DRUGS & BEYOND

Utrecht University

Honours Programme HPPS Pharmaceutical Sciences

ISSUE 5
06/2021

GENE EDITING

CONTENTS

LETTER TO THE COMMUNITY

MIRTHE

Dear HPPS Community,

Welcome to the fifth edition of Drugs and Beyond! For this issue we have delved into the world of genetic editing. We will start this issue with an introduction on genes and their history, followed by more information about different gene editing techniques, both existing ones and new techniques. We will also shed some light on ethical considerations and current legislation with respect to genetic editing since this is a rather complex ethical issue.

As usual, we will also discuss some interesting internships that are being carried out by members of the HPPS community, related to the subject of this edition. To end it all in a fun way, we have added a little quiz at the end for this edition, so keep that in mind while reading!

We hope that you will enjoy this issue, and that you will join us for the next issue!

The Drugs and Beyond team:

Job, Jaap, Jenny, Jamie, Jacqueline, Carlon, Mirthe, Stephanie

Introduction Gene and History

JENNY, STEPHANIE

The gene is a basic physical unit of inheritance. Genes are passed from parents to children, and decide which features and traits are inherited from ancestors. Genes are arranged on chromosomes which consist of a long string of DNA (deoxyribonucleic acid). There are about 20.000 different genes in the human body. [1]

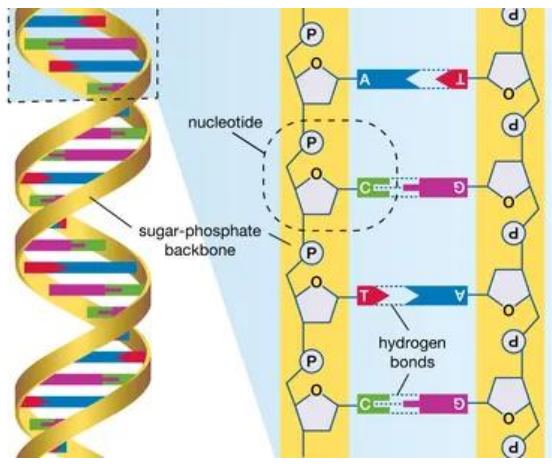
In 1857, an experiment with grain plants was done by Gregor Mendel that led to an increased interest in the study of genetics. Growing thousands of pea plants for 8 years, all these experiments led to the first belief about genetics, they exist! He died in 1884, but until today his experiments still form the basis for genetic research. Mendel was the first person to distinguish dominant and recessive traits. He also formed a basic idea of heterozygote and homozygote as well as the difference between genotype and phenotype. [2]

Nuclei were discovered in 1869 by Frierdich Miescher. He was able to isolate a pure sample of DNA from the sperm of a salmon. Chromosomes were observed for the first time in 1848 by Wilhelm Hofmeister during a cell division experiment. Wilhelm Roux guessed in 1883 that that chromosomes are the carriers of inheritance. In 1910 Thomas Hunt Morgan showed that genes reside on specific chromosomes. With this knowledge he and his students made the first genetic map for a fruit fly. [2]

The two sequencing technologies that were classified as the first-generation sequencing technologies are from Sanger and Maxam-Gilbert, who started DNA-sequencing with a publication of their techniques in 1977. Sanger's sequencing technique is known as the dideoxy-nucleotide method. One strand of double stranded DNA is used as a template to be sequenced. This double stranded DNA is made using chemically modified nucleotides called dideoxy-nucleotides and they are marked for each DNA base. The dideoxy-nucleotides are used for elongation of nucleotides. After this process of the elongation, the obtained DNA fragments are ended and separated by their size. This sequencing method was widely used for three decades and is still used today for single or low-throughput DNA sequencing.

The technique of Maxam-Gilbert is known as the chemical degradation method. The technique relies on the cleaving of nucleotides by chemicals and is most effective with small nucleotide polymers. The reaction leads to a series of marked fragments that can be separated according to size. The Sangers method favors the Maxam-Gilbert method because the last one is considered dangerous as it uses toxic and radioactive chemicals. [3]

In 2005 a new generation of sequencing technologies was invented. The basic characteristics of the second-generation sequencing methods are: millions of short reads in parallel, the speed up of sequencing, the low cost of sequencing and the direct detection of the sequencing output. Sequencing by ligation and sequencing by synthesis are both approaches of short read sequencing. A few examples of second-generation sequencing methods are Roche/454 sequencing, ion torrent sequencing, illumina/solexa sequencing and ABI/SOLiD sequencing. [3]



https://kids.britannica.com/students/article/DNA/398123

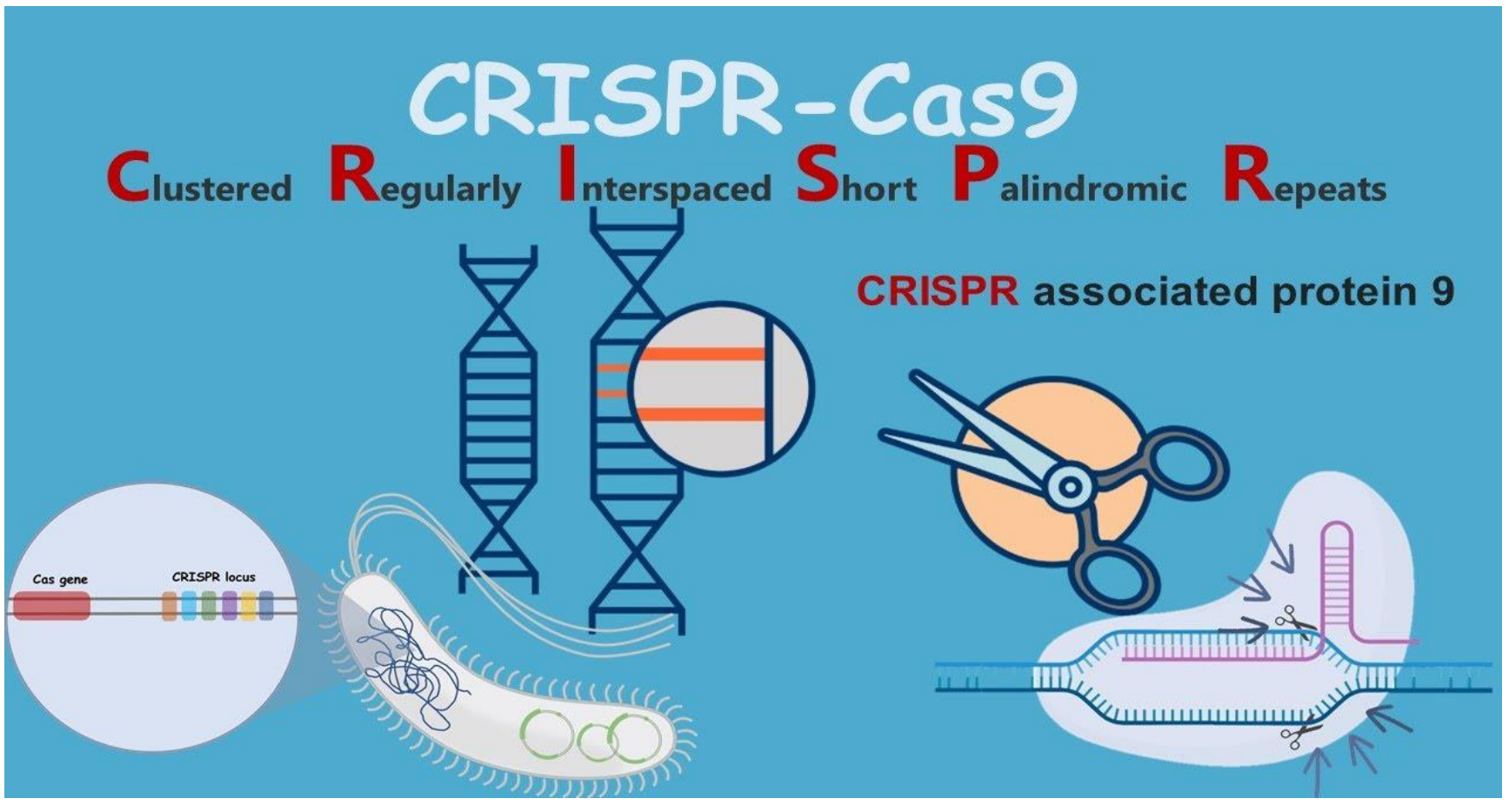
Another new generation of DNA sequencing was developed because second generation sequencing technologies generally require PCR (Polymerase Chain Reaction) amplification steps, which are expensive and take a lot of time and genomes are overly complex with many repetitive areas that the second-generation sequencing technologies are not able to solve.

The third-generation sequencing technology generates low sequencing cost and easy sample preparation. Next to this, they are able to produce long reads exceeding several kilobases for the resolution of the assembly problem and repetitive region of complex genomes. The two main third generation techniques are single molecule real time sequencing and the synthetic approach that rely on existing short reads technologies. The first of these two are the most widely used. [3]

1. Gene [Internet]. [cited 2021 Feb 7]. Available from: https://www.genome.gov/genetics-glossary/Gene

2. Gene History [Internet]. [cited 2021 Feb 7]. Available from: https://www.news-medical.net/life-sciences/Gene-History.aspx

3. Kchouk M, Gibrat JF, Elloumi M. Generations of sequencing technologies: from first to next generation. Biol Med (Aligarh). 2017;09(03).



CRISPR-Cas 9

JAAP

Gene editing is a group of technologies that have made it possible to add, change or delete genetic material. One of the most important new developments in gene editing is clustered regulatory interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein 9 (Cas9). The CRISPR/Cas9 technique has become very popular since it has shown to be faster, cheaper, more accurate and more efficient than previous techniques.

Discovery of CRISPR/Cas

In 1987 a research team in Japan described a series of short, direct repeats interspaced with short sequences in the genome of Escherichia Coli. These were later described with the term CRISPR and observed in a variety of bacteria and archaea. In 2007, the first proof was found that CRISPR-Cas is an adaptive defense mechanism in Streptococcus Thermophilus that uses antisense RNAs. Later, CRISPR-Cas also showed DNA targeting activity in Staphylococcus Epidermidis.

Functional CRISPR/Cas loci consist of identical repeats with invader DNA-targeting spacers that encode the mature CRISPR RNA (crRNA) and an operon of Cas genes. This adaptive immunity pathway of CRISPR is divided into three parts. Firstly, a short sequence of DNA of the invader is inserted as a spacer. Secondly, individual crRNA with a repeat part and an invader-targeting spacer portion is maturated from pre-crRNA after transcription. Lastly, Cas proteins cleave foreign nucleic acid at sites complementary to the crRNA sequence. There are three different CRISPR-Cas system types, type I,II and III. Type I and III are relatively complicated and use a complex set of Cas proteins. However, type II uses only a single protein for DNA recognition and cleavage. This makes type II interesting for biotechnological purposes. In this type II system trans-activating crRNA (tracrRNA) was reported to be essential. [Doudna, Jennifer A., and Emmanuelle Charpentier. "The new frontier of genome engineering with CRISPR-Cas9." Science 346.6213 (2014).]

