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Bachelor's thesis Biology; Biomedical Sciences

The Interplay Between Host Genetics, the Gut Microbiome, and Rheumatoid Arthritis; A Literature Review.

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Summary

Rheumatoid arthritis (RA) burdens approximately 18 million individuals globally, by causing severely debilitating joint inflammation. This literature review aims to comprehensively explore the intricate relationships among host genetics, the gut microbiome GM, and RA pathogenesis. By exploring this interplay in more depth, this review aims to establish a solid foundation of up-to-date insights, propose hypotheses, and identify future research avenues. Host genetics have been found to significantly contribute to RA heritability, with HLA-DRB1 and PTPN22 genes being implicated in contributing genetic predisposition. Furthermore, a bidirectional relationship between the host genetics and GM has been observed and pre-dispositioned individuals show distinct GM compositions compared to healthy individuals, even before the onset of RA. Dysbiosis in the GM, commonly observed in RA patients, may contribute to chronic inflammation and RA disease progression. Specific microbes like *Prevotella spp.*, particularly, *P. copri,* have been associated with early-RA and disease severity. This highlights the plausible role in RA pathogenesis. These findings illustrate the importance of unraveling the interplay between host genetics, GM, and RA, in its sequential developmental phases. Through a thorough analysis and comparisons made between current literature, multifaceted mechanisms underlying RA pathogenesis will be discussed. Additional insights, specifically on the oral microbiome and individual contributing factors like epigenetics, diet, and smoking can aid in further elucidating RA pathogenesis. Incorporating new research methodologies and taking the hypotheses outlined in this literature review into account can enhance future research on RA, potentially contributing to the development of enhanced RA therapeutics and ultimately reducing the global prevalence of RA.

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Introduction

What could be the potential reasons behind some individuals developing Rheumatoid Arthritis (RA) while others remain unaffected? Ongoing research is aimed at elucidating the reasons and mechanisms behind this severely debilitating auto-immune disease, which is characterized by inflammation in the lining of the synovial joints, referring to areas where bones move against each other.

What happens in RA is that the immune system loses its ability to distinguish between healthy cells and invading pathogens. This dysregulated immune response results in an attack on the body's healthy cells and tissues, leading to severe, progressive damage and ultimately weakening the body (Autoimmune Diseases, n.d.). Research suggests that disturbances of the gut microbiome, known as dysbiosis, possibly contribute to RA by triggering immune dysregulation (Rooney et al., 2020).

More specifically, various immune cells including B-cells, T-cells, and macrophages are thought to be involved in the state of chronic inflammation observed in RA (Yap et al., 2018). B-cells become autoreactive, targeting, and destroying self-antigens, while T-helper and T-helper 17 cells secrete pro-inflammatory chemokines and cytokines inducing inflammation. Additionally, macrophages can undergo a phenotypic transformation from an anti-inflammatory to a pro-inflammatory state, contributing to the secretion of cytokines that promote joint destruction (Yap et al., 2018). These immune dysfunctions accumulate and collectively result in chronic systemic inflammation in RA.

In addition to inflammation of the synovial joints, individuals experience painful progression of bone and cartilage breakdown, ultimately resulting in joint deformation (Mayo Clinic, 2023). In the early stages of RA, the initial symptoms will manifest in the smaller joints of the hands and feet. When the disease progresses, inflammation will extend to the lining of the larger joints, including the wrists, ankles, knees, elbows, shoulders, and hips, thereby, contributing to progressive joint damage and large physical impairment to the individual (Mayo Clinic, 2023). Moreover, individuals with RA commonly experience a diminished health-related quality of life (HRQoL) due to pain and impaired physical functioning. These limitations not only affect daily life but also impact social interactions and the ability to work (Strand & Khanna, 2010).

In 2019, the World Health Organization (WHO) stated that 18 million individuals globally were affected by RA. There was an increased prevalence of RA found among females, and 55% of them were aged over 55 years old. This suggests that individuals at an older age are at a higher risk of developing RA (World Health Organization, 2023).

It has been a challenge for scientists to further unravel the pathology and aetiology of RA, which is inherently a polygenic disease. Polygenic refers to the fact that a disease can result from a combination of individual or multiple interacting genes ultimately leading to the development of RA (Wells et al., 2019). Based on current research, it is believed that genetic and environmental factors and their interactions significantly contribute to triggering the development of RA and

determine the severity. Genetic heritability is estimated to contribute approximately 60% to the development of RA, supported by twin studies (Jahid et al., 2023). Therefore, the remaining 40% can be attributed to non-genetic factors. Studies have revealed associations between RA and specific environmental factors such as diet, tobacco smoking, the microbiome, and an individual's overall lifestyle, including a lack of physical activity – affecting the development and progression of RA (Shekhar et al., 2023). Particularly, the gut microbiota (GM) has been described to be a centre where signals regarding the immune system, environmental factors, and genetics all interact with one another (Wells et al., 2019).

The GM encompasses all microbes in the gastrointestinal tract (GI tract) and host genetics play a substantial role in shaping the GM composition. Genetics can even impact the heritability of specific microbial species in the gut (Bubier et al., 2021). Moreover, the GM holds approximately 70-80% of all immune cells in the body (Wiertsema et al., 2021). Researchers have found that GM has been associated with inducing systemic inflammation, which is what occurs in RA (Lin et al., 2023). Therefore, the interplay between the gut microbiome, host genetics, and RA is very complex but fascinating to uncover.

The Global Burden of Diseases, Injuries, and Risk Factor Study (GBD) in 2021 developed an estimation model for RA prevalence in 2050, based on obtained data from population-based studies and medical claims data. GBD foresees an increase in prevalence of RA up to 31.7 million individuals with RA globally by 2050 (Black et al., 2023). This growing global burden is urgent to tackle. Currently, there is no curative treatment available for RA. However, medication can aid in managing symptoms and disease progression. Early diagnosis and timely treatment initiation are important in attenuating the progressive- and chronic nature and the severity of RA (Black et al., 2023).

Hence, the main objective of this literature review is to summarize the latest research findings regarding the interplay of genetics, the gut microbiome, and rheumatoid arthritis. This comprehensive review can be used as a foundation for future research avenues on this topic. Obtaining a better understanding of this interplay can ultimately contribute to reducing the prevalence of RA, broadening our comprehension of the underlying mechanisms, and potentially enhancing treatment approaches for individuals affected by this autoimmune disease.

