"All disease begins in the gut" – the role of the intestinal microbiome in ankylosing spondylitis

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Abstract

Ankylosing spondylitis is a chronic debilitating arthritis with a predilection for the axial skeleton. It has a strong genetic predisposition, however, the precise pathogenetic mechanisms involved in its development have not yet been fully elucidated. This has implications both for early diagnosis and also effective management. Recently, alterations in the intestinal microbiome have been implicated in disease pathogenesis. In this review we summarise studies assessing the intestinal microbiome in ankylosing spondylitis pathogenesis, in addition to syntheising the literature exploring the postulated mechanisms by which it exerts its pathogenic potential. Finally we will review studies analysing manipulation of the microbiome as a potential therapeutic avenue in ankylosing spondylitis management.

Keywords: (Ankylosing Spondylitis); (Spondyloarthropathy); (intestinal microbiome); (intestinal microbiota); (intestinal dysbiosis); (pathogenesis).

Key Messages:

- AS is a complex debilitating arthropathy, whose pathogenesis is not yet fully elucidated.
- Advancements in analytical methods have enhanced understanding of the intestinal microbiome, and its pathogenic potential in AS.
- The role of the intestinal microbiome in AS likely involves a complex interplay of genetic, immune mediated and microbial metabolic dysfunction.

Introduction

Ankylosing Spondylitis (AS) is an insidiously progressive, chronic, immune mediated arthritis, characterised by inflammation of the axial skeleton, with early involvement of the sacroiliac joints (1). Its prevalence ranges from 0.2% to 1.6% depending on the geographical location, and population studied (2), with 90% of patients developing symptoms before 40 years of age (3).

AS is the prototype of a class of seronegative spondyloarthritides (SpA), which also include reactive arthritis, psoriatic arthritis, arthritis associated with inflammatory bowel disease and undifferentiated spondyloarthritis (4). The pathogenesis of AS has not been yet been fully elucidated. Human Leukocyte Antigen B27 (HLA-B27), present in up to 90% of those with AS, is a major risk factor for the development of the disease (3). However, despite extensive research, the
precise pathogenic mechanism by which HLA-B27 is involved in AS development remains unclear, and genetic predisposition alone fails to adequately explain AS pathogenesis. This paucity of information concerning the causality of AS development has catalysed an expanding field of research analysing alternative pathogenic mechanisms and predisposing factors. One such area under investigation is the intestinal microbiota, or more precisely intestinal dysbiosis. Greater than 2000 years ago, Hippocrates supposedly declared that “all disease begins in the gut”, and most certainly in recent years there has been an explosion of scientific literature pioneering the intestinal microbiome as crucial to the pathogenesis of a number of systemic autoimmune and inflammatory disorders (5). Interest in the pathogenic potential of the intestinal microbiota in AS stems from evidence that approximately 60% of those with AS have subclinical intestinal inflammation (6), with a further 4 to 16% developing clinically evident inflammatory bowel disease (IBD) (7) thus establishing involvement of the gastrointestinal tract in disease pathogenesis. Furthermore, the implication of a number of bacterial flora, including salmonella, shigella, yersinia and campylobacter, in the initiation of reactive arthritis (8) provides further evidence of a link between the intestinal microbiota and SpA. In addition to this, Klebsiella pneumoniae, an intestinal bacterium with both commensal and pathogenic potential, has long been implicated as a precipitating factor for AS (9), further strengthening the association of the intestinal microbiota and AS pathogenesis.

This review article aims to delineate the current understanding of the microbiome in AS disease pathogenesis, in addition to summarising potential biological mechanisms by which it exerts its pathogenic potential. To conclude, with a greater understanding of the pathogenic role of the intestinal microbiome, we will explore methods by which the intestinal microbiome can be exploited for therapeutic benefit in AS management.

