*Nature Reviews Rheumatology* | Published online 18 May 2017; doi:10.1038/nrrheum.2017.76

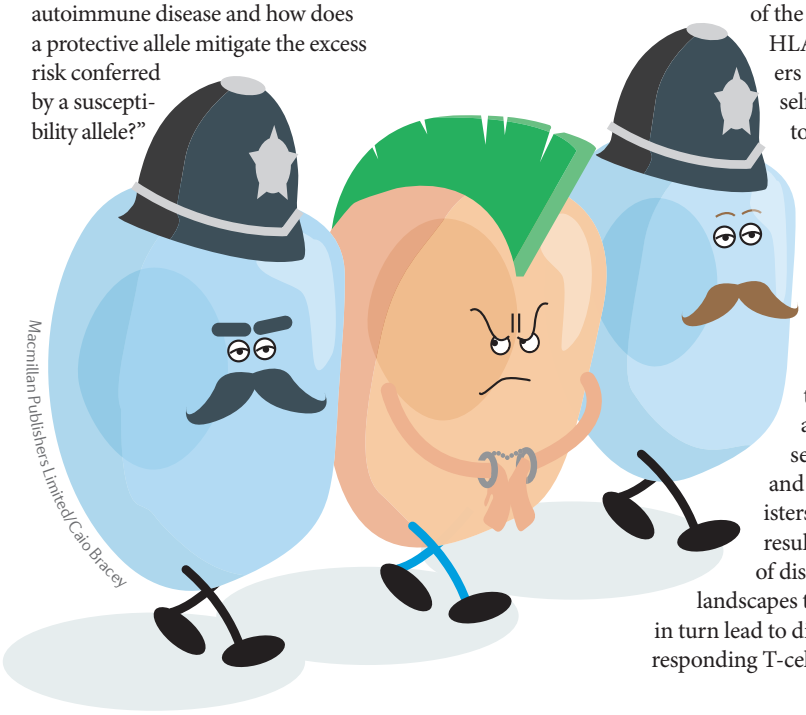
AUTOIMMUNITY

# HLA-mediated protection in Goodpasture disease

A new study provides insights into the molecular mechanisms of HLA-mediated susceptibility and protection in Goodpasture disease (GPD). This rare renal disorder is characterized by autoimmune responses to the non-collagenous domain of the α3 chain of type IV collagen (α3(IV) NC1). Expression of HLA-DR15 is associated with an increased risk of GPD, but this risk is reduced to baseline in the presence of the protective HLA allele HLA-DR1.

"The basis of the loss of immunological tolerance in most autoimmune diseases lies in the inheritance of HLA alleles that modulate the risk of autoimmunity," explains researcher Richard Kitching. "In this study we used GPD to address two long-standing questions — what is the basis of HLA-mediated protection in autoimmune disease and how does a protective allele mitigate the excess risk conferred by a susceptibility allele?"

> "HLA allelic polymorphisms result in structurally important differences in presentation of the critical T-cell epitope of the disease-causing autoantigen"



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The researchers focused their investigations on the immunodominant T-cell epitope of α3(IV) NC1, $\alpha3_{135–145}$. Using HLA-DR15-$\alpha3_{135–145}$ tetramers, they showed that $\alpha3_{135–145}$-specific CD4$^+$ T cells were 100-fold more frequent in the peripheral blood of HLA-DR15$^+$ patients with GPD than in HLA-DR15$^+$ healthy controls. In seven of the eight patients, these epitope-specific cells were mainly conventional FOXP3$^-$ T cells. Consistent with a protective role of HLA-DR1 in GPD, immunization with $\alpha3_{135–145}$ led to renal infiltration of $\alpha3_{135–145}$-specific CD4$^+$ T cells and the development of GPD in HLA-DR15$^+$ transgenic mice, but did not induce proinflammatory autoreactivity to $\alpha3_{135–145}$ in HLA-DR15$^+$DR1$^+$ transgenic mice.

To determine the molecular basis of the protective effect of HLA-DR1, the researchers characterized the self-peptide repertoires of HLA-DR1 and HLA-DR15 allomorphs from naive HLA-DR1$^+$ and HLA-DR15$^+$ mice and identified consensus peptide-binding motifs. They report that these HLA alleles have distinct self-peptide repertoires and $\alpha3_{135–145}$-binding registers. These differences result in the presentation of distinct peptide-HLA landscapes to T cells, which in turn lead to differences in the responding T-cell repertoires.

Further investigations indicated that $\alpha3_{135–145}$-specific CD4$^+$ T cells from transgenic mice that express HLA-DR15, HLA-DR1 or both HLA alleles have functional phenotypic differences. In HLA-DR15$^+$ mice, the majority of these cells were conventional Foxp3$^-$ T cells that secreted proinflammatory cytokines, whereas in HLA-DR1$^+$ mice the majority of the epitope-specific CD4$^+$ T cells were Foxp3$^+$ regulatory T ($T_{reg}$) cells that secreted tolerogenic cytokines. HLA-DR15$^+$DR1$^+$ mice expressed both conventional and regulatory $\alpha3_{135–145}$-specific CD4$^+$ T cells. Analysis of the phenotypes of $\alpha3_{135–145}$-specific CD4$^+$ T cells from healthy people who were either homozygous for HLA-DR1 or HLA-DR15, or were heterozygous for these HLA alleles, gave similar results.

Finally, the researchers showed that $\alpha3_{135–145}$-specific CD4$^+$ $T_{reg}$ cells generated in the context of HLA-DR1 suppress autoreactive conventional $\alpha3_{135–145}$-T cells generated in the context of HLA-DR15, so maintain self-tolerance to $\alpha3_{135–145}$ and protect against the development of GPD.

"HLA allelic polymorphisms result in structurally important differences in presentation of the critical T-cell epitope of the disease-causing autoantigen," concludes Kitching. "These differences result either in the generation of conventional autoantigen-specific CD4$^+$ T cells (in the context of the risk allele, HLA-DR15) or of dominantly protective antigen-specific $T_{reg}$ cells (when the epitope is presented by the protective HLA allele, HLA-DR1). These findings provide a structural and mechanistic basis for understanding HLA-mediated susceptibility and protection in autoimmune disease."

