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Targeting Genetic and Epigenetic Mechanisms for Precise Treatment in Rheumatoid Arthritis

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Report title: Targeting Genetic and Epigenetic Mechanisms for Precise Treatment in Rheumatoid Arthritis

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Abstract:

Rheumatoid Arthritis (RA) is a chronic inflammatory disease that imposes a significant burden on the affected individual and society. Disease progression mainly affects the diarthrodial joints, causing swelling, pain, deformation and functional limitations to the patient. The burden of RA is trending upwards globally with Ireland exhibiting the highest age standard rate in the world of 30 per 100,000 people. The goal of disease remission is achievable in up to 50% of patients with the current standard of care. Genetic susceptibility is a primary factor in RA development, particularly due to variants within the human leukocyte antigen (HLA) region. Concordance rates in twins demonstrate genetic risk but their low values suggest that other regulatory mechanisms are also involved. Emerging research highlights DNA methylation as a critical epigenetic modification contributing to disease progression. These modifications may impact RA through their influence on fibroblast-like synoviocytes (FLSs), which evade immune recognition and interact with T-cells. Further research may also reveal novel biomarkers and more targeted therapies to improve RA treatment outcomes.

List of abbreviations:

ABT	Abatacept
ACPA	Anti-citrullinated protein antibodies
bDMARDs	Biologic disease modifying anti-rheumatic drugs
csDMARDs	Conventional synthetic disease modifying antirheumatic drugs
DNMT	DNA methyltransferases
FLS	Fibroblast-like synoviocyte
HLA	Human leukocyte antigen
IL	Interleukin
MMP	Matrix metalloproteinase
NGS	Next generation sequencing
OR	Odds ratio
PAD	Peptidylarginine deiminases
RA	Rheumatoid Arthritis
RF	Rheumatoid factor
SAM	S-adenosylmethionine
SAT	Spermine N1-acetyltransferase
SE	Shared epitope
TAD	Topologically associated domains
TNF-α	Tumour necrosis factor alpha
TNFi	Tumour necrosis factor inhibition

1. Introduction

1.1. Global burden of rheumatoid arthritis

Rheumatoid Arthritis (RA) is characterised by chronic inflammation that primarily affects the joints. It involves interplay between immune cells and pro-inflammatory cytokines that helps sustain inflammatory responses at the molecular level. Disease progression results in bone and cartilage destruction, joint deformities, disability and low quality of life [1]. RA is a key global issue, and its burden has increased considerably over the last few decades (Fig. 1). Improved diagnostics has contributed to this increase, but the age standard rate is still expected to trend upwards until 2030, emphasising an urgent need to enhance current treatment strategies [2].