Methodology

Relevant literature pertaining to the intestinal microbiome and ankylosing spondylitis was identified by keyword searches of Medline (via Pubmed) and Embase (OVID) from inception until February 1, 2021. Keywords employed included: (ankylosing spondylitis); (axial spondyloarthritis); (spondyloarthropathy); (intestinal microbiome); (intestinal microbiota); (intestinal dysbiosis) and (pathogenesis). In addition, manual searches of reference lists from primary articles was performed. Articles published in English were included, and there was no study type restrictions.
Review

1. The intestinal microbiome

The intestinal microbiota describes the highly diverse microbial flora including commensal, symbiotic and pathogenic microorganisms inhabiting our intestine, whilst the intestinal microbiome, refers to their collective genome and gene products (10).

The microbiota is predominantly composed of bacteria, with Firmicutes and Bacteroidetes representing the two most abundant phyla in healthy adults (11). An increasing body of evidence has confirmed that the intestinal microbiome is essential for health, exerting pleiotropic roles in immune system modulation, nutrition and metabolism (12). Due to these essential functions, the intestinal microbiota has been coined the "microbial organ" (13). Intestinal dysbiosis is a consequence of altered diversity, composition or function of the intestinal microbiota (14). Such intestinal microbiota dysregulation has been implicated in the initiation and perpetuation of a myriad of autoimmune and inflammatory diseases, including the spondyloarthritides (5).

2. Analysis of the intestinal microbiome

The analysis of the intestinal microbiome has undergone a significant paradigm shift in recent decades and correspondingly our understanding of the microbiome and its role in health and disease has increased. In the 1970s, Carl Woese analysed the 16S ribosomal RNA (16S rRNA) genes of prokaryotes, and successfully elucidated microbial phylogeny, creating the field of molecular phylogenetics and later catalysing the concept of metagenomics (15). Prior to the work of Woese, the study of the microbiome was significantly limited given the inability of more than 70% of bacteria to be readily cultured (11). The 16S rRNA genes have highly conserved regions common to most bacteria, interspersed with hypervariable regions unique to individual bacterial taxa (16). 16S rRNA gene-sequencing uses primers which target the highly conserved sequences within the hypervariable regions, enabling polymerase chain reaction (PCR) amplification and subsequent sequencing, taxonomic assignment and community comparisons of bacterial species (17). Following sequencing, highly similar sequences are categorised into a single group referred to as operational taxonomic units (OTUs), which are then either compared to existing reference sequences enabling taxonomic assignment, or alternatively they are compared based on sequence similarity, referred to as "de novo OTU clustering" (18). The latter, although sometimes used for taxonomic assignment, is
more frequently employed in community diversity studies. The 16S rRNA sequencing approach is the most commonly employed method for microbiome analysis, owing to its relatively low cost and large body of archived reference data, thus supporting large scale microbiome analyses. However, it has a number of shortcomings which must be considered. Firstly, taxonomic classification is reliant on the quality of the pre-existing reference database employed. This precludes characterisation of previously unknown microbiota, in addition to the increased potential for inaccurate taxonomic classification. There is a further risk of inaccurate taxonomic classification due to chimera formation or intrinsic error rate of sequencing (19). However, undoubtedly, one of the biggest limitations of this method is the inability of OTUs to allow for accurate identification at the species level (20).

Metagenomic sequencing, an alternative method of microbiome analysis, captures not just fragments of genes, but instead focuses on the entire genome (21). One such approach is that of high throughput whole genome shotgun sequencing (WGS), which uncovers the complete genetic information of microbes, providing specific taxonomic information down to the species level, in addition to describing functional profiling and enzymatic capabilities (22). However, WGS is expensive and time consuming requiring extensive data analysis (23).

Where metagenomics has the potential to describe the potential functional capabilities of the intestinal microbiome, methodology is expanding to analyse the actual functional activity of a chosen intestinal microbiome. Metatranscriptomic methods analyse the RNA transcribed by the microbiota (24), whilst metabolomic and metaproteomic approaches, describe the metabolites, and proteins, respectively, present in the microbiome (25, 26). These evolving areas in microbiome analysis are providing further insight into the relationship of the gut microbiome with the host, and although somewhat in its infancy, the “multiomic” approach is providing increased understanding of the gut-host symbiotic interaction central to health, and also disease states.

