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The Contribution of Genetic Testing to The Early Detection and Prevention of Ischemic Stroke

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ABSTRACT

Cerebrovascular accident, more commonly known as stroke, is defined by the National Health Institute as an incident that occurs when blood flow to the brain is blocked. The main symptom of stroke is a sudden numbness in the face, arm, or leg, especially on one side of the body. Other common symptoms include sudden confusion, trouble seeing, or dizziness. Depending on how the blood flow to the brain is limited, there are two main classifications of stroke: hemorrhagic and ischemic. Ischemic stroke, which makes up roughly 80% of all cases, occurs when blood flow to the brain is abruptly interrupted, resulting in a sudden loss of function. A study showed that heritability accounted for around 37.9% of all cases of ischemic stroke. Hence, the more urgent need to be able to detect, prevent and cure ischemic stroke, while adhering to strict moral and ethical principles. This literature review will explore how genetic testing and engineering can be used to detect, prevent and cure ischemic stroke. Through the analysis of genetic mutations linked to ischemic stroke, genetic engineering can identify the condition early and create personalised prevention approaches. Thus, genetic testing holds the potential to enhance the well-being of individuals who are vulnerable to or impacted by ischemic stroke.

Introduction

Stroke is defined by the American Stroke Association as a disease that shows evidence of cell death in the brain, spinal cord, or the eye with or without the presence of clinical symptoms [1]. Stroke is often considered amongst the top 5 leading causes of death [2], affecting 15 million people worldwide, as of 2023. Of this group of people, 5 million cases often result in death, and another 5 million are permanently disabled due to the effects of stroke. Although stroke has been researched to be more prevalent in people over 40 years old, it is not uncommon for children with predeposited sickle cell anaemia to also suffer from stroke [3].

Stroke is a disease whose risk increases with age. Other such factors that affect the distribution of cases of stroke include gender, with women having a lower self-adjusted stroke incidence than men. Apart from uncontrollable factors, such as genes, to reduce the risk of stroke it is often advised by doctors to stay away from frequently doing activities that can increase blood pressure or increase the risk of cholesterol build up. Amongst the many factors to pertain to, the most important ones are arguably keeping a healthy weight, staying away from smoking, and limiting the intake of alcohol [4].

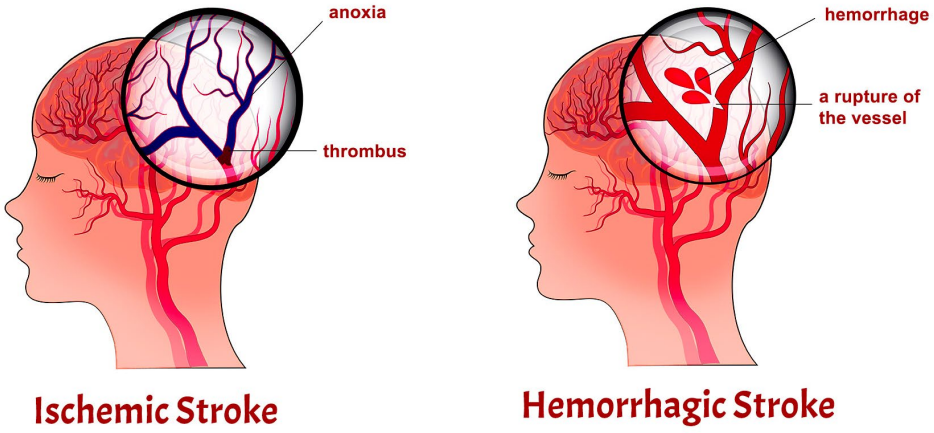


Figure 1. A diagram highlighting the two main types of stroke, and how each stroke occurs in the human body [5]

As highlighted in Figure 1, stroke can be categorised into two broad subgroups: ischemic and hemorrhagic stroke. Ischemic stroke occurs when there is a blockage of a vessel, usually with cholesterol. Hemorrhagic stroke on the other hand, occurs where there is a rupture in the blood vessel.