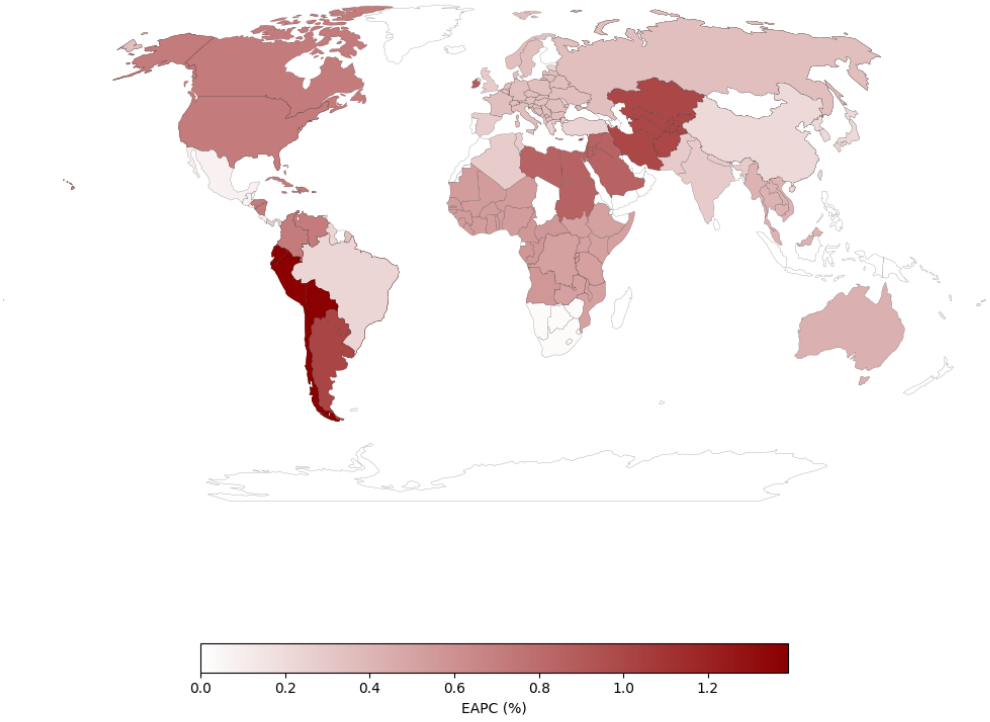

Fig 1. Estimated annual percentage change (EAPC) of RA incidence (1990-2019). *The EAPC in over 200 countries is represented on a colour gradient for the magnitude of change. Countries with no available data were excluded and are visible in white. The EAPC for the incidence of RA globally was 0.3%, dark red countries (over 0.4%) are considered statistically significant. Figure created with python.*

1.2. Current treatment strategy

Early intervention and aggressive therapies improve patient outcomes by up to 30% [3]. Standard treatment involves the use of conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) such as methotrexate and antimalarial agents. Methotrexate is the first-line therapy for most RA patients, and achieves a response rate of 41% on the American College of Rheumatology scale indicating at least 50% improvement [4]. Various combination therapies involving csDMARDs may be considered for patients who do not respond adequately to methotrexate [4]. Many contemporary RA treatment strategies now involve the use of biological disease modifying anti-rheumatic drugs (bDMARDs), and this has led to dramatic improvements. Nevertheless, RA medication selection is often guided by clinician experience and a degree of trial and error since precise biomarkers for predicting treatment responses are still lacking [5].

1.3. Avenues of improvement

RA is a complex disease with an aetiology that is not fully understood. Twin studies have been valuable for distinguishing between genetic and external factors in disease development. RA concordance rates are less significant than other autoimmune diseases, implying a dominant influence of other determinants like environmental factors and epigenetic modifications [6]. However, genetic variants appear to play a critical role once chronic inflammation has been activated. Genotyping these variants could open up the possibility for more personalised medicine, although further research is needed to validate this potential [4]. New therapeutic approaches and diagnostic biomarkers may emerge as epigenetics gains more attention in RA pathogenesis research.

2. Molecular Mechanisms of Pathogenesis

2.1. Immune cell activation

RA occurs when the immune system mistakenly attacks healthy tissue, leading to a cascade of responses. Immune cells including B cells, T cells, and macrophages accumulate in the synovial membrane and fluid, increasing levels of proinflammatory cytokines and causing progressive cartilage damage [7]. Macrophages are responsible for the production of proinflammatory cytokines like interleukin 6 (IL-6), IL-1 and tumour necrosis factor alpha (TNF-α) which are known to play pivotal roles in chronic inflammation [3]. B cells are central to the pathogenesis of RA as the source of rheumatoid factor (RF) and anti-citrullinated

protein antibodies (ACPAs). They also perpetuate T cell activation through their expression of proinflammatory cytokines [3].

2.2. Anti-citrullinated protein antibody mechanism of action

ACPAs produced by the B cells can react with various citrullinated autoantigens. Citrullination is a post-translational modification catalysed by peptidylarginine deiminases (PADs) which convert a charged arginine to a neutral citrulline [4]. Domains consisting of citrullinated fibrinogen and ACPA have been found in almost 70% of ACPA-positive patients. These complexes can stimulate Fcγ receptors on macrophages which then induce secretion of proinflammatory cytokine such as TNF-α (Fig. 2)[4].

