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Using CRISPR-Cas9 Gene Editing Methods to Create Novel Diagnostic Exams

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Abstract

In order to monitor the spread of diseases globally, diagnostic testing is essential. It consists of three main stages: detection, analysis, and outcomes. The diagnosis of many infectious diseases is based on symptoms, which can frequently overlap between infections and result in incorrect diagnoses. For many illnesses, conventional antibody testing is quite slow and not particularly economical. Using blood or urine samples from patients, CRISPR-based diagnostics could detect the disease-specific DNA sequences in less than a day. The type of disease might then be quickly identified using this data, and the appropriate course of treatment could be started. There is currently a CRISPR-based influenza diagnostic available. Research has shown that Cas9 is useful in differentiating between the virus's strains. The next stage would be to modify this test to make it easier to use than the PCR techniques that are now in use. As a result of the flu's symptoms' resemblance to those of other respiratory illnesses, misdiagnosis rates of influenza would decline.

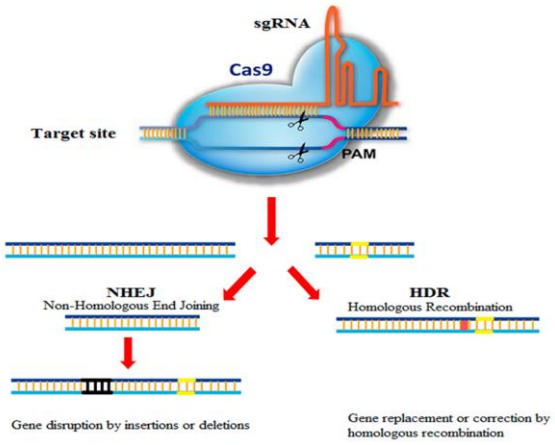
The traditional ways of diagnosing different diseases are covered in this literature review, along with an analysis of how CRISPR technology can improve the detection of tests that are now on the market. This review focuses on non-communicable diseases (diabetes and cancer) and communicable diseases (dengue, influenza, and HIV) using data and research that is already available.

Novel approaches in the field of molecular diagnostics have been introduced as a result of recent developments in the genomic sciences. The application of CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats)-based gene editing to the development of quick, inexpensive, and extremely sensitive diagnostic tests for the identification of communicable and non-communicable diseases is one of the most promising future directions. The way diagnostic tests are performed could be completely changed by CRISPR technology, creating a whole new set of opportunities that the world's health desperately needs.

Keywords*: CRISPR technology, molecular diagnosis.*

1. Introduction

These days, CRISPR-associated Cas9 proteins and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) represent viable genetic engineering tools. Because of its extensive variety of applications in numerous disciplines, it has generated discussions and had a significant impact on gene editing. Numerous researchers have been motivated to explore the bacterial origin and its adaptive immune system as a fresh approach to gene manipulation. It modifies the site-specific DNA cleavage mechanism and has revolutionized the field of genomics. When compared to other techniques like TALENs or zinc finger nucleases, the discovery of CRISPR has made it possible to perform targeted gene editing very easily and affordably. While the traditional PCR can identify the antigen, its specificity is limited. This is where the real use of gene editing to improve diagnostic techniques will happen. Gene editing offers new approaches to make the tests considerably simpler to perform and highly selective toward the antigen or target gene, paving the way from the days of RT-PCR and outdated molecular biology diagnostic tests to the most cutting-edge molecular assays. The fundamentals of gene editing, such the introduction of gene knockout and knockin, have already influenced the creation of diagnostic techniques for some diseases, and with the recent discoveries of CRISPR, they can only improve. The method of site-specific cleavage in CRISPR allowed for the development of highly specific tests for the detection of a particular gene or antigen. The gene can inactivate itself and stop functioning as a result of Cas9's cleavage at the target sequence. This offers a novel approach to disease diagnostics in which the gene responsible for a particular ailment may be identified, and PCR can then be used to determine whether the antigen is still present. One application of the CRISPR process is the PCR-based identification of the p53 tumor suppressor gene in cancer.



1.1. Overview of gene editing using CRISPR-Cas9

Since the discovery of CRISPR, gene editing in mammalian cells has been easier, more affordable, and more accurate. A gene's DNA can be altered in a variety of ways within a cell. The simplest and most popular method of gene editing involves making double-strand breaks in the desired gene and depending on the DNA repair mechanism found naturally in cells. If a DNA template is not supplied, the repair machinery will repair the break, frequently with alterations like insertions or deletions. One can insert the template's sequence at the site of the double-strand break in the genome by supplying a certain DNA template together with the CRISPR RNA. The targeted DNA may be precisely and smoothly integrated using this technique. The discovery that CRISPR can modify genes opens up new avenues for research in numerous fields. Primarily, CRISPR can be utilized to simulate diseases, investigate the consequences of particular mutations, and most significantly, discover possible treatments.

The bacterial immune defense mechanism known as CRISPR, or clustered regularly interspaced short palindromic repeats, was created for uses other than immune defense. CRISPR has been modified and used to alter genes in mammalian cells in Allison and John Doench's lab. The unique feature of the bacterial CRISPR system is that it cleaves invasive DNA or RNA inside the bacterial cell under the guidance of tiny RNAs. The RNA is changed to a synthetic, user-defined RNA in

the CRISPR adaptation for genome editing, which is then used to induce a double-strand break at a particular site in the genome. "The Many Ways to Repair Broken DNA" provides an overview of the state of knowledge on double-strand breaks in DNA as well as the techniques and precision of repair in mammalian cells.