Although many precautions can be taken to minimise the risk of experiencing stroke, some factors, like gene mutations, are something that can not be controlled at will. Through genome testing, many single nucleotide polymorphisms (a genetic variant in one base position in a typical genome sequence) have been discovered that can lead to stroke. Knowing such mutations can allow the editing of the gene to prevent the mutation from occurring. In the case that the gene does mutate, it can be promptly mended, to prevent the risk of developing ischemic stroke.

As shown in Figure 2 below, this literature review will focus on how known mutations can be identified, and how early detection of possible genetic variations is able to minimise the number of patients that have to suffer major consequences of stroke, by genetic mutation of the gene.

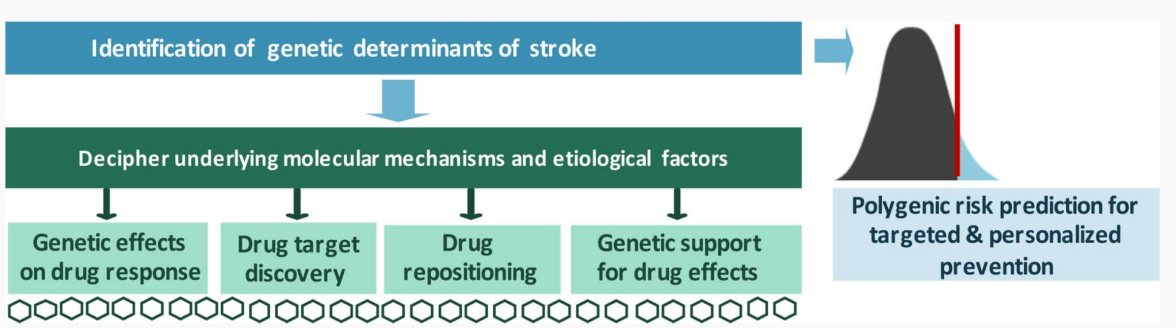


Figure 2. Outline of the 3 steps that leads from identification to the prevention of ischemic stroke in patients [6]

Detection of Ischemic Stroke Risk Through Genetic Testing

There are two methods of gene testing that are often used to test people; namely: molecular testing and cytogenetic testing [7]. For the purpose of testing and detecting whether a person has a higher risk of getting ischemic

stroke, this literature review will discuss how different types of molecular testing, a method focusing on individual bases to detect abnormalities in the DNA sequence, can be used.

The first method that will be discussed is Whole Genome Sequencing (WGS), a process that determines the order of nucleotide bases in the genome of an organism [8]. Being able to analyse entire genomes, WGS is one of the most comprehensive methods to identify known and new types of mutations. Unlike targeted methods of genome sequencing, WGS captures both small and large variants in the genome [9]. Such examples range from detecting single nucleotide deletions to detecting structural variants of a DNA.

As illustrated by Figure 3 below, Whole Genome Sequencing is primarily conducted in a four step process. The first step is known as DNA shearing, which is the unwinding and cutting of a DNA molecule to pieces small enough to be processed and read by the sequencing machine. Known as DNA barcoding, the second step involves adding tags to identify where each cut of DNA belongs in a sequence of bases. DNA sequencing uses a machine to identify the 4 bases that make up each sheared DNA fragment. This allows for scientists to conduct final data analysis to compare the copied sequence to the template genome, and detect variations that can possibly indicate a disease causing mutation[8].

