Sains Malaysiana 53(8)(2024): 1843-1858
http://doi.org/10.17576/jsm-2024-5308-10

Establishing the Association between Ankylosing Spondylitis and Its Comorbidities Based on Their Shared Pathways

(Penentuan Asosiasi antara Ankylosing Spondylitis dan Komorbiditinya Berdasarkan Tapak Jalan Sepunya)

ALHASSAN USMAN BELLO[1], SARAHANI HARUN[1], NOR AFIQAH-ALENG[2], RAJALINGHAM SAKTHISWARY[3] & ZETI-AZURA MOHAMED-HUSSEIN[4,5,*]

[1]*Institute of Systems Biology (INBIOSIS), Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[2]*Institute of Climate Adaptation and Marine Biotechnology (ICAMB), Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia*
[3]*Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, Hospital Canselor Tuanku Muhriz, 56000 Kuala Lumpur, Malaysia*
[4]*UKM Medical Molecular Biology Institute, UKM Medical Centre, Jalan Yaacob Latiff, 56000 Cheras, Kuala Lumpur, Malaysia*
[5]*Department of Applied Physics, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*

Received: 30 March 2024/Accepted: 25 June 2024

ABSTRACT

Ankylosing spondylitis (AS) is an autoimmune and inflammatory arthritis associated with various comorbidities, such as axial spondyloarthritis (axSpA), cardiovascular disease (CD), Guillain-Barre syndrome (GBS), rheumatic fever (RF), and vasculitis (Vs). The co-occurrence of these comorbidities underlies the molecular mechanisms of complex biological interactions shared by dysfunctional pathways. We used network biology and computational methods to establish association between biological processes and molecular mechanisms in AS and its comorbidities. The findings showed significant association between twelve shared pathways in AS and its comorbidities. These shared pathways are associated with pathobiological processes, such as immune responses, inflammatory responses and cellular signaling responses, in AS and its comorbidities. Nine of these pathways are involved in signaling, two are involved in the metabolic processes, and one is involved in the regulatory processes in AS and its comorbidities. In conclusion, this work highlights specific and common shared pathways in AS and its comorbidities. These findings provide information on key shared pathways that can be used to explain the pathobiological processes of AS and its comorbidities and can help in therapeutic discovery towards accurate diagnosis and effective treatment.

Keywords: Ankylosing spondylitis; comorbidities; network biology; protein-protein interaction; shared pathways

ABSTRAK

Ankylosing spondilitis (AS) adalah penyakit artritis auto-imun dan keradangan yang berkait dengan pelbagai komorbiditi seperti spondiloartritis paksi (axSpA), penyakit kardiovaskular (CD), sindrom Guillain-Barre (GBS), demam reumatik (RF) dan vaskulitis (Vs). Kewujudan bersama komorbiditi ini mendasari mekanisme molekul bagi interaksi biologi kompleks yang dikongsi oleh tapak jalan tidak berfungsi. Pendekatan jaringan biologi dan pengkomputeran telah digunakan untuk menunjukkan hubungan antara proses biologi dan mekanisme molekul dalam AS dan komorbiditinya. Hasil kajian ini menunjukkan hubungan yang signifikan antara dua belas tapak jalan sepunya dalam AS dan komorbiditinya. Tapak jalan sepunya ini dikaitkan dengan proses patobiologi seperti tindak balas imun, tindak balas keradangan dan tindak balas pengisyaratan sel dalam AS dan komorbiditinya. Sebanyak sembilan daripada tapak jalan ini terlibat dalam pengisyaratan, dua terlibat dalam proses metabolik dan satu tapak jalan terlibat dalam proses pengawalaturan dalam AS dan komorbiditinya. Kesimpulannya, kajian ini menyerlahkan tapak jalan sepunya khusus dan umum dalam AS dan komorbiditinya. Penemuan ini memberikan maklumat mengenai tapak jalan sepunya yang boleh digunakan untuk menerangkan proses patobiologi AS dan komorbiditinya serta boleh membantu dalam penemuan terapeutik ke arah diagnosis yang tepat dan rawatan yang berkesan.

Kata kunci: Ankylosing spondylitis; interaksi protein-protein; jaringan biologi; komorbiditi; tapak jalan sepunya

INTRODUCTION

Ankylosing spondylitis is an inflammatory condition that leads to vertebral fusion in the spine. This fusion reduces the flexibility of the spine and may cause a hunched stance (Tan et al. 2021). The mechanisms underlying the root cause of AS remain limited due to the complex and multifaceted interactions between molecular pathways (signaling, regulatory, and metabolic pathways) that trigger autoimmune and inflammatory processes. AS complexity is believed to be contributed by the molecular association between shared pathways (Kasher et al. 2022). Molecular associations at the pathobiological level resulted from shared biological pathways that underlie AS complexity (Kasher et al. 2022). When different protein that functions in the same biological process trigger different signal responses in the same pathway (Zhang, Liu & Zhang 2021), it may cause changes to cell function in a different way. A biological pathway is a chain of interactions between molecules in a cell that results in a specific change in the cell. This interaction turns genes on and off, causes a cell to move, or starts the production of new molecules like proteins. These processes provide information sharing among molecular components and pathways within the cells. Communication between molecules via a specific pathway can be shared over short or long distances, where cells transmit signals to adjacent cells to repair localised damages (Yates 2021). In protein-protein interaction (PPI) network, a disease pathway is a collection of interdependent proteins whose abnormal interactions result in a disease phenotype that is influenced by shared molecular signals at the specific degree (Alanis-Lobato & Schaefer 2020). Finding out which pathways are shared between AS and its comorbidities might help us understand the association between them and how they trigger molecular functions that encourage innate autoimmune responses.

From the clinical research, AS patients are found to have one or more comorbidities (Cella et al. 2022; Kim et al. 2022; Yang et al. 2022). Medical Subject Headings (MeSH) define comorbidities as 'the presence of co-existing or additional diseases with reference to early diagnosis or with reference to the index condition'. Comorbidity is a medical condition that manifests simultaneously as a result of ongoing inflammation or its treatment (López-Medina & Molto 2020). Axial spondyloarthritis is reported to affect 10-15% of individuals with AS (Singh & Magrey 2020). Furthermore, rheumatic fever (11.4%), cardiovascular disease (22.8%), and vasculitis (12.8%) are the most prevalent comorbidities in AS patients (Coulson et al. 2021). Similarly, other studies also found that Guillain-Barre syndrome (22%), cardiovascular disease (21%), rheumatoid arthritis (15.7%), osteoporosis (10.7%), and ischemic heart disease (10%) were the most prevalent comorbidities in AS (England et al. 2023; Kaur, Mittal & Singhdev 2021; Rebordosa et al. 2022).

The pathobiology of AS and its comorbidities is still unclear. Based on earlier studies carried out by the authors of this study, multiple biological pathways were found to be associated with AS and its comorbidities through AS-related proteins. However, the biological mechanism and the degree to which these pathways are connected or shared between AS and its comorbidities are still not clear. Two or more comorbidities can interact *via* a specific or shared pathway to create diseasome in patients. A diseasome is a collection of all diseases and health conditions that affect an organism, with specific reference to its biological aspect (Wysocki & Ritter 2011). The pathway shared by AS and its comorbidities and the association between the shared pathways are still unidentified. Thus, this study was conducted to determine the association between AS and its comorbidities based on their shared pathways.

MATERIALS AND METHODS

CONSTRUCTION OF INTERACTIONS IN AS-COMORBIDITY NETWORK

The two comorbidities are said to be associated if they share similar biological or inflammatory related pathways. This pathway sharing is defined between AS and any of its identified comorbidities. A total of 22,219 interaction data associated with AS and its comorbidities are obtained from the PathCards database (v5.18.1073.0). In addition, a total of 1,434 protein-disease associations and 1,290 disease-disease associations were obtained from the DisGeNET database (v5.0). The comorbidities associated with AS and these pathways are axial spondyloarthritis, rheumatic fever, cardiovascular disease, vasculitis, and Guillain-Barre syndrome. The information on these comorbidities was obtained from Human Protein Reference Database (HPRD; v9.0) and was used to construct the human protein-protein interaction network (HPPIN). The methods for the comorbidity network analysis used were according to Choudhary et al. (2023). The degree of the connection between two comorbidities in the diseasome was measured using the molecular comorbidity index (MCI) based on Sunc $MCI_{com1,com2}$mugam (2016), which is expressed as:

$$= \frac{|(\mathrm{ASrp}_{\mathrm{Com1}} \cap \mathrm{ASrp}_{\mathrm{Com2}}) \cup (\mathrm{ASrp}_{\mathrm{Com1-Com2}}) \cup (\mathrm{ASrp}_{\mathrm{Com2-Com1}})|}{|(\mathrm{ASrp}_{Com1} \cup \mathrm{ASrp}_{Com2})|} \quad (1)$$

ASrp represents AS related proteins. $\mathrm{ASrp}_{\mathrm{Com1}}$ and $\mathrm{ASrp}_{\mathrm{Com2}}$ are the proteins associated with comorbidity1 and comorbidity2, respectively. $\mathrm{ASrp}_{\mathrm{Com1/Com2}}$ are ASrp associated with comorbidity1 that show interactions with ASrp connected with comorbidity2 (vice versa for $\mathrm{ASrp}_{\mathrm{Com1 \rightarrow Com2}}$). $\cap$ symbol is the intersection function, indicating the number of common ASrp between the comorbidities, while function imply the total number of

ASrp involving in both comorbidities. The equations within the vertical bars represent their cardinality.

