Tampereen yliopisto

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RISK FACTORS OF INFLAMMATORY BOWEL DISEASE

Faculty of Medicine and Health Technology
Bachelor's thesis
April 2022

ABSTRACT

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Bachelor's thesis
Tampere University
Biotechnology and Biomedical Engineering
April 2022

Inflammatory bowel disease (IBD) is a chronic immune-mediated condition of the gastrointestinal tract. IBD includes Crohn's disease (CD), ulcerative colitis (UC) and inflammatory bowel disease unclassified (IBDU) which serves as a diagnosis when condition cannot be classified as CD or UC according to pathological features. The prevalence of IBD is highest in urban areas of Western countries. Nevertheless, the incidence has been increasing significantly in Eastern Europe and Asia while in Western countries the rates are stabilised over years. The aetiology of IBD and cause of high morbidity remain unknown, yet several risk factors have been identified. This thesis reviews the IBD associated factors and their potential mechanisms in pathogenesis.

Environmental factors are believed to trigger onset of IBD in genetically susceptible individuals due to alterations in immune response against gut microbiota. Over 200 genetic risk loci has been associated with IBD, but for most of these the causal gene or variant conferring risk is yet to be identified. However, known variants are located in genes controlling microbial sensing and clearance along with regulation of immune responses against microbes. In addition, epigenetic modifications, such as DNA methylation and alterations to microRNA (miRNA) expression, have been observed in IBD patients. Not all individuals susceptible to IBD develop the disease, indicating that additional environmental factors and alterations to gut microbiota are involved.

Disruption of the gut microbiota or dysbiosis has been described in IBD patients. Dysbiosis includes decreased bacterial diversity as abundance of commensal bacteria is reduced. In turn, loss of their protective functions may allow increased abundance of pathogenic bacteria. It is also hypothesised that under certain circumstances commensal bacteria can become pathogenic and disrupt the intestinal homeostasis. Such bacteria are called pathobionts. It is unclear whether dysbiosis is a cause or a consequence of IBD. Regardless, early life factors influence the development of microbiota composition and adulthood exposures may as well cause alterations.

An environmental trigger is considered to be essential for the onset of IBD as the increasing incidence could not be explained solely with genetics. Thus, it is suggested that the environmental factors associated with Western lifestyle and urbanisation have a critical role in IBD pathogenesis. This is supported by the high prevalence in Western countries and subsequent emergence of IBD in recently industrialised countries. IBD associated environmental factors may contribute to onset through alterations to the gut microbiota. Factors that promote changes include smoking, diet and use of antibiotics. Moreover, heterogeneity of the disease could indicate different risk factors behind each phenotype hence complicating the attempts to determine aetiology of IBD.

Keywords: Inflammatory bowel disease, Risk factors, Genetic factors, Gut microbiota, Environmental factors

TIIVISTELMÄ

Jenni Rapakko: Tulehduksellisille suolistosairauksille altistavat tekijät
Kandidaatin tutkielma
Tampereen yliopisto
Bioteknologia ja biolääketieteen tekniikka
Huhtikuu 2022

Tulehdukselliset suolistosairaudet (IBD, inflammatory bowel disease) ovat ruoansulatuskanavan kroonisia immuunivälitteisiä sairauksia, joihin kuuluvat Crohnin tauti (CD, Crohn's disease), haavainen paksusuolentulehdus (UC, ulcerative colitis) ja luokittelemattomaton koliitti (IBDU, inflammatory bowel disease unclassified). IBDU toimii diagnoosina tapauksissa, joissa sairautta ei voida luokitella CD:ksi tai UC:ksi patologisten piirteiden perusteella. IBD:n vallitsevuus on suurinta länsimaiden kaupunkialueilla. Siitä huolimatta ilmaantuvuus on lisääntynyt merkittävästi Itä-Euroopassa ja Aasiassa, kun taas länsimaissa luvut ovat vakiintuneet vuosien saatossa. IBD:n etiologiaa ja korkean sairastuvuuden syytä ei tiedetä, mutta useita riskitekijöitä on tunnistettu. Tässä työssä tarkastellaan IBD:hen liitettyjä tekijöitä ja niiden mahdollisia vaikutusmekanismeja taudin synnyssä.

Ympäristötekijöiden uskotaan aiheuttavan IBD:n puhkeaminen geneettisesti alttiissa yksilöissä, kun tapahtuu muutoksia immuunivasteessa suoliston mikrobiomia kohtaan. Yli 200 geneettistä riskilokusta on yhdistetty IBD:hen, mutta useimmista näistä riskin aiheuttavaa geeniä tai varianttia ei ole vielä tunnistettu. Tunnetut variantit kuitenkin esiintyvät geeneissä, jotka ohjaavat mikrobien tunnistusta ja tuhoamista sekä säätelevät immuunivastetta niitä kohtaan. Lisäksi IBD-potilailla on havaittu epigeneettisiä muutoksia, kuten DNA-metylaatiota ja vaihtelua mikroRNA:n (miRNA, microRNA) ilmentymisessä. Kaikki IBD:lle alttiit yksilöt eivät sairastu, mikä viittaa ympäristötekijöiden ja suoliston mikrobiomin osallisuuteen.