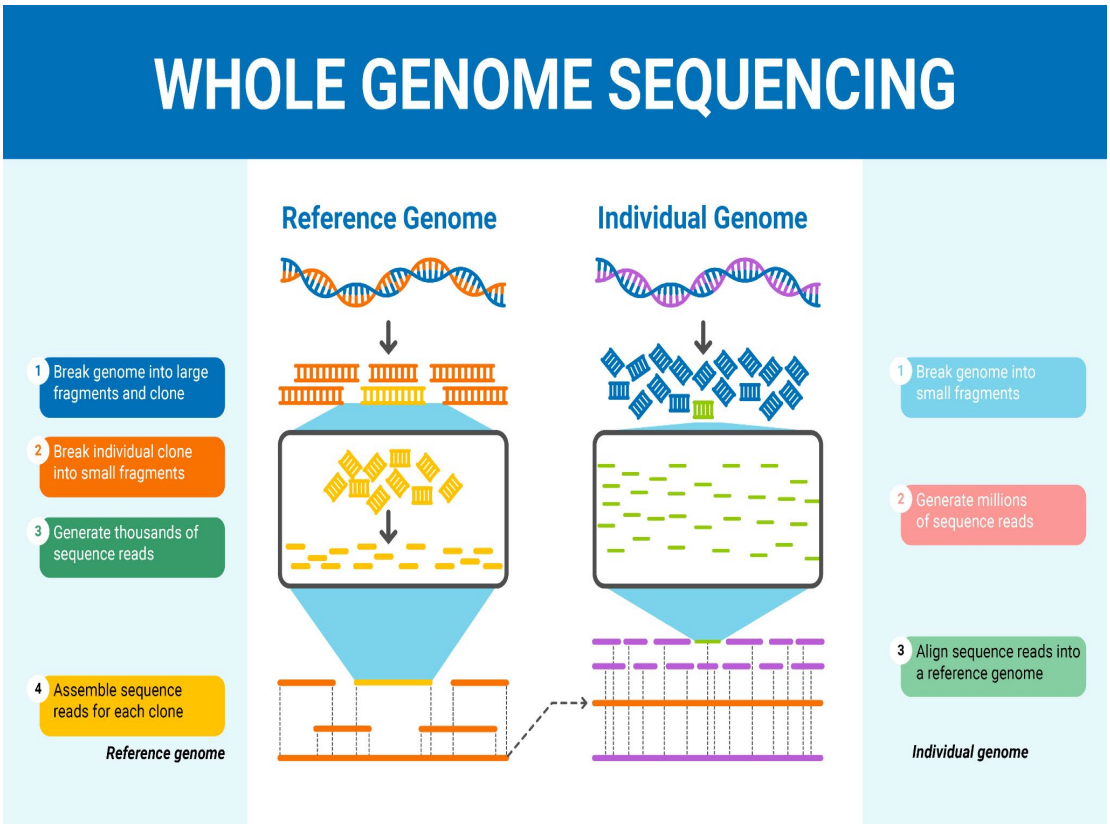
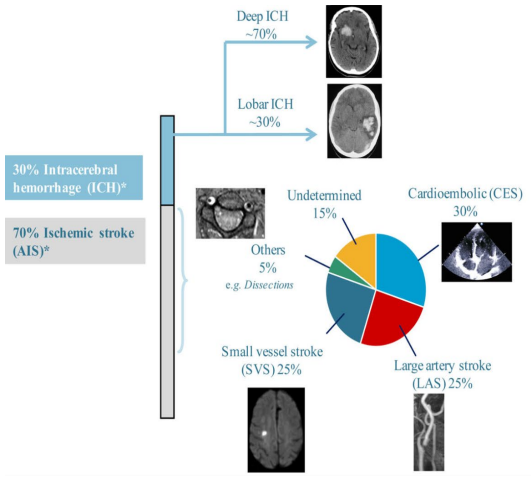


Figure 3. Process of Whole Genome Sequencing outlined in a diagram

The data collected from WGS experiments and studies all over the world are collected in a centralised database. One of the most prominent experimental methods is called the Genome Wide Association Studies (GWAS), which aims to identify mutations that are related to ischemic stroke. This method studies the entire genome, looking for small mutations, often referred to as "single nucleotide polymorphism" (SNPs). Using the collected data, scientists and researchers are able to identify SNPs that are more frequently associated with a certain disease, and hopefully treat the patient before they are affected by the disease. GWAS is especially useful in studying complex diseases in which multiple genetic mutations can contribute to a person's risk of

developing the disease, and works by testing for differences in the allele frequency of genetic variants between individuals with similar personal backgrounds [10]. As illustrated in Figure 4, GWAS is able to identify a wide number of genes and the possible effects that each of them may have to influence ischemic stroke.



