Sickle Cell Disease: Symptoms, Molecular Basis, and Treatments

Brooks J Rady

October 17th, 2019

1 Introduction

Sickle Cell Disease (SCD) is a chronic, mostly recessive, genetic condition suffered by 3.1 million people world-wide with 460 million more being asymptomatic gene carriers [9]. Though modern medicine has vastly improved the life expectancy of individuals with SCD, it remains just 48 years — if left untreated, it drops to only 14 years [16]. SCD also imposes a sizable economic burden: SCD hospitalisations in the United States cost $780 million in 1996 alone [1].

Sickle cell is most prevalent in Africa, but is also endemic to southern Europe and India [6]. It's no coincidence that these regions are ones in which malaria is common. While developing sickle cell disease is often lethal, possessing the sickle cell trait confers a significant resistance to malaria, giving an evolutionary edge to carriers of the sickle cell trait (Figure 6) [2, 5, 19].

The symptoms of SCD are numerous and diverse, but most patients suffer from acute pain, anaemia, and an increased risk of infection. Other symptoms include ischaemia induced tissue damage and an increased risk of clotting events leading to stroke, acute chest syndrome, and embolism conditions [19].

While SCD can arise from a number of genotypes — all containing at least one copy of the sickle cell gene and another mutant b-globin [1] — this paper will focus on sickle-cell anaemia (SCA) in which two copies of the sickle cell gene are present. It will begin by describing the structure and function of haemoglobin before diving into the molecular basis of SCA and its symptoms. Prior to concluding, the diagnosis and treatment of the disease will be explored.

2 Haemoglobin

2.1 Structure & Function

Haemoglobin is the protein responsible for oxygen transport in most animals. It can account for the majority of the mass of an erythrocyte, but is not often found within the blood plasma [11].

Haemoglobin is a tetramer, composed of four polypeptide chains each containing a prosthetic group. This group, a haem, is formed when a protoporphyrin IX molecule binds a ferrous ion (Fe^{2+}) and is what allows haemoglobin to interact with oxygen (Figure 2) [11].

All naturally-occurring haemoglobins contain two a-chains, each 141 residues long, and a pair of secondary globin chains 146 residues in length [11].

In healthy adults, these secondary chains are b-globins. This form of haemoglobin is referred to as adult haemoglobin or HbA [11]. Foetuses and newborns, on the other hand, possess a so-called foetal haemoglobin (HbF). This form of haemoglobin substitutes the b-chain of HbA with a g-chain. While HbF mostly disappears a few months after birth, small concentrations can persist into adulthood [11].

2.2 Chemistry & Bonds

Within the oxygen-binding site of haemoglobin, there exist several strictly conserved histidine residues. One of these residues, called the proximal histidine, forms a dative bond to the iron of the haem molecule, fixing it in place [10].

A second, distal histidine sits on the opposite side of the haem and serves to stabilize (via hydrogen bonding) the superoxide ion of oxyhaemoglobin [10, 11].

Importantly, the four sub-units of haemoglobin are held together by malleable salt-bridges that allow for conformational changes to occur upon oxygen binding [10].

