The potential role of bacteria associated with the development of ulcerative colitis and Crohn's disease's etiology.

Bachelor's Thesis Life Sciences – WBBY901-05

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Abstract

Our microbiota is the result of millions of years of co-evolution and adaptation. The human microbiota is the composition of multiple microorganisms and consists of the 10 to 100 trillion symbiotic microbial cells possessed by each person, primarily bacteria in the gut. The relation between human health and the gut microbiota is being progressively recognised. It contributes largely to our health and to diseases, especially diseases that occur in the gastrointestinal tract. Due to this, having knowledge of which particular bacteria and microorganisms in the microbiota contribute to certain diseases is cardinal in order to identify and solve complications which can be life-threatening. Developments in genome sequencing technologies and bioinformatics have now enabled researchers to study these microorganisms and their function and microbe-host interactions in an elaborate manner both in health and diseases . Inflammatory bowel diseases (IBD) are chronic disabling gastrointestinal disorders that harshly impact almost every aspect of an affected individual's life. In Europe, there is an estimated number of 2.5 - 3 million people newly affected by IBD each year. The incidence and prevalence of IBD is increasing with the highest annual incidence of Crohn's disease being in North America (20.2 per 100,000 person-years). Therefore, the aim of this paper is to identify species of bacteria that are associated with the development of IBD and to decipher if there is a similar correlation with these specific bacteria in other diseases. The most commonly found bacteria that are associated with IBD patients are members of the bacterial families *Ruminococcaceae, Lachnospiraceae, Clostridiaceae* and *Coriobacteriaceae* . Currently there is a low number of viable IBD patient samples with visible and culturable microbes to research, which is the main problem and limits future implications to finding the unknown cause. More research and further experimental implications are still necessary to detect whether specific bacterial species significantly contribute to the diseases.

Chapter 1: General Introduction to IBD and scope of thesis

Inflammatory Bowel Disease

Inflammatory bowel diseases are chronic relapsing disorders and mainly describe two conditions which involve inflammation in the gut, Ulcerative colitis (UC) and Crohn's disease (CD) (Zhang, 2014) . Both diseases have their own specific challenges and side effects however with current anti TNF treatment patients can still have a good quality of life. The prevalence of the disease is higher in childhood than adulthood and will most likely become an extensive issue henceforward (le Berre et al, 2020). The diseases are similar however, CD and UC have very distinct pathobiology in regard to the spatial distribution and penetrance of inflammation along the intestine (Baumgart & Sandborn, 2007). Crohn's disease affects the mouth, anus, and the layers of the intestine whereas ulcerative colitis is a relapsing non-transmural disease that affects the mucosal layer of the colon and is limited to the colon (Baumgart & Sandborn, 2007).

According to a comprehensive study, the first symptoms of both disorders include vomiting, anemia, bloody diarrhea, pediatric growth disorders, abdominal pain and arthritis (Seyededian, 2019). Individuals who suffered an acute infection are more prone to develop UC or CD in the future. Ulcerative colitis is associated with rectal bleeding, severe abdominal pain and diarrhea, while in CD there is a risk of bleeding but in very severe cases only. Due to this, people with UC more often suffer from anemia and iron deficiencies whereas those with CD suffer from vitamin D and folate deficiencies (Seyededian, 2019).

In some instances in UC the mucus from the colon is red with severe inflammation and small ulcers growing around the wall. If UC prognosis gets worse benign tumors may start to develop which can become cancerous overtime. UC can also impact the colon muscle layer which is known as toxic megacolon, characterized by an overly dilated colon, resulting in abdominal distension or shock (Burgmann et al, 2006) .

Crohn's disease is usually accompanied with mucosal constriction and fistulas. The inflamed ulcers may develop into fistulas. This is caused by the intestinal mechanical obstructions due to swelling and scarring. Similarly to UC, CD patients are prone to cancer. Their risk of developing gastrointestinal and colorectal cancers is much higher (Fornaro et al, 2009). Also, the differentiation of immune protection cells is not similar in UC and CD patients. CD4+lymphocytes with Th1 phenotype are dominant in the mucosa of patients with CD whereas patients with UC have a mucosa that is dominated by CD4+ lymphocytes with a Th2 phenotype (Podolsky, 2002).