Examples of genes with evidence for causal implication

Gene	Stroke type	GWAS	Mono-genic stroke	Experi-mental data	Likely mechanism
COL4A1/2	AS, SVS, ICH	●	●	●	impaired collagen deposition in vessel walls
PITX2	AIS, CES	●		●	cardiac development, electrical and structural remodelling
PHACTR1	CeAD	●		●	unknown
HTRA1	SVS	●	●	●	dysregulation of TGF-beta signaling by HTRA1 LOF
FOXF2	AS, AIS, SVS	●	⦸	●	differentiation defect vascular mural cells in brain
HDAC9	LAS	●		●	regulator of atherosclerotic plaque stability

Figure 4. Chart showing genes that have found to be commonly associated with the development of stroke

There are multiple polymorphisms of different genes and sections of those genes that have been identified to have a large influence on the diagnosis of ischemic stroke. A common example of a gene that can increase the risk is the PITX2 gene, which is a common factor for cardiac development, electrical and structural remodelling. On the PITX2 gene, there are many possible mutations that can lead to ischemic stroke [11]. The SNP variant rs6817105 within the chromosome 4q25 has been associated with an increased risk in patients of over 65 years of age, whilst SNPs on rs 6817105, 131443308 and 6843082 have all been shown to increase the risk of ischemic stroke in men [12].

The second molecular testing method is the “Fluorescence in situ hybridization”, more commonly known as FISH. Although scientists commonly regard it as a cytogenetic method of gene testing, the FISH method traces and detects the location of a specific DNA on a chromosome, making it more similar to a molecular method [13].

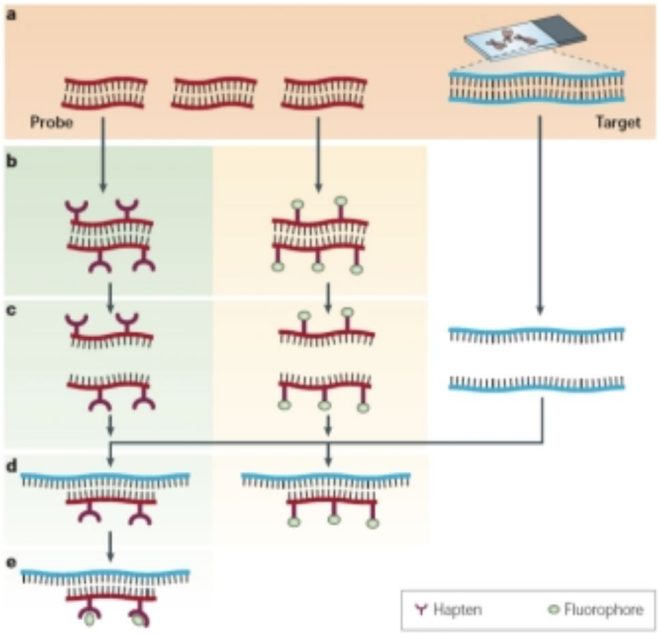


Figure 5. A step by step diagram showing the preparation process of FISH to detect changes in the DNA sequence [14]

The FISH method uses a fluorescent probe to target specific locations of chromosomes, by binding to specific parts of those chromosomes. For FISH to work, however, an identical copy of the probe sequence must be made. Hence why the process must be conducted *in vitro*. As shown in Figure 5, following the making of an identical, fluorescent probe sequence, it first needs to be denatured, to ensure that it can form new hydrogen bonds with the original template chromosome. Changes in the fluorescent probe sequences will result in coloured signals, which can be detected using a light or fluorescent microscope [14].

In situ methods such as FISH are important in the clinical identification and diagnosis of chromosomal disorders that may lead to possible illnesses. Chromosomal disorders that FISH can detect include deletions, duplications and translocations.