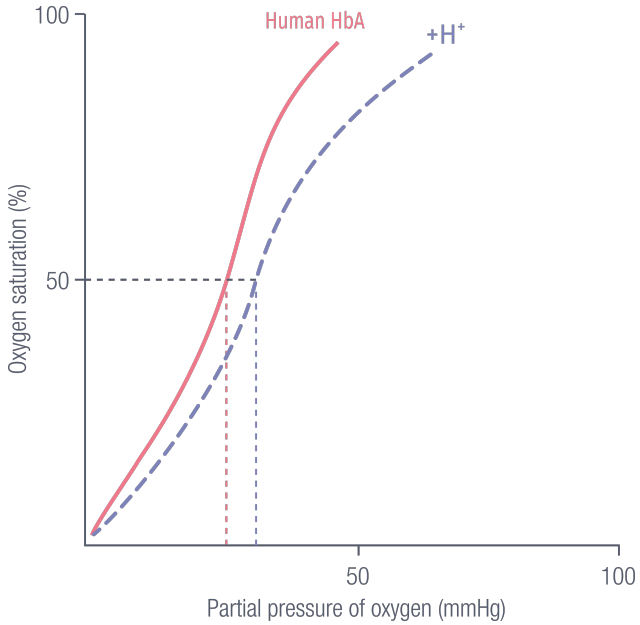
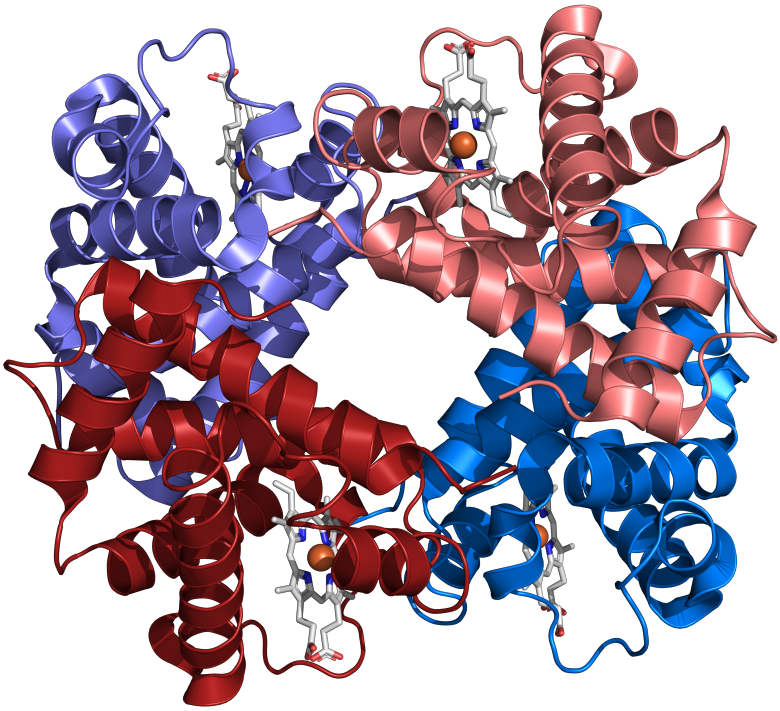


Figure 1: A Pair of Oxygen-Haemoglobin Dissociation Curves Demonstrating the Bohr Effect
A standard measure of oxygen affinity is p_{50} (the partial pressure at which haemoglobin is 50% saturated with oxygen). The *larger* the p_{50}, the *weaker* the affinity for oxygen. Here, the curve for haemoglobin at low pH, shows the reduced affinity for oxygen as predicted by the Bohr effect. Graph adapted from Marengo-Rowe [11].

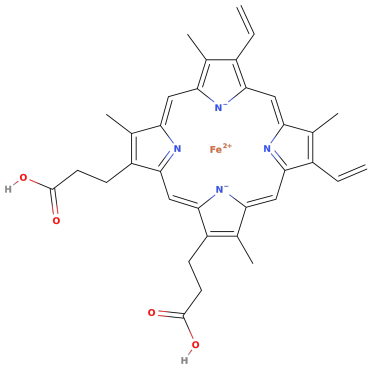
2.3 Oxygen Binding & The Bohr Effect

When the partial pressure of O_2 is relatively high, the ferrous ion of each haem reversibly binds an oxygen, becoming a ferric ion and forming superoxide [11].

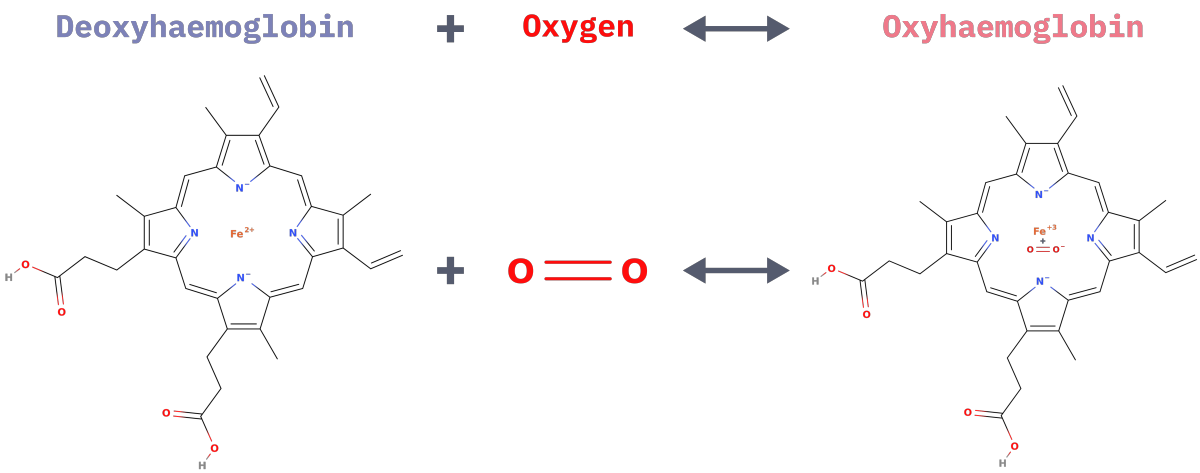
Oxygen binding is a cooperative process — once one oxygen is bound, it pulls on the distal histidine and rearranges



