NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

CRISPR-Cas9 and Sickle Cell Anemia

by
Elizabeth R. Everman
School of Biological Sciences
University of Oklahoma, Norman, OK

Preparation

To prepare for this case study, review the following interactive CRISPR-Cas9 module from HHMI BioInteractive:

- HHMI BioInteractive. (2023). CRISPR-Cas9 mechanism and applications. [Interactive module]. HHMI BioInteractive. <https://www.biointeractive.org/classroom-resources/crispr-cas9-mechanism-applications>

Next, listen to (or read the trasncript of) the following two NPR stories:

- Stein, R. (2019, December 25). A young Mississippi woman's journey through a pioneering gene-editing experiment. [Webpage]. NPR.org. <https://www.npr.org/sections/health-shots/2019/12/25/784395525/a-young-mississippi-womans-journey-through-a-pioneering-gene-editing-experiment>
- Stein, R. (2023, March 16). Sickle cell patient's success with gene editing raises hopes and questions. [Webpage]. NPR.org. <https://www.npr.org/sections/health-shots/2023/03/16/1163104822/crispr-gene-editing-sickle-cell-success-cost-ethics>

Watch the first five minutes of the following video that explains the mechanism of hemoglobin switching and the CRISPR gene edit used in the clinical trial that is discussed in this case study.

- "A Closer Look at How CASGEVY Works." Produced by Vertex Pharmaceuticals Incorporated. Running time: 12:06 min. <https://player.vimeo.com/video/919517749>

To review additional representations of the process, or if the above video is replaced or no longer available, the following videos are also recommended:

- "Treating Sickle Cell Disease with Genome Editing." Produced by Nucleus Medical Animation, 2024. Running time: 2:16 min. <https://player.vimeo.com/video/721532544>
- "New CRISPR-Based Sickle Cell Treatment, Explained." Produced by STAT, 2023. Running time: 2:06 min. <https://youtu.be/2sAGtqm3o1g>
- "CRISPR Gene-Editing Treatment for Sickle Cell Disease Explained." Produced by UMass Chan Medical School, 2024. Running time: 2:50 min. <https://youtu.be/BNP4hnMmQUw>

Abbreviations Used in Ths Case Study

The following is a list of abbreviations it will help you to be familiar with:

crRNA:	CRISPR-derived RNA	*RBC:*	red blood cell
GMO:	genetically modified organism	*SCA:*	sickle cell anemia
HDR:	homology-directed repair	*SCT:*	sickle cell trait
LCR:	locus control region	*SNP:*	single nucleotide polymorphism
NHEJ:	non-homologous end joining	*tracrRNA:*	transactivating RNA
PAM:	protospacer adjacent motif	*VOC:*	vaso-occlusive crisis

Introduction – A Review of Gene Editing

Various techniques are used to edit the genomes of organisms. These techniques can result in changes that affect single genes or entire genomes. The extent of the change depends on the approach that is used. Answer the two following questions to review material with which you should already be familiar before beginning this case study.

Questions

1. What gene editing techniques(s) are more likely to result in many changes across an entire genome?

2. What gene editing techniques(s) are more likely to result in targeted changes?

This case study focuses on one gene editing technique and its use in finding a cure for a genetic disease. CRISPR-Cas9 was originally discovered in bacteria and has been adapted as a gene editing tool that can be used to target and edit specific sections of the genome. This precision combined with our expanding knowledge of the structure and function of genetic sequences has opened the door to a wide range of potential applications in the fields of molecular genetics and personalized medicine.

To prepare for this case study, you listened to Victoria Gray's story. NPR reported on her experiences as a person living with sickle cell anemia (SCA) and the toll that it has taken on her health and her ability to achieve her goals for herself, her family, and her career. As we examine the genetic basis of SCA, the mechanism of CRISPR-cas9, and the clinical trial for a gene-editing cure for SCA, put yourself in Victoria's place and think about her perspective.

Question

3. The use of CRISPR gene editing for SCA is a new treatment. Would you be excited about a new treatment option, or would you be concerned? How has the media, your education, or your upbringing influenced your first thoughts?

The goals of this case study are to enable you to describe the conditions that lead to sickle cell anemia and sickle cell trait, apply the concepts of genetic equilibrium to human populations that are undergoing selection for malaria resistance, and to recognize the steps in harnessing the CRISPR gene editing mechanism from its evolved role in bacteria. You will combine these ideas by examining the first FDA-approved gene editing approach to curing a genetic disease.