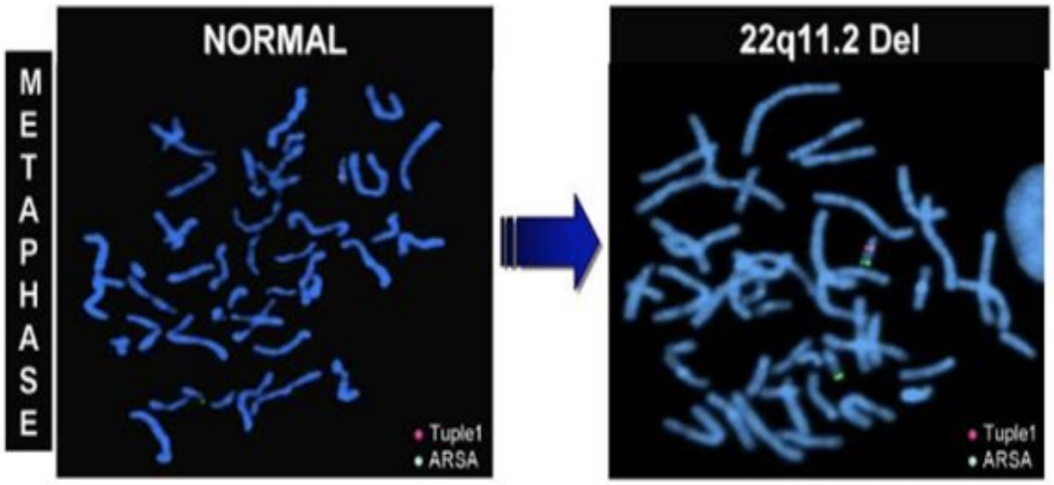


Figure 6. An example of FISH being used to detect a deletion in chromosome 22

In Figure 6, we can see that red is used to identify Tuple 1, and green is used to identify ARSA - a type of protein, to analyse a deletion in chromosome 22. The hybridization probes corresponded to 3 individual separate segments of this chromosome, where deletion is suspected to take place. Following the attachment, the result illuminates certain parts of the chromosome have been deleted.

The FISH method can be used for the presence of polymorphisms in the angiotensin converting enzyme (ACE) [15]. The ACE gene is located on chromosome 17q23.3 [15] and is associated with the development of hypertension, atherosclerosis and other cardiovascular diseases. A deletion/insertion polymorphism of a 287-bp fragment of intron 16 has been identified as a genetic factor that can lead to the development of ischemic stroke. FISH can be used to detect the initial deletion of part of chromosome 17, and diagnose the patient as being prone to developing ischemic stroke [15].

The last method to test genetics is the Polymerase Chain Reaction, or more commonly known as PCR. Being one of the most widely used techniques to genetically test an individual, the PCR method is able to produce billions of copies of an individual's DNA, enabling scientists to either use the PCR method in conjunction with another form of genetic testing, or only using PCR to search for a specific gene in a sequence of DNA.

The initial stage of producing a countless number of copies can be broken down into 3 main steps: denaturation, annealing, and extension, as outlined in Figure 7. To denature DNA molecules, the reaction temperature is increased to 95℃, which breaks the hydrogen bonds between the base pairs, resulting in two separate single strands. Following this step, to allow forward and reverse primers to bind to the antisense and sense strands, respectively, the temperature of the reaction is decreased to 5℃. In this step, DNA polymerase is also attached to the individual strands, which allows the final step, extension, to occur. In order to optimise the extension of the primers, the temperature is raised to 72℃. The cooling and heating of the reaction allows the optimum conditions to be maintained individually, without any concern that it will affect other stages in the process [16].

