IL-17-producing cells in ankylosing spondylitis patients show gender-based differences in gene expression

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Abstract

Objective

Gender has been shown to impact disease expression in ankylosing spondylitis (AS) and Th17 cells play a key role in AS pathogenesis. To better understand what Th17-associated immune pathways are different between men and women, we compared the transcriptome of IL-17-enriched peripheral blood mononuclear cells (PBMCs) in male and female AS patients, with a particular focus on inflammatory cytokine genes.

Methods

PBMCs were collected from 10 female and 11 male AS patients at the Clinical Research Unit of MetroHealth Medical Center. IL-17-enriched PBMCs were isolated and stimulated with CytoStim. RNA-sequencing (RNA-seq) was performed on the samples, and the data were analysed using iPathwayGuide. Inflammatory markers and genes related to Th17 differentiation and function were identified based on previous studies.

Results

RNA-seq identified 12,893 genes with 2,851 genes with p-values <0.05 with distinct patterns of gene expression between male and female AS patients. TGF-β, PGE2, and S100 proteins were significantly upregulated in males. Levels of IL-12B, a Th17 inducer, were lower in males compared to females. Additionally, receptors of IL-6, 12, 23, TGF-β, and PGE2 were downregulated in males, except for IL-17RC, which was upregulated. Genes involved in Th17 differentiation showed differential expression between genders, with elevated expression of BATF, SOCS1, NKD2, and ARID5A in men and decreased expression of FOXO1.

Conclusion

Transcriptomic analysis revealed that male AS patients exhibit distinct expression patterns of IL-17 pro-inflammatory genes, which may contribute to the phenotypic differences observed between genders in AS.

Key words

ankylosing spondylitis, sex characteristics, interleukin-17, gene expression profiling

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Introduction

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease that predominantly involves the spine and sacroiliac joints, but may involve peripheral joints as well (1). The characteristic features of the disease are chronic back pain (CBP), peripheral joint arthritis, enthesitis, dactylitis, and extra-articular manifestations, including inflammatory bowel disease (IBD), psoriasis and acute anterior uveitis (2). Estimated prevalence of axSpA in the United States stands at 0.9–1.4% (3-6). Depending upon the presence of radiographic sacroiliitis, axSpA is divided into radiographic axSpA (r-axSpA), or non-radiographic axSpA (nr-axSpA).

Gender has been shown to impact disease expression. Most autoimmune diseases predominantly affect females, indicating a female bias (7-10). However, the opposite is seen in AS which is historically considered a disease of men with male to female ratio of 3:1 and has a strong association with HLA-B27 (7, 9). Men with AS are more likely to develop axial involvement and radiographic joint damage (11-14), while women have delayed onset of disease (11, 12, 15), higher symptomatic burden (16, 17), more peripheral manifestations of axSpA such as arthritis and enthesitis (18), slower progression of structural damage (15, 17, 19) and delayed response to treatment (16). Unlike r-axSpA, there is only few sex differences in patient characteristics and prevalence in nr-axSpA, and recent difference reported include significantly lower response rate to TNF inhibitors in women than in men (20). The IL-23/IL-17 axis has emerged as a critical pathway in the pathogenesis of spondyloarthritis and new biologic therapies are being developed to target this pathway (21, 22). IL-23 signalling promotes $CD4^+$ Th17 cell differentiation, resulting in increased IL-17A production (22). IL-17A is a member of IL-17 cytokine family that includes IL-17A-F, with a role of IL-17A and IL-17F implicated in the pathogenesis of inflammation (22, 23). IL-17A-F activate pathways which lead to transcriptional upregulation and release of proinflammatory cytokines such as IL-1β, IL-6, GM-CSF, G-CSF, and tumor necrosis factor alpha (TNF-α), chemokines, antimicrobial peptides, and tissue matrix metalloproteinases (24, 25). However, inhibitors of IL-23, IL-12, or IL-6 failed to show clinical efficacy in AS (25-28), suggesting that the IL-17 production in AS may be independent of IL-23. Interestingly, IL-17 pathway has been highlighted as one of the key differences in both immunologic and gene expression patterns of men and women with AS (29). Men with AS, but not women, were found to have higher levels of IL-17A and Th17 cells in peripheral blood than healthy controls (30, 31). Additionally, men with AS have higher circulating levels of TNF-α and IL-18, while women had significantly higher levels of IL-6 in the peripheral blood (30, 32). Male patients with AS also showed alterations in gene expression compared with healthy controls that were not observed in female patients AS, such as up-regulation of immune sensors, autophagy-related genes, myeloid-associated genes, and certain proteases (*ADAM8, CTSA,* and *CTSB*), but downregulation of lymphocyte-regulating genes such as *CD7, SKAP1, SLAMF6,* and *SH2D1A* (29).