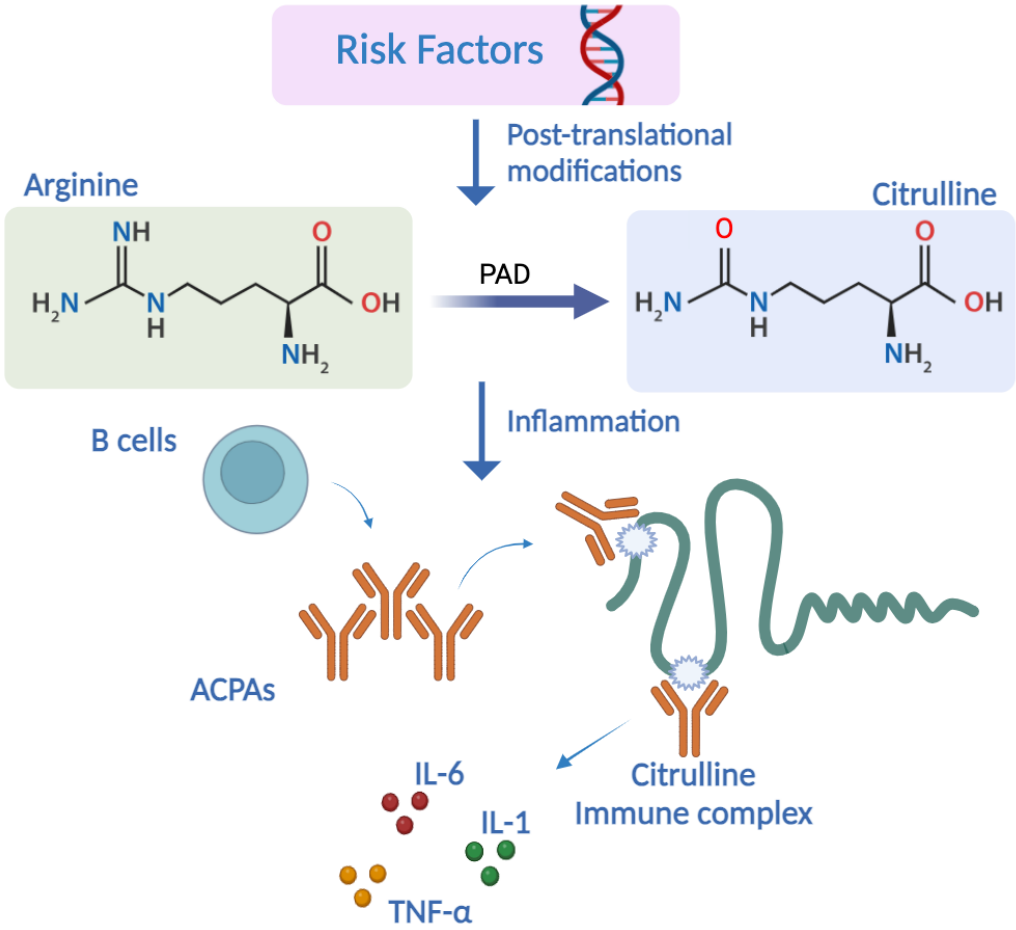

Figure 2. Activation of ACPAs through citrullination. *Genetic and environmental risks trigger the post-translational modification of arginine residues by PAD. ACPAs are produced by B cells which interact with citrullinated protein to initiate an inflammatory cascade. Pro-inflammatory cytokines critical to the progression of RA are released, including IL-6 and TNF-α. Figure created with biorender.com.*

2.3. Role of Fibroblast-like synoviocytes

Fibroblast-like synoviocytes (FLSs) are specialised cells residing in the lining layer of the joint. They play an important role in maintaining joint nutrition and lubrication by producing synovial fluid, a viscous liquid which reduces friction between cartilage allowing for smooth joint movement [8]. Pathogenic FLSs display features reminiscent of tumour cells [9]. T cells activate apoptosis-resistant FLSs during inflammation, and the FLSs become key effector cells in the hyperplastic, inflamed and aggressive mass of synovial tissue extending from the RA synovium [1]. FLSs secrete cytokines and proteases which contributes to the destructive environment. This unique aggressive phenotype invades the extra cellular matrix, ultimately resulting in joint destruction and RA symptoms [3]. This sequence of events has been confirmed by isolating FLSs from RA patients and demonstrating their ability to degrade cartilage in immunodeficient mice [7].

3. Genetic Susceptibility

3.1. Dominant genetic influence

The genetic influence of RA is still debated with an estimated range of 12-58% heritability for RA patients who test positive to RF and ACPA [10,11]. Intensive genetic studies have been carried out in search of pathogenetic insights and prognostic biomarkers. Genome wide association studies identified over 100 genetic loci associated with RA risk. Next generation sequencing (NGS) combined with functional annotation techniques are now being used to unravel the roles of these in disease development [4].

The human leukocyte antigen (HLA) system on chromosome 6 (Fig. 3) is as a dominant genetic contributor in RA with a Japanese RA population study attributing 9% of the phenotypic variance to HLA genes [12]. HLA genes are vital to the immune response as the binding groove in *HLA-DR* molecules is essential for antigen presentation [4]. The highly polymorphic *HLA-DRB1* gene within the DR sub-region (Fig. 3) plays a key role in the pathogenesis of RA. *HLA-DRB1* encodes a protein that presents antigens to immune cells, with certain alleles having stronger links to elevated risk [4].