(a) Cartoon Representation of Deoxyhaemoglobin



(b) Structural Formula of Haem



(c) Haem Oxygenation Reaction

Figure 2: The Natural Structure and Function of Haemoglobin
(a) The tetrameric structure of deoxyhaemoglobin is shown with its two a-globins in red and its two b-globins in blue. The grey porphyrin rings are haem and have had their central irons coloured orange. Made using PyMol and crystal structure data from PDB entry 4HHB [4]. **(b)** The structural formula of haem is shown here in the ferrous (Fe^{2+}) oxidation state. The oxygen-containing vinyl groups are hydrophilic and protrude from the haemoglobin structure. Structure acquired from PubChem [17]. **(c)** The reaction between haem and molecular oxygen is shown, highlighting the chemical process that converts deoxyhaemoglobin to oxyhaemoglobin.

the salt-bridges holding haemoglobin together. This facilitates the transition between the R (high-affinity) and T (low-affinity) states [10, 11]. It's this cooperation that's responsible for the sigmoidal curves of Figure 1.

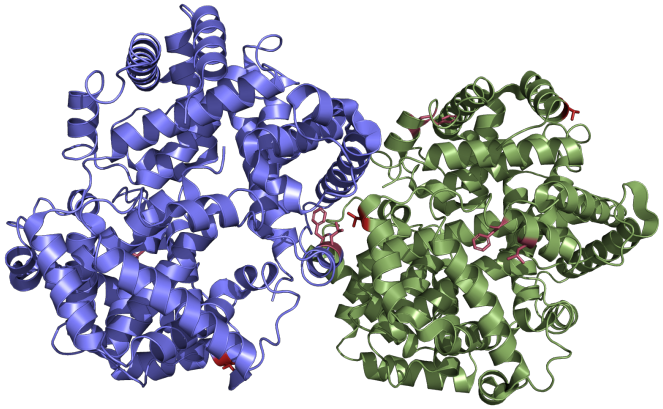
As the pH of blood changes, the histidines within haemoglobin are protonated and deprotonated, modulating their affinity for oxygen [15]. Lower pH means a lower oxygen-affinity. This effect helps haemoglobin deliver oxygen to the tissues that need it most. CO_2 produced during respiration reacts with water to form carbonic acid, which goes on to lower the pH and trigger O_2 release [15].

3 Molecular Basis

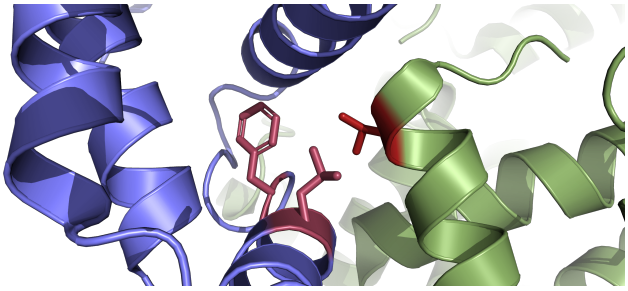
3.1 Pathogenesis

3.1.1 Genetic Origin

Sickle Cell Disease is caused by a missense mutation in the 17^{th} base of the b-globin gene that swaps its 6^{th} residue from a glutamic acid to valine [2, 19]. Valine, unlike glutamic acid, has a hydrophobic side-chain which subtly alters the chemistry of the b-globin chain (Figure 5, 1-2). This mutated b-globin goes on to form a complex with one other b and two other a chains, becoming a mature sickle haemoglobin (HbS) [2].



(a) Hydrophobic Interactions Between Two HbS Molecules



(b) Close-Up of Molecular Interactions

Figure 3: The Molecular Interactions Responsible for HbS Polymerisation
(a) The hydrophobic surface-residues (shown in red) of two sickle haemoglobins (green and blue) can be seen interacting with each other. **(b)** A closer view makes it clear to see the hydrophobic pocket formed by Phe 85 and Leu 88 on the blue chain and the mutant valine protruding from the green chain. Both images were made using PyMol and crystal structure data from PDB entry 2HBS [8].