To better understand what Th17-associated immune pathways are implicated in the phenotypic difference between men and women, we proposed that mRNAs are differentially expressed in IL-17-producing cells between males and females with AS and that disparately regulated genes in males and females subsequently contribute to the difference in disease phenotype. We thus compared the transcriptome of IL-17-enriched PBMCs in male and female AS patients in this study.

Materials and methods

Patient recruitment, demographics, and disease activity

The patients and healthy controls consented to participate, and the study was approved by the Institutional of Research Ethics Board at MetroHealth Medical Center in Cleveland, Ohio. All parts of research were performed in accordance with relevant guidelines/ regulations including the Declaration of Helsinki. Inclusion criteria for the study

participants included being ≥18 years of age with radiographic sacroiliitis as defined by the modified New York classification criteria for AS (33). Patients with history of (i) malignancy in the last 5 years; or (ii) other rheumatic autoimmune diseases; and/or (iii) chronic viral infections like hepatitis B and hepatitis C and HIV were excluded from the study. Patients were matched for age and sex with healthy controls (hospital staff and volunteers) with no medical or autoimmune conditions. Twenty-one patients (11 males and 10 females) and 8 controls (4 males and 4 females) were consecutively recruited and included in the study, and PBMCs (5 ml) were collected. At the baseline visit, data collected included demographics (age, sex, weight, and height), HLA-B27 status. The disease-specific patient-reported outcomes (PRO) used were the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) (34) and the Bath Ankylosing Spondylitis Functional Index (BASFI) (35). Patient's global assessment (PGA) was determined by asking the patients to consider their disease activity in the past 48 hours. Routine assessment of patient index data 3 (RAPID3) scores was calculated as the sum of the three rheumatoid arthritis (RA) core data set measures: physical function (FN), pain, and a patient global estimate (PATGL) (36, 37). Physician Global Impression (PGI) was determined by the treating rheumatologist. HLA -B27, and C-reactive protein (CRP) were measured using routine laboratory methods. Erythrocyte sedimentation rate (ESR) was calculated by the local lab, and was used to determine Ankylosing Spondylitis Disease Activity Score with ESR (ASDAS-ESR) (38, 39). Information regarding the presence or history of uveitis as well as on the use of TNF blockers were obtained from the electronic medical record (EMR), and the collected data were recorded in RedCap database at MetroHealth Medical Center.

Table I. Demographics and disease activity in the patient cohort.

Parameters	Female (n=9) Mean ± SD	Male (n=11) Mean ± SD	*p*-value (two-tailed)
Race	7W, 2AA	10W, 1AA	
Age	49.78 ± 17.04	45 ± 13.16	0.49
HLA-B27+ (n)	67% (6)	82% (9)	
Uveitis (n)	33% (3)	55% (6)	
TNF inhibitor (n)	56% (5)	45% (5)	
BASDAI	3.69 ± 3.03	3.00 ± 2.14	0.56
BASFI	3.10 ± 2.23	3.58 ± 2.55	0.66
ASDASESR	2.89 ± 1.12	2.29 ± 1.16	0.26
Rapid 3	9.59 ± 8.14	10.07 ± 6.87	0.89
Patient Global	3.44 ± 3.17	4.18 ± 3.25	0.62
Physician Global	3.89 ± 3.48	4.09 ± 2.95	0.89
ESR	29.5 ± 15.44	18.91 ± 15.67	0.15
CRP (mg/dL)	0.6 ± 0.1 (n=7)	1.5 ± 1.6 (n=8)	0.16

ASDAS-ESR: Ankylosing Spondylitis Disease Activity Score; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index. W: White; AA: African American. *p*-value (two-tailed) by unpaired t-test using mean, SD, and sample size (n).