Consecutive phases of RA development

RA has a sequence of phases in disease development, including a preclinical and clinical stage. Individuals can have a genetic predisposition to RA without exhibiting clinical symptoms, composing an ‘at-risk’ group. Notably, within this cohort, some individuals may exhibit detectable markers such as anti-citrullinated protein antibodies (ACPA) or rheumatoid factor (RF) autoantibodies, though not all individuals progress to clinical RA (Greenblatt et al., 2020).

The ‘pre-clinical’ stage represents individuals who are in the earliest detectable phase of RA and possess the previously mentioned markers in addition to a genetic predisposition. Despite having the indicators, clinical symptoms are still absent during this phase (Greenblatt et al., 2020).

Once clinical symptoms manifest and a formal diagnosis of RA is made, an individual progresses into the ‘early-RA’ stage (Greenblatt et al., 2020). This phase rapidly follows the onset of initial clinical manifestations of RA.

Furthermore, the distinct ‘new-onset RA’ stage encompasses individuals experiencing recent clinical symptoms, irrespective of the timing of diagnosis. This stage may also include symptom intensification or episodic flare-ups (Stack et al., 2013). The distinction between the ‘early-RA’ and ‘new-onset RA’ groups is solely based on the timing of diagnosis concerning the onset of initial clinical symptoms, highlighting the nuances in disease progression.

Mapping out these consecutive phases of RA not only facilitates gaining more understanding of the disease progression but also offers new insights into the intricate interplay among genetics, the GM, and RA pathogenesis. Moreover, such insights can enable more precise targeting of interventions trying to attenuate the progressive nature of RA.

The genetic makeup of RA

RA is a complex, polygenic disease, indicating that various genetic variants collectively contribute to the onset of disease. Genetic factors interact with environmental influences, thus complicating the understanding of RA onset. Consequently, elucidating the underlying genetic mechanisms of RA is important for a greater understanding of RA aetiology.

GWAS

Genome-wide association studies (GWAS) are an important research tool for studying polygenic diseases like RA. GWAS is designed to test for millions of genetic variants across the genome of an individual. Its main objective is to identify alleles of genetic variants that exhibit statistically significant correlations with specific traits or diseases, such as RA (Uffelmann et al., 2021).

SNPs

Polymorphisms in the RA population are variations in specific genes that add to the complexity of the disease (Mikhaylenko et al., 2020). A relevant type of polymorphism in RA is a single nucleotide polymorphism (SNP), which is a small variation in a single nucleotide base of the DNA, consequently contributing to genetic variation. SNPs can be further associated with specific risk loci, which are physical locations on a chromosome. By linking SNPs to these risk loci, GWAS can facilitate gaining further insight into the genetic mechanisms associated with RA (Uffelmann et al., 2021). SNPs are commonly used genetic tools in RA research, allowing insight into how the genome potentially influences the onset, further development, and severity of RA (Collins, 2021).

Interestingly, approximately 60% of RA-associated SNPs are in context-specific gene enhancers, pivotal in regulating gene expression in response to specific environmental contexts (Messemaker et al., 2015). Considering RA's polygenic nature and intricate interplay between genetics and environmental factors, it is plausible that SNPs may influence or be influenced by gene expression regulation in RA. Consequently, these SNPs could directly or indirectly contribute to dysregulating gene expression, thereby contributing to RA pathogenesis.

HLA associations

Genetics plays a significant role in RA, accounting for a heritability of approximately 60%. The most strongly established genetic risk factor is the human leukocyte antigen-DRB1 (HLA-DRB1) gene, where RA-associated SNPs can arise. HLA-DRB1 is located on chromosome 6 (Wells et al., 2019). It is thought that HLA-DRB1 contributes to approximately 30-50% of the genetic risk for the onset of RA (Inoue et al., 2023). Within HLA-DRB1, specific amino acid positions 11, 71, and 74 compose the shared epitope (SE) phenomenon, having the largest association with a heightened RA risk. The shared epitope hypothesis proposes that the shared amino acid sequences, occurring in positions 11, 71, and 74, among the HLA-DBR1 alleles increase the genetic risk for RA and disrupt the immune system, resulting in an autoimmune response against self-antigens, thereby contributing to RA pathogenesis (Wells et al., 2019) (Cha et al., 2024).

The HLA-DRB1 allele is part of the major histocompatibility complex (MHC) class II, pivotal in the immune system's antigen presentation to T cells. The MHC complex distinguishes between harmful/foreign and self-antigens, giving rise to the appropriate immune response (HLA-DRB1 Gene: MedlinePlus Genetics, n.d.). Despite everyone having the HLA-DRB1 gene, there are numerous allele variants such as HLA-DRB1*04:01, HLA-DRB1*04:08, HLA-DRB1*01:01, HLA-DRB1*10:01 and more that exhibit genetic variation within the HLA-DRB1 gene.

Shared epitopes may disrupt MHC II presentation, crucial for proper immune responses. More specifically, the interaction between shared epitopes and the MHC II complex may interrupt antigen presentation or alter the peptide binding affinity of the MHC II complex, dysregulating immune responses and resulting in autoimmunity (Maria-Nefeli Tsetseri et al., 2023).

Non-HLA associations

In addition to the MHC-associated HLA-DRB1 gene, several non-HLA genetic risk factors are associated with RA onset. Among these, the PTPN22 (Protein tyrosine phosphatase, non-receptor type 22) gene is the second most significant genetic risk factor for RA. PTPN22 has been observed in other autoimmune disorders besides RA, like type 1 diabetes. Its link to immune dysregulation is established and is theorized to exacerbate immune dysregulation by impacting T- and B-cells and macrophage activity, thereby contributing to systemic inflammation seen in RA (Tizaoui et al., 2021). Furthermore, STAT4, IRF5, and CCR6 are non-HLA genes that possess SNPs associated with RA susceptibility (Kurkó et al., 2013).