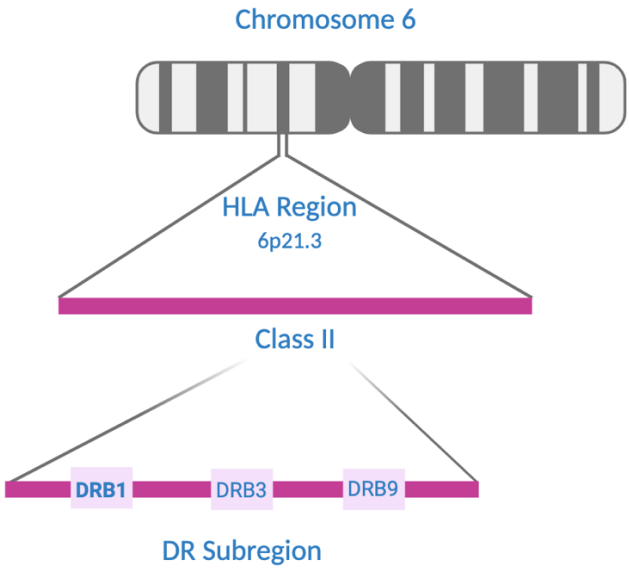

Figure 3. Gene organisation of the HLA region. *The HLA region on the short arm of chromosome 6 encodes three classes of genes, with the class II genes DRB1, DRB3 and DRB9 being crucial in immune response. HLA-DRB1 is involved in the pathogenesis of RA. Figure created with biorender.com*

3.2. Risk factors in HLA-DRB1

The "shared epitope" (SE) hypothesis for conferring susceptibility identifies common features between different variants of the *HLA-DRB1* gene by categorising alleles based on the presence of an RAA motif at site 72-74 [13]. "X" alleles lacking the motif are unlikely to cause any RA risk, while "S" alleles containing the motif can be further subdivided. Lysine (K) at position 71 confers the highest risk. Glutamine (Q) and arginine (R) at position 70 is linked to higher risk compared to the protective effect of aspartic acid (D) [14]. SE *HLA-DRB1* variants exhibit antigen binding grooves that can accommodate citrullinated peptides. An immune response is triggered through their presentation to T cells which recognise them as foreign leading to ACPA production. The presence of the SE in patients is associated with highly invasive and detrimental RA [13].

Further advancements in *HLA-DRB1* research have highlighted risk factors beyond the SE. The amino acid valine at position 11 has been identified as a profound biomarker, and a unique haplotype with valine at position 11, lysine at position 71 and alanine at position 74 is now recognised as having the strongest association with RA development [4]. Position 11 appears to play a role in structuring the internal groove unlike traditional SE alleles that only alter the shape of the external binding sites. This discovery expands the understanding of RA pathogenesis by pinpointing a novel genetic configuration and its impacts [4].

3.3. Biomarker treatment

Many RA treatment strategies now incorporate the use of bDMARDs and have led to dramatic improvements in patient symptoms [4]. For example, abatacept (ABT) works to inhibit T cell co-stimulation, while tumour necrosis factor inhibitor (TNFi) targets pro-inflammatory cytokines like TNF-α to block their action [5]. Several studies have evaluated the effectiveness of ABT in relation to the *HLA-DRB1* locus [5,15]. *HLA-DRB1* alleles were determined from patient peripheral blood samples using NGS combined with linkage and bioinformatic tools. Carriers of *HLA-DRB1*04:05* demonstrated a 60% improvement in simple disease activity index scores after three months of ABT treatment, compared to 29% for non-carriers. Notably, *HLA-DRB1*04:05* alleles encode valine at position 11, which highlights its potential utility as a predictive biomarker [5].

This was further supported by a study comparing treatment responses to ABT and TNFi in patients with specific *HLA-DRB1* variants. SE-positive patients in the TNFi treated group were less likely to respond favourably than SE-negative patients with odds ratio (OR) of 0.57 [15]. Analysis of amino acid positions 11, 71 and 74 revealed promising insights for potential drug targeting biomarkers, although SE-positive patients treated with ABT did not show significant improvements. Both SE-positive and SE-negative patients with valine at position 11 showed significant positive responses with ORs of 6.46 and 5.17, respectively. Patients with the VRA haplotype encoding valine at position 11, arginine at 71 and alanine at 74 also responded positively with OR of 4.56 [15]. These results highlight the potential of genetic screening to enhance RA patient outcomes. However, further studies with larger cohort sizes are needed to confirm these findings [15].

4. Epigenetic Mechanisms in Rheumatoid Arthritis

4.1. Epigenetics in rheumatoid arthritis pathogenesis

Epigenetics encompasses the cellular changes in gene function that occur without altering the DNA sequence itself [16]. Epigenetic mechanisms are sensitive to external stimuli like infections, smoking and environmental agents, and can act as triggers for individuals who are genetically susceptible to RA by altering their epigenetic profile [8]. DNA methylation, histone modifications, lncRNAs, and miRNAs have been highlighted as the main epigenetic contributors to RA, with most data derived from DNA methylation studies on synovial tissue and FLS cells [1].

