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CRISPR Cas9 and its Uses in Bowel Disease: A Review

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Abstract: ***CRISPR Cas9 is a groundbreaking genome editing tool that allows precise modifications of genes, offering significant potential in treating hereditary diseases. This paper explores the application of CRISPRCas9 in improving gut health, specifically focusing on Crohns disease, ulcerative colitis, coeliac disease, and malrotation. Gut diseases, which affect digestion and nutrient absorption, pose serious health challenges. Despite ongoing research, there remains a considerable gap in effective treatments. CRISPRCas9 provides a promising avenue for genetic intervention, potentially revolutionizing the management and treatment of these complex diseases.***

Keywords: CRISPR Cas9, gut health, Crohns disease, ulcerative colitis, genetic therapy

1. Introduction

CRISPR cas9, or clustered regularly interspaced short palindromic repeat (CRISPR) and their associated protein (Cas - 9) is a genome - editing tool that can be used to replace, add, or remove specific genes. This technology is being heavily researched, with specific interest in its potential uses relating in treating or curing hereditary diseases.

The gut is a vital organ, comprising of the small intestine and the large intestine. It plays a vital role in digestion and absorption of nutrients and water, making a healthy gut necessary for survival. Gut diseases occur in every population, and cause discomfort and pain, sometimes even requiring surgery.

This paper will focus on the question "What is the role of CRISPR Cas9 as a genetic tool and how is it being applied in gut disease to improve health?" focusing on 4 specific diseases or abnormalities: Crohn's disease, ulcerative colitis, coeliac disease, and malrotation. Crohn's disease and ulcerative colitis are both conditions that fall under inflammatory bowel diseases, or IBD. Coeliac disease is a gluten intolerance, where the symptoms range from.

While research is being conducted, and progress has been made, gut disease remains poorly understood and there is still immense potential for improved diagnosis and treatment.
III. Gut disease and its effect on populations

Coeliac Disease:
When ingesting gluten, Coeliac disease causes an immune response in genetically susceptible individuals, ranging from severe malabsorption to non - symptomatic presentations. The cause of the response is unknown [1], but the incidence of coeliac disease has been rising [2]. This is attributed partly to a rise in detection, but there has also been a real increase in incidence, unrelated to disease detection [2]. This could be a result of environmental factors promoting an increase in intolerance to dietary gluten [2].

Coeliac disease is one of the most common autoimmune disorders, with it's prevalence ranging from 0.5 - 1% of the population [3]. It is hard to detect as there is a very wide range of clinical presentations. Abdominal pain and growth issues are common presentations in children, while anemia, osteoporosis, and recognition at endoscopy performed for GERD are seen as modes of presentation in adults [4].

Coeliac disease is a unique condition, since we know exactly what the key genetic components are. Human leukocyte antigen (HLA) - DQ2 and HLA - DQ8, the auto - antigen involved (tissue transglutaminase (tTG)), and the environmental trigger which is gluten are all well researched and known [3]. Gluten is a relatively recent addition to the diet, and is a digestion - resistant protein. One issue with research into coeliac disease is the lack of an appropriate animal model for research, as it is nearly impossible to replicate human presentations of coeliac disease [3]. As with many autoimmune diseases, coeliac prevalence has increased recently, though the reason behind this is not confirmed. There are theories that suggest the increase is due to an increase in exposure to pathogens, which other theories suggest that the increase is due to an increase in hygiene, and therefore a decrease in exposure to pathogens [3].

Similar to other autoimmune diseases, Coeliac is highly related to genetics, and has a strong hereditary component. This can be seen by its high familial occurrence, and how the disease is often presented in two monozygotic twins [3].

In terms of clinical presentation, celiac disease is more frequently diagnosed in females as compared to males, and it is diagnosed at all ages. It if often diagnosed around the age of two, as gluten is introduced to the diet. It is also often diagnosed from the ages of 20 - 30. Celiac disease can develop at any age, and some studies have reported the loss of gluten tolerance in adulthood. In most cases, however, Celiac develops before the age of 10. Celiac is more common in women than in men, however men are more likely to remain undiagnosed. Celiac occurrence also differs between country and geographical location, one cause of this potentially being dietary differences. There are also racial and ethnic differences in celiac disease prevalence.

When evaluating CD, there are two different categories patients can be placed under, symptoms displaying active CD and the presence of associated CD conditions [3].