Part I – Genetics of Sickle Cell Anemia and Sickle Cell Trait

Sickle cell anemia is caused by a mutation in a single gene. The gene codes for a subunit of hemoglobin, the multi-subunit protein in red blood cells that binds and carries oxygen. A single nucleotide mutation in the β hemoglobin gene results in a single amino acid change in the β subunit, which in turn causes the hemoglobin molecules to stick together and change the shape of the red blood cells (RBCs) (Ramadas & Sparkenbaugh. 2023). Figure 1 below shows the mutation. Read the caption for Figure 1 for more detail on how the mutation results in the sickle cell shape.

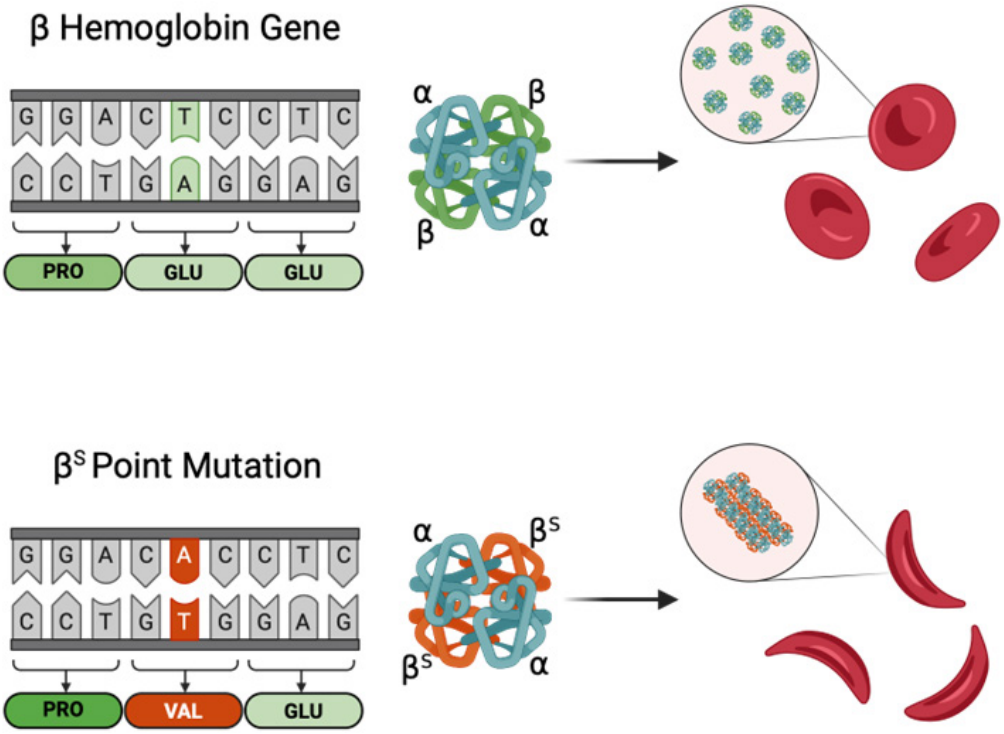

Figure 1. Hemoglobin proteins have four subunits (two α and two β subunits). A single nucleotide polymorphism (SNP) occurred in the gene that codes for the β hemoglobin subunit. While SNP mutations have the potential to be silent, this SNP mutation resulted in a nonsynonymous change to the amino acid sequence. Switching the amino acid sequence from a glutamate to a valine amino acid causes a structural change in the β hemoglobin subunit. Normally hemoglobin is evenly dispersed throughout the red blood cell, allowing the red blood cell to take on a disc shape. The mutated hemoglobin tends to clump together in rigid rafts. This rigidity causes the red blood cell to stiffen and take on a sickle shape. *Credit:* Figure adapted from Ramadas and Sparkenbaugh (2023) and recreated in BioRender.

Question

1. What type of mutation results from this single nucleotide change? (Recall that single nucleotide changes can result in silent, missense, or nonsense mutations.)