IBD-potilailla on kuvattu häiriöitä suoliston mikrobiomissa eli dysbioosia. Dysbioosiin liittyy bakteerien monimuotoisuuden väheneminen kun kommensaalisten bakteerien määrä pienenee. Niiden suojaavien toimintojen menetys voi puolestaan mahdollistaa patogeenisten bakteerien lisääntymisen. On myös oletettu, että tietyissä olosuhteissa kommensaaliset bakteerit voivat muuttua patogeenisiksi ja siten häiritä suoliston homeostaasia. Näitä bakteereja kutsutaan englannin kielisellä termillä pathobionts. On epäselvää, onko dysbioosi IBD:n syy vai seuraus. Joka tapauksessa varhaisessa elämässä koetut ympäristötekijät vaikuttavat mikrobiomin koostumuksen kehittymiseen, ja aikuisiän altistuminen voi myös aiheuttaa muutoksia.

Laukaisevaa ympäristötekijää pidetään välttämättömänä IBD:n puhkeamiselle, sillä lisääntyvää ilmaantuvuutta ei voida selittää pelkästään geneettisillä tekijöillä. Näin ollen ehdotetaan, että länsimaiseen elämäntapaan ja kaupungistumiseen liittyvillä ympäristötekijöillä on tärkeä rooli IBD:n synnyssä. Tätä tukee korkea vallitsevuus länsimaissa ja myöhempi IBD:n ilmaantuvuuden kasvu hiljattain teollistuneissa maissa. IBD:hen yhdistetyt ympäristötekijät voivat vaikuttaa sairauden puhkeamiseen suoliston mikrobiomin muutosten kautta. Muutoksia edistäviä tekijöitä ovat muun muassa tupakointi, ruokavalio ja antibioottien käyttö. Lisäksi sairauden heterogeenisyys voisi viitata siihen, että kunkin fenotyypin taustalla on eri riskitekijöitä, mikä vaikeuttaa entisestään IBD:n etiologian määrittämistä.

Avainsanat: Tulehdukselliset suolistosairaudet, Riskitekijät, Geneettiset tekijät, Suoliston mikrobiomi, Ympäristötekijät

ACKNOWLEDGEMENTS

This thesis is a part of my Bachelor of Science studies in the degree programme in Biotechnology and Biomedical Engineering at Tampere University.

I would like to express my gratitude to my supervisor, Pia Oksanen, MD, PhD, for the valuable feedback and guidance during the writing process. Also, thank you to my family and friends who offered support and advice throughout the process.

Tampere 26.4.2022

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CONTENTS

1. INTRODUCTION

Inflammatory bowel disease (IBD) is an immune-mediated condition characterised by chronic inflammation involving gastrointestinal tract (Clarke and Chintanaboina 2019). IBD includes subtypes Crohn's disease (CD), ulcerative colitis (UC) and less common inflammatory bowel disease unclassified (IBDU) (Thurgate et al. 2019). IBDU serves as a diagnosis for patients who have chronic colitis with characteristic clinical and pathological features of IBD which do not allow classification into CD or UC (Thurgate et al. 2019, Venkateswaran, Weismiller and Clarke 2021). IBD diagnosis is primarily based on clinical symptoms, characteristic endoscopic findings and histology (Ungaro et al. 2017, Agrawal et al. 2021). However, clinical presentation of IBD is heterogenous with varying location and severity of inflammation between patients (Clarke and Chintanaboina 2019). In addition, a patient may have extraintestinal manifestations (EIMs) that affect organs outside the gastrointestinal tract (Rogler et al. 2021).

IBD is most prevalent in Western countries (Clarke and Chintanaboina 2019). To illustrate, in Finland the prevalence of IBD was as much as 972 per 100,000 inhabitants in 2020 (Kontola et al. 2022). The incidence and prevalence rates have been increasing worldwide (Jussila et al. 2013b, Ungaro et al. 2017) although the incidence has increased more significantly in Eastern Europe and Asia compared to Western countries (Jussila et al. 2012).

Long-lasting and uncontrolled inflammation can lead to complications which have a substantial impact on the quality of life and may eventually result in surgery (Torres et al. 2017, Ungaro et al. 2017). The quality of life may be affected by EIMs as well, which frequently require specific treatment (Rogler et al. 2021). Expensive medical therapy and surgery with increasing morbidity of IBD (Jussila et al. 2012) causes considerable burden and costs to healthcare system (Ramos and Papadakis 2019).

IBD aetiology is unknown but it is proposed that the disease develops in individuals with genetic susceptibility who are exposed to an environmental factor leading to an abnormal immune response against gut microbiota (Turpin et al. 2018). Several genetic and environmental factors as well as alterations in the gut microbiota have already been associated with IBD, the most prominent of which are presented in this thesis. However, given the burden to an individual and the healthcare, further research is needed to determine the mechanisms by which the risk factors contribute to IBD pathogenesis, and to develop methods to reduce high morbidity and to treat patients more effectively.

2. INFLAMMATORY BOWEL DISEASE

2.1 Definition of inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic inflammatory condition mediated by the abnormal activity of immune system (Clarke and Chintanaboina 2019). The disease involves mainly the gastrointestinal tract but a patient may also have extraintestinal manifestations (EIMs) affecting organs outside the gastrointestinal tract (Rogler et al. 2021). IBD includes Crohn's disease (CD), ulcerative colitis (UC) and inflammatory bowel disease unclassified (IBDU) (Thurgate et al. 2019). CD and UC are the most prevalent subtypes while IBDU is less common. Although IBD can be divided into different subtypes, the disease is heterogenous and its clinical presentation may vary from patient to patient depending on the location and severity of the inflammation. (Clarke and Chintanaboina 2019)

2.1.1 Crohn's disease

Crohn's disease (CD) is one of the main types of IBD which can affect any area of the gastrointestinal tract, most commonly the terminal ileum of the small intestine. CD is characterised by transmural inflammation and altering periods of remission and recurrence. Normal mucosa is often found between inflamed areas. (Torres et al. 2017, Clarke and Chintanaboina 2019)