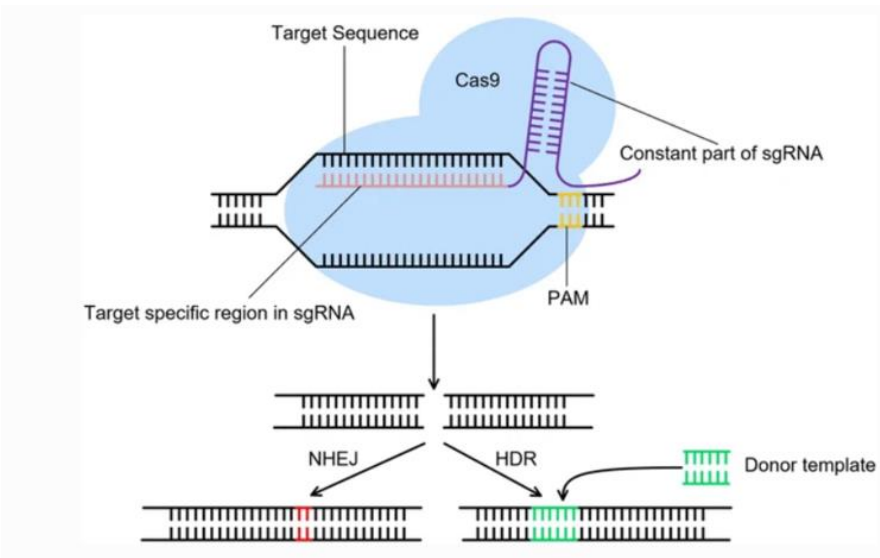


Figure 1: An illustration of genome editing with CRISPR-Cas. Showing both the non-homologous end joining (NHEJ) and the Homology directed repair (HDR).

Biomedical purposes of CRISPR/Cas9

CRISPR/Cas9 gene-editing techniques use this type II system. The technique consists of two parts: single guide RNA (sgRNA) and Cas9 endonuclease. sgRNA is a fusion of earlier explained tracrRNA and crRNA. It consists of two parts: a constant part and a 5'-end 20-nt altered part. The constant part forms a scaffold that Cas9 can bind to in order for it to form the complex and cut. The 5'-end 20-nt part can be altered to target different DNA sites by being complementary to it. The target DNA has to contain a specific sequence, the adjacent protospacer motif (PAM). Without this usually short sequence directly adjacent to protospacer, the Cas9 will not cut. The protospacer directly adjacent to the PAM is complementary to the 5'-end 20-nt sequence of the engineered sgRNA.

The 5'-end 20-nt sequence binds to the protospacer, and the Cas9 binds with PAM to make a double-strand break (DSB). After this, the DNA repair system of the cells will cause one of two things at this specific site. One option is occurrence of non-homologous end joining (NHEJ). This will lead to possible sequence insertions or deletions, which can cause the gene to lose its functionality. Otherwise homology-directed repair (HDR) can occur. With HDR, a piece of DNA will fill the gap of the cut, as can be seen in figure 1. This DSB can cause gene loss of function, but it is also possible to repress or activate specific genes using CRISPR-Cas9. Gene repression is done by making a Cas9 that has no cleaving ability to bind with the transcription factor binding site. Gene activation can be achieved by fusion of inactive Cas9 to the transcriptional activation domain. [Cui, Yingbo, et al. "Review of CRISPR/Cas9 sgRNA design tools." *Interdisciplinary Sciences: Computational Life Sciences* 10.2 (2018): 455-465.]

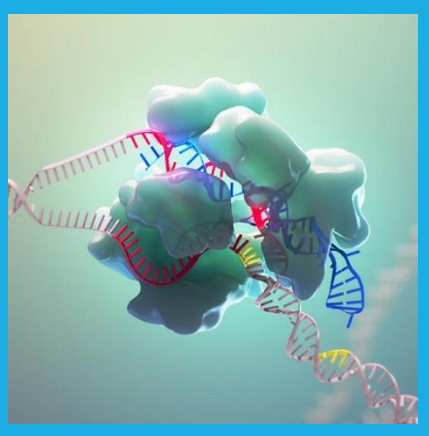
Varanda, C. M. R., Félix, M. do R., Campos, M. D., Patanita, M., & Materatski, P. (2021). Plant Viruses: From Targets to Tools for CRISPR. *Viruses*, *13*(1), 141. https://doi.org/10.3390/v13010141

The main advantage of CRISPR-Cas systems is that they can genetically modify an organism without leaving any foreign DNA behind. Furthermore, its versatility and simplicity of programming are also two big advantages that set it apart from other gene-editing techniques. Unlike other gene-editing techniques in CRISPR/Cas only the recombinant RNA sequence has to be changed. There are still some issues regarding the implementation of CRISPR-Cas. Delivery of CRISPR-Cas components into the target cells remains one of the main issues in gene editing. This issue is being addressed in research by the development of shorter guide RNA-coding sequences and smaller Cas endonucleases.

Another issue is that there are only 20 programmable bases in Cas9. The PAM sequence has to be within ten bases from the base target for the process to work optimally.

Extending PAM preferences and identifying new CRISPR endonucleases have been ways for scientists to try and circumvent this problem. (Waddington, Simon N., et al. "A broad overview and review of CRISPR-Cas technology and stem cells." Current stem cell reports 2.1 (2016): 9-20.)

Furthermore, there are many ethical concerns when working with gene editing. In the chapter “Ethics and Legislation” we will delve more into this.



Gene Editing Techniques

JACQUELINE

Currently, there are many different gene editing techniques available, ranging from fairly simple to highly complex systems. Some of these, displayed in Figure 1, will be discussed in more detail below.

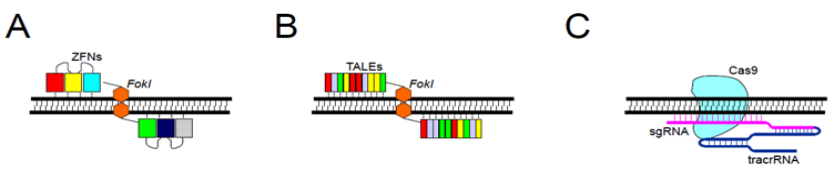


Figure 1. Editing nucleases. A. ZFNs – two discrete ZFNs recognize and bind to specific sites at opposite DNA strands; assembled FokI dimer specifically cleaves target DNA. B. TALENs – two discrete TALENs recognize and bind to specific sites at opposite DNA strands; assembled FokI dimer specifically cleaves target DNA. C. In the CRISPR-Cas9 system, the DNA site is recognized by base complementarity between the genomic DNA and sgRNA, associated with tracrRNA, and loaded into Cas9 nuclease, which performs DNA cleavage. [6]

Restriction enzymes are the original genome editor. [2] Their discovery and characterization in the late 1960s and early 1970s by molecular biologists Werner Arber, Hamilton O. Smith, and Daniel Nathans allowed scientists to cut DNA at precise locations.[3][4] This opened the world of gene editing, allowing them to cut, isolate and recombine fragments of DNA. Restriction enzymes are not commonly used for genome editing these days, because they are limited by the sequence they recognize. Furthermore, the sequence may also exist on the genome outside the target area. However, the enzymes are still widely used in a laboratory setting because they are very cost-effective and do not require the designing of a guide RNA like with CRISPR.

In 1985, the first endonucleases, **Zinc-finger nucleases (ZFNs)** were discovered.[2] These ZFNs (figure 2A) had the advantage of being much more precise in targeting the site that needed to be edited than the earlier restriction enzymes. They are entirely artificial and composed of FokI restriction endonucleases that are joined with zinc-finger-binding domain protein. [1] The protein-binding domain locates the three base pairs that compose the splice site, after which the restriction endonuclease cuts it. While this was an improvement upon the restriction enzymes, it was still prone to off-target mutations and difficult to construct and therefore not widely adopted. [5]

ZFNs were followed up by **TALENs** (figure 2B), Transcription activator-like effector nucleases. These were structurally similar to ZFNs in that they both use the FokI restriction endonuclease, but they differ in their protein-binding domain. TALE-binding domains consist of a series of repeat domains, each ~34 residues in length, and can recognize a single base pair, thus improving specificity. TALENs, however, did not improve much on the costs and difficulties involved in construction of the system, which hindered its use.

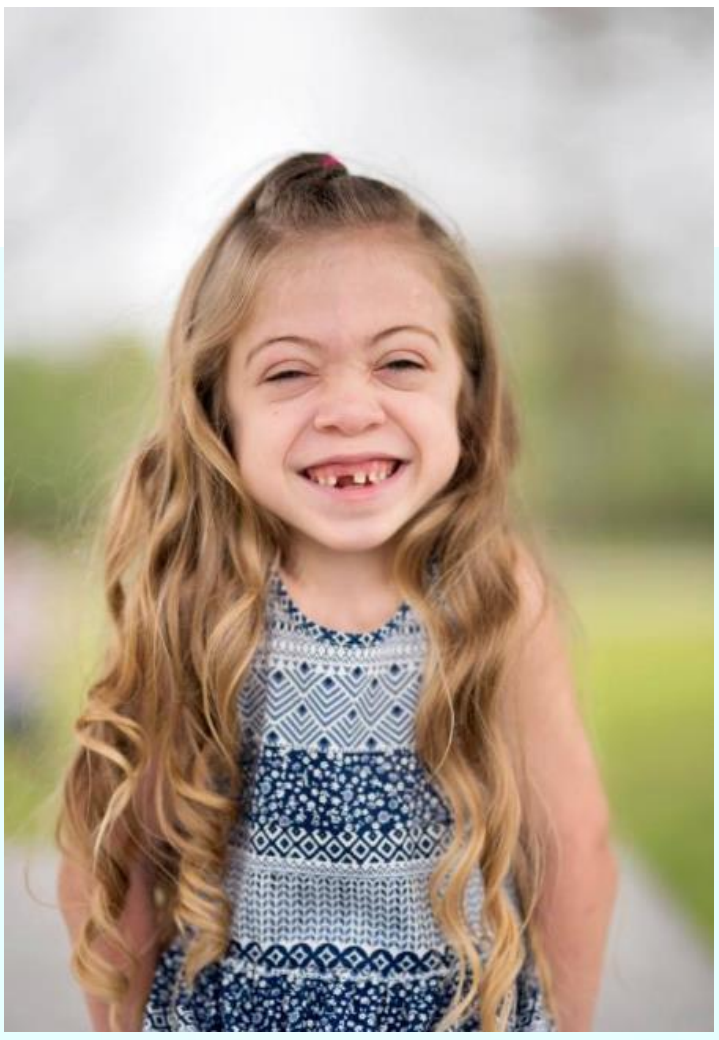
The **CRISPR-cas9** system (Figure 2C) is based on the bacterial adaptive immune system. More on the structure and working mechanism can be read in the section ‘CRISPR-cas9'. This technique did gain widespread popularity because of its specificity, simplicity, and cost-effectiveness. The discovery of this method has opened doors to experiments and therapeutics that were previously thought to be impossible, and one can only wonder what is next.

