Gene Editing with CRISPR-Cas – A Cure for Severe Sickle Cell Anemia?¹

Victoria Gray had severe sickle cell anemia with repeated painful crises when sickled red blood cells blocked her circulation. She said, "The pain is excruciating. It's like being in a car accident and having lightning in your chest. It's a pain that makes a grown woman like me scream."

Since many sickle cell patients don't survive past their 40s, Victoria Gray worried that she wouldn't live to see her children grow up. "It's horrible ... knowing that I could have a stroke or a heart attack ... at any time," Gray said.

1a. Figure A shows the effects of the normal hemoglobin allele, and figure B shows the effects of the sickle cell hemoglobin allele. Inside a cell, the sequence of nucleotides in a gene specifies the sequence of amino acids in a protein by two processes. Write the names of these two processes between the DNA and the protein in figure B.

1b. Explain how the sickle cell hemoglobin allele can result in pain.

¹ By Dr. Ingrid Waldron, Dept Biology, Univ Pennsylvania, © 2021. This Student Handout and Teacher Notes (with background information and instructional suggestions) are available at https://serendipstudio.org/exchange/bioactivities/GeneEdit.
Because her sickle cell anemia was so severe and none of the usual treatments provided lasting relief, Victoria Gray volunteered to be the first patient in a clinical trial of a gene-editing treatment. To develop this treatment, medical scientists adapted molecules that bacteria use to defend themselves against viral infections. The figure below shows that a viral infection can kill a bacterium.

2a. Label the bacterial DNA and the viral DNA in drawing 2 above.

2b. The virus hijacks bacterial molecules and organelles to make new viruses. For example, bacterial _____________ and _____________ are used to make viral proteins.

   (amino acids / lipids)  (mitochondria / ribosomes)

To learn how bacteria defend themselves against viral infection, watch the first 2 minutes and 40 seconds of the video, “What is CRISPR-Cas?” [https://www.youtube.com/watch?v=52jOEPzhpzc](https://www.youtube.com/watch?v=52jOEPzhpzc).

3a. What is Cas? How does Cas help to defend a bacterium against viral infection?

3b. How does an RNA guide help a bacterium fight a repeat infection by the same virus?

3c. What could go wrong if Cas were active without needing any guide RNA?
For a brief introduction to how medical scientists are using CRISPR-Cas9 to develop treatments for genetic diseases like sickle cell anemia, watch “CRISPR Explained”, available at https://www.youtube.com/watch?v=UKbrwPL3wXE.

4. How can doctors use the CRISPR-Cas9 system to cut DNA in a specific location to inactivate a specific gene? Describe the role of the guide RNA and the role of the cas9 enzyme.

The gene therapy for Victoria Gray stimulated her body to produce fetal hemoglobin. Before birth, the fetus produces fetal hemoglobin, which has a stronger affinity for oxygen so the fetal blood can absorb oxygen from the mother’s blood. To understand why scientists thought that increased production of fetal hemoglobin would reduce Gray’s symptoms, read the summaries of earlier research in the box below.

Excerpts from “Sickle cell gene therapy to boost fetal hemoglobin: A 70-year timeline of discovery” (https://answers.childrenshospital.org/sickle-cell-gene-therapy-bcl11a-timeline/)

In 1948, the “pediatrician Janet Watson noted that children who later developed sickle-cell disease had few sickled red blood cells when they were newborns. She tied this to the greater concentration of fetal hemoglobin” in the blood of newborns.

Research during 1972-1994 showed that some sickle cell patients had mutations that resulted in continued production of fetal hemoglobin after birth. These sickle cell patients had milder sickle cell disease and lived longer.

A 1995 study found “that hydroxyurea, which reactivates fetal hemoglobin production, reduces the number and severity of sickle-cell attacks in adults.... But hydroxyurea can cause toxicity and helps only about half of all patients.”

Studies in 2008-2011 showed “that inactivating the BCL11A gene restarts fetal hemoglobin production.” Researchers could “correct sickle-cell disease in mice by inactivating BCL11A, allowing fetal hemoglobin to be made.”

5. In the box, underline the research evidence that supports the conclusion that production of fetal hemoglobin decreases the severity of sickle cell disease. In the space below, summarize an argument that supports this conclusion.
This figure shows that the BCL11A protein binds to the DNA at the beginning of the fetal hemoglobin gene and turns off transcription of the fetal hemoglobin gene.

6. Flowchart A shows how transcription of the fetal hemoglobin gene is turned off in children and adults. Flowchart B shows what happens if cells are treated with CRISPR-Cas9 that cuts the BCL11A gene so the cells can’t make functional BCL11A protein. Explain how this CRISPR-Cas9 treatment could help patients with severe sickle cell anemia.

To learn how CRISPR-Cas9 is being used in the experimental treatment of severe sickle cell anemia, view the first 11 minutes of “How Gene Editing Is Curing Disease”, available at https://www.youtube.com/watch?v=ezfwqmKC9Uc.

7. Based on this video, summarize additional information about how Victoria Gray’s severe sickle cell anemia was treated.

During the year after Victoria Gray received the experimental gene editing treatment, her health improved dramatically. She did not have any painful sickle cell crises or any hospitalizations. She said “It’s hard to put into words the joy that I feel – being grateful for change this big. It’s been amazing.”

To learn about other possible uses of CRISPR-Cas and related ethical controversies, view “Gene Editing and CRISPR – How far should we go?”, which is a little more than halfway down at http://scienceovereverything.com/2017/09/18/crisprcas9-gene-editing/.

8. How do you think gene editing should be regulated? What safeguards and limitations would you recommend? Explain your reasoning.