Sickle cell anemia is a recessive trait, meaning that only individuals who are homozygous for the allele (Hb^SHb^S) that leads to mutant hemoglobin have the disease. Sickle cell trait is a codominant trait at the molecular level and is observed in individuals who are heterozygous carriers (Hb^AHb^S). Both the Hb^A and Hb^S alleles are expressed, and their respective subunits are used to assemble the hemoglobin protein. In an individual with sickle cell trait, hemoglobin could contain two functional β subunits, two mutant β subunits, or a combination of the functional and mutant subunits. By random chance, most hemoglobin is expected to have a combination of the functional and mutant subunits. Each RBC has a mix of wild-type and combination hemoglobin, which allows them to retain their normal, round shape. However, under certain environmental conditions, the presence of the mutant β hemoglobin can cause the RBCs to change from disc to sickle shape.

Question

2. What proportion of the hemoglobin would be expected to contain the mutant β hemoglobin subunit in a carrier with sickle cell trait?

In the early 20th century, researchers in the United States began studying sickle cell anemia, and one of the first steps in understanding the disease was to determine the frequency of the allele in human populations (Allison, 2002). Researchers noted that the disease predominantly affected individuals of African descent as well as in individuals with Greek and Mediterranean ancestry. In the 1950s, a study in Kenya led by Dr. Tony Allison examined blood samples from hundreds of individuals. There was no genetic test for sickle cell at the time, but carriers could be identified with a simple test run on a drop of blood (Allison, 2002). Because each of the RBCs of individuals with sickle cell trait contain some of the affected hemoglobin, RBC sickling can be induced by exposing the RBCs to a low oxygen environment. When hemoglobin loses the oxygen it is carrying under low oxygen conditions, HB^S changes conformation and becomes stickier. The hemoglobin starts to clump, and the cells start to sickle (Allison, 2002).

Question

3. Why would a recessive allele that causes a disease continue to exist in a population? Try to come up with a few reasons why a disease-causing allele would not be effectively removed by natural selection.

In Dr. Allison's study, it quickly became apparent that the mutant allele was much more common in the humid coastal regions of Kenya and along a large inland lake (Allison, 2002). The mutant allele became much less common in the drier, higher elevation inland region of Kenya.

Question

4. Why would an environmental cline in humidity help explain the geographic pattern in mutant β subunit allele frequency?

Part II – Revisiting CRISPR-Cas9

Sickle cell anemia was first reported and described in 1910 when Dr. James Herrick observed sickle shaped blood cells in a student at Chicago (Savitt, 2010). It wasn't until the 1960s that treatment of SCA patients began with blood transfusions. Since the 1980s, more treatments have become available that range in effectiveness and cost. Most recently, gene editing techniques are being developed as potential cures for SCA. One of these recent treatments (Casgevy) involves the use of CRISPR gene editing (Frangoul et al., 2020).

CRISPR was first discovered by microbiologists and geneticists interested in understanding how bacteria fend off and fight infections from bacteriophages (Gostimskaya, 2022). In the bacterial genome, CRISPR is a genetic locus that encodes two functional products: the Cas proteins and crRNA. These functional products recognize invading viral DNA and destroy it, and this stops the virus from entering the lytic or lysogenic cycle.

Questions

1. In what cells were CRISPRs originally discovered and what is their purpose in that type of cell?

2. What is the role of each of the functional products produced at the CRISPR locus?
   a. Cas proteins:

   b. crRNA:

After discovering how CRISPR can destroy viral DNA by targeting specific sequences that match part of the surveillance complex, Dr. Jennifer Doudna and Dr. Emmanuelle Charpentier realized that this molecular editor could be adapted to target any sequence (Gostimskaya, 2022). They developed a simplified version of the adaptive immune CRISPR response seen in bacteria so that it could be directed to edit or break any known sequence in the genome.

Questions

3. CRISPR-Cas9 causes double stranded breaks in the target DNA. What are the two mechanisms in eukaryotic cells that repair double stranded DNA breaks?

4. What mechanism of DNA repair would use donor DNA as a template?

5. Find an example of CRISPR used in basic research. Try searching CRISPR in scholar.google.com. Include the title, authors, and date for the example you find.

Part III – CRISPR-Cas9 Gene Editing and Sickle Cell Anemia

Treatment for individuals with sickle cell anemia spans everything from medication to help manage pain to frequent blood transfusions that replenish the supply of normal RBCs that can carry oxygen to all tissues in the body. Despite these treatments, patients live limited lives avoiding activities that would place strain on the ability of their cells to carry oxygen. In the NPR story, you heard about Victoria Gray, the first person to receive CRISPR-Cas9 gene editing as a cure for a genetic disease. Victoria's treatment has allowed her to completely change the way she has been living her life. The key to her treatment was to make an edit to the genome of her hematopoietic stem cells that would influence the function of hemoglobin in her blood.

