Image generated through Midjourney.

The promise of genome engineering in Africa

In 2012, whilst studying the basic mechanisms of how bacteria defend themselves against viruses (some blue-sky basic science), Emmanuelle Charpentier and Jennifer Doudna identified a method for genome editing. Less than a decade later in 2020, they were awarded the Nobel Prize in Chemistry for the development of this method – which the scientific community now refers to as CRISPR genome editing. This began a gene editing revolution that is still ramping up, and that Africa and South Africa are actively taking part in. But a lot more can and should be done on the continent.

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Genome editing tools were already in existence – and several have the ability to home in on a very specific DNA sequence (think of it like a GPS location system) and then edit that specific piece of DNA to make any change you want (Figure 1). The enormity of Charpentier and Doudna's discovery lay in the simplicity of implementing this tool in any lab that had

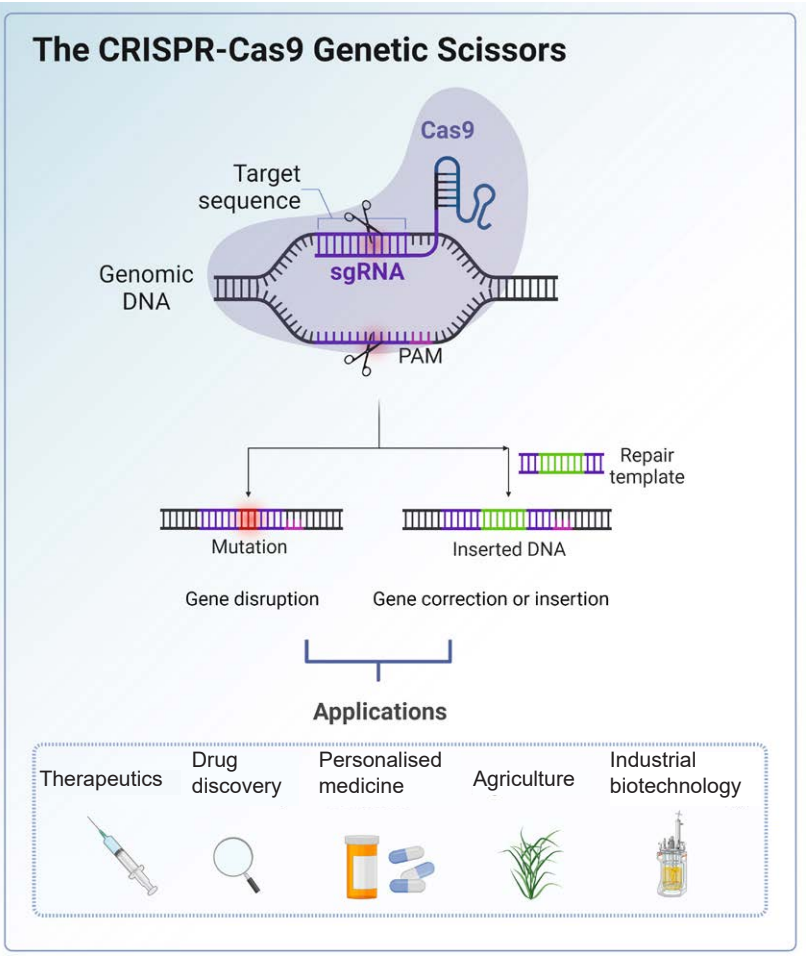

Figure 1: How CRISPR-Cas9 works and its application in bio-innovation

Cas9 is directed by guide RNAs (sgRNA) to a specific DNA sequence containing a recognition site (PAM) for Cas9 to cut the DNA sequence and create a break. This break is repaired by the cell, which may lead to mutations to disrupt the function of a gene, or it may use a similar DNA sequence (repair template) and insert this into the break site to correct gene function. (Created with BioRender.com).

robust molecular biology tools, opening a Pandora's box of scientific research questions to investigate and therapeutic avenues to pursue.

Disease-in-a-dish models

Say, for example, you want to understand the role of a unique African genetic variant and how it predisposes our population to disease, scientists can use CRISPR tools to create that variant in cells in a dish and investigate what this mutation does, from our immune responses to infectious disease and our predisposition to Parkinson's or our risk factors for cancer. Genome engineering has created highly advanced 'disease-in-a-dish' modelling, transforming the scientific landscape and opening avenues for understanding population group-based genetic predisposition.

Catapulting gene therapy

Whilst the Human Genome Project revolutionised scientific discovery in identifying disease-causing genes or specific mutations, until recently, traditional gene therapy strategies that sought to repair these mutations had to contend with issues of inefficiency (i.e. not enough cells in the body corrected, so only a small improvement in the disease) or unsafe gene correction (gene modifications in the wrong place). CRISPR genome engineering has thrust gene therapy back into the fast lane by ensuring that specific safe sites of the genome are corrected – minimising unwanted 'off-target' effects where incorrect changes to the genome are made by accident.