Although the exact cause is still a mystery, the conditions result from genetic and environmental interactions contributing to an immune response which in turn causes gastrointestinal inflammation (Seyededian, 2019). Other factors such as diet, geographical residence, smoking, vaccinations, stress, antibiotics and breastfeeding etc. also potentially contribute to incidence of the disease (Molodecky & Kaplan, 2010) . The most believed hypothesis of IBD pathogenesis as of now is that the gut microbiota is triggered by a deviant immune response from these factors (Matsuoka & Kanai, 2015). Different gut bacterial species adhere to the gut

mucosa using their virulence factors to invade the mucosal epithelial cells, this results in inflammation mediated by the production of tumor necrosis factor-α (TNF-α) by monocytes and macrophages. TNF-α plays a pivotal role in a variety of immunomodulatory reactions, and is associated with IBD via induction of cell apoptosis and necroptosis (Ruder, 2019). Despite the gut having a coherent symbiotic relationship with the innate and adaptive immune system, in dysbiosis microbes remain cryptic and cause ulcers see Figure 1 for UC and Figure 2 for CD (Geva-Zatorsky, 2017).

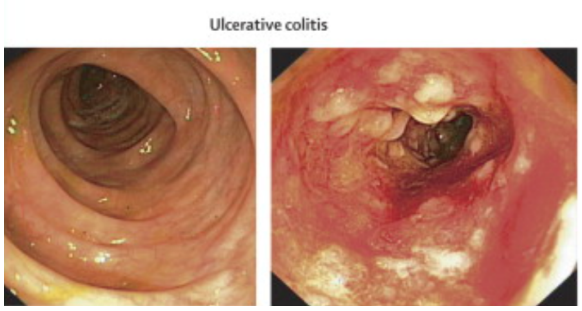


Figure 1: Ulcerative Colitis: image 1 Transverse colon in patient in remission, image 2 Descending colon with irregular surface. (Baumgart & Sandborn, 2007)

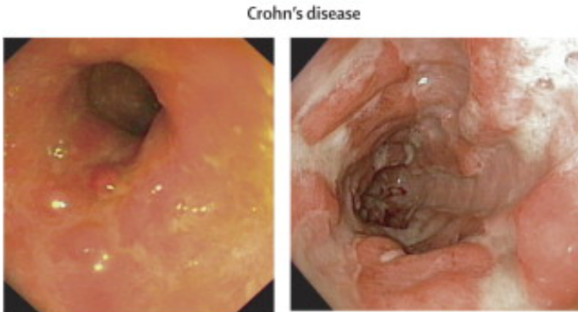


Figure 2: Crohn's disease: image 1 Terminal ileum, image 2 Sigmoid colon with linear and serpiginous ulcerations. Retrieved from (Baumgart & Sandborn, 2007)

Like many diseases, genetics can play a vital role in IBD. Around 20-25% of individuals are diagnosed with IBD before the age of 16 years. This age of onset suggests that genetic features play a role. These genetic variants disrupt the epithelial barrier, induce hyperinflammation and disrupt the T- and B-cell selection and activation (Loddo & Romano, 2015). Multiple studies have highlighted the prevalence of the disease in the twins, the role of ethnic racial contrasts in IBD, hereditary factors and genetic syndromes. The risk percentage of developing IBD in the first-degree relatives of the patient was 7%. Contrastingly, Dickinson et al. depicted that families with IBD history have a higher prevalence of the disease occurring in young people (Dickinson, 1980). Hayman et al. studied the effect of genetic relationships within twins, the study showed that the effect of genetic relationships in twins was larger in CD than UC, however there was still a hereditary correlation present (Hayman, 2005). Environmental factors such as breastfeeding, antibiotics, smoking and oral pills are risk factors that affect both diseases.

Additionally, Reactive oxygen species produced by specific bacteria (ROS) are also majorly involved in aspects of intestinal inflammation responses (Guy Le, 2010). Superoxide dismutase 3 (SOD+) , which is also known as extracellular superoxide dismutase, is an enzyme that eliminates reactive oxygen species (Taq, 2021). It has been reported that SOD3 exerts anti-inflammatory abilities in several immune disorders. However, the effect of SOD3 and the underlying mechanism in inflammatory bowel disease (IBD) have not yet been further exhibited. According to a study done in 2010, using mice and murine

Crohn's disease models by using superoxide dismutase this reduces the level of inflammation. The study of the cytokines also showed that the mice that received CAT+ and SOD+ bacterial strains diminished the severity of the inflammation with IFN intestinal levels similar to the control mice (Guy Le, 2010) However the human colon is more anaerobic than the mouse colon therefore this creates a limit to the studies.