IL-17 secretion assay - cell enrichment and detection

PBMCs (30 ml) were obtained in the Clinical Research Unit at MetroHealth Medical Center using BD Vacutainer CPT Cell Preparation tubes. The PBMCs obtained were stimulated with CytoStim for 4 hours (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). After stimulation, the cells were processed through the interleukin IL-17-phycoerythrin (PE) cytokine secretion assay enrichment kit (Miltenyi Biotec) as per manufacturer's instructions. The IL-17 positive cells were counted and verified for high expression levels of IL-17 by RT-PCR, and total RNA was extracted as per Takara kit (Takara Biotech, Japan).

Next generation RNA-sequencing

Changes in mRNA expression were identified using next generation RNA-seq. RNA-seq projects were carried out as follows: total RNA extraction, sample quality control (QC) test, library preparation, sequencing by synthesis, and bioinformatic analysis. To proceed, 500 ng of RNA from each sample was sent to Novogene Bioinformatics for sequencing. These data were analysed in the context of pathways obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Release 96.0+/11-21, Nov 20) (40, 41), gene ontologies from the Gene Ontology Consortium database (2020-Oct14) (42, 43), miRNAs from the miRBase (MIRBASE version: v. 22.1,10/18) and TARGETSCAN (Targetscan version: Mouse:7.2, Human:7.2) databases (44-49), network of regulatory relations from BioGRID: Biological General Repository for Interaction Datasets v. 4.0.189. Aug. 25th, 2020 (50), chemicals/drugs/toxicants from the Comparative Toxicogenomics Database July 2020 (51), and diseases from the KEGG database (release 96.0+/11-21, Nov 20) (40, 41).

Statistical analysis

The RNA-seq data were analysed using Advaita Bio iPathwayGuide (52), and relative fold (logFC) was calculated using female AS patient values as baseline. Genes were identified using a threshold of 0.05 for statistical significance (*p*-value) using false discovery rate (FDR)-adjusted *p*-values by Benjamini-Hochberg correction, and with absolute log fold change of at least 0.6. Proinflammatory cytokines and genes involved in Th17 differentiation, signalling, and function were identified and assessed based on prior Th17 transcriptome studies using human cells (53-56). Heat maps were generated with ClustVis (57). Where appropriate, two-tailed student t-test was used to compare two means, and *p*-values were calculated using the GraphPad QuickCalcs Web site: http://www.graphpad.com/quickcalcs/ConfInterval1.cfm (accessed May 2022). The results are presented as frequency (%) or mean with standard deviation (SD). One-way ANOVA followed by Tukey's multiple comparisons test and two-tailed t-tests were performed using GraphPad Prism Software v. 9.0.0 for Windows, GraphPad Software, San Diego, California USA.

