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Genetic cause and therapy of sickle cell disease

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Abstract. Sickle cell disease (SCD) is an influential genetic disorder on a global scale. The sickle cell population has been a large collective in recent years. Vaso-occlusion, membrane injury, and cardiovascular complications were discovered to result from its presence. There is currently no treatment that can completely cure SCD. Using a method of literature review, this paper discusses the history, pathology, and potential treatments. A point mutation of the sixth amino acid in the β-hemoglobin gene (HBB) is the genetic etiology of sickle cell disease. The replacement of glutamic acid with valine reduces the oxygen-carrying capacity of erythroid. Even though the cause of SCD is evident, the treatments, including blood transfusion, hydroxyurea, gene-editing, and GBT440 agent therapy, are still uncertain. Further research and testing are required to reduce the morbidity of SCD, and additional efforts are required to increase the rate of SCD cure.

Keywords: sickle cell disease, genetic cause, β-hemoglobin gene.

1. Introduction

Sickle cell disease (SCD) is an influential genetic disorder on a global scale. According to the Global Burden of Disease Study, 3,2 million persons suffer from sickle cell disease and 176,000 die as a result of diseases related to sickle cells [1]. The population of sickle cell anemia was 50,000 thirty years ago, and it appears to be increasing [2]. As the global SCD community continues to expand, SCD continues to garner considerable interest. In 1910, a physician from Chicago discovered SCD, and it was later discovered that the disease causes decreased oxygen circulation in the human body. During this period, only fundamental drugs and pain management are administered. Better treatments were not found until 1957, when the etiology of SCD was identified.

A point mutation of the sixth amino acid in the β-hemoglobin gene (HBB) is the genetic etiology of sickle cell disease. The replacement of glutamic acid with valine reduces the oxygen-carrying capacity of erythroid. Deoxygenated sickle hemoglobin polymerizes, resulting in erythrocyte deformation, hemolysis, anemia, vaso-occlusive episodes, end-organ injury, and a decreased life expectancy [3]. In addition, SCD is associated with multiple alternative mutations of the -hemoglobin gene, which are known as sickle cell anemia, sickle cell trait, HbSC disease, HbSE disease, -thalassemia, etc. [4].

Transfusion, pain management, hydroxyurea, and bone marrow or stem cell transplantation are modern treatments for SCD [3]. Even though there are numerous treatments for SCD, there is no definitive cure. To cure the disease, scientists are devising additional treatments, such as CRISPR-CAS9 gene editing. Using methods of literature review and analysis, this paper will provide a summary of the history, genetic causes, and treatments of sickle cell disease to support researchers.

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2. History of sickle cell disease

A physician from Chicago initially identified sickle cell disease. Herrick reported the first case of SCD in 1910, which involved a 20-year-old black male from Grenada. He presented to the clinic with a body that was feeble and ill, as well as scars around his legs. He stated that numerous other symptoms manifested in the preceding years. He suffered from palpitations, shortness of breath, yellow-tinged irises, purulent discharge from the right ear, and ankle pain. He had recently been coughing and suffering from a mild chill and fever. The physical examination revealed that he was a robust young man, with the exception of a few unusual symptoms. His membranes contained mucus and his cervical, axillary, inguinal, and epitrochlear lymph nodes were enlarged. In addition, the previously mentioned scars had a diameter of 3 centimeters and a total of approximately 20 round scars. Additionally, it was observed that his heart was enlarged on the left side and that there was inflammation throughout his thorax. His average temperature was 100 degrees Fahrenheit and his average pulse rate was 80. Several testings had been done on him. In every blood examination, the percentage of hemoglobin increased. Polychromatophilia, which indicates an abnormally high number of stained cells, manifested itself. The red corpuscles had slender, elongated, sickle-shaped, and crescent-shaped forms, and were unusually flexible. Significant blood-stained mucus was detected in stool examination. Although he has undergone these procedures, his condition has not yet been identified. He felt improved after four weeks, but it turned out that his symptoms quickly returned. It was hypothesized that his condition was caused by syphilis, intestinal parasite infection, or coal-tar preparations [5].