As previously mentioned, the incidence rate of the disease is high and is increasing worldwide. For that reason, presently it is crucial to investigate the cause in order to tackle the rising incidences. Over 1 million individuals in the United States and 2.5 million in Europe are estimated to have IBD. In regions with newly industrialized areas such as Asia, South America and the Middle East there is a rising prevalence (Kaplan, 2015) . The prevalence of the disease is mostly seen in regions in Europe and North America whereas the regions considered as 'low risk' are Asian and Japan (Ananthakrishnan, 2015). The disease usually begins in early adulthood and most cases are reported between the ages 15-35 years. Studies, have indicated that patients who are diagnosed before 10 years develop a varied phenotype of the disease. In addition, it is estimated that about 20% - 25% of patients with IBD are diagnosed in childhood and adolescence (Uhlig, 2014) . Between 2% and 14% of patients with CD show a previous family history of the condition however this is not usually the case with UC (Ananthakrishnan, 2015).

There is currently a lack of research performed on the cause of IBD and information on how microorganisms and bacteria in the gut impact individuals. Previous reports have stated that specific bacteria are negatively associated with health commonly found in UC and CD patients (Gacesa & Kurilshikov, 2022) . Thereby, in this paper these specific bacteria are investigated: *Enterocloster bolteae, Eggerthella species and Ruminococcus gnavus .* Our hypothesis is that these bacteria contribute more to the disorders than what has been observed and we question ourselves if there is a connection with other diseases.

Chapter 2: The importance of the microbiome in IBD

The human gut is the largest organ of the body and in the gut microorganisms with their genes and products create an environment we call the microbiome. The living microbes, we call the microbiota, reach high numbers in the gastrointestinal tract to approximately 10^{14} . (Stojonov, 2020) the microbiome plays an essential role to our health, intestinal homeostasis, pathogen protection, development, and begins to change from the moment of birth (Stojanov, 2020). In addition, an infant's microbial colonization strongly depends on the mode of birth delivery and can contribute to health factors later in life (Ursell et al, 2012). The presence of pathogens and diverse changes in the gut is interrelated with immunomodulatory and metabolic reactions. The impact of the microbial composition of our gastrointestinal tract is becoming apparent and has attracted numerous attention. Many disease factors are now associated with an unbalanced gut microbiota that we call a dysbiosis of the gut (Verdu, 2016). The microbiome and changes to gut microbiota not only affects gastrointestinal diseases, but also obesity, diabetes, liver diseases, cancer and even neurodegenerative diseases (Cani, 2018) . Although the universal main cause of UC and CD has not yet been identified, comprehensive studies executed highlight the role of environmental elements, genetic factors and the microbiota (Baumgart & Sandborn, 2007). The microbiota composition varies between IBD patients and healthy people.

The gut microbiota in healthy subjects depicts minor temporal changes, while the gut microbiota in IBD patients is unstable. The composition of the gut microbiota differs between active and quiescent stages (Ursell, 2012). Gut microbiota consists of bacteria, yeasts, viruses and sometimes even parasites. Bacteria in the gut are represented by over 1000 species from the six dominant phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Firmicutes and Bacteroidetes are the two most common in the gut (Rinninella et al, 2019). The ratio between these two phyla has been associated with homeostasis and if the ratio alters this can lead to disruptions and pathologies as seen in Figure 3. Increases in specific Bacteroides species and decreases in Firmicutes can contribute to bowel inflammation, while the opposite contributes to obesity, which can in turn result in IBD (Stojanov et al, 2020). Firmicutes include mostly gram-positive bacteria with rigid walls that are primarily from the genera *Clostridium, Bacillus, Lactobacillus, Enterococcus, and Ruminococcus* (Rinninella et al, 2019) whereas the Bacteroidetes consist of mainly Gram-negative bacteria, predominantly from the genera *Bacteroides*, *Parabacteroides*, and *Prevotella* (Gibiino et al, 2018). Therefore, balancing the intestinal F/B ratio is an important aspect of maintaining gut function and preventing disorders such as UC and CD.