*Ellen F. Carney*

This article originally appeared in *Nature Reviews Nephrology* (doi:10.1038/nrneph.2017.71).

**ORIGINAL ARTICLE** Ooi, J. D. *et al.* Dominant protection from HLA-linked autoimmunity by antigen-specific regulatory T cells. *Nature* http://dx.doi.org/10.1038/nature22329 (2017)

*Nature Reviews Rheumatology* | Published online 25 May 2017

# IN BRIEF

## SPONDYLOARTHROPATHIES

### Abatacept treatment for PsA

The efficacy of abatacept, a drug that blocks T cell co-stimulation, has been investigated in a randomized, double-blind, placebo-controlled, phase III trial involving patients with psoriatic arthritis (PsA). The investigators randomly assigned patients with PsA to receive either 125 mg of abatacept weekly or placebo for 24 weeks. The ACR20 response rate at 24 weeks was higher in the abatacept group compared with the placebo group (39.4% **versus** 22.3%, respectively), whereas there was no difference between the groups in the incidence of adverse events. Treatment with abatacept resulted in a modest improvement of skin lesions.

**ORIGINAL ARTICLE** Mease, P. J. *et al.* Efficacy and safety of abatacept, a T-cell modulator, in a randomised, double-blind, placebo-controlled, phase III study in psoriatic arthritis. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210724 (2017)

## AUTOINFLAMMATION

### Canakinumab effective in HIDS treatment

In an open-label study involving nine patients with hyper-IgD and periodic fever syndrome (HIDS), the anti-IL-1β monoclonal antibody canakinumab treatment significantly reduced the frequency of attacks compared with the historical period during which patients received no drug treatment other than NSAIDs and/or steroids (median number of attacks per patient of 0 **versus** 5, respectively). A total of 14 serious adverse events were observed in four patients with HIDS during canakinumab treatment.

**ORIGINAL ARTICLE** Arostegui, J. I. *et al.* Open-label, phase II study to assess efficacy and safety of canakinumab treatment in active hyperimmunoglobulinemia D with periodic fever syndrome. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40146 (2017)

## CONNECTIVE TISSUE DISEASES

### Mycobacterial infection and Sjögren syndrome

In a Taiwanese population-based study involving 5,751 patients with newly diagnosed Sjögren syndrome and 86,265 individuals without Sjögren syndrome, a history of nontuberculous mycobacterial infection (NTM), but not tuberculosis infection, was associated with an increased risk of Sjögren syndrome. In subgroup analyses, the association remained statistically significant among patients aged 40–65 years, as well as in female patients and those without bronchiectasis. The authors speculated that shared immunological pathways between the two conditions could explain this association.

**ORIGINAL ARTICLE** Chao, W.-C. *et al.* Association between a history of mycobacterial infection and the risk of newly diagnosed Sjögren's syndrome: a nationwide, population-based case-control study. *PLoS ONE* http://dx.doi.org/10.1371/journal.pone.0176549 (2017)

## RISK FACTORS

### Susceptibility allele identified in RHD

A genome-wide association study in 2,852 individuals from eight Oceanian countries — where rheumatic heart disease (RHD) is highly prevalent — identified a genetic polymorphism in the immunoglobulin heavy chain (IGH) locus that is associated with increased RHD susceptibility. This finding was consistent across distinct ancestral groups such as Melanesians, Polynesians and South Asians. The investigators found that each copy of the *IGHV4-61*02* allele conferred a 1.4-fold increased risk of RHD.

**ORIGINAL ARTICLE** Parks, T. *et al.* Association between a common immunoglobulin heavy chain allele and rheumatic heart disease risk in Oceania. *Nat. Commun.* http://dx.doi.org/10.1038/ncomms14946 (2017)

*Nature Reviews Rheumatology* | Published online 11 May 2017; doi:10.1038/nrrheum.2017.73

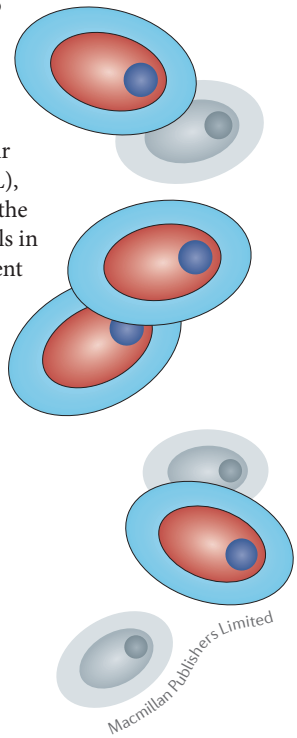
OSTEOARTHRITIS

# Removing old chondrocytes to combat disease

Senescent cells accumulate in a variety of tissues as the body ages, releasing a selection of pro-inflammatory factors that can contribute to tissue damage. In patients with osteoarthritis (OA), senescent cells exist in cartilage tissue, but their role in disease progression is poorly understood. Now, new research reveals a potential pathogenic role for senescent cells in OA that can be countered by depleting these cells from affected joints.

The researchers behind this new study utilized the p16-3MR transgenic mouse, which enables the luciferase-based tracking and ganciclovir-induced selective killing of cells expressing *Cdkn2a* (also known as *P16ink4a*), a gene upregulated in senescent cells. By monitoring *Cdkn2a*-expressing cells in mice that have undergone surgery on their anterior cruciate ligament (ACL), a model of post-traumatic OA, the research team observed that cells in the joint tissues became senescent following injury and secreted pro-inflammatory factors. Removing these senescent cells reduced pain and the accrual of cartilage erosion, as well as increasing cartilage development.