In 1927, it was discovered that hemoglobin sickling is the consequence of deoxygenation. The hemoglobin is deformed by deoxygenation until it is reoxygenated [6]. In 1949, it was determined that SCD is a genetic disorder. Pauling and his colleagues conducted an electrophoretic comparison of normal and sickle hemoglobins as part of an experiment. The experiment was divided into three groups: hypoxic hemoglobins, uncombined ferrohemoglobins, and hypoxic hemoglobins and dithionite ions. They discovered that sickle hemoglobin contained two to four times more net positive charges per molecule than normal hemoglobin. Thus, a distinction was made between normal hemoglobin and sickle hemoglobin, indicating that hemoglobin within erythrocytes may be the cause of SCD. Nonetheless, the precise cause of the sickling process remained unknown [7].

Vernon Ingram, Ph.D., was the first to demonstrate the biochemical cause of sickle cell disease after extensive investigation [8]. In 1957, he demonstrated the chemical distinction between normal and sickle cell hemoglobin. Normal and abnormal hemoglobin are digested with tryptic enzymes in his experiment, and the separated peptides are displayed on a sheet of paper. In one direction, he employed electrophoresis, and in the other, partition chromatography. Except for the hemoglobin S 4th peptide, all peptides have the same electrophoretic and chromatographic signature, as demonstrated by paper chromatography. Hemoglobin A (normal) contained more glutamic acid than hemoglobin S (sickled). Following hydrolysis in 12 N hydrochloric acid and X-ray crystallography, it was determined that the disease is caused by the replacement of glutamic acid with valine in the hemoglobin cell, and that the entire hemoglobin S molecule contains two or three fewer carboxyl groups than the hemoglobin A molecule. Vernon Ingram also stated that the inability to distinguish between normal hemoglobin and sickle hemoglobin is due to the inability of qualitative amino acid analysis to distinguish between the two because they have identical structures and only a single peptide is altered [8].

3. Pathology of sickle cell disease

3.1. Genetics of SCD

SCD is caused by a point mutation in the beta hemoglobin gene's (HBB) amino acid chain. It is a single A-T mutation in the amino acid codon at position 6 that replaces a glutamic acid residue with a valine residue and converts the normal β-globin chain into a sickled β-globin chain [8]. Adult hemoglobin (HbA) is composed of two β-globin chains and two β-globin chains, whereas sickled hemoglobin (HbS) is the result of a mutation. Upon deoxygenation, the HbS polymerizes and forms a crystal solution equilibrium in which the solid phase is composed of aligned filaments and the liquid phase is composed

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of single hemoglobin molecules [9]. Deoxygenation also causes the sickling phenomenon of β hemoglobin, and the reduced portion of hemoglobin is shown to be in a crystalline state, which is a solid-state and increases blood viscosity [10]. Therefore, the polymerization of HbS is the primary cause of the pathology of SCD.

One of the factors that explains why polymerization of HbS is crucial to the pathology of SCD is its solubility. As depicted in reference [11], two phases of HbS solution exist at equilibrium, one containing fibers and the other HBB [11]. When HbS is polymerized, the increase in intracellular viscosity results in molecules that are less flexible, which is detrimental to the human body due to the decreased oxygen circulation [11]. The solubility of deoxygenated HbS is only 0.02 that of deoxygenated HbA, according to the reference [10]. Consequently, HbS is less flexible than HbA, resulting in several complications of SCD, including vaso-occlusion.

3.2. Possibility of inheriting SCD

Inherited blood disorders like sickle cell disease are quite prevalent, and a kid must have a pair of sickled genes from each parent in order to develop the disease or become a carrier. When both parents are carriers of SCD, or when one parent has the disease and the other is a carrier, the disease is inherited. Carriers do not have maladies themselves, but they can pass on the sickle cell trait to future generations. If both parents are sickle cell disease carriers, there is a 25% chance that their child will not develop sickle cell disease or become a carrier, a 25% chance that their child will develop sickle cell disease, and a 50% chance that their child will become a carrier.