Question

1. Which gene was targeted through CRISPR-Cas9 in Victoria's gene editing treatment?

CRISPR gene editing can be used in a couple of obvious ways to modify the gene product that is produced. Through homology-directed repair, CRISPR can make a cut and exchange a short section of a nonfunctional gene so that a functional product can be made and through non-homologous end joining CRISPR can make a cut in a functional gene to make it nonfunctional. However, it is important to remember that genes are not active in isolation—often a cascade of other genes and proteins interact to produce the functions and phenotypes we observe. Even though the β hemoglobin gene is the source of the disease in sickle cell patient, CRISPR was used to modify expression of fetal hemoglobin—a gene that is normally silenced shortly after birth.

A process called hemoglobin switching is responsible for the transition in expression of fetal hemoglobin to adult β hemoglobin (Liu et al., 2018; Liu et al., 2021; Stamatoyannopoulos, 2005). During gestation, fetal hemoglobin and β hemoglobin are expressed, and these subunits are used to make the hemoglobin protein. Expression of fetal hemoglobin is controlled by an interaction between the locus control region (LCR) and a transcription factor (BCL11A) that is expressed near the end of the gestational term (Liu et al., 2018).

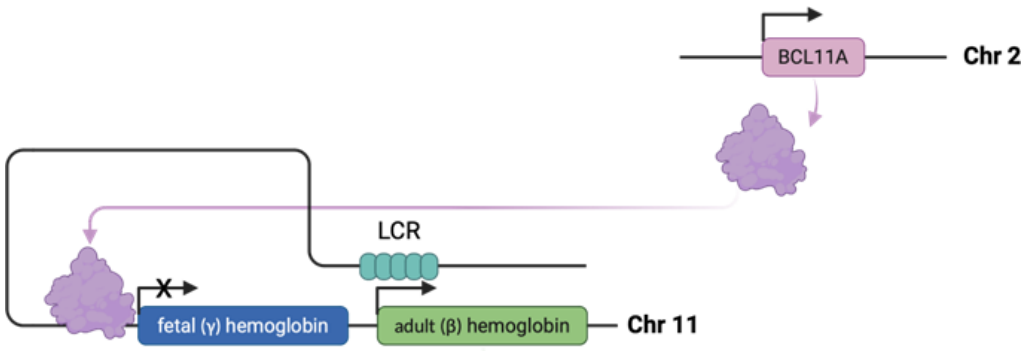

Figure 2. The LCR (locus control region) has a higher affinity for fetal hemoglobin, and during gestation when BCL11A is not expressed, LCR increases expression of fetal hemoglobin. Expression of BCL11A near the end of gestation and from birth on leads to hemoglobin switching by binding to the proximal control elements upstream of fetal hemoglobin. The physical blockage of the BCL11A protein prevents the LCR from associating with fetal hemoglobin. Instead, LCR associates with adult b hemoglobin and leads to the expression of this gene from birth onwards.

In class, we went into more detail on the specific edits that were used to cause fetal hemoglobin to be expressed in adult RBCs. The CRISPR modification was actually made to a cis regulatory element present between two exons of the *BCL11A* gene, not to fetal hemoglobin itself. We walked through the figure below showing this process in class.

Question

2. Write a caption for the figure below (Figure 3) that explains the edit that was made by CRISPR and why this leads to expression of fetal hemoglobin in adult cells.