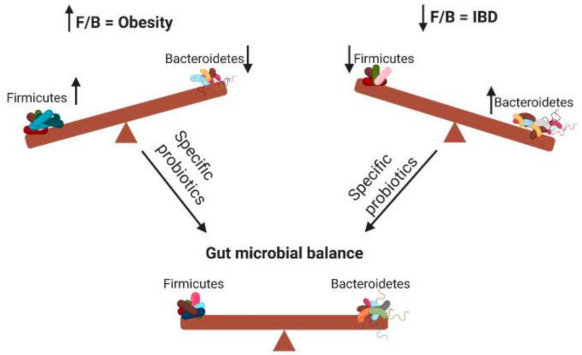


Figure 3: Ratio changes of Firmicutes and Bacteroidetes can cause Inflammatory Bowel Disease. ↑ means increase; ↓ means decrease. Retrieved from (Stojanov et al, 2020)

Researchers have carried out comparison studies of the microbial profiles of patients and healthy people, significant differences were seen however no coherent links to the cause have been obtained. Heymen et al. suggested that it could be a disruption of the mucous system that increases the immunological response rate in the human microbiota or the change of the gut microbiota composition which stimulates the pathologic response. Others have pointed out that pathogenicity of IBD depends on the microbiota of the intestine, the patient's susceptibility and mucosal immunity (Sultan, 2021). In an experiment, Polovsky observed that the levels of Bacteroidetes in patients with CD are higher in comparison to healthy people, while *Lactobacillus* and *Bifidobacterium* decreased. According to reports, *Enterobacteria* levels and specifically *Escherichia. coli* increased significantly in patients with CD. In a comprehensive study of that, Marteau et al. depicts that the levels of *E. coli* and Bacteroides in the gut were higher in people with IBD (Sultan, 2021) .

To date, there have been no specific pathogens that have successfully proved all of Koch's postulates. However, there are specific bacteria which are constantly recurring within IBD patients. In general, common pathogenic bacteria causing IBD are *Salmonella, Shigella, Yersinia, Aeromonas, Campylobacter, E. coli, Clostridium difficile and Mycobacterium tuberculosis* (Seyededian, 2019). According to a recent study, the species *E. bolteae, an unclassified Eggerthella species and R. gnavus,* were quite prevalent in both UC and CD. These taxa showed a negative correlation with health and species richness within patients (Gacesa & Kurilshikov, 2022) .

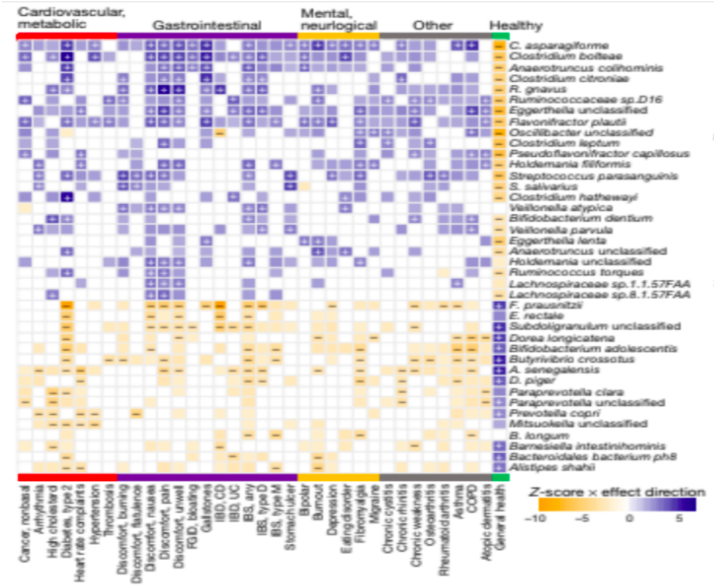


Figure 4: Microbiome signatures of health and diseases. Retrieved from (Gacesa & Kurilshikov, 2022)

Chapter 3: Negatively associated bacterial species with health.

Enterocloster bolteae

E. bolteae is a Gram positive, anaerobic, rod shaped, and spore-forming bacterium from the genus *Clostridium* and belongs to the family *Lachnospiraceae.* E. bolteae which was previously reclassified from the name *Clostridium bolteae* is known for being a benign member of the gut microbiome (Haas et al 2020). Previous analyses have reported *Clostridium* in IBD patients from two different families *Lachnospiraceae* and *Clostridiaceae* see **Figure 5** (Rodriguez et al, 2020) .