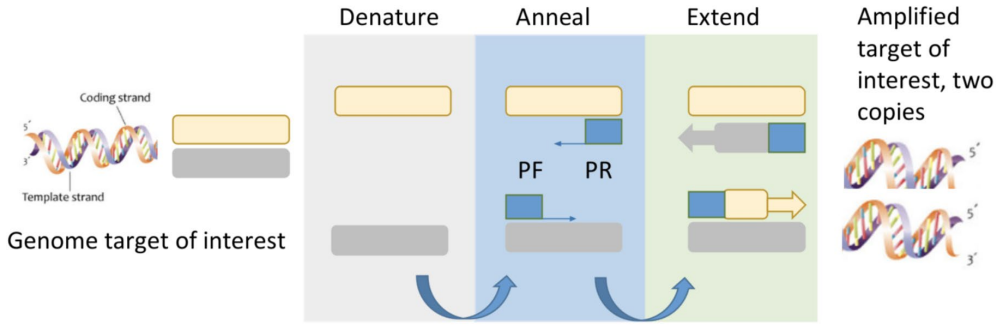


Figure 7. A diagram representation of the multiplication of genes using PCR

The first way in which PCR can be used to directly test the genes of a patient, and look for a specific pattern, codon, or gene abnormality within the genome. However, in order to detect a gene mutation in the gene sequence, the mutation must already be known, otherwise, it's impossible to determine whether the patient is at risk of a future disease or not. PCR allows researchers to examine the genes of a person. For example, due to the COVID-19 pandemic, the science world was met with leaps and bounds in the development of the PCR testing method, to make sure it is an affordable and accessible method to diagnose a patient.

The second way is to use PCR in association with another type of gene sequencing model, such as whole genome sequencing. PCR is an extremely useful technique to mass produce identical copies of a gene, which would otherwise be almost impossible to extract from a patient. The mass production enables multiple

clinical trials to be run simultaneously to detect any abnormalities, and ensure that the results produced in each trial are concordant with each other.

Disorder	Gene	Inheritance	Stroke Mechanism	Clinical Manifestation	Diagnostic Test
CADASIL	*NOTCH3*	Autosomal dominant	SVD	Migraine with aura, recurrent strokes	Molecular genetic tests, skin biopsy
CARASIL	*HTRA1*	Autosomal recessive	SVD	Recurrent strokes, vascular dementia, severe back pain, premature alopecia	Molecular genetic tests
Fabry's disease	*GAL*	X-linked	Large-artery disease, SVD	Neuropathic, abdominal pain, angiokeratoma, renal and cardiac failure,	Molecular genetic tests, α galactosidase activity
MELAS	mtDNA	Maternal	Complex (microvascular and neuronal factors)	Seizures, headache, ataxia, hearing loss, muscle weakness	Muscle biopsy, mutational analysis of mtDNA
RVCL	*TREX1*	Autosomal dominant	SVD	Visual loss, migraines, cognitive impairment, strokes	Molecular genetic tests

Figure 8. A table outlining monogenic disorders that include stroke as a potential clinical manifestation

The PCR method can be used to closely examine NOTCH3 (located in chromosome 19), which is a gene that is commonly thought to be associated with CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy). Clinical testing has yielded that the NOTCH3 gene has a 95% certainty that the specific gene has links to causing ischemic stroke. To be more precise, scientists have discovered that SNP ID's, rs3815188 and rs 1043994, on the NOTCH3 gene are significantly associated with lacunar stroke, one of 5 types of ischemic stroke. Other research has shown that presence of a NOTCH3 variant in a patient's gene can yield a two to threefold chance of experiencing stroke. Other genes discovered to be associated with the development of ischemic stroke, as outlined by Figure 8, have varying effects on different groups of patients, depending on nationality, age and gender [17].

To detect an abnormality in the NOTCH3 gene, PCR can be used to first mass produce the molecular section. After the leading and lagging strands are split, primers can be attached, to look for a SNP in known areas of NOTCH3 that is associated with stroke, such as rs 3815188 or rs 1043994. If no abnormalities are yielded, the patient would not be at risk of ischemic stroke.

The three methods of molecular testing that were outlined in this literature review are only a few options to many other ways that mutations in a patient's gene can be detected. Although many more options exist, WGS, FISH and PCR were chosen to be discussed to highlight their benefits, such as how each is able to detect mutations to varying levels of detail. Not only this, but the three methods outlined in this literature review have been proven to be useful and widely used in the detection of gene mutations that can lead to stroke. Recent improvements in the capabilities of conducting genetic testing has been pivotal in ensuring that common pathogens or mutations can be found more readily. The early detection of gene variations can play a pivotal role in preventing the illness before it even happens, minimising the number of annual patients that have to risk death, or permanent disabilities.