> “Removing these senescent cells reduced pain and the accrual of cartilage erosion”



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Following ACL surgery in young (10 week-old) p16-3MR mice, senescent cells accumulated in the superficial layer of cartilage and in the synovium and secreted a range of pro-inflammatory cytokines and matrix-degrading enzymes. Selective depletion of the *Cdkn2a*-expressing senescent cells by ganciclovir not only inhibited articular cartilage erosion and reduced the expression of pro-inflammatory factors, but also increased the production of type II collagen and aggrecan, suggestive of cartilage repair. However, depletion of senescent cells did not rescue the damage accrued in the subchondral bone.

By contrast, aged (19 month-old) p16-3MR mice exhibited more severe OA than their young counterparts following ACL surgery. Senescent cells were distributed throughout the cartilage in aged mice, rather than primarily in the superficial zone, and depletion of these cells reduced disease activity without creating the pro-regenerative environment observed in young mice.

The researchers replicated the ganciclovir-induced depletion of senescent cells in young p16-3MR and C57BL mice following ACL surgery using the senolytic agent UBX0101, which selectively kills senescent cells. Removal of senescent cells by UBX0101 mirrored the effects observed upon depletion of senescent cells in young p16-3MR mice by ganciclovir; articular cartilage erosion, pain and levels of pro-inflammatory factors were reduced and cartilage production increased, but subchondral bone was unaffected.

“We are now looking more into the impact of these cells in older animals and the impact of trauma and age. Also, we are looking at how these cells [recruit] immune cells to the area of damage and the role of these immune cells in disease progression,” states Jennifer Elisseeff, corresponding author on the study. Looking to the future, the researchers also hope to determine the optimal drug and dosage for selectively clearing senescent cells from the cartilage of patients with OA.

*Joanna Collison*

**ORIGINAL ARTICLE** Jeon, O. H. *et al.* Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat. Med.* http://dx.doi.org/10.1038/nm.4324 (2017)
**FURTHER READING** Loeser, R. F. *et al.* Ageing and the pathogenesis of osteoarthritis. *Nat. Rev. Rheumatol.* **12**, 412–420 (2016)

*Nature Reviews Rheumatology* | Published online 1 Jun 2017; doi:10.1038/nrrheum.2017.89

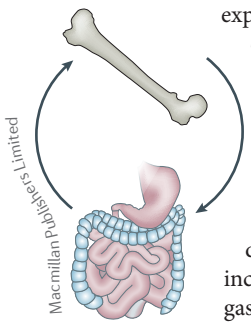
SPONDYLOARTHROPATHIES

# Gut–bone crosstalk in HLA-B27 rats

> Treatment with broad-spectrum antibiotics … reduced the number of CD43$^{lo}$ monocytes

HLA-B27, a known genetic risk factor for ankylosing spondylitis, is associated with spondyloarthritis-like symptoms in HLA-B27 transgenic rats that begin in the gut and spread to the joints. New research is beginning to unravel the links between gut inflammation and arthritis in this model of ankylosing spondylitis, with the microbiota thought to be pivotal in controlling disease progression.

"It is now appreciated that dysbiosis and gastrointestinal inflammation are key components of HLA-B27$^{+}$ spondyloarthropathies, but we still do not understand how the various factors initiate or contribute towards the multisystemic inflammatory-driven pathology," explains Carl Goodyear, corresponding author on the new study. "Human HLA-B27 transgenic rats display a plethora of the pathological aspects of the spondyloarthropathies, including dysbiosis, gastrointestinal inflammation and skeletal changes. They represent an excellent model for investigating the interaction and/or contribution of the various biological systems," he continues.




Previous studies revealed that germ-free HLA-B27 transgenic rats do not develop gut or joint pathologies, indicating a clear association between the gut microbiota and pathogenesis. Taking this concept forward, Goodyear and colleagues found that reducing gut inflammation in HLA-B27 transgenic rats by treating them with broad-spectrum antibiotics affected circulating levels of pro-inflammatory cytokines, chemokines and osteoclast precursor cells.

HLA-B27 transgenic rats had increased numbers of CD43$^{lo}$ conventional monocytes in their blood and bone marrow compared with wildtype and HLA-B7 transgenic rats. These CD43$^{lo}$ monocytes express CC-chemokine receptor 2 (CCR2), which is involved in migration from the bone marrow to the bloodstream, and represent the main population of osteoclast precursors. Treatment with broad-spectrum antibiotics not only reduced gut inflammation in these rats, but also reduced the number of CD43$^{lo}$ monocytes in the bone marrow and bloodstream to levels comparable with wildtype animals. High levels of CCL2, the ligand for CCR2, and IL-1α in the blood of HLA-B27 transgenic rats were also reduced by antibiotic treatment.

"The most significant finding of our study is the direct link between the gastrointestinal environment and how it moulds the central and peripheral myeloid compartment, which can contribute to the additional pathological aspects associated with spondyloarthropathies. Importantly, this includes controlling cellular distribution, migration, and differentiation," states Goodyear.

*Joanna Collison*

**ORIGINAL ARTICLE** Ansalone, C. *et al.* Gut inflammation in HLA-B27 transgenic rats alters the monocyte compartment and its osteoclastogenic potential. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40154 (2017)
**FURTHER READING** Ranganathan, V. *et al.* Pathogenesis of ankylosing spondylitis — recent advances and future directions. *Nat. Rev. Rheumatol.* **13**, 359–367 (2017)

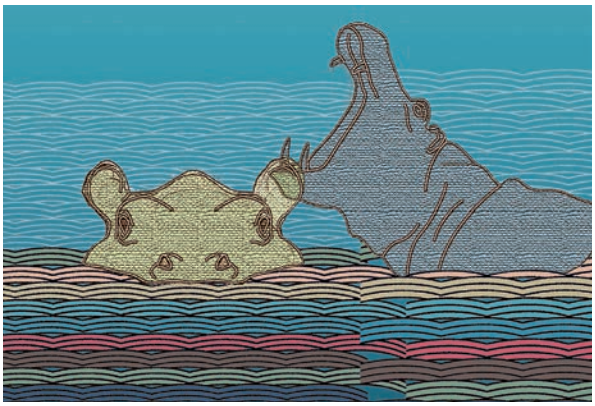
*Nature Reviews Rheumatology* | Published online 25 May 2017; doi:10.1038/nrrheum.2017.82