1.2. Diagnostic testing's significance in healthcare

It becomes crucial to make an accurate diagnosis of the illness when someone becomes ill. The findings of the diagnostic tests are used to decide the treatment plan. Diagnostic testing is typically done on patients in laboratories to learn more about their health. Diagnostic tests play a critical role in a patient's care since they give the physician important information that helps him plan the patient's management. Results from diagnostic tests are essential not only for making recommendations about a diagnosis but also for tracking a patient's ongoing health, reaction to therapy, and advancement in the healing process. An inaccurate diagnosis may result in inadequate patient care, exacerbating the patient's condition. This is so because a diagnosis is the process of determining the kind and origin of a disease. This is not an easy, one-step procedure; rather, it is an abstract concept. Many times, a diagnosis consists of a sequence of stages used to determine what is wrong. Since the symptoms and indicators of several health issues sometimes overlap, diagnostic tests must be done to rule out certain conditions while ruling in others. Additional testing is typically required as a result of the results of a diagnostic test. As such, the sensitivity and specificity of a diagnostic test are critical. The genuine positive rate is called sensitivity, and the true negative rate is called specificity. These ideas stem from the test findings, which can be either positive or negative depending on whether the health issue is actually present or absent. Moreover, the field of diagnostic testing is one that is fast developing. More precise and timely results can now be obtained, rendering traditional tests obsolete. The use of genetic testing to identify a medical condition is one instance of this. This stands in stark contrast to earlier techniques; a recent comparison of the bone marrow biopsy from the same patient utilizing both old and current techniques serves as an illustration of this. Myelodysplastic syndrome is the condition for which this patient was identified; conventional testing procedures produced conflicting results. As a result, the comparison was created to examine how testing techniques changed throughout time in order to determine the diagnosis. The more recent flow cytometry technique proved to be more accurate in this instance, and genetic testing can provide even more precise diagnosis information.

1.3. CRISPR-Cas9's potential for developing diagnostic tests

The development of diagnostic tests may undergo a revolution with the use of CRISPR-Cas9 gene editing. Nowadays, PCR (polymerase chain reaction) is used in molecular diagnostics to amplify DNA and find mutations. This is not optimal because it takes a long time, costs a lot of money, and has poor sensitivity and specificity. Sequencing is expensive and unsuitable for large-scale public screening. Such tests are just not practical for the majority of global healthcare markets, and they are ineffective in many high-burden low-income countries (HBLC). A low-cost, user-friendly point-of-care test that is comparable to the quick diagnostics for infectious diseases is actually what's required. It is possible to develop quick and accurate diagnostics for any genetic condition or tendency using the CRISPR-Cas9 technology. Recently, a research team from MIT and Harvard devised a diagnostic device that can identify specific DNA targets using Cas12a and a paper strip. Activated upon encountering the target sequence, Cas12a cleaves all single-stranded DNA randomly. Although this is far from ideal for gene editing, the team recognized they might use Cas12a's collateral activity to make a powerful diagnostic tool. A guide RNA was used to instruct the enzyme so that it would only cleave a particular sequence of the viral genome. When this was found on the paper strip, adding a drop of dengue virus-containing urine caused the strip to appropriately show a positive result. They also demonstrated how

this approach could be readily modified to identify host DNA sequences linked to illness and how it could be used to identify sequences suggestive of harmful bacteria in a patient's blood. This technology could significantly improve the state of healthcare worldwide and is a significant step forward in the development of low-cost point-of-care tests for a wide range of disorders.

2. Overview of CRISPR-Cas9 Gene Editing Principles

A cell fixes a break in DNA by using a known DNA sequence as a template since DNA double helix strands are constantly broken and reconnected at different times. At the break point, a short DNA sequence can be added or removed by taking advantage of this feature. Scientists can alter the gene at a particular gene site to produce a new trait by doing this.

The CRISPR-Cas9 system is based on its capacity to identify dangerous virus DNA and cut the DNA strands to prevent the virus from functioning. Similarly, researchers have been able to engineer the Cas9 protein's RNA to detect a specific DNA sequence found in a plant or animal. The targeted DNA is then cleaved by the Cas9 protein.

A couple of recent discoveries in the realm of gene editing, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas9 (CRISPR-associated protein 9) systems, have generated a lot of excitement in the scientific community. This is a straightforward gene editing technique that was borrowed from bacteria's inherent defense systems to combat viruses. This technique has been utilized for many different things, including as altering the DNA of plants and animals and maybe serving as a disease diagnosis tool.

2.1. The CRISPR-Cas9 system explained

A crucial part of bacteria's adaptive immune system is played by crispr. It is made up of a sequence of short spacer sequences that are derived from prior exposure to bacterial viruses interspersed among repeating DNA sequences. The Crispr-Cas system is made up of Cas proteins and short RNA sequences that are produced from the spacer sequences. In the event of another infection, the spacer sequences—which take the form of an RNA transcript—are used to identify and render certain viral DNA or RNA inactive. It is well known that a tracrRNA-crRNA duplex is responsible for the Cas9 system's ability to identify foreign DNA. To cleave foreign DNA or RNA, Cas enzymes are employed for their nuclease activity. Nuclease activity uses the guidance of the RNA transcript to break double-stranded DNA at locations identified by the PAM (protospacer adjacent motif) of foreign DNA in the type II Crispr system, where it is sufficient to use Cas9 alone for foreign DNA inactivation. By doing this, the foreign DNA is kept from becoming infected further and the host is protected from similar viral attacks in the future. The ability of bacteria to retain a memory of the virus is essential to the entire process of developing immunity because it enables the quick deactivation of the foreign DNA during successive attacks. This technique provides a helpful comparison to the way that scientists are currently editing individual genomes with Crispr.