Moreover, GWAS has revealed further insights into the genetic mechanisms underlying RA by identifying 100 non-MHC genetic loci found to be associated with other immunological processes that contribute to the development of RA (Jiang & Alfredsson, 2020). Intriguingly, risk loci that have been identified to date merely account for 15% of the genetic contribution to RA development, implying that many risk loci are yet to be discovered (Wells et al., 2019).

Genetic risk and predisposition

Individuals with an inherent genetic predisposition, such as having the HLA-DRB1 gene, show an increased susceptibility to RA based on their genetic makeup. However, it is important to mention that genetic predisposition does not imply that an individual will develop RA, but rather that the likelihood of developing RA is higher due to genetic risk factors.

Polygenic risk score (PRS)

The polygenic risk score (PRS) is a quantitative tool used to assess an individual's genetic risk based on their genetic predisposition to a particular disease. This predisposition is determined by an individual's risk alleles, often SNPs, detected by a GWA study. The reliability of PRS prediction can be enhanced by integrating additional risk factors that are established to contribute to developing RA (CDC, n.d.). For instance, tobacco smoking, diet, age, and gender can be incorporated into the PRS calculation.

Furthermore, covariates are independent variables, which are commonly integrated into forming the PRS. This is done to limit bias caused by confounding factors or affecting the PRS. For example, in a study conducted by Wells et al. (2020), they investigated the associations between the gut microbiota and genetic predisposition to RA in individuals without the disease. They generated a PRS for RA and used the covariates BMI, age, and sequencing depth to account for unseen observations that may influence the results of their research.

In short, PRS serves as an important tool for predicting disease development, severity, and individual treatment response, thereby helping to increase our understanding of RA aetiology and progression (CDC, n.d.).

Serum Classification in RA

To gain more insight into RA and its underlying mechanisms, it is beneficial to classify RA into its subtypes, namely seropositive and seronegative RA. Seropositive RA is characterized by an individual testing positive for the presence of rheumatoid factor (RF) and/or anti-citrullinated protein antibodies (ACPAs) in the blood serum. Whereas seronegative RA has undetectable levels of RF or ACPA (Salma et al., 2020).

In a study conducted by Selma et al. (2020), individuals with seropositivity showed significantly higher levels of RA-related clinical symptoms, including joint inflammation, joint destruction, and deformation compared to seronegative individuals in the study. However, it is notable that 90% of the individuals taking part in the study were seropositive and 10% were seronegative (n=294). An explanation for the seronegative cohort being significantly smaller in size could be attributed to the fact that generally, an approximation of 30% of the individuals affected by RA have undetectable levels of RF and ACPAs, thus are seronegative (Kolarz et al., 2021).

Autoantibodies in RA

Rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPAs) are autoantibodies and fungate as serum hallmarks for RA, detectable in approximately 70-80% of individuals affected by this autoimmune disease (Okada et al., 2019). Moreover, they are both associated with specific genetic risk loci in RA (Wells et al., 2020).

In the context of RA, RF targets the tail of the antibody IgG, known as the Fc region. The Fc portion of the antibody is important for binding to immune cells and activating immune responses. Consequently, if RF disrupts the Fc region, this can cause inflammation and immune dysregulation, such as a loss of tolerance to self-antigens (i.e., citrullinated antigens) (Tiwari et al., 2020). Moreover, RF in a disease context is not solely associated with RA; it is also observed in other (non-)autoimmune diseases (Yap et al., 2018)

ACPAs target citrullinated proteins, which occur when arginine (amino acid) is converted into citrulline by the enzyme peptidyl arginine deiminase (PAD), a calcium-dependent enzyme. This post-translational modification is irreversible and occurs mainly in the central nervous system (CNS). Citrullination has been correlated to autoimmune diseases such as RA. In the context of autoimmunity, citrullinated proteins have been found to be more likely to trigger inflammatory responses compared to non-citrullinated proteins (Jin et al., 2023).

The origin of RF remains unknown and the reason why ACPAs specifically target citrullinated proteins in RA is not elucidated. Nonetheless, detectable levels of RF and ACPA in the serum are strongly linked to clinical symptoms of RA (Kolarz et al., 2021). Strikingly, it has been found that an individual can even have detectable RF and ACPAs in their serum before any clinical RA symptoms manifest (Okada et al., 2019).

Host genetics, PRS, and the gut microbiome

Understanding genetic predispositions and risks is important for uncovering the complex aetiology of RA, with the PRS being a valuable tool in quantifying genetic susceptibility. The PRS integrates information from various genetic variants associated with RA risk, and serological markers like RF and ACPA are also considered.

The latest research highlights the importance of studying the interplay between host genetics and environmental factors (Jiang & Alfredsson, 2020). In particular, the exposome, such as the gut microbiome, encompasses external as well as internal factors that can influence health and disease in an individual's body. Therefore, the gut microbiome (GM) has emerged as an important environmental component in RA pathogenesis and is a growing field of research.

For instance, a study conducted by Wells et al. (2020) explored the association between the GM and genetic risk for RA in individuals without the disease. The PRS was based on 233 SNPs associated with RA identified through GWAS. This study focused on a cohort of twins with a high PRS but no RA diagnosis. The cohort was carefully selected to isolate genetic factors associated with RA and minimize confounding influences. The obtained findings were validated in another

cohort, the SCREEN-RA patients, being first-degree relatives of patients with RA carrying SE risk alleles. This validation was done to enhance the reliability of the twin's results.

The study identified the bacteria *Prevotella spp.*, showing a positive association with RA. The presence of *Prevotella spp.* in the GM of individuals without RA, but with a high PRS for RA, suggested there is an association between host genetics and GM composition before the manifestation of clinical RA symptoms (Wells et al., 2020). Essentially, this study highlights the potential role of gut microbiome composition in the early stages of RA pathogenesis and that it is essential to study the interplay between host genetics and gut microbiome.

The gut microbiome in RA

The gut microbiome (GM) encompasses a dynamic population of microorganisms consisting of various bacteria, viruses, fungi, and archaea that play a crucial role in maintaining a healthy microbiome, important for overall health and homeostasis. The gastrointestinal tract (GI tract) is the largest mucosal surface of the microbiome and is the host of the highest concentration of microorganisms. The GM is subjected to many internal and external influences, including environmental or genetic factors, which shape the composition and function of the commensal microbes within the GI tract (Maria-Nefeli Tsetseri et al., 2023). Accordingly, the GM varies strongly among individuals.

