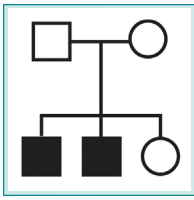


Inheritance and Mutations in a Single-Gene Disorder

hhmi | BioInteractive

Activity
Educator Materials

OVERVIEW

This activity supplements the short film *Genes as Medicine*, which describes how scientists developed a gene therapy to treat an inherited form of blindness called Leber congenital amaurosis (LCA). In Part 1 of this activity, students analyze pedigrees from a published scientific study to determine that LCA has an autosomal recessive inheritance pattern. In Part 2, students explore some of the mutations that cause LCA and discover that individuals with LCA can carry two different disease-causing alleles. In Part 3, they reflect on how LCA can be treated using gene therapy.

KEY CONCEPTS

- Pedigrees can be used to infer the inheritance pattern of single-gene traits.
- An inherited disease can be caused by mutations in a gene that result in a protein with an altered function or no function at all.
- An individual with a recessive trait may have two different mutant alleles for the corresponding gene.

STUDENT LEARNING TARGETS

- Describe the relationship between genotype and phenotype.
- Use a pedigree to infer genotypes and determine the most likely inheritance pattern of a single-gene trait.
- Analyze amino acid sequence data to identify mutations.

CURRICULUM CONNECTIONS

Standards	Curriculum Connection
NGSS (2013)	HS-LS1-1, HS-LS3-1; SEP2, SEP6
AP Bio (2015)	3.A.1, 3.A.3, 3.C.1; SP1, SP6
IB Bio (2016)	3.1, 3.4, B.4
Common Core (2010)	ELA.RST.9–12.4, ELA.RST.9-12.7 Math.S-IC.2; MP2, MP4
Vision and Change (2009)	CC3; DP3

KEY TERMS

allele, dominant, gene sequencing, gene therapy, genotype, homozygous, heterozygous, Leber congenital amaurosis (LCA), pedigree chart, phenotype, recessive

TIME REQUIREMENTS

One 50-minute class period if the film is watched outside of class. Two 50-minute class periods may be needed to watch the film in class and/or review basic genetics concepts.

SUGGESTED AUDIENCE

- High School: Biology (AP/IB)
- College: Introductory Biology, Introductory Genetics

PRIOR KNOWLEDGE

Students should be familiar with:

- basic genetics terms such as gene, allele, mutation, and genotype
- using pedigrees to determine patterns of inheritance for single-gene traits (in particular, autosomal dominant, autosomal recessive, and X-linked recessive patterns)

- predicting genotypes using Mendelian genetics (e.g., by using simple Punnett squares with dominant and recessive traits)
- the fact that proteins are made up of amino acids, which can be designated by three-letter abbreviations
- how changes in DNA sequences (mutations) can alter the amino acid sequence of a protein, which may in turn change the protein's structure and prevent it from functioning properly

MATERIALS

- access to the short film *Genes as Medicine*
- copies of the "Student Handout"

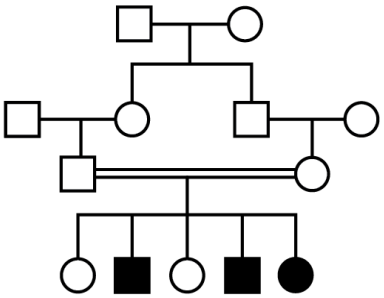
BACKGROUND

This activity uses real pedigrees and sequence data from published scientific papers. More information on the research used in each part of the activity is provided below.

Part 1

This part of the activity uses a series of pedigrees originally published by Morimura et al. (1998). These pedigrees show families with LCA patients from the United States and Canada. Mutations in the *RPE65* gene accounted for about 16% of the LCA cases. All of the individuals with LCA were either recessive homozygotes or compound heterozygotes. (For more information on these terms, see Part 2 of the "Background" below.)

Another study by Li et al. (2009) shows pedigrees of families with LCA patients from Saudi Arabia, which could be used to supplement the Part 1 activity. Some of the marriages in these families are consanguineous, meaning that they are between two closely related individuals. Such marriages are indicated by a double horizontal line, like the marriage between first cousins in the pedigree shown to the right. You may opt to show some of these pedigrees to your students and ask them to infer why homozygous recessive traits, such as LCA, are more common in families with consanguineous marriages.



Part 2

This part of the activity uses data from Russell et al. (2017), which describes the results of the gene therapy trial featured in the *Genes as Medicine* film. Students examine RPE65 protein sequences from two female patients who participated in the trial, a 9-year-old and an 11-year-old. Note that the sequences shown in the "Student Handout" are shortened to focus on the regions around the mutations; UniProt provides the full RPE65 protein sequence (533 amino acids total).