A further consideration in microbiome analysis concerns the type of sample analysed. The majority of studies examining the intestinal microbiota analyse faecal samples, given their ease of obtainment versus mucosal samples from intestinal biopsies. However, using faecal samples alone fails to account for the spatial heterogeneity of distinct microbial habitats along the intestinal tract (27). In particular, ileal inflammation is common in AS, however, the ileal microbiome analysed using mucosal samples obtained at biopsy differs significantly from the microbial profile of faecal samples (28). This is an important consideration when planning a study examining the intestinal microbiota in
those with AS, especially to ensure reproducibility and authenticity of findings. Future studies examining intestinal microhabitats is essential.

3. Evidence of Altered Intestinal Microbiota in AS

To date, no distinct AS microbiome signature has been characterised. However, through the advancements in microbiome analysis outlined above, it has been demonstrated that the intestinal microbiota composition of those with AS is altered versus healthy controls. There is variation in the nature of this intestinal microbial dysbiosis between studies dependent on the study population, mode of microbiome analysis and type of sample analysed (29). Table 1 highlights findings of human studies to date displaying altered intestinal bacterial composition in those with AS.

4. The Intestinal Microbiome and AS pathogenesis

Whilst there is strong evidence to implicate changes in the intestinal microbiome in AS disease pathogenesis, biological mechanisms by which it contributes to AS development are still under investigation. Multiple postulations, including interplay with genetics, altered intestinal epithelial and mucosal barrier with associated immune dysregulation, and altered bacterial function with dysregulation of microbial metabolites have been proposed in an attempt to link the intestinal microbiome with the pathogenesis of AS.

4.1 HLA-B27 and the microbiome

The association of the major histocompatibility complex class I (MHC-1) human leukocyte antigen-B27 (HLA-B27) with AS is well established. Up to 90% of patients with AS possess the HLA-B27 allele (3). However, only 5% of the healthy population who are positive carriers will develop AS (30). Despite intensive research, the precise pathogenic role of HLA-B27 in AS remains unknown. However, several hypotheses have been interrogated including arthritogenic peptide presentation, cell surface HLAB27 dimer recognition by NK receptors and HLA-B27 misfolding with subsequent activation of proinflammatory endoplasmic reticulum stress (31).

Recently, an alternative hypothesis, linking the interaction between the HLA-B27 allele and the intestinal microbiota, coined “B-27 shaped flora”(8), and the increased risk of development of AS was proposed. Evidence for this concept is supported by the demonstration of HLA-B27 transgenic rats, raised under germ free conditions failing to develop an arthritic phenotype (32). Interestingly,
once recolonised with commensal microbiota, over 80% of the HLA-B27 transgenic rats, developed both arthritis and colitis (33), establishing a causative association between the HLA-B27/microbiota interaction and disease penetrance. Furthermore, the substantial influence that HLA-B27 allele carriage alone has on alteration of the intestinal microbiome of healthy individuals without disease was recently described (34). One may thus postulate that HLA-B27 dependent intestinal dysbiosis potentially occurs prior to AS phenotypic development, and not merely as a consequence of disease, thus playing a potential role in AS disease initiation. However, evidence to date is circumstantial, and the precise mechanistic role of HLA-B27 in the development of gut dysbiosis and the subsequent development of AS requires further investigation.