African relevance

The sub-Saharan ethnolinguistic population groups of Black African ancestry represent the greatest genetic diversity in the world as a consequence of representing the origin of mankind. Therefore, genetic variations (or disease-causing mutations) that are rare or absent in other population groups, such as those of European descent, are often common in African populations. Consequently, the use of genome engineering strategies to investigate or target specific genetic variants should not be based on population groups in the Global North. Such strategies are critically needed to apply African solutions to African disease burdens against an African genetic background. Unfortunately, only 2% of the

world's CRISPR gene editing papers to come from the entire African continent – serving as a stark reminder of how far behind the continental uptake of this incredible tool is (Figure 2).

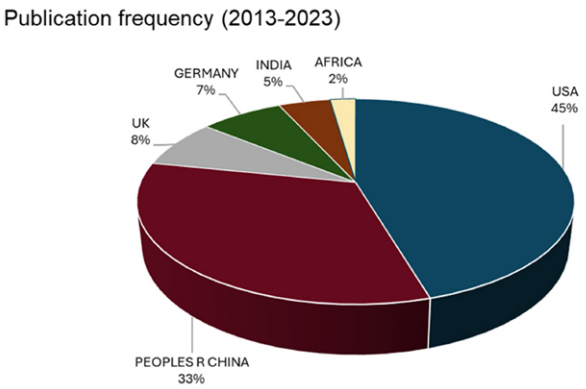

Figure 2: The distribution of CRISPR gene editing publications by the top five contributing countries compared to the African continent to this field in the years 2013–2023

What investigations (human health) do we apply genome engineering to in South Africa?

However, there are several groups promoting the use of CRISPR genome engineering on the African continent and especially here in South Africa. Dr Kristie Bloom and colleagues at the Antiviral Gene Therapy Research Unit at the University of the Witwatersrand, were one of the first research groups in the world to show the promise of genome engineering in combating an infectious disease – hepatitis B virus. Our group at the CSIR has been working with several collaborators using CRISPR genome engineering to modify African stem cell models to investigate immune responses to infection and mitochondrial disease within the context of an African genetic background to understand the role of unique African mutations in disease pathogenesis.

Heading: A call for African genetic representation

Perhaps one of the most exciting avenues where we will impact on the health of the African population is in targeting Sickle Cell Disease (SCD) which mostly affects the African population (ranging between 10% and 40%). In this disease, the β-globin gene has a mutation which leads to 'sickle' shaped red blood cells (Figure 3). Recently, the FDA approved the first CRISPR-based treatment, where a patient's bone marrow stem cells are harvested, CRISPR engineered to activate a backup γ-globin gene, and reintroduced into the patient via a stem cell transplant.

