HIGH SCHOOL EDITION
Journal of Student Research
Volume 13 Issue 2 (2024)

Title: Personalized Medicine in Crohn's Disease

Abstract: *Crohn's disease* is an autoimmune disease that has no complete cure. Although the disease has no cure, there are many different ways to treat it. The cause of Crohn's disease is not entirely known yet. However, researchers suspect that a mutation in the NOD2 gene causes Crohn's, and they have found many statistics to prove that the Nucleotide Binding Oligomerization DomainCcontaining 2 (NOD2) is the reason for Crohn's. Many other genes contribute to the cause of Crohn's, but for this research paper, NOD2 is the main focus. NOD2 is a crucial gene in the immune system; because of its vital role, a mutation in this gene can have many effects. This paper will first discuss Crohn's and why NOD2 is essential. A popular and emerging topic in the field of medicine is personalized medicine. Personalized medicine is using or changing an individual's gene to help treat or cure the disease the individual is fighting. This paper will explore the details of personalized medicine and its different types. Personalization transforms the field of healthcare since perspectives are changing. Instead of everyone getting the same medication no matter how their body reacts to it, personalized medicine can provide a unique and personal treatment for the disease. To conclude, the paper will address and analyze the effect of personalized medicine on Crohn's disease and its impact.

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Discipline: Personalized Medicine, IBD, Gene Therapy, CRISPR-CAS9

Keywords: Inflammatory diseases, Gene therapy, Personalized Medicine, CRISPR-CAS9, Gene Editing

Personalized Medicine in Crohn's Disease

INTRODUCTION

Over three million people in the United States suffer from Inflammatory Bowel Disease, more commonly known as IBD. IBD can is caused by inflammation in the gastrointestinal(GI) tract. The specific cause of this inflammation is unknown, but many researchers have found that it could be because of a weakened immune system. However, another reason for the inflammation is environmental triggers and inherited diseases [1]. IBD is an extensive disease; two types are the most common: Crohn's Disease and Ulcerative Colitis. To identify the two types, doctors use endoscopy or colonoscopy to identify Crohn's and Ulcerative Colitis. Medications or removing parts of the intestinal tract cannot cure these two diseases.

This sole reason makes Crohn's and Ulcerative Colitis a disease that a person has to deal with their entire life. Among the two, Crohn's Disease is one of the most common types of IBD [2].

Crohn's is an issue that has no known cure, but many treatments and medications can help improve the symptoms. Advancing technology could allow researchers to find a cure for Crohn's by using personalized medicine. Personalized medicine is the process of using one's genes and proteins to help find a potential treatment or cure for a disease they are affected with. Personalized medicine has many benefits, and one of them is that it allows doctors to prevent diseases instead of having to treat them.

CROHN'S DISEASE

Currently, the cause of Crohn's disease is not fully understood. Many researchers believe a combination of genetic, environmental, and lifestyle factors causes it: smoking, unhealthy diets, lack of sanitation, and many other factors. Doctors can use stool tests, blood tests, upper GI endoscopy/enteroscopy, capsule endoscopy, or colonoscopy to diagnose Crohn's. In the endoscopy process, a long tube with a camera is passed through an opening in the body [4]. This process can help view the inflammation in the upper part of the GI. In a colonoscopy, the doctor uses the tube to view the lower part of the intestine, the colon, or the ileum (small intestine). Lastly, in capsule endoscopy, the patient is given a capsule with a camera to take pictures of the digestive tract. The pictures are sent to a transmitter for the doctor to observe and identify symptoms [5].