IMMUNOLOGY

# Hippo signalling influences T cell fate

> TAZ...is required for the differentiation of ... $T_H17$ cells, but inhibits the differentiation of ... $T_{reg}$ cells

The Hippo signalling pathway is primarily known for its roles in tissue homeostasis, organ size regulation and tumour suppression. However, emerging evidence implicates key components of this pathway in T cell development and function. New research published in *Nature Immunology* now reveals that Hippo signalling is an important regulator of the reciprocal differentiation of T helper 17 ($T_H17$) cells and regulatory T ($T_{reg}$) cells, two T cell subsets with prominent roles in autoimmunity. Geng *et al.* show that a downstream effector of the Hippo signalling pathway, the transcriptional coactivator TAZ (also known as WW domain-containing transcription regulator protein 1), is required for the differentiation of inflammatory $T_H17$ cells, but inhibits the differentiation of immunosuppressive $T_{reg}$ cells.



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TAZ expression was substantially enriched in $T_H17$ cells, and expression of the gene encoding TAZ was higher in memory $CD4^+$ T cells isolated from patients with rheumatoid arthritis, Sjögren syndrome or inflammatory bowel disease than in cells from healthy individuals. Conversely, TAZ deficiency protected mice from $T_H17$ cell-driven autoimmune diseases (experimental autoimmune encephalomyelitis and a T cell transfer model of colitis). Mechanistically, TAZ promoted $T_H17$ differentiation and function by directly binding to and activating RORγt (considered to be the master transcriptional regulator of $T_H17$ cells) and blocking the activity of FOXP3 (the $T_{reg}$ cell master regulator, which has an inhibitory effect on RORγt). TAZ also impaired $T_{reg}$ cell development by disrupting the acetylation of FOXP3 by the histone acetyltransferase Tip60 (also known as KAT5), thus targeting FOXP3 for proteasomal degradation.

When Hippo signalling is inactivated, TAZ translocates to the nucleus, where it binds to and activates TEAD family transcription factors, leading to transcription of genes related to cell proliferation. Geng *et al.* demonstrated that $T_{reg}$ cell-polarizing conditions induced high levels of expression of the transcription factor TEAD1, which disrupted the interaction of TAZ with RORγt, FOXP3 and Tip60 by binding TAZ with higher affinity, thereby promoting $T_{reg}$ cell differentiation.

Notably, knockout of TAZ or overexpression of TEAD1 induced $T_{reg}$ cell differentiation, whereas expression of a transgene encoding TAZ or disruption of Hippo signalling promoted $T_H17$ cell differentiation. Together, the findings highlight the critical role of Hippo signalling in T cell homeostasis.

*Sarah Onuora*

**ORIGINAL ARTICLE** Geng, J. *et al.* The transcriptional coactivator TAZ regulates reciprocal differentiation of $T_H17$ cells and $T_{reg}$ cells. *Nat. Immunol.* http://dx.doi.org/10.1038/ni.3748 (2017)

*Nature Reviews Rheumatology* | Published online 8 Jun 2017; doi:10.1038/nrrheum.2017.94

SPONDYLOARTHROPATHIES

# Targeting IL-17 in refractory PsA

According to new findings from the SPIRIT-P2 phase III clinical trial, ixekizumab is superior to placebo treatment in improving the signs and symptoms of psoriatic arthritis (PsA) in patients who have previously shown an inadequate response to TNF inhibition. Ixekizumab treatment was associated with improvements in patient-reported physical and mental outcomes, and with the resolution of dactylitis in this difficult-to-treat patient group.

Ben Gingell/Alamy Stock Photo

The term 'inadequate responders' refers to those patients who are either refractory to TNF inhibitor therapy, have demonstrated loss of efficacy of therapy, or are intolerant to TNF inhibitors. Such patients are generally more difficult to treat than biologic-naive patients, with the current alternative treatment options having lower levels of efficacy in inadequate responders. Ixekizumab selectively targets IL-17A, a pro-inflammatory cytokine thought to be involved in the pathogenesis of PsA, and is hence a promising alternative to TNF inhibition.

In this study, patients with active PsA who have had inadequate response to TNF inhibition were recruited from 109 centres across multiple countries and randomly assigned to receive either placebo (n = 118), 80 mg ixekizumab every 4 weeks (n = 122) or 80 mg ixekizumab every 2 weeks (n = 123), following an initial starting dose of 160 mg ixekizumab. Significantly more patients achieved the primary endpoint of at least 20% improvement in the ACR response criteria (ACR-20) at week 24 in either ixekizumab treatment group compared with the placebo group ($P$<0.0001).

> “Targeting IL-17A represent an alternative strategy to TNF inhibition in patients with active PsA”

Consistent with the role of IL-17A in the immune response to extracellular pathogens, a higher proportion of patients receiving ixekizumab treatment reported *Candida* infection than in the placebo group. Significantly more patients in the ixekizumab treatment group also reported mild-to-moderate injection site reactions. However neither of these adverse events were generally associated with study discontinuation.

These findings are consistent with previous studies of this IL-17A inhibitor, and support the role of IL-17A in PsA pathogenesis. Targeting IL-17A could represent an alternative strategy to TNF inhibition in patients with active PsA.

*Jessica McHugh*

**ORIGINAL ARTICLE** Nash, P. *et al.* Ixekizumab for the treatment of patients with active psoriatic arthritis and an inadequate response to tumour necrosis factor inhibitors: results from the 24-week randomised, double-blind, placebo-controlled period of the SPIRIT-P2 phase 3 trial. *Lancet* http://dx.doi.org/10.1016/S0140-6736(17)31429-0 (2017)
**FURTHER READING** Lubberts, E. The IL-23–IL-17 axis in inflammatory arthritis. *Nat. Rev. Rheumatol.* **11**, 415–429 (2015).