A strong bidirectional interaction exists between the GM and its host, which significantly influences the host's health status or susceptibility to disease. In the case of disease, dysbiosis of the microbiome is a commonly observed characteristic, which refers to an imbalance or disruption in the population of microorganisms of the microbiome (Dieterich et al., 2018). This can have profound consequences for the hosts' physiological state. As previously mentioned, the gut microbiome harbours a significant amount of the body's immune cells and dysbiosis can lead to dysfunctional immune responses, potentially contributing to the onset and progression of RA (Dong et al., 2023).

Intestinal dysbiosis and inflammation

A healthy human gut hosts approximately 35,000 bacterial species (Jeyaraman et al., 2023). Dysbiosis is common among individuals affected by RA, disrupting their microbial composition, characterized by an abundance or deficiency of species that reside in the gut. This imbalance can impact the gut epithelium, a main component of the intestinal barrier responsible for maintaining gut homeostasis between commensal and foreign substances, and pathogenic microorganisms (Audo et al., 2022).

Crucial components of the gut epithelium are the tight junctions, which are specialized proteins that safeguard proper selective intestinal permeability (IP) and ensure a strong barrier structure (Stolfi et al., 2022). In the context of RA, dysbiosis can disrupt the gut barrier, leading to an increase in IP allowing an increased passage of unwanted antigens such as toxins, pathogens, or food particles across the gut barrier (Figure 1). This translocation of antigens can trigger inflammation and lead to an upregulation of HLA class II molecules (Stolfi et al., 2022) (Berryman et al., 2023). Consequently, antigen-presenting cells (APCs) in the gut mucosa encounter a greater antigen load, leading to the upregulation of HLA-class II molecules and subsequent activated immune responses. The increase in immune responses can eventually impact the microbial composition of the GM, sustain dysbiosis, increase inflammation, and contribute to immune dysregulation, thereby potentially aggravating RA's autoimmune nature (Berryman et al., 2023). Currently, it is hypothesized that chronic inflammation at mucosal sites, including the gut, lungs, and oral cavity contributes to immune dysregulation and the onset of autoimmunity through loss of tolerance for self-antigens (Gilbert et al., 2024).

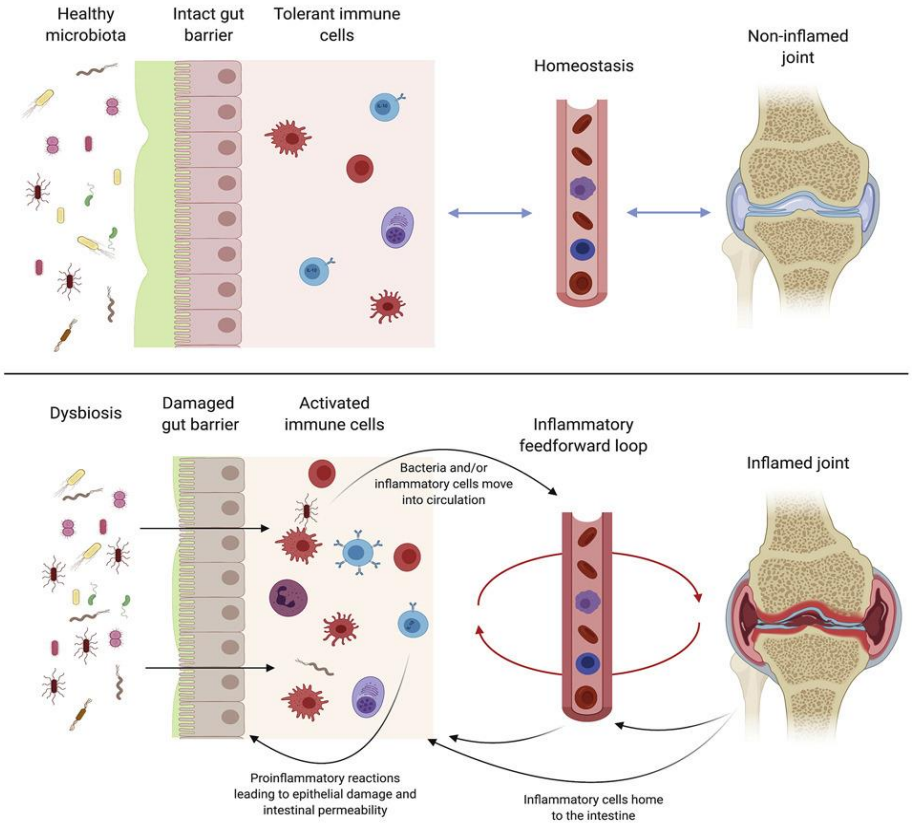

Figure 1. This illustrates a comparison between a healthy gut microbiome versus gut dysbiosis, featuring dysbiosis, a damaged gut barrier, activated immune responses, and a proposed inflammatory feedforward loop within the context of RA. This graphic represents a hypothetical mechanism contributing to the development of RA pathogenesis; however, it is important to note the exact underlying mechanism has not yet been elucidated (Matei et al., 2021).

In addition, there is evidence suggesting that heightened IP may allow the inflow of the PAD enzyme into the gut, which initiates the citrullination of proteins. As mentioned before, initiating protein citrullination correlates with RA symptoms. Excessive PAD enzyme activity may lead to hyper citrullination, resulting in high ACPA production marking the onset of autoimmunity and contributing to RA pathogenesis and progression of the disease (Blenkinsopp et al., 2023). In concordance, dysbiosis and an increase in IP correlated to RA have been supported by research (Blenkinsopp et al., 2023).

Gut microbes associated with RA

More present-day studies have started exploring the interplay among the gut microbiome, genetic factors, and RA, providing new insights into the associations between specific microbial communities and the progression of RA.

Studies have consistently shown that individuals at risk for RA often have a distinct gut microbiome composition compared to healthy individuals (Rooney et al., 2023). Wells et al. (2020) explored the gut microbiota in genetically predisposed individuals in the absence of RA. They found an abundance of *Prevotella_7* in the GM of these individuals, linked to the SE hypothesis of HLA-DRB1 (Figure 2). Their phylogenetic and community relationship analysis within the *Prevotellaceae* family indicated that there might be a biological linkage between *Prevotella_7* and *Prevotella_9*, based on the frequently observed clustering of these species (Wells et al., 2020).