3.1.2 Sickle Cell Formation

In oxyhaemoglobin, this mutation is silent and leads to no ill-effects as the rogue valine residue has no other hydrophobic groups to interact with. The issue arises when deoxyhaemoglobin is formed. In the deoxyhaemoglobin, a second hydrophobic patch is naturally present on the b-chain (formed by a phenylalanine and leucine in positions 85 and 88 respectively). This hydrophobic patch is then free to interact with the mutant valine on other HbS molecules (Figure 3). When enough deoxyhaemoglobin is present within an erythrocyte, these two sites can form hydrophobic bonds and spur the HbS to polymerise (Figure 5, 2-3) [2, 19].

This polymerisation eventually results in the formation of stiff haemoglobin fibres within the erythrocyte [2]. As the haemoglobin is precipitated out of the cytosol, the osmotic pressure within the cell drops and it begins to lose water. Large fibres can even damage the plasma membrane, leading to the dysfunction of the cell's sodium-potassium pumps [10]. This further drops the solute concentration within the erythrocyte and accelerates the dehydration process. The membrane then shrivels around the polymerised haemoglobin fibres, forming SCA's characteristic sickle-shape (Figure 5, 5-6) [7, 19].

3.2 Molecular Basis of Symptoms

3.2.1 Acute Pain

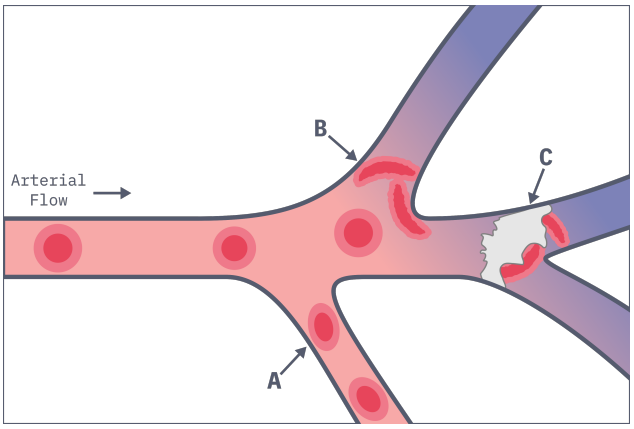


Figure 4: Sickle Cell Induced Vaso-Occlusion
(A) Healthy erythrocytes are flexible and can easily squeeze into small capillaries. **(B)** Sickled cells are both stiffer and "stickier" than healthy cells. They can become lodged in the capillaries and interrupt circulation. **(C)** The sickle cells blocking the capillaries induce an immune response and are cleared by a phagocytic cell (shown here in white).

The recurring pain experienced by patients with SCA is thought to be the result of vaso-occlusion. During vaso-occlusion, stiff, sickled cells become lodged in capillaries leading to ischaemia (a lack of oxygen in the tissues) which manifests as an intense pain (Figure 4) [19].

3.2.2 Anaemia

Sickle Cell Anaemia is a form of haemolytic anaemia resulting from the destruction of erythrocytes by the immune system (10% of the total population every 24 hours [20]). When sickled cells become trapped in the capillaries, they trigger an immune response in which the leukocytes lyse the sickle-cells in an attempt to restore circulation. This leads to a lack of oxygen-carriers in the blood [2].

In the event of a splenic sequestration crisis (SSC), this anaemia can quickly worsen into a life-threatening condition. In a SSC, vaso-occlusion blocks blood from flowing out of the spleen. This results in the spleen swelling significantly and the number of erythrocytes in circulation plummeting [1].