*Nature Reviews Rheumatology* | Published online 1 Jun 2017; doi:10.1038/nrrheum.2017.90

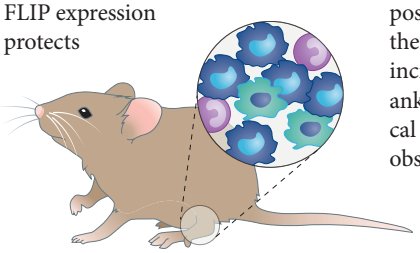
EXPERIMENTAL ARTHRITIS

# FLIPping the switch on macrophages

> the number of F4/80$^{hi}$ macrophages and the level of expression of F4/80 inversely correlated with joint swelling

Reducing the expression of anti-apoptosis molecule FLICE-like inhibitory protein (FLIP, also known as CASP8 and FADD-like apoptosis regulator) in macrophages could protect against inflammatory arthritis, according to new findings published in *Arthritis & Rheumatology*. Deleting FLIP in macrophages exacerbated the early stages of serum transfer-induced arthritis in mice, but attenuated the later peak and resolution stages. "Suppression of FLIP in macrophages might be an effective strategy to increase anti-inflammatory macrophages and to treat inflammatory conditions, such as rheumatoid arthritis," states corresponding author Richard Pope.

Previous work by Pope's group demonstrated that FLIP expression protects macrophages from Fas ligand-mediated apoptosis, leading to the hypothesis that deletion of FLIP in myeloid cells would reduce the number of synovial tissue macrophages by increasing apoptosis of these cells, thereby protecting against arthritis. To investigate this hypothesis, Pope and colleagues generated mice that do not express FLIP in their myeloid cells (Flip$^{f/f}$LysM$^{c/+}$ mice). The total numbers of macrophages in the joints were unchanged in these mice compared with their littermate controls, and 9 days after arthritis induction, they showed similar levels of apoptosis and necrosis in synovial macrophages in their ankles compared with their littermate controls.



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Flip$^{f/f}$LysM$^{c/+}$ mice had modestly increased joint swelling at 4 days post-injection compared with their littermate controls, as well as increased inflammation around the ankle joints as assessed by histological analysis. However, the reverse was observed at 9 days following arthritis induction. Surprisingly, the number of resident macrophages in the synovium was increased at day 9, corresponding to an increase in a population of F4/80$^{hi}$ macrophages. These macrophages had an M2-like phenotype, with increased expression of *IL10* and *RETNLA* mRNA and decreased expression of *NOS2* mRNA compared with F4/80$^{lo}$ macrophages. Furthermore, the number of F4/80$^{hi}$ macrophages and the level of expression of F4/80 inversely correlated with joint swelling.

Pope and colleagues speculate that the Flip$^{f/f}$LysM$^{c/+}$ mice had improved resolution of arthritis due to these anti-inflammatory macrophages, and that FLIP suppresses the transition of monocytes to suppressive macrophages. "In future studies we will examine the mechanism(s) by which the level of FLIP regulates macrophage polarization," concludes Pope.

*Jessica McHugh*

**ORIGINAL ARTICLE** Huang, Q. Q. *et al.* Increased F4/80$^{hi}$ macrophages is associated with suppression of serum transfer induced arthritis in mice with Flip reduced in myeloid cells. *Arthritis Rheumatol.* http://dx.doi.org/10.1002/art.40151 (2017)

PHARMACOTHERAPY

# Biosimilar switching — "To set a form upon desired change"

*Jonathan Kay and Kevin L. Winthrop*

The highly anticipated NOR-SWITCH trial results provide valuable information for patients and physicians concerned about the effects of switching between a biologic agent and a biosimilar product. However, the possibility of frequent switches, potentially involving more than one biosimilar, raises more questions.

*Refers to* Jørgensen, K. K. *et al.* Switching from originator infliximab to biosimilar CT-P13 compared with maintained treatment with originator infliximab (NOR-SWITCH): a 52-week, randomised, double-blind, non-inferiority trial. *Lancet* http://dx.doi.org/10.1016/S0140-6736(17)30068-5 (2017)

With biosimilars now available to treat patients with rheumatologic diseases, the question remains as to whether patients whose disease is well-controlled by treatment with a bio-originator can be transitioned to its biosimilar without a loss of efficacy or an increased risk of adverse events. To address this concern, the Norwegian government funded the NOR-SWITCH study to assess the effect of transitioning from the bio-originator infliximab (Remicade; Janssen Biotech) to the biosimilar infliximab CT-P13 (Remsima, Celltrion; Inflectra, Pfizer) on efficacy and safety in the various indications for which both therapies have been approved. Data from this first 'real-world' double-blind, randomized controlled trial of transitioning from a bio-originator to its biosimilar have been eagerly awaited, both by proponents of biosimilars looking forward to positive findings and sceptics concerned that the findings might be applied inappropriately to support policies that are financially advantageous to payers but not in the best interest of patients. Jørgensen and colleagues now have published the results of this clinical trial in *The Lancet*[1].

NOR-SWITCH was a 52-week double-blind, non-inferiority, phase IV clinical trial that randomly allocated 481 patients with Crohn's disease ($n = 155$), ulcerative colitis ($n = 93$), spondyloarthritis ($n = 91$), rheumatoid arthritis ($n = 77$), psoriasis ($n = 35$) or psoriatic arthritis ($n = 30$), each of whom had been on stable treatment with the bio-originator infliximab for at least 6 months, to either continue treatment with the bio-originator or transition to the biosimilar infliximab CT-P13 (REF. 1) (FIG. 1). The primary endpoint was disease worsening by week 52, as determined by disease-specific composite measures and/or agreement between the investigator and the patient that increased disease activity necessitated a "major change in treatment" (REF. 1). This study demonstrated non-inferiority of transitioning from the bio-originator to the biosimilar, as compared with continuing treatment with the bio-originator, for the aggregate of enrolled patients with various diseases.