NETWORK AND TOPOLOGICAL ANALYSIS

The larger connected component was extracted from AS PPI network and utilised for all other network analyses. Kitsak et al. (2022) method was used to compute the shortest path and clustering coefficient, and Cytoscape v3.9.1 was used to visualise the network. Network of comorbidities was created with each disease represented by a node, and an edge between two comorbidities showed that they share at least one pathway. The default DisGeNET (D) scores were computed to determine the strength of the edges (relationship) between two comorbidities d_1 and d_2 using Li and Agarwal (2009) method below:

$$D_{d1,d2} = -log_{10}[\max(\sqrt{\mathcal{P}_{d1,i} \times \mathcal{P}_{d2,i}})] \quad (2)$$

i ξ pathways associated with two comorbidities d_1 and d_2,

where $\mathcal{P}_{d1,i}$, $\mathcal{P}_{d2,i}$, represent p-value for the association between d_1 and pathway i, d_2, and pathway i, respectively. For an edge to be included in the final network, the D score had a default cut-off of $-log_{10}$ (0.01). The topological distribution of AS comorbidities was determined by within comorbidity distance (WCD). The WCD for each comorbidity was computed as the mean of the length of the shortest path between pairs of comorbidities. The WCD for a comorbidity m is computed as:

$$WCD_m = \frac{\sum d(i,j)}{\frac{n(n-1)}{2}}, i, j \xi m \quad (3)$$

where n represents the number of comorbidities and n(n-1)/2 is the total number of unique comorbidity pairs for m, $d(i,j)$ indicates the shortest path distance between comorbidity i and j. The comorbidity nodes and pathways were randomly done, and WCDs were re-computed in order to evaluate the statistical significance of WCD ($p<0.01$).

The degree of connectivity and Edge Percolated Component (EPC) for comorbidity networks were computed using cytoHubba (Chin et al. 2014).

i. Degree (Deg)

$$\mathrm{Deg}(d)=|\mathrm{N}(d)| \quad (4)$$

ii. Edge Percolated Component (EPC)

Each edge in an interaction network X' is assigned a removing probability p. X' is an attainment of this probability. A node connected in X' has $d_{mn} = 1$, whereas a node not connected in X' has $d_{mn} = 0$. In EPC connectivity, m and n are connected by d_{mn} over attainments, where K_{mn} is their average. The size of EPC including node m, T_m, is defined as the sum of K_{mn} over nodes n. The score of node m is represented by

$$\mathrm{EPC(m)}=1|\mathrm{n}|\sum \mathrm{K_{mn}} \quad (5)$$

The comorbidity networks were extracted from giant PPI of interacting ASrp and pathways by randomly and individually allocating values between 0 and 1 to cluster of edges, and edges that connected random numbers were eliminated i.e., $0 \leq \text{limit} \leq 1$.

FUNCTIONAL ENRICHMENT ANALYSIS

Functional analysis of the comorbidity associations was performed to determine the significance biological functions associated with each comorbidity and its pathways involved. A functional enrichment analysis was performed utilising Reactome pathway database (v86) and Panther classification system (v16). Human biological pathway unification (PathCards) database was used for annotations of the biological and inflammatory pathways. The biological processes shared by the AS comorbidities was measured with Jaccard index (JI):

$$\mathrm{JI\_1_{Com1,Com2}} = \frac{|\mathrm{BF}_{Com1} \cap \mathrm{BF}_{Com2}|}{|\mathrm{BF}_{Com1} \cup \mathrm{BF}_{Com2}|} \quad (6)$$

$$\mathrm{JI\_2_{Com1,Com2}} = \frac{|\mathrm{Pathways}_{Com1} \cap \mathrm{Pathways}_{Com2}|}{|\mathrm{Pathways}_{Com1} \cup \mathrm{Pathways}_{Com2}|} \quad (7)$$

JI determines the degree of similarity between AS and its comorbidities. Com1 and Com2 represent AS and any of its comorbidities. Biological function (BF) of Com1 and Com2 represents the biological processes involved in AS and any its comorbidities. The pathways for Com1 and Com2 are the biological pathways where ASrp associated with the comorbidities are shared. All pathways and interactions networks were visualized using Cytoscape v3.9.1.

BONFERRONI CORRECTION

We used ClueGo algorithm to conduct the statistical analysis and Bonferroni method to control the group-wise error rate within a cluster. A network was determined at a significance level *t* if there were K variables (i.e., a pathway or comorbidity); each variable was calculated at *t*/*K*. Thus, only the nodes that have p-values of *t*/*K* were considered significant in the network. False positives were controlled at *t* by a probability function.

PAIRWISE CORRELATION NETWORK

Shared network was constructed using pairs of shared pathways in shared comorbidities that have significant pairwise correlations. Each node in this paired correlation network represented a pathway or comorbidities, and the connections between the interactions showed the significant correlations between them. Pair correlation of a pathway or comorbidity could be either positive or negative, indicating that the interactions between shared pathways among comorbidities may be significantly stronger or weaker. Pairwise correlation on shared network was computed using the approach described by Tam, Chang and Hung (2013) which determines the correlation coefficient using D = n(n-1) potential nodes of the n-shared network. Since there are a total of n(n-1)/2 pairs of shared pathways (i, j), thus, the n(n-1)/2 coefficient matrices E for shared comorbidities are computed.

DETERMINATION OF ASSOCIATIONS' STRENGTH BETWEEN SHARED PATHWAYS

Association correlational approach was used to determine the strength of associations between shared pathways. The interaction strengths between domains (domain-domain interactions) were computed from MCI of the shared pathways and the comorbidity pairs, using the following association approach (Sprinzak & Margalit 2001), which is based on the binary interaction between shared pathways' information and allots a score to each domain pair (S_m , S_n). The probability of interacting pathways (correlation score) for S_m , S_n is computed as

$$\text{ASSOC}\,(S_m\,, S_n) = \frac{A_{m,n}}{B_{m,n}} \tag{8}$$

where $B_{m,n}$ denote the number of pathway pairs [scores obtained from Human Integrated Protein-Protein Interaction Reference (HIPPIE) (http://cbdm-01.zdv.uni-mainz.de/~mschaefer/hippie/index.php)] containing domain pairs (S_m,S_n). The denote the number of interacting comorbidities pairs containing domain pairs (S_m,S_n).

COMORBIDITY-PATHWAY ASSOCIATION

The comorbidity-pathway association was performed using pathway-based methods (Li & Agarwal 2009; Yu & Gao 2017), such as a one-sided Fisher's Exact test that is to determine whether there is an overlap between a disease and a pathway, and FDR Benjamini–Hochberg technique which is used to adjust the p-values. Comorbidity-pathway pairs with adjusted p-value of 0.05 were gathered. All comorbidities were then examined for pathway association after being randomly assigned. The background distribution was created by repeating this procedure ten times. In cases where a comorbidity was associated to multiple pathways, the biological association of those pathways were assessed to generate a comorbidity-pathway association (CPA) score:

$$\text{CPA} = 1 - \frac{\sum_{i=j} \frac{\mathcal{P}_i \cap \mathcal{P}_j}{\min\,(\mathcal{P}_i, \mathcal{P}_j)}}{\frac{n(n-1)}{2}} \tag{9}$$

$\mathcal{P}_i, \mathcal{P}_j \xi$ pathways associated with a comorbidities, where n indicates the number of pathways associated with a comorbidity, n(n-1)/2 is the total number of distinctive pathway pairs, $\mathcal{P}_i/\mathcal{P}_j$ indicates number of ASrp shared by pathways $\mathcal{P}_i$ and $\mathcal{P}_j$, and min($\mathcal{P}_i$,$\mathcal{P}_j$) indicates the size of the smaller pathway between $\mathcal{P}_i$ and $\mathcal{P}_j$. A high CPA score suggests a group of pathways have a high degree of biological connection. It is equivalent to 1 when there are no overlaps between the pathways and 0 when there are no pathways at all. The pathway content index (PCI) is computed as:

$$\text{PCI} = \frac{|\mathcal{P}|}{\frac{X(\mathcal{P})}{Y(\mathcal{P})}} \tag{10}$$

X($\mathcal{P}$) represents the overall number of ASrp from set $\mathcal{P}$, and Y($\mathcal{P}$) represents the number of distinctive ASrp from set $\mathcal{P}$, where $\mathcal{P}$ is a set of pathways connected to a comorbidity. When the connected pathways are entirely redundant, the PCI = 1, and when there is no pathway redundancy among comorbidities, it equals the size of $\mathcal{P}$. A similar assessment, the CCI (comorbidity content index), was used to quantify the function of associated comorbidities when a pathway is associated to many comorbidities.