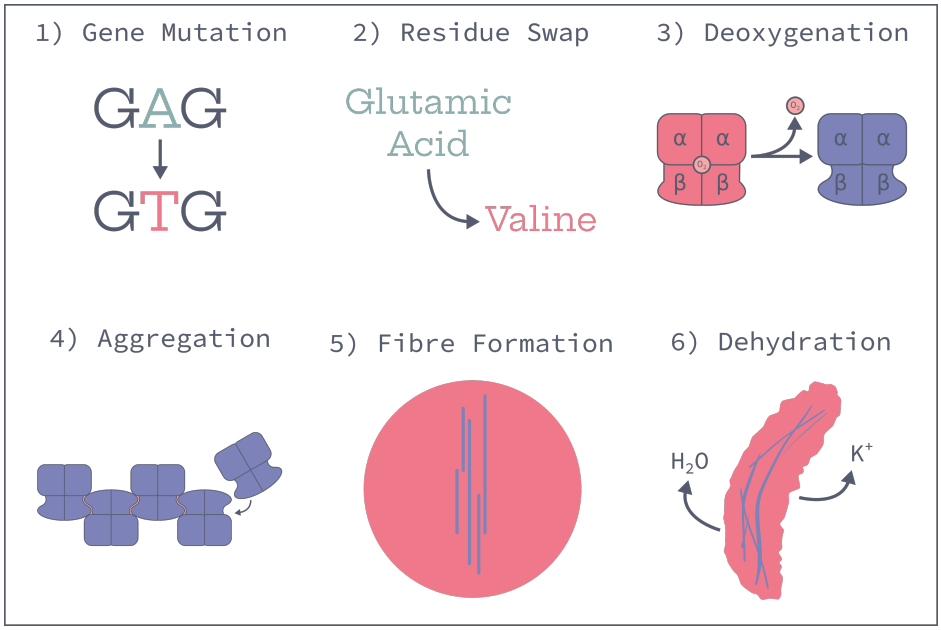


Figure 5: An Overview of Sickle Cell Formation
(1) A missense mutation occurs in the 17^{th} position of the b-globin gene, changing out an adenine for a thymine. **(2)** This codon change replaces the 6^{th} residue in the polypeptide chain, a glutamic acid, with a hydrophobic valine. **(3)** After the haemoglobin loses its oxygen, it undergoes a conformational change that results in the formation of a hydrophobic pocket on the surface of the protein. **(4)** The newly exposed pocket then interacts with the mutant valine of another b-chain, forming a polymer. **(5)** As polymerisation continues, large, stiff fibres form within the erythrocyte. **(6)** The precipitation of haemoglobin from the cytosol results in a drop in osmotic pressure. The loss of potassium ions serves to further reduce intracellular solute concentrations. As a result, water flows out of the cell and the erythrocyte takes on a shrivelled, sickled appearance.

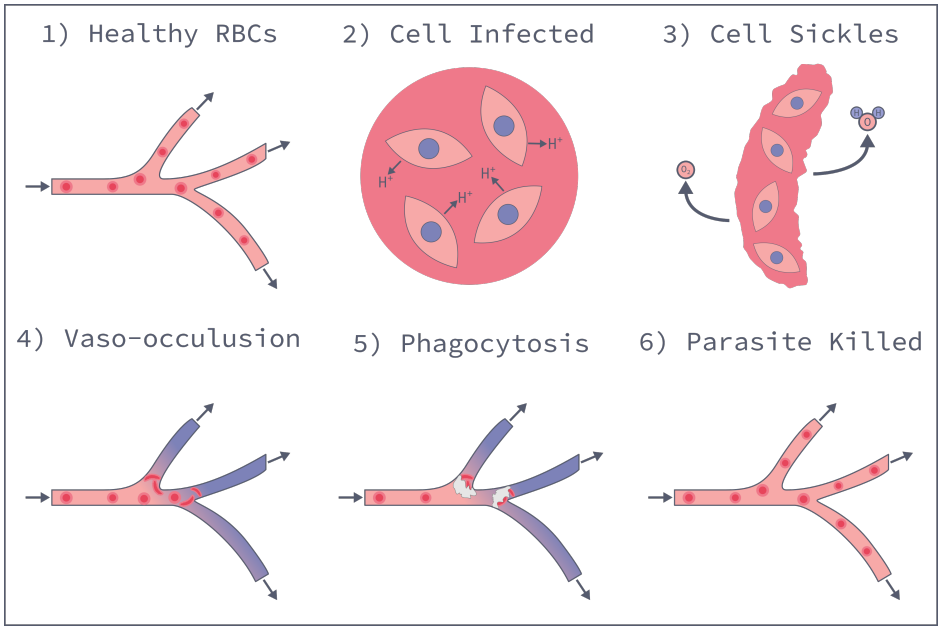


Figure 6: A Proposed Mechanism for Malarial Resistance in Sickle Cell Trait
(1) In humans with sickle cell trait, both HbA and HbS exist in a balance. The presence of non-aggregating HbA usually prevents haemoglobin fibres from forming and protects cells from sickling. **(2)** When the malarial parasite (*Plasmodium falciparum*) infects an erythrocyte and begins to metabolise, it secretes lactic acid as a byproduct. This lowers the pH of the cytosol. **(3)** As the pH drops, the Bohr Effect encourages the dissociation of haemoglobin and oxygen. As progressively more deoxyhaemoglobin is formed, a critical threshold is crossed and the HbS within the cell begins to aggregate despite the presence of HbA. This leads to the formation of a sickle-cell with the parasite trapped inside. **(4)** This sticky and inflexible sickle-cell can then become caught in capillary beds and lead to vaso-occlusion. **(5)** The lack of oxygen in the tissue then incites an immune response in which phagocytic cells lyse the sickle-cells blocking circulation. **(6)** When the sickle cells are destroyed, so too are the parasites trapped within them. This means that not only is circulation restored, but the malarial parasites are killed before they have the opportunity to spread.