4.2 Alteration of the Intestinal Epithelial Barrier

The intestinal epithelium plays a key role in tissue homeostasis, functioning as an effective physical and biochemical barrier against both pathogenic and commensal microorganisms (35). Tight junctions form connections between adjacent intestinal epithelial cells, and tightly regulate the paracellular movement of water, ions and solutes across the epithelium (36). Of note, under normal circumstances these tight junctions preclude the passage of bacteria, pathogens and toxins (36). If the integrity of the tight junction is compromised, there is a corresponding increase in intestinal permeability, leading to a “leaky gut” phenomenon (37). Such dysregulation of tight junctions and subsequent increased intestinal permeability has been demonstrated to be increased in both AS patients, and their first degree relatives (38). The disruption of intestinal epithelial integrity, dysbiosis and intestinal inflammation is likely closely interrelated both temporally and spatially. Studies in HLA-B27 rat models suggest that both intestinal inflammation and impaired intestinal barrier function develop simultaneously (39). Whether the integrity of the intestinal epithelium is compromised by intestinal inflammation, or whether dysbiotic changes that precipitate epithelial breakdown, such as the intestinal bacterial upregulation of the tight junction modulator zonulin (40), culminate in intestinal inflammation, or perhaps an interplay of both, are responsible for increased intestinal permeability remains to be elucidated.

It is postulated that once there is an increase in intestinal permeability, increased translocation of intestinal microbes to the systemic circulation is facilitated, with the subsequent priming of immunological reactions, such as leukocyte recruitment and activation and release of soluble mediators (10, 41). This theory is supported by the demonstrated high levels of lipopolysaccharide (LPS), a bacterial endotoxin in the serum of those with AS (42). Furthermore, translocated intestinal
microorganisms, may themselves precipitate an inflammatory cascade at extra-intestinal sites, as
evident in endotoxin induced uveitis (43).

4.3 Microbiota Induced Immune Dysregulation

There is an increasing body of evidence to suggest that alterations in intestinal microbiota
composition lead to dysregulation of the mucosal immune balance, with implications in AS
pathogenesis. The interleukin-23 (IL-23)/ T-helper cell 17 (Th17) signalling axis has been
demonstrated as central in the pathogenesis of AS (44). Th17 cells are a subtype of effector T cells,
with a proposed crucial role in immunological defence against microbial infections (45). The
differentiation of Th17 cells is stimulated by a number of cytokines, with IL-23 in particular
implicated in driving its pathogenic potential through the expression of the key transcription factor
ROR-\(\gamma\)t (46).

Th17 subsequently mediates its effects through the release of cytokines, including IL-17, of which IL-
17A is the signature cytokine of the lineage (47). The pathogenic potential of this type 17 response is
evident in the DBA/1 murine model, which under normal circumstances spontaneously develops AS-
like enthesitis, however upon neutralisation of IL-17A fails to do so (48).

Its role is further supported in human studies, by the demonstration of increased levels of both IL-17
and IL-23 in the serum of those with AS (49), in addition to increased numbers of circulating Th 17
cells in those with AS (50). Furthermore, the serum IL-17 levels of patients with AS, have been shown
to correlate closely with their Bath AS Disease Activity Index (BASDAI) score, further implicating IL-17
in disease activity, but also catalysing interest in its future potential as a disease biomarker (51).

Excitingly, IL-17A has also been successfully exploited as a therapeutic target, with the monoclonal
antibody targeting IL-17A secukinumab, demonstrating efficacy in the management of AS (52, 53).
One caveat to IL-17 antagonism is its associated increased risk of IBD exacerbation(54). This
highlights the differences in the immunopathogenic pathways driving these conditions, despite
their high degree of co-familiality. Furthermore, quite surprisingly, two clinical trials (55, 56) evaluating IL-
23 inhibition as a therapeutic target in AS failed to achieve their primary endpoints. This has
stimulated further research into the precise pathogenic role of IL-23 in AS, particularly its varying
degrees of significance at different sites of inflammation, including the axial skeleton and entheses
(57).
Furthermore, what exactly precipitates IL-23/Th17 axis activation remains under investigation. Several immunological studies have postulated a role of the intestinal microbiota as a pathogenic link between IL-23/Th17 and AS development. One hypothesis suggests a link with intestinal villi Paneth cells, that specialise in the secretion of antimicrobial peptides (AMPs) including defensins, lysozymes and cathelicidins.

