other hand, Individual B IV-9 inherited two chromosomes with a C at position 14 of Sequence 1, which is the allele associated with lactose intolerance. Individual B IV-9 is homozygous for the recessive lactose-intolerance alleles.

7. *The mutation associated with lactose tolerance keeps the lactase gene turned on in adults. Based on the sequence and pedigree data, what can you infer about the regulation of the lactase gene in Individual A IV-3? Explain.*

Individual A IV-3 is lactose tolerant, according to the pedigree. This individual has two copies of the T variant at position 14 of Sequence 1, which is associated with lactose tolerance. We can infer that in this individual the lactase gene continues to be active into adulthood.

REFERENCES

Enattah, N. S., *et al*. 2002. Identification of a variant associated with adult-type hypolactasia. *Nature Genetics* 30:233-237.
Sahi, T. 2001. Genetics and epidemiology of adult-type hypolactasia with emphasis on the situation in Europe. *Scandinavian Journal of Nutrition* 45:161-162.
Ingram C.J.E., *et al*. 2009. Lactose digestion and the evolutionary genetics of lactase persistence. *Human Genetics* 124: 579.

AUTHORS

Written by Paul Strode, PhD, Fairview High School, and Laura Bonetta, PhD, HHMI

Edited by Susan Dodge

Reviewed by Paul Beardsley, PhD

Field Tested by Donald R. Kirkpatrick, Marion High School; Ellen Perry, Connetquot High School; Jack Saffer, Central Islip High School; Jen Stites, John Hancock College Prep High School; Jennifer Walters, Corona del Mar High School; Karin Marcotullio, Ballston Spa High School; Linda Ciota, St. John the Baptist; Mary Wuerth, Tamalpais High School; Sarah Freilich, Kehillah Jewish High School; Valerie May, Woodstock Academy

DECODING A PEDIGREE

SYMBOLS

Circles indicate females.

An unfilled symbol represents someone without the trait being studied.

Thus, this symbol represents a female without that trait.

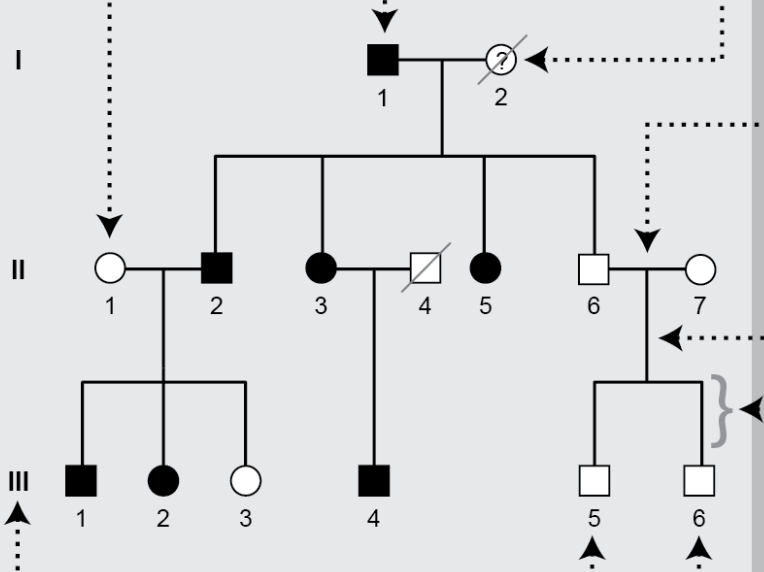
Squares indicate males.

A filled symbol represents someone who exhibits the trait being studied.

Thus, this symbol represents a male with that trait.

A question mark indicates that the person's trait is unknown.

A diagonal line indicates that the person is deceased.



RELATIONSHIPS

A horizontal line between two symbols connects two parents.

Vertical lines connect parents to children.

Horizontal brackets connect siblings.

NUMBERS

Roman numerals (I, II, III, ...) represent generations.

Arabic numerals (1, 2, 3, ...) identify each person in a generation.

Individuals in pedigrees are sometimes identified by the generation number followed by the individual number.

Thus, this is individual III-6, or the sixth individual in the third generation of this family.