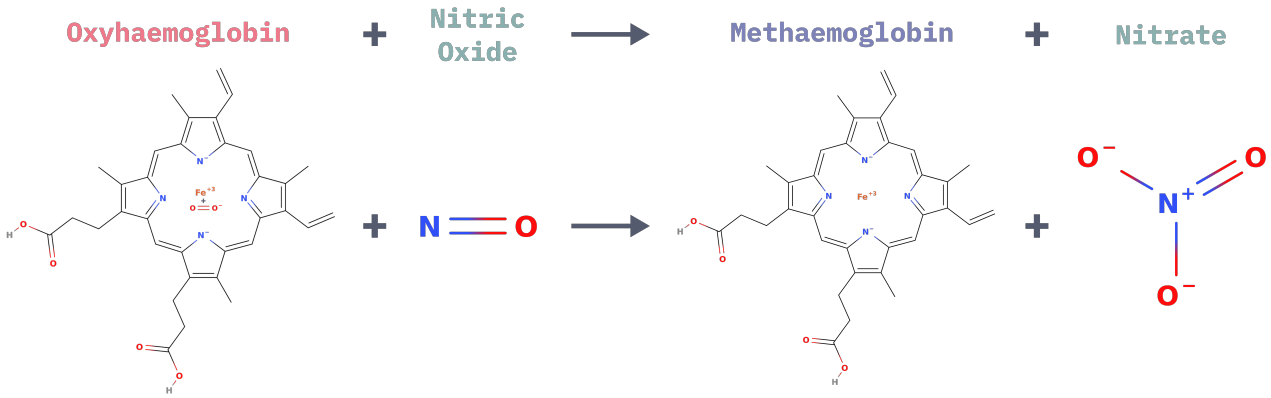


Figure 7: The Oxyhaemoglobin-Driven Nitric Oxide Elimination Pathway
Free oxyhaemoglobin in the plasma can react with dissolved nitric oxide to form methaemoglobin and nitrate. Methaemoglobin is similar to deoxyhaemoglobin, but its iron ions are in the ferric (Fe^{3+}) oxidation state, so it's unable to bind and transport oxygen. The elevated rates of haemolysis in SCA patients leads to increased concentrations of free oxyhaemoglobin and, consequentially, greater rates of NO elimination.

3.2.3 Clotting Disorders

The lysis of sickle-cells releases large amounts of haemoglobin into the plasma (up to 30g per day [20]) where it can oxidise a number of molecules — molecules like nitric oxide (NO) [12, 19].

Free haemoglobin reacts with NO to form nitrate and methemoglobin 1,000 times more rapidly than the haemoglobin stored in erythrocytes (Figure 7) [20]. In healthy individuals, haptoglobin binds and pacifies oxyhaemoglobin before it can cause much damage, but the rapid rate of haemolysis in individuals with SCA means this protection is quickly overwhelmed [20]. Just 6µM haemoglobin in the plasma is enough to nullify the positive effects of nitric oxide [20].

NO is an important vasodilator and downregulates the cell-adhesion molecule sVCAM-1. Elevated levels of this molecule create a "stickier" endothelium more prone to vaso-occlusion and clot formation [12, 19, 20]. In the absence of NO, hypertension quickly develops and the blood takes on a hypercoagulable state. This dramatically increases the risk of vasculopathies like acute chest syndrome and stroke [19].

3.2.4 Immune Suppression

The increased susceptibility to infection is a multifactorial symptom thought to be caused in part by impaired circulation and micronutrient deficiencies [5]. The impaired circulation is a product of the aforementioned vasculopathies, but the mechanism of micronutrient loss remains an area of active study. It has been proposed that a combination of ischaemic damage to intestinal mucosa, and an elevated rate of metabolism (to support the production of new erythrocytes) are to blame [3].

4 Diagnosis & Treatment

4.1 Diagnosis

In many developed countries, testing for SCA is now done during neonatal screenings and DNA tests can even be carried out prenatally if the parents are carriers [19].

4.1.1 Haemoglobin Tests

While many options exist for differentiating between HbA and HbS, isoelectric focusing (IEF) is often a good balance between cost and sensitivity [1] — though cellulose acetate electrophoresis and cation exchange HPLC are viable alternatives [21].