The clinical presentation of CD is diverse which results from the varying location, severity, and behaviour of the disease. The most common symptoms include abdominal pain, diarrhoea and weight loss along with fatigue. (Torres et al. 2017, Clarke and Chintanaboina 2019) Rectal bleeding may occur if colon is affected by the disease. Additionally, up to half of patients have EIMs which frequently involve skin, joints or eyes. (Torres et al. 2017)

Chronic inflammation in CD can lead to complications such as strictures, fistulas and abscesses and eventually result in surgery (Torres et al. 2017, Clarke and Chintanaboina, 2019). Bowel damage described is present in about 20% of patients at diagnosis. Approximately a third of CD patients have perianal disease which presents as fistulas and skin or anal canal lesions, such as ulcers. (Torres et al. 2017). Perianal disease increases the risk of anal cancer in addition to substantially high risk of small bowel cancer in CD (Jussila et al. 2013a).

2.1.2 Ulcerative colitis

Ulcerative colitis (UC) is the second main type of IBD. UC affects the colon, typically starting from the rectum and extending to proximal segments. Depending on the population, the entire colon is

involved in 15-35% of cases. Likewise CD, also UC has periods of remission and recurrence. However, the inflammation is mucosal and continuous without normal mucosa between affected areas. (Ungaro et al. 2017, Clarke and Chintanaboina 2019)

The clinical presentation of UC might vary due to extent of inflammation. A patient commonly suffers from bloody diarrhoea, cramps, tenesmus and incontinence. Almost every tenth patient with UC limited to the rectum or the left side of the colon might experience constipation. Approximately a third of patients have EIMs. (Ungaro et al. 2017)

Structural and functional damage can develop to the colon due to chronic inflammation, for example strictures and anorectal dysfunction. Additionally, UC patients have increased risk of colorectal cancer, especially those with long-lasting and uncontrolled inflammation. (Ungaro et al. 2017)

2.1.3 IBD unclassified

Inflammatory bowel disease unclassified (IBDU) is the third subtype of IBD which constitutes approximately 5-15% of all cases. IBDU patients have chronic colitis with clinical and pathological features that are characteristic for IBD but do not allow the condition to be classified as CD or UC. IBDU can serve as a provisional diagnosis since the disease eventually evolves to CD or UC in some patients. Former term indeterminate colitis (IC) is still used interchangeably with IBDU. (Thurgate et al. 2019, Venkateswaran, Weismiller and Clarke 2021)

IBDU symptoms are very similar to those in UC as they mainly affect same locations of gastrointestinal tract (Thurgate et al. 2019), although the disease tends to be more extensive and severe in IBDU (Venkateswaran, Weismiller and Clarke 2021). Symptoms include abdominal pain or cramps, diarrhoea with blood in stool and weight loss. Compared to UC, IBDU tends to relapse more frequently and patients have increased risk of colectomy and colorectal cancer. (Thurgate et al. 2019, Venkateswaran, Weismiller and Clarke 2021)

2.2 Diagnostics

IBD is a heterogenous disease with varying clinical presentation which can make the diagnosis challenging and cause delays (Agrawal et al. 2021). Diagnosis of IBD is based on a set of clinical symptoms, biomarkers, endoscopic findings and histology (Torres et al. 2017, Ungaro et al. 2017, Agrawal et al. 2021). Laboratory results typically show thrombocytosis, anaemia, and signs of malabsorption as well as hypoalbuminaemia in severe disease. The concentration of acute phase proteins, C-reactive protein (CRP) in particular, may be increased. (Torres et al. 2017, Ungaro et al. 2017) Other conditions, such as cancer and infection, are excluded before the definitive IBD diagnosis is established (Lamb et al. 2019).

The Montreal classification is used to categorise IBD patients according to the features of each phenotype to ensure appropriate decisions regarding treatment. Classification of CD patients takes into account the disease location and behaviour whereas UC patients are classified by the extent and severity of the disease. (Lamb et al. 2019)

Stool biomarkers, including faecal calprotectin (FC), are non-invasive and specific for intestinal inflammation (Ungaro et al. 2017, Lamb et al. 2019) which is why they are widely used at diagnosis of IBD and later to monitor disease activity (Torres et al. 2017). FC is tested in a stool sample and its concentration correlates with increased amount of neutrophils in the intestine (Torres et al. 2017, Ungaro et al. 2017). FC can be utilized to assess whether endoscopy is necessary and to exclude IBD since the disease is very unlikely in patients with a normal level of FC (Ungaro et al. 2017, Lamb et al. 2019). It should be noted that FC does not distinguish between different causes of intestinal inflammation hence it is not specific for IBD (Ungaro et al. 2017, Agrawal et al. 2021).

Endoscopy with biopsies is the primary investigation method for suspected IBD. An ileocolonoscopy is usually recommended to be able to examine both the colon and the terminal ileum. (Ungaro et al. 2017, Lamb et al. 2019) Endoscopic findings in CD include serpiginous ulcerations and cobblestone-like appearance produced by nodular mucosa (Torres et al. 2017). As for UC, typical findings are erythema, granularity and ulcerations surrounded with inflamed mucosa (Ungaro et al. 2017). An upper endoscopy, also called esophagogastroduodenoscopy, is performed only in patients whose symptoms indicate involvement of the upper gastrointestinal tract (Torres et al. 2017, Ungaro et al. 2017). Small bowel capsule endoscopy is less invasive compared to traditional endoscopy techniques and it produces high resolution images (Lamb et al. 2019) but it should be reserved for patients with IBDU or suspected IBD and who do not show typical findings on conventional endoscopic examination (Torres et al. 2017).