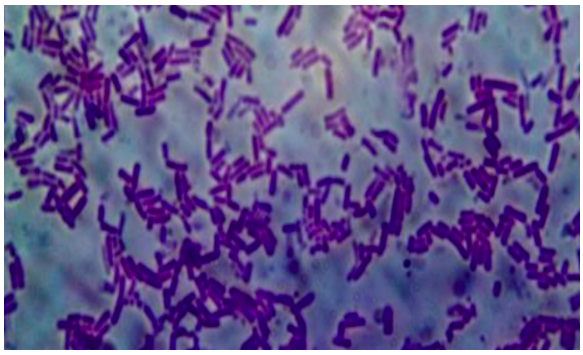


Figure 5: Rods overview of *Clostridium. Bolteae* Retrieved from (Satar, Al Thwani, 2018)

NF-κB is a crucial regulator of immune function and an important target for UC and CD treatment (Giri et al 2022). *E. bolteae* enriched microbial species in the gut are mostly positively involved in NF KB signaling suppression (Giri et al, 2022) and sugar turnover, i.e. fructose, mannose, and galactose metabolism (Sankarasubramanian & Ahmed, 2020) . They are enriched in CD more than in UC and are known to use the above sugars and metabolize them into glyceraldehyde-3 phosphate more rapidly than other bacteria, which is a key metabolite of the glycolytic pathway, the principal energy-generating mechanism in the human body. NF-κB signaling is an important regulator in the immune system and activates the antigen-presenting cells and effector leukocytes. In a healthy individual's gut, NF-κB activation is tightly regulated (Renner and Schmitz, 2009), According to a study done using a cell-free culture supernatant from *E. bolteae* AHG0001 strain suppressed the Nf-kb activation. NF-κb signaling contributes significantly to multiple host responses underlying the pathogenesis of IBD (Giri et al, 2022).

E. Bolteae can be detrimental and affect UC and CD by its virulence factors. Gut microbes have the ability to convert human bile acids which are now being called microbially conjugated bile acids (MCBAs) by deconjugation, dehydroxylation, dehydrogenation, and epimerization of the cholesterol core (Garcia et al, 2022. Bile acids are important factors in lipid regulation, as well as glucose and energy metabolism (McGlone & Bloom, 2019). *E. bolteae* is said to be a main producer of these MCBAs, however the phenomena through which the microbes conjugate them remains a mystery but scientists believe it could be comparable to the amino acid N-acyltransferase mechanism. An abundance of these MCBAs cause gut disturbances and play a part in other relevant diseases such as colorectal cancer. Reactive oxygen species can also contribute to inflammation however with the use of superoxide dismutase (SOD+) the effects can be scavenged.

Eggerthella Species

Eggerthella lenta is an anaerobic, Gram positive bacilli, rod - shaped, non spore-forming bacterium and is a common gut commensal like *Clostridium, Propionibacterium, Bifidobacterium, Eubacterium, Lactobacillus.* It is part of the family *Coriobacteriaceae* and the phylum Actinobacteria see **Figure 6**. Its identification is often a challenge due to the bacteria's slow growing nature and Gastrointestinal diseases and malignancy are the most common causes for bacteremia from this organism (Thota et al, 2011). Furthermore, they reside in the colon ,contributing to IBD and *Eggerthella* bacteremia numbers are increasing in regards to morbidity and mortality. (Thota et al, 2011)

From previous studies only three cases of inflammatory bowel disease have reported *Eggerthella* strains in the feces samples. The first report of its first association with Crohn's disease was recently released. An observation was done on a 21 year old female patient, according to the study, her microbiota showed a high abundance of *E. lenta*. Theoretically, it is logical for *E. lenta* translocation to go from the gut to blood due to the wall inflammation, it is not known yet whether this occurs with greater frequency or contributes to excess morbidity in Crohn's disease. *Eggerthella* isolation from blood cultures in a previously healthy patient should prompt looking for any underlying diseases of the GI tract and malignancy (Thota et al, 2011).
Even though there have not been many cases of *E. lenta* reported in UC and CD specifically , the bacteria has been indicated as the cause of a polymicrobial bloodstream infection and a serious pathogen in gastrointestinal pathology (Liderot, Larsson, Boräng, & Ozenci, 2010). It induces intestinal Th17 activation by lifting inhibition of the Th17 transcription factor through cell and antigen independent mechanisms, which causes inflammation and ulcers in the gut lining (Alexander & Yan Ang et al, 2021). By using clinical technologies such as MALDI-TOF, antibiotics against this strain could be developed to limit the GI inflammation.