Although both patients have LCA, their sequences reveal that they have different mutations in *RPE65*. In general, an individual with a recessive trait, such as LCA, must have two recessive alleles for the corresponding gene. If these two recessive alleles have the *same* mutation in the gene, the individual is **homozygous recessive** for that gene. If the two recessive alleles have *different* mutations in the gene, the individual is **compound heterozygous** for that gene. The 9-year-old patient has the same mutation in both *RPE65* alleles, so she is homozygous recessive. Both the 11-year-old patient and Molly from the *Genes as Medicine* film have different mutations in each *RPE65* allele, so they are compound heterozygous.

TEACHING TIPS

- More background information can be found in the Activity for *Genes as Medicine* "Educator Materials."
- To save in-class time, consider having students watch the first part of *Genes as Medicine* (up to time 8:47) for homework before class. They can also complete Part 3 of the activity for homework after class.

- You may want to have students work in pairs or small groups to complete this activity.
- Students may ask if pedigrees will still be useful as human genomes become cheaper and easier to sequence. You may wish to discuss the differences between pedigree analysis and gene sequencing and have students think about when to use each method. For example:
  - **Pedigrees** are used to determine whether a trait shows a clear pattern of inheritance and whether it is likely to be associated with a single gene, as demonstrated in **Part 1** of the activity. Pedigrees:
    - help determine the mode of inheritance of a trait
    - typically focus on the presence or absence of the trait over multiple generations.
    - can be done even without DNA samples from all the individuals (some of whom may be deceased)
    - can inform which individuals to sequence in a family (for example, to assess risks of transmitting a disease)
  - **Gene sequencing** is used to identify the mutations associated with a specific trait, as demonstrated **in Part 2** of the activity. Sequencing:
    - provides a genotype to go along with a phenotype but does not determine mode of inheritance
    - may focus on candidate genes already known to cause the trait in other individuals
    - requires DNA samples from the individuals to sequence
    - could target unaffected relatives of an affected individual to understand the risk of developing or passing on the trait
  - In addition, some genetic conditions are caused by chromosome abnormalities. Both pedigree and karyotype analysis, rather than gene sequencing, are used to understand the causes of such conditions and to determine who is at risk for transmitting them.
    - For example, Down syndrome (DS) can be caused by nondisjunction of chromosome 21 or a Robertsonian translocation (specifically, fusion between chromosome 21 and an autosome). Karyotyping can be used to determine whether DS is caused by a translocation, and pedigree analysis can then be used to discern potential carriers of the translocation.
- If your class has already covered different types of mutations, consider asking students to determine whether the mutations shown in Part 2 are **missense** (one amino acid is swapped for another), **nonsense** (one amino acid is swapped for a "stop" codon), or **silent** (no amino acid change). Missense and nonsense mutations are also known as **nonsynonymous**, whereas silent mutations are known as **synonymous**.
- Students may have the misconception that every gene associated with a genetic disease has only two possible alleles: one unmutated/"normal"/wild-type allele and one mutated/disease-causing allele. DNA sequencing has shown that there are many alleles in the population for any single DNA sequence.
  - Genes can have more than one "normal" allele, since many mutations do not have a noticeable effect.
  - For many genetic diseases, there are many disease-causing alleles with different mutations. For example, more than 1,700 different mutations in the *CFTR* gene can cause cystic fibrosis.
  - An example of a genetic disease with only one disease-causing allele is sickle cell anemia, a common form of sickle cell disease. All individuals with sickle cell anemia have the same point mutation in the gene *HBB*: a missense mutation that changes the sixth amino acid of one of the subunits of hemoglobin (beta-globin) from glutamic acid to valine. This particular change in beta-globin causes the protein to form fibers and red blood cells with a sickled shape. Other mutations can occur in this gene, but only this mutation is known to be associated with this particular phenotype.

ANSWER KEY

PART 1: Determining LCA's Pattern of Inheritance

1. Imagine you're a doctor treating a patient with severe vision issues. What questions might you ask to determine whether these issues are more likely to be inherited or caused by environmental factors?
   Answers will vary. Sample questions include:
   - *Were you born with vision issues, or did they start more recently?*
   - *Were your eyes exposed to any chemicals, bright lights, or trauma before your vision issues started?*
   - *Does anyone else in your family have vision issues too?*

In a 1998 study, scientists analyzed a series of pedigrees showing the family histories of LCA patients. Some of their pedigrees are shown in the following figures. Squares represent males, circles represent females, and shading indicates that an individual has LCA.