RESULTS AND DISCUSSION

ASSOCIATION BETWEEN AS AND ITS COMORBIDITIES

The disease-disease association and protein-disease association information was obtained from the DisGeNET database of human gene-disease associations for the AS comorbidities [AS, axial spondyloarthritis (axSpA), rheumatic fever (RF), cardiovascular disease (CD), vasculitis (Vs), and Guillain-Barre syndrome (GBS). Table 1 presents the results of functional mapping of the ASrp and their relationship with the comorbidities based on disease-disease and protein-disease associations. A total of 1,434 and 1,290 interactions for protein-disease associations and disease-disease associations, respectively, were obtained. The protein-disease association score (Score pda) showed a strong association between six AS

comorbidities. Most of the AS-related proteins associated with these comorbidities were cytokines, chemokines, interleukins, and growth factors. These ASrp were signaling proteins, enzymes, receptors, nucleic acid-binding proteins, metabolites, kinases, epigenetic regulators, hormones, transcription factors, and immune responses. The results also showed that the disease specificity index for the protein (DSIp) and the disease pleiotropy index for the protein (DPIp) were higher and similar among comorbidities.

TABLE 1. Statistical analysis for the shared pathways

Key comorbidities	Associated comorbidities	Shared pathways	Pairwise correlation	p-value	Bonferroni correction
AS	axSpA	Innate Immune System	1.0016	0.001	0.999
		Canonical Wnt Pathway	1.0024	0.001	0.999
	CD	PI3K-Akt signaling pathway	1.0032	0.001	0.999
		Oxidative damage response	1.005	0.001	0.999
	GBS	IL-6 family	1.0053	0.001	0.999
		Oxidative damage response	1.0053	0.001	0.999
	RF	Metabolism of proteins	1.0053	0.014	0.986
		IL-1 family signaling	1.0053	0.001	0.999
	Vs	B Cell Receptor Signaling Pathway	1.0053	0.001	0.999
		PI3K-Akt signaling pathway	1.0071	0.001	0.999
axSpA	AS	Canonical Wnt Pathway	-1.0071	0.001	0.999
		Innate Immune System	1.0088	0.001	0.999
	RF	Metabolism of proteins	1.0098	0.011	0.989
		IL-1 family signaling	1.0101	0.001	0.999
	Vs	PI3K-Akt signaling pathway	1.0103	0.001	0.999
		B Cell Receptor Signaling Pathway	1.0103	0.016	0.984
CD	AS	Oxidative damage response	1.0137	0.001	0.999
		PI3K-Akt signaling pathway	1.0154	0.001	0.999
	GBS	IL-6 family	1.0169	0.001	0.999
		Oxidative damage response	1.0238	0.001	0.999
GBS	AS	IL-6 family	1.0286	0.001	0.999
		Innate Immune System	1.0286	0.001	0.999
	CD	Cytokine Signaling in Immune system	1.0286	0.013	0.987
		PI3K-Akt signaling pathway	1.0286	0.001	0.999
Vs	AS	PI3K-Akt signaling pathway	1.0286	0.001	0.999
		Extracellular matrix organization	-1.0326	0.001	0.999
	axSpA	B Cell Receptor Signaling Pathway	1.0351	0.001	0.999
		Cytokine Signaling in Immune system	1.0373	0.001	0.999

AS=ankylosing spondylitis; axSpA=axial spondyloarthritis; CD=cardiovascular diseases; GBS= Guillain-Barre syndrome; RF= rheumatic fever; Vs=vasculitis. The significant is $P<0.05$

AS and its key comorbidities, such as axSpA, RF, CD, Vs, and GBS, have been found to biologically and statistically link through several shared pathways, suggesting their involvement in several biological processes (e.g., acute inflammatory response, multi-functional enzymatic activity in inflammation, cartilage development, tissue remodeling, and skeletal system development) that contribute to the difficulties in diagnosing AS accurately and providing effective treatments. Key shared mechanisms in comorbidities were associated with common shared pathways (both biological and inflammatory). Clinical research has shown that the coexistence of these comorbidities contributes to the complexity of the diagnosis and treatment processes; for example, treating one comorbidity will interfere with the treatment of another comorbidity (Choudhary et al. 2023; Singh & Magrey 2020). This coexistence showed that the comorbidities were associated via common pathways. Seronegative spondyloarthropathies, including AS and axSpA, have been found to be comorbid in the same patients (Khan 2023). Psarelis et al. (2017) reported GBS comorbid with AS in anti-TNF-α treatment. Similarly, Nygaard et al. (2023) reported long-term cardiovascular diseases in patients diagnosed with vasculitis. Weber et al. (2023) have indicated an association between systemic vasculitis and coronary artery microvascular (cardiovascular) dysfunction in patients with AS. All these reports provide strong evidence of the biological association between these comorbidities through their shared pathways.

COMORBIDITIES-PATHWAY MAPPING

Mapping of the pathways-related data produced overall 22,219 pathways from PathCards. A total of 65 sub-pathways and 22,154 other pathways (overall = 22,219) connected with these sub-pathways were found to be associated with AS and its comorbidities. The inflammatory-related pathways were compared with biological pathways. On average, 50% of pathways from each comorbidity were statistically associated with biological pathways (p-value < 0.01), suggesting the possibility that the associated pathways can be used to functionally characterise comorbidities. The comorbidity-pathway associations were significantly distributed (p-value < 0.01). Comorbidities were connected to several pathways (mean = 100), and the pathways were connected to six comorbidities. For each comorbidity, the fraction of pathways (mean < 65) associated with comorbidities was statistically mapped. Collectively, these results implied that six comorbidities associated with various pathways were related and consistent with the sub-pathways.

The information from disease-disease associations, protein-disease associations, and associated pathways was used to construct the AS-comorbidities interactome network (Figure 1). The network was built by 22,219 (i.e., 65 + 22,154) connections between related pathways (denoted by interacting edges), 2,724 connections between (PDA = 1434 + DDA = 1290) related diseases (denoted by yellow nodes), and 1,076 ASrp (denoted by black nodes) connected to the six comorbidities (i.e., AS, axSpA, RF, CD, GBS, and Vs). Figure 2 displays the AS PPI network, which consists of 409 nodes and 2,247 edges, which were then refined using MCODE to illustrate the 1,235 interactions of the highly associated diseasome. The comorbidities were illustrated in different colours, while the lines represent the shared pathways involved. The application of group attribute layout and MCODE clustering on the network created different comorbidity clusters such as AS, axSpA, RF, CD, GBS, and Vs and their shared pathways. The network showed the interactions between different comorbidities. The non-interacted nodes were clustered separately under the main network. All the comorbidities appeared to be interconnected and interrelated across the topology.

The findings from pathway analysis showed that AS, axSpA, RF, CD, GBS, and Vs were associated with twelve key shared pathways, and nine of these pathways were involved in signaling, two pathways were involved in metabolic processes, and one pathway was involved in the regulatory processes in biological systems. These biological processes were involved in the dysfunctional and dysregulated processes among these comorbidities in AS patients. The innate immune system played a crucial role in the development of autoimmunity. It is the first non-specific mechanism for biological defence. The activation of this pathway started with the pattern recognition of biological molecules expressed in innate immune cells that were bound to the extracellular matrix (Mantovani & Garlanda 2023). Pattern-recognition receptors were involved in this process of immune response which detected negative signals (Tian et al. 2023). Innate immune signaling performs a variety of functions in response to a wide range of different infections. Uncontrolled immunological responses result in negative effects that trigger dysfunctional processes (Łukawska, Polcyn-Adamczak & Niemir 2018). The innate immune system triggers autoimmunity via B lymphocyte (B cell) activation to produce antibodies, which triggers T lymphocytes (T cells) to fight the antigens (Rubtsova et al. 2017). The innate regulators (NK cells, Tregs, basophils, NODs, neutrophils, PRRs, macrophages, mast cells, eosinophils, and DCs) of these processes regulate different signaling pathways. This implied that the innate immune system pathway played a role in a network of interactions with extracellular matrix organisation and B cell receptor signaling processes to trigger the autoimmune activities between the comorbidities.