Biopsies taken during the endoscopic procedure are examined for histological features typical of the disease. Crypt distortion occur in both subtypes, but granulomatous inflammation is characteristic to CD while basal plasmacytosis and mucus depletion are seen in UC. (Lamb et al. 2019)

Cross-sectional imaging methods, such as CT-enterography and MR-enterography, are increasingly used in CD diagnosis and management to evaluate the extent of disease and possible complications (Torres et al. 2017). Considering UC, the above-mentioned methods are not specific enough to be used in diagnosis (Ungaro et al. 2017).

2.3 Epidemiology

IBD is most prevalent in Western countries, especially in urban areas compared to rural areas (Clarke and Chintanaboina 2019). The highest incidence and prevalence rates are found in Europe and Canada. In addition, incidence is also high in Australia and prevalence in the USA. (Torres et

al. 2017, Ungaro et al. 2017) Within Europe, higher incidence rates of IBD have been noted in northern countries (Jussila et al. 2013b, Ungaro et al. 2017, Clarke and Chintanaboina 2019). There is no significant difference in prevalence between the sexes globally (Torres et al. 2017, Ungaro et al. 2017).

Finland has high incidence and prevalence rates of IBD, especially of UC (Jussila et al. 2012, Jussila et al. 2013b). In 2007, the incidence of IBD was 36.8 per 100,000 inhabitants, of which UC accounted for 27.4 and CD for 9.4. The incidence of UC was therefore almost three times that of CD. The IBDU cases were included in the incidence rate if UC. (Jussila et al. 2012) In 2008, the prevalence of IBD was 595 per 100,000 inhabitants. The prevalence of CD showed no significant difference between geographical regions, while the prevalence of UC increased from southern Finland to north. (Jussila et al. 2013b)

The incidence and prevalence rates of IBD have been increasing worldwide (Jussila et al. 2013b Ungaro et al. 2017). The increase in incidence has been more significant in Eastern Europe and Asia than in Western countries where the rates have mostly stabilised or increased only slightly. In Finland the incidence of UC increased by as much as 25% from 2000 to 2007 while the incidence of CD remained stable over the period. (Jussila et al. 2012) Furthermore, the prevalence of IBD became almost threefold between 1993 and 2008 (Jussila et al. 2013b). The rates continue to rise as in 2020 the incidence of IBD was already 48.1 per 100,000 inhabitants and the prevalence was 972 per 100,000 (Kontola et al. 2022).

3. RISK FACTORS

3.1 Genetic factors

Over 200 genetic risk loci which are significantly associated with IBD have been identified through genome-wide association studies (GWAS) (Cohen et al. 2019, Meddens et al. 2019). A risk locus may have multiple candidate genes or genetic variants, and for most of the identified loci it is not clear which gene or variant accounts for the increased risk of IBD (Uniken Venema et al. 2017). However, many of known variants are located in genes that control microbial detection and response of immune and intestinal cells to microbes (Cohen et al. 2019). Moreover, the identified genes affect epithelial barrier function, autophagy and endoplasmic reticulum (ER) stress, for instance (Turpin et al. 2018, Ramos and Papadakis 2019).

The majority of associated loci is shared between CD and UC, suggesting that their pathogenesis is partly overlapping (Turpin et al. 2018, Meddens et al. 2019). Interestingly, some shared loci can increase the risk of one subtype but be protective for another (Uniken Venema et al. 2017). Additionally, around 50% of IBD risk loci are associated with other immune-mediated and inflammatory diseases (Ramos and Papadakis 2019).

A heritable component to IBD has been found, with around one in ten patients having family history of IBD. However, heredity seems to have a greater influence on the development of CD than UC. (Turpin et al. 2018) Not everyone carrying IBD-associated variants develop the disease and genetics could not explain the increasing incidence alone, proposing environmental factors and alterations to immune response against gut microbiota are also required (Jussila et al. 2012, Turpin et al. 2018, Cohen et al. 2019).

3.1.1 Genetic risk loci

Proteins encoded by IBD associated genes are involved in one of three pathways: Microbial recognition, autophagy or regulation of inflammatory responses (Table 1). The genetic variants in these genes either increase the risk for IBD or are protective against it. For example, *CARD9* variant c.IVS11+1G>C is protective while variant rs10870077 of the same gene increases the risk. (Uniken Venema et al. 2017)

NOD2 (nucleotide-binding oligomerization domain containing 2) was the first gene to be associated with increased risk of CD (Cohen et al. 2019). The gene encodes for an intracellular pattern recognition receptor which is most expressed in macrophages, T cells and Paneth cells of small intestine epithelium. NOD2 detects bacteria by the peptidoglycan component muramyl dipeptide (MDP), and activates NK-κB and MAPK leading to the production of pro-inflammatory cytokines. (Turpin et al. 2018) Three identified *NOD2* variants, fs1007insC, R702W and G908R, are all located in the leucine-rich repeat (LRR) (Uniken Venema et al., 2017). The LRR domain mediates MDP binding, proposing that the increased risk may be due to a loss of microbial sensing (Turpin et al. 2018).

ATG16L1 (autophagy related 16 like 1) and *IGRM* (immunity-related GTPase family M) encode proteins involved in autophagy, and they are associated with increased risk of CD (Cohen et al., 2019). Autophagy is used by cells for lysosomal degradation of cytosolic debris and organelles as well as intracellular pathogens (Turpin et al. 2018, Cohen et al. 2019).