Prevention of Ischemic Stroke Through Gene Editing

Once the predetermined gene that could greatly influence the suffering from stroke has been found, gene editing methods can be used to alter the gene, or mutation from happening. Gene editing is a type of genetic engineering where specific DNA can be intentionally modified in living cells.

Figure 9. An image of a simple CRISPR/cas9 machine[18]

In the scientific community, the most common method of gene editing today is the CRISPR/cas9 method. This method of gene editing uses a machine, illustrated in Figure 9, to cut out a specific gene out of the entire sequence. The machine has two main components to it that work in conjunction with each other to be able to cut out a subsection of the gene to edit it. The first part of the machine is the homing mechanical part, which is often referred to as the CRISPR part of the machine. The second part of the machine is the cas9 enzyme, which is used in this machine as a form of molecular scissor to target a section of the DNA [20].

The process of cutting out a specific gene out of the DNA sequence follows 3 main steps, namely: recognition, cleavage and repair. The recognition step of the process involves using RNA to read genetic material on the DNA of an organism. The RNA molecule is used to locate a specific location in the gene where editing needs to take place, and then leads the cas9 enzyme to this specific location that has to be edited. Following the recognition of the location in the nucleus, the cas9 enzyme will make a cut in the specific location in the DNA. This cut discontinues the long chain DNA molecule, resulting in a short break. The natural response of the cell is to repair this break, after it detects the issue. Natural reparation of the cut in the DNA will be done by glueing the two cut ends of the DNA chain, which usually is able to disable the entire gene. [20]

However, scientists that made the cut in the DNA may choose to insert or change a single nucleotide or chemical within the genome, depending on the aim of the investigation. For example, following a cut made by CRISPR, scientists may choose to insert a fluorescent probe to tag the DNA through the FISH method. Following the insertion, scientists may be able to detect a deletion polymorphism of a 287-bp fragment of intron 16. Once the gene variation has been detected, scientists are able to conduct CRISPR again to cut another location, or insert another nucleotide to prevent the deletion from happening.

Discussion into Moral and Ethical Concerns

Genetic testing, a technique that is commonly practised on humans, incurs massive controversial debates within the STEM community. Some scientists argue that the widespread use of genetic testing can benefit mankind, whilst others argue that it provides more disadvantages than advantages.

The first general question raised by the scientific community is whether or not genetic testing should be practised on embryos and/or small children. According to the Office of the High Commissioner for Human Rights (OHCHR), every child and embryo has the rights to human dignity, which can be summarised to the right of reverence, respect, and protection towards each person, as a free being with a unique history. Testing the genome of an embryo would infringe on this foetal right that embryos are supposedly meant to have, as the process may intrude on personal information. In addition, every child has the right to autonomy and self-determination, meaning that everyone is entitled to make decisions that concern their body for themselves.

Not just this, but there are many medical cases where the opinions of children are rendered useless. The most prominent example is vaccination. Every year, many newborns around the world are exposed to a series of vaccinations. Arguably, vaccinations benefit children, as it provides the basic foundation of their immune system to develop. However, just like gene therapy, newborn vaccination is done against the will and decision of the child, yet people still do it, because of the benefits that closely follow suit. It seems arbitrary to draw the line at gene therapy, when it can also provide countless benefits to an individual, and wider society.

Figure 10. A chart showing a change in eye colour from before, and one year after gene therapy was conducted [21]

As shown in Figure 10, modern technology has rapidly accelerated to the point where the most minute details, such as eye or hair colour can be changed at will. However, since it breaches the rights of foetuses and humans, it is generally perceived to be morally unethical to alter an insignificant feature such as hair or eye colour. If no decision was made by the patient undergoing a transformation to switch eye or hair colour, there are no justifiable reasons that the change should be made. However, in the case of a life threatening condition, such as ischemic stroke, the majority of patients would gladly accept and undergo treatment of their disease.