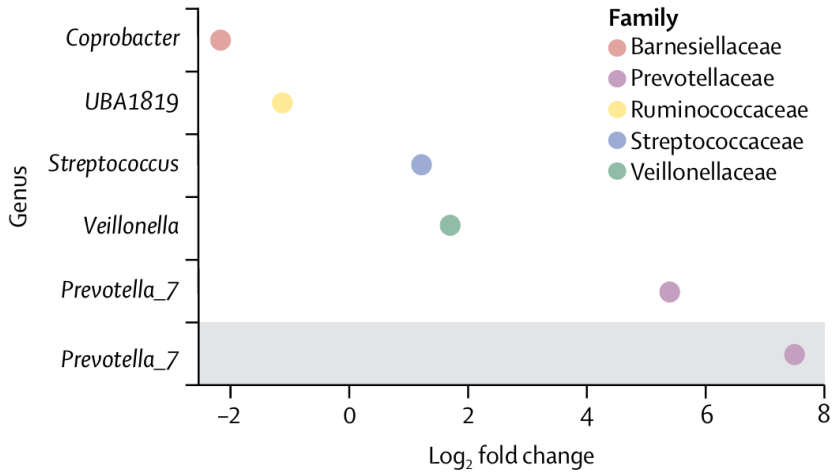

Figure 2. This graph represents the abundance of various bacterial genera found in the gut microbiota of the participants in the study by Wells et al. (2020). The Log2 fold scale indicates that among all participants Prevotella_7 was found to be the highest in abundance. However, in individuals without RA associated symptoms (grey area) but positively associated with HLA-DRB1 SE positivity, Prevotella_7 was the only genus to remain in abundance among these individuals.

P. copri, a species within the *Prevotella_9* cluster, is hypothesized to play a role in RA and is associated with inflammatory conditions and disease severity. One study accentuates the potential involvement of *Prevotella* species (*Prevotella spp.*) in the early developmental stages of RA (Wells et al., 2020). In concordance, Jeyarman et al. (2023) posit that *P. copri* abundance is observable in early-RA individuals, stimulating Th17 cells that are involved in pro-inflammatory immune responses and contributing to RA pathogenesis.

Overall, these findings suggest that genetic predisposition for RA can influence the gut microbial composition, even in the absence of disease. It is suggested that *Prevotella spp.* may primarily contribute to the early developmental phases of RA (Wells et al., 2020).

In addition to investigating GM dysbiosis, scientists are also exploring how dysbiosis can disrupt the gut-bone axis. This axis is crucial for maintaining overall health and involves bidirectional communication between the gut and bones, but also encompasses muscles, surrounding tissues, and joints. Disruption may contribute to the onset of chronic diseases, such as RA, and again highlights the importance of maintaining a healthy GM (Jeyaraman et al., 2023).

Rooney et al. (2020), in contrast to the study by Wells et al. (2020), investigated GM perturbations in individuals predisposed to RA and positive for ACPA. They found no abundance of *Prevotellaceae* in their GM composition; however, they did find there to be a distinct GM composition compared to healthy individuals. The genetically predisposed cohort exhibited an increase in *Lachnospiraceae, Helicobactereae, Ruminocaccaceae, Erisipelotrichaceae,* and *Bifidobacteriaceae*. In particular, *Erisipelotrichaceae* and *Helicobactereae* were highly immunogenic, stimulating IgA secretion and resulting in elevated serum IgA levels among genetically predisposed individuals (Rooney et al., 2020).

IgA, closely associated with mucosal tissues, plays a pivotal role in maintaining their homeostasis (Patel & Jialal, 2023). Increased IgA levels in at-risk individuals suggest that mucosal tissues, such as the gut, contribute to the development of RA and its autoimmune nature (Rooney et al., 2023).

While genetically predisposed individuals exhibited dysbiosis while maintaining healthy microbial richness, some participants advanced to an early stage of RA, showing dysbiosis and a decreased microbial richness compared to healthy controls. This suggests dynamic changes in microbial communities during the transition from genetic predisposition to early RA. The exact mechanism underlying this transition remains unknown (Rooney et al., 2023).

Among individuals progressing to an early-RA stage, a phylogenetic link revealing shared microbial taxa before the onset of RA, occurring approximately 188 days before the disease was a compelling discovery. This finding suggests that rare microbial taxa potentially contribute to the onset of RA, as they differ from the taxa found in abundance in the at-risk cohort (Rooney et al., 2023).

Additional evidence supporting the potential role of rare microbial taxa comes from a study by Chen et al. (2016), describing a microbial profile for disease prediction by identifying rare lineage intestinal microbes (RLIM) that are uncommon in a healthy GM. However, in the context of RA, there appears to be an increase in RLIM, including *Eggerthella, Collinsella*, and *Faecalibacterium*. Specifically, *Collinsella* abundance was higher in individuals affected by RA, associated with increased IP and potentially impacting RA severity (Chen et al., 2016).

In line with Chen et al. (2016), a literature review by Berryman et al. (2023) explored the interaction between other autoimmune diseases, HLA class II variants, and gut bacteria positively associated with RA. This included *Faecalibacterium, Eggerthella, Collinsella*, and *Prevotella copri*. Interestingly, *Prevotella copri* was also found to be positively associated with type 1 diabetes (T1D), another autoimmune disease. This finding highlights the complex interplay between

autoimmune disorders and emphasizes the interconnectedness of microbes underlying the intricate nature of autoimmunity.

Oral microbiome in RA

The Mucosal Origin Hypothesis suggests a linkage between chronic inflammation of the gut and oral cavity, leading to immune dysregulation. Consequently, there is a particularly growing interest in exploring the oral microbiome and its relationship with the GM and RA (Gilbert et al., 2024). Interestingly, following the gut microbiome, the oral microbiome represents the second most bacterial-rich environment, encompassing approximately 700 bacterial species (Corrêa et al., 2019).

RA patients commonly present with periodontitis (PD), a serious gum disease, which is characterized by dysbiosis of the oral microbiome. The pathogen *Porphyromonas gingivalis (P. gingivalis)* gives rise to PD (Cheng et al., 2020).