ATG16L1 is recruited to the plasma membrane by activated NOD2 upon bacterial entry (Uniken Venema et al. 2017). *ATG16L1* variant T300A results in increased cleavage of the gene product by caspase-3 reducing its activity (Cohen et al. 2019). CD associated genetic variants of *ATG16L1* and *NOD2* disturb their interaction leading to diminished killing of pathogens by autophagy and inappropriate cytokine responses (Uniken Venema et al. 2017, Cohen et al. 2019).

IRGM regulates autophagy by indirectly activating AMP-activated protein kinase (AMPK), which along with IRGM activates autophagy regulators, including Unc-51 like autophagy activating kinase 1 (ULK1) and beclin 1. IRGM gathers the above-mentioned autophagy regulators with ATG16L1 to form the autophagy machinery. Furthermore, IRGM interacts with NOD2 which enhances its polyubiquitination and stabilizes the autophagy machinery. IRGM can be down-regulated by microRNA-196 (miRNA-196). However, causal variant c.313C>T alters the miR-196 binding site to inhibit regulation which consequently affects autophagy. (Uniken Venema et al. 2017)

Table 1. IBD associated genes and their identified variants. (Uniken Venema et al. 2017, Cohen et al. 2019)

Pathway	**Gene**	**Variant(s)**	**Effect**
Microbial sensing	*NOD2*	fs1007insC, R702W, G908R	Risk for CD
Autophagy	*ATG16L1*	T300A	Risk for CD
	IRGM	c.313C>T	Risk for CD
Regulation of inflammatory responses	*IL23R*	R381Q, G149R, V362I	Protective for IBD
	CARD9	rs10870077	Risk for IBD
	RNF186	A64T	Risk for UC
		R179X	Protective for UC
	PRDM1	S354N	Risk for CD
		rs7746082	Risk for CD
		L450F	Protective for UC
		rs6911490	Risk for UC
	HLA-DRB1	-	Risk for IBD

Several IBD associated genes are involved in regulation of inflammatory responses. For example, *IL23R* (interleukin 23 receptor) has three variants protective for IBD, R381Q, G149R and V362I. *IL23R* encodes for a receptor of pro-inflammatory cytokine interleukin 23 (IL23). Binding of IL23 to its receptor eventually leads to activation of signal transducer and activator of transcription 3 (STAT3). STAT3 promotes transcription of other pro-inflammatory cytokines which are essential for differentiation of $CD4^+$ T cells into T helper 17 (Th17) cells. The protective variant R381Q causes a loss of IL23R function, reducing the amount of Th17 cells which mediate inflammation. (Uniken Venema et al. 2017)

CARD9 (caspase recruitment family member 9) gene product is an adaptor protein critical for immune response against bacteria and fungi through activation of interleukin 22 (IL22) (Uniken Venema et al. 2017, Cohen et al. 2019). The *CARD9* variant rs10870077 confers risk of IBD by causing deficient CARD9 function (Uniken Venema et al. 2017). Additionally, *CARD9* risk variants are associated with reduced production of aryl hydrocarbon receptor (AHR) ligands by bacteria thus inhibiting IL22 pathway and leading to susceptibility to fungal infections (Uniken Venema et al. 2017, Cohen et al. 2019).

RNF186 (ring finger protein 186) encodes for a ring finger E3 ubiquitin ligase which regulates ER stress induced apoptosis. *RNF186* variant A64T associated with increased risk of UC is located in the domain with E3 ubiquitin ligase activity. A protective variant R179X has also been identified. Unfortunately, the mechanisms by which these variants are involved in the IBD pathogenesis remain to be solved. (Uniken Venema et al. 2017)

PRDM1 (positive regulatory domain 1) encodes a zinc finger containing transcription repressor called PRDM1 or B-lymphocyte-induced maturation protein 1 (BLIMP1). The protein regulates differentiation of B cells into plasma cells and their immunoglobulin secretion. The *PRDM1* variant S354N has been identified to increase risk of CD, and variant L450F offers protection against UC. Two other variants, rs7746082 and rs6911490, confer risk of IBD by reducing *PRDM1* expression: the first is associated with CD and the second with UC. (Uniken Venema et al. 2017)

Lastly, variants in *HLA* (human leukocyte antigen complex) gene encoding major histocompatibility complex (MHC) class II molecules have been associated with IBD. MHC class II molecules are involved in antigen presentation to immune cells hence these risk variants increase inappropriate recognition and immune response to antigens. (Cohen et al. 2019) Particularly alleles of *HLA-DRB1* gene, most notably *HLA-DRB1*01:03* and *HLA-DRB1*07:01*, are strongly associated with an increased risk of IBD (Cleynen et al. 2016). Nonetheless, further studies on HLA variants are needed to understand their role in IBD pathogenesis (Cohen et al. 2019).

3.1.2 Epigenetics

Epigenetics refers to alterations in phenotype that occur independently of modifications to DNA sequence and can be inherited to daughter cells. Environmental factors and microbiota are known to influence epigenetic modifications which are likely to contribute to IBD pathogenesis. (Turpin et al. 2018) Epigenetic modifications include differences in DNA methylation which have been observed in IBD patients (Mentella et al. 2020).

In addition, microRNAs (miRNA) can mediate epigenetic modifications by regulating gene expression (Ramos and Papadakis 2019). Abnormal miRNA expression has been noted in IBD patients.