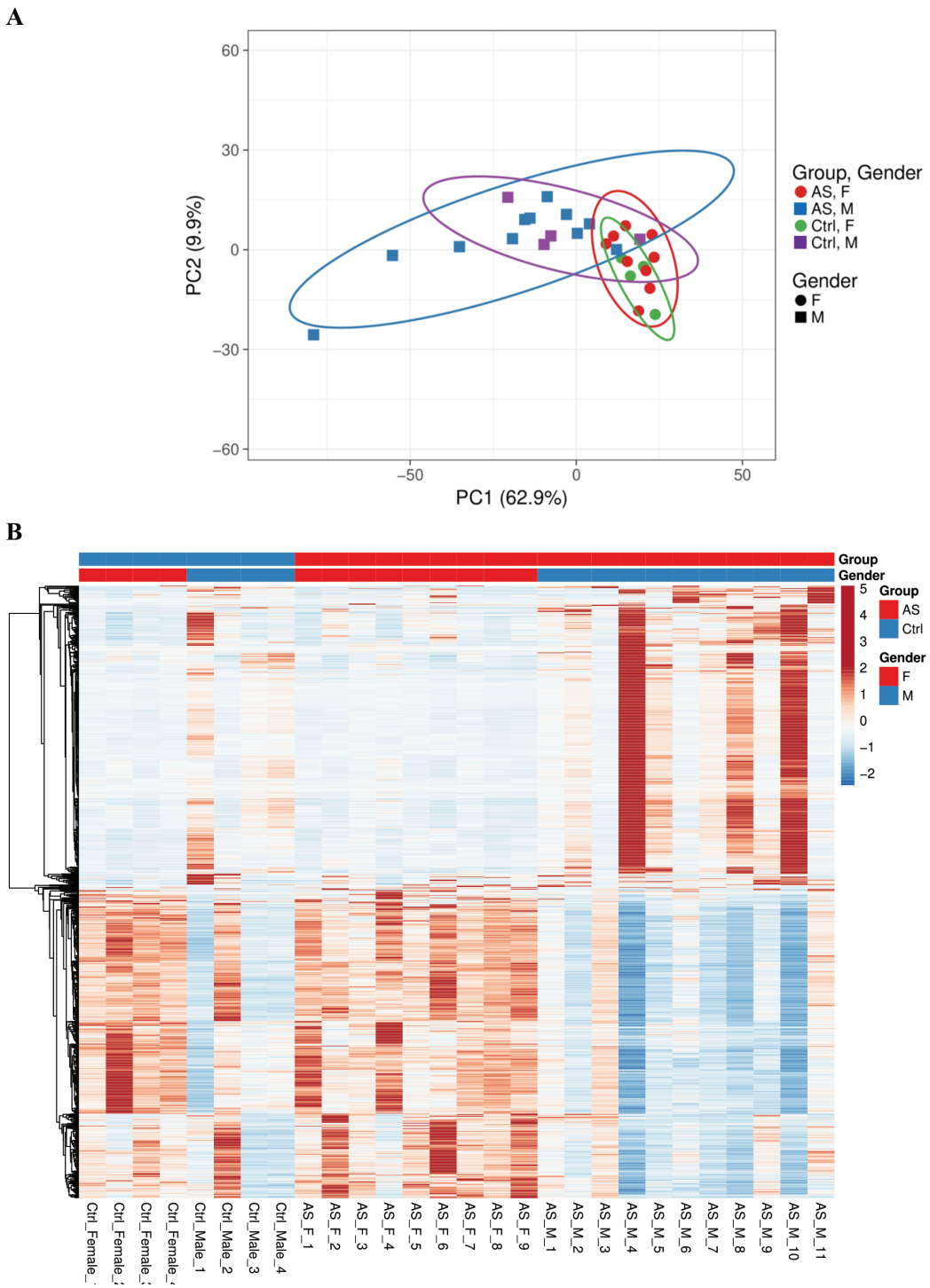

Fig. 1. RNA-seq analysis of peripheral blood mononuclear cells (PBMCs) from healthy and ankylosing spondylitis (AS) patients which were enriched for IL-17 expression.
A: Principal Component Analysis (PCA) of healthy control and AS patients by gender.
B: Heatmap of total gene analysis.
n=4 per gender group for healthy controls, n=9 of AS female and n=11 of AS male patients were included in the analysis, using AS female values as baseline. The PCA and Heatmaps were generated with ClustVis publicly available from https://github.com/taunometsalu/ClustVis

Results

Male and female patients with AS: baseline characteristics and AS disease severity

In this pilot study, 9 female patients and 11 male patients with AS were included in the final analysis. The mean age of females at the time of diagnosis was approximately 50 years, while in males it was 45 (Table I). Four female and four male controls included were matched by gender, race, and age. The mean age of the 8 controls were 51±9.1 and consisted of 6 white, one subject self-identified as White-Hispanic, and one as of African-American origin. Consistent with previous findings (5, 58, 59), more men were positive for HLA-B27 (82% *vs.* 67% in women) and higher proportion of men had uveitis (55% *vs.* 33% in women) (60, 61). However, in our study cohort, women did not have a significantly higher burden of disease by validated questionnaires that assess disease, functional activity and PRO in AS, and were more likely to be treated with TNF inhibitor (56% *vs.* 45%).