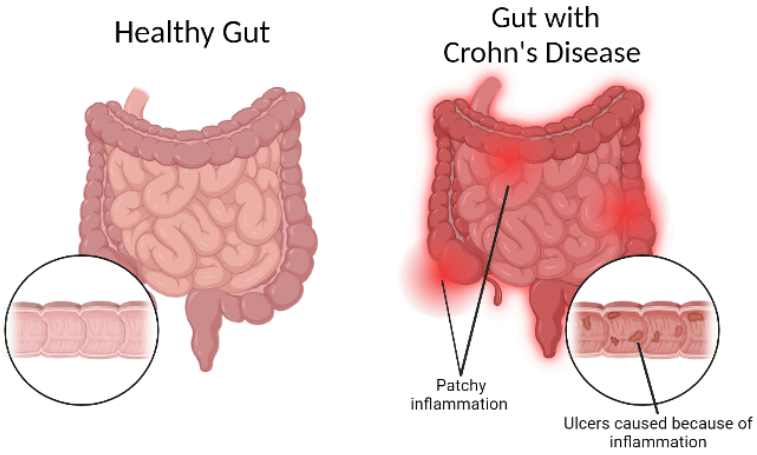
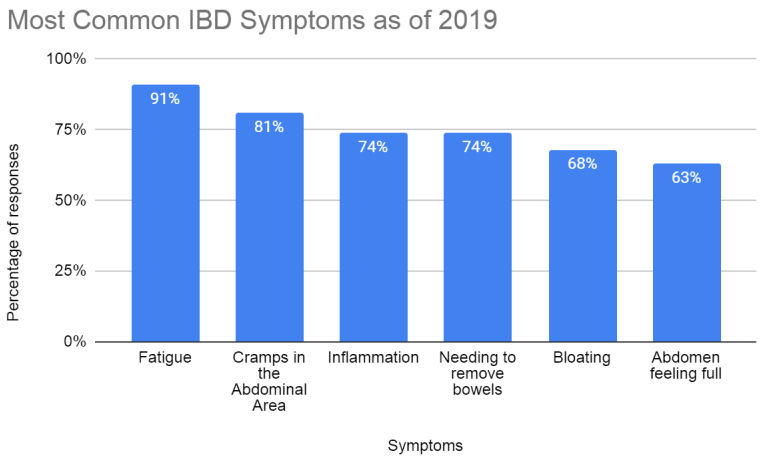


Figure 1: Normal and healthy gut (left) and gut infected with Crohn's disease with patchy inflammation and ulcers (right). Created and copyrighted by Divyasree Prem Sankar

An individual affected by Crohn's can experience many symptoms; some of the most common ones are cramping, weight loss, appetite loss, and fevers. These symptoms occur in random bursts throughout an individual's lifetime. Crohn's causes chronic inflammation in the gastrointestinal tract (GI), especially in the small intestine and small parts of the large intestine. It occurs in small patches which form near healthy tissues and can go through many layers of walls in the intestinal tract. The tissues with this inflammation can become swollen and develop ulcers (open sores). Besides ulcers and swollen tissue, inflammation can cause chronic bleeding, leading to anemia and a lack of blood cells in a person's body. Apart from anemia, fistula and abscess are two things that can show up because of Chrons. The fistula is a connection created when the ulcers break through the intestinal wall and form pathways between the intestines, other parts of the intestine, or other structures located nearby, and abscesses are pockets filled with pus and are infectious [2]. The disease can also impact the structure of the intestine and cause it to thicken areas of the intestine, causing a blockage, which can disrupt the processes in the intestines [3].

Figure 2: A survey was conducted to see which symptom was the most common among patients as



of 2019. Source [Statistica]. Graph created and Copyrighted by Divyasree Prem Sankar

Crohn's disease is an autoimmune disorder. This means that the body's immune system attacks healthy tissue since the body thinks that the tissue is dangerous [6]. This autoimmune response is caused because

of bacteria in the digestive system that triggers it. The triggered immune system can cause the inflammation that occurs in Crohn's [7]. Researchers have found four central genes related to Crohn's: NOD2, ATG16L1, IL23R, and IRGM. All four genes play an extremely vital role in the immune system and are crucial for autophagy. If there is a mutation in these genes, it can disrupt both these vital processes [2]. The gene that is most commonly related to Crohn's is NOD2. Researchers have found that a frameshift mutation in NOD2 causes Crohn's disease.

NOD2

NOD2 is an abbreviation for nucleotide-binding oligomerization domain containing 2. This gene is located on chromosome 16, specifically, 16q12.1 [11]. It is composed of three domains: CARD (caspase activation and recruitment domains), NOD (nucleotide-binding oligomerization domain), and LRR (leucine-rich repeat) [18].