For example, miRNA-21 expression is elevated in pro-inflammatory Th17 cells. (Mentella et al. 2020)

3.2 Gut microbiota

The human gut microbiota is a community of bacteria, fungi, viruses, archaea, and eukaryotic microbes inhabiting the gastrointestinal tract. The gut microbiota varies in different regions of the intestines in an individual and microbial assembly differs greatly between individuals. (Ananthakrishnan et al. 2018) Research of the gut microbiota has mainly focused on bacteria (Turpin et al. 2018), and the total number of different bacterial species in the human gut is estimated to be at 500-1000 (Mentella et al. 2020). Nevertheless, Firmicutes and Bacteroidetes make up the majority of intestinal bacteria in a healthy individual (Sartor and Wu 2017, Lee and Chang 2021). Members of these two phyla produce short-chain fatty acids (SCFAs) from dietary fibres. SCFAs serve as an energy source for colonic epithelial cells and regulate intestinal homeostasis. (Lee and Chang 2021)

Dysbiosis or disruption of normal gut microbiota composition have been associated with IBD (Ananthakrishnan et al. 2018, Cohen et al. 2019). It is unclear whether these changes are a potential cause of IBD or simply a consequence as, for example, inflammation can affect the microbiota (Turpin et al. 2018). In addition, genetic factors influence the composition of gut microbiota, and IBD associated gene variants affect immune responses to microbes (Sartor and Wu 2017). For example, C11orf30-LRRC32 locus is involved in cell-cell signalling pathway and its variant rs2155219 is associated with an increased risk of IBD. This variant in question was found to correlate with the abundance of Proteobacteria, which potentially contribute to the IBD pathogenesis. (Turpin et al. 2018)

A stable gut microbiota composition is gained by 3 years of age (Turpin et al. 2018) thus environmental factors affecting microbiota during early life, for instance mode of birth and breastfeeding, have an important role (Ananthakrishnan et al. 2018). The gut microbiota remains fairly stable in adulthood unless a major perturbing factor occurs, such as an illness or excessive use of antibiotics (Turpin et al. 2018).

3.2.1 Intestinal bacteria

The reduced bacterial diversity of gut microbiota is characteristic to IBD patients (Sartor and Wu 2017). Regarding to this, decreased abundance of bacteria with protective abilities, mostly Firmicutes and Bacteroidetes, is observed compared to healthy individuals (Turpin et al. 2018, Ramos and Papadakis 2019). These changes in bacterial diversity may expose the host to pathogens and

pathobionts (Ananthakrishnan et al. 2018), such as Proteobacteria and Actinobacteria which are increased in patients with IBD (Turpin et al. 2018).

Additionally, reduced diversity leads to loss of beneficial functions of commensal bacteria. Normally these support intestinal homeostasis, and include digestion, pathogen limitation and barrier function. Disruption of homeostasis allows increased abundance of pathogenic bacteria which can amplify existing inflammation and thus contribute to complications and further decline commensal bacteria. (Lee and Chang 2021)

It is likely that IBD is not caused by a pathogen but by a pathobiont, a commensal microbe that can become pathogenic under certain circumstances. Pathobionts would not cause the disease in a healthy individual who is not susceptible to IBD. For example, *Bilophila wadsworthia* is a sulphite-reducing commensal bacteria also found in healthy individuals although it has been associated with IBD. Regardless, both the loss of commensal bacteria and bloom of pathobionts seem to at least maintain, if not cause, the inflammation. (Lee and Chang 2021)

It is worth mentioning that the composition of gut microbiota can differ between active CD and UC. For example, the abundance of *Haemophilus* genus is found to be decreased in CD yet increased in UC. (Turpin et al. 2018)

As mentioned above, commensal bacteria of the Firmicutes phylum are reduced in IBD (Table 2). *Faecalibacterium prausnitzii* produces butyrate, a SCFA with anti-inflammatory properties. (Turpin et al. 2018) SCFAs promote differentiation of regulatory T cell (Treg cell) and downregulate inflammatory signalling pathways (Cohen et al. 2019) but reduction of *F. prausnitzii* results in a loss of these functions and disrupts intestinal homeostasis (Lee and Chang 2021). However, loss of a specific function may be more relevant than reduction of a certain species as decreased abundance of two other butyrate-producing species, *Roseburia hominis* and *Eubacterium rectale,* has the same effect as *F. prausnitzii* (Lee and Chang 2021).

Bacteroidetes is another phylum with anti-inflammatory properties and decreased abundance in IBD. Yet, commensal *Bacteroides fragilis* is increased in IBD patients despite being low abundant in healthy individuals. Therefore, it is considered to be a potential pathobiont involved in IBD pathogenesis. (Lee and Chang 2021) *B. fragilis* activates Tregs that produce anti-inflammatory Interleukin 10 (IL10). However, this protective function is lost if ATG16L1 and NOD2 mediated autophagy is defective, possibly explaining the pathogenicity in IBD patients. (Sartor and Wu 2017, Cohen et al. 2019)

Adherent-invasive *Escherichia coli* (AIEC) with the ability to adhere to intestinal epithelial cells has increased abundance in IBD patients. Additionally, AIEC are able to replicate in macrophages and prevent phagocytosis. (Lee and Chang 2021) Together with *Bifidobacterium adolescentis,* AIEC activates pro-inflammatory Th17 cells thus promoting inflammation (Cohen et al. 2019). In turn

Enterococcus faecalis is believed to contribute to IBD pathogenesis by inducing disease promoting immune responses and epithelial barrier damage (Sartor and Wu 2017). Finally, the production of reactive oxygen species is elevated during inflammation which may increase the abundance of resistant bacteria, such as *Salmonella typhimurium* and other aerobic microbes (Turpin et al. 2018).