4.2. DNA methylation mechanism of action

DNA methylation is a crucial epigenetic modification that influences gene expression. It operates in multicellular eukaryotes by adding methyl groups to cytosine bases in CpG islands which are disproportionably located within promoter regions [1]. DNA methyltransferases (DNMTs) facilitate this process through methylation of the fifth carbon of the cytosine ring, using S-adenosylmethionine (SAM) as the methyl donor [3]. Methylation of the promoter can suppress gene expression through inhibition of transcription factor binding by assisting in heterochromatin formation [3].

4.3. DNA methylation in fibroblast-like Synoviocytes

Differentially methylated CpG islands have been identified in 3470 loci across more than 1200 genes in the FLSs associated with the RA phenotype (Fig. 4) [17]. This defining epigenetic dysregulation is significantly influenced by the chronic inflammatory environment which is rich in cytokines [8]. A key point of study has been the hypomethylated genome where the methylation of CpG islands has been decreased. This global hypomethylation is hypothesised to be related to a deficiency of SAM [1]. A study inducing an increased activity of a pro-inflammatory cytokine central to joint inflammation, IL-1β, raised the polyamine level by enhancing spermine N1-acetyltransferase (SAT) activity [18]. This increased SAT activity consumes more SAM for polyamine recycling, reducing its availability to DNMTs. This results in the global hypomethylation state driving disease progression [18].

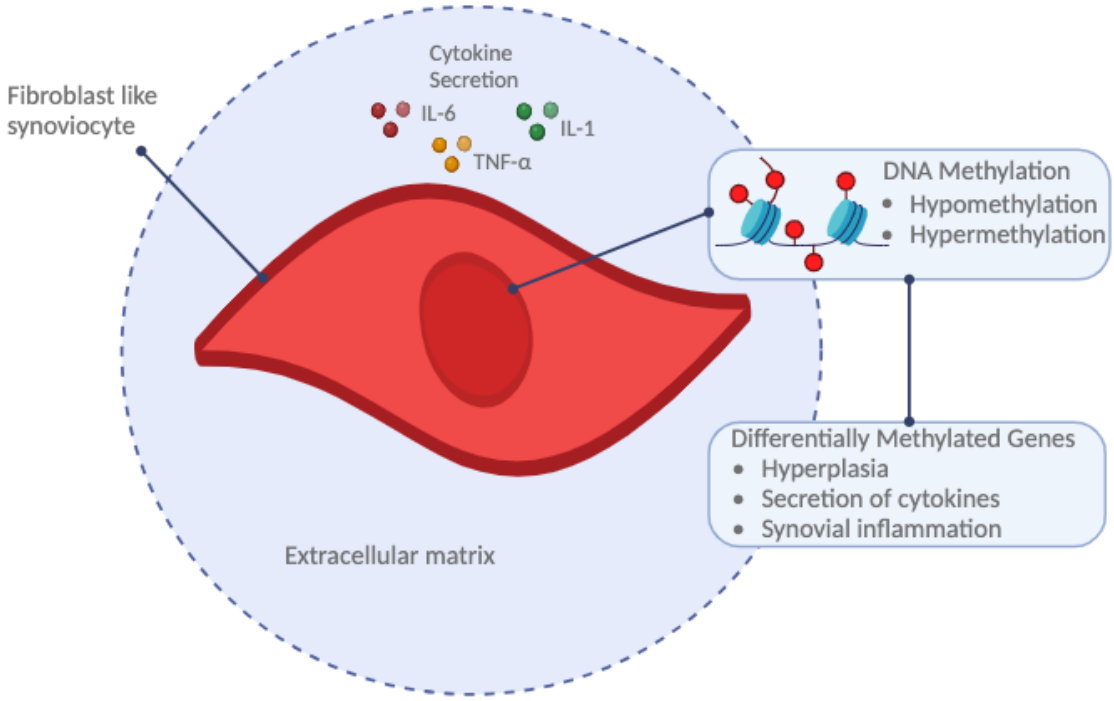

Figure 4. DNA methylation in RA FLSs. *Genes within the FLS cells are differentially methylated by removal and addition of methyl groups, converting a normal FLS into a hyperplastic phenotype. Pro-inflammatory cytokines are produced and drive the destructive environment that eventually leads to cartilage damage. Created with biorender.com.*

Mapping the epigenetic landscape with and without macrophage stimulation revealed altered topologically associated domains (TADs) in FLSs. TADs are regions where genes are typically co-regulated, and promoters and enhancers interact [19]. An average of 4,100 TAD boundaries were identified in FLS samples and most remained unchanged after treatment with TNF [19]. However, only 17% of the genes demonstrated the expected inverse relationship between gene expression and DNA methylation within the altered TADs [17]. Methylation changes exclusive to transcription start sites and 5' untranslated regions showed differential gene expression [8]. These genes were connected to pathways involved in RA pathogenesis including cell-cell, cell-matrix interactions, and cell recruitment [19]. For example, analysis of differentially methylated regions revealed an over-representation of AP-1 transcription factor binding sites in FLSs associated with RA [8]. The chromatin regions rearranged to reveal the enriched sites after treatment with TNF. The FOS component of AP-1 is linked to the production of matrix metalloproteinases (MMPs), including MMP2 which causes enhanced migration and invasiveness of RA [9].