These AMPs are produced following exposure to pathogenic micro-organisms (pathobionts) and have an essential role in modulating microbial composition and enteric pathogen invasion (58). The AMP defensin has proven to be crucial to the defence against pathobionts and modulation of microbiota composition (59). Defensins exert their effect via chemoattraction of macrophages, T lymphocytes and mast cells (59), in addition to the production of proinflammatory cytokines and chemokines (60). Murine models deficient in alpha defensin compared with those overexpressing the human Paneth cell alpha defensin 5 (DEFA-5), demonstrated significantly different microbiota composition (61). Furthermore, those with overexpression of DEFA-5 display reduction in segmented filamentous bacteria (SFB) colonisation, and subsequently reduced Th17 skewing (61). SFB are commensal bacteria that induce IL-17 (13). Murine models lacking SFB have reduced levels of IL-17, and a subsequent increased susceptibility to infection, particularly with the pathobiont Citrobacter spp (13). However, restoration of SFB in these murine models is associated with a corresponding increase in the intestinal production of IL-17, and a heightened resistance to infection (62). Interestingly, the levels of Paneth cell derived DEFA-5 have been demonstrated to be increased in the terminal ileum of AS patients with acute intestinal inflammation (63). Thus, one may postulate that activation of Paneth cells by intestinal pathobionts, precipitates the activation and release of AMPs, such as DEFA-5, with subsequent further alteration of the intestinal microbiota resulting in immune system activation, implication of the Type 17 immune response and development of AS.

An alternative hypothesis involves the microbiota induced activation of Mucosal-associated invariant T (MAIT) cells. IL-17 produced by Paneth cells in the gut, has been shown to activate MAIT cells in those with AS (64). MAIT cells are innate like lymphocytes with anti-bacterial potential, which when activated induce a rapid immunological response with the production of pro-inflammatory cytokines, including both IL-17 and TNF-alpha (65). Notably, germ free mice display an absence of MAIT cells (66), whilst riboflavin metabolites of bacteria and fungi have been shown to activate MAIT cells (40). Furthermore, MAIT cells have been demonstrated to be elevated in the serum of AS patients (64). Thus one may postulate that MAIT cells, released secondary to dysbiosis, stimulate an aberrant immunological response and thus AS pathogenesis. However, evidence for both of these
theories remains circumstantial, and significant additional research is required to elucidate the precise role of the intestinal microbiome-immune axis in AS pathogenesis.

4.4 Microbiota Metabolic Function

Intestinal microbial metabolites are essential for host homeostasis, and similarly intestinal dysbiosis is associated with significant alteration in the gut metabolic profile (67). Such dysregulation of the gut “metabolome” has been implicated in the pathogenesis of AS, with HLA-B27 expression in murine models shown to dramatically alter the intestinal metabolic profile (68).

One such association is the finding of increased levels of sulphate reducing bacteria in the faecal samples of those with AS (69). Sulphate reducing bacteria catalyse the reduction of inorganic sulphate to hydrogen sulphide (70), and increased levels of this metabolic product have been demonstrated in the intestinal lumen of those with AS (69). Whilst a convincing association has been drawn, undeniably further studies are required to establish a causative relationship, in addition to further identifying the precise pathogenic mechanism these sulphate reducing bacteria play in disease development.

Butyrate, a short chain fatty acid (SCFA) intestinal metabolite has also been implicated in AS pathogenesis (71). It is normally found in high concentrations in the intestinal tract, where it is the end product of microbial fermentation of indigestible polysaccharides (72). SCFAs play a crucial role in defence against infection and inflammation via recruitment and maturation of various subsets of immune cells, in addition to mediating host-microbe interactions (73). One method by which they exert their function is via the G-coupled protein receptors (GPR), namely GPR-41 and GPR-43, thus inhibiting histone-deacetylases (HDAC), modulating host gene expression and inducing autophagy (74). Interestingly, mouse models deficient in GPR-43 display exacerbated or unresolving inflammation in models of colitis and arthritis, however demonstrate full resolution of their inflammatory response, with increased production of inflammatory mediators and immune cell recruitment, upon activation of GPR-43 by SCFAs (75). Reduced levels of butyrate metabolism have recently been identified in AS gut microbiota (76), and correspondingly reduced levels of several species capable of producing SCFAs such as, Eubacterium hallii and Faecalibacterium prausnitzii, were also demonstrated in the microbiota of AS patients (76). This provides further evidence for the potential role of altered intestinal microbial metabolites in the pathogenesis of AS, however further mechanistic studies are required.
5. Potential Therapeutic Strategies

The established link between the intestinal microbiome in AS disease pathogenesis has catalysed much research in the area of microbiota modulation as a therapeutic target in AS management.