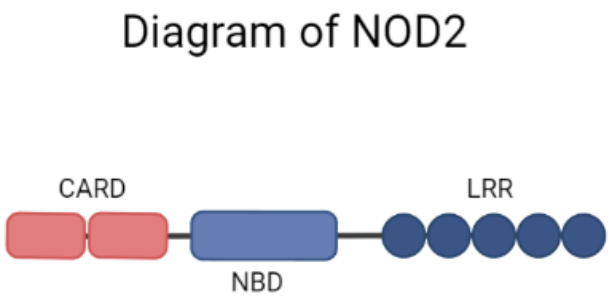


Figure 3: This shows a simple diagram of the NOD2 gene and what it is composed of. Image created and copyrighted by Divyasree Prem Sankar

This gene plays an extremely vital role in the immune system and helps the body protect itself from diseases. NOD2 is found in Paneth cells, which help protect the intestinal wall from infections. Paneth cells are cells that are found in the intestinal lining. NOD2 is vital because it helps the immune system respond correctly by recognizing pathogens. With it, the immune system would respond to unknown invaders correctly. This shows that a mutation in this gene can cause many obstacles in the immune system's response. When the immune system does not respond correctly, it can lead to autoimmune diseases, where the body attacks healthy tissues instead of infected tissues. NOD2 helps the immune

system by activating nuclear factor-kappa-B, a protein complex. This specific protein complex helps manage the genes that control the immune system and inflammatory reactions. Apart from the immune system, NOD2 also plays a vital role in autophagy and apoptosis, two essential processes in maintaining homeostasis. Autophagy is the process cells use to destroy foreign invaders and protect them from diseases and infections. It also plays an important role in apoptosis, when cells destroy themselves [12].

As indicated earlier, mutations in NOD2 can cause autoimmune diseases; one example is Crohn's. Researchers have found that three primary variants of NOD2 can cause Crohn's. The three are a frameshift (insertion) mutation in NOD2, a conversion from glycine to arginine, and a conversion from arginine and tryptophan [8, 14]. Glycine, arginine, and tryptophan are all crucial amino acids needed for the body. Glycine has an anti-inflammatory property, arginine helps the body build proteins, and tryptophan is needed to produce and maintain the proteins [19, 20, 21]. Patients with Crohn's who have a NOD2 mutation have shown that many parts of their immune system have declined in how they respond. For example, the production of alpha defense was shown to have reduced in these patients. Alpha defenses are crucial in the immune system since they help fight bacteria. An individual with a mutation in NOD2 has a very high chance of getting affected by Crohn's disease because of the significance NOD2 has in the immune system [14].

PERSONALIZED MEDICINE

A new topic that has been emerging in the past few years is personalized medicine. The idea of personalized medicine is pretty simple. It is when a person's genes and proteins are used or modified to find ways to prevent or treat diseases. These treatments help provide new ways to treat patients since the treatment would be more customized. This is crucial since it can focus on preventing disease rather than trying to find a way to cure it when one person is affected. A benefit of this approach is that many healthcare costs can be reduced. Personalized medicine is beneficial since a medicine that works for one person might not work for another person. Medicine given to patients must be able to help with the disease they are fighting and not make it worse since everyone has different genes; even if two people

have the same disease, they have different variations that make up the disease. This slight variation can cause them to react differently to medicines. Personalized medicine is safer since there is a better selection of medicine, and it can also provide an opportunity to improve the development of new drugs in the future.