Diagnosis requires either the demonstration of small intestinal villous atrophy on biopsy [3], which is the erosion of the microvilli present on the wall of the small intestine, or an unequivocal response to a gluten - free diet [3]. In the case of children, duodenal biopsies can often be avoided in the

presence of strict symptomatic criteria [3].

The most common treatment course is a strict adhesion to a gluten - free diet, however in some cases symptoms are recurrent or persistent [1]. Both a persistent response and difficulty adhering to a gluten - free diet have resulted in research into pharmacologic therapies, some of which are currently undergoing human trials [1].

Crohn's Disease:

Crohn's disease is a chronic spontaneous inflammatory bowel disease of the gut, with symptoms including skip lesions, which are patches of inflammation, and transmural inflammation, which causes the bowel wall to thicken [5]. It primarily affects the gastrointestinal tract, ranging from the mouth to the anus [5]. Crohn's disease could be a result of genetic susceptibility, environmental factors, and altered gut microbiota, leading to dysregulated immune responses [6].

The most common localization for intestinal Crohn's disease is the terminal ileum and ileocecal area [7]. The presenting symptoms depend on the individual, but often include diarrhea, abdominal pain, weight loss, nausea, vomiting, and in certain cases fevers or chills [5]. Extraintestinal manifestations, which occur outside the gastrointestinal tract, may develop, including anemia, mouth sores, inflammation of your eyes or joints (arthritis), osteoporosis, skin conditions, cancer, and liver conditions [5]. The liver conditions include fatty liver disease (an excess of fat deposited in the liver), or drug - induced liver injury, caused by the medications prescribed to treat the inflammation from Crohn's.

There are three main phenotypes of Crohn's: inflammatory, stricturing, and penetrating disease, and patients can have one or more of these phenotypes presenting throughout their disease course [5]. Patients often present only with inflammatory symptoms, but this phenotype can later progress to the stricturing or penetrating phenotype. The stricturing phenotype indicates a narrowing of the lumen of the intestine [5]. The inflammation caused by Crohn's disease leads to the formation of scar tissue, which is not as flexible as regular tissue, and therefore it behaves differently. When there is scar tissue in the intestine, the lumen is affected, and it may become narrowed or obstructed (blocked). The narrowed part of the lumen is called a stricture. The waste material can't pass through the lumen easily, which can lead to pain, bowel obstructions, or other complications. Strictures tend to be more commonly associated with Crohn's disease than with ulcerative colitis, however they can occur in both forms of IBD.

Pharmacologic therapy is usually the first - line method for disease management, depending on the severity of the symptoms. There is no cure, however there are different types of pharmacologic treatment, such as anti - inflammatory drugs or immunosuppressants [5]. The first step is often anti - inflammatory pharmacologic therapy, such as corticosteroids, which help reduce inflammation. Previously oral 5 - aminosalicylates were widely used, however now they are considered to be of little benefit. Immunosuppressants are also used, as they reduce inflammation by targeting the immune system. Examples of a commonly used immunosuppressant are zathioprine (Azasan, Imuran) and mercaptopurine (Purinethol, Purixan), however these treatments often have side effects, such as reduced resistance to infection and inflammation of the liver [8]. The goal of the medication is controlling inflammation and clinical remission; however, it is estimated that around one in four patients eventually need surgery. Other treatments used also include pain - relievers, biologics (therapies that targets proteins made by the immune system), anti - biotics, and anti - diarrheals. Around 80% of Crohn's patients need surgery at some point during their lives, however, surgery is not curative, and the risk of disease recurrence is up to 40% [7]. The most common surgical options in colonic CD are total proctocolectomy (TPC) with permanent ileostomy, segmental bowel resection, subtotal colectomy. TPC completely removes all colonic and rectal disease and avoids the use of a potentially diseased anus [7].

Some patients have recurring periods of active inflammation, and they can present with bloating, diarrhea (can include mucus and blood), fever, weight loss, and anemia. In particularly severe cases, conditions such as perianal abscess (a collection of pus in confined to the perianal area), or perianal Crohn disease can be seen [7]. If the flare - ups are also originating from the small bowel, Crohn's may present with diarrhea, malabsorption, weight loss, abdominal pain, and anorexia [7].

