Year 13 student Eleanor Page completed this research project independently. It is always wonderful to see such commitment and curiosity. Well done, Eleanor.

How can Genetic diseases be treated?

The Problem

Around 1% of the world population are affected by single-gene hereditary diseases alone. Some of the most common genetic diseases are dispositions like Down Syndrome (affecting 0.1% of the population), Thalassemia (0.043% of children are born with this annually), and Cystic Fibrosis(affecting 0.001% of the world's population). For centuries, diseases have been passed down from generation to generation, with no treatment or remedy in sight, yet now, scientific discoveries are allowing for the potential cures to these conditions to be trialled.(3)

These diseases impair the lives of the people afflicted greatly, and cannot be prevented by any normal means such as vaccine, or antibiotics. The patients are afflicted from birth, as the diseases are caused by mutations in the DNA of the parent, which is then passed down to the child either due to the disease being a dominant characteristic in the DNA or due to both parents being carriers of the disposition. As often the parents are unaware of carrying the disease, as they are not affected by it, and so it can be an unexpected occurrence in the child – these diseases can often cause miscarriages, stillbirths, or the child to die shortly after birth, which can be incredibly hard for the parents.(4)

Up until recently, these diseases have been inaccessible to medicine – as the source of the symptoms are part of the gene code, it could not be eliminated as a pathogen, and the body had nothing to recognise as a foreign entity and so fight off. For a long time, nothing could be done, but now at least there are methods of managing these diseases to ease the lives of those afflicted, but a working 'cure' has yet to be used generally.

The Solution

A relatively recent discovery has been made with a process called CRISPR gene editing – CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats(1). This is a process whereby the immune system of specific bacteria is utilised to 'cut' DNA. This is made up of two components – CRISPR-associated (Cas) nuclease, which is essentially the 'cutting tool', and a sequence of RNA (gRNA) that guides the nuclease to the desired location on the DNA strand. This was discovered while observing interactions between bacteria and bacteriophages, when it was found that as part of the bacteria's immune response, it would cut the DNA strands of the invading viruses, therefore disabling them. This discovery was made by Dr. Jennifer Doudna and Dr. Emmanuel Charpentier, and their paper detailing how the bacteria's immune responses could be used for gene editing was published in 2012, and they received a noble prize for their work in 2020. From the release of the paper, other scientists took over in making this a working tool – first, testing on eukaryotic cells and the discoveries of new variants of Cas, then work showing that it could be used on human cells, on to uses in other organisms. This first originated in 1993, when Dr. Fransisco Mojica identified repetitive palindromic repeats separated by sections of other genetic material in the genomes of bacteria, and named these sections CRISPR as parts of the bacterial immune system.

It was only in 2007 that this CRISPR system was actually tested for usefulness of any kind, and clinical trials began in 2019.(2)

The clinical trials were performed on patients with sickle cell disease – the cells were extracted and edited outside of the body, then infused back in – a process now known as gene therapy, restoring fetal haemoglobin, and eliminating the need for a functional copy of adult haemoglobin. After those trials were successful, a CRISPR treatment was injected directly into human patients in 2020, and this technique has since been used to treat hereditary blindness. The CRISPR systems in bacteria use the Cas nucleases, enzymes that can bind and create double stranded breaks in DNA sequences – when a virus invades the bacterium, it uses these enzymes to cut out a specific part of the virus' DNA – a protospacer – which is then stored in the bacterium's genome alongside the protospacers of any other viruses that had previously invade, creating an immune memory, so that if any of the same virus types attack again, the bacterium can use recognise it and disable it using Cas9. Cas9 is the specific 'cutting tool', and is directed by specific RNA guides. However, to use this, there must be a specific sequence in the DNA of the target organism, called a PAM, or protospacer adjacent motif – only when both the target site and the PAM is recognised, can the enzymes create the double stranded break in the DNA. This then disables the virus, as it has no DNA repair mechanisms of its own. In treatment, the gRNA combination required to guide the Cas9 enzyme has been modified so that only one guide is needed, simplifying the process greatly. After the cut has taken place, DNA repair pathways can be utilised - one of two processes can be used - Non-Homologous End-Joining (NHEJ), which can be used to knock out or disable a gene in the DNA sequence, and Homology-Directed Repair (HDR) which can be used to insert a gene or specific section of genetic material into the DNA strands.(2)

In 2021, a trial was conducted into a treatment for transthyretin amyloidosis, type of neurodegenerative disease and showed promising results, as well as developments into paediatric cancer treatments. Although still in the early stages of clinical trials, this has the potential to be used to treat certain types of cancer, and many different genetic diseases, and has already been utilised in the recent coronavirus pandemic to identify the virus. This can also be used to identify genetic and infectious diseases, and was recently used in combination with new technologies utilising transistors and graphene into a chip capable of identifying pathogenic single nucleotide polymers, which make up 50% of all disease causing mutations in humans.(2)

<u>Ethical disputes</u>

As the basis of this treatment is editing the genes of humans, it leads into the debate over the idea of 'designer babies' – that a technology like this could potentially be used in the future not only for curing disease, but eventually develop into eliminating traits seen as undesirable, or more cosmetic than medical alterations. That a technology like this which is seen as revolutionary in the medical field could evolve into something negative if used poorly.