IEF works by running haemoglobin samples through a gel containing a pH gradient. This causes the samples to migrate until they reach the pH representing their isoelectric point (pI) at which they have no net charge [21]. As HbS is missing a glutamic acid, it's pI will differ from that of HbA, allowing the two to be visually separated. IEF can separate molecules differing in pI by as little as 0.02 pH [21].

4.1.2 DNA Tests

If prenatal testing is required, or additional verification is needed, a DNA based screening can be performed. There exist a number of methods employing ARMS-PCR, allele specific oligonucleotides, and restriction enzymes [13].

ARMS-PCR is a relatively simple method that leverages primers designed to amplify the sickle cell allele, but not the wild-type gene [13]. A second pair of primers is then used to create a control fragment. The PCR products are subsequently separated via gel electrophoresis and any samples containing the sickle-cell allele will show a second visible band (in addition to the control) [13].

4.2 Treatment

4.2.1 Hydroxyurea

Hydroxyurea (HU) is a cytotoxic drug that induces the expression of HbF in adults. As HbF contains no faulty b-chains, fetal haemoglobin does not aggregate or lead to the formation of sickle-cells, thus preventing most SCA symptoms [2, 5, 6]. In small doses (∼20mg / day), HU shows little toxicity and substantially reduces HbS aggregation. While many mechanisms have been proposed to explain how HU induces the expression of HbF, a scientific consensus has yet to be reached [18].

In addition to increasing the production of HbF, HU has been found to generate NO in the blood plasma — further combating the excessive clotting exhibited by patients with SCA [19].

4.2.2 Blood Transfusions

Blood transfusions are a common, quick, and relatively safe treatment for SCA [5]. While this works well for a while, repeat transfusions can put the patient at risk of an iron overload. Excess iron can damage organs like the liver and the heart, but can be treated using iron chelating drugs that capture and flush iron from the body [5].

4.2.3 Bone Marrow Transplant

Currently, the only known cure for SCA is a bone-marrow transplant. By replacing the haematopoietic cells in the marrow, the patient's HbS can be replaced with the donor's HbA [5].

The biggest issue with this treatment is histocompatibility. While it has an 82-86% cure rate, it also has a mortality rate of 7%. Even if the transplant goes well, the patient often relies on long-term immunosuppressants [5].

5 Conclusion

While SCA is one of the better studied heritable diseases, it's localisation in underdeveloped countries means that treatment options are limited. Fortunately, SCA treatment lends itself well to several emerging technologies: gene therapy and induced pluripotent stem cells (iPSCs). At the moment, haematopoietic cells are difficult to culture, so gene therapy tends to be carried out *in vivo* using lentiviral vectors [5]. While this works decently (there is even an ongoing clinical trial in France [19]), there is a risk of off-target mutations within the patient's genome. However, by culturing iPSCs from the patient and modifying them *in vitro*, both the issue of histocompatibility and unintended mutations could be eliminated as the gene-edits could be verified before the stem cells are transplanted into the patient [14]. While SCA remains a complex condition and it's effects on the immune system and nitric oxide regulation are just beginning to be understood, science continues to deepen our understanding of the disease and reveals how to better treat patients who are suffering.

References

[1] A. Ashley-Koch, Q. Yang, and R.S. Olney. Sickle Hemoglobin (Hb S) Allele and Sickle Cell Disease: A HuGE Review. American Journal of Epidemiology *151*.9 (May 1, 2000), 839–845. ISSN: 0002-9262, 1476-6256. DOI: 10.1093/oxfordjournals.aje.a010288.

[2] J.M. Berg et al. Biochemistry Eighth edition. (New York: W.H. Freeman & Company, a Macmillan Education Imprint, 2015). 1 p. ISBN: 978-1-4641-2610-9.

[3] L.H. Dekker et al. Micronutrients and sickle cell disease, effects on growth, infection and vaso-occlusive crisis: A systematic review. Pediatric Blood & Cancer *59*.2 (Aug. 2012), 211–215. ISSN: 15455009. DOI: 10.1002/pbc.24163.

[4] G. Fermi et al. The crystal structure of human deoxyhaemoglobin at 1.74 A resolution. Journal of Molecular Biology *175*.2 (May 15, 1984), 159–174. ISSN: 0022-2836. DOI: 10.1016/0022-2836(84)90472-8.