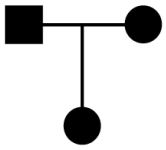


Figure 1. A pedigree of a family that has individuals with LCA. Adapted from Morimura et al. (1998).

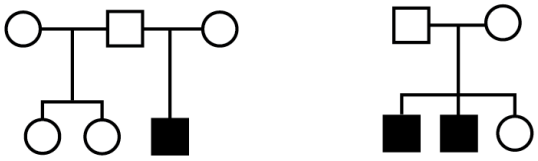


Figure 2. Two pedigrees of families that have individuals with LCA. Adapted from Morimura et al. (1998).

2. Based *only* on the pedigree in **Figure 1**, can you determine whether LCA is or isn't inherited according to an autosomal dominant, autosomal recessive, or X-linked recessive pattern? Explain your answer.
   You can't rule out any of these inheritance patterns based only on this pedigree. Everyone in the family has LCA, which would be possible for autosomal dominant, autosomal recessive, and X-linked recessive patterns.

3. Based on the pedigrees in **Figure 2**, can you determine whether LCA is or isn't inherited according to an autosomal dominant, autosomal recessive, or X-linked recessive pattern? Explain your answer.
   You can determine LCA <u>isn't</u> inherited according to an autosomal dominant pattern. This is because in both families, the parents do not have the disease, but some of their children do. If LCA was autosomal dominant, at least one of the parents would have LCA too, since they must have a disease-causing allele to pass on to their children. Autosomal recessive and X-linked recessive patterns are both still possible.

Figure 3 shows the pedigrees of seven families in the 1998 study. The scientists used these pedigrees to determine the inheritance pattern for LCA.

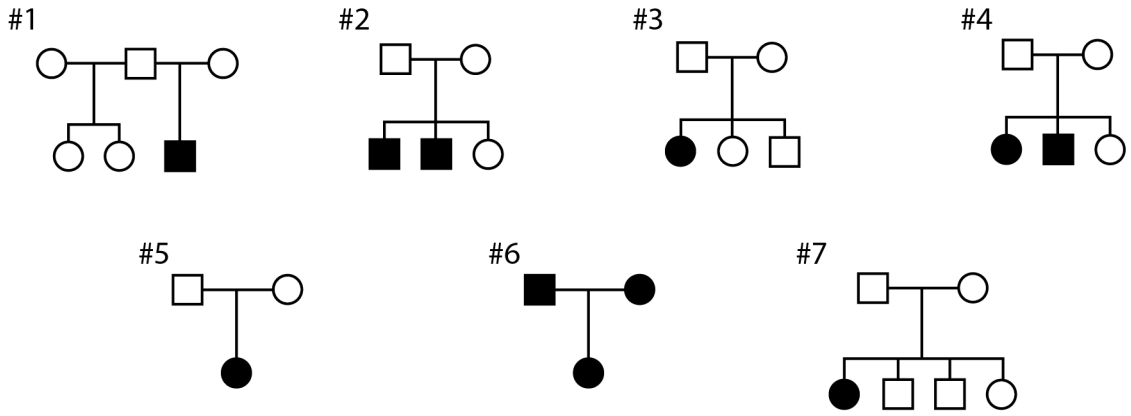


Figure 3. Seven pedigrees of families that have individuals with LCA. Adapted from Morimura et al. (1998).

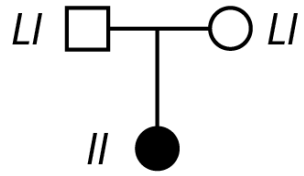
4. Based on the pedigrees in **Figure 3**, is LCA inherited according to an autosomal dominant, autosomal recessive, or X-linked recessive pattern? Use evidence from the pedigrees to support your claim, making sure to explain the evidence that rules out the inheritance patterns you didn't choose.
   It is very likely that LCA has an autosomal recessive inheritance pattern. It can't be autosomal dominant because parents without LCA (in all of the families except #6) still have children with it. It also can't be X-linked recessive because of the inheritance patterns seen in Families #3, #4, #5, and #7. If LCA were X-linked recessive, any female with LCA must have received disease-causing alleles from both of her parents. This means that her father must also have LCA, since males have only one copy of the X chromosome. But in these families, there are females with LCA that have fathers <u>without</u> LCA, which is impossible under X-linked recessive inheritance.