The idea of personalized medicine was introduced to the public by an article in 1999. The article "New Era of Personalized Medicine: Targeting Drugs for Each Unique Genetic Profile" was written by Robert Langreth and Micheal Waldholz. The article stated that having more than one type of medicine to treat patients was ineffective since the drug worked for only 50%-70% of patients. The two authors emphasized the idea of disease heterogeneity and genetic variability. They discussed how a diagnostic test could help physicians identify which patients would benefit from certain drugs and which would not. The FDA approved the first idea of a diagnostic test, and in 1998, these treatments could be performed on breast cancer patients with tumors expressing the HER2 protein. An immunohistochemical test used to detect patients expressing HER2 was also approved during this time. This test helped select patients that would respond to treatment with trastuzumab, a drug used to deal with breast cancer that is HER2 positive. This made trastuzumab the first targeted cancer drug. Another significant milestone for personalized medicine was met when a drug to help fight leukemia was introduced. Researchers have found and learned more about ways to incorporate personalized medicine into everyone's lives. They have found that more drugs must be developed to be effective and safer since diseases are very diverse. Although the idea of personalized medicine was introduced 24 years ago, there still needs to be a clear definition for it [29].

Personalized medicine is a unique field of research with many different types. Gene therapy is one of the many different types. Gene therapy can do three things:

- Replace a diseased gene with a healthy one.
- Inactivate the disease-causing gene.
- Introduce a new gene to support and help the body treat the disease.

Scientists extract stem cells from a patient and modify them to help the patient.

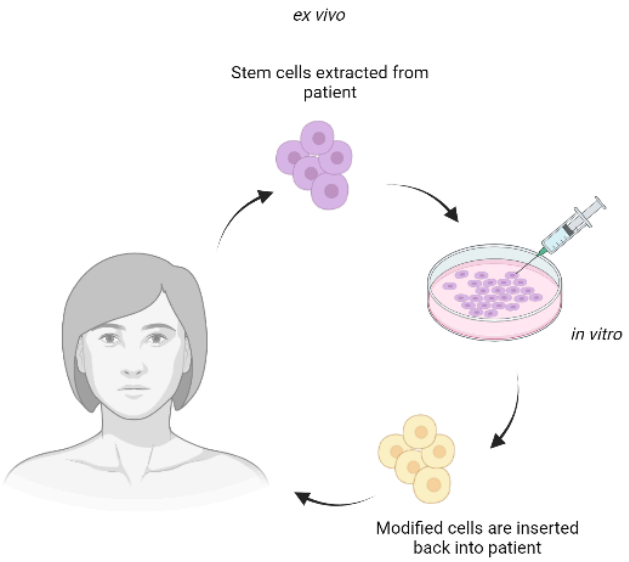
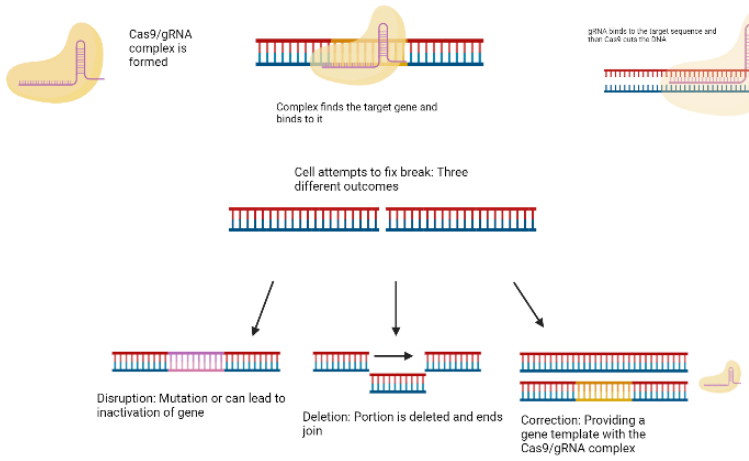


Figure 4: This diagram shows how gene-therapy works. Diagram created and copyrighted by Divyasree Prem Sankar

There are many types of gene therapy, and the most popular type is CRISPR-Cas9 (CRISPR). Gene editing is a unique field of technology that can provide a chance to change an organism's DNA. With the help of CRISPR-Cas9, genetic material can be added, removed, or even altered at specific destinations when instructed. The main reason CRISPR-Cas9 is so popular is that it is shown to be more efficient and accurate than any other technique. CRISPR-Cas9 is based on bacteria's ability to defend themselves from viruses because of how they have evolved. CRISPR stands for "clustered regularly interspaced short palindromic repeats," and Cas9 is a DNA-cutting molecule and can also be known as a nuclease.