Immune system dysregulation is also involved in CD. Innate lymphoid cells (ILCs) contribute to the maintenance of intestinal barrier integrity. The innate lymphoid cell (ILC) family contains natural killer (NK) cells, ILC1, ILC2 and ILC3, which participate in immune responses to virus, bacteria, parasites and transformed cells [11].

Malnutrition is one of the most common results of Crohn's disease, detected in 65 - 75% of patients [12]. This can be the result of reduced intestinal absorption, gut microbiota changes, and the loss of appetite. Deficiencies such as vitamin A, D, and folate are among the most common [12]. Patients with extensive bowel resection treatment may have increased malabsorption of vitamin B12, as well as vital nutrients such as magnesium, zinc, and iron causing deficiency in these nutrients. In children, energy can be diverted from growth to disease activity, therefore children are advised to increase their caloric intake [12].

Diets are often recommended, to make sure all the vital nutrients are consumed. Liquid diets, enteral nutrition, and parenteral nutrition are used, usually during a relapse, to induce remission. Enteral nutrition excludes all solid food while ensuring the necessary number of calories are consumed. This treatment lasts from 6 - 8 weeks. EN is administered orally, as a drink, powder, dessert - like snack, or via a feeding tube. Enteral nutrition combined with a regular diet can also be used as maintenance diet during remission [12].

Ulcerative Colitis:

Ulcerative colitis is a chronic inflammatory disease affecting the colon, which is the longest part of the large intestine [13]. IBD goes through periods of active disease and remission. In times of active disease, there is inflammation that affects the surface layer (mucosal layer) of the intestine, which can cause

ulcers [13]. Patients with ulcerative colitis have mucosal inflammation starting in the rectum that can extend to neighbouring parts of the colon [13]. It often presents as bloody diarrhoea, and diagnosis is made by a colonoscopy and histological findings. There are multiple factors playing a role in the cause of the disease, including genetic predisposition, epithelial barrier defects, dysregulated immune responses, and environmental factors [13], such as diet, particularly western diet influences, and medication [14].

There are three different diagnoses for ulcerative colitis: proctitis, left - sided colitis, and extensive colitis, with each diagnosis representing a spectrum of disease characterized by increased area affected [13]. Proctitis is inflammation in the lining of the rectum and affects 30 - 60% of patients. The symptoms include rectal bleeding, tenesmus, and urgency, and up to 10% of patients with proctitis or left - sided colitis suffer from paradoxical constipation [13]. The next diagnosis is left - sided colitis, where the inflammation extends from the rectum up through the sigmoid and descending portions of the colon [13].

Extraintestinal manifestations occur in up to a third of patients suffering from ulcerative colitis, and up to a quarter have extraintestinal manifestations before the diagnosis[13]. Peripheral arthritis, which is inflammation or degradation of the large joints in the body such as the knees or elbows, is the most common extraintestinal manifestation [13].

Treatments for the disease include 5 - aminosalicylic acid drugs (Mesalamine), steroids, and immunosuppressants to target both inflammation and immune dysregulation [15]. Some cases require a colectomy, a surgical procedure to remove all or part of the colon. The range of pharmacologic treatments for ulcerative colitis is expanding, and multiple drugs with different targets will be released in upcoming years [15]. The new development of biologics and Janus kinase (JAK) inhibitors might be able to induce and maintain remission [15].

With new understanding regarding the complexity of the causes of ulcerative colitis, new treatments, such as anti - tumour necrosis factor treatment have substantially improved outcomes [15]. Previously, treatments focused on symptomatic relief, but now with the recent developments, long - term health [15].

Similarly to the goals for Crohn's disease, the main aim of treatment is to induce and maintain remission, with long - term goals including preventing disability, colectomy, and colorectal cancer. Remission includes improvement in bowel habits and cessation of rectal bleeding.

Patient Experience or Implications of IBD:

Patients' lives can be severely impacted by inflammatory bowel disease, as the disease has social, financial, and psychological effects [16]. Challenges of living with IBD are subjective, and there is a lack of suggestions to improve patient experience. Studies assessing patients' persepectives about IBD care focus on the need for added psychological and social support, such as increased awareness [16].

A study was conducted, asking patients' how many symptoms, and which ones were experienced over the last week [17]. A lack of energy was identified as the most life - affecting symptom, along with bowel symptoms of urgency, diarrhea, feeling bloated, and flatulence. In terms of psychological burdens, feelings of worry were the most intrusive and most common [17].