Table 2. Alterations in the abundance of IBD associated bacterial species. (Sartor and Wu 2017, Turpin et al. 2018, Cohen et al. 2019, Lee and Chang 2021)

Increased in IBD	**Reduced in IBD**
Bilophila wadsworthia	*Faecalibacterium prausnitzii*
Bacteroides fragilis	*Roseburia hominis*
Adherent-invasive *Eschriachia coli*	*Eubacterium rectale*
Bifidobacterium adolescentis	
Enterococcus faecalis	
Salmonella typhimurium	

3.2.2 Nonbacterial gut microbiota

Besides alterations of bacterial composition, it has been suggested that fungi, viruses and archaea are involved in IBD pathogenesis (Ananthakrishnan et al. 2018). Fungi make up only a small part of the gut microbiota but the repeated use of antibiotic favours fungal overgrowth. This discovery supports the theory that bacteria and fungi have a competitive relationship in the gut. (Sartor and Wu 2017) The diversity of fungi is found to be decreased in IBD patients as two phyla, Ascomycota and Basidiomycota, seem to dominate (Cohen et al. 2019).

Members of the *Candida* genus predominate in the human gastrointestinal tract (Sartor and Wu 2017). Especially *Candida albicans* and *Candida tropicalis* have increased abundance in IBD patients (Sartor and Wu 2017, Lee and Chang 2021). Also, a commensal skin yeast *Malassezia restricta* has been found in increased amounts in the intestinal mucosa of patients with CD. In contrast, *Saccharomyces cerevisiae* shows decreased abundance. In fact, anti-*Saccharomyces cerevisiae* antibody (ASCA) acts as a biomarker for CD. ASCA targets the cell wall of *S. cerevisiae* and its generation indicates fungal invasion of intestinal epithelial cells. (Lee and Chang 2021)

The bacterial composition of the gut microbiota is affected by viruses, more specifically by bacteriophages (Sartor and Wu 2017). Bacteriophages are the most common viruses of human gastrointestinal tract and they infect bacteria and archaea (Lee and Chang 2021). Lytic cycle of bacteriophage leads to viral replication and eventually to lysis of bacterial cell. In turn, integration of viral

genetic material intro bacterial genome through lysogenic cycle alters the function of bacteria. IBD patients show increased viral diversity with higher abundance of bacteriophages belonging to Caudovirales. (Sartor and Wu 2017) Not much is known of archaea and their contribution to IBD pathogenesis, but they seem to be less abundant during active disease (Lee and Chang 2021).

3.3 Environmental factors

The rapidly increasing incidence of IBD worldwide suggests the importance of environmental factors associated with Western lifestyle and urbanisation in IBD pathogenesis, for example Western diet, use of antibiotics and pollution in an urban environment. This is supported by the emergence of IBD in developing countries in recent decades, in parallel with their industrialisation. (Ananthakrishnan et al. 2018) Additionally, early life factors, such as breastfeeding and hygiene, influence the development of the immune system, and together with adulthood exposures they may contribute to the risk of IBD (van der Sloot et al. 2017, Ananthakrishnan et al. 2018). Potential environmental risk factors of IBD are presented in Table 3 and discussed below.

Table 3 Overview of potential environmental factors contributing to IBD pathogenesis. (van der Sloot et al. 2017, Ananthakrishnan et al. 2018, Ramos and Papadakis 2019, Mentella et al. 2020)

Environmental factor	**Role in CD**	**Role in UC**
Lifestyle		
Smoking	Risk	Protective
Diet & nutrients		
Dietary fibre	Protective	No association
Omega-3 PUFA	Protective	Protective
Omega-6 PUFA	Risk	Risk
Animal protein	Risk	Risk
Zinc	Protective	Protective
Vitamin D	Protective	Protective
Medications		
Antibiotics	Risk	Risk
NSAIDs	Risk	Risk
Geographic location		
Pollution	Risk	Risk
Hypoxia	Risk	Risk

3.3.1 Smoking

Smoking is a widely studied environmental factor associated with increased risk for CD in both current and former smokers. Interestingly, current smoking has shown protective effects on UC although former smoking increases the risk significantly. Thus, it has been concluded that smoking mainly delays the onset of UC. On the other hand, smoking has been associated with milder symptoms and fewer recurrences in patients with UC. Passive cigarette smoke exposure has shown no association with IBD. (van der Sloot et al. 2017)

Smoking affects the immune responses and contribute to inflammation in IBD by altering production of pro-inflammatory cytokines (van der Sloot et al. 2017). Additionally, cigarette smoke promotes changes in the gut microbiota and epithelial barrier integrity, as well as being toxic to immune cells and mucus producing epithelial cells (van der Sloot et al. 2017, Ramos and Papadakis 2019). However, the protective mechanisms of current smoking in UC remain unclear (van der Sloot et al. 2017).

3.3.2 Diet and nutrients

Diet can alter the composition of the gut microbiota eventually leading to abnormal intestinal immune response and inflammation (Ananthakrishnan et al. 2018). Indeed, certain dietary factors have been associated with IBD (Turpin et al. 2018).

High consumption of dietary fibre has been inversely associated with risk of CD (Ramos and Papadakis 2019). Metabolism of fibre into SCFAs by intestinal bacteria could explain the protective effect as SCFAs preserve intestinal homeostasis with their anti-inflammatory properties (Ramos and Papadakis 2019, Mentella et al. 2020). Especially fibre from fruits reduced the risk of CD while fibre from whole grains was not associated with the risk (Ananthakrishnan et al. 2018).