3.3. Fetal hemoglobin

Even though the sickled hemoglobin gene may be transmitted from parents to embryos, the disease will not affect embryos. The β-globin gene is not expressed during the fetal period; instead, γ-globin is expressed. In this period, feral hemoglobin (HbF) is produced. Due to the expression of BCL11A, a transcription factor that represses γ-globin in adults, it dissipates during adulthood. However, it was discovered that adults with HbF into maturity have minimal or no SCD [12].

4. Complications and damages of SCD

4.1. Vaso-occlusion

Vaso-occlusion is one of the most prevalent complications associated with SCD. During the deoxygenation of HbS, erythrocytes transform into a less flexible sickle shape and can obstruct the microvasculature. However, the severity of vaso-occlusive crises (VOCs) varies between patients. The blood flow rate in the microvasculature and the delay time between deoxygenation and polymerization of HbS play a role in some way [13]. Moreover, the abnormal adhesion of erythrocytes and endothelium impacts the severity of volatile organic compounds [14].

4.2. Membrane damage

SCD can damage multiple cell membranes, including the membrane of red blood cells (RBCs) and human pulmonary artery endothelial cells (HPAECs). HbS has been shown to be more susceptible to oxidative stress than HbA. Oxidative stress is a result of heme release and subsequent iron accumulation intracellular, with heme being a damage-associated molecular pattern and the byproduct of Hb oxidation. In the experiment conducted by Jana et al., HO-1 and H-ferritin were measured to ascertain the oxidative stress caused by HbS and HbA. The data demonstrated that HbS increased both HO-1 and H-ferritin. An increase in oxidative stress may result in inflammation, tissue and DNA damage, as well as a possible circular disorder. Incubating HbS with cultivated HPAECs for 6 hours, Jana's team discovered a significant change in dextran permeability. In contrast, HbA did not exhibit any significant permeability modifications. This alteration in permeability may result in the departure of cells from blood vessels into other body tissues [15].

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4.3. Cardiovascular complications

Anemia and HbS polymerization resulting from SCD are the root cause of cardiovascular complications. Both chronic anemia and HbS polymerization reduce the blood's oxygen-carrying capacity due to the inflexibility of the erythrocyte [11]. As a result, the need for blood circulation in the body increases, resulting in an increase in cardiac output to maintain a sufficient circulation [16]. A high cardiac output may result in a state of volume overload [17]. A volume overload state can cause venous return, increased filling pressures, and high-output cardiac failure in SCD [18].

5. Therapies

The initial recorded case of SCD was treated with rest, nourishing diet, and iron iodide syrup [5]. Until the 1960s, when blood transfusions were administered to SCD patients, SCD treatment consisted solely of complications management. A children's facility in Michigan initiates a transfusion program in 1969. 15 individuals, ranging in age from 2 to 20 years, with SCD and a CNS infraction opted for partial exchange or packed cell transfusions to lower their HbS levels. 14 individuals were cured of CNS infractions after treatment, while one girl had a probable recurrence but was cured after a second transfusion [19].

Then, stem cell transplantation as a treatment for SCD was discovered. An eight-year-old girl received a bone marrow transplant from her brother, as reported by Johnson and his colleagues. The daughter received cyclophosphamide and a bone marrow transplant to convert sickle-cell anemia to sickle-cell trait. Even though bone marrow transplantation increases HbA, there are complications, particularly graft-versus-host disease [20].

To explore additional SCD treatment options, additional therapies were developed. In 1997, hydroxyurea was authorized for the treatment of SCD in adults [21]. In addition, researchers are devising an increasing number of technologically-based treatments, including gene editing. In addition to blood transfusion and stem cell transplant, additional SCD treatments rely on an increase in HbF. Moreover, it was discovered that co-inheritance of α-thalassemia protects against the complications of SCD [22]. As more potential treatments for SCD are discovered, SCD patients are given greater hope.