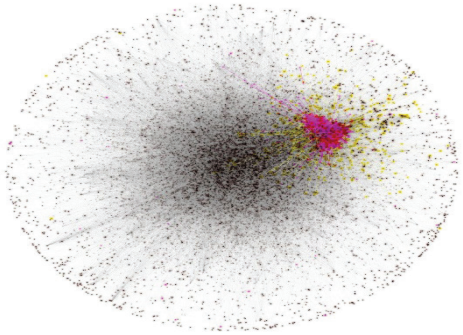

FIGURE 1. AS Comorbidities and Pathway Interactome (AS PPI Network). The pink colour represents a cluster of AS comorbidities, while the yellow colour denotes ASrp. The dark black spot represents the counts of associated pathways in AS and its comorbidities. The PPI network was constructed using data from protein-disease associations and the associated pathways

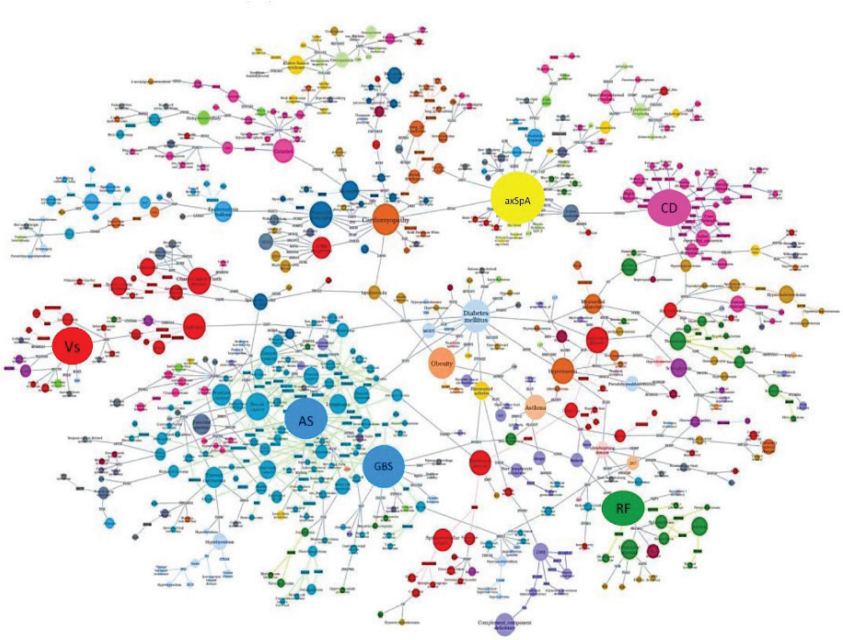

FIGURE 2. The Clusters of Human Diseasome. The comorbidities are represented by different colours, while the lines represent the shared pathways. Blue = AS, Yellow = asSpA, Pale Blue = GBS, Red = Vs, Green = RF, Pink = CD. Note: AS=ankylosing spondylitis; axSpA=axial spondyloarthritis; CD=cardiovascular diseases; GBS= Guillain-Barre syndrome; RF= rheumatic fever; Vs=vasculitis

THE ASSOCIATION BETWEEN COMORBIDITIES THROUGH THEIR SHARED PATHWAYS AND SPECIFIC PATHWAYS

Shared pathways, either in a similar or different direction, can be an underlying mechanism for the dysfunctional processes encouraging comorbidity coexistence and their pathobiology. Shared pathways include biological and inflammatory pathways that function holistically and in tandem in biological systems. In order to determine the associations between comorbidities through their common linker, we first determined the shared pathways and the common pathways for each comorbidity using PPI analysis. Figure 3(A) illustrates the AS diseasome and its associating pathways. AS diseasome was associated with 22 shared pathways (red edges). Meanwhile, 23 other pathways (pink edges) were specific to AS. The interactions between shared and specific pathways may contribute to negative or positive responses in disease pathobiological processes. Figure 3(B) depicts the axSpA diseasome and its associating pathways. The axSpA diseasome was associated with 12 shared pathways (red edges) that can be used to describe the pathobiological processes in axSpA, while 11 other pathways (pink edges) were specific to axSpA only.

Figure 4(A) displays the CD diseasome and the pathways that connect it to other diseases. Eight of these pathways (shown by red edges) were shared with other comorbidities, while the other twelve were unique to CD. The interactions between shared and common pathways can be used to describe the pathobiological processes in CD and its comorbidities. The GBS diseasome and its associating pathways are shown in Figure 4(B). There were nine pathways associated with the GBS diseasome (red edges) that were shared with other comorbidities. However, ten other pathways were common to the GBS diseasome, which interact with the shared pathways to produce comorbid pathobiological processes.

Figure 5(A) shows the RF diseasome and its associating pathways. The RF diseasome was found to be associated with 11 shared pathways (red edges). However, 11 other pathways were found to be common to RF. It was possible that the interactions between the shared pathways and these other pathways contributed to positive or negative comorbid pathobiological processes. Figure 5(B) illustrates the Vs diseasome and its associating pathways. The Vs diseasome was found to be associated with 16 shared pathways (red edges). However, 11 other pathways were found to be common to Vs. It is possible that the interactions between the shared pathways and these other pathways contribute to positive or negative comorbid pathobiological processes.

The findings of this study also showed strong connections between the extracellular matrix (ECM), innate immune system, cytokine signaling in the immune system, IL-1 family signaling, and IL-6 family signaling shared pathways. ECM was a complex network made up of a variety of multidomain macromolecules arranged in a cell-tissue-specific pattern (Zhang, Liu & Zhang 2021). The mechanical properties of cells were influenced by the ECM that linked them together to produce a structurally stable complex. The ECM appeared to be a structurally supportive protein that supports cell survival and other biological functions. For example, collagen, laminin, and fibronectin were a few of the complex proteins that make up the ECM and were essentially for tissue growth. The spatial organization of ECM proteins significantly affected cell contact guidance (Szabo & Momen-Heravi 2020). In the skeletal muscles, collagen makes up the majority of ECM proteins (Zhang, Liu & Zhang 2021). In addition to many bioactive ECM breakdown products that affected cells, ECM appeared to be a main and great source of cytokines (including IL-6, IL-1, IL-2, IL-3, IL-17, IL-23) and growth factors (Boehme & Rolauffs 2018). This can explain the association between IL-6 family signaling, IL-1 family signaling, and extracellular matrix organization pathways, which were shared among the comorbidities. These pathways were discovered to connect: AS and Vs via extracellular matrix organization; GBS, CD, and axSpA via cytokine signaling in the immune system; AS, GBS, and CD via IL-6 family signaling; AS, RF, and axSpA via IL-1 family signaling. Both the IL-6 family signaling pathway and the IL-1 family signaling pathway were inflammatory cytokines that trigger innate immune responses, likely triggering autoimmune activities. This might be because IL-1 produced from the ECM and other immune cells provided immunity to the cells, increasing the release of defensins, which create a feedback-like reaction within the cells. Proinflammatory cytokines (including IL-1, IL-6) were involved in both the initiation and effector stages of the innate immune response pathways. Moreover, a complex of proteins known as the inflammasome, which might be made up of caspase-1, ASC, and NLR, was activated during autoimmune responses. Following their activation, they cleave pro-IL-1β, pro-IL-18, IL-37, and IL-12, allowing for their maturation and release (Li, Guo & Bi 2020). Several inflammatory functions and processes involve the initiation of inflammasomes.

ESTABLISHING SHARED PATHWAYS THROUGH TOPOLOGICAL AND STATISTICAL ANALYSIS

The interactions between shared pathways among comorbidities (Figure 6(A)) showed significant interactions among key shared pathways and comorbidities across the network. AS (number of associated comorbidities = 5, number of associated pathways = 8) and axSpA (number of associated comorbidities = 3, number of associated pathways = 6) had the most shared pathways. Six of the comorbidities were associated to the same signaling