Stress has been linked to gastrointestinal disorders since the 1930s, with depression being twice as common in IBD individuals [17]. IBD has a very unpredictable nature accompanied by frequent relapses, worsening the emotional toll the disease can take on one's mental wellbeing. To adjust, patients must make significant lifestyle changed and find suitable coping strategies. Some patients opt for maintaining low baseline stress levels through meditation, yoga, or other types of exercise, while others opt for counseling.

There are also financial burdens when dealing with IBD, as the cost of treatment has substantially increased over the past two decades. The cost increase is attributed to new treatments, mainly biologics [16]. Since IBD is becoming more and more common, the psychological and financial burden is becoming a bigger issue [16].

Malrotation:

Malrotation is a congenital anomaly, where the rotation of the embryonic gut is disrupted in the uterus. Rotational anomalies often remain asymptomatic throughout a person's life, meaning that often, malrotation goes undetected. Most cases present themselves in the first year of life, however, some cases are diagnosed in children or adults. The incidence of mid - gut malrotation is estimated to be around 1 in 6000 live births [19]. Through colorectal screening, it is estimated that around 0.17% of adults have intestinal malrotation [18].

This is a rare anomaly in adults, therefore diagnosis in children and adults is often delayed. As a result of the incorrect rotation of the embryonic gut, two variations develop, Ladd's bands or a narrow mesenteric base [19].

The clinical presentation for malrotation is often similar for most cases [20]. In neonatal malrotation, in around one - third of the cases, malrotation presents with volvulus (an obstruction caused by the twisting of the stomach or intestine). The symptoms of volvulus include vomiting and severe pain [20].

In asymptomatic adults, the anomaly is usually discovered through tests for other purposes.

The symptoms often experienced include intermittent abdominal pain, occurring for periods and then pausing, which occurs in around 40% of patients [21]. Then, on and off vomiting, followed by weight loss due to a lack of proper nutrition. Food intolerance, and chronic diarrhea are also reported symptoms [21].

Some adults report severe intermittent pain and vomiting, which may be due to periodic volvulus, creating a blockage for a period of time [21]. This makes the anomaly hard to diagnose, as the intestine can revert back to its normal position and pain can subside before being tested.

10 - 15% of adults present with acute midgut volvulus, a life - threatening anomaly. These patients experience severe pain,

nausea, vomiting, hematemesis, hematochezia, or hemodynamic instability. If acute midgut volvulus is presented, the patient may be immediately taken into emergency surgery [21].

CRISPR

How does it work?

Clustered regularly interspaced short palindromic repeat (CRISPR) and their associated protein (Cas - 9) is the most accurate and efficient method used to edit genomes in all living things [22]. It can be used to remove, replace, or add a sequence of bases. CRISPR evolved naturally in bacteria and archaea as a defense mechanism against phage infection and plasmid transfer, and it forms the backbone of CRISPR - Cas9 genome editing technology [22]. Many diseases have a genetic component, with some caused by mutations in only one gene.

Gene - editing technology is being developed to control diseases at a genetic level, in vivo. The two necessary components in the CRISPR - Cas9 system are the Guide RNA, which recognizes the sequence to be edited, (gRNA) and the CRISPR - associated Cas9 proteins, which break the DNA. There are three steps in the process: recognition, cleavage, and repair. The target sequence of the genome is determined beforehand [22]. During the recognition step, the gRNA finds the target sequence, using a landmark sequence known as the protospacer adjacent motif (PAM) sequence, in the specific gene needed, recognizing it through a complementary base pair, the fundamental unit of double - stranded nucleic acids. Then, the Cas - 9 nuclease makes double - stranded breaks at this 3 - base pair site [22].

CRISPR is utilized in many disciplines, such as medicine, agriculture, and biotechnology [22]. For example, in agriculture, CRISPR is being used to design new grains, which improves their nutritional value. In the medical industry, it is being further researched in the areas of cancer, HIV, and gene therapy, for example sickle cell disease, cystic fibrosis, and Duchenne muscular dystrophy [22].

DNA is the carrier of information across generations of reproducing organisms, containing the genetic code that determines which proteins an organism produces [23]. DNA is a dual - linear polymer in a double helix, made up of the 4 different bases (A, C, T, G). Different genes code for different proteins, of which there are estimated to be between 20, 000 and 25, 000. Changes in the sequence of bases in a gene translate to different proteins being created, leading to changes in the real world. This relationship can be exploited and used to modify genes.