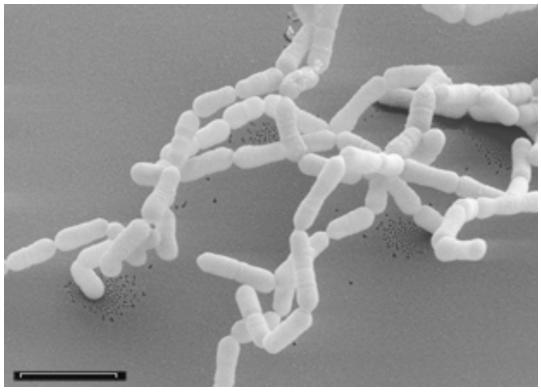


Figure 6: Overview of *Eggerthella* species. Retrieved from (Wade et al, 1999)

Ruminococcus gnavus

Ruminococcus species were also reported to be found in two different families in IBD, which were *Ruminococcaceae* in UC and *Lachnospiraceae* in CD. R. gnavus is an anaerobic, gram-positive bacterium found in the gut microbiome as seen in **Figure 7**. It has been a prevalent microbe found in nearly 90% of people's intestines and IBD patients, particularly those with CD. *R. gnavus* synthesizes and secretes a complex glucomannan polysaccharide with a rhamnose backbone and glucose side chains as seen in **Figure 8**. The glucomannan is a repeating unit of five sugars with a linear backbone formed from three rhamnose units and a short sidechain composed of two glucose units. The rhamnose backbone is made from 1,2- and 1,3-linked rhamnose units, and the sidechain has a terminal glucose linked to a 1,6-glucose. This glucomannan induces inflammatory cytokines (Henke & Kenny, 2019).

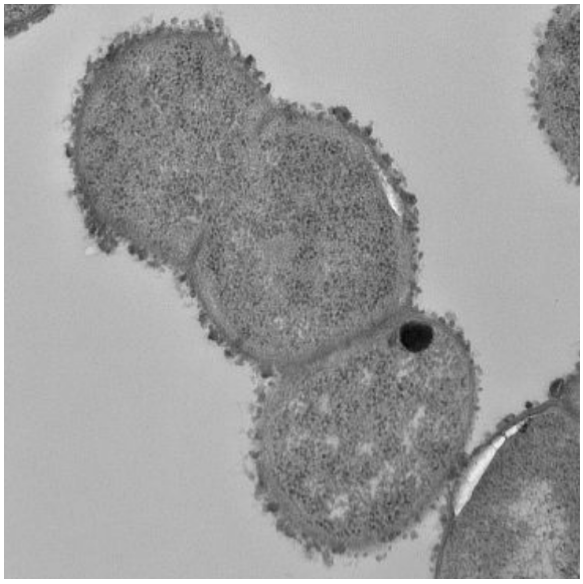


Figure 7: Electron microscopic view of *Ruminococcus gnavus*. Retrieved from (Henke, 2019)

Previous studies show that two distinct unidentified clades of *R. gnavus* were observed in CD patients and were associated with increased disease (Hall & Yassour, 2017).

Several studies link an increased relative abundance of *R. gnavus* to increased prevalence of CD (Henke & Kenny, 2019) (Hall & Yassour, 2017). The disease associations described above suggest that *R. gnavus* produces tiny molecules that directly induce an inflammatory response by innate immune cells. Through bioactivity-guided fractionation, we found that *R. gnavus* produces a potent, inflammatory polysaccharide seen in **Figure 8**. The *R. gnavus* glucorhamnan then induces secretion of TNFα in a dose-dependent manner (Henke & Cassily, 2019). *R. gnavus* is also involved in the development of other gastrointestinal disorders and causes vomiting and diarrhea.