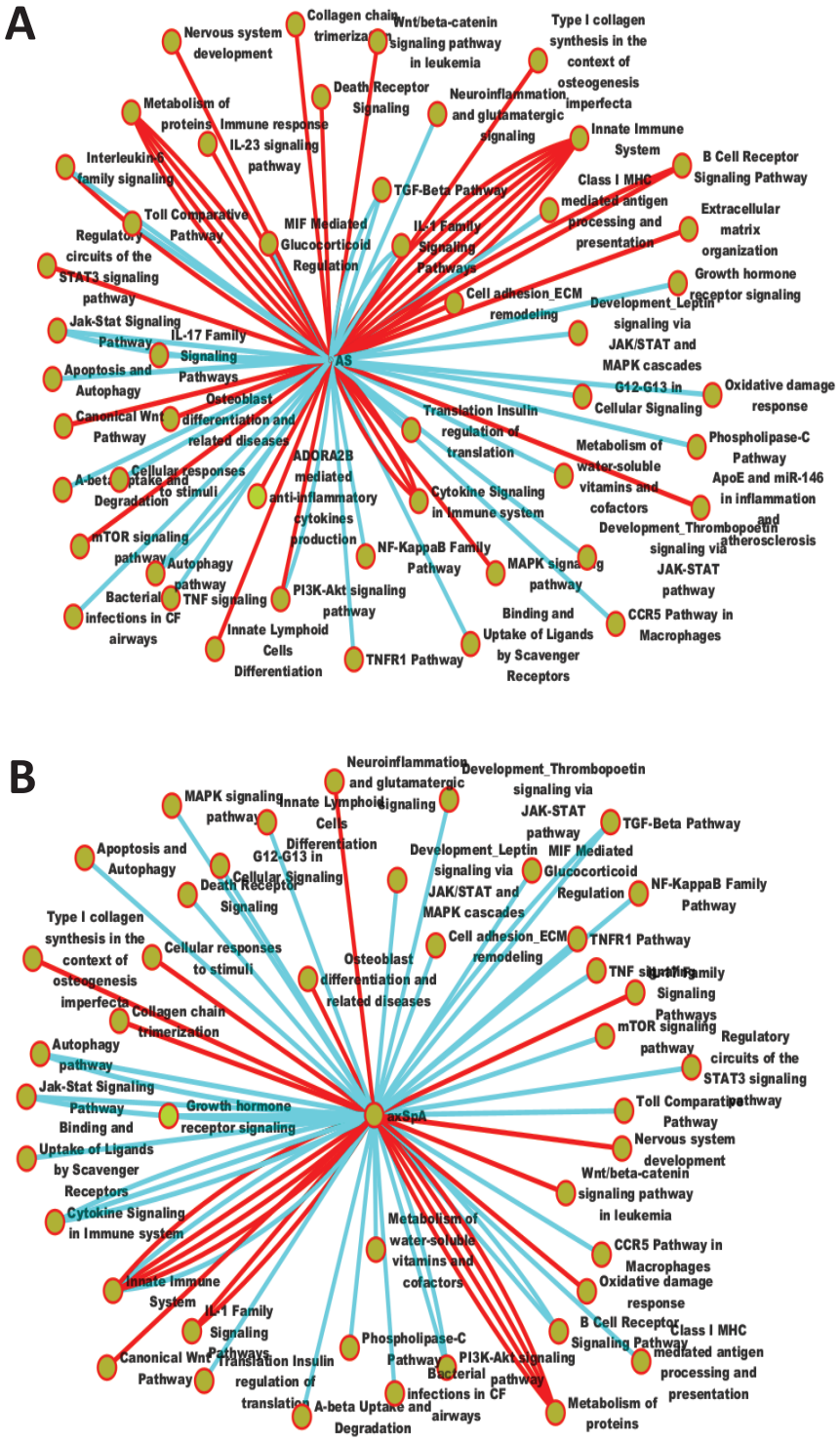

FIGURE 3. Diseasome network in AS and axSpA. (A) The AS diseasome and linking pathways. Green circle nodes and black texts indicate pathways involved in AS; red edges indicate common pathways shared by AS and its comorbidities; and cyan edges indicate pathways specific to AS with indirect interactions. (B) The axSpA diseasome and linking pathways. Green circle nodes and black texts indicate pathways involved in axSpA; red edges indicate common pathways shared by axSpA and other comorbidities; and cyan edges indicate pathways specific to axSpA with indirect interactions

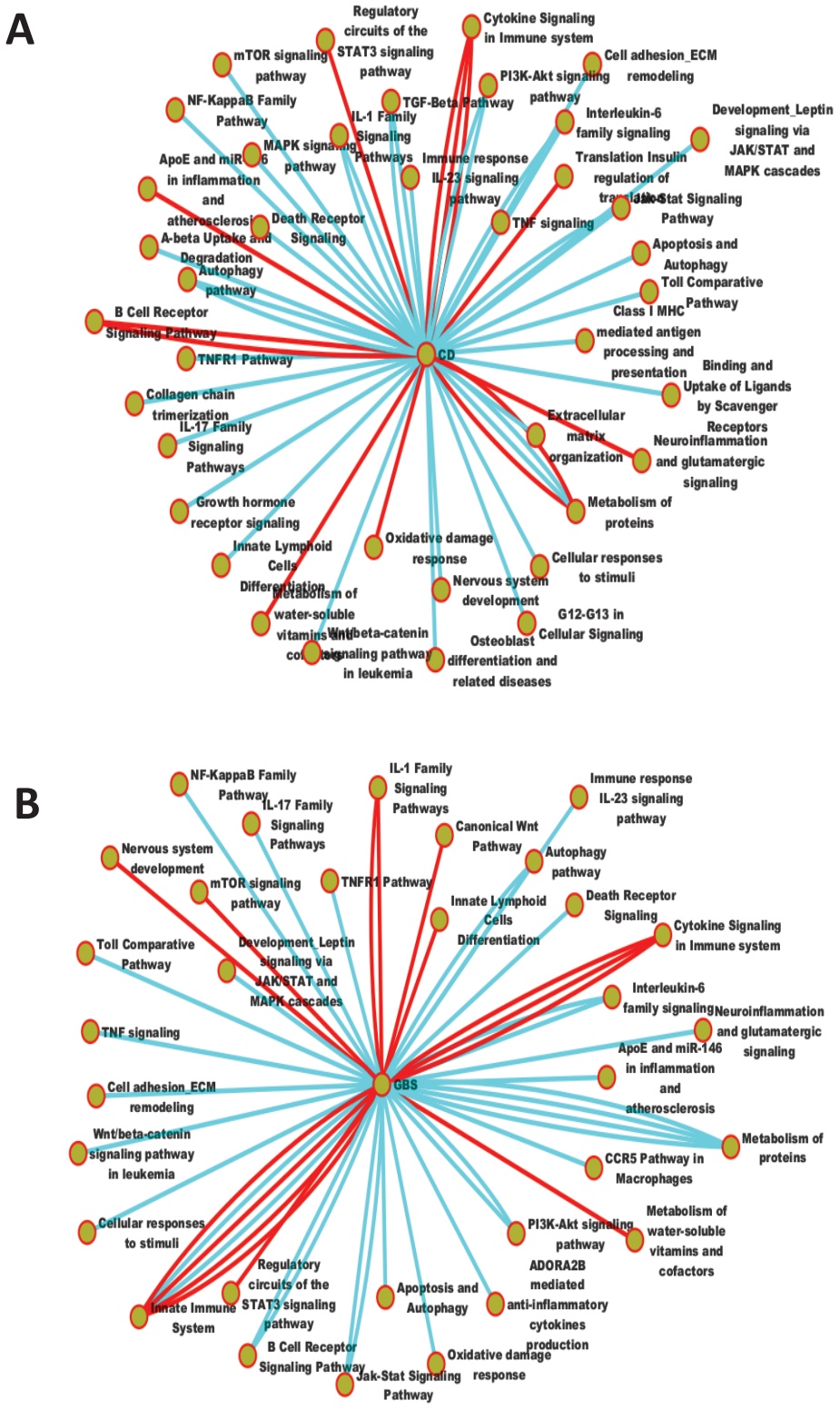

FIGURE 4. Diseasome network in CD and GBS. (A) CD diseasome and linking pathways. Green circle nodes and black texts indicate pathways involved in CD; red edges indicate shared pathways shared by CD and other comorbidities; and cyan edges indicate pathways specific to CD with indirect interactions. (B) GBS diseasome and linking pathways. Green circle nodes and black texts indicate pathways involved in GBS; red edges indicate common pathways shared by GBS and other comorbidities; and cyan edges indicate pathways specific to GBS with indirect interactions