2.2. Mechanisms of CRISPR-Cas9 gene editing

The Cas9 protein causes a double-stranded break in the DNA after the RNA finds its target. In order to allow the DNA to enter the protein and create an R-loop structure, the Cas9 protein undergoes a conformational shift. The target DNA strand and the displaced non-target DNA strand are the two distinct DNA strands in this R-loop arrangement.

The relatively ease and efficiency of CRISPR-Cas9 gene editing makes it a groundbreaking tool in the field of molecular biology advancements. An RNA-guided DNA

endonuclease enzyme called Cas9 is a component of the immune system of Streptococcus pyogenes. The Cas9 protein uses a synthesized RNA molecule to precisely cleave targets when infection is present.

Step 1: RNA-directed DNA binding: Using base pairing between the RNA and DNA, two RNA molecules called tracrRNA and crRNA drive the Cas9 protein to the DNA target.

The RNA-guided DNA endonuclease enzyme Cas9, which was discussed in the previous section, has been discovered to be essential to the mechanism of gene editing by the CRISPR-Cas9 system. The mechanism of CRISPR-Cas9 gene editing consists of three key stages:

3. Benefits and restrictions of the CRISPR-Cas9 method

Variation The accuracy and effectiveness of the CRISPR-Cas9 system have been lauded, in contrast to less specific gene alteration approaches. Off-target effects are negligible as compared to alternative techniques. At the chosen gene region, the Cas9 enzyme specifically breaks single or double strands, which are then repaired by the cell via non-homologous end joining. Small insertions or deletions at the location may result from this, and a frameshift mutation may be created that effectively knocks out the gene. By providing an exogenous repair template, homology-directed repair—an additional repair pathway—can be employed to produce specific mutations or gene insertions. Though there is still much to learn about this relatively new technology, it is evident that the CRISPR-Cas9 system holds great promise for advancing genetic research and offering practical treatments for hereditary illnesses. These results are based on several research that successfully show how to employ CRISPR-Cas9. In a 2014 study, for instance, the symptoms of a severe form of muscle wasting disease called Duchenne's muscular dystrophy—caused by a hereditary deficit of the protein dystrophin—were successfully ameliorated in a mouse model. After just one injection, the mice's dystrophin protein-encoding gene was successfully altered at several locations. The accomplishment of a 2015 Chinese study, which revealed the first genetically altered human embryo ever, is equally astounding. They tried to alter the gene that causes the potentially lethal blood condition β-thalassemia. Due to a high rate of off-target mutations, this study demonstrated that the technique is still dangerous for use on people, but it does suggest that the possibility of designer babies and the permanent eradication of some genetic illnesses may one day become a reality.

Procedure for Developing Diagnostic Tests

Using CRISPR-Cas9 to generate illness models is one way the technology may be used in the early stages of the development of a gene-based diagnostic tool. This entails developing an animal or cellular model that contains the genetic mutation thought to be the illness's etiology. Gene editing or knockout can help identify the genetic marker that the diagnostic test is currently targeting in in vitro experiments. Compared to earlier gene-based diagnostic techniques that might have looked at a gene with several different functions being expressed, this approach is more focused. This procedure offers a positive and negative control for the genetic test in addition to facilitating a better knowledge of the gene. As a result, the disease model can be applied to a broader range of diagnostic examinations, including those that track the advancement of the disease or the effectiveness of treatment.

A CRISPR-Cas9-based diagnostic test's nature allows it to be used to a variety of test models in a range of illness categories. This advances CRISPR diagnostics from less sophisticated, all-inclusive testing techniques to the creation of tests that pinpoint certain genomic alterations or markers.

A number of requirements must be met in order for a diagnostic test, such as a point of care (PoC) test, to be developed. Initially, the test needs to look for a certain genetic and/or protein marker. A test may also be symptomatic from a clinical perspective, which means it is associated with a general state of

illness. Additionally, the test must provide comprehensive and detailed diagnostic information because in certain instances, the results may not be definitive. In light of the intended usage and the state of medical technology, the test must also be economical and feasible.

3.1. Procedures for creating diagnostic examinations

Using an illustration from Nakamoto's identical study, S. et al. used an immunoblot and a Northern Blot analysis of gene expression to examine the differences in Ptbp isoforms in an attempt to diagnose the liver ailment. Later, through trial and error with a gene editing test, it was discovered that Ptbp isoforms can be identified by varying the mRNA sequence with varying outcomes for the protein product. This made it possible to perform a straightforward reverse transcriptase polymerase chain reaction on the gene's mRNA, cDNA, and DNA sequencing. This was a more reliable and repeatable approach that was also easier to use for changing the patient's care. The fourth step would entail validation and optimization. Prior to the diagnostic test being used in a clinical setting, this would be the last evidence. This further illustrates the previously stated function of CRISPR-Cas9 at every stage.

In this step, the effectiveness of the CRISPR-Cas9 gene editing technology would be shown. The Nakamoto, S. et al. used TALEN and CRISPR-Cas9 to make a modest deletion on the polypyrimidine tract-binding protein (Ptbp) gene in order to separate the Ptbp isoforms and determine the difference between near-end-stage liver disease and chronic hepatic illness in rats. When compared to the current rat liver condition testing, this represents a significant advancement. The current testing necessitates comparing the Ptbp gene expression in rats and humans using RT-PCR and Northern Blot methods. Even though these methods can identify problems, it would not be possible to modify the patient's care. Gene mutations can occasionally lead to changes in gene expression, which can be used to infer improved markers. By using this strategy, you will skip to the third step and come back to the second step later. In the third step, the optimum analysis methods and testing platform are chosen in order to determine whether the markers are present or absent.