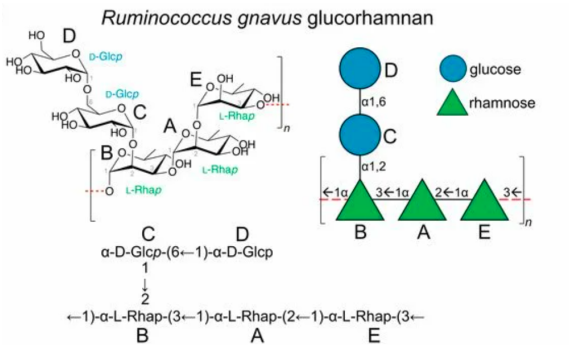


Figure 8 : *The purified polysaccharide is a glucorhamnan, Retrieved from* (Henke & Cassily, 2019)

Chapter 4 :

Summary and Perspectives

In addition to confirming the known microbiome associations of Ulcerative Colitis and Crohn's disease, our results highlight the importance of the microbiome involvement over a long period of time, beginning from birth to the onset of IBD . It is vital when studying possible causations of such gastrointestinal diseases, while other factors such as environmental, diet, genetic, and psychological emotions do play a factor, the gut microbiome stood at the forefront of our focus. After all, the lining of the gut is usually the key site where inflammation occurs. Both diseases usually evolve with a remitting and relapsing course in patients. Due to this, determining the inflammatory state is a crucial start for the assessment of disease activity, cause and (future) improved therapies (Vilela et al, 2012).

This study has investigated the alterations in the gut microbiome leading to IBD. These changes may be able to help implicate new therapeutic strategies, perhaps taking the approach of targeting the specific disease linked bacteria observed.

Recently, antibody neutralization studies have shown the important role of TNF in IBD patients especially in CD patients . Anti TNF drugs such as Infliximab and Adalimumab use mechanisms of action including neutralizing the TNF-α, reverse signaling, cytotoxicity and apoptosis. X
They diminish the inflammation by depleting the overexpression of the TNF-α, by inhibiting binding to the receptors, by binding soluble and transmembrane TNF-α which results in a blockage of proinflammatory signals. Anti TNF treatment has also been depicted in vitro to induce cytokine suppression via reverse signaling. This compelling phenomenon occurs when the cell-surface bound precursor to TNF binds to anti-TNF and acts as a ligand and triggers cell activation, cytokine suppression or apoptosis of the cell bearing the cell-surface bound precursor (Eissner et al 2000) (Eissner at al, 2004) . Anti TNF treatment also induces apoptosis of activated T lymphocytes (Hove et al, 2022), countering a mechanism in CD, where mucosal T cell proliferation exceeds T cell apoptosis (Den Brade et al, 2007). Anti-TNF therapies with an Fc region (e.g. infliximab and adalimumab) are also able to induce antibody-dependent cell mediated and complement dependent cytotoxicity (Mitoma et al, 2018). By targeting the inhibition of TNF-α this can improve the health of patients and results in remission of IBD (Pache, 2009).

The administration of probiotics, prebiotics, a combination: synbiotics, fecal transplantation, and bacterial consortium transplantation have all been tested methods to modulate the gut microbiota. These probiotics have been promising in modulating the gut microbial, decreasing blood cholesterol levels and targeting the local infections (Jakubczyk et al, 2020. New probiotics targeting the F/B ratios and the specific bacterial species could benefit patients (Gagliardi & Totino , 2018).

Additionally there is a co - morbidity between psychological effects, early infancy and IBD. It is known that chronic IBD stimulates negative psychological emotions like stress, anxiety and even depression. However, it is unknown if this response is a biological response that may be an active inflammatory state of IBD intersecting with the pathobiology of what mediates mood

and anxiety disorders (Bernstein, 2016). Studies have shown that adequate nutrition and breast milk protects a baby from gastrointestinal infections as they help in the development of the mucosal system (Carr et al, 2021). It is also seen that infants with infectious diarrhea entail a higher risk of developing UC or CD, this could be a future key point microbiologists could work on. By identifying the specific recurring harmful microbes within the infants' diarrhea this could assist in preventing the diseases.