Another pro-inflammatory cytokine, IL-17A, was linked to the activation of the NF-κB pathway which increases expression of MMP2 in FLSs (Fig. 5) [9]. This suggests the activity of synergistic signalling to reshape the epigenetic landscape through DNA methylation. IL-1B induced hypomethylation and TNFs chromatin rearrangement may 'prime' the promoter regions of genes like MMP2, while IL-17A drives the transcriptional machinery resulting in high expression. This repeated stimulation of macrophages changes the epigenetic landscape and induces reprogramming by imprinting in FLSs which can be recalled when the same stimulus is applied again [8].

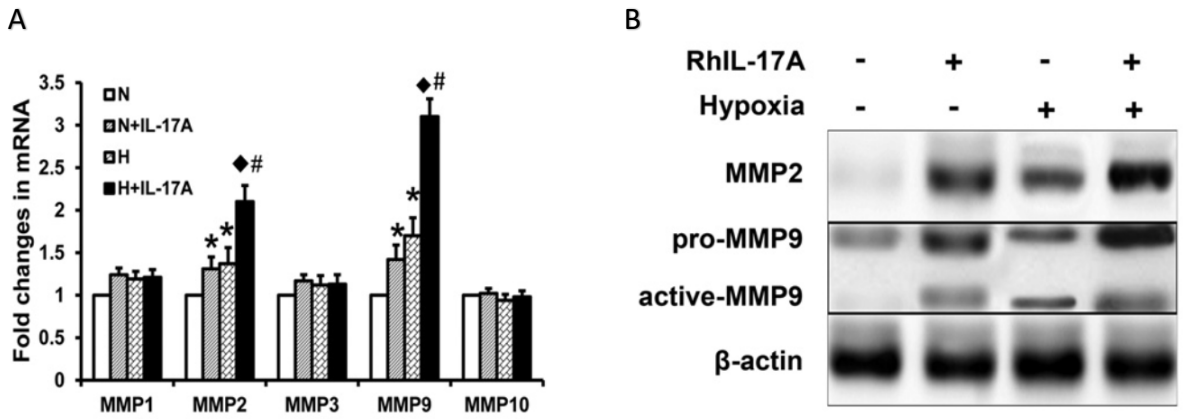

Figure 5. Influence of IL-17A on MMP production. *MMP expression was examined using qRT-PCR. (**A**) The fold change of mRNA expression from four groups was tested with and without IL-17A, under hypoxic and normal conditions. MMP2 is upregulated by IL-17A under both conditions. (**B**) MMP2 was detected in FLS cells under IL-17A stimulation. Adapted from [9].*

4.4. Dynamic nature of methylation in fibroblast-like synoviocytes

DNA methylation in FLSs is a dynamic process which changes throughout the course of the disease. Variation in the status of DNA methylation may reflect different disease stages like in cancer. The global hypomethylation could be associated with early disease onset, while hypermethylation in latter stages may target specific promoters such as those of tumour suppressor genes [1]. Epigenetic imprinting is the preservation of a specific phenotype, through stable and heritable epimutations that can be activated under certain environmental conditions. Assuming imprinting of the RA phenotype by macrophages occurs, it could be triggered at any time between embryonic development to disease flares, in response to stimuli such as high-intensity muscle contractions, smoking, and viral infections [8]. Understanding epigenetic modifications in the early disease stages of RA and gaining insights into the invasive growth of FLSs may aid in early diagnosis and open the way for direct therapeutic targeting.

5. Future Perspectives

Testing for specific *HLA-DRB1* amino acid positions holds promise in improving treatment personalisation and cost optimisation for RA. Studies show that patients respond better to certain treatments based on specific amino acid combinations which highlights the potential clinical utility of biomarkers [15]. Using genotyping to guide the administration of bDMARDs rather than relying on clinical experience could drastically improve patient outcomes by reducing selection errors and enabling earlier, more effective treatment. This field of genomic medicine has progressed rapidly, particularly in oncology, where genetic mutations in tumours are used to influence treatment decisions. This approach could be used in RA, though more research is needed to confirm its efficacy across diverse populations [4]. Genetic variability and population specific predispositions that influence treatment response must be considered.