IL-17-expressing PBMCs in male and female AS patients demonstrate differential gene expression

Principal component analysis (PCA) of PBMCs enriched for IL-17 in healthy

controls (n=4 per female and male) and AS patients (n=9 in female and n=11 in male) (Fig. 1A) revealed that male patients with AS show more drastic difference in gene clusters in the diseased state compared to that of healthy controls, while female patients with AS showed little change in the overall gene expression pattern between healthy *versus* diseased state. This could be more visualised in the heatmap of significantly upregulated or downregulated genes comparing healthy and AS groups, subcategorised by gender (Fig. 1B). The transcriptomics analysis was done using raw data from Novogen Bioinformatics, and no genes were removed from the analysis. Using the iPathway software, various pre-set biological processes, pathways, and cellular functions were explored, but this approach did not reveal notable differences between male *versus* female subjects. However, looking at the whole gene set globally, male and female patients with AS seemed to have a distinct pattern of IL-17-associated gene expression. This was consistent with previous finding which showed that female patients with AS had relatively few uniquely expressed genes (30). This included upregulation of genes involved in adhesion, vacuole/autophagy, myeloid cell, wound healing/coagulation, osteoclast differentiation, and MAPK signalling pathways, and downregulation of genes involved in protein translation and ribosome-related pathways (30). In the current analysis, we demonstrate that in addition to the baseline higher frequency of IL-17-expressing cells and expression of IL-17A (30), the gene expression pattern of PBMCs themselves enriched for IL-17 is also distinct.

Table II. Cytokines and effector genes that were significantly changed with IL-17-expressing PBMCs of male patients with AS, using female AS patient values as baseline. Only genes with *p*-value <0.05 are shown in the table below.

Gene name	Log FC	Fold change	*p*-value
IL6R	-0.95	0.52	0.01
IL6ST	-1.64	0.32	0.00
IL12B	-1.80	0.29	0.04
IL12RB2	-1.61	0.33	0.00
IL23R	-1.89	0.27	0.03
TGFBR1	-0.94	0.52	0.01
TGFBR2	-0.91	0.53	0.02
TGFBR3	-1.37	0.39	0.00
PTGER2	-0.76	0.59	0.04
CCR6	-1.10	0.47	0.03
CCL24	-1.43	0.37	0.04
TNFAIP6	-1.20	0.44	0.00
TNFRSF10B	-0.92	0.53	0.02
TNFSF14	-1.05	0.48	0.03
TNFSF8	-1.33	0.40	0.03
TNFRSF9	-0.78	0.58	0.04
IL17RC	1.71	3.28	0.01
CLTA	0.94	1.92	0.01
TGFB1	1.16	2.24	0.04
S100A2	2.18	4.52	0.01
S100A4	1.58	3.00	0.04
S100A6	1.96	3.88	0.02
S100A8	1.21	2.31	0.03
S100A9	1.33	2.52	0.03
S100A10	1.08	2.12	0.03
S100P	3.12	8.66	0.00
PGE2 (PTGES2)	1.60	3.04	0.00
PTGES	3.76	13.53	0.00
CCL17	1.71	3.27	0.00
TNFRSF18 (AITR)	2.20	4.58	0.00
TNFAIP8L2 (TIPE2)	1.66	3.16	0.00
C1QTNF6	2.07	4.19	0.00
C1QTNF1	1.26	2.40	0.00
TNFRSF14	1.29	2.44	0.01
TNFRSF4	1.93	3.81	0.01
TNFRSF25	1.11	2.16	0.01

IL-17-expressing PBMCs in male vs. female AS patients show differential expression of TGF-β, PGE-2, and S100 proteins and may express higher levels of Th17 differentiation genes

To further investigate the difference in IL-17-induced gene expression, we generated a list of 72 inducers and effectors of the Th17 pathway and 157 genes involved in Th17 differentiation based on previous Th17 transcriptome studies using human cells (53-56), to be checked against 12,893 gene list generated by the RNA-Seq. Looking at Th17 inducers, there was no difference in *IL-1, 6, 8, 13, 17, 21, 22, 23,* or *26*, while *IL-12B* was expressed at lower levels in males (Table II; Fig. 2A). Interestingly, receptors of IL-6, 12, 23, TGF-β and PGE2 were also down-regulated in males compared to females except for *IL-17RC*, which was upregulated (Table II; Fig. 2B).