Diet with high fat intake is considered a significant risk factor for the development of IBD. In particular, the role of polyunsaturated fatty acids (PUFAs) in IBD pathogenesis has received attention. There is evidence that omega-3 PUFAs confer protection against IBD while omega-6 PUFAs induce inflammation. Ratio of omega-3 and omega-6 PUFAs determine the state of homeostasis. Hence, the high incidence in Western countries could be explained by a typical high omega-6 to omega-3 ratio in Western diets. (Mentella et al. 2020)

Additionally, high intake of animal protein from fish and meat is associated with increased risk of IBD. However, mechanisms by which animal protein contribute to IBD pathogenesis is unknown. (Mentella et al. 2020)

Various micronutrients act as coenzymes involved in regulation of intestinal homeostasis and immune responses. Zinc, for instance, is a cofactor of intestinal metalloproteinases whose deficiency might reduce epithelial barrier integrity and confer risk for IBD. Conversely high zinc intake is found to be a protective factor in IBD. (Ananthakrishnan et al. 2018) The same applies to vitamin D, which downregulates the inflammatory response trough activation of the vitamin D receptor in on T helper 1 (Th1) cells. (van der Sloot et al. 2017)

Lastly, emulsifiers present in processed foods might contribute to pathogenesis of IBD. These detergent-like molecules, such as carboxymethyl cellulose (CMC) and polysorbate 80 (P80), can induce damage in the intestinal epithelium and alter the gut microbiota, which may result in inflammation. (Ananthakrishnan et al. 2018, Mentella et al. 2020) Other food additives in processed foods that promote inflammation include maltodextrin used as thickener, artificial sweeteners and food colorants like titanium dioxide (Mentella et al. 2020).

3.3.3 Medications

Antibiotics are common in Western countries and they are increasingly used in developing countries as well. The use of antibiotics has been associated with increased risk of IBD, especially the exposure to metronidazole and fluoroquinolones in early life. The risk seems to increase with a rise in antibiotic dispensations. (Ananthakrishnan et al. 2018) Use of antibiotics during 2 years preceding the diagnosis was associated with paediatric CD in Finland. No significant association was found with paediatric UC. The risk of CD increased with more frequent use of antibiotics, particularly cephalosporins. (Virta et al. 2012)

Susceptibility to IBD due to the use of antibiotics is likely a result of a disruption in the composition of the gut microbiota. Alterations in microbial communities may enable the colonisation by pathogenic bacteria while interfering the development and function of the intestinal immune system hence increasing the risk. (van der Sloot et al. 2017)

The increased risk of IBD is also associated with frequent use of non-steroidal anti-inflammatory drugs (NSAIDs) (Ananthakrishnan et al. 2018). The risk of UC increases almost twofold when NSAIDs are used for at least 15 days per month, whereas the increase in risk of CD is more subtle. Overall, the risk of developing the disease increases with higher frequency and longer duration of use. (van der Sloot et al. 2017)

3.3.4 Geographic location

Several components of air pollution in urban environments have been associated with IBD (Ananthakrishnan et al. 2018). For example, individuals living in regions with high nitrogen dioxide (NO_2)

concentrations were at higher risk for CD whereas high concentrations of sulphur dioxide (SO_2) increased the risk of UC (van der Sloot et al. 2017, Ananthakrishnan et al. 2018). Fine particulate matter is an exception as exposure was inversely associated with the risk of IBD (Ananthakrishnan et al. 2018). Nevertheless, total pollutant emissions correlate with increased risk of IBD and hospitalisation due to disease (van der Sloot et al. 2017, Ananthakrishnan et al. 2018).

Hypoxic conditions, such as high altitude, induce inflammation and increase levels of inflammatory markers, mainly interleukin 1 receptor antagonist (IL1ra), IL6 and CRP (van der Sloot et al. 2017, Ananthakrishnan et al., 2018). Hypoxia-inducible factor (HIF) is activated in hypoxic conditions, after which it upregulates the transcription of proteins involved in angiogenesis, for instance. Increased expression of HIF has been observed in patients with IBD. (Ananthakrishnan et al. 2018) Additionally, it has been shown that patients experiencing recurrences fly or travel more frequently over 2000 metres above the sea level when compared to patients in remission. However, the role of hypoxia in development of the disease is unclear and needs further evaluation. (van der Sloot et al. 2017, Ananthakrishnan et al. 2018).

4. CONCLUSIONS

The incidence and prevalence of IBD are ever-increasing but the underlying cause is to this day unknown. The prevalence is highest in Western countries although the rise in incidence has recently been more significant in countries which have industrialised during the past few decades. These observations highlight the importance of environmental factors associated with Western lifestyle and urban environments, most importantly omega-6 PUFAs and meat in diet, pollutant emissions and antibiotics. Environmental factors may contribute to the inflammation through altering the composition of the gut microbiota.

Dysbiosis in the gastrointestinal tract include decreased abundance of commensal, potentially protective bacteria. Reduction of commensal species and loss of their beneficial functions may allow pathogens to invade and disrupt the intestinal homeostasis. However, it is believed that pathobionts have the most significant impact in individuals genetically susceptible to IBD. In fact, dozens of genetic loci have been associated with increased risk of IBD. Identified gene variants cause alterations to immune responses against intestinal microbes thus promoting dysbiosis. Heterogeneity of the disease could indicate differential risk factors contributing to each phenotype, as have been seen with separate genetic risk loci for CD and UC.

IBD causes decreased quality of life in patients and substantial burden and costs to healthcare, with expensive medical therapy and surgery. Hence, further research is necessary to determine aetiology of IBD and to develop methods to reduce morbidity.

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