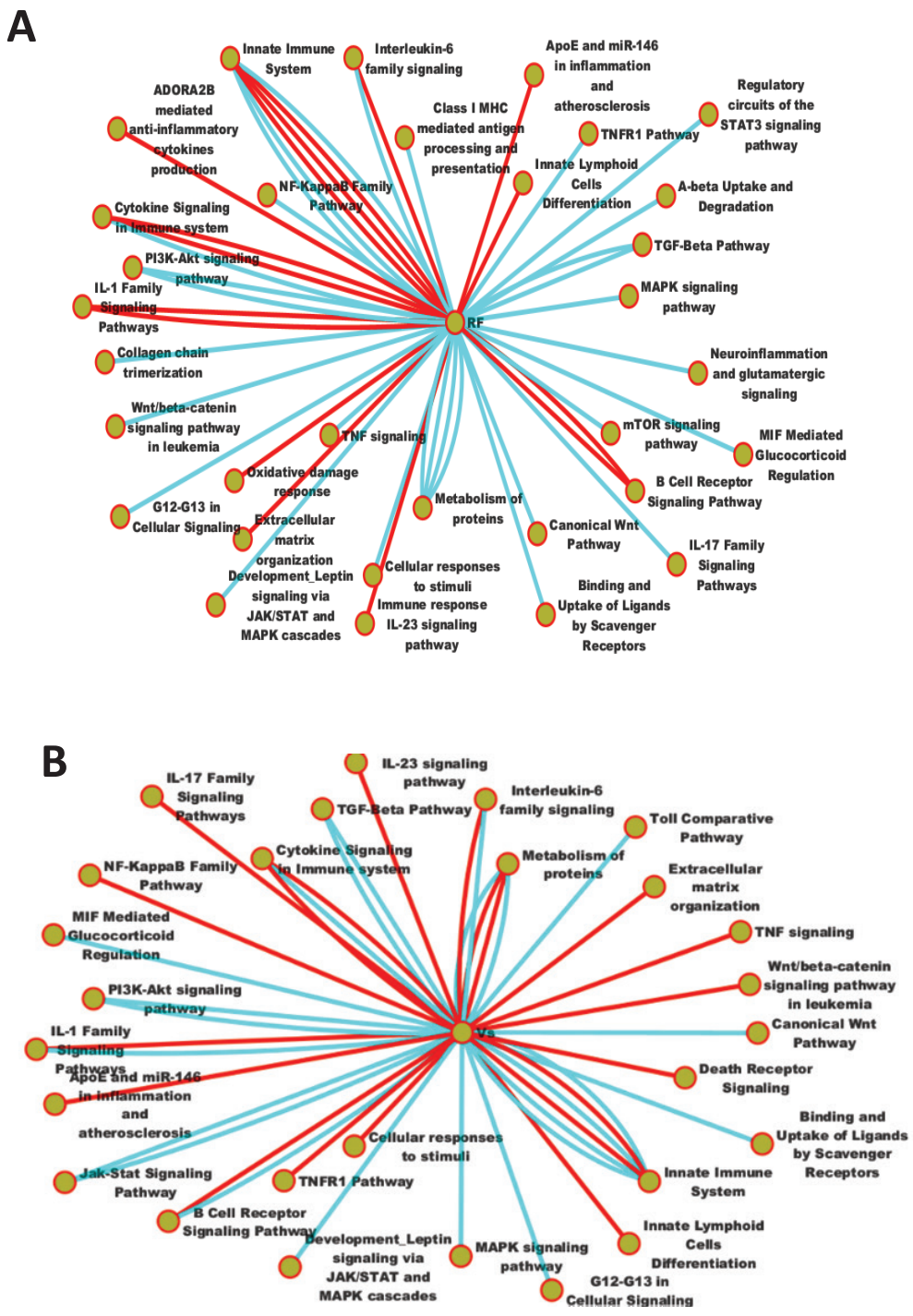

FIGURE 5. Diseasome network in RF and Vs. (A) RF diseasome and linking pathways. Green circle nodes and black texts indicate pathways involved in RF; red edges indicate common pathways shared by RF and other comorbidities; and pink edges indicate pathways specific to RF with indirect interactions. (B) Vs diseasome and linking pathways. Green circle nodes and black texts indicate pathways involved in Vs; red edges indicate common pathways shared by Vs and other comorbidities; and cyan edges indicate pathways specific to Vs with indirect interactions

pathways. These were the PI3K-Akt pathway (n = 6), the oxidative damage response (n = 4), the innate immune system (n = 3), the B cell receptor signaling pathway (n = 3), and the IL-6 family (n = 3). The interactions among these shared pathways contribute to the mechanisms that promote negative or positive dysfunction and dysregulation activities.

Further topological analysis was carried out to determine the degree of association between pathways and comorbidities. Figure 6(B) displays the result of the topological association between shared pathways and comorbidities. The topological analysis showed that there were stronger associations between shared pathways and comorbidities than between EPC and shared pathways. The higher the degree of connectivity among nodes, the greater the associations between them. However, the EPC showed the probability of connectivity and interaction between edges (centrality) that were less related. The higher the EPC, the less the association between connected edges and nodes. Thus, the result showed a stronger association between shared pathways and comorbidities. Table 1 presents the statistical analysis for shared pathways. The pairwise correlation revealed strong associations (p-value ≤ 0.001) between shared pathways and their corresponding comorbidities. The Bonferroni correction showed a low group-wise error across all shared pathways and that the shared pathways were closely associated.

Shared protein metabolism pathways were associated with cytokine signaling in the immune system, innate immune system, IL-1, and IL-6 pathways, which was critical in connecting AS and its comorbidities. Additionally, the metabolites produced in this process serve as signaling molecules that directly control inflammatory reactions. Granulocytes and monocytes, which trigger innate immune responses, tightly entwined their ECM metabolism. This process promotes ECM disruption, weakening, and damage (Zhang, Liu & Zhang 2021). Granulocytes and monocytes originate from hematopoietic stem cells in the bone marrow. This might be because the machinery controlling mRNA in skeletal muscle in the spine, through activated cellular pathways such as JAK-STAT signaling and the canonical Wnt pathways, may receive signals to control protein metabolism. Protein structure could be altered by metabolic changes caused by inflammatory events in the cells. The metabolism may regulate changes in muscle mass by controlling the production and breakdown of muscle protein. All these shared pathways in protein metabolism showed the strength of the molecular comorbidity association between the shared pathways in AS and its comorbidities. Thus, these processes might help explain some of the dysfunctional mechanisms that activate spinal immobilization and erosion in AS.

THE STRENGTH OF ASSOCIATION AMONG THE COMORBIDITIES IN THE SHARED PATHWAYS

Based on the pathway overlap finding, the strength of connection and association between two shared pathways in the diseasome was determined, which can help us understand the nature of the relationships and their functions. Table 2 illustrates the strength of comorbidity associations between shared pathways, where the ones with higher comorbidity associations in order of their strength were the innate immune system (94.2%), extracellular matrix organization (95.6%), IL-6 family signaling (95.8%), and B cell receptor signaling pathway (96%). The order of strength also includes the PI3K-Akt signaling pathway (96.7%), the metabolism of proteins (96.9%), the Canonical Wnt Pathway (97.3%), the JAK-STAT Signaling Pathway (97.7%), and cytokine signaling in the immune system (97.9%). Lastly, the order of strength includes oxidative damage response (98.2%), death receptor signaling (98.3%), and IL-1 family signaling pathways (98.4%). These results indicated that the strength of comorbidity associations between shared pathways can be associated with the dysfunctional pathways in the network. The strength and closeness could also define possible mechanisms of autoimmune responses in AS through the connected shared pathways.

The JAK-STAT signaling pathway was crucial for biological and inflammatory responses via the cytokine signaling pathway between the comorbidities. The JAK-STAT signaling system appeared to be a network of protein interactions that played a role in a number of cellular functions, including cell division, tumor development, and cell death (Xin et al. 2020). The pathway transmits information from chemical signals outside of a cell to the cell nucleus, leading to transcription for gene activation. JAKs, signal transducers and activators of transcription proteins (STATs), and receptors were essential components of JAK-STAT signaling (Hu et al. 2021). AS, axSpA, RF, CD, GBS, and Vs were autoimmune diseases caused by the dysfunctional and dysregulated JAK-STAT signaling (Guo et al. 2021; Malemud 2018; Tzeng, Chyuan & Lai 2021; Xue et al. 2023). The JAK-STAT pathway was also associated with the TGF-beta pathway, which appeared to be the key pathway associated with HLA-B27 protein (Grandon et al. 2019; Tran et al. 2023). This protein was also associated with interleukin in AS, which seemed to be one of the main ASrp found in AS comorbidities. The existence of HLA-B27 on cells (such as white blood cells, which can be both T cells and B cells) could cause the immune system to attack the healthy cells. As a result, immune-mediated or autoimmune conditions like AS, axSpA, and RF might develop (Appel et al. 2004; Grandon et al. 2019). Changes made to the receptors let the intracellular JAKs that were connected to them phosphorylate and start the binding of ligands from outside the cell (Seif et al. 2017).