It is important to note that in addition to Th17 cells, there are other IL-17-producing cell types in response to IL-23 (62), such as γδ T cells, natural killer (NK) cells, mast cells, neutrophils, and innate lymphoid cells, which amplify Th17 responses (23). While we do not know the exact constitution of IL-17 producing cells within the PBMCs included in the current study, it seems that molecules that are involved in the amplification of IL-17 responses, do not differ among male and female patients with AS. On the other hand, genes involved in Th17 differentiation, such as *BATF, SOCS1, NKD2*, and *ARID5A*, were noticeably elevated in men with AS compared to females (Table III; Figure 2C) while *FOXO1*, which inhibits Th17 development by directly repressing *RORC* and *IL-23R* (66, 67), was decreased. Interestingly, genes involved in the differentiation of type 1 regulatory T cells (Tr1) such as *AHR* (68) and *BLIMP1* (69) were also significantly downregulated in men (Table III). Therefore, despite decreased in *IL-23R* expression, men with AS may have an increased propensity to generate Th17 cells with the same stimuli compared to women due to higher induction of Th17-associated differentiation genes.

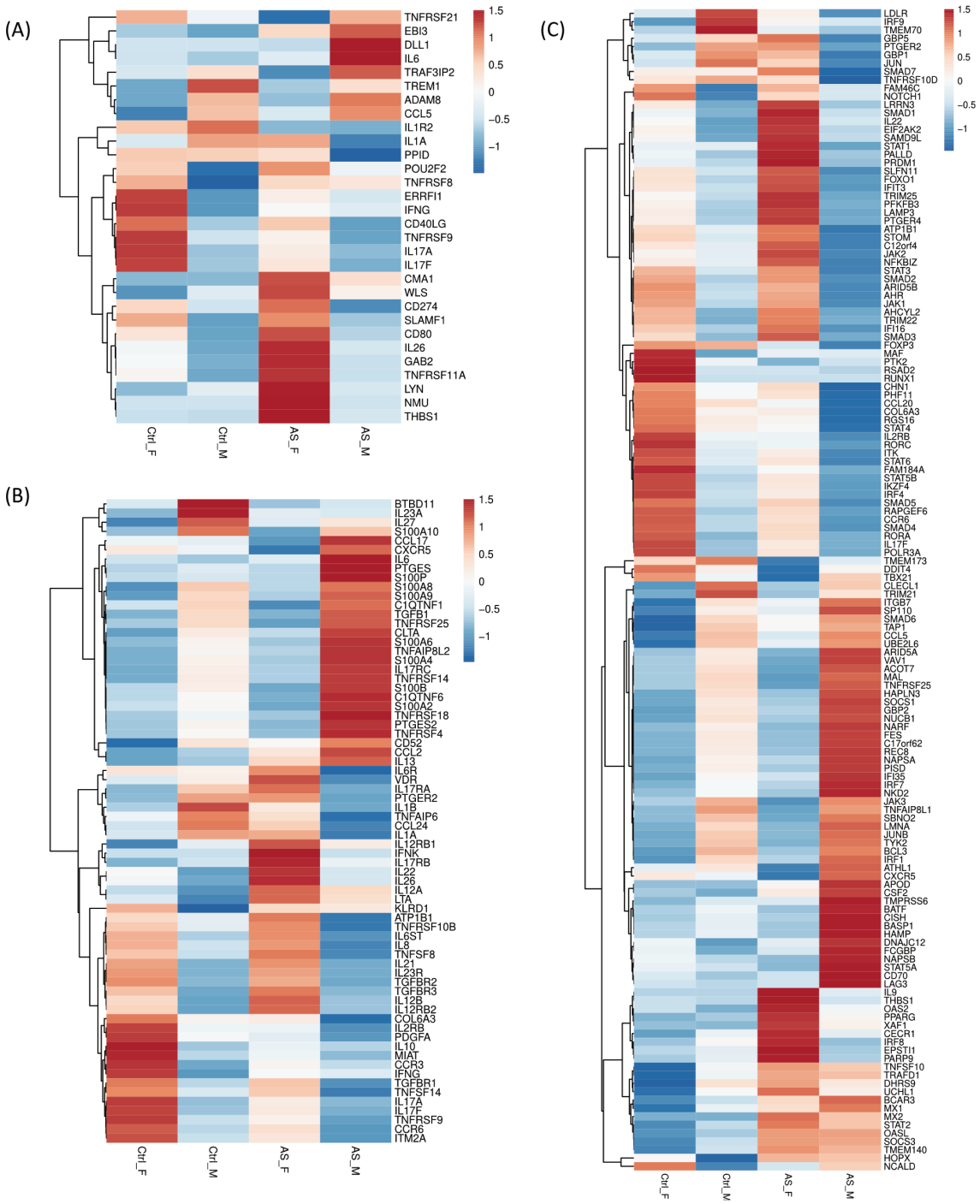

Fig. 2. Heatmaps of genes in IL-17-expressing cells, comparing average values of healthy controls subdivided by gender (Ctrl_F = female healthy controls; Ctrl_M = male healthy controls) and patients with AS also separately analysed by gender (AS_F = female AS patients; AS_M = male AS patients), looking at various cytokines (**A**), IL-17 pathway inducers and effectors (**B**), and Th17-associated differentiation genes (**C**). The Heatmaps were generated with ClustVis publicly available from https://github.com/taunometsalu/ClustVis