5.1 Antimicrobials

Antimicrobial use is associated with a significantly altered taxonomic, genomic and functional ability of intestinal microbiota (77). Exploitation of this common side effect, by the therapeutic application of antimicrobials to alter intestinal luminal microbiota composition was thus explored.

Sulfasalazine, a disease modifying antirheumatic drug, composed of an antimicrobial sulfapyridine, in addition to salicylate (78). One of its mechanistic properties includes alteration of the intestinal microbial flora (79), with one study demonstrating decreased numbers of non-sporing anaerobes associated with its use (80). In the management of AS, it is associated with improved early morning stiffness and reduced erythrocyte sedimentation rate in addition to efficacy in the management of peripheral, but not axial disease (81, 82).

An alternative antimicrobial agent demonstrating promise in the management of AS is Moxifloxacin, a fluoroquinolone antibiotic, with both gram positive and negative action (83). Its use was shown to be associated with a marked sustained reduction in erythrocyte sedimentation rate and C-Reactive protein in those with AS (83). Furthermore, in mouse models, the antimicrobial Rifaximin was also effective in halting AS progression and modulating intestinal microbial composition (84).

Undoubtedly, evidence of the application of these antimicrobial agents provide promise for the potential role of antimicrobials in targeting the intestinal microbiome in those with AS.

5.2 Diet, Probiotics and Prebiotics

Dietary intake has the potential to change the composition of intestinal microbiota, thus altering immune homeostasis (85). Whilst still an area of evolving research, dietary modifications such as a low starch diet, is associated with symptomatic benefit in those with AS, in addition to reduction in pharmacological therapy requirement (86). Intestinal microbiota rely on dietary starch for growth.
(86), and thus, by inference, reduction in starch intake may modulate the intestinal microbiome, with potential benefit in AS.

In recent years, there has been an explosion of research analysing the application of probiotics and prebiotics in a myriad of inflammatory and autoimmune disorders, including AS. Probiotics are combinations of beneficial live microorganisms, whereas prebiotics work to alter the structure and metabolism of beneficial commensals already present in the intestinal microbiota (87). Both probiotics and prebiotics strive to improve intestinal microbial health, strengthening the epithelial barrier and also modulating immune responses (87). Promising results were observed in HLA-B27 transgenic rat models, with Lactobacillus rhamnosus showing benefit in preventing colitis (88), in addition to prebiotic treatment demonstrating efficacy in reducing colitis (89). However, human studies have been less successful with two randomised trials, demonstrating no significant difference between probiotic use versus placebo in those with spondylarthritis (90, 91). Further studies are thus warranted, to identify the optimal probiotic / prebiotic combination capable of modulating the intestinal microflora in those with AS.

5.3 Faecal Microbiota Transplantation

Faecal Microbiota Transplantation (FMT) involves the transfer of stool from a healthy donor, with a relatively stable intestinal microbiota composition, to the intestine of the recipient (92). The proposed role of FMT is to restore a normal intestinal microbiome to the recipient, with subsequent modulation of immune homeostasis (92). It has demonstrated therapeutic benefit in the management of refractory Clostridium Difficile infections (93), and also in inflammatory bowel disease (94). However, its application has yet to be elucidated in AS. Notably, FLORA (NCT03058900), an ongoing double blind, placebo controlled randomised control trial is evaluating the application of FMT in the treatment of peripheral psoriatic arthritis (95). This should provide valuable information for the potential use of FMT in intestinal microbial manipulation in patients with spondylarthritis, including AS. Additionally, further research is required to fully characterise the safety of FMT, in addition to optimal methods of delivery (12). Although in its infancy, FMT is an exciting potential therapeutic avenue in the management of AS associated intestinal microbiome dysregulation.