5.1. Blood transfusion

The purpose of blood transfusion is to increase the ratio of HbA to HbS in order to increase the oxygen-carrying capacity of red blood cells and decrease blood viscosity. However, HBB levels cannot exceed 10 g/dL, as this will result in complications for the patients [23]. There are two varieties of blood transfusion: simple transfusion and exchange transfusion. Red cell exchange (RCE), one of the exchange transfusions, has several advantages over basic transfusion. The duration of the procedure ranges from 90 to 120 minutes. In addition, it prevents excess iron [24]. Blood transfusions prevent both primary and secondary strokes in patients with SCD [25].

5.2. Hydroxyurea

The synthesis of HbF by hydroxyurea increases the quantity of fetal hemoglobin in patients, thereby reducing the frequency of pain crises. HbF, which lacks ϐ-globin, prevents a red blood cell from sickling. The patient's hydroxyurea dosage would depend on their weight. In the investigation conducted by Charache et al., hydroxyurea was administered to 148 male and 151 female SCD patients. The outcome indicated that fewer patients had chest syndrome and had reduced annual crisis rates. There were no adverse complications observed [26].

5.3. Gene-editing

Even though gene editing treatments are not extensively used in SCD patients, researchers continue to work on developing a suitable clinical treatment. Numerous gene-editing therapies are based on the premise that HbF inhibits SCD. Clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9, which enables targeting of insertions and deletions at a specific DNA site, was utilized in the previous two methodologies. One of the treatments is to reduce BCL11A expression in hematopoietic

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stem and progenitor cells (HSPCs), the cells from which blood cells are derived, including the erythroid-specific enhancer region of BCL11A. By employing this technique to patients, it was determined that their HbF levels have significantly increased. However, adverse events such as sepsis in the presence of neutropenia, cholelithiasis, and abdominal pain were also reported [3]. Hereditary persistence of fetal hemoglobin (HPFH) results in a high level of HbF expression throughout life. In this instance, CRISPR-Cas9 modifies the human blood progenitors to mutate the 13th nucleotide of HBG1/HBG2, which are two paralogous γ-globin genes. These mutations enhance adult γ-globin expression. As RBCs contain more HbF, they are able to inhibit the sickling process of SCD [27].

5.4. Agent treatment (GBT440)

New anti-sickling and anti-polymerization agent GBT440 (voxeloter) is undergoing clinical evaluation. The agent attempts to decrease blood viscosity by inhibiting HbS polymerization. In Dufu et al.'s experiment, RBC filterability was used to ascertain RBC deformability. The deoxygenated SCD blood did not move efficiently through the gel, whereas the deoxygenated SCD blood with GBT440 did. Under deoxygenated HbS conditions, the GBT440 agent was able to maintain RBC deformability. In addition, using a cone viscometer to evaluate the effects of GBT440 on the viscosity of deoxygenated SCD blood at various shear rates reveals that the viscosity decreases as the shear rate increases. Consequently, GBT440 is capable of reducing the hyperviscosity of deoxygenated SCD blood [28].

6. Conclusion

In this paper, the history of SCD and its treatment, the disease's pathology, and the theories behind several treatments are discussed. It is known that the molecular etiology of genetix is an amino acid point mutation at position 6. In addition, it is known that there are numerous potential treatments for this disease, such as increasing the amount of HbF, editing the mutated gene, or increasing the amount of oxygen in the human body. However, this paper's content is not the most comprehensive. Although the researchers has the concept of the pathology of SCD, correlations between complications and SCD still remains unknown. It is neccesary to obtain the physiological conditions of HbS, HbF, and HbA, including solubility, delay times, and so on. Moreover, in recent years, an increasing number of solutions have been developed, and the methods presented here are merely an introduction. Plausible areas that worth to be developed include BCL11A factor, lessen in blood viscosity, HbS polymerization inhibition, and RBC formability. In order to be implemented on humans, potential treatments must also undergo clinical testing after all the theory-based experiments. As a prevalent genetic disorder, SCD continues to affect a significant proportion of the population. To discover a solution to the current situation, additional research is necessary.

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