A perfect diagnostic test should be able to determine if a disease or condition exists or not, influence how the patient manages their condition, be readily applicable, and be reasonably priced in relation to the expenditures of healthcare. In light of this idealism, the development process need to comprise a sequence of actions that are painstakingly organized and carried out. Finding the illness or condition for which a test is being developed is the first stage in the process. A great deal of genes could be deleted or altered by using CRISPR-Cas9 genome-wide screens in order to study how certain genes affect the onset of a certain illness or condition. This is particularly helpful in ascertaining the biological markers, the genotype of the illness or condition, and the adjustments that should be made to the patient's treatment plan. The best biological markers that most accurately reflect the patient's health change in comparison to a patient who does not have the disease or condition are chosen in the second phase.

3.2. CRISPR-Cas9 function in every stage of test development

Advances in the creation of diagnostic tests, facilitated by the application of gene therapy and editing technologies like CRISPR-Cas9, have made tests that are more accurate, dependable, and economical possible. A streamlined form of the bacterial antiviral defense system serves as the foundation for the novel gene editing technique known as CRISPR-Cas9. It employs guide RNAs (gRNA) to target a particular DNA region using the Cas9 enzyme. At this moment, Cas9 cleaves the DNA in order to disable, fix, or alter the gene. There are numerous uses for this method in the creation of diagnostic exams. The availability of a straightforward yet effective method for modifying DNA sequences has sped up progress in many contemporary biological domains. Though

additional research is still needed to bring CRISPR-Cas9 to this point, it has the potential to change the development of diagnostic tests that can help identify future targets and clarify the path of disease. Target identification is the first and most crucial stage in the development of a diagnostic test. This is the identification of a particular genetic or molecular alteration that is the cause of a disease. This makes it possible to comprehend the disease's course and outward manifestations while also identifying potential treatment targets. Finding the single nucleotide polymorphism (SNP) in the HFE gene, which causes hereditary hemochromatosis, is one example. This stage can be limited by the technology available and is frequently highly challenging. Because CRISPR-Cas9 is a gene editing tool, it might potentially address this stage by modifying specific DNA sequences and seeing how they affect cells to generate disease models. One example is a study that modeled graft versus host disease using immortalized human T cells. This was accomplished by introducing indels into the Pd1 gene, a mutation that prevents pathogenic T-cells from differentiating into effector cells. With CRISPR-Cas9, this stage in disease modeling—which would typically take several years—can be greatly accelerated and has the potential to improve our understanding of a wide range of diseases.

3.3. Problems and factors to take into account when using CRISPR-Cas9 for diagnostic testing

Developing animal models could offer a chance to test the diagnostic technique practically and contribute to the assessment of its utility. Phenotype selection problems would be particularly challenging for diagnostic tests for complicated diseases with numerous genetic causes or diseases with poorly understood genetic causes. A test for early-stage cancer that aims to identify a disease state in patients who are not yet aware they have it could be one concerning example. In order to identify the best-case phenotype, the CRISPR technique may be able to induce and reverse cancer in animal models, but this would be a risky and expensive tactic. A diagnostics project can potentially come to an end if a known, precise phenotype of the disease cannot be achieved.

Selecting the right phenotype can be a big problem when developing diagnostic tests. More complex tests would require a prompt and precise diagnosis of a specific illness state, whereas simpler tests, such as PCR to ascertain the presence of a particular gene, are less exacting in this regard. It would be preferable to develop animal models of the disease and treat them with the gene silencing technique before testing for the anticipated genetic or phenotypic alterations in cases where the disease has a known genetic cause and phenotype.

utilizing CRISPR-Cas9 gene editing methods to create novel diagnostic examinations. We will talk about the potential difficulties in using CRISPR-Cas9 in a diagnostic test in this chapter. We'll talk about the possible roadblocks at each stage and offer methods to check if they are real problems.

4. CRISPR-Cas9 Applications in Diagnostic Testing

Originating from its innate bacterial immune system, CRISPR-Cas9 is a genomic tool that is being used more and more to edit the human genome in order to contribute to the development of innovative new technologies. CRISPR-Cas9 provides an adaptable tool for genetic modification that opens up a plethora of new possibilities for its application in all those research, diagnostic, and therapeutic domains. The CRISPR system is far faster and less expensive than earlier genetic modification techniques. It has been utilized to produce mice with altered genes unique to a number of human illnesses, enabling a greater use of mouse models in the study of various illnesses. The capacity of CRISPR technology to quickly and effectively identify genetic changes in humans is highly attractive for use in diagnostic testing. It can identify and fix faults thanks to cutting-edge DNA. 'Gene drive' systems that bias a particular gene's inheritance to nearly 100% can be created with CRISPR. In the near future, CRISPR may be widely utilized in

diagnostic testing for the identification of genetic diseases. Although CRISPR-Cas9's capacity to detect genetic abnormalities efficiently currently marks a substantial advancement, its true potential rests in its potential to evolve the detection of genetic illnesses. Conventional techniques for genetic disease diagnosis have been costly, time-consuming, and frequently unreliable. By capturing an image of the chromosomes and organizing them according to size, a process known as karyotyping, for instance, can be used to diagnose genetic conditions like Down's syndrome. The treatment becomes more invasive and expensive due to the need for fetal tissue and a higher-resolution picture. Certain protein abnormalities and sickle cell anemia can be diagnosed with DNA hybridization methods. In order to determine whether any anomalies exist, this procedure entails analyzing DNA or RNA and comparing it to a complementary sequence. This method is ineffective for large-scale testing due to its high costs and potential for erroneous results. A potential new technique for diagnosing Down syndrome is described in an article by Myriam Revillion and Francois Nattier that was published in BioMed Research International. This method would use CRISPR-Cas9 to identify the Down syndrome-specific gene from a mother's blood sample. The efficiency and safety of testing for expectant moms are increased by the simple blood sample collection process that can be used to screen for any genetic diseases.