Captivatingly, a study by Smit et al. (2012) discovered a higher prevalence of severe PD among RA patients, indicating a potential correlation between its severity and RA, as RA patients exhibited a heightened immune response against *P. gingivalis* compared to healthy controls (Smit et al., 2012). These findings could potentially be attributed to the fact that RA is characterized by systemic inflammation. For instance, the inflammatory environment in RA could impact the oral microbiota, thereby inducing dysbiosis (Corrêa et al., 2019). Subsequently, dysbiosis of the oral microbiome can alter its microbial composition, and as demonstrated by Cheng et al. (2020), dysbiosis is associated with PD. Corrêa et al. (2019) observed that specific microbes linked with PD were also present in RA patients with an altered GM composition, offering important insights into the interplay between PD, the oral microbiome, the GM, and RA.

Cheng et al. (2020) additionally examined dysbiosis in the oral microbiome of individuals positive for ACPA and at risk for developing RA. They showed a reduction in oral microbial richness compared to individuals with early RA and healthy controls (Audo et al., 2022). Specifically, ACPA-positive at-risk individuals showed an abundance of *P. gingivalis*, which is implicated in inducing citrullination of proteins, thereby increasing ACPA production.

Supporting evidence by Jeyaraman et al. (2023) suggested a positive correlation between *P. gingivalis* and increased ACPA concentrations detected in the serum of individuals affected by RA, indicating its potential role in oral dysbiosis and RA onset (Cheng et al., 2020).

Lastly, an increased abundance of *Prevotella* in the GM, suspected to contribute to RA initiation, has also been observed in studies exploring the oral microbiome. Two independent studies supported these findings, reporting that individuals genetically predisposed or affected by RA exhibited a relatively high abundance of *Prevotella*, specifically in saliva samples (Kroese et al., 2021) (Tong et al., 2020).

Discussion & Conclusion

This literature review was carried out to explore the complex relationship between gut microbiome, host genetics, and Rheumatoid Arthritis, and provide a comprehensive overview of recent research findings, including suggestions for future research. By analysing these components and their interplay, this review aims to contribute to elucidating the intricate mechanisms critical to RA pathogenesis.

Insight into the multifaceted aetiology of RA can be gained by examining other autoimmune conditions, such as Multiple Sclerosis (MS) and Type 1 Diabetes (T1D), on grounds of commonalities among these diseases such as chronic inflammation, heritability factors, microbial associations, dysbiosis, and similar proposed pathogenic mechanisms (Kuhlmann & Antel, 2023) (Berryman et al., 2023) (Hedström et al., 2021) (Ordoñez-Rodriguez et al., 2023) (Zajec et al., 2022).

Genetics and gut microbiome in RA

Highly polymorphic HLA genes are associated with various autoimmune diseases, including MS and T1D, that have certain genetic variations in common with RA. For example, DT1 and MS both have associations with HLA-DRB1 gene variants (Berryman et al., 2023) (Hedström et al., 2021). Intriguingly, it is proposed that gene variations linked to autoimmune risk can impact the GM composition long before the onset of another autoimmune disease and RA (Berryman et al., 2023) (Rooney et al., 2020) (Wells et al., 2020).

A causal relationship between genetic predisposition to RA and dysbiosis of the GM, leading to inflammation and RA onset, remains undefined. It remains ambiguous whether genetic factors directly cause GM dysbiosis, thereby triggering inflammation and ultimately RA onset, or if genetic predisposition influences the initial GM composition, making individuals more susceptible to dysbiosis. Additionally, it is important to consider the possibility of a bidirectional relationship between GM dysbiosis and inflammation and that they may exacerbate each other, thereby contributing to RA pathogenesis. The exact sequence of events and the nature of the relationships are yet to be discovered.

Furthermore, it is essential to consider various environmental influences such as medication, diet, microbial pathogens, and smoking which can also significantly impact the GM, regardless of a genetic predisposition.

Exploring the GM in RA

Investigations into the relationship between the GM and disease progression in RA have grown significantly. The GM, harbouring many immune cells, has demonstrated various potential roles in disease development.

Modern findings when studying individuals with pre-existing GM dysbiosis, hypothesized that dysbiosis increases the IP of the gut, consequently upregulating the antigen load within the GM. Subsequently, molecular mimicry within the GM could trigger autoimmune responses, resulting in

chronic inflammation leading to damage to the synovial lining, characteristic of RA (Lin et al., 2023). Molecular mimicry refers to pathogenic molecules resembling the body's natural molecules, which results in cross-reactivity between pathogens and the body's molecules, provoking autoimmune responses (Moten et al., 2022).

But what ultimately leads to the development of dysbiosis in the GM, and which microbes play a large role? One of the main hypotheses regarding the origin of GM dysbiosis in RA is a genetic predisposition to the disease, supported by a well-established relationship between the host and GM composition (Dieterich et al., 2018).

However, other factors like medication may also contribute to GM dysbiosis. The GM plays a significant role in drug metabolism, with evidence indicating that gut microbial communities have the potential to alter the chemical composition and efficacy of oral medication (Zimmermann et al., 2019). Based on this evidence, it is plausible to hypothesize a bidirectional relationship between GM and medication giving rise to GM dysbiosis.

In the context of genetic predisposition to RA contributing to GM dysbiosis, studies by Wells et al. (2020) and Jeyarman et al. (2023) identified an abundance of *Prevotella spp.* in the GM of genetically predisposed individuals and early-RA individuals. Particularly, *P. copri* has emerged as an interesting microbe for studying the association between the GM and RA.

Wells et al. (2020) propose that *P. copri* in early-RA patients may perpetuate inflammation. Moreover, they hypothesize that the inflammatory milieu induced by RA may stimulate the multiplication of *P. copri*, potentially adapting to growing in an inflamed gut environment. *P. copri*, encompassing pro-inflammatory characteristics, could exacerbate inflammation in RA and sustain dysbiosis within the GM.

Further investigation into the role of *Prevotella* species, particularly *P. copri*, may provide more insights into its influence on the GM development of RA. Understanding its exact mechanism is essential for uncovering its contribution to RA pathogenesis. Notably, *P. copri* was also associated with T1D and other immune diseases, suggesting that it may be involved in a larger autoimmune mechanism (Berryman et al., 2023).

