INTRODUCTION

Some diseases are caused by the environment. For example, exposure to chemicals or extremely bright lights can cause certain forms of blindness. But other forms of blindness are inherited, meaning that they are passed on from one generation to another. In this activity, you will learn about a young woman, Molly Troxel, who has an inherited form of blindness called Leber congenital amaurosis (LCA). The activity explores how LCA is inherited, the mutations that cause it, and how the disease can be treated to help Molly and other patients.

PART 1: Determining LCA's Pattern of Inheritance

Watch the short film *Genes as Medicine* until time **1:42**. You will meet Molly and learn about what causes her blindness. Use what you learn from the film to answer the following questions.

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?

Some inherited diseases, including LCA, may be caused by mutations in a single gene. Pedigrees can be used to determine the patterns of inheritance for these diseases.

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.

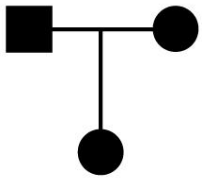

Figure 1. A pedigree of a family that has individuals with LCA. Adapted from Morimura et al. (1998).

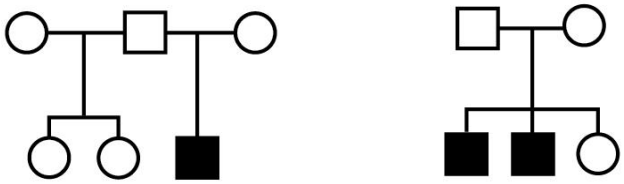

Figure 2. Two pedigrees of families that have individuals with LCA. Adapted from Morimura et al. (1998).

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.

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.

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

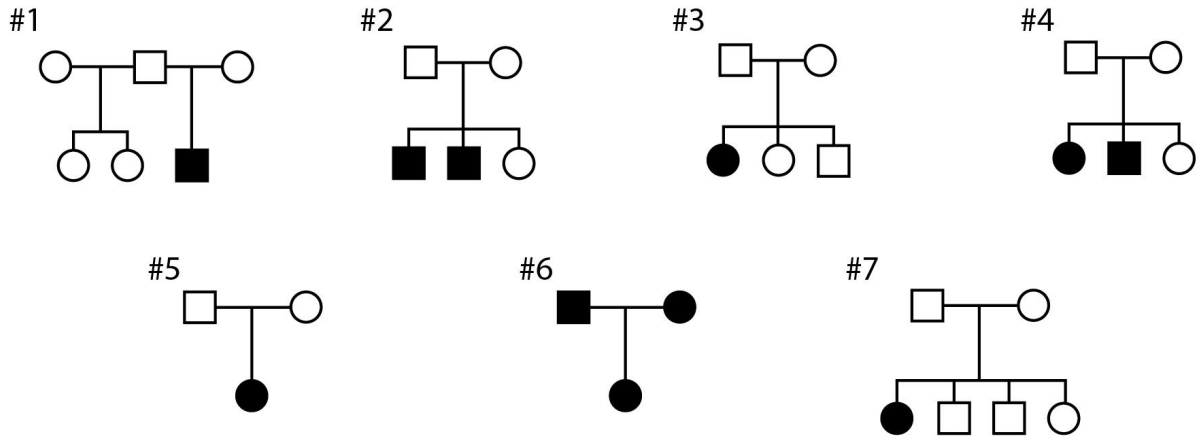

Figure 3. Seven pedigrees of families that have individuals with LCA. Adapted from Morimura et al. (1998).

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.

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.

   b. What are the possible genotypes of the female child *without* LCA? Explain your answer.

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

Many genes contain instructions for making proteins, which have important functions in the body. Mutations, changes in a gene's DNA sequence, can affect these proteins and cause inherited diseases, such as LCA.

Continue watching the *Genes as Medicine* film until time **2:44**. Figure 4 shows two illustrations from the film.

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?

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.

9. Use the diagram you drew to explain why Molly's parents do not have LCA, but Molly does.

Continue watching the *Genes as Medicine* film to time **8:47**.

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?

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.

Scientists can use gene sequencing to identify mutations that cause disease. Molly's LCA, for example, was found to be due to mutations in the gene *RPE65*. This gene codes for a protein, RPE65, that plays an important role in the eye.

Tables 1 and 2 compare parts of the unmutated RPE65 amino acid sequence with those of two LCA patients: a 9-year-old and an 11-year-old. The tables show sequences for the proteins produced by each *RPE65* allele in both patients. The "…" indicate amino acids in the full protein sequences that are not included in the tables.

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).

Use Tables 1 and 2 to answer the questions below.

12. Circle the mutations in each *RPE65* allele for both patients.
13. Does each patient have the same mutation in both of their alleles? Explain your reasoning.

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.

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.

    b. Based on Table 2, predict the *RPE65* genotypes of the parents of the 11-year-old patient.

PART 3: Using Gene Therapy to Treat LCA

Continue watching the *Genes as Medicine* film **to the end**. As shown in the film, scientists treated Molly's blindness with gene therapy. To do this, they used a modified virus to deliver copies of the unmutated *RPE65* gene into cells in Molly's eye.

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.

RPE65 is not the only gene that, when mutated, can cause LCA. Mutations in other genes that code for proteins involved in vision can also cause this type of blindness.

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.