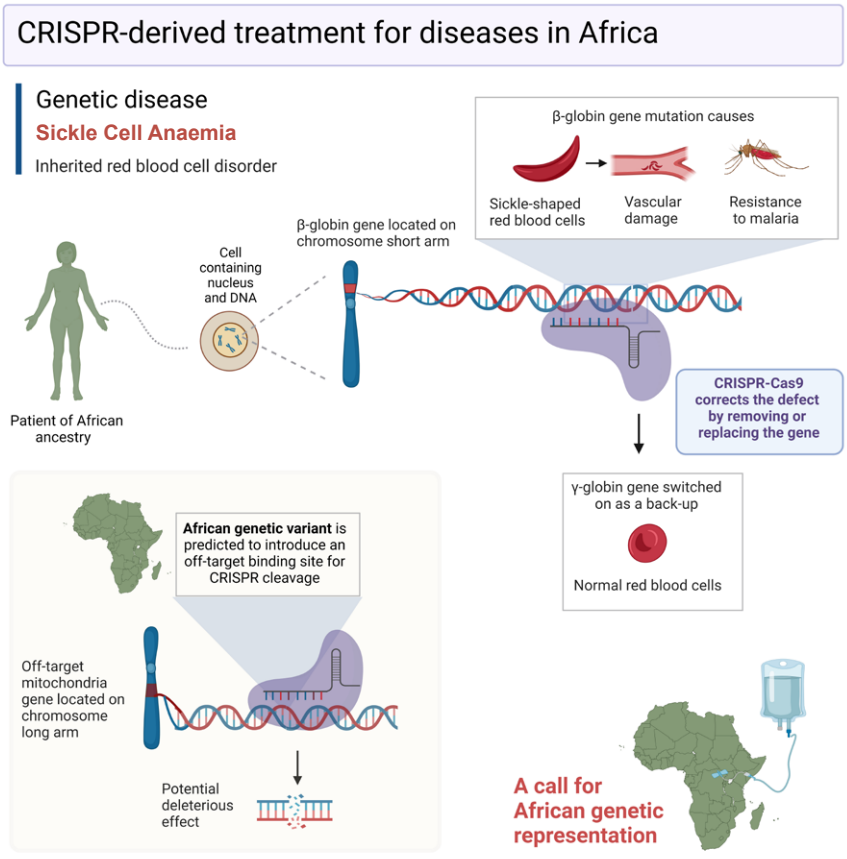

Figure 3: CRISPR-Cas9-based gene therapy for the treatment of sickle cell disease

CRISPR-Cas9 targets the defective β-globin gene in patients with the disease to activate the γ-globin gene, which restores normal red blood cells. However, a genetic variant in African individuals is a potential off-target site for the guide RNA, which directs CRISPR-Cas9 to a gene found in the mitochondria. (Created with BioRender.com).

Terminology

What is the genome? It is the instruction manual of an organism, and the letters are the nucleotide (A, C, G and T) sequences of DNA, which form words to represent the gene.

What is genome editing/engineering? DNA, which is present in the genome of living cells, such as bacteria and human cells, is changed by inserting, deleting, replacing or modifying its code.

What is CRISPR? Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats are DNA sequences found in bacteria and Archaea (prokaryotes that survive in extreme environments with different cell wall components from bacteria). Researchers have repurposed CRISPR as a gene editing tool.

What is Cas protein? CRISPR **AS**sociated protein is an enzyme (nuclease) whose purpose is to cut any nucleic acid such as DNA from viruses with the aim of building immune memory so that when it re-encounters the virus, Cas9 nuclease will destroy viral DNA.

What is single guide RNA? A short RNA sequence that works like GPS coordinates to direct the Cas nuclease to cut double-stranded DNA.

How does the Cas nuclease know where to cut? Besides the gRNA that directs it to the DNA sequence, the DNA sequence itself has a protospacer adjacent motif (PAM). Cas binds to the DNA sequence by recognising the PAM, the gRNA matches to the DNA sequence to unwind the double-strand DNA sequence and Cas9 cuts three nucleotide bases before the PAM.

What is an off-target? Other DNA sequences that have the PAM recognised by Cas and almost match the complete gRNA sequence, causing Cas to cut DNA at this unintended sequence.

What is a genetic variant? A change in the DNA sequence in each of our human genomes that may have arisen due to changes in the environment with the aim of improving the survival of a population. Some genetic variants are useless or neutral, while others can cause disease, such as sickle cell disease.

What is the haemoglobin gene? A protein containing iron in red blood cells that delivers oxygen to tissue. In adults, the haemoglobin gene codes for four protein subunits (two units of β-globin and two units of α-globin). In a newborn, the haemoglobin gene codes for four protein subunits (two units of γ-globin and two units of α-globin).

What is the therapy for sickle cell disease? To increase the amount of foetal haemoglobin, as it makes sickle cell disease milder and increases the life expectancy of patients.

This is game-changing for many of those patients for whom appropriately matched stem cell donors cannot be found. However, a recent study evaluating the rare but possible off-target effects of this CRISPR tool has suggested that one in 20 African patients harbours a genetic variant in an important mitochondrial gene which may be affected by this targeted treatment.

Summary

Whilst this article provides further evidence of the cautionary tale of Africa not taking up genome engineering tools, the impact this will have on so many patients should be applauded and acknowledged. The time is ripe for young African scientists to get involved in this exciting field.

VIDEO: How CRISPR works, explained in two minutes (Courtesy, STAT YouTube channel): https://www.youtube.com/watch?v=A5rUC6NiQfo

Article by Dr Shivani Goolab and Dr Janine Scholefield of the Bioengineering and Integrated Genomics Group at the Council for Scientific and Industrial Research (CSIR).

Ka 2020, Emmanuel Charpentier le Jennifer Doudna ba ile ba fiwa Sefoka sa Nobel sa tlhabollo ya go rulaganya genome ya CRISPR. Se se thomile phetogo ya go rulaganya dikarolwana tša leabela yeo Afrika le Afrika Borwa di tšeago karolo ka mafolofolo go yona.Eupša tše ntši tše ntši di ka dirwa ebile di swanetše go dirwa mo kontinenteng. Nako e butšwitšego gore boramahlale ba bafsa ba Afrika ba tsenele tšhemo ye e kgahlišago.

Translated into North Sotho by A/Prof. Walter Matli