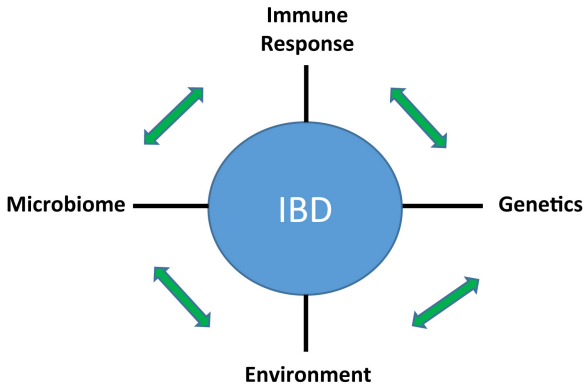
Therefore, extended research should focus on these topics in order to improve the natural measures that can be taken to prevent such chronic illnesses. By comparing associations between a healthy person's microbiome and a patients, we identified common signals and bacteria that are for gut dysbiosis.

The existence of shared bacterial species and dysbiosis depicts considerable implications for microbiome research and microbiota-targeting diagnostics and therapies. Shared dysbiosis implies that the gut microbiome is a biomarker of general health (Gacesa & Kurilshikov, 2022). Interestingly, we also observed that these disease-linked *Enterocloster, Ruminococcus and Eggerthella species* are positively correlated with cardiovascular metabolic and other gastrointestinal diseases. Type 2 Diabetes, High cholesterol and IBS were among those. Perhaps, could this suggest that there is a common causal link. Type 2 diabetes is a serious and common chronic disease resulting from a complex inheritance-environment interaction along with other risk factors such as obesity and sedentary lifestyle (Wu, 2014) . With that, high cholesterol is also a result of lifestyle choices such as diet and exercise (Hou, 2021). Similarly, to IBD, Irritable bowel syndrome (IBS) is an extremely common and debilitating chronic functional gastrointestinal disorder. It is now evident that microbial factors play key roles in IBS pathophysiology (Pimentel, 2020). Diet and environmental factors also contribute to its development, therefore these shared causes of the diseases suggest that the unknown mechanism behind UC and CD could simply just be more general such as environmental changes exposed to the host and various lifestyle factors affecting individuals over time . Previously, the regions where IBD is most prevalent were mentioned, in regions in the Western world (Europe & North America) eating disorders, unbalanced eating patterns, subsequent abnormal weights and unhealthy dieting is more common there than the Eastern/Mediterranean side (Nasser, 2009). Individuals living in the Western world also tend to smoke and adhere to bad lifestyle choices moreover than the East. Perhaps, this indicates that the personal decisions individuals make for themselves contribute to their development of IBD and possibly by living in areas where these elements are more accessible to people individuals become more prone to the diseases (Nasser, 2009). Eating cruciferous vegetables is hypothesized to be a protective method as they contain a relatively high abundance of fiber and phytochemicals (i.e glucosinolates). These decrease the high levels of gut microbial composition and prevent bacteria from producing isothiocyanates which are also carcinogenic factors. It becomes increasingly vital to incorporate diets with whole foods and cruciferous vegetables, fabricate their lifestyle choices and avoid the habits that the Western world has

generated, this in turn could be a prevention strategy.

CD and UC are complex diseases and are increasing in prevalence. This gives rise to a great challenge for medical specialists in terms of management as the etiology remains unknown. The purpose of this study was to examine the extent to which specific bacterias, *E. bolteae, E. lenta and R. gnavus* could be the ultimate cause of UC and CD and the ties IBD has with other diseases. Our results showed that the bacteria species *R. gnavus, E. Bolteae* and certain *Eggerthella* species are frequently occurring in patients as well as the usual commensal bacteria. They do further assist in causing negative inflammatory responses. We showed that each bacteria had a significant impact in contributing to the diseases by their phenomenons they use which inflame the gut. However, our observation in correlation to other similar diseases is that the cause is primarily a combination associated with environmental and genetic relatedness corroborates which affects and alters the microbiome. Hence, showing a combination of risk factors as the cause as seen in **Figure 9.** (Combination of risk factors for the development of IBD (Glassner et al, 2020))

FIG 9:



This gut microbial alteration could in turn cause inflammation when bacterial species adhere and invade the mucosal epithelium tissues. Potentially, this could be where the previously mentioned bacteria cause further inflammation, their mechanisms initially start off (i.e *R. gnavus* emitting TNFa) and grow within the environment. More studies on a larger scale with a numerous number of patients are needed in order to prove that those specific bacteria alone have a strong correlation in regard to the exact cause.

With that being said, the associated bacteria mentioned previously could be resulting from the IBD rather than the exact cause, however further and extensive research is needed to decipher this.

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