4.1. Identification of genetic illnesses

For certain illnesses, postnatal diagnosis is sufficient and offers more advantages than prenatal diagnosis, which is invasive, expensive, and time-consuming. prompt start of therapy, as well as more chances for family members to think through the ramifications of a positive diagnosis. NGS techniques are not economical for regular genetic diagnosis because there are over 2000 distinct mutations for cystic fibrosis. Even though genome editing, gene therapy, and repair have been successfully tested for cystic fibrosis and beta-thalassemia, these treatments can only be used on afflicted patients whose genetic status is known. Genome editing and gene therapy are becoming more feasible due to the high degree of confirmed diagnosis shown in many hereditary diseases using linkage analysis or direct mutation scanning. Compounds containing C-CPE may facilitate absorption. Therefore, a quick and accurate diagnostic test is required to assess the possibility of in-vitro mutation repair. As an illustration, consider the severe combined immunodeficiency illnesses, which, in the absence of gene therapy, can be effectively treated with bone marrow transplants if the fetus is screened for genetic disorders and the affected persons' somatic cell mutations are verified. While preimplantation genetic diagnosis (PGD) and fetal diagnosis are also seen as kinds of genetic manipulation, they do not provide therapeutic choices for the sick individual. Hurler syndrome is a fast-moving field of gene therapy and pro-drug activation with potential SDT. Affected individuals have bone marrow transplants, and blood stem cell-carried gene addition and enzyme substitution have been tested. This kind of treatment offers options for treatment for sick individuals, not merely gene therapy for future generations, and could be tracked by genetic testing. The Perth-based business "Zucero Therapeutics" was said to have acquired a fast-track designation for its gene therapy, which is based on reactivating the synthesis of the missing enzyme for Hunter syndrome, a closely related mucopolysaccharidosis, from the US FDA at the beginning of 2020. For those who are impacted by these disorders, this kind of development holds out a lot of potential. However, in order to establish eligibility and oversee the course of treatment, a trustworthy test is required. The ability to treat hemoglobinopathies and thalassemia by activating fetal hemoglobin is probably going to be one of the most significant advances in genome editing during the next five years. Therefore, it is necessary to diagnose those who are impacted and to keep an eye on this kind of gene therapy. Thus, applying CRISPR-Cas9 gene editing techniques to the creation of novel diagnostic tests is a promising step toward treating genetic illnesses; however, the review does not address possible SDT.

4.2. Identifying infectious illnesses

Since symptoms of HCV can take time to manifest, individuals are frequently unaware that they have the infection until severe liver damage has progressed. Patients awaiting therapy would benefit greatly from an easy-to-use test that could reliably identify whether a patient has HCV. More CRISPR-Cas9 gene editing experiments will probably be conducted in the future for different gene-specific strains of infectious diseases, as this is just the beginning.

Using a knock-out strategy, one gene was targeted and edited in different HCV-infectious cell cultures to determine the sequence's importance for the translation of viral proteins and the viability of HCV in the cell. This allowed for the detection of HCV-specific gene sequences and the development of a CRISPR-based test for the virus. High rates of modification in wild-type HCV were achieved by step-by-step alterations of the guide RNA and trans-activating RNA (tracrRNA) sequences. This technique effectively generated mutations in the HCV genome's 5'-NTR and E1 regions. It might be helpful in the future for the development of HCV gene therapy and could contribute to our understanding of how the HCV gene functions. However, as of right now, the CRISPR-based approach has been made possible through the creation of mutant HCV-infectious cultures using an Alt-R CRISPR Cas9 system and specialized gRNA. This made it possible to successfully edit more than half of the mutant HCV cells, demonstrating that it can be a useful testing technique.

The lethal virus known as hepatitis B can cause both acute and chronic liver damage. Over 2 billion people are thought to have been infected with HBV, and in the next 20 years, 15–25% of those cases are expected to result in liver illness such hepatocellular carcinoma or death. These estimates are from the World Health Organization. When HBV was first discovered, it was frequently too late to treat the infection because the illness frequently showed no symptoms or just moderate ones. As a result, liver failure frequently happened before the virus was discovered. Because of this, the majority of HBV infections are not aware that they have the virus. Since HBV can spread through blood and other body fluids, it is crucial to test blood donors for the virus since they may be able to infect others. One significant step in stopping the virus's spread would be the development of an efficient test to identify whether a patient has HBV. The same is true with HCV, which causes chronic liver disorders and is also transmitted by blood.

The genetic diversity of infectious pathogens presents the biggest obstacle to the diagnosis of infectious diseases. By identifying certain strains of a pathogen and then creating a diagnostic test that can precisely identify the strain in a patient sample, CRISPR-Cas9 has the potential to solve this issue. One recent example of a successful strain-specific gene discovery is the creation of a CRISPR-based technique for the detection of the hepatitis B and C viruses (HBV and HCV).