Current diagnostic approaches of RA typically involve error-prone physical examination followed by blood tests for comprehensive analysis. Common biomarkers like RF and ACPA often lack specificity and reproducibility in the early stages of RA [3]. Methylation marks detectable in liquid biopsies via cell-free DNA can offer a valuable diagnostic tool, and this method has shown diagnostic accuracies of up to 90% in cancers [20]. These tests are minimally invasive and cost effective [20]. New DNA methylation biomarkers may be identified as our understanding of the epigenetic landscape in RA pathogenesis grows, potentially transforming early RA management. Leveraging these revolutionary oncologic approaches in combination with comprehensive data of the genetic and epigenetic modifications involved in RA will help to pioneer a better treatment and diagnostic strategy for patients.

References:

- of special interest

●● of outstanding interest

1. Karami J, Aslani S, Tahmasebi MN, Mousavi MJ, Sharafat Vaziri A, Jamshidi A, Farhadi E, Mahmoudi M: **Epigenetics in rheumatoid arthritis; fibroblast-like synoviocytes as an emerging paradigm in the pathogenesis of the disease**. *Immunol Cell Biol* 2020, **98**:171-186.

This review highlights the novel understanding of RA pathogenesis. It compares the role of epigenetic mechanisms and their interactions with FLSs which are shown to contribute to RA. ●

2. Cai Y, Zhang J, Liang J, Xiao M, Zhang G, Jing Z, Lv L, Nan K, Dang X: **The Burden of Rheumatoid Arthritis: Findings from the 2019 Global Burden of Diseases Study and Forecasts for 2030 by Bayesian Age-Period-Cohort Analysis**. *J Clin Med* 2023, **12**:1291-1302.

This study uses data from the Global Burden of Diseases Study 2019 to analyse the global burden of RA. Key findings include a gradual increase in the age-standardized prevalence and incidence. ●

3. Ciechomska M, Roszkowski L, Maslinski W: **DNA Methylation as a Future Therapeutic and Diagnostic Target in Rheumatoid Arthritis**. *Cells* 2019, **8**:953-969.

This paper emphasises epigenetic therapies, specifically targeting DNA methylation for improving the diagnosis and treatment of RA. They suggest the use of cell-free methylated DNA as a non-invasive biomarker for RA. ●

4. Wysocki T, Olesinska M, Paradowska-Gorycka A: **Current Understanding of an Emerging Role of HLA-DRB1 Gene in Rheumatoid Arthritis-From Research to Clinical Practice**. *Cells* 2020, **9**:1127-1158.

The *HLA-DRB1* gene plays a role in RA development through its influence on the structure of *HLA-DR* molecules and their binding affinity. Further research into the *HLA-DRB1* gene is emphasised to better understand RA and develop new personalized treatment approaches.

5. Inoue M, Nagafuchi Y, Ota M, Tsuchiya H, Tateishi S, Kanda H, Fujio K: **Carriers of HLA-DRB1*04:05 have a better clinical response to abatacept in rheumatoid arthritis**. *Sci Rep* 2023, **13**:15250.

6. Aslani S, Rezaei R, Jamshidi A, Mahmoudi M: **Genetic and epigenetic etiology of autoimmune diseases: lessons from twin studies**. *Rheumatology Research* 2018, **3**:45-57.

This paper explores the use of twin studies to understand the genetic and environmental factors contributing to autoimmune diseases. It highlights that while monozygotic twins share identical genetic backgrounds, they still exhibit differences in disease susceptibility, suggesting the role of environmental influences.

7. Lefevre S, Knedla A, Tennie C, Kampmann A, Wunrau C, Dinser R, Korb A, Schnaker EM, Tarner IH, Robbins PD, et al.: **Synovial fibroblasts spread rheumatoid arthritis to unaffected joints**. *Nat Med* 2009, **15**:1414-1420.

8. Ospelt C: **Site of invasion revisited: epigenetic drivers of joint destruction in RA**. *Ann Rheum Dis* 2023, **82**:734-739.

The paper presents a better understanding of SF activation and heterogeneity through new analytical methods and the increased availability of synovial biopsies. It integrates both established and emerging evidence providing comprehensive information of the processes involved in the expansion of invasive synovial tissue in RA. ●●

9. Li G, Zhang Y, Qian Y, Zhang H, Guo S, Sunagawa M, Hisamitsu T, Liu Y: **Interleukin-17A promotes rheumatoid arthritis synoviocytes migration and invasion under hypoxia by increasing MMP2 and MMP9 expression through NF-kappaB/HIF-1alpha pathway**. *Mol Immunol* 2013, **53**:227-236.

