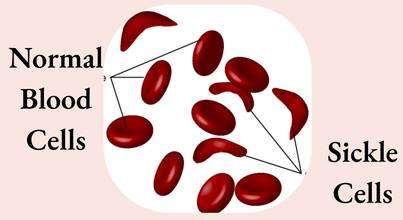
Correcting a Single Mutation: Precision Gene Editing for Sickle Cell Disease

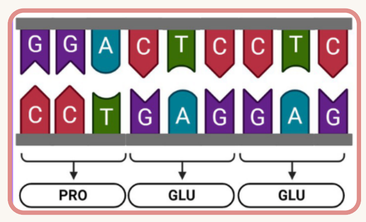
Avanthika Krishna, Grade 9, Lynbrook High School

Background

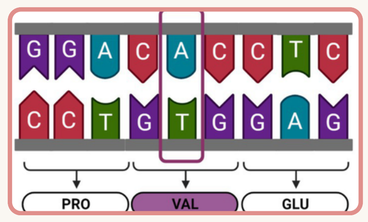
Sickle Cell Disease is one the most prevalent and rapidly-growing genetic diseases, nearly 8 million people suffering worldwide.

- Caused by a **point mutation in the gene encoding the subunit of hemoglobin, called β-globin**. This abnormal beta-globin is called sickle hemoglobin (HbS).
- Sickle hemoglobin polymerization occurs, where **deoxygenated sickle hemoglobin aggregate into helical fibers.**
  - damages the erythrocyte membrane and destroying red blood cells
- Abnormal signals such as **phosphatidylserine** are exposed on membrane
  - This triggers the immune system, **activating monocytes, macrophages, and neutrophils**.
- Sickle cell disease causes **pain crises, organ damage, risk for stroke, and lung problems.**





HBB Gene (Beta-globin)

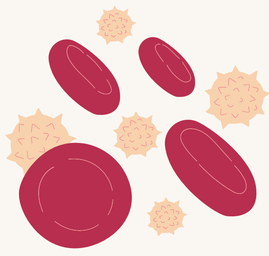


HBBSS Gene (Mutated Beta-globin)

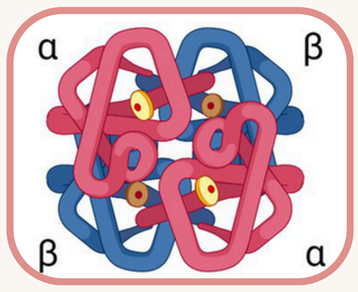
Molecular Basis of Sickle Cells

Sickle Cell Disease is caused by a single base mutation that causes sickle cells.

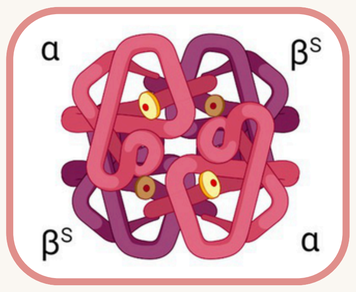
- It is generated from the **β^S allele (defective β-globin subunit) and is called the sickle β-globin subunit.**
  - **replaces glutamic acid with valine** at position 6, producing hemoglobin S.
- The mutation causes hemoglobin to polymerize, stiffening the erythrocytes.
  - causes the red blood cells to become rigid and adopt a sickle shape
- Because of **sickle hemoglobin polymerization**, damage to the erythrocyte membrane, and **intravascular hemolysis**, where red blood cells are destroyed directly in the bloodstream occur.



HbAA Molecule



HbSS Molecule



Fetal Hemglobin

Fetal hemoglobin (HbF) is produced in children and researchers discovered that children are more resistant to sickle cell disease due to its presence in their blood. This is because HbF inhibits the polymerization of sickle hemoglobin, due to its hybrid tetramer structure (two alpha-globin and two gamma- globin polypeptide chains). HbF also reduces the HbS concentration in RBCs, decreasing the chance of polymerization.The BCL11A gene inhibits production of gamma-globin, the main component of fetal hemoglobin.

BCL11A Gene Editing via CRISPR-Cas9

BCL11A is a repressor of gamma-globin gene expression, which is predominantly expressed during fetal hemoglobin.

- Scientists have utilized **CRISPR and Cas9 technologies** to delete this gene in order to promote the production of fetal hemoglobin
- Although researchers have utilized several different ways, the most widely used approach, as shown by a study by Khosravi, et. al is by targeting the **erythrocyte enhancer**.
- Using CRISPR-Cas9, the **shortest deletion of the GATA1 motif** was created inside the erythrocyte enhancer of the BCL11A gene
- This distorts the expression of the gene directly in the erythroid lineage.
- Targeted genome editing of a small 200 bp segment of the human BCL11A gene within the second intron resulted in **higher production of gamma-globin, creating fetal hemoglobin, allowing more resistance towards sickle cell disease**.

Technological Timeline

1949-1956

Linus Pauling learns that **oxygen carrying hemoglobin has a different chemical structure** in people with sickle-cell disease. Vernon Ingram and Cambridge University identifies the **genetic difference** between sickle hemoglobin and regular hemoglobin.

1998

FDA approves **hydroxyurea**, oral chemotherapy medication that inhibits DNA synthesis, as a sickle cell drug.

2008

A study led by **Orkin and Joel Hirschhorn**, from the Boston Children's and the Broad Institute suggested that genetic variants of BCL11A affect fetal hemoglobin production.

2011

Orkin and his colleagues test their theory by curing sickle cell disease in **mice** by **inactivating BCL11A gene,** promoting the production of fetal hemoglobin.

2013-2015

Daniel Bauer finds a region of the BCL11A gene called the **enhancer region**, which provided a **safer target** for inducing the production of fetal hemoglobin, and using CRISPR genetic editing, his team identified a discrete site where most activity occurs.

2018-2022

Opened clinical trials opened with positive results, resulting in therapy approvals and **now available to all people with sickle cell disease!**

Future Applications

Successful clinical trials has proved that CRISPR-Cas9 technology can be effectively used for precision genome editing for sickle cell disease. A study by Swetha Battini from the University of Connecticut suggests that future research will most likely focus on **optimizing RNA guide design** and **improving editing efficiency** to ensure **sustainability of the HbF production**. In addition to sickle cell disease, these methodologies could be applied to other hemoglobinopathies, such as various anemias. A part from conditions within the Hematopoietic Stem and Progenitor Cells (HSPCS), **CRISPR-Cas9 technology shows potential in genetic disorders such as cancer, neurological, and metabolic diseases**.

Scan here to get full references!

References

Akinsheye, Idowu, et al. "Fetal Hemoglobin in Sickle Cell Anemia." Blood, vol. 118, no. 1, July 2011, pp. 19–27, https://doi.org/10.1182/blood-2011-03-325258.

Carrocini, Gisele Cristine de Souza, et al. "What Influences Hb Fetal Production in Adulthood?" Revista Brasileira de Hematologia E Hemoterapia, vol. 33, no. 3, 2011, pp. 231–36, https://doi.org/10.5581/1516-8484.20110059. Accessed 2 Nov. 2019.

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