Dysbiosis is also observed in other autoimmune conditions such as MS. Notably, GM dysbiosis can disrupt and dysregulate metabolites, including short-chain fatty acids (SCFA), abundant in the GI tract. Dysregulation of SCFAs has been linked to inflammatory responses, contributing to the development and progression of autoimmune diseases (Ordoñez-Rodriguez et al., 2023).

In RA, the interplay between genetic predispositions and environmental triggers, inducing gut dysbiosis may contribute to inflammation through the dysregulation of SCFAs. Alternatively, dysregulation of SCAFs could serve as an early indicator of RA, influenced by genetic factors and alterations in the GM composition that induce inflammation and contribute to RA development (Ordoñez-Rodriguez et al., 2023).

Lastly, recent evidence suggests that epigenetic modifications may impact genes that regulate immune responses and alter the GM composition (Barik & Bhatt, 2021). Modifications such as

DNA methylation, regulation of non-coding RNAs, and histone modifications, potentially influence RA disease development (Chen et al., 2022). Though provokingly, evidence suggests epigenetics is one of the primary causes of RA onset (Barik & Bhatt, 2021). Given these findings, future research could potentially discover more about the interconnectedness of epigenetics, inflammation, and GM.

Over time, progress has been made in discovering potential bidirectional relationships between the GM, inflammation, and RA. However, as for now, these relationships are theoretical. Hypotheses suggest that dysregulated immune responses may influence the composition of the GM, contributing to dysbiosis. Conversely, dysbiosis may exacerbate inflammation and autoimmunity. Accordingly, the exact mechanisms and sequence of events underlying these hypotheses and their contribution to GM dysbiosis, and RA pathogenesis remain unestablished. Future research should prioritize studies aimed at establishing causality between all factors.

The role of the oral microbiome in RA

The involvement of the oral microbiome is gaining more attention in RA research. Dysbiosis within the oral microbiome, found to be characterized by an abundance of *P. gingivalis* and *Prevotella,* has particularly been observed among individuals with a genetic predisposition for RA. Strikingly, the presence of these microbes is suggested to be associated with the onset of RA, with both microbes presenting links to the presence of ACPA, a serum hallmark in RA.

Specifically, *P. gingivalis* has been linked to periodontal disease (PD) and various other inflammatory diseases. It is suspected that *P. gingivalis* can translocate to the gut, survive, and proliferate there due to its virulence mechanisms, allowing it to survive in high acidity levels in the gut (Chopra et al., 2023). Consequently, this translocation, in addition to *P. gingivalis* being able to alter other microbes in the gut, may contribute to alterations of the GM. Ultimately, leading to dysbiosis and subsequent inflammation, contributing to the onset of RA (Chopra et al., 2023). However, the causal relationship between the oral microbiome, GM, and RA still needs to be unravelled and it remains plausible that dysbiosis of the oral microbiome is a consequence rather than a cause of RA.

Moving on, regarding the undefined relationship between the oral microbiome and RA, it could be valuable to further explore the interplay between genetics, and the oral microbiome in RA. Researchers have investigated common genetic predispositions, involving the HLA-DRB1 and PTPN22 genes, which are implicated in upregulated susceptibility to RA and PD. PTPN22 has inhibitory effects on T-cell signalling, contributing to immune dysregulation and autoimmunity (Schulz et al., 2020).

Specifically, the rs2476601 T allele within the PTPN22 gene has been demonstrated to function as an independent risk contributor in RA and is associated with increased susceptibility to RA, specifically in participants with European ancestry. Furthermore, Schulz et al. (2020) demonstrated its association with increased susceptibility and independent contribution to PD susceptibility in RA patients. These findings support the studies observing a high prevalence of PD among RA patients.

These contemporary findings stimulate the speculation that the rs2476601 T allele's role in predisposing individuals to PD and RA is commonly occurring among RA patients. This genetic predisposition could contribute to the onset of RA and PD, potentially triggering oral dysbiosis and subsequent inflammation, thus contributing to the exacerbation of RA pathogenesis.

Treatment implications in RA

RA patients require an extensive selection of drugs to manage their RA, which highlights the need to understand the bidirectional relationship between therapeutic drugs and the impact on the RA patient, given the significant role of the GM in drug metabolism. Evidence indicates that distinct gut microbial communities in RA patients can modify the chemical composition of oral drugs and potentially influence their efficacy (Zimmermann et al., 2019).

In another context of potential RA treatment, a parallel comparison can be made with T1D. The immune system targets pancreatic β-cells, resulting in insulin deficiency and pancreatic inflammation (Zajec et al., 2022). Similarly, in RA, synovial fibroblasts (FLS), crucial for maintaining proper joint function, become altered, contributing to joint inflammation and tissue damage (Németh et al., 2022).

Intercommunication between immune cells and FLS results in modified FLS behaviour and morphology. It is plausible that synovial FLS, like β-cells in T1D, play a significant role in RA development, though their main involvement in RA pathogenesis is unclear. Moreover, epigenetic, or metabolic impacts may influence FLS behaviour and morphology in RA (Németh et al., 2022). Investigating the underlying mechanism of FLS transitioning can help elucidate the development of autoimmunity in RA and investigate if FLS might be a potential target in RA therapeutics.

Strengths and limitations in RA research methodology

Regarding RA research, there is an increasing amount of research dedicated to exploring the complex interplay between host genetics, the gut microbiome, and RA. While investigations into potential relationships run, various methodologies are utilized to study these intricate mechanisms. Particularly, GWAS and the use of PRS are emerging tools offering valuable insights into the genetic foundation of RA.

The cross-sectional study by Wells et al. (2020) employed GWAS to identify associated SNPs and conducted microbiota profiling to study the relationship between genetics and the gut microbiome. Moreover, incorporating PRS combined with the validation cohort SCREEN-RA nicely represents how techniques can provide in-depth insights using these valuable tools. Future studies could benefit from using large cohort groups to enhance statistical power, a PRS encompassing many specific risk factors, and the inclusion of other cohorts to validate findings.

Nevertheless, there is still much research and improvement to be made regarding RA. Namely, there is some persisting variability and discrepancy among studies on RA. For instance, Wells et al. (2020) found an abundance of *Prevotella spp.* in the GM of at-risk individuals, specifically linked to HLA-DRB1 variants, while Rooney et al. (2020) reported finding no *Prevotella spp.* abundance but did observe a distinct GM composition in the at-risk cohort. This discrepancy

underscores the complexity of the disease, particularly within the context of the GM because of its high variability and intricacy among individuals.

Supplementary challenges stem from heterogeneity within cohort group selection and study design in RA research, disallowing straightforward comparison of findings across studies and inferring ideas. Standardizing inclusion criteria, particularly in at-risk cohorts, is pivotal for reducing discrepancies and enhancing the validity and reliability of study outcomes. In addition, deciphering the uni- or bidirectional nature of relationships in RA pathogenesis remains a challenge, further complicated by the genetic polymorphism in RA.

Moving forward, the implementation of longitudinal studies for integrating genetics and the GM, or studying the mechanisms underlying the GM hold promise in unravelling the mechanisms contributing to the progression of RA from at-risk, pre-clinical to the onset of RA. Such investigations are crucial for enhancing insight into the dynamic mechanisms of RA pathogenesis (Maria-Nefeli Tsetseri et al., 2023) (Zajec et al., 2022) (Kuhlmann & Antel, 2023). Furthermore, randomized controlled trials (RCTs) allow researchers to investigate the efficacy of interventions, like investigating the influence of the diet in at-risk and pre-clinical individuals progressing to RA in more depth, to observe how the diet may affect the development of RA. Similarly, investigating the impact of RA therapeutics on the gut and oral microbiome in at-risk and pre-clinical individuals progressing to RA, can facilitate long-term tracking and generate valuable data for the impact of RA treatment and possibly provide new insights for disease management (Ibrahim et al., 2016).

While mouse studies offer some insights into RA pathology, there must be caution regarding the direct translatability to humans, particularly concerning the interplay between genetics and GM (Wells et al., 2019). Moreover, rare microbial taxa have been speculated to contribute from pre-clinical RA to early-RA. Thus, rare taxa also call for longitudinal studies combined with microbial profiling, to provide insights into disease progression.

Concluding remarks

Ultimately, this literature review aspired to thoroughly explore the intricate interplay among host genetics, the gut microbiome, and Rheumatoid Arthritis. By analyzing existing literature, this review has highlighted the multifaceted mechanisms underlying RA pathogenesis, including insights into the oral microbiome and the individual. During investigation, it became evident that these factors also interact with the components of the primary scope (Figure 3).

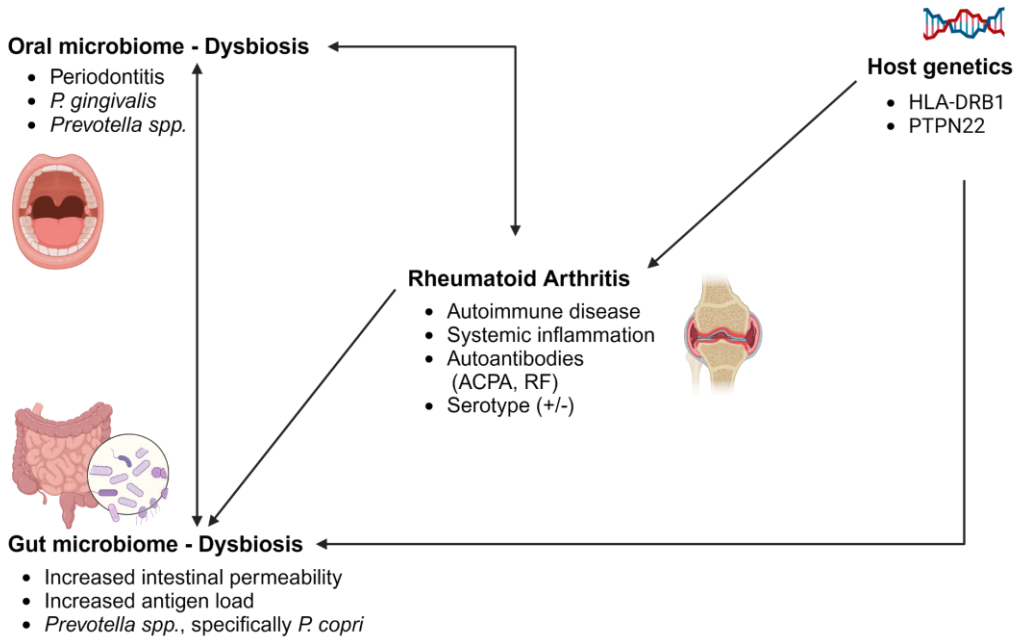

Figure 3. This self-generated graphical abstract illustrates the potential intricate relationships between host genetics, Rheumatoid Arthritis, the gut microbiome, and the oral microbiome, based on the literature review. Relevant characteristics of the individual factors are highlighted in bullet points.

Recent studies have employed in-depth methodologies like GWAS and PRS to uncover the connections between Rheumatoid Arthritis and the gut microbiome and should continue to do so. Moreover, there is a need for further exploration into the influence of factors like diet, smoking, molecular mimicry, drug metabolism, and epigenetics on host genetics, RA, and GM, while examining their relationships with the oral microbiome and each other in more detail.

Incorporating longitudinal studies and RCTs into future research can enhance our understanding of the mechanisms and microbial changes during each consecutive phase of RA. By implementing these recommendations, future research can continue to build a solid foundation for the development of therapeutics in RA, ultimately improving patient outcomes and alleviating RA's global prevalence.

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Afterword

Driven by my initial fascination with the gut microbiome, I decided to incorporate it into my bachelor's thesis to gain more in-depth knowledge. Throughout the process of writing the literature review, I greatly enjoyed investigating all the associations between the gut microbiome, host genetics, and Rheumatoid arthritis (RA). It has taught me that there is a lot more to be discovered about the interplay in RA pathogenesis, but mostly, that the gut microbiome has a profound impact on an individual on countless other health aspects beyond RA.

After completing the thesis, I can state that I have improved learning how to read through a lot of scientific literature, practice critical thinking, and suggest future research avenues. The thesis has given me more insight into what I wish to do in my future career.

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