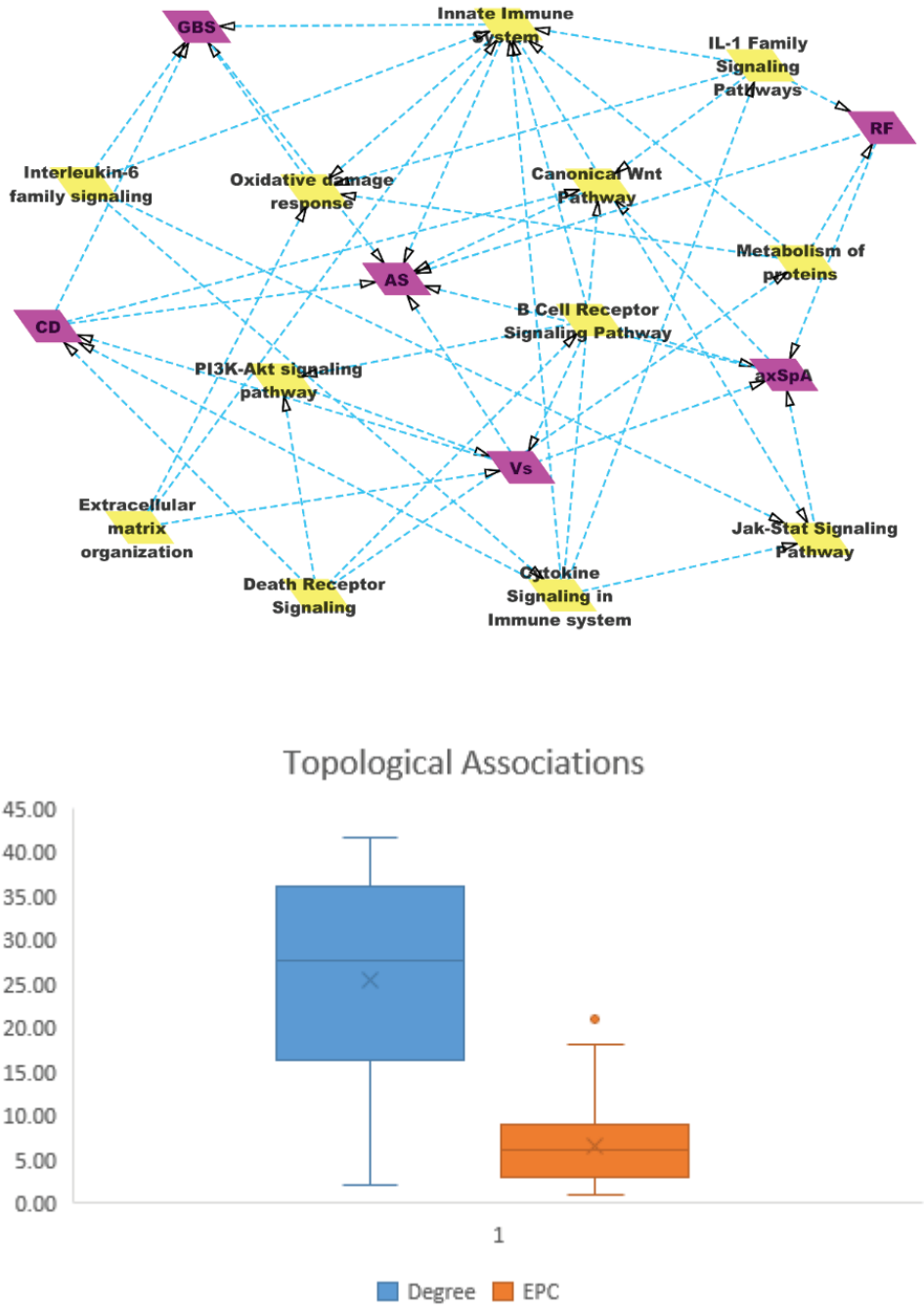

FIGURE 6. The relationship between the comorbidities. (A) The interactions between shared pathways among comorbidities. (B) Topological association between shared pathways and AS comorbidities. Note: Pink nodes represent the comorbidities, yellow nodes represent shared pathways, pale blue edges represent interactions, and arrows represent the direction of association. AS=ankylosing spondylitis; axSpA=axial spondyloarthritis; CD=cardiovascular diseases; GBS= Guillain-Barre syndrome; RF= rheumatic fever; Vs=vasculitis; EPC=Edge Percolated Component

TABLE 2. Strength of comorbidities associations between shared pathways

Shared pathway	MCI	HIPPIE score	p-value
B cell receptor signaling pathway	96.0%	0.93	0.001
Canonical Wnt Pathway	97.3%	0.91	0.001
Cytokine signaling in the immune system	97.9%	0.89	0.001
Death receptor signaling	98.3%	0.8	0.001
Extracellular matrix organization	95.6%	0.88	0.001
IL-1 family signaling pathways	98.4%	0.93	0.001
IL-6 family signaling	95.8%	0.9	0.001
Innate immune system	94.2%	0.95	0.001
JAK-STAT Signaling Pathway	97.7%	0.88	0.001
Metabolism of proteins	96.9%	0.95	0.001
Oxidative damage response	98.2%	0.86	0.001
PI3K-Akt signaling pathway	96.7%	0.89	0.001

The closeness of MCI (yellow bar) to shared pathways indicates strong association. The closeness of MCI to HIPPIE scores (green bar) indicates weak association. Low percentage shows better strength. HIPPIE scores are based on longer paths in a network of nodes closeness. MCI = molecular comorbidities index; HIPPIE = Human Integrated Protein-Protein Interaction Reference

The oxidative damage response and canonical Wnt signaling pathways were associated with death receptor signaling pathways, and shared between AS, axSpA, RF, CD, GBS, and Vs. The oxidative stress response is associated with these comorbidities when cellular defense mechanisms were incapable of managing the presence of ROS. ROS seemed to be connected to a process that activates apoptosis without the use of caspase (Poprac et al. 2017; Xiang et al. 2020). These comorbidities interact based on their pathways and functions. Apoptosis can change structural proteins in the oxidative damage response and canonical Wnt signaling pathways. This could stop the protein signal and cause more complicated biological functions. This process results in cell death and might activate mechanisms responsible for spine eroding, potentially leading to further cell death. These pathways involved in protein carbonylation might be triggered by ROS, which could directly oxidise amino acids associated with the structural proteins (Ghosh & Shcherbik 2020). Damage to complex proteins appeared to be a negative regulator of the canonical Wnt signaling pathway because it encouraged cytoplasmic β-catenin mutilation when Wnt signaling was not likely to be present.

CONCLUSION

Twelve shared pathways have been identified as linkers between AS and its comorbidities such as axSpA, RF, CD, GBS, and Vs and can be used to explain their underlying mechanisms. MCI analyses showed strong association between these shared pathways. Information on shared pathways between AS, axSpA, RF, CD, GBS, and Vs can be used to explain the pathobiology of AS and its comorbidities, assisting in accurate diagnosis and effective treatment. The findings of this study could aid biological and clinical research in understanding the role of biological pathways in complex mechanisms of AS pathobiology to enhance clinical treatment, drug target actions, and biomarker discovery. Furthermore, it provides high-throughput biological information on significant comorbidities associated with AS and their common pathways, which could be crucial in investigating dysregulation and malfunctioning proteins in the inflammatory pathways in any of AS comorbidities.

ACKNOWLEDGEMENTS

This work has no funding support. AUB acknowledges The Islamic Development Bank (IsDB) for his PhD scholarship. The APC is paid using TAP-K007217 awarded to ZAMH.

REFERENCES

Alanis-Lobato, G. & Schaefer, M.H. 2020. Generation and interpretation of context-specific human protein–protein interaction networks with HIPPIE. *Protein-Protein Interaction Networks*. *Methods in Molecular Biology* 2074: 135-144.

Appel, H., Neure, L., Kuhne, M., Braun, J., Rudwaleit, M. & Sieper, J. 2004. An elevated level of IL-10-and TGFβ-secreting T cells, B cells and macrophages in the synovial membrane of patients with reactive arthritis compared to rheumatoid arthritis. *Clinical Rheumatology* 23: 435-440.

Boehme, K.A. & Rolauffs, B. 2018. Onset and progression of human osteoarthritis - Can growth factors, inflammatory cytokines, or differential miRNA expression concomitantly induce proliferation, ECM degradation, and inflammation in articular cartilage? *International Journal of Molecular Sciences* 19(8): 2282.

Cella, D., Lenderking, W.R., Chongpinitchai, P., Bushmakin, A.G., Dina, O., Wang, L. & Navarro-Compán, V. 2022. Functional assessment of chronic illness therapy-fatigue is a reliable and valid measure in patients with active ankylosing spondylitis. *Journal of Patient-Reported Outcomes* 6: 100.

Chin, C.H., Chen, S.H., Wu, H.H., Ho, C.W., Ko, M.T. & Lin, C.Y. 2014. CytoHubba: Identifying hub objects and sub-networks from complex interactome. *BMC Systems Biology* 8(Suppl 4): S11.

Choudhary, S., Khan, N.S., Verma, R., Saxena, P., Singh, H., Jain, A.K. & Kumar, N. 2023. Exploring the molecular underpinning of psoriasis and its associated comorbidities through network approach: Cross talks of genes and pathways. *3 Biotech* 13(5): 130.

Coulson, D.J., Bakhashab, S., Latief, J.S. & Weaver, J.U. 2021. MiR-126, IL-7, CXCR1/2 receptors, inflammation and circulating endothelial progenitor cells: The study on targets for treatment pathways in a model of subclinical cardiovascular disease (type 1 diabetes mellitus). *Journal of Translational Medicine* 19(1): 140.

England, B.R., Yang, Y., Roul, P., Haas, C., Najjar, L., Sayles, H. & Mikuls, T.R. 2023. Identification of multimorbidity patterns in rheumatoid arthritis through machine learning. *Arthritis Care and Research* 75(2): 220-230.

Ghosh, A. & Shcherbik, N. 2020. Effects of oxidative stress on protein translation: Implications for cardiovascular diseases. *International Journal of Molecular Sciences* 21(8): 2661.

Grandon, B., Rincheval-Arnold, A., Jah, N., Corsi, J.M., Araujo, L.M., Glatigny, S. & Breban, M. 2019. HLA-B27 alters BMP/TGFβ signalling in drosophila, revealing putative pathogenic mechanism for spondyloarthritis. *Annals of the Rheumatic Diseases* 78(12): 1653-1662.

Guo, X., Ji, J., Zhang, J., Hou, X., Fu, X., Luo, Y. & Feng, Z. 2021. Anti-inflammatory and osteoprotective effects of chikusetsusaponin IVa on rheumatoid arthritis via the JAK/STAT signaling pathway. *Phytomedicine* 93: 153801.