[5] Q. Fernandes. Therapeutic strategies in Sickle Cell Anemia: The past present and future. Life Sciences *178* (June 2017), 100–108. ISSN: 00243205. DOI: 10.1016/j.lfs.2017.03.025.

[6] A.J.F. Griffiths et al. Introduction to genetic analysis Eleventh edition. OCLC: ocn900650999. (New York, NY: W.H. Freeman & Company, a Macmillan Education imprint, 2015). 868 pp. ISBN: 978-1-4641-8804-6.

[7] A.R. Hargens et al. Sickle-cell hemoglobin: fall in osmotic pressure upon deoxygenation. Proceedings of the National Academy of Sciences *77*.7 (July 1, 1980), 4310–4312. ISSN: 0027-8424, 1091-6490. DOI: 10.1073/pnas.77.7.4310.

[8] D.J. Harrington, K. Adachi, and W.E. Royer. The high resolution crystal structure of deoxyhemoglobin S. Journal of Molecular Biology *272*.3 (Sept. 26, 1997), 398–407. ISSN: 0022-2836. DOI: 10.1006/jmbi.1997.1253.

[9] S.L. James et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet *392*.10159 (Nov. 2018), 1789–1858. ISSN: 01406736. DOI: 10.1016/S0140-6736(18)32279-7.

[10] K. Kaushansky, ed. *Williams hematology*. Ninth edition. (New York: McGraw-Hill, 2016). 2 pp. ISBN: 978-0-07-183300-4.

[11] A.J. Marengo-Rowe. Structure-Function Relations of Human Hemoglobins. Baylor University Medical Center Proceedings *19*.3 (July 2006), 239–245. ISSN: 0899-8280, 1525-3252. DOI: 10.1080/08998280.2006.11928171.

[12] K. Matsubara et al. Nitric Oxide and Reactive Oxygen Species in the Pathogenesis of Preeclampsia. International Journal of Molecular Sciences *16*.3 (Mar. 2, 2015), 4600–4614. ISSN: 1422-0067. DOI: 10.3390/ijms16034600.

[13] J.M. Old. "DNA Diagnosis of Hemoglobin Mutations". R.L. Nagel. Hemoglobin Disorders, vol. 82. (New Jersey: Humana Press, Mar. 4, 2003), pp. 101–116. ISBN: 978-1-59259-373-6. DOI: 10.1385/1-59259-373-9:101.

[14] E.P. Papapetrou. "Gene and Cell Therapy for -Thalassemia and Sickle Cell Disease with Induced Pluripotent Stem Cells (iPSCs): The Next Frontier". Gene and Cell Therapies for Beta-Globinopathies, P. Malik and J. Tisdale, ed. Vol. 1013. (New York, NY: Springer New York, 2017), pp. 219–240. ISBN: 978-1-4939-7299-9. DOI: 10.1007/978-1-4939-7299-9_9.

[15] M. Perutz et al. Identification of residues contributing to the Bohr effect of human haemoglobin. Journal of Molecular Biology *138*.3 (Apr. 1980), 649–668. ISSN: 00222836. DOI: 10.1016/S0022-2836(80)80022-2.

[16] O.S. Platt et al. Mortality In Sickle Cell Disease – Life Expectancy and Risk Factors for Early Death. New England Journal of Medicine *330*.23 (June 9, 1994), 1639–1644. ISSN: 0028-4793, 1533-4406. DOI: 10.1056/NEJM199406093302303.

[17] PubChem. *Ferroprotoporphyrin IX*.

[18] G.D. Pule et al. A systematic review of known mechanisms of hydroxyurea-induced fetal hemoglobin for treatment of sickle cell disease. Expert Review of Hematology *8*.5 (Sept. 3, 2015), 669–679. ISSN: 1747-4086, 1747-4094. DOI: 10.1586/17474086.2015.1078235.

[19] D.C. Rees, T.N. Williams, and M.T. Gladwin. Sickle-cell disease. The Lancet *376*.9757 (Dec. 2010), 2018–2031. ISSN: 01406736. DOI: 10.1016/S0140-6736(10)61029-X.

[20] C.D. Reiter et al. Cell-free hemoglobin limits nitric oxide bioavailability in sickle-cell disease. Nature Medicine *8*.12 (Dec. 2002), 1383–1389. ISSN: 1078-8956, 1546-170X. DOI: 10.1038/nm1202-799.

[21] H. Wajcman and K. Moradkhani. Abnormal haemoglobins: detection & characterization. The Indian Journal of Medical Research *134* (Oct. 2011), 538–546. ISSN: 0971-5916.