5. Recall that a person typically has two copies, called **alleles**, of every gene. One or both of these alleles may be mutated in certain individuals. Use what you know about LCA's inheritance pattern to write the genotypes for **Family #4 in Figure 3**, using *L* to represent an unmutated allele and *l* to represent a mutant, disease-causing allele for the LCA-related gene.

   a. What are the possible genotypes of both parents in this family? Explain your answer.
      Since LCA is autosomal recessive, the children with LCA must both have two mutant alleles, one from each parent. Since neither parent has LCA, they must each have one unmutated allele (L) and one mutant allele (l). So the parents' genotypes are both Ll.

   b. What are the possible genotypes of the female child *without* LCA? Explain your answer.
      The child without LCA must have at least one unmutated allele (L), so her genotype could be LL or Ll.

6. Although Molly has LCA, her parents do not. Draw a pedigree for Molly and her parents using the same style as in the previous pedigrees. Write the genotypes for each person using *L* and *l* as before.



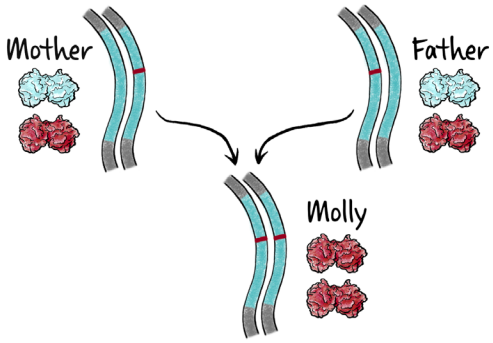
PART 2: Examining Mutations That Cause LCA

Figure 4. Illustrations comparing unmutated and mutated, disease-causing copies of a gene, and their resulting proteins.

7. Based on the film, what is the difference between the two proteins shown in Figure 4?
   The protein resulting from the mutated, disease-causing copy of the gene has a different function or no function at all compared to the protein resulting from the unmutated copy of the gene.

8. Consider what mutations Molly and her parents may have in the gene related to Molly's LCA. Using Figure 4 as a guide, draw the gene's two alleles and their resulting proteins for both of Molly's parents and Molly.

Answers will vary. As shown in the example diagram to the right, students may draw identical mutations for both of Molly's parents and alleles. They will learn later that Molly's parents and alleles actually have different mutations in the LCA-related gene.



9. Use the diagram you drew to explain why Molly's parents do not have LCA, but Molly does.
   Molly's parents each produce some "normal" protein for the LCA-related gene, which must be enough to help their eyes function and avoid LCA. However, Molly does not produce any "normal" protein for this gene. All of her protein is mutated and probably has a different function/no function at all, which would lead to LCA.

10. Look at the diagram shown at time 8:26 of the film. What are the similarities and differences between this diagram and the one you drew in Question 8?
    Answers will vary depending on what students drew in Question 8. Many likely drew the same mutation for both of Molly's parents and alleles. The diagram at time 8:26 in the film shows that Molly's parents actually have different mutations in the LCA-related gene, so Molly has a different mutation in each allele.

11. Is Molly homozygous or heterozygous for the *RPE65* gene? Are her alleles for RPE65 both dominant, both recessive, or does she have one of each? Explain your answers.
    Molly is heterozygous because she has two different alleles for the gene. Both of her alleles are recessive. (If either allele was dominant, at least one of her parents would also have LCA.)

12. Circle the mutations in each allele for the patients in the tables.
    Mutations are bolded and highlighted in yellow below.

Table 1: Partial RPE65 protein sequence (amino acids 41–60) for the 9-year-old LCA patient.

Unmutated Protein Sequence	START…Ser-Leu-Leu-Arg-Cyc-Gly-Pro-Gly-Leu-Phe-Glu-Val-Gly-Ser-Glu-Pro-Phe-Tyr-His-Gly…STOP
Patient's Allele 1 Protein Sequence	START…Ser-Leu-Leu-**Gln**-Cyc-Gly-Pro-Gly-Leu-Phe-Glu-Val-Gly-Ser-Glu-Pro-Phe-Tyr-His-Gly…STOP
Patient's Allele 2 Protein Sequence	START…Ser-Leu-Leu-**Gln**-Cyc-Gly-Pro-Gly-Leu-Phe-Glu-Val-Gly-Ser-Glu-Pro-Phe-Tyr-His-Gly…STOP

Table 2. Partial RPE65 protein sequence (amino acids 61–70 and 291–300) for the 11-year-old LCA patient.