Hu, X., Li, J., Fu, M., Zhao, X. & Wang, W. 2021. The JAK/STAT signaling pathway: From bench to clinic. *Signal Transduction and Targeted Therapy* 6(1): 402.

Kasher, M., Williams, F.M., Freidin, M.B., Malkin, I., Cherny, S.S. & Livshits, G. 2022. Understanding the complex genetic architecture connecting rheumatoid arthritis, osteoporosis and inflammation: Discovering causal pathways. *Human Molecular Genetics* 31(16): 2810-2819.

Kaur, H., Mittal, G.K. & Singhdev, J. 2021. Guillain–Barré syndrome unmasking asymptomatic peripheral spondyloarthritis. *Indian Journal of Medical Specialities* 12(1): 37.

Khan, M.A. 2023. *Axial Spondyloarthritis and Ankylosing Spondylitis*. 6th Ed. Oxford: Oxford University Press.

Kim, H.A., Lee, E., Park, S.Y., Lee, S.S. & Shin, K. 2022. Clinical characteristics of patients with psoriatic spondylitis versus those with ankylosing spondylitis: Features at baseline before biologic therapy. *Journal of Korean Medical Science* 37(33): e253.

Kitsak, M., Ganin, A., Elmokashfi, A., Cui, H., Eisenberg, D.A., Alderson, D.L. & Linkov, I. 2022. Finding shortest and nearly shortest path nodes in large substantially incomplete networks. *Diseases* 78(12): 1653-1662.

Li, Y. & Agarwal, P. 2009. A pathway-based view of human diseases and disease relationships. *PLoS ONE* 4(2): e4346.

Li, Z., Guo, J. & Bi, L. 2020. Role of the NLRP3 inflammasome in autoimmune diseases. *Biomedicine and Pharmacotherapy* 130: 110542.

López-Medina, C. & Molto, A. 2020. Comorbidities management in spondyloarthritis. *RMD Open* 6(2): e001135.

Łukawska, E., Polcyn-Adamczak, M. & Niemir, Z.I. 2018. The role of the alternative pathway of complement activation in glomerular diseases. *Clinical and Experimental Medicine* 18: 297-318.

Malemud, C.J. 2018. The role of the JAK/STAT signal pathway in rheumatoid arthritis. *Therapeutic Advances in Musculoskeletal Disease* 10(5-6): 117-127.

Mantovani, A. & Garlanda, C. 2023. Humoral innate immunity and acute-phase proteins. *New England Journal of Medicine* 388(5): 439-452.

Nygaard, L., Polcwiartek, C., Nelveg-Kristensen, K.E., Carlson, N., Kristensen, S., Torp-Pedersen, C. & Gregersen, J.W. 2023. Long-term cardiovascular outcomes and temporal trends in patients diagnosed with ANCA-associated vasculitis: A Danish nationwide registry study. *Rheumatology* 62(2): 735-746.

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C.J. & Valko, M. 2017. Targeting free radicals in oxidative stress-related human diseases. *Trends in Pharmacological Sciences* 38(7): 592-607.

Psarelis, S., Hajineocli, A.P., Hadjicosta, E., Elliott, H.S.A. & Johnson, P. 2017. Is secukinumab a safe alternative treatment for ankylosing spondylitis with Guillain Barré syndrome after anti-TNF-α treatment? Case report and literature review. *Clinical Rheumatology* 36(5): 1197-1199.

Rebordosa, C., Farkas, D.K., Montonen, J., Laugesen, K., Voss, F., Aguado, J. & Ehrenstein, V. 2022. Cardiovascular events and all-cause mortality in patients with chronic obstructive pulmonary disease using olodaterol and other long-acting beta2-agonists. *Pharmacoepidemiology and Drug Safety* 31(8): 827-839.

Rubtsova, K., Rubtsov, A.V., Thurman, J.M., Mennona, J.M., Kappler, J.W. & Marrack, P. 2017. B cells expressing the transcription factor T-bet drive lupus-like autoimmunity. *The Journal of Clinical Investigation* 127(4): 1392-1404.

Seif, F., Khoshmirsafa, M., Aazami, H., Mohsenzadegan, M., Sedighi, G. & Bahar, M. 2017. The role of JAK-STAT signaling pathway and its regulators in the fate of T helper cells. *Cell Communication and Signaling* 15(1): 23.

Singh, D.K. & Magrey, M.N. 2020. Racial differences in clinical features and comorbidities in ankylosing spondylitis in the United States. *The Journal of Rheumatology* 47(6): 835-838.

Sprinzak, E. & Margalit, H. 2001. Correlated sequence-signatures as markers of protein-protein interaction. *Journal of Molecular Biology* 311(4): 681-692.

Sundarrajan, S. & Arumugam, M. 2016. Comorbidities of psoriasis-exploring the links by network approach. *PLoS ONE* 11(3): e0149175.

Szabo, G. & Momen-Heravi, F. 2020. Extracellular vesicles and exosomes: Biology and pathobiology. In *The Liver: Biology and Pathobiology,* edited by Arias, I.M., Alter, H.J., Boyer, J.L., Cohen, D.E., Shafritz, D.A., Thorgeirsson, S.S. & Wolkoff, A.W. New York: John Wiley & Sons Ltd. pp. 1022-1027.

Tam, G.H.F., Chang, C. & Hung, Y.S. 2013. Gene regulatory network discovery using pairwise Granger causality. *IET Systems Biology* 7(5): 195-204.

Tan, S., Bagheri, H., Lee, D., Shafiei, A., Keaveny, T.M., Yao, L. & Ward, M.M. 2022. Vertebral bone mineral density, vertebral strength, and syndesmophyte growth in ankylosing spondylitis: The importance of bridging. *Arthritis & Rheumatology* 74(8): 1352-1362.

Tian, X., Nanding, K., Dai, X., Wang, Q., Wang, J. & Fan, L. 2023. Pattern recognition receptor mediated innate immune response requires a Rif-dependent pathway. *Journal of Autoimmunity* 134: 102975.

Tran, T.M., Gill, T., Bennett, J., Hong, S., Holt, V., Lindstedt, A.J. & Colbert, R.A. 2023. Paradoxical effects of endoplasmic reticulum aminopeptidase 1 deficiency on HLA–B27 and its role as an epistatic modifier in experimental spondyloarthritis. *Arthritis & Rheumatology* 75(2): 220-231.

Tzeng, H.T., Chyuan, I.T. & Lai, J.H. 2021. Targeting the JAK-STAT pathway in autoimmune diseases and cancers: A focus on molecular mechanisms and therapeutic potential. *Biochemical Pharmacology* 193: 114760.

Weber, B., Wallace, Z.S., Parks, S., Cook, C., Huck, D.M., Garshick, M. & Di Carli, M. 2023. Association between systemic vasculitis and coronary microvascular dysfunction in the absence of obstructive coronary artery disease. *Circulation: Cardiovascular Imaging* 16(1): e014940.

Wysocki, K. & Ritter, L. 2011. Diseasome: An approach to understanding gene-disease interactions. *Annual Review of Nursing Research* 29: 55-72.

Xiang, Q., Cheng, Z., Wang, J., Feng, X., Hua, W., Luo, R., Wang, B., Liao, Z., Ma, L., Li, G., Lu, S., Wang, K., Song, Y., Li, S., Wu, X., Yang, C. & Zhang, Y. 2020. Allicin attenuated advanced oxidation protein product-induced oxidative stress and mitochondrial apoptosis in human nucleus pulposus cells. *Oxidative Medicine and Cellular Longevity* 2020: 6685043.

Xin, P., Xu, X., Deng, C., Liu, S., Wang, Y., Zhou, X. & Sun, S. 2020. The role of JAK/STAT signaling pathway and its inhibitors in diseases. *International Immunopharmacology* 80: 106210.

Xue, C., Yao, Q., Gu, X., Shi, Q., Yuan, X., Chu, Q. & Li, L. 2023. Evolving cognition of the JAK-STAT signaling pathway: Autoimmune disorders and cancer. *Signal Transduction and Targeted Therapy* 8(1): 204.

Yang, W., Rong, L., Xu, Q., Fu, X., Deng, X., Hu, A. & Jiang, Y. 2022. Identification of symptom clusters in patients with ankylosing spondylitis. *International Journal of Rheumatic Diseases* 25(10): 1137-1144.

Yates, D. 2021. A shared pathway? *Nature Reviews Neuroscience* 22(6): 325.

Yu, L. & Gao, L. 2017. Human pathway-based disease network. *IEEE/ACM Transactions on Computational Biology and Bioinformatics* 16(4): 1240-1249.

Zhang, W., Liu, Y. & Zhang, H. 2021. Extracellular matrix: An important regulator of cell functions and skeletal muscle development. *Cell and Bioscience* 11: 65.

*Corresponding author; email: zeti.hussein@ukm.edu.my