This research paper investigates polyamine metabolism in RA, specifically in cells stimulated by IL-1B. The study found that IL-1B increased the activity of SAT which may contribute to the pathogenesis of RA.

10. Knevel R, Grondal G, Huizinga TW, Visser AW, Jonsson H, Vikingsson A, Geirsson AJ, Steinsson K, van der Helm-van Mil AH: **Genetic predisposition of the severity of joint destruction in rheumatoid arthritis: a population-based study**. *Ann Rheum Dis* 2012, **71**:707-709.

This study found an association between the degree of relatedness among patients and the similarity in their joint destruction rates, suggesting that genetic factors play a considerable role in RA. Heritability estimates range from 45% to 58%.

11. Svendsen AJ, Kyvik KO, Houen G, Junker P, Christensen K, Christiansen L, Nielsen C, Skytthe A, Hjelmborg JV: **On the origin of rheumatoid arthritis: the impact of environment and genes--a population based twin study**. *PLoS One* 2013, **8**:e57304.

12. Okada Y, Suzuki A, Ikari K, Terao C, Kochi Y, Ohmura K, Higasa K, Akiyama M, Ashikawa K, Kanai M, et al.: **Contribution of a Non-classical HLA Gene, HLA-DOA, to the Risk of Rheumatoid Arthritis**. *Am J Hum Genet* 2016, **99**:366-374.

13. Karami J, Aslani S, Jamshidi A, Garshasbi M, Mahmoudi M: **Genetic implications in the pathogenesis of rheumatoid arthritis; an updated review**. *Gene* 2019, **702**:8-16.

14. du Montcel ST, Michou L, Petit-Teixeira E, Osorio J, Lemaire I, Lasbleiz S, Pierlot C, Quillet P, Bardin T, Prum B, et al.: **New classification of HLA-DRB1 alleles supports the shared epitope hypothesis of rheumatoid arthritis susceptibility**. *Arthritis Rheum* 2005, **52**:1063-1068.

This paper proposes a new classification system to better understand the role of *HLA-DRB1* alleles on RA risk, based on the amino acid sequence at position 70-74. The study tested this new classification in two groups of RA patients and found that it accurately predicted the risk of developing RA based on the presence of the SE RAA sequence at position 72-74. ●

15. Cha S, Bang SY, Joo YB, Cho SK, Choi CB, Sung YK, Kim TH, Jun JB, Yoo DH, Lee HS, et al.: **Association of HLA-DRB1 locus with treatment response to abatacept or TNF inhibitors in patients with seropositive rheumatoid arthritis**. *Sci Rep* 2024, **14**:6763.

This research paper examines the relationship between the *HLA-DRB1* gene and the effectiveness of two treatments, ABT and TNFi. The study found that patients with valine at position 11 of the *HLA-DRB1* gene demonstrate a significantly better response to ABT. ●●

16. Mueller AL, Payandeh Z, Mohammadkhani N, Mubarak SMH, Zakeri A, Alagheband Bahrami A, Brockmueller A, Shakibaei M: **Recent Advances in Understanding the Pathogenesis of Rheumatoid Arthritis: New Treatment Strategies**. *Cells* 2021, **10**:3017-3048.

17. de la Rica L, Urquiza JM, Gomez-Cabrero D, Islam AB, Lopez-Bigas N, Tegner J, Toes RE, Ballestar E: **Identification of novel markers in rheumatoid arthritis through integrated analysis of DNA methylation and microRNA expression**. *J Autoimmun* 2013, **41**:6-16.

18. Furumitsu Y, Yukioka K, Yukioka M, Ochi T, Morishima Y, Matsui-Yuasa I, Otani S, Inaba M, Nishizawa Y, Morii H: **Interleukin-1beta induces elevation of spermidine/spermine N1-acetyltransferase activity and an increase in the amount of putrescine in synovial adherent cells from patients with rheumatoid arthritis**. *J Rheumatol* 2000, **27**:1352-1357.

19. Ge X, Frank-Bertoncelj M, Klein K, McGovern A, Kuret T, Houtman M, Burja B, Micheroli R, Shi C, Marks M, et al.: **Functional genomics atlas of synovial fibroblasts defining rheumatoid arthritis heritability**. *Genome Biol* 2021, **22**:247.

20. Draskovic T, Hauptman N: **Discovery of novel DNA methylation biomarker panels for the diagnosis and differentiation between common adenocarcinomas and their liver metastases**. *Sci Rep* 2024, **14**:3095.