The chosen non-inferiority margin of 15% was based on the equivalence margin of 15% used in the phase III PLANETRA study, in which CT-P13 was compared to bio-originator infliximab for the treatment of rheumatoid arthritis with the primary end point of an ACR20 response at 30 weeks[2]. However, in the NOR-SWITCH study this specific non-inferiority margin was arbitrarily extrapolated to a heterogeneous mix of disease-specific composite measures. Ideally, non-inferiority margins would have been derived individually for each disease-specific outcome measure from the difference between active drug and placebo in a meta-analysis of placebo-controlled clinical trials of the bio-originator in that disease, and then combined, based upon the anticipated number of subjects with each disease that were to be enrolled[3]. However, enrolment in NOR-SWITCH was competitive and not stratified by diagnosis, precluding the prediction of the number of patients with each disease that would be enrolled and the calculation of a non-inferiority margin appropriate to the entire patient population. Thus, the relevance of the chosen non-inferiority margin to all patients enrolled, only 16% of whom had rheumatoid arthritis, is uncertain.

NOR-SWITCH was not powered to compare the two treatment strategies in patients with any one disease. In all but the spondyloarthritis group, the lower end of the 95% confidence intervals for disease worsening in each individual disease group crossed the non-inferiority margin of 15%. However, the lower end of the 95% confidence intervals for disease worsening in the combined group of all six diagnoses was −12.7% and fell within the predefined non-inferiority margin. Similar proportions of patients in each group developed treatment-emergent adverse events (TEAEs), serious adverse events and TEAEs

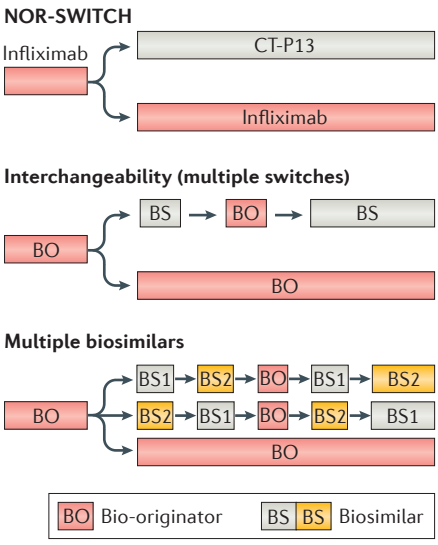


Figure 1 | **Clinical trials are needed to explore the effects of switching repeatedly between a bio-originator and its biosimilar or between multiple biosimilars.** The NOR-SWITCH study evaluated the transition from infliximab to its biosimilar CT-P13.

resulting in discontinuation of the study drug; trough drug concentrations and the incidence of neutralizing anti-drug antibodies were also similar in the two groups. Thus, the results of the NOR-SWITCH study support the practice of transitioning patients with stable disease activity from bio-originator infliximab to CT-P13 and provide further reassurance regarding the use of approved biosimilars. However, the results of NOR-SWITCH cannot yet be generalized to other biopharmaceuticals and their biosimilars or to repeated back-and-forth switching between bio-originator infliximab and the biosimilar CT-P13, although the high degree of similarity between a biosimilar and its bio-originator makes it unlikely that such frequent switching would diminish efficacy or increase the risk of adverse events or immunogenicity.

To support the practice of switching back-and-forth repeatedly between a bio-originator and its biosimilar, a clinical trial must include multiple switches (FIG. 1), as in the design for a prospective, controlled switching study proposed in January 2017 by the FDA[4]. The randomized, controlled phase III clinical trial (EGALITY) that compared the etanercept biosimilar GP2015 (Erelzi; Sandoz) to bio-originator etanercept (Enbrel; Amgen and Pfizer) in patients with active plaque psoriasis incorporated three transitions between the two products, each of 6 weeks' duration, after assessment of the primary endpoint at 12 weeks[5]. The incidence of treatment-related TEAEs, serious adverse events and study discontinuation due to TEAEs was similar among patients who switched back-and-forth between the bio-originator and the biosimilar and among those who continued either the bio-originator or the biosimilar throughout the study. Nevertheless, when multiple biosimilars of a given bio-originator become available, it will be virtually impossible to reproduce in a clinical trial the variety of switches that will occur in clinical practice.

The designation of 'interchangeability' is an issue faced primarily in North America. The Biologics Price Competition and Innovation Act of 2009 defines an interchangeable biosimilar as one that "may be substituted for the reference product without the intervention of the healthcare provider who prescribed the reference product" (REF. 6). In Canada, interchangeability is determined by provincial pharmacy boards rather than by Health Canada[7]. In the European Union, the European Medicines Agency does not have the authority to designate a biosimilar as being interchangeable; rather, this judgment must be made by regulatory agencies in each member state[8]. In France, a pharmacist can substitute a prescribed biopharmaceutical with a biosimilar for patients who are naive to the bio-originator, as long as the prescribing health-care provider has not indicated that such substitution is prohibited[9]. However, in most other member states of the European Union, although use of a biosimilar is encouraged for treatment-naive patients, automatic substitution of a biosimilar for the bio-originator is prohibited.

Nonetheless, 'nonmedical switching' from a bio-originator to its biosimilar might still occur, even without a designation of interchangeability, when a payer mandates substitution of the biosimilar for its bio-originator for financial reasons. This is the case in Norway[1], where all patients initiating treatment with infliximab must be administered the lower-cost biosimilar, and in Denmark[10], where the government has mandated that all patients treated with infliximab be transitioned to the biosimilar. When nonmedical switching takes place, the prescribing health-care provider is required to authorize the substitution before a pharmacist can dispense the alternative drug. Because of economic considerations, payers in the USA now mandate nonmedical switching between different drugs in a given class, such as TNF inhibitors, and probably will encourage nonmedical switching from bio-originators to their biosimilars if the cost of the biosimilar is lower.