Along with Cas9, CRISPR technology also uses gRNA (guide RNA). The gRNA binds to the Cas9, becoming a Cas9/gRNA complex. The Cas9/gRNA complex goes to the DNA and binds with it to start the editing process. Then the RNA pairs with the targeted region, and Cas9 cuts the DNA. After the DNA is cut, the cell recognizes this and initiates a process to fix the break in the DNA. During this process, three things can occur. A disruption occurs in the DNA, causing the gene to change or inactivate. Deletions can occur when two Cas9/gRNA complexes cut two targeted parts of the DNA to delete a section, and the two cut ends eventually join together. Corrections can be made when a DNA template is

provided with the Cas9/gRNA complex. In this process, the cell uses the template provided to repair the break made by the Cas9/gRNA complex. The reason scientists use CRISPR is for them to understand



mutations and inherited diseases.

Figure 5: A diagram of how CRISPR works. Diagram created and copyrighted by Divyasree Prem Sankar

Advancements in CRISPR can also provide researchers with ways to prevent and treat diseases. Another part of personalized medicine is cell therapy. Cell therapy uses living cells as a drug to treat many types of diseases, including autoimmune ones. Cell therapy introduces new cells to the body to help treat the disease, and this is different from gene therapy since gene therapy modifies the genes. These different methods to treat Crohn's can help reduce inflammation and return the body to homeostasis [10, 26-28].

PERSONALIZED MEDICINE IN CROHN'S AND LONG-TERM EFFECTS

Currently, personalized medicine is not being used in hospitals every day because of the need for more information it has. However, as more research is being done, it could be the future. A lot of the treatments for IBD now are all short-term. Advances in the field are being made since more research institutes can gain funding. Gene therapy and cell therapy are the methods that have been researched the most for IBD.

To begin with, for gene therapy to be used in IBD, there needs to be a vector. A *vector* is a vehicle created to deliver genetic material into a cell [31]. These vectors can be viral or non-viral. If a viral vector is chosen, they need to be changed so that they do not spread too much, and they have to be viral enough to affect the cells and help them. In this scenario, the IBD viral vectors must target the gut. A vector that could be useful for Crohn's is the lentivirus since it does not damage the linings of the GI or go beyond a certain point in the tract. The issue with using these vectors is that they cause cancer development, and more exploration needs to be done since safe vectors need to be found [17]. Instead of a lentiviral vector, an adenoviral one can be used. However, an adenoviral vector also has its negative impacts. An AAV (Adeno-associated virus)vector can be used from the viral vectors. This vector targets the GI and provides hope for gene therapy in Crohn's [17]. There needs to be more testing done on the AAV vectors since there has yet to be known data about the usage of these vectors. It may seem like all hope is gone for gene therapy in Crohn's, but it is only the beginning. As more research is being done on this topic, it is still a possible solution for Crohn's. They are moving on to Cell therapy which can provide more solutions. For Crohn's, more T cells can be introduced to the GI so that the gut can be more tolerant and prevent the inflammation from spreading. When the spread of inflammation is prevented, the body can return to homeostasis. T cells are a crucial part of the immune system, and they can help the body fight infection. Other than T cells, Mesenchymal stromal cells (MSCs) can also help the cell therapy process. MSCs are cells that play an essential role in many things, including inflammation. Some of their effects can be anti-inflammatory and useful in Crohn's disease [17]. MSCs help the immune system operate and can be found in the bone marrow. Like all other methodologies, using MSCs also comes with risks, and more research is needed. Van der Marel S et al. suggest that both cell therapy and gene therapy should be used when using personalized medicine in IBD (can be viewed here [17])

CONCLUSION

Implementing the different ideas of personalized medicine into a complex disease like Crohn's can be challenging. Based on the research done in this paper, it provides a guiding point and tells us that

personalized medicine could be the route for Crohn's disease and other types of autoimmune diseases. Although the impact of personalized medicine now might not be that big, in the future personalized medicine in Crohn's has a huge opportunity to grow and become more permanent. Personalized medicine not only provides us hope for Crohn's, but it can also provide us hope to find treatments for many other diseases.

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