A second area of contention is whether certain people would be disadvantaged from the widespread use of gene therapy. The first ever case of gene therapy on a human being was recorded to be on September 14, 1990, by French Anderson [22]. Evidently, gene therapy is still in its very early stages of development, with few cases of successful clinical trials. Gene therapy is thus extremely costly, and can cost anywhere between $1 million and $2 million per trial [23].

Very few individuals are able to afford this high cost of treatment, resulting in some saying that gene therapy should not be used, as it favours the rich over the poor. An equal opportunity is not given to everyone, and thus poses the issue of gene therapy not being ethical. Arguably, the rich would have a better standard of living, as they are the ones that are able to afford gene therapy and try to alter certain characteristics for the better.

However, one could also pose the counterargument that all medicine is like that. According to the WHO, of the 8 billion people on the planet, 2 billion still don't have access to fundamental [20] access to healthcare; the market for any type of medicine already favours the rich over the poor.

Lastly, there is the issue of gene discrimination. Gene therapy can be used to produce an extensive information sheet that delves into a patient's personal medical background, as well as potential risks. In the event that the data is leaked, there could be an abuse of data. For example, an insurance company may charge clients that have a higher risk of ischemic stroke more than they would charge a client with a lower risk. Ultimately, this will result in more disparities within the healthcare system.

Not only this, but if gene therapy becomes more widespread, employers and large corporations may ask to see the medical background of a candidate. Undoubtedly, those with a genetic background more closely associated with an illness that can prove to be fatal, or result in a disability will not be as highly valued as those that can work for a long time and stay healthy.

The debate around whether or no gene therapy should be conducted revolves around many central ideas that involve the invasion of human rights, issues of privacy and putting certain groups of people at more of a disadvantage in society than others. Although such issues are posed, the first thing that has to be considered is the potential number of people that can be saved by the widespread commercialization of genetic testing and editing.

Conclusion

The implications of stroke are getting more widespread, simultaneously killing an ever growing number of people. Stroke is a disease that is not only caused by human lifestyle, but can also be inherited as part of a genetic disorder. Often, it is hard to detect whether or not a patient is prone to developing this disease because there are usually multiple genes that can affect a person, as already investigated within this paper. Although there are ways to predict the possibility of developing ischemic stroke, the most promising technique still remains to be cytogenetic testing. Some of the tests that can be done include:

1. Whole Genome Sequencing, which allows the entire genome of a patient to be analysed and checked for any SNP within multiple chromosomes that was previously recorded to have resulted in ischemic stroke in other patients. For this technique to work, it does not require prior knowledge of specific mutations, as the goal is to try to find new mutations that can lead to ischemic stroke. The most common type of WGS is the GWAS method, which can be used to detect abnormalities in specific genotypes.
2. Fluorescent in Situ Hybridisation (FISH), which detects deletions within chromosomes and marks with bioluminescent probes that can be identified using an electron or fluorescent microscope. Since this technique focuses on one specific area of a gene, it often requires prior knowledge of a gene variation that can lead to the development of ischemic stroke.
3. PCR method, which can make millions of copies of copies of a patient's database to analyse in conjunction with other gene testing methods, or have them compared to detect any mutations.

Regardless of the method, gene testing often involves invading the patient's privacy, raising moral and ethical concerns on whether it should be practised or not. Questions such as exploitation of data, breach of human rights and disparities to access plague the medical world, as scientists often indulge themselves into

heated debate. However, what is often overlooked is the fact that much of the medical world is already unequal, with injustice clearly visible in many aspects of it. As such, even if it initially benefits a small population of people, if genetic testing is able to save the life of someone with a known terminal illness, it seems more ethical to save them from it, rather than have to make them suffer through it.

It is hoped that further research into the genes and epidemiology of stroke is able to ensure that less and less people experience stroke due to genetics. Research is still being done on how to effectively alter the genes of patients to allow them to be rid of genetic disorders.

Acknowledgments

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