Regarding effectors of IL-17-induced pathways, *TGF-β, PGE2,* and *S100* proteins including *S100A2, 4, 6, 8, 9, 10*, and *SP100P* were highly upregulated in males with AS compared with females (Table II; Fig. 2B). PGE2 is known to upregulate osteogenic bone morphogenetic protein 2 (BMP-2) (70) which induces osteoblast differentiation from precursor cells and *in vitro* osteogenesis (71, 72). It is also downregulates Wnt/β-catenin inhibitors, including dickkopf-1 and sclerostin, which negatively regulate AS bone formation (73, 74). Additionally, EP4, which is one of the four PGE2 receptors (EP1-4), has been found to be associated with AS (75) and it is uniquely upregulated in Th17 cells, where it drives Th17 cell development and further promotes EP4 expression in a positive feedback loop in AS (76). EP4 expression levels have been shown to correlate with high AS disease activ-

ity (76). Therefore, male patients with AS may have different mechanisms of AS pathogenesis and progression than females via upregulation of *PGE2* and *S100A* genes.

Discussion

It has already been known that men with AS, but not women, have more IL-17-expressing cells and higher levels of IL-17A in peripheral blood than healthy controls (30, 31). Similarly, Gracey et al. data of whole blood RNA, male AS patients also showed alterations in gene expression compared with healthy controls that were not observed in female AS patients. Such as up-regulation of immune sensors, autophagy-related genes, myeloid-associated genes, and certain proteases (*ADAM8, CTSA,* and *CTSB*), but downregulation of lymphocyte-regulating genes such as *CD7, SKAP1, SLAMF6,* and *SH2D1A* (30). In the present study, we specifically show that PBMCs from male and female AS patients stimulated with IL-17 also show a differential gene expression pattern, which may offer insights into molecular mechanisms behind why AS presents differently by gender. In-depth transcriptomic analysis of the IL-17 expressing PBMCs in males versus female AS patients revealed that males may have a higher propensity to produce Th17 cells by higher levels of transcription factors that are associated with Th17 differentiation, despite having lower transcriptional levels of *IL-23R*. Genome-wide association studies (GWAS) have shown that single nucleotide polymorphism (SNPs) in the IL-23R are a susceptibility factor for ankylosing spondylitis (77). However, the associated relative risks are moderate to low, and there is currently lack of functional evidence on how IL-23 exactly contributes to the pathobiology of AS (27). Cytokines with well-understood mechanisms may in fact behave differently depending on the interacting cytokine networks or contacts (78, 79). For example, δ1 population of enthesis resident γδ T-cells lack IL-23R expression and thus only δ2 cells upregulate IL-17A in response to IL-23 (80). Therefore, spinal inflammation in AS, especially in males, may not exclusively depend on IL-23. Additionally, downregulation of receptors for IL23 and IL-6 in males in this study might be due to the lack of efficacy of IL-23 inhibitors and IL-6 in AS.