1. Khan SH. Genome-Editing Technologies: Concept, Pros, and Cons of Various Genome-Editing Techniques and Bioethical Concerns for Clinical Application [Internet]. Vol. 16, Molecular Therapy - Nucleic Acids. Cell Press; 2019 [cited 2021 Jan 16]. p. 326–34. Available from: https://doi.org/10.1016/j.omtn.2019.02.027.
2. Genome Editing Techniques: The Tools That Enable Scientists to Alter the Genetic Code [Internet]. [cited 2021 Jan 16]. Available from: https://www.synthego.com/blog/genome-editing-techniques
3. Restriction Enzymes Spotlight [Internet]. [cited 2021 Jan 16]. Available from: https://www-nature-com.proxy.library.uu.nl/scitable/spotlight/restriction-enzymes-18458113/
4. Restriction enzyme. In: Encycolpaedia Brittanica [Internet]. Encyclopedia Brittanica; 2020 [cited 2021 Jan 16]. Available from: https://www.britannica.com/science/restriction-enzyme
5. Gaj T, Sirk SJ, Shui SL, Liu J. Genome-editing technologies: Principles and applications. Cold Spring Harb Perspect Biol [Internet]. 2016 [cited 2021 Jan 16];8(12). Available from: /pmc/articles/PMC5131771/?report=abstract
6. CRISPR-Cas9, TALENs and ZFNs - the battle in gene editing | Proteintech Group [Internet]. [cited 2021 Jan 16]. Available from: https://www.ptglab.com/news/blog/crispr-cas9-talens-and-zfns-the-battle-in-gene-editing/

Casus - Gene therapy for Maroteaux-Lamy Syndrome

JENNY, CARLON

Maroteaux-Lamy Syndrome is type VI of the 60 inherited lysosomal storage disorders, which are also called mucopolysaccharidoses (MPS). Mucopolysaccharidosis type VI (MPS VI) patients suffer from a deficiency or malfunction of specific lysosomal enzymes. Lysosomes serve as the most important digestive system within cells, digesting metabolites such as carbohydrates. Defects in these lysosomal enzymes can lead to anomalous build-up of certain complex carbohydrates, also known as glycosaminoglycans (GAGs), in for example joints and skeletons. These build-ups can extend further to other parts of the body, such as the respiratory system, central nervous system, and liver. Consequently, cells, tissues and other organ systems can be damaged. The syndrome is inherited as an autosomal recessive disorder and is characterized by mutations in the Arylsulfatase B (ARSB) gene, of which more than 220 different mutations have been identified. These mutations result in insufficient activity of lysosomal enzyme arylsulfatase B, which in turn increases GAG-levels. Symptoms, onset and progression of MPS VI differ extremely per patient. There are cases of MPS VI which progress rapidly and there are cases of MPS VI which progress relatively slowly. Besides onset, there are also variations in symptoms. Examples include skeletal malformations, clouding of the cornea, pulmonary problems, hydrocephalus, and hepato- and splenomegaly [1,2].



MPS VI is characterized by many different mutations in the ARSB gene which result in insufficient activity of lysosomal enzyme N-acetylgalactosamine 4-sulfatase (arylsulfatase B, ARSB). This enzyme is responsible for the degradation of mucopolysaccharides within lysosomes by catalyzing the hydrolysis of the sulfate groups of GAGs, especially the sulfate groups of dermatan sulfate and chondroitin-4-sulfate [3]. As a consequence of the malfunctioning enzyme, dermatan sulfate and chondroitin-4-sulfate accumulate and elevated urinary excretion of ARSB enzyme substrates is observed. The accumulated lysosomal GAGs cause injuries to the cell, resulting in a broad spectrum of clinical phenotypes [4].

An example of current treatment for MPS VI patients is hematopoietic stem cell transplantation (HSCT). HSCT is the transport of either autologous, syngeneic or allogeneic multipotent hematopoietic stem cells that are obtained from umbilical cord blood, peripheral blood or from the bone marrow [5]. HSCT is a treatment for multiple MPS type patients and is based on the transplantation of donor stem cells into the bone marrow of the MPS patient. HSCT is currently seen as a treatment option for MPS VI patients. The effectiveness depends on multiple factors, such as the age of the patient, disease stage, preparative regimen and the type of donor.

Currently, HSCT has shown some improvements in the quality of life in MPS VI patients [6]. Another treatment option is the drug Naglazyme. Naglazyme is one of the standards of care for MPS VI and was authorized in 2005 by the EMA. Naglazyme is an enzyme replacement therapy, given to patients who are missing this particular enzyme.

The API of Naglazyme is galsulfase, which is the copy of the human enzyme N-acetylgalactosamine 4-sulfatase. Naglazyme improves the breakdown of GAGs and helps to stop the buildup in the cells [7–9].

Currently, a research group is performing a study for the treatment of MPS VI using adeno-associated viruses (AAV). The AAV is used for delivery of the healthy ARSB gene to the cells of the liver. This allows the cells can than produce healthy protein and reduce the severity of the disease. The results of this clinical trial are expected in April 2022 [10]. Another type of therapy that is currently under investigation for different types of inherited diseases is germline therapy. Germline therapy is a therapy in which DNA is transferred to a cell for reproduction, such as sperm cells, and egg cells. This therapy could prevent diseases that are inherited from generation to generation and so the parents could possibly get a ‘healthy’ child, even when they are at risk from giving the gene to their infants. At this moment germline therapy is not allowed, because of ethical considerations and the risk still outweighs the benefits of this treatment. But maybe in the future, this technique will eliminate the disease altogether [7,9].

References:

1. Maroteaux Lamy Syndrome - NORD (National Organization for Rare Disorders) [Internet]. [cited 2021 Mar 21]. Available from: https://rarediseases.org/rare-diseases/maroteaux-lamy-syndrome/

2. Valayannopoulos V, Nicely H, Harmatz P, Turbeville S. Mucopolysaccharidosis VI. Orphanet J Rare Dis. 2010;5:5.

3. Tomanin R, Karageorgos L, Zanetti A, Al-Sayed M, Bailey M, Miller N, et al. Mucopolysaccharidosis type VI (MPS VI) and molecular analysis: Review and classification of published variants in the ARSB gene. Hum Mutat. 2018;39:1788–802.

4. Simonaro CM, Haskins ME, Schuchman EH. Articular chondrocytes from animals with a dermatan sulfate storage disease undergo a high rate of apoptosis and release nitric oxide and inflammatory cytokines: a possible mechanism underlying degenerative joint disease in the mucopolysaccharidoses. Lab Invest. 2001;81:1319–28.

5. Mahla RS. Stem cells applications in regenerative medicine and disease therapeutics. Int J Cell Biol 2016;2016:6940283. https://doi.org/10.1155/2016/6940283.

6. Analysis of factors affecting development of carpal tunnel syndrome in patients with Hurler syndrome after hematopoietic cell transplantation. Khanna G, Van Heest AE, Agel J, Bjoraker K, Grewal S, Abel S, Krivit W, Peters C, Orchard PJ Bone Marrow Transplant. 2007 Mar; 39(6):331-4.

7. Naglazyme | European Medicines Agency n.d. https://www.ema.europa.eu/en/medicines/human/EPAR/naglazyme#product-information-section (accessed March 21, 2021).

8. Is germline gene therapy ethical? | Debates | yourgenome .org n.d. https://www.yourgenome.org/debates/is-germline-gene-therapy-ethical (accessed March 21, 2021).

9. Wolf DP, Mitalipov PA, Mitalipov SM. Principles of and strategies for germline gene therapy. Nat Med 2019;25:890–7. https://doi.org/10.1038/s41591-019-0473-8.

10. Gene Therapy in Patients With Mucopolysaccharidosis Disease - Tabular View - ClinicalTrials.gov n.d. https://clinicaltrials.gov/ct2/show/record/NCT03173521?cond=Maroteaux+Lamy+Syndrome&draw=2&rank=10 (accessed March 29, 2021).

"World's first gene-edited babies created in China, claims scientist" was one of the headlines in The Guardian on November 28, 2018. [1] On this day a revolutionary step was made in science and ethics, the birth of Lulu and Nana. The pseudonymously named twins were the result of an experiment conducted by Chinese scientist He Jiankui. Using oocytes and sperm cells from selected couples, where the male was HIV-positive, Dr. He claimed his research was aiming to prevent HIV in new-born babies.

One of the best documented genetic mutations in disease regulation is the CCR5-Δ32 HIV resistance allele. Absence of this allele is associated with a substantial risk of disease progression when diagnosed with HIV. Heterozygous carriers for the allele have shown to inherit the risk of HIV infection which is why many couples "…had lost hope for life" in the eyes of Dr. He. [2][3] By using *in vitro* fertilization the Chinese scientist created embryos which he then genetically modified with CRISPR-Cas9 to deactivate the CCR5 gene. Deletion of the last 32 nucleotides will cause translation to stop too early producing a non-active version of the chemokine receptor CCR5. The reduced expression of CCR5 prevents HIV from entering macrophages and producing copies of the virus. [2]

Objectively this seems like an innovative way to prevent HIV in newborn babies and at first sight seems to be with good intentions. Though, Dr. He received a lot of criticism for his actions. Scientists were angry because they believed Dr. He did not see the moral and ethical implications of his work. Before gene-editing, the embryos were healthy and free of disease. Applying this technique may be able to reduce the risk of HIV infection in a later stage of the baby's life, but it does come with a catch. Gene editing is still underdeveloped and potentially leads to off-target mutations which can cause genetic problems like cancer development. Besides, HIV prevention and treatment has evolved significantly and provides many ways in which people can protect themselves. Prevention can be simple like protected sex, but even if someone carries the virus there are effective treatments against it. [1] Therefore, many people believe Dr. He's actions were unethical because he introduced an irrevocable gene edit, that can be passed down for generations and could ultimately alter the human species, while other effective treatments were available.

A couple of months later, effects of this experiment started to show in all of China. Suddenly new headlines stated that the whole incident was the work of "Chinese scientists" forgetting that there was only one person behind all this. Quickly all gene editing studies coming from Chinese research institutes were tainted due to this one scandal. Almost all of these studies were focused on somatic cells, of which genetic mutations are not passed on to future generations, and not on germline cells. Even so, people see 'genome editing' and immediately think it is the same kind of research that Dr. He was doing, without knowing the difference between a germline and somatic cells. With the risk of being bombarded with criticism many Chinese researchers decided to stop ongoing CRISPR-Cas9 research. [4]

The Chinese Academy of Medical Sciences are opposed to any clinical operation of human embryo genome editing for reproductive purposes in violation of laws, regulations, and ethical norms in the absence of full scientific evaluation". [5]

Early December 2019 his research was published, and the results showed something dissimilar from what the Chinese scientist had previously claimed. Scientists revealed that the results stated a different mutation was made on the CCR5 gene. Instead of creating the HIV resistant allele, he created edits in the gene that were never seen or studied before. Later that month he was sentenced to 3 years in prison for "illegal medical practices" and "…violating a government ban by carrying out his own experiments on human embryos…". [6][7]

Ever since this incident, the debate has been sparked on where the 'red line' lies with regards to gene editing. Due to there being doubts on whether the laws and regulations for gene editing were strict enough at the time, people are questioning if He Jiankui was solely to blame for his actions. [8] After the birth of Lulu and Nana many countries reevaluated and rewrote their regulations on experimentation with gene editing techniques. Most countries have prohibited the use of CRISPR-Cas9 on germline engineering or restricted it to *in vitro* research. They have drawn the line at modifying a gene that can change the DNA passed down to entire generations. However, the genetic modification of somatic cells is now making rapid development and a lot of research is being conducted on making the technology more specific, to avoid off-target effects. The technology is now entering clinical trials and is expected to revolutionize medical treatment for many current and future diseases. [9]

References:

1. "World's First Gene-Edited Babies Created in China, Claims Scientist." *The Guardian*, Guardian News and Media, 26 Nov. 2018.
2. de Silva E, Stumpf MP. HIV and the CCR5-Δ32 resistance allele. FEMS microbiology letters. 1 Dec. 2004.
3. Belluck, Pam. "Chinese Scientist Who Says He Edited Babies' Genes Defends His Work." *The New York Times*, The New York Times, 28 Nov. 2018.
4. Cohen, Jon. "The Long Shadow of a CRISPR Scandal." *Science*, American Association for the Advancement of Science, 2 Aug. 2019.
5. Wang C, Zhai X, Zhang X, Li L, Wang J, Liu DP. Gene-edited babies: Chinese Academy of Medical Sciences' response and action. The Lancet. 5 Jan. 2019.
6. "China Jails 'Gene-Edited Babies' Scientist for Three Years." *BBC News*, BBC, 30 Dec. 2019.
7. Normile, Dennis. "Chinese Scientist Who Produced Genetically Altered Babies Sentenced to 3 Years in Jail." *Science*, American Association for the Advancement of Science, 30 Dec. 2019.
8. Cohen, Jon. "The Untold Story of the 'Circle of Trust' behind the World's First Gene-Edited Babies." *Science*, American Association for the Advancement of Science, 1 Aug. 2019.
9. Grand Moursel L, Visser M, Servant G, Durmus S, Zuurmond AM. CRISPRing future medicines. Expert Opinion on Drug Discovery. 3 Apr. 2021.

Ethics & Legislation – Should gene editing be formalized?

JOB

The hottest topic around CRISPR/Cas9 technology right now is maybe not how it works and what it can be used for, but more if application of this technology should be allowed in germline gene engineering practices. Countries all around the world have different views on the legislation of this technology and laws and regulations have therefore changed over the years. Before 2018, countries were quite lackadaisical with monitoring gene editing experiments, creating loopholes in the system. Due to this, the Chinese scientist He Jiankui was able to advance with his designer baby experiment. After the controversy surrounding this issue, many countries rewrote their laws on gene editing experiments and enforced a clear set of rules to be followed by scientists when working with this technology. 24 countries now prohibit the editing of germline genes by law and 9 other countries in their guidelines.[1] In Europe, the Oviedo Convention, stated in Article 13 that: "An intervention seeking to modify the human genome may only be undertaken for preventive, diagnostic or therapeutic purposes and only if its aim is not to introduce any modification in the genome of any descendants".[2] Many European countries deem this article to be too strict, considering the growing knowledge within this field of study, and have therefore asked for a reconsideration. Evidence shows that potential exceptions will be made regarding article 13 and that germline editing might be allowed in very extreme cases, provided that:

I. No alternatives for correcting the recognized abnormalities.

II. The purpose is to relieve severe human suffering.

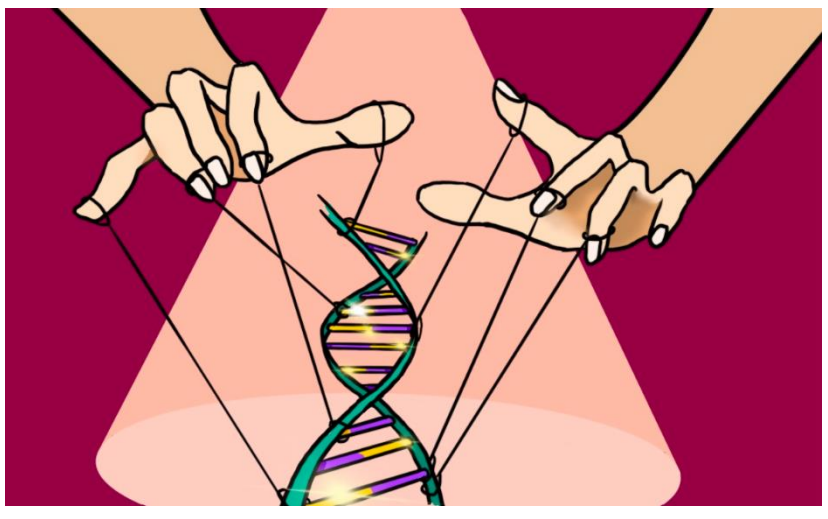
III. Strict standards of safety and reliability are to be met[3].

Why is it that countries are now all of a sudden being so strict when it comes to germline gene editing? Like most new treatments also gene editing therapies need an ethics chapter in their approval statement from drug regulation agencies. Unlike most treatments, the chapter is multiple times longer in gene editing therapies than the average approved drug. The reason being that the very "thing" that makes us "us" is being modified and could potentially be passed on to future generations. It is therefore interesting to dig a bit deeper into the ethical issues that arise in the germline gene editing discussion and what the arguments for and against are regarding this topic.

The procreative beneficence principle states that: "couples should select the child, of the possible children they can have, who is expected to have the best life, or at least as good a life as the others, based on the relevant, available information". In-vitro fertilization (IVF) is allowed in many countries with the option for pre-implantation genetic diagnosis (PGD). Even based on sex an embryo selection can be made if it is strictly for medical purposes. If opted for IVF, in these countries, people are given the option to choose between embryos that either have or don't have the gene for certain diseases, like for example asthma. Through the principle of procreative beneficence, it seems logical that the couple would choose the embryo with the genetic makeup that reduces the chance of asthma development. We know that asthma reduces the quality of life in the child, and medical treatments for it can cause severe side effects in the long run. With the information and knowledge that we have, we are giving the child the best life possible.[4] Using techniques like PGD lets you choose the genetic makeup of your child and generations to come, why aren't we allowing CRISPR/Cas9 on germline genes then? Why shouldn't we normalize something that seems so obvious?

The same principle can be applied from a non-medical standpoint. Take an example given by a famous British philosopher, Parfit. A poor country does not have enough energy to provide power to the population during a cold winter. The government has decided to open an old and unsafe nuclear reactor, giving the country a bit more power. A couple of months later the nuclear reactor melts down and causes a radiation leak. The only effect the meltdown has is that children born shortly after are born with early childhood malignancies. Most people would argue that the decision made by the government was wrong and could have been foreseen. They should have sacrificed the extra power during the winter for the safety of the newborns. Based on the information the government had, they should have chosen to leave the nuclear reactor closed. In the case of gene editing, we have the techniques and information to improve quality of life in people with a risk of developing a certain disease.[4] Using the same logic as in the nuclear reactor example, it would then be wrong not to genetically edit an embryo to provide it with the best quality of life, right?

Gene editing on genes not causing a certain type of disease is usually where the line is drawn. However, besides the fact that the principle of procreative beneficence can be used to support non-disease gene editing too, there are some more fundamental points that need to be considered. It is argued that for a liberal democracy it is important to provide people with general purpose means. These include the ability to hear and see. But also, the ability to concentrate, engage with and be empathetic towards others. Should the insurance of providing children with these characteristics be limited to education and parenting if we have

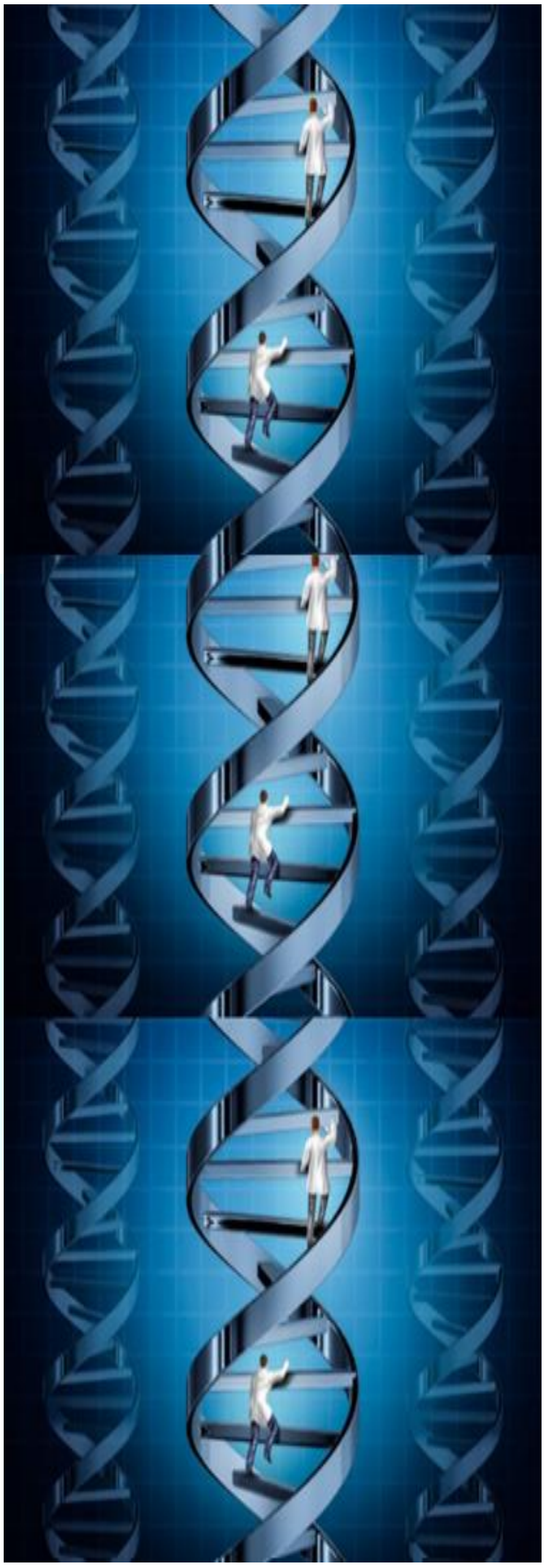


other available techniques? For example, if you have a reduced capacity for memory and this can only be improved slightly through other treatments (not enough to give you a memory comparable to the rest of the population). Going to the supermarket twice because you forgot a pack of butter wastes time, during which you could have done other things. Forgetting a compass/phone/map on a long hike can be fatal. For an improvement in quality of life (which is the same reason for which we cure diseases) and providing general purpose means these genes should be altered.[4]

It is never as simple as just looking at “the positive side of things”, because with big important decisions like these there will always be cons. It should be about reflecting on these cons and figuring out how to minimize them. A concern with human gene editing is the fact that it is a powerful tool and can be used in a lot of good, but also a lot of bad. If put into the wrong hands the creation of edited babies can be made under racial and prejudicial thoughts. We should look at the past and learn from earlier generations’ mistakes and make decisions based on previous experiences. Is it a good idea to let the population of a still very prejudiced world choose what their offspring’s traits and characteristics should be? And with germline gene editing even change traits and characteristics of future generations? Or should we let natural selection do its thing for a little while longer while we work on more pressing issues to fix?

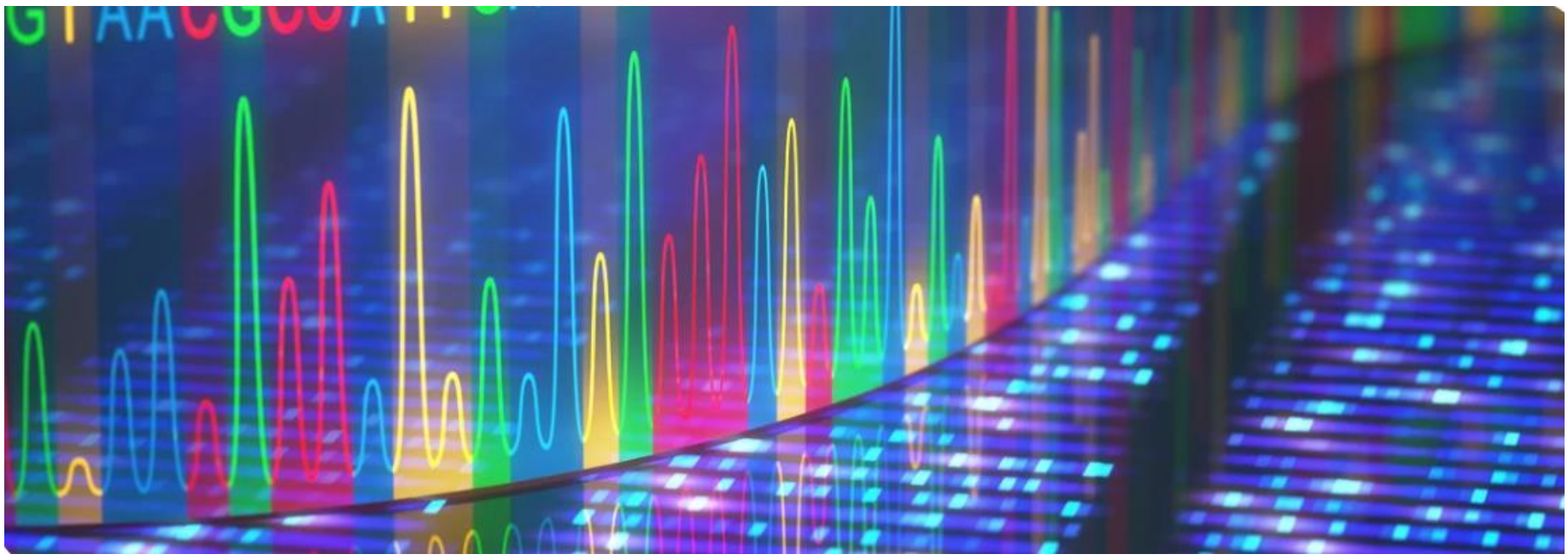
The same point can be made about genetically manipulating disease genes. Selecting for people with or without disabilities may translate into something about the worth of the lives of people with disabilities.[5] Because these disabilities would then become rare, they are more prone to be seen as “weird” or “different”.

Another argument for not starting with genetic manipulation is that you are working with genetic material. It shouldn’t be taken lightly in any extent. Crispr/Cas9 introduces irreversible changes in your genetic code. If some kind of mutation happens to the gene during the process or the introduced gene is misplaced a couple of nucleotides it could induce an effect that the patient needs to live with forever. In one treatment the life of a patient and their offspring (if they wanted to have offspring, this is now also something they need to reconsider) can turn out for the worst. Are we really ready to introduce this new powerful tool to the medical world?



References:

1. Ayanoğlu, Fatma Betül, et al. “Bioethical Issues in Genome Editing by CRISPR-Cas9 Technology.” *Turkish Journal of Biology*, vol. 44, no. 2, 2 Apr. 2020, pp. 110–120., doi:10.3906/biy-1912-52.
2. de Lecuona, Itziar, et al. “Gene Editing in Humans: Towards a Global and Inclusive Debate for Responsible Research.” *The Yale Journal of Biology and Medicine*, YJBM, 19 Dec. 2017.
3. Yotova, Rumiana. “Regulating genome editing under international human rights law” *International and Comparative Law Quarterly*, vol. 69, no. 3, 3 July 2020, pp. 653–684., doi:10.1017/s0020589320000184.
4. Savulescu, Julian. “Procreative Beneficence: Why We Should Select the Best Children.” *Bioethics*, vol. 15, no. 5-6, 2001, pp. 413–426., doi:10.1111/1467-8519.00251.
5. Agar, Nicholas. “Genius Sperm, Eugenics and Enhancement Technologies.” *Liberal Eugenics*, 7 Aug. 2004, pp. 1–19., doi:10.1002/9780470775004.ch1.



Future techniques

JENNY

The current method of treating an infection is to give a broad spectrum of antibiotics until the infection is identified to subsequently switch to a more specific treatment. This is an effective way of treatment for antibiotic susceptible bacteria, but it is inadequate for antibiotic resistant infections. This way of treatment also increases antibiotic resistance and prolongs the hospital stay of the patient. The current method for identification of pathogens is a culturing method, which takes several days. For this reason, a new technique in which pathogens are faster identified is needed. Previous efforts using DNA sequencing to target and identify organisms, by variable region sequencing, has not yielded reliable species-level identification due to target selection and technical limitations. However, last year the technology advanced by making NGS (Next Generation Sequencing) a feasible option for many hospitals. [1]

Two main approaches of NGS are the single molecule real time sequencing approach (SMRT) and the synthetic approach that relies on existing short reads technologies (Moleculo). The most widely used approach of these two is SMRT. The sequencing that has been used in this approach is the pacific biosciences and Oxford nanopore sequencing. [2]

Pacific biosciences sequencing uses fluorescent labelling. It uses a structure composed of many SMRT cells; each cell contains nanostructures that are tens of nanometers in diameter. During the sequencing reaction, the DNA fragments are incorporated by the DNA polymerase with fluorescent labeled nucleotides. The detection of the labeled nucleotides makes it possible to determine the DNA sequence. [2]

Another type of NGS is the Oxford nanopore sequencing (ONT). With this technique the first strand of a DNA molecule is linked to its complementary strand by a hairpin. The DNA fragment is then passed through a protein nanopore. When the fragment is translated through the pore, it generates an ionic current caused by differences in the moving nucleotides. The variation of ionic current is recorded progressively on a graphic model and then interpreted to identify the sequence. [2]

NGS is also used for the prediction of diseases by using for example PCR (Polymerase Chain Reaction) based technologies, but also non-PCR techniques can be used. An example of such a non-PCR technique is the sequence capture approach to isolate huge or profoundly scattered areas from a pool of DNA. Both PCR and non-PCR techniques can be used for variant detection, parental diagnosis, circulation tumor analysis, pharmacogenomics, and gene expression regulation. However, in the prediction of human diseases a lot more aspects need to be considered than only DNA. [3]

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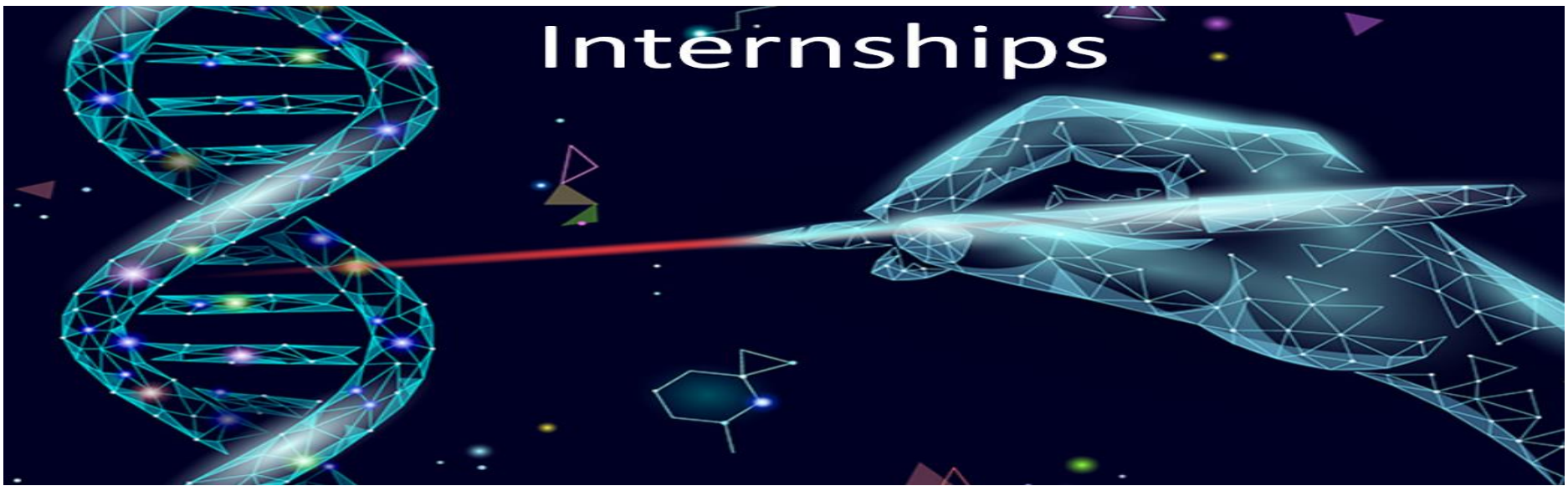
To conclude, using NGS, we will be able to predict diseases, but it also has a considerable influence on the diagnosis. The use of NGS will increase the accuracy of the diagnosis of infections and it also makes it possible to diagnose it earlier. [4] For this reason, NGS will be used widely in medical care in the future. This will help decrease the costs of our health care. Besides, NGS will contribute to fighting bacterial resistance. Overall, NGS will make our health care a lot more efficient.

References:

1. Ellis JE, Missan DS, Shabilla M, Martinez D, Fry SE. Rapid infectious disease identification by next-generation DNA sequencing. J Microbiol Methods. 2017;138:12–19.
2. Kchouk M, Gibrat JF, Elloumi M. Generations of sequencing technologies: from first to next generation. Biol Med (Aligarh). 2017;09(03).
3. Lokuge C. Review of Prediction of Human Diseases using DNA Sequencing Technologies.
4. View of Applied Use of Next Generation DNA Sequencing for Infectious Disease [Internet]. [cited 2021 Feb 21]. Available from: https://esmed.org/MRA/mra/article/view/1645/1721



Internship Nanette Becht: Developing a fluorescence method to track the cellular uptake of Cas9 in HEK293T and HepG2 cells.

CARLON

The Nobel Prize winning genome editing technique CRISPR/Cas9 makes use of the enzyme Cas9 that cleaves DNA while adaptable guide RNA offers the ability to target any part of the genome. This offers a method for gene editing in which virtually any gene can be altered. Direct administration of CRISPR/Cas9 is limited as it is a bacterial complex that quickly induces immune responses. Delivery formulations such as viral vectors, nanoparticles or cell-penetrating peptides can be used to safely deliver CRISPR/Cas9 to cells or tissue in the human body. Currently, research into these delivery formulations is booming although they are mostly compared on gene editing output, instead of Cas9 uptake.

In this study, the free amines on Cas9 were labelled with the fluorescent AF-647. This enabled the tracking of cellular Cas9 uptake using a confocal microscope. Cas9 uptake was studied in HEK293T and HepG2 cells, which are immortalized kidney and liver cell lines. The uptake of Cas9 was determined to be time and concentration dependent. Various cell-penetrating peptides and lipid-nanoparticles were compared on delivery abilities. Cell-penetrating peptides proved to give the highest increase of nuclear Cas9 uptake.

Internship Thomas Rouw: Lipid nanoparticle mediated CRISPR/Cas9 delivery to Jurkat T cells as precursor model for human CAR T cell therapy.

CARLON

The discovery of CRISPR/cas9 has accelerated the application of gene editing because the mechanism is able to precisely edit and modify basically any location in the genome. Gene editing applications can nowadays be applied in cancer immunotherapy which is considered more often as a promising alternative to conventional highly invasive and unspecific cancer therapies. Immunotherapy is a broad term for different forms of cancer treatment which all rely on the ability of our own immune system to tackle cancer cell progression. One of the immunotherapy classes that has reached the clinic is called chimeric antigen receptor (CAR)-T cell therapy. In this therapy, T cells are manipulated ex vivo to express CARs that specifically recognize tumor antigens and initiate productive anti-tumor cell responses.

The FDA has approved two CAR T-cell therapies in which T cells are transduced with a retroviral or lentiviral vector system. Using viral transduction is restricted by immunogenicity and moreover viral vector production is costly and requires a complex synthesis. Non-viral delivery systems such as lipid nanoparticles (LNPs) represent an efficacious approach to overcome the challenges faced using viral delivery. In this study, ionizable cationic LNPs were used to deliver CRISPR/Cas9 components to Jurkat T cells to explore an alternative method to viral vector delivery used in CAR T cell therapy. The Jurkat T cell line is an immortalized T cell line commonly utilized to study T cell behavior. The results demonstrate that the LNP mediated CRISPR/Cas9 RNP delivery was not able to knock down the T cell receptor expression in the Jurkat T cells. Moreover, it was demonstrated that only a small amount of Cas9 protein was transfected into the Jurkat T cells. Nevertheless, results show that the weight ratio between lipids and sgRNA is important for the efficacy of the Cas9 protein encapsulation by the ionizable LNPs. Collectively, this research indicates that the LNP delivery system, combined with CRISPR/Cas9, needs further optimization before it offers a clinical viable alternative to optimize the therapeutic potential of ex vivo CAR T cell therapy.

Meet the Expert-Drug delivery systems and the potential of CRISPR/Cas9

CARLON

Prof. Enrico Mastrobattista is an expert in pharmaceutical biotechnology and delivery at Utrecht University Faculty of Science. He is the head of the research group Pharmaceutics at the Utrecht Institute for Pharmaceutical Sciences, where they work on developing drug delivery systems. Prof. Mastrobattista's expertise is the delivery of biopharmaceutics. In this interview, he gives some more insights into the current ideas and challenges concerning drug delivery systems. The interview then focuses more specifically on the novel research of using CRISPR/Cas9 as delivery systems.

Current ideas for delivery systems

''If you have the ideal drug substance with good absorption, distribution and stability, there is not much you need to do in terms of delivery. Unfortunately, this is not always the case and most of the time a delivery system is needed, especially for biopharmaceutics. For example, nucleic acids are mostly active inside the cell. Unmodified nucleic acids are degraded during administration, so without a delivery system they cannot be used therapeutically. Often a delivery system is based on a nanoparticle. These are little particles in the nano scale that contain the drug substance and allow the drug to enter the cell. These nanomedicines can be chemically modified to functionalize the surface. Structures can be added to the particle to allow them to stick specifically to the desired cell type. This way you create a target delivery system. However, drug delivery is more than that. You can also think of drugs that are effective in the body but give a lot of side effects. The drug delivery system is then used to avoid toxic effects on healthy tissues, by specific targeted delivery. Nanomedicines can be used for this purpose as well, as is done for example with Doxil, a drug against cancer. This drug contains liposome-encapsulated doxorubicin, which would be toxic if it were administered in a free form. Within the nano-scaled liposome, this toxicity can be avoided, and treatment can be better tolerated.'

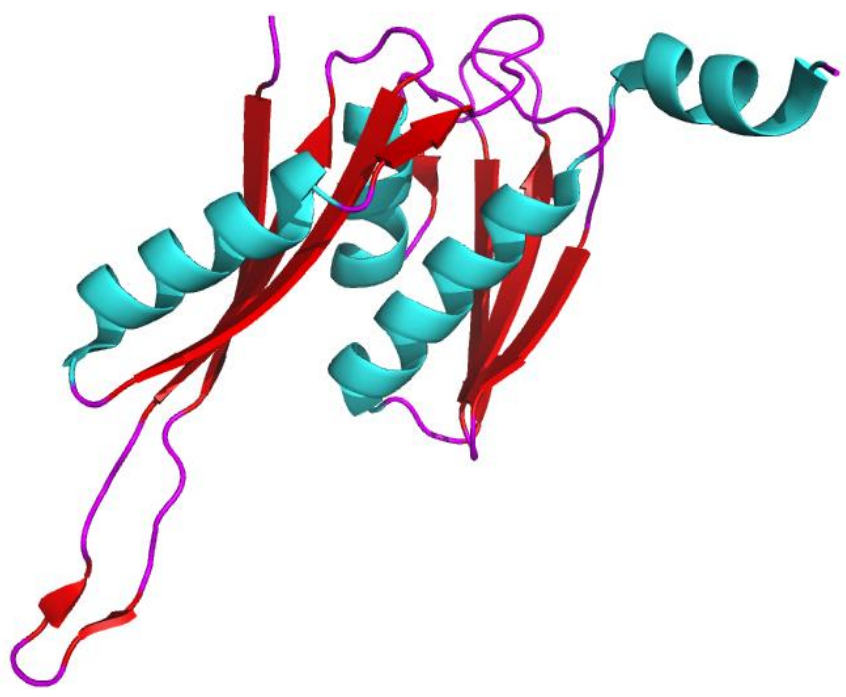
Challenges with development of delivery systems

''The biggest challenge is to reach an effective concentration of the drug in free form inside the target tissue. When you design a delivery system, you want to create a stable system that does not fall apart during the transport, but once at the target site the drug needs to be released. It depends on the properties of the drug if the delivery should take place outside or inside the target cells. The system needs to sense when it is in the right environment to consequently release the drug. Finding that balance is the most challenging part of delivery systems. Another big issue is being able to reach the target cells.

Current technologies allow precise local injections, making this issue solvable, although this might not always be the most patient-friendly solution. In short, the delivery issues can be overcome, but solving toxicity issues of a drug is more of a challenge, especially in oncology.''

Ongoing research

''In my group, we work on delivering CRISPR/Cas9 as a ready-made ribonucleoprotein particle, which can be seen in the figure. To get CRISPR/Cas9 activated in cells, the Cas9 nuclease and a piece of RNA that associates with this nuclease are needed. This combination needs to form a ribonucleoprotein complex in the cell. Most strategies so far deliver genetic information encoded in a plasmid or viral vector. This can be done by administering the genetic material to the patients directly or by modifying the cells ex vivo. Once active in the cell, the Cas9 nuclease and the piece of RNA are produced. The downside of this system is longevity of expression of the Cas9 nuclease as it can be quite toxic. Cas9 cuts your DNA at a specific sequence, but some places in the genome have approximately the same sequence, which can cause an incorrect cut in the DNA. This is called an off-target effect. The longer the Cas9 is present, the more likely it is that such off-target effects occur. Systems that deliver Cas9 as a gene construct have been improved by research, resulting in a lot of ways nowadays to make the cutting more specific. Within my research group, we are focusing on delivering the Cas9 protein ready-made, so it will stay active in the cell for only a couple of hours, which is enough for on-target effects. After those couple of hours, by following this approach, we hope to limit off-target effects as much as possible. The aim is to encapsulate this ready-made ribonucleoprotein in a synthetic vector. In our case we aim to produce a lipid nanoparticle filled with the protein-RNA complex to target progressive familial intrahepatic cholestasis, a metabolic liver disease. We have now reached the stage where we can show the gene editing in cells in vitro, so our next step would be to test this therapy in healthy mice.”



Interesting diseases to study the application of CRISPR/Cas9

''In gene therapy research you go for something that you think is feasible. Anything you can reach by local injection is feasible, but there are also diseases that are not feasible, such as diseases which spread throughout different tissues.
The easiest ones are diseases that allow you to take the cells out of the body and modify them ex vivo. 90% of CRISPR/Cas9 clinical trials are based on this principle and many companies are working on this approach, for example Editas Medicine and Intellia Therapeutics.

Metabolic liver diseases are feasible in gene therapy research, as the majority of lipid nanoparticles are taken up by cells in the liver. This makes the liver a natural target for nanomedicines. As mentioned before, we focus on progressive familial intrahepatic cholestasis. This disease is caused by a mutation in transporter molecule ABCD4 that pumps out phospholipids from hepatocytes into the bile duct. If the transporter of phospholipids is malfunctioning, bile acids are damaging the bile duct, resulting in liver cirrhosis. Patients suffering from this disease already need a liver transplant at a young age. Within our research group we are working on this interesting disease to hopefully try to correct this mutation and cure the disease.'

CRISPR/Cas9 in the clinic

''It will still take quite some time before we can really perform gene editing in the clinic. It would be the easiest route to modify embryos, but that is not allowed yet. Not all side effects, safety aspects and long-term effects are known yet, so clinical trials need to be performed first. In principle, CRISPR/Cas9 technology would allow for the correction of any genetic disease at embryo level, but it should be considered that you are changing genetic information from unborn children, which results in an ethical discussion. My personal opinion is that it is still too early right now to perform gene editing, since there is just not enough knowledge yet. I do think that at a certain stage, maybe in 10-20 years, we can be confident that the therapy is safe in adults. At that moment, I think it is time to look at deadly diseases from which patients suffer a lot and die within a few years. Of course, this will remain an ethical discussion with many questions and that is the reason we should start the discussion now. So, when the time is there, we already have a plan as society.'

Future research

''Besides gene correction, CRISPR/Cas9 can also be used to add additional genetic information that can be of therapeutic value. A lot of patients use biopharmaceutics, which need to be given lifelong via injections. Maybe that will change if we solve the oral availability of drugs, but for now this is not the case. You could think about modifying for example liver cells, so they produce the protein themselves. This is a permanent modification which gives the patient the therapeutic dose of the protein lifelong. Maybe in the future we can go even further and control how much of the protein is produced. This is a research field I am interested in for the future.''

Achievements so far

''The thing that I am most proud of is not a specific publication. Everything that we do as a group is just a tiny contribution to the field. Science is moving forward, but you do this as a scientific community. It is also about bringing people together. For example, collaborations with the industry. I have been coordinating a project which involves different academic groups in Europe, and we did some very interesting things together that were quite successful. That is something that I am very proud of.''

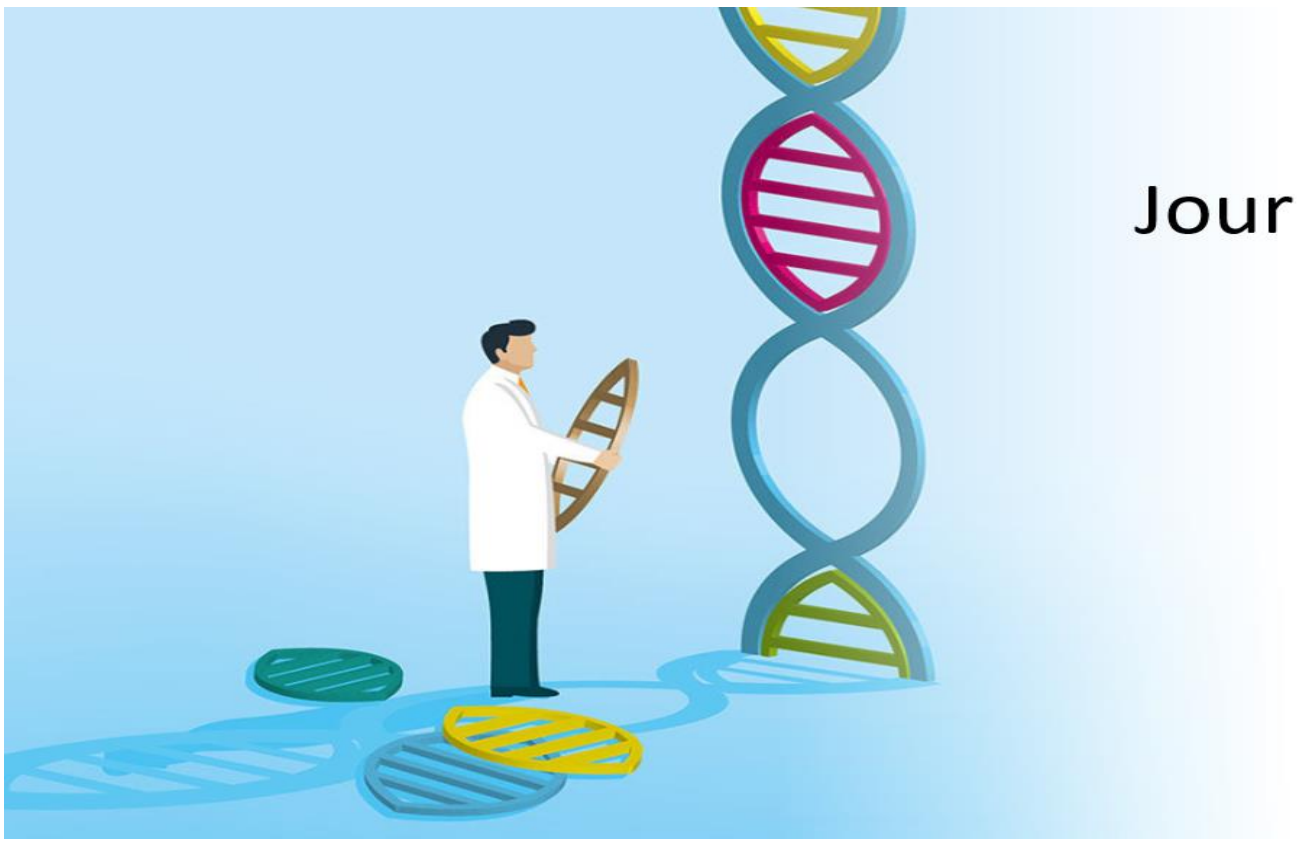
Suggested readings

Several papers of prof. Mastrobattista can be found below.

1) *Wilbie, Danny, Walther, Johanna & Mastrobattista, Enrico (18-06-2019). Delivery Aspects of CRISPR/Cas for in Vivo Genome Editing. Accounts of Chemical Research, 52 (6), (pp. 1555-1564) (10 p.)*

2) *Oude Blenke, Erik, Evers, Martijn J W, Mastrobattista, Enrico & van der Oost, John (04-08-2016). CRISPR-Cas9 gene editing - Delivery aspects and therapeutic potential. Journal of Controlled Release*

3) *Aldosari, Mohammed H., de Vries, Robert P., Rodriguez, Lucia R., Hesen, Nienke A., Beztsinna, Nataliia, van Kuilenburg, André B.P., Hollak, Carla E.M., Schellekens, Huub & Mastrobattista, Enrico (01-04-2019). Liposome-targeted recombinant human acid sphingomyelinase - Production, formulation, and in vitro evaluation. European Journal of Pharmaceutics and Biopharmaceutics, 137, (pp. 185-195) (11 p.)*



Journal Journey

Did you enjoy reading this issue? Go on a journey through this journal to find the answers to the questions.

1. What is the oldest gene editing technique?

A CRISPR-cas9

B ZFNs

C Restriction enzymes

D TALENs

2. In which country was the first Designer Baby created?

A Japan

B United States of America

C Finland

D China

3. What is **not** a second-generation sequencing method?

A Dideoxynucleotide method

B Roche/454 sequencing

C Ion torrent sequencing

D ABI/SOLiD sequencing

4. What does NGS stand for?

A New Gene System

B Next Generation Sequencing

C Novel Genetic Strategy

D Next Genetic Science

5. What are variances in the giraffe genome linked to according to latest research?

A Skin pattern

B Weight

C Height

D Teeth

6. What unknown molecule did Emmanuelle Charpentier unexpectedly discover while studying *Streptococcus pyogenes?*

A Cas9

B ERAP1

C XNa

D tracrRNA

7. What is the biggest challenge while developing a gene therapy?

A Developing the delivery system

B Reach an effective concentration in the body

C Generating the right GOI

D Creating a ready-made ribonucleoprotein particle

8. Which enzyme is lacking in MPS VI patients?

A Arysulfatase B

B α–L-iduronidase

C Glucocerebrosidase

D Sphingomyelinase

ANSWERS:

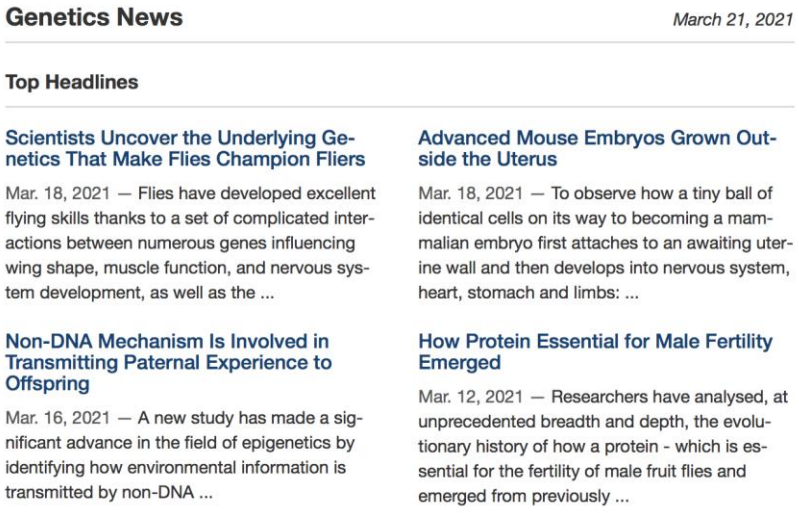
8A 7B 6D 5C 4B 3A 2D 1C

How many did you get right? :)

Latest News Genes

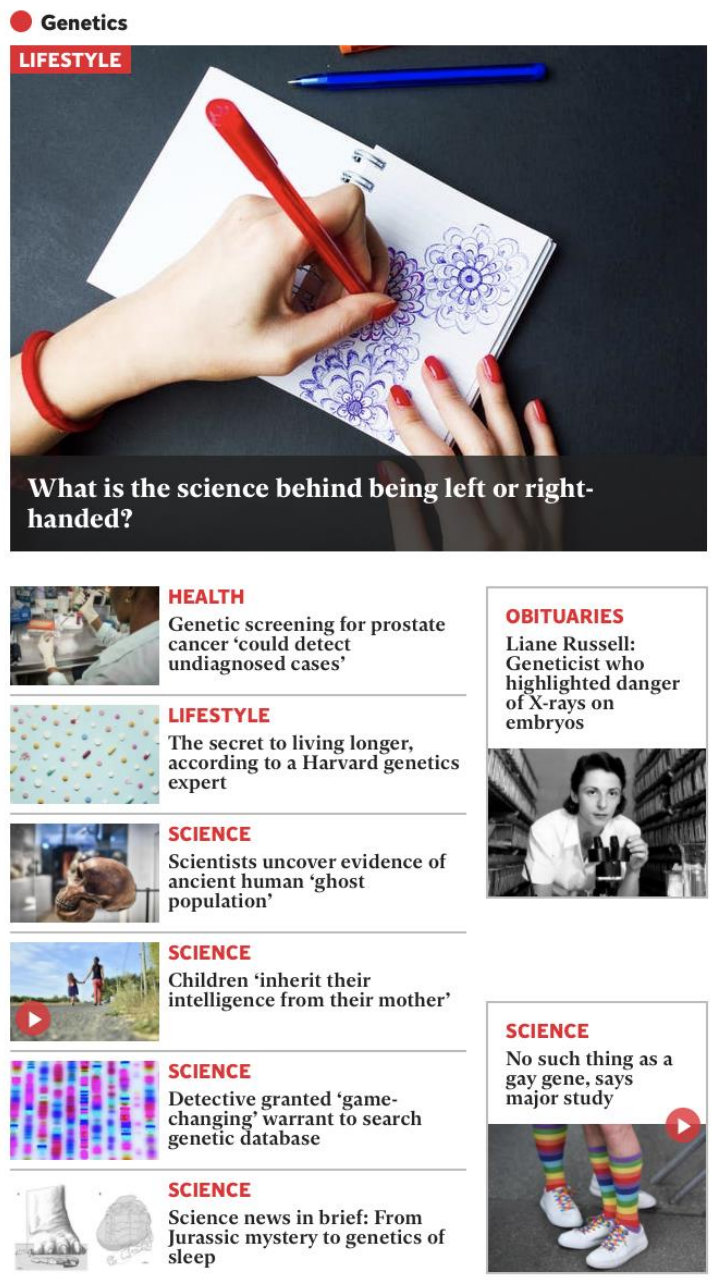
STEPHANIE

The world of genetics is constantly being developed further and further. Research is being done while you read this! This means that the information that you have obtained in this journal could have been elaborated in a month, year and so on. If you have become really interested in genetics after reading this journal and would like to stay up to date about the latest news on genes, it is worth keeping an eye out for ScienceDaily. They are a source for the latest research news, and they have a tab about genetics news! Currently the latest news tabs are:

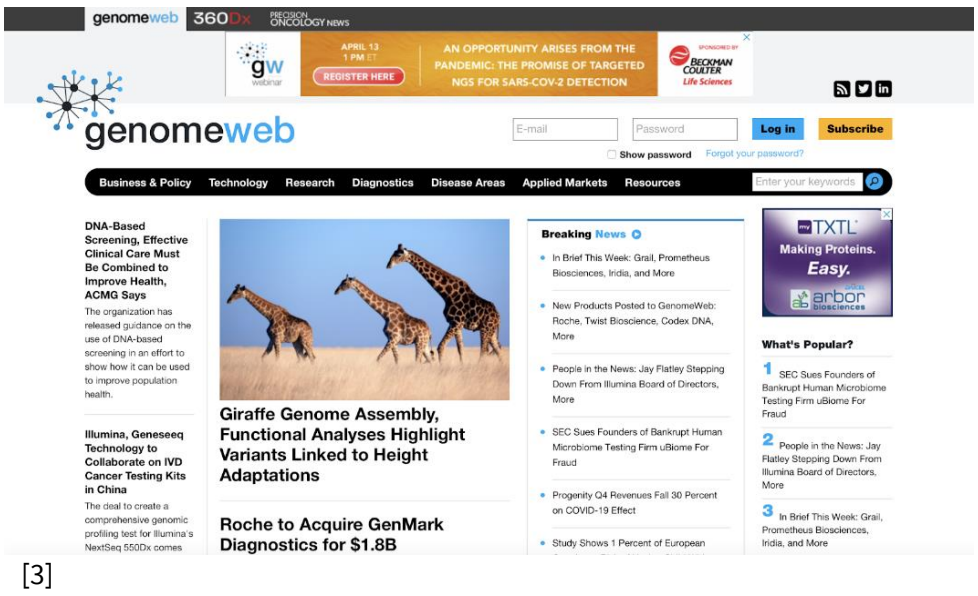


[1]

You will also find up to date information on Independent, which is a website that shares latest worldwide news. They have a section about genetics that keep you up to date about news involving genetics and lifestyle, genetics and science, genetics and health and much more!



Lastly, there is one more site that has a lot of up-to-date news about genetics which I would like to share with you: Genomeweb. The website talks only about genetics.



[3]

They cover the world of genetics involving a lot of different topics such as technology, research, disease areas and much more.

These three sites are very informative about the latest news about genes, so look at them occasionally if you want to stay up to date about genetics!

References:

1. *Genetics News: https://www.sciencedaily.com/news/plants_animals/genetics/*
2. *Genetics https://www.independent.co.uk/topic/genetics*
3. *Genomeweb https://www.genomeweb.com/?gclid=CjwKCAjwgOGCBhAlEiwA7FUXkpgNrQKVYGZgx-SXcFYhAE5N127x4vQVGLdPNidTKDNaK4_tkzc9iRoCk3MQAvD_BwE*

Nobel prize

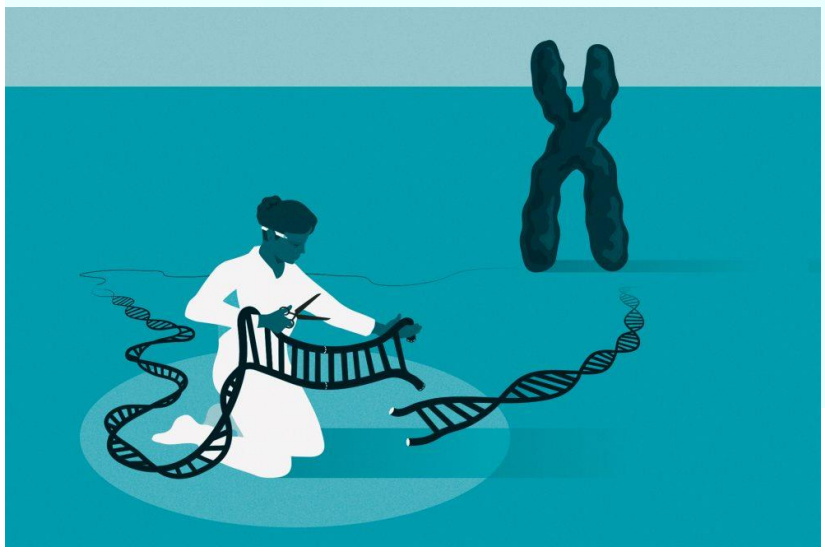
JACQUELINE

Genetic scissors: a tool for rewriting the code of life.

Emmanuelle Charpentier and Jennifer A. Doudna have discovered one of gene technology's sharpest tools: the CRISPR/Cas9 genetic scissors. Using these, researchers can change the DNA of animals, plants and microorganisms with extremely high precision. This technology has had a revolutionary impact on the life sciences, is contributing to new cancer therapies and may make the dream of curing inherited diseases come true.

Researchers need to modify genes in cells if they are to find out about life's inner workings. This used to be time-consuming, difficult and sometimes impossible work. Using the CRISPR/Cas9 genetic scissors, it is now possible to change the code of life over the course of a few weeks.

"There is enormous power in this genetic tool, which affects us all. It has not only revolutionised basic science, but also resulted in innovative crops and will lead to ground-breaking new medical treatments," says Claes Gustafsson, chair of the Nobel Committee for Chemistry.



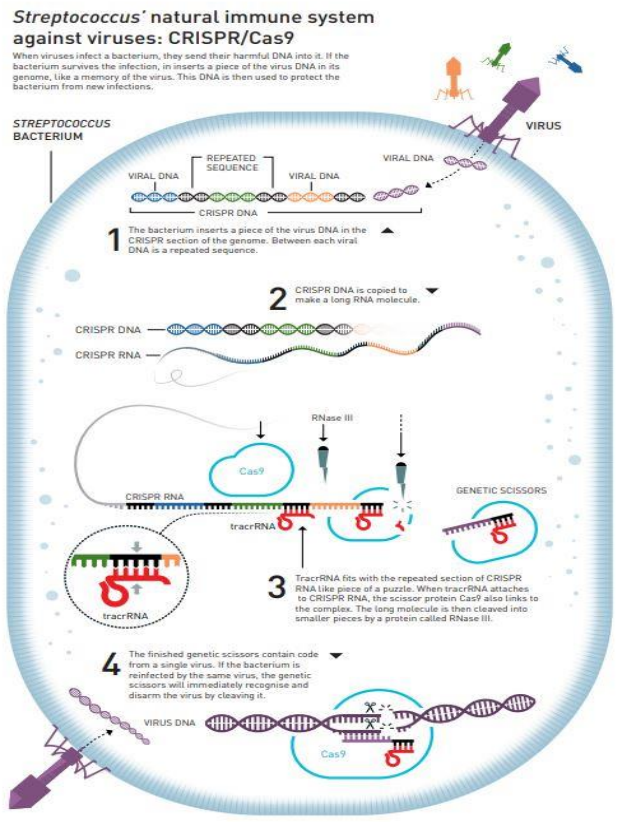
[1]

As so often in science, the discovery of these genetic scissors was unexpected. During Emmanuelle Charpentier's studies of *Streptococcus pyogenes*, one of the bacteria that cause the most harm to humanity, she discovered a previously unknown molecule, *tracrRNA*. Her work showed that tracrRNA is part of bacteria's ancient immune system, *CRISPR/Cas*, that disarms viruses by cleaving their DNA.

Charpentier published her discovery in 2011. The same year, she initiated a collaboration with Jennifer Doudna, an experienced biochemist with vast knowledge of RNA. Together, they succeeded in recreating the bacteria's genetic scissors in a test tube and simplifying the scissors' molecular components, so they were easier to use.

In an epoch-making experiment, they then reprogrammed the genetic scissors. In their natural form, the scissors recognize DNA from viruses, but Charpentier and Doudna proved that they could be controlled so that they can cut any DNA molecule at a predetermined site. Where the DNA is cut it is then easy to rewrite the code of life.

Since Charpentier and Doudna discovered the CRISPR/Cas9 genetic scissors in 2012 their use has exploded. This tool has contributed to many important discoveries in basic research, and plant researchers have been able to develop crops that withstand mold, pests and drought. In medicine, clinical trials of new cancer therapies are underway, and the dream of being able to cure inherited diseases is about to come true. These genetic scissors have taken the life sciences into a new epoch and, in many ways, are bringing the greatest benefit to humankind.



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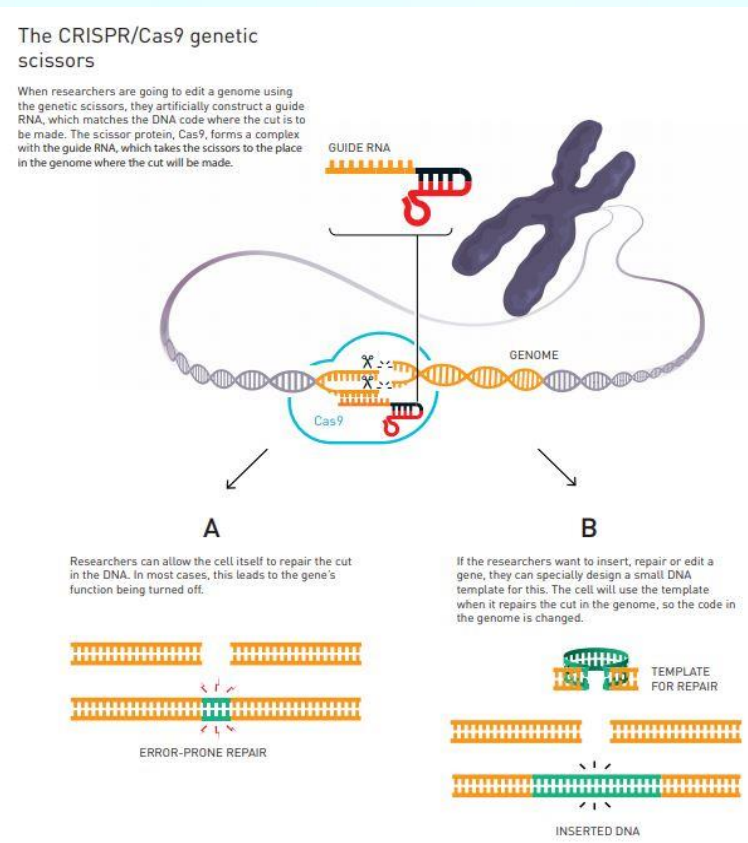

[1]

References:

1. Press release: The Nobel Prize in Chemistry 2020. NobelPrize.org. Nobel Media AB 2021. Wed. 6 Jan 2021. <https://www.nobelprize.org/prizes/chemistry/2020/press-release/>