Taking into consideration the subtle differences in structure between a bio-originator and its biosimilar and the limited amount of clinical data that is acquired before biosimilar approval, randomized, controlled clinical trials of transitioning from a bio-originator to its biosimilar, such as NOR-SWITCH, are necessary to reassure patients and health-care providers that biosimilars will perform similarly to their reference products. However, these studies must be designed so that the chosen non-inferiority margin is relevant to not only one, but all of the diseases studied. Regardless, long-term safety data must be collected after approval of the biosimilar to ensure that its long-term safety profile is similar to that of its bio-originator.

The NOR-SWITCH study serves as a prototype for future studies that should be conducted to demonstrate the efficacy and safety of transitioning from the bio-originator to other biosimilars. Although the results of NOR-SWITCH might assuage potential concerns regarding 'switching' patients once from bio-originator infliximab to the infliximab biosimilar CT-P13, uncertainty remains regarding the long-term safety and efficacy of multiple switches back-and-forth between a bio-originator and its biosimilar or among multiple biosimilars. These concerns will need to be addressed in clinical trials using other study designs or by assessing real-world data collected in clinical practice settings and maintained in registers.

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1. Jørgensen, K. K. *et al.* Switching from originator infliximab to biosimilar CT-P13 compared to maintained treatment with originator infliximab (NOR-SWITCH): a 52-week randomised double-blind non-inferiority trial. *Lancet* http://dx.doi.org/10.1016/S0140-6736(17)30068-5 (2017).
2. Yoo, D. H. *et al.* A randomised, double-blind, parallel-group study to demonstrate equivalence in efficacy and safety of CT-P13 compared with innovator infliximab when coadministered with methotrexate in patients with active rheumatoid arthritis: the PLANETRA study. *Ann. Rheum. Dis.* **72**, 1613–1620 (2013).
3. Kay, J. & Isaacs, J. D. Clinical trials of biosimilars should become more similar. *Ann. Rheum. Dis.* **76**, 4–6 (2017).
4. US Food & Drug Administration. Guidance for industry: considerations in demonstrating interchangeability with a reference product, draft guidance. *FDA* http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM537135 (2017).
5. Griffiths, C. E. *et al.* The EGALITY study: a confirmatory, randomised, double-blind study comparing the efficacy, safety and immunogenicity of GP2015, a proposed etanercept biosimilar, versus the originator product in patients with moderate to severe chronic plaque-type psoriasis. *Br. J. Dermatol.* **176**, 928–938 (2017).
6. Biologics Price Competition and Innovation Act of 2009, 119 United States Code, §7001-7003, https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/UCM216146.pdf (2010).
7. Klein, A. V., Wang, J. & Bedford, P. Subsequent entry biologics (biosimilars) in Canada: approaches to interchangeability and the extrapolation of indications and uses. *GaBI J.* **3**, 150–154 (2014).
8. Thimmaraju, P. K., Rakshambikai, R., Farista, R. & Juluru, K. Legislations on biosimilar interchangeability in the US and EU — developments far from visibility. *GaBI Online* http://www.gabionline.net/Sponsored-Articles/Legislations-on-biosimilar-interchangeability-in-the-US-and-EU-developments-far-from-visibility (2015).
9. GaBI Online — Generics and Biosimilars Initiative. France to allow biosimilars substitution. *GaBI Online* http://www.gabionline.net/Policies-Legislation/France-to-allow-biosimilars-substitution (2014).
10. Lunddahl, B. Pharmacovigilance on biologicals and biosimilars: a Danish perspective. *GaBI J.* **5**, 123–124 (2016).

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The title of this piece quotes Sonnet 89 by William Shakespeare.

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OSTEOARTHRITIS

# Nerve ablation — a new treatment for OA pain?

*David A. Walsh*

Pain in osteoarthritis (OA) can be resistant to the medical and surgical treatments currently in use. Local denervation of the arthritic joint could offer a new approach to relieve OA pain, if the results of a new cryoneurolysis clinical trial are confirmed.

*Refers to* Radnovich, R. *et al.* Cryoneurolysis to treat the pain and symptoms of knee osteoarthritis: a multicenter, randomized, double-blind, sham-controlled trial. *Osteoarthritis Cartilage* http://dx.doi.org/10.1016/j.joca.2017.03.006 (2017)

Osteoarthritis (OA) is a common disease for which current analgesic treatments are, for many patients, inadequate, owing to either low efficacy or the risk of adverse events. OA therefore represents a huge burden on individuals, society and healthcare systems. OA pain results from multiple complex mechanisms, both within the joint and in the central nervous system. In a new study, Radnovich *et al.*[1] report that ablation of a sensory nerve innervating the knee joint (the infrapatellar branch of the saphenous nerve) through cryoneurolysis can reduce pain in patients with knee OA.

Pain on joint movement underlines the importance of biomechanical factors in mediating OA pain. Reducing sensory input from the joint can improve pain, even when central augmentation is present[2]. Clinical trials of antibodies against β-nerve growth factor (β-NGF)[3] have highlighted the contribution of peripheral sensitization to OA pain; β-NGF is generated by inflamed tissues, including osteoarthritic synovium and subchondral bone, increasing the sensitivity of peripheral nerves and thereby exacerbating pain[4]. Other pharmacological treatments targeting the OA joint, including intra-articular injections of glucocorticoids or topical NSAIDs, can also reduce OA pain.

Joint replacement surgery can substantially reduce OA knee pain in people with advanced structural disease, probably by shielding nerves in the subchondral bone from mechanical and biochemical stimuli in the joint. The prosthesis replaces a function fulfilled in the normal joint by an intact articular cartilage and subchondral bone plate. An alternative approach to preventing nociceptive transmission from the joint might be to disrupt the afferent nerves, for example by cryoneurolysis[1] (that is, 'freezing' of the target nerve) or radiofrequency ablation[5], techniques that are already well established in other areas of pain medicine.