Conclusion

Despite Hippocrates declaration greater than 2000 years ago that “all disease begins in the gut”, understanding the elaborate, highly intricate and dynamic relationship between the intestinal
microbiota, health and disease pathogenesis is only in its infancy. The intestinal microbiome plays a crucial role in gut homeostasis, and as outlined throughout, dysregulation of this has significant implications for immune system modulation and AS disease pathogenesis. Although alteration of the microbiota in those with AS is established, studies have to date unfortunately failed to identify a consistent uniform alteration. This could be accounted for by study heterogeneity, including the type of sample analysed, the method of analysis, the diversity in severity of AS, and the influence of pharmacological treatment, in addition to potential bias from external factors altering the microbiota such as age, sex, ethnicity, diet and BMI. The significant advancements in analytical methods, in addition to accounting for such biases as outlined above, could provide more reproducible data to establish a microbial signature unique to AS. Whilst we explored potential biological mechanisms by which the intestinal microbiome may participate in disease pathogenesis, the evidence remains circumstantial and further studies are required to establish the precise pathogenic contribution of dysbiosis to AS development. It is an extremely exciting time in intestinal microbiome research, and the potential for exploitation of its dysregulation as a therapeutic target provides substantial impetus for further animal and human studies.

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Table 1: Human studies depicting altered intestinal bacterial composition in ankylosing spondylitis compared to healthy controls.

| Study Reference | Study Population | Sample Analysed | Analysis Method | Increased Microbiota Abundance AS | Decreased Microbiota Abundance AS |
|-----------------|------------------|-----------------|-----------------|-----------------------------------|-----------------------------------|
| (96)            | Italy            | Terminal ileal biopsies | 16S rRNA sequencing | Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Porphyromonadaceae, Bacteroidaceae families | Veillonellaceae, Prevotellaceae families |
| (97)            | China            | Faecal samples | WGS | Prevotella spp – Prevotella copri, Bifidobacterium spp- Bifidobacterium Bifidum | Bacteroides spp |
| (98)            | Sweden           | Faecal samples | 16S rRNA sequencing | Proteobacteria, Enterobacteriaceae, Bacilli, Streptococcus species, Actinobacteria | Bacteroides, Lachnospiraceae |
| (99)            | China            | Faecal samples | 16S rRNA sequencing | Prevotella, Dialister, Comamonas, Collinsella, Streptococcus, Alloprevotella | Eubacterium ruminantium, Ruminococcus gravis, Lachnospira, Bacteroides |
| (100)           | China            | Faecal samples | 16S rRNA sequencing | Bacteroidetes, Megamonas, Dorea, Blautia | Lachnospira Ruminococcus Clostridium |
| (101)           | China            | Faecal samples | 16S rRNA sequencing | Proteobacteria Enterobacteriaceae | Bacteroidetes |
| (102)           | China            | Faecal samples | WGS | Clostridiales bacterium, C. bolteae, C. hathewayi | Bifidobacterium adolescentis, Coprococcus comes, Lachnospiraceae |
| (103)           | China            | Faecal samples | WGS | Flavonifractor plautii, Oscillibacter, Parabacteroides Distasonis, Bacteroides Nordii | Enterococcus Faecium, Eubacterium hallii, Coprococcus catus, Faecalibacterium prausnitzii, Coprococcus eutactus |
| (76)            | China            | Faecal samples | WGS | Bacteroides coprophilus, Parabacteroides distasonis, Eubacterium Sireaeum, Acidaminococcus fermentans, Prevotella copri | Enterohubacter Faecium, Eubacterium hallii, Coprococcus catus, Faecalibacterium prausnitzii, Coprococcus eutactus |

AS – Ankylosing Spondylitis; HC – Healthy control; WGS – whole genome metagenomic shotgun sequencing; UC – ulcerative colitis