4.3. searching for indicators of cancer

Breast cancer and cervical cancer are the most common cancers among women, accounting for about 500,000 deaths worldwide each year. A sometimes disregarded component of cancer diagnosis is the diagnosis made prior to the onset of symptoms in the patient. In terms of molecular diagnosis, this entails screening for precancerous lesions and, more crucially, altered genes that are known to cause cancer. That is addressed in a 2015 publication from the University of California, Los Angeles titled "Functional Analysis of a Novel Genome-Wide Association Study that involved CRISPR Disruption of CTCF in Lung Cancer". The study highlights the promising application of CRISPR in creating models that indicate the location and potential causation of cancer gene alterations. This can be applied to the development of diagnostic tests that identify gene alterations that cause cancer in populations predisposed to particular cancer types. This would improve the chances of cancer patients surviving by substantially assisting in the identification of malignancy before symptoms appear.

This section discusses the application of CRISPR-Cas9 in the development of diagnostic tools for cancer, one of the most common diseases in contemporary civilization. The first study to be mentioned used lung cancer cells from humans. In order to determine if the lung cancer cells would grow more aggressive, the scientists used a technique called gene disruption of the p53 gene. A test group of mutant p53 cells and a control group of wild-type p53 cells were established, and it was determined that the mutant p53 cells were more aggressive. Following that, the p53 gene in the mutant cells was sequenced. The results were validated, revealing a single pyrimidine deletion in the gene's coding region. Given that the disruption of this gene resulted in cancer cells becoming more malignant, this demonstrated the p53 gene's role as a tumor suppressor. This approach does pave the way for the use of CRISPR-Cas9 in identifying particular cancer-causing genes and the consequences of altering those genes, even though it was not a diagnostic test.

4.4. Finding uncommon disease diagnoses

Targeted genome editing has made the Type IV Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 Endonuclease System a leader in the field. The use of CRISPR-Cas9 in many different sectors has become exciting as a result; nonetheless, the subject where the CRISPR-Cas9 system has the most potential for rapid effect is probably diagnostic testing. Using CRISPR-Cas9 gene editing techniques to create tests for infectious diseases has been a popular issue in the scientific community ever since Saurabh Dube and his colleagues initially proposed the idea of using CRISPR machinery as a tool to detect human pathogen infections. This concept makes sense since the CRISPR-Cas9 system can be repurposed to identify the same DNA sequences in a host sample, even though it was originally created to target infections' DNA sequences in order to defeat them. Although there are many possible uses for this concept, a test for malaria in human patients is one of the paper's main examples. PCR-based testing is the gold-standard diagnostic method currently used for malaria. Using this technique, a patient's blood sample is taken, DNA is extracted, and the DNA is then amplified to a detectable amount using polymerase chain reaction (PCR).

5. Case Studies: Diagnostic Tests for CRISPR-Cas9

In CS3, the possibility of using CRISPR-Cas9 to identify a genetic disorder in a fetus was examined. There is a considerable risk of miscarriage associated with the more invasive methods used to diagnose genetic disorders in pregnancy today. To alter the accepted standard of treatment, it was believed that a highly sensitive and non-invasive test like CRISPR-Cas9 would be necessary.

The goal of CS2 was to demonstrate the potential of CRISPR-Cas9 for bacterial identification. Given the established efficacy of the type II CRISPR-Cas9 system in producing knockout mutations in target genes, it was thought that this kind of detection test would be most appropriate for identifying pathogenic germs found in sources like blood or CSF. Antibiotic-resistant Staphylococcus aureus was used in the test prototype in order to distinguish it from its non-resistant counterpart.

The possibility of using CRISPR-Cas9 for Alzheimer's disease diagnosis was demonstrated by CS1. This disease is currently incurable and can only be definitively diagnosed post-mortem. Since the causative mutations could be directly targeted and on-target detection would be necessary in the absence of a disease manifestation, it was thought that this would be a "ideal" disease to diagnose using CRISPR-Cas9. Thus, it was determined that a platform employing genome-wide association at a single nucleotide polymorphism, followed by targeted PCR on those sites containing the SNPs, would be a feasible test. It was believed that this test could revolutionize the way this condition is identified, despite its comparatively high cost.

It was astonishing how inexpensively and effectively the CRISPR-Cas9 technology could be used, especially considering its enormous

potential for diagnostic testing. In order to determine the best way to use this system, a number of case studies were conducted while keeping in mind the vast range of diagnostic tests that may make use of it. When compared to existing methods (typically off-target and/or less sensitive immunological detection), researchers found that CRISPR-Cas9 use could benefit from on-target and sensitive detection for any test that required identifying a nucleic acid, which includes most genetic diseases and many cancers. Three case studies were deemed to be indicative of the possibilities for tests based on CRISPR-Cas9; these were:

5.1. Case study 1: Early Alzheimer's disease detection using a CRISPR-Cas9-based assay

The development of anti-dementia therapeutics severely depends on the early detection of Alzheimer's disease (AD), even prior to the development of amyloid plaques and neurofibrillary tangles. But the clinical diagnosis techniques used today—which mostly rely on cognitive testing and cross-sectional imaging—are typically not sensitive to early AD and moderate cognitive impairment (MCI). These tests frequently yield normal results for patients with MCI and early AD, and people with depression—which can also result in cognitive function deficits—may be mistakenly labeled with early AD. For the early diagnosis of AD, therefore, a sensitive and accurate test is necessary. In light of this, an in vitro CRISPR-Cas9 system presents itself as a desirable option that may fulfill these diagnostic requirements. Three stages comprise the creation of this system: the first is the discovery of SNPs unique to AD that both raise the risk of the disease and provide protection against it. This should be possible because there are seven distinct SNPs that are known to exist, at least one of which is seen in 80% of individuals with late-onset AD and the majority of which are not found in the general population. The SNP must then be used as a target, and a technique that can distinguish the SNP in a DNA sample and modify the DNA if it is the target SNP needs to be created. A DNA strip like the one Gootenberg et al. reported, which is used to identify dengue and Zika viruses in patient samples, might be utilized to accomplish this. Even if this is successful, there is still a chance that patients with the SNP but no AD will receive false positive results, or patients with AD in its early stages may receive false negative results. Finding the modified DNA and accurately and specifically distinguishing it from non-edited DNA thus constitute the final step. According to Gaudelli et al.'s research, modified Cas9 enzymes have the ability to specifically cleave and mutate one of two DNA strands. As a result, these enzymes could be employed to edit the SNP during the third phase. Additionally, during the same phase, the edited DNA could be detected through the selective action of these enzymes on the targeted mutation.