Additionally, while Th17 cells are the main cell type to produce IL-17 (23), it is important to note that there are other IL-17 producing cell types, including CD+8 Tc17 cells, natural killer T cells, T Ψ/δ cells, group 3 innate lymphoid cells (ILC3) and natural Th17 cells (81). Macrophages and microglia have also been shown to produce IL-17 (82-84). For our RNA-seq analysis, we isolated PBMCs from whole blood of healthy controls and AS patients; however, we did not perform flow cytometry to identify the exact constituents of the mononuclear cells. Similarly, while it is well recognised that male AS patients typically exhibit a higher prevalence of HLA-B27 positivity and uveitis compared to their female counterparts, whether this disparity is linked to other gene expressions remains a topic that warrants independent investigation. It is important to note that our current pilot study did not account for these variations, which could be considered a limitation of our research. It is possible that Th17 cells were over-represented in the male patients, given their pre-existing higher frequency at baseline. Also, the observation of decreased *IL-23R* expression in male patients is intriguing, considering that *IL-23R* polymorphisms have a strong association with AS and exert functional effects on T-cell immune response (63-65). Specifically, loss of function polymorphisms such as R381Q IL23R is associated with decreased IL-23-dependent IL-17 production and a lower percentage of circulating Th17 and Tc17 cells (64). IL-17 inhibitors such as secukinumab and ixekizumab have been shown to significantly improve patient's Assessment in Spondyloarthritis international Society 20 (ASAS20) response (85). However, AS pathogenesis may also be mediated by other downstream pathways such as S100 proteins and prostaglandins than by commonly known cytokines such as IL-6, and IL-23. S100 proteins are part of one of the largest subgroups of the calcium-binding cytosolic protein family expressed in many tissues in humans. The S100 protein family consists of 25 known members (86, 87). They have a broad range of intracellular and extracellular functions encompassing the regulation of cell apoptosis, proliferation, differentiation, migration, energy metabolism, calcium balance, protein phosphorylation, and inflammation, where they trigger inflammatory response through interacting with receptors RAGE and TLR4 (87). These findings may suggest new therapeutic target for AS and help us understand why targeting IL-6, IL-12, or IL-23 alone has had limited clinical efficacy. In our study, for example, *TGF-β, PGE2,* and *S100* proteins including *S100A2, 4, 6, 8, 9, 10,* and *S100P* were highly upregulated in men, which may offer insights into phenotypic differences between male and female patients with AS.

Women with AS tend to have more prevalent or severe extra-articular presentations than men, including inflammatory bowel disease, psoriasis, enthesitis, and dactylitis (5, 29, 58). This may be related to imbalances of Th1/Th2 and Th17/Treg ratios that result from differing propensities to Th17 differentiation. Increase in Th1/Th2 ratio and activity has been associated with increased disease severity in AS (88, 89) while high Th1/Th17 cell ratio has also been associated with more disease (90). Thus, the delay in diagnosis and progression of disease in women with AS may be partially attributed to this overall change the immune system composition compared to men. More studies focusing on the changes in these immune dynamics caused by increased IL-17 axis in male patients may be helpful to better understand the gender difference in clinical manifestation of AS. For example, Th17 differentiation is intrinsically associated with iTreg cells given their shared TGFβ signalling, and Th17 differentiation is also associated with Th22 subsets given their shared IL-6 signalling (91). Additionally, despite distinct proximal signalling events that induce Th17 differentiation, chronic stimulation of Th17 cells via T-cell receptor

(TCR) or pro-inflammatory cytokines can convert mature Th17 cells to "Th1-like" cells, as their late developmental axis of Th17 overlaps with Th1 cells (91). Studying the effect (or possibly the cause) of altered immune cell ratios may also include investigating roles of previously under-studied immune molecules and players in AS, such as S100 proteins, which are starting to gain attention (92). Additionally, in this study, the disease activity and severity as measured by validated questionnaires were comparable in both male and female cohorts. However, given the differing disease progression in the two genders, a widely applicable, objective staging system available for AS (93) may be useful to ensure comparisons at similar stages of disease between genders.

Conclusions

Overall, in the present study we demonstrate that male and female patients with AS show differential gene expression patterns in IL-17-expressing PBMCs. Genes involved in Th17 differentiation, notably *BATF, SOCS1, NKD2,* and *ARID5A* were elevated in men compared to women, while cytokines and receptors which were known to amplify the IL-17 response were comparable. Moreover, there was no difference in *IL-1, 6, 8, 13, 17, 21, 22, 23,* or *26,* except lower levels of *IL-12B* in males. Instead, *TGF-β, PGE2,* and *S100* proteins including *S100A2, 4, 6, 8, 9, 10,* and *S100P* were highly upregulated in men, but IL-23R and IL-6R were downregulated. Future studies should focus on the effect of increased propensity to Th17 differentiation in men on the immune cell ratios and identification of disease stage system. Recognising differences in the immune response between genders may be helpful in better understanding the molecular mechanism behind gender bias in the clinical manifestation of AS.

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