Unmutated Protein Sequence	START…Phe-Asp-Gly-Gln-Ala-Leu-Leu-His-Lys-Phe…Ile-Ala-Asp-Lys-Lys-Arg-Lys-Lys-Tyr-Leu…STOP
Patient's Allele 1 Protein Sequence	START…Phe-Asp-Gly-Gln-Ala-Leu-Leu-**Tyr**-Lys-Phe…Ile-Ala-Asp-Lys-Lys-Arg-Lys-Lys-Tyr-Leu…STOP
Patient's Allele 2 Protein Sequence	START…Phe-Asp-Gly-Gln-Ala-Leu-Leu-His-Lys-Phe…Ile-Ala-Asp-Lys-**STOP**

Source: Data from Russell et al. (2017).

13. Does each patient have the same mutation in both of their alleles? Explain your reasoning.
    No. The 9-year-old patient has the same mutation in each allele, but the 11-year-old patient has a different mutation in each allele.

14. Based on your answers to Questions 11 and 13, which patient's genotype for *RPE65* is more similar to Molly's? Explain your answer.
    The 11-year-old patient's genotype is more similar to Molly's, because they both have a different mutation in each of their RPE65 *alleles/are heterozygous for this gene. The 9-year-old patient, on the other hand, has the same mutation in each of their* RPE65 *alleles/is homozygous for this gene.*

15. Assume that the parents of both patients do not have LCA.
    a. Based on Table 1, predict the *RPE65* genotypes of the parents of the 9-year-old patient.
    Neither parent has LCA yet has a child with LCA. So each parent must have one unmutated RPE65 *allele and one mutant* RPE65 *allele. Based on Table 1, these parents have the same mutation in their mutant* RPE65 *allele. This mutation is an Arg-to-Gln substitution, which is shown in both of the patient's alleles.*

    b. Based on Table 2, predict the *RPE65* genotypes of the parents of the 11-year-old patient.
    Again, neither parent has LCA yet has a child with LCA. So each parent must have one unmutated RPE65 *allele and one mutant* RPE65 *allele. Based on Table 2, these parents have different mutations in their mutant* RPE65 *alleles. One parent's mutation is a Tyr-to-His substitution, which is shown in the patient's Allele 1. The other parent's mutation is a deletion of the end of the protein, which is shown in the patient's Allele 2.*

PART 3: Using Gene Therapy to Treat LCA

16. Like Molly, the LCA patients in Tables 1 and 2 have nonfunctioning RPE65 proteins. However, their mutations in the *RPE65* gene may be different from Molly's. If so, could the gene therapy that helped Molly work for either of these patients? Justify your answer.
    Yes, this gene therapy should work for anyone with a nonfunctioning RPE65 protein. This is because the gene therapy works by delivering a "normal" allele of the RPE65 *gene, which would allow any patient with a nonfunctioning* RPE65 *protein to produce a functional* RPE65 *protein.*

17. Do you think that the same gene therapy that helped Molly could also help LCA patients with mutations in genes other than *RPE65*? Explain your reasoning.
    No, the gene therapy Molly used works by helping the body produce functional RPE65 protein. So it can only help patients with a nonfunctioning RPE65 protein, not patients with mutated versions of other proteins. These patients could be treated with a different type of gene therapy, one that delivers a "normal" version of the gene for their mutated protein.

REFERENCES

Morimura, Hiroyuki, Gerald A. Fishman, Sandeep A. Grover, Anne B. Fulton, Eliot L. Berson, and Thaddeus P. Dryja. "Mutations in the *RPE65* gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis." *Proceedings of the National Academy of Sciences* 95, 6 (1998): 3088–3093. https://doi.org/10.1073/pnas.95.6.3088.

Li, Yumei, Hui Wang, Jianlan Peng, Richard A. Gibbs, Richard Alan Lewis, James R. Lupski, Graeme Mardon, and Rui Chen. "Mutation survey of known LCA genes and loci in the Saudi Arabian population." *Investigative Ophthalmology and Visual Science* 50, 3 (2009): 1336–1343. https://doi.org/10.1167/iovs.08-2589.

Russell, Stephen, Jean Bennett, Jennifer A. Wellman, Daniel C. Chung, Zi-Fan Yu, Amy Tillman, Janet Wittes, et al. "Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with *RPE65*-mediated inherited retinal dystrophy: A randomised, controlled, open-label, phase 3 trial." *The Lancet* 390, 10097 (2017): 849–860. https://doi.org/10.1016/s0140-6736(17)31868-8.

AUTHOR

Kathryn S. Jones, Howard Community College, MD
Edited by Vincent Buonaccorsi, Juniata College, PA; Esther Shyu, HHMI; Stephanie Keep, consultant
Illustrations by Li Yao, HHMI