An important goal with studying human genetics is to identify which specific DNA sequences and genes influence biological traits, especially regarding disease genetics. Many diseases have large genetic components, such as cystic fibrosis, or specific types of cancer. And almost all genetic diseases are influenced by genetic variation. With the exponential growth in technology, data, and analytical tools, understanding of the complex mechanisms contributing to phenotypes and genotypes has vastly improved [24].

The connection established between genes and diseases, specifically rare diseases, has rapidly been applied to genomic technologies. There are now specific genetic tests for almost all clinical presentations related to alleles with a large impact, as well as more specific tests to sort through longer lists of genes. Symptomatic individuals and relatives are now often tested in many medical specialities [24].

Both targeted gene tests (tests which look at a subset of 100 - 500 genes) as well as genome sequencing (looks at all genes) are used, however, an advantage with genome sequencing is the ability to reevaluate specific sequences. This is especially helpful in the case of newer discoveries identifying different causal genes.

Ethics on CRISPR

CRISPR is a powerful genome editing tool, with many applications that could revolutionize the medicine, biotechnology, and pharmaceutical industries. However, as with any tool that directly alters the human body and potentially changes thousands of lives, there is a moral implication.

What ethical considerations should be had when using CRISPR, and how should we strictly regulate the use of it?

CRISPR appears to offer considerable promise in a wide variety of disease contexts. For example, around the world, at more than 15 clinical trials— focused on multiple myeloma, esophageal, lung, prostate, and bladder cancer, solid tumors, melanoma, leukemia, human papilloma virus, HIV - 1, gastrointestinal infection, β - thalassemia, sickle cell disease, and other diseases— involving CRISPR applications have been developed [25]. Moreover, as of May, 2018, in China at least 86 individuals have had their genes altered as part of clinical trials [25].

One issue with genome editing is the possibility of the edited genomes being passed down to the next generations, and so forth. U. S. federal funding cannot be used on research involving human embryos, however, following more research focused on assessing risk potential, as well as firm reasons for doing so, and considerable oversight. heritable genome - editing trials might be permitted. With gene editing such as CRISPR, there are a range of results and uses possible, and it is important to minimize risk while maximizing benefit.

Finally, the skeptical view is that even if the genome is edited as expected and the desired functional output is achieved at the time, the complex relationship between genetic information and biological phenotypes is not fully understood. This means that even if the genome is edited as planned, and the direct consequences are as planned, there may be long - term consequences, or off - target side - effects. As the edited genes can be passed forth, these side effects could affect hundreds of individuals. There are also concerns regarding the on - target efficiency, incomplete editing, and inaccurate genome editing. However, as the technology improves and gets revised, these problems become more negligible.

One recent controversy regarding CRISPR Cas9 application is China editing the genes of embryos, where in November

2018, media from all over the world reported that two twin girls had been born with modified genes to make them HIV immune. He Jiankui disabled the CCR5 gene that enables the HIV infection, however, there was no evidence provided to support this experiment [26]. This controversy sparked debate, and arguments both for and against the use of CRISPR on embryos were made [26].

CRISPR is an efficient, cheap way to edit genes, however, with the lack of understanding of the human genome, and the possible effects of gene - editing, there could be consequences not fully understood by genetic engineers.

Monitoring the use and application of gene - editing technology is important, to make sure individuals are not misusing or weaponizing the technology. Strict standards and criteria must be met before a project is allowed to continue.

2. Conclusion

This paper compiled research on the question “What is the role of CRISPR Cas9 as a genetic tool and how is it being applied in gut disease to improve health?”. It compiled reviews on the topics of clinical presentations, current treatment plans, patient experience, and future directions for different bowel diseases and anomalies. The gut diseases covered were Crohn’s disease and ulcerative colitis, both inflammatory bowel diseases, as well as malrotation, a congenital anomaly, and lastly, Celiac disease, a gluten intolerance. While the bowel diseases and abnormalities discussed in this paper are not fully determined by genetics, there is a genetic component, meaning that the CRISPR cas9 technology could be applied in these fields.

Gut health is extremely vital, however, it is an area of medicine and biotechnology that is not as heavily researched as it is often overlooked. With the development of CRISPR Cas9 gene - editing, the approach to treatments and cures for bowel diseases could be completely changed, and progress could skyrocket.

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