It is used in stem cell technology, and is therefore included in the debate surrounding it – this is because stem cells, being able to differentiate into any cell needed is incredibly useful to the medical community, but the main source of them is from embryos, which is highly controversial. However, recent developments into CRISPR technology has allowed for the production of induced Pluripotent Stem Cells (iPSCs) to become far easier. iPSCs are adult cells (usually blood or skin cells) that have been essentially reprogrammed to be pluripotent (can differentiate into any type of cell). Before CRISPR, it was relatively difficult to reprogram and grow these cells, and CRISPR has proved to not only help in the engineering process of these cells, but also to be more efficient than previous gene editing processes. Therefore CRISPR has also been able to reduce the need to use embryonic

stem cells as well as treat genetic diseases – these iPSCs have been used to treat genetic diseases in trials like the sickle cell disease trial mentioned earlier. CRISPR has also recently been used to create hypoimmunogenic cells that can be used to treat any patient, which can be used in immunotherapy, a treatment for cancer.(2)

On balance, with the right monitoring and use of this technology, all ethical concerns can be managed, and its usefulness may still outweigh any further issues.

Economic impacts

As mentioned earlier, this technology makes the production of treatments like iPSCs far more efficient, and can be used to identify both genetic and infectious diseases in humans – this will save medical research facilities and companies substantial amounts of money, and this is still being worked on, with hopefully more developments to come, with the potential to cure many human diseases and save endangered species all around the globe.(2)

Alternative solutions

For conditions involving the formation of blood cells like sickle cell disease, bone marrow transplants are also an option – bone marrow is gathered from the healthy donor, and given over to the patient, allowing the formation of normal blood cells, and if performed early enough in life, can help prevent further complications and painful episodes, but this can only be used if the disease is spotted quickly enough. The donation could also be rejected by the patient's immune system, and they may have to be put on immunosuppressants until the body accepts the donation or for the rest of their lives - however, this can make the patient more susceptible to disease and infections.(4)

Another potential option is screening and blood test before birth – this does not address the problem, but avoids it, as it prevents a child being born with the disease. However, this has come under ethical questioning, as that child will not be born simply because they have the disease – this is a form of management, not treatment, but does reduce the number of patients with the disease. Screening can also be used later in life to check for conditions like cancer and late-onset versions of the diseases, so they can be spotted as quickly as possible and then provided with suitable medical care to make the condition easier for the patient – again, this is not a cure, but instead management.(4)

As these diseases are often part of a group of conditions called inborn errors of metabolism, the symptoms can often be prevented by a change in diet or replacement of the enzymes that are missing. These result from genetic changes that mean that certain enzymes are not produced, so certain materials cannot be broken down. An example of this is enzyme replacement therapy – the enzymes not produced naturally by the patients' body are either gathered from donors or made artificially and then introduced to the patient. A change in diet can be helpful, as it excludes the material that cannot be broken down as the patient is lacking the enzymes that would usually do this. (4)

Overall, this revolutionary CRISPR process is likely to improve the lives of thousands of people around the world and provide a potential cure to diseases previously considered untreatable. With the right application of this technology, many genetic diseases could be considered eradicated in the near future, and could provide a solution to ethical qualms surrounding other medical processes, clearing the way for more research and development in the general field. This means that genetic diseases may soon have the potential to be truly treated instead of avoided, and has given procedures like tissue and blood donations a new potential. CRISPR technology also has the

potential to improve recent technologies like immunotherapy and STEM cell use. This process is on track to save medical research providers both time and money in the long run, and if used correctly, can be a positive influence on the world.

References

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Source Evaluation

My first source is the article *Recent Advances in the CRISPR Genome Editing Tool Set* (source 1) - this is from the Experimental and Molecular Medicine Journal – its purpose is to provide high-quality scholarly information, the collaborative authors are well-respected experts in this subject, and they have given evidence of all the sources they have used, and it was released within the last five years. Any statistics or information used is backed up with scientific evidence and sources, and the article has gone through a review process, and the authors have declared no conflict of interest. It may not be completely up-to-date, as this was published in 2019, but the information it has is backed up and evidenced, with no bias or conflict of interest and clearly accredited authors. Overall, this is a very reliable source with relevant information.

My second source is the webpage *The Ultimate Guide To CRISPR: Mechanism, Applications, Methods & More* (source 2) – this is from the company Synthego, a genome engineering platform – this means that they will have some bias, as they are trying to sell you the idea that CRISPR is amazing and will change the world, as that is what their company specialises in. However, their article has been created extremely recently, although no sources or authors are listed on the page. They have used scientific evidence to back up most of their claims, and most of the article is factual information instead of opinion. The information is relevant and recent, although it ahs a bias and no author or sources are listed – overall, this is a relatively reliable source.