Health care for OA pain has largely been provided through physiotherapy, medication or surgery. Nerve blockade, if successful, could allow the expansion to OA of treatment modalities that are being used in other forms of chronic pain, including lower back pain[6]. Radiofrequency denervation of the medial branches of spinal nerve posterior rami (generally referred to as 'medial branch blocks') has evidence of analgesic efficacy from multiple clinical trials for the relief of lower back pain thought to originate from the facet joints. Evidence from these trials led the UK National Institute for Health and Care Excellence (NICE) to recommend radiofrequency denervation for the treatment of selected cases of lower back pain[6]. Might similar approaches also be successful for knee OA?

Radnovich *et al.*[1] reported the results of a randomized sham-controlled trial of ablation of the infrapatellar branch of the saphenous nerve in 180 patients with knee OA pain. The authors reported greater improvements in pain scores at day 30 in individuals who received active treatment compared with sham-treated individuals. The infrapatellar branch of the saphenous nerve innervates structures in the anterior compartment of the knee, but is only one part of the complex innervation of the knee. Pain originating from the tibiofemoral joints or subchondral bone might be expected to respond less well to a single nerve lesion. Appropriately, Radnovich *et al.* selected patients with pain on climbing stairs or pain on rising from a seated position, activities that stress the patellofemoral joint. For this patient group, the benefits of cryoneurolysis were comparable to those of other available nonsurgical treatments[1]. However, cost-effectiveness analysis is not available to help determine where denervation might fit within existing treatment pathways, and generalization of the findings of this study to patients with tibiofemoral pain might not be justified.

> “Cryoneurolysis induces Wallerian degeneration of axons without killing the nerve's cell body in the dorsal root ganglion”

OA knee pain is typically persistent, such that long-term treatment might be required. Cryoneurolysis induces Wallerian degeneration of axons without killing the nerve's cell body in the dorsal root ganglion. Nerves therefore regenerate over time following cyroneurolysis. The data presented by Radnovich *et al.* show clinically important benefits of treatment with cryoneurolysis compared with a sham procedure at 30 days[1]. However, beyond 30 days the mean effects were no longer at the threshold for clinical importance and lost statistical significance after 90 days. The authors suggest that patients could undergo repeated cryoneurolysis as needed, although this repetition would inevitably increase treatment costs. Moreover, cryoneurolysis is used to generate neuropathic pain in animal models[7] and the current study was not powered to exclude clinically important adverse events of single or repeated nerve injury.

The results presented by Radnovich *et al.*[1] are very encouraging, but do require independent replication. Subsequent to NICE's

review of the literature for radiofrequency denervation in lower back pain, further randomized trials have been published with apparently contradictory results[8]. Generalizability cannot be assumed from the results of a single study, even if well-conducted and involving multiple centres. Small variations in the randomization process can combine to influence trial outcome; for example, a higher proportion of patients who exhibit severe structural changes, high pain scores or other unmeasured characteristics could be assigned to one group (such as the sham group) than another. Selective publication of positive results, which might benefit the interests of journals or academic, professional or commercial groups, is well recognized; studies of cryoneurolysis that are truly independent of those who might benefit financially or professionally from a positive trial will be difficult to achieve. However, encouraging initial findings should not be ignored.

Contextual effects, as demonstrated in placebo or sham groups, are often greater than the additional benefit provided by the specific intervention for OA pain[9]. In this new trial[1], sham treatment offered 60–70% of the clinical benefit of cryoneurolyisis, even during the first 30 days following treatment. This finding reflects both the potential importance of treatment context as well as the rather limited additional benefit derived from specific treatments. Non-blinded trials and cohort studies almost always overestimate specific treatment effects, but interventions such as cryoneurolysis are notoriously difficult to fully blind. Successful cryoneurolysis, for example, causes inflammation and swelling, and should produce sustained cutaneous sensory disturbance given the functional anatomy of the infrapatellar branch of the saphenous nerve.

> “In light of positive preliminary data, further research might lead to cryoneurolysis entering routine clinical practice”

Pain remains a major concern for patients with OA, and better, safer and more effective treatments that maintain quality of life into old age are urgently needed. Local denervation procedures have the potential to benefit people with arthritic pain, might avoid some of the systemic toxicity of parenteral medications and could reduce the need for arthroplasty. In light of positive preliminary data, further research might lead to cryoneurolysis entering routine clinical practice. Before then, however, patient subgroups who are likely to benefit from this treatment need to be better defined, effects of repeated or sequential treatments need to be established and the cost-effectiveness of this approach needs to be demonstrated.

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1. Radnovich, R. *et al.* Cryoneurolysis to treat the pain and symptoms of knee osteoarthritis: a multicenter, randomized, double-blind, sham-controlled trial. *Osteoarthritis Cartilage* http://dx.doi.org/10.1016/j.joca.2017.03.006 (2017).
2. Gwilym, S. E., Filippini, N., Douaud, G., Carr, A. J. & Tracey, I. Thalamic atrophy associated with painful osteoarthritis of the hip is reversible after arthroplasty: a longitudinal voxel-based morphometric study. *Arthritis Rheum.* **62**, 2930–2940 (2010).
3. Schnitzer, T. J. & Marks, J. A. A systematic review of the efficacy and general safety of antibodies to NGF in the treatment of OA of the hip or knee. *Osteoarthritis Cartilage* **23** (Suppl. 1), S8–S17 (2015).
4. Mapp, P. I. & Walsh, D. A. Mechanisms and targets of angiogenesis and nerve growth in osteoarthritis. *Nat. Rev. Rheumatol.* **8**, 390–398 (2012).
5. Bellini, M. & Barbieri, M. Cooled radiofrequency system relieves chronic knee osteoarthritis pain: the first case-series. *Anaesthesiol. Intensive Ther.* **47**, 30–33 (2015).
6. Ward, S. *et al. Low back pain and sciatica in over 16s: assessment and management. NICE guideline NG59* (National Institute for Health and Care Excellence, 2016).
7. Ju, H., Feng, Y., Gao, Z. & Yang, B. X. The potential role of nerve growth factor in cryoneurolysis-induced neuropathic pain in rats. *Cryobiology* **65**, 132–138 (2012).
8. van Tilburg, C. W. J., Stronks, D. L., Groeneweg, J. G. & Huygen