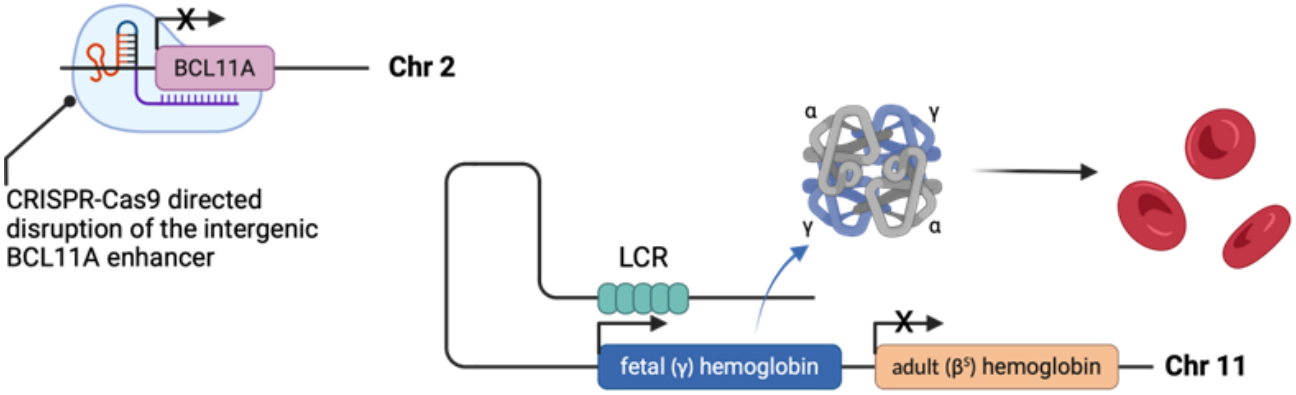

Figure 3. [SUPPLY CAPTION]

Part IV – Gene Editing and Ethical Considerations

The discovery and development of the CRISPR-Cas9 gene editing technique has had a transformative effect on the progress toward a deeper understanding of gene function and in moving our society toward a future where personalized medicine and cures for previously uncurable diseases is an ever more likely reality. However, there are significant ethical considerations that need to be kept in mind. Consider the following questions in small groups. Pick a question you want to focus on for the next ten minutes. We will regroup as a class and discuss each of the questions in turn.

Questions

1 How do we decide when gene editing is justified? Are only disease-causing genes candidates for gene therapy? What about edits to the genome that could be argued to improve quality of life?

2. Many genetic diseases are influenced by more than one or a few genes. For example, diabetes is a threshold trait that is controlled by potentially hundreds of genes. Is gene editing a viable approach for all diseases with a genetic basis? Should gene editing be an option even if it is only possible to change the severity or pace of progression of a disease?

3. Victoria Gray was part of a clinical trial for the sickle cell gene therapy treatment. Unfortunately, this treatment is very expensive. Who should be responsible for paying for others who are candidates for this treatment?

References

Allison, A.C. (2002). The discovery of resistance to malaria of sickle-cell heterozygotes. *Biochemistry and Molecular Biology Education* 30(5): 279–87. <https://doi.org/10.1002/bmb.2002.494030050108>

Frangoul, H., et al. (2020). CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. *New England Journal of Medicine* 384(3): 252–60. <https://doi.org/10.1056/NEJMoa2031054>

Gostimskaya, I. (2022). CRISPR-Cas9: a history of its discovery and ethical considerations of its use in genome editing. *Biochemistry (Moscow)* 87(8): 777–88. <https://doi.org/10.1134/S0006297922080090>

Liu, N., V.V. Hargreaves, Q. Zhu, J.V. Kurland, J. Hong, W. Kim, F. Sher, C. Macias-Trevino, J.M. Rogers, R. Kurita, Y. Nakamura, G.-C. Yuan, D.E. Bauer, J. Xu, M.L. Bulyk, & S.H. Orkin. (2018). Direct promoter repression by *BCL11A* controls the fetal to adult hemoglobin switch. *Cell* 173(2): 430–42. <https://doi.org/10.1016/j.cell.2018.03.016>

Liu, N., S. Xu, Q. Yao, Q. Zhu, Y. Kai, J.Y. Hsu, P. Sakon, L. Pinello, G.-C. Yuan, D.E. Bauer, & S.H. Orkin. (2021). Transcription factor competition at the γ-globin promoters controls hemoglobin switching. *Nature Genetics* 53: 511–20. <https://doi.org/10.1038/s41588-021-00798-y>

Ramadas, N., & E.M. Sparkenbaugh. (2023). The APC-EPCR-PAR1 axis in sickle cell disease. *Frontiers in Medicine* 10: 1141020. <https://doi.org/10.3389/fmed.2023.1141020>

Savitt, T.L. (2010). Tracking down the first recorded sickle cell patient in Western medicine. *Journal of the National Medical Association* 102(11): 981–92. <https://doi.org/10.1016/S0027-9684(15)30723-9>

Stamatoyannopoulos, G. (2005). Control of globin gene expression during development and erythroid differentiation. *Experimental Hematology* 33(3): 259–71. <https://doi.org/10.1016/j.exphem.2004.11.007>

Internet references accessible as of December 12, 2024.