5.2. Case study 2: Rapid detection of antibiotic-resistant microorganisms using a CRISPR-Cas9-based test

The current techniques for identifying bacteria involve cultivating the organism and gauging how well various drugs work to eradicate the infection. Standard in vitro procedures may not always be able to culture the bacteria, and the process itself may take up to 48 hours. When an infection is not identified right away, broad-spectrum medicines are frequently used, which increases the risk of selecting for resistant variants of the infection. Finding and identifying bacterial genes linked to antibiotic resistance through hybridization methods has been one approach. Although this approach is quicker than culture-based techniques, it necessitates the creation of tests specific to each resistance gene and might not detect resistance brought about by novel mutations. A novel solution to this problem is provided by CRISPR-Cas9 gene editing, which works by essentially "training" the Cas9 protein to identify antibiotic-resistant DNA sequences from any type of bacterium and then using that knowledge to enable the quick identification of resistant strains. By developing a CRISPR-Cas9 system that can precisely target and cleave the DNA sequences of antibiotic-resistant tuberculosis strains, Gentleman et al. have demonstrated proof of principle.

The World Health Organization has warned that the advent of antibiotic-resistant bacteria

could mean "the end of modern medicine as we know it." For this reason, it is critical to quickly identify strains of bacteria that are resistant to antibiotics. This is now accomplished by cultivating bacteria, subjecting them to a range of antibiotics, and determining which of the medications they are resistant to. It can take up to 48 hours to complete this laborious operation, and occasionally the bacteria themselves cannot be cultivated. A practical response to this expanding problem is the creation of a quick diagnostic test for the detection of antibiotic-resistant bacteria, and CRISPR-Cas9 gene editing is leading the way in this area.

5.3. Case study 3: Non-invasive prenatal diagnosis using a CRISPR-Cas9-based technique

Creating diagnostic tests for infectious diseases and genetic disorders is a versatile use of the CRISPR-Cas9 system. This section outlines the potential application of the CRISPR-Cas9 system to create a non-invasive prenatal diagnostic test for β-thalassemia, a genetic condition that is prevalent in numerous populations. To avoid thalassemia, molecular diagnostics and gene editing are employed. This test can assist couples who are at risk of having a child affected by β-thalassemia and can be helpful in the early detection of an affected fetus during the first trimester of pregnancy. For at-risk couples, the usual course of action to avoid having an affected child born is prenatal diagnosis and subsequent abortion of the diseased pregnancy. Parents experience emotional distress and guilt as a result of this. We are offering families a different, less stressful option that gives them hope in avoiding having a child with the condition. This is accomplished by providing a fetus with an option through early detection of the ailment and subsequent in-utero treatment aimed at curing the fetus. While β-thalassemia is classified as a serious condition, there are numerous additional genetic abnormalities as well as other types that, while not fatal, can nonetheless have an impact on a person's quality of life. Due to the non-invasive nature of these tests, high-risk couples with a variety of genetic abnormalities can use them to determine their alternatives and receive an early diagnosis of an affected fetus.

6. Prospects and Difficulties for the Future

CRISPR-Cas9 is currently only used to demonstrate genetic alterations in vitro, despite its potential to change diagnostic tests. Creating a quick, user-friendly diagnostic test in a clinical context still needs a lot of effort. Using CRISPR-Cas9 to attach mutant DNA sequences preferentially and then indicate the mutation is one such technique. By combining different designed RNA with dCas9, this has been accomplished. A recent and intriguing discovery involves the utilization of Cas9's collateral activity to cut ssDNA, thereby establishing a DNA mismatch repair (MMR) toehold that triggers the fluorescent reporter of viral infection. It is anticipated that CRISPR-Cas9 diagnostic testing will be possible in a clinical context as the dCas9 system develops. A perfect diagnostic test would be able to distinguish between cancer, hereditary problems, and infectious diseases. It frequently takes a long time to cultivate bacteria or viruses in order to diagnose infectious disorders. Gootenberg et al. used the sensitivity of Cas13 in a recent investigation to identify the Zika virus in patient samples and distinguish it from dengue sickness. This might result in the development of quick testing for infectious diseases based on nucleic acids. Owing to CRISPR-Cas9's enormous potential, diagnostic tests will probably be modified for use in a range of clinical contexts.

6.1. Prospects for improving CRISPR-Cas9 diagnostic assays

Modifying the Cas nuclease to increase the range of modifications it may cause is one of the most important recent advancements in genome editing. The tendency of the double-strand break (DSB) that the CRISPR-Cas system causes to be repaired by non-homologous end-joining is a significant disadvantage in comparison to other nuclease-based genome engineering platforms like zinc-finger nucleases and transcription activator-like

effector nucleases. Small insertions and deletions occur at the break site as a result, which can result in "nulomorphs," which are hard to describe and could not have the desired effect. A homologous DNA template is used in homology-directed repair, an alternate technique for DSB repair, to "repair" the break. To introduce desired sequence adjustments, use this. Hence, modifying the nuclease to produce a "nickase," a single-stranded DNA break that can only be fixed by HDR, or single-stranded DNA repair, was a milestone for the CRISPR-Cas system. This was initially shown by Jinek et al., who used the CRISPR-Cas system to induce a site-specific DSB in eukaryotic cells for the first time. Using CRISPR-Cas alone or in combination with other DNA modification technologies is another way to expand the scope of modifications that can be made. Hu et al. recently demonstrated DiTA-CRISPR, a fusion of CRISPR-Cas9 and DNA adenine methyltransferase that enables programmable conversion of adenine to inosine.

The development of instruments able to introduce a double-strand break at a particular DNA region is essential for effective genome engineering. Earlier methods frequently depended on simpler RNA-based systems and specially-made proteins. On the other hand, genome editing has seen a revolution thanks to the recent invention of the CRISPR-Cas system. This is a streamlined approach of targeting double-strand breaks using RNA programming. It is simple to employ, readily available, and usually consists of a fusion of CRISPR RNA and trans-activating CRISPR RNA called a "single-guide RNA" molecule that instructs the Cas endonuclease to produce a site-specific DSB.

6.2. Challenges with regulations and ethical issues

Three phases of evaluation are outlined by Gantz and Bier for gene-drive-based techniques, with a focus on eliminating disease vectors. These entail getting opinions from a range of specialists and stakeholders, then iteratively reworking the suggested intervention in light of the input received. For all hereditary illnesses, it is imperative that comparable procedures be used for gene editing and diagnostics, along with transparent public information sharing regarding the advantages, drawbacks, and alternative approaches to disease control. Rules should be based on the state of science at the time they are created and should be flexible enough to accommodate new discoveries. The success of this endeavor hinges on the intricate interactions among scientists, physicians, funding agencies, ethical committees, government agencies, general public sentiment, and the perspectives of individuals impacted by particular illnesses.

There is a tangible sense of enthusiasm about the advancements in CRISPR gene editing and diagnostic techniques. However, it needs to be weighed against a thorough comprehension of the moral and societal ramifications of this technology. Fears of eugenics have put CRISPR methods under intense scrutiny, and our current understanding of genetics is far too primitive to allow us to predict the full effects of gene modification on an individual and future generations. This is despite the allure of simple fixes for genetic diseases and novel detection capabilities. The safety and effectiveness of gene editing in disease models must be thoroughly validated before CRISPR-based diagnostic tests move forward with human trials and use. Additionally, there are still a number of unanswered questions regarding the unintended consequences of curing disease and the off-target effects of CRISPR methods that need to be resolved.

6.3. Future Development: Cooperation and Multidisciplinary Approaches

The development of diagnostic tests is the outcome of cooperation and coordination between many government, business, and academic entities. Academic experts in the fields of genetics, molecular biology, and clinical sciences will be important in the development and validation of CRISPR-Cas9 diagnostic tests at the fundamental research level. These scientific disciplines' multidisciplinary research facilities will be able to create customized tests for diseases and verify their usefulness against existing diagnostics. Funding for these developments

comes from the government and charitable groups, and the public health sector frequently collaborates with industry and academic researchers to enhance diagnostics for diseases like HIV and tuberculosis. Industry alliances can range in size from tiny startups to major biotech or pharmaceutical firms. Although partnerships with larger corporations are typically necessary to get a CRISPR-Cas9 diagnostic test from research to clinical trials and into the marketplace, small enterprises play a crucial role in advancing breakthroughs toward diagnostic testing. An instance of the latter would be the recent collaboration between academic researchers and The Innovative Medicines Initiative, an alliance between the European Union and the European pharmaceutical industry, with the goal of creating a tuberculosis diagnostic test utilizing alternative technologies, the results of which will be relevant to CRISPR-Cas9 diagnostic tests in the future.

7. Conclusion

The ethical concerns and environmental effects of the CRISPR/Cas9 system's ongoing use must also be kept in mind. This method may potentially be utilized not only to provide diagnostic tools but also to perform gene therapy directly on patients in a diagnostic context. But even though this seems very cutting edge, novel, and unsettling, humans have been tampering with our own DNA for a very long time, and this type of gene therapy is just more straightforward than earlier approaches. But this is insufficient justification to accept it and go on. Numerous genes have several effects, frequently in distinct locations. These genes cannot be "turned off" easily; many cuts and guide RNAs are needed, which raises the possibility of off-target consequences and leaves the work partially completed. Currently, efforts are being made to reduce off-target impacts. Additionally, there's a chance that fresh mutations will emerge at the repair site; the long-term consequences of these changes are unknown. Finally, stem cell research has shown that learning how to do something typically requires first learning how not to do it. This could be extremely risky when it comes to gene therapy. Having said that, a clear risk-benefit ratio for many genetic disorders will emerge as techniques advance, and CRISPR/Cas9 gene therapy may become a practical therapeutic alternative. Transgenic organisms may also have effects on the environment that go far beyond those of the diagnostic instrument itself. With current technology, it is possible to introduce a desired DNA sequence into any creature and use the CRISPR/Cas9 system to insert that sequence into the genome at a specific spot. Anything that can be done will be done, according to the cause-and-effect biology concept. A reasonably dispensed-with diagnostic tool could pave the way for gene-driven transgenic organism propagation aimed at directly treating disease-specific regions. To determine what constitutes an appropriate degree of intervention and what constitutes unacceptable dangerous forays into the unknown, similar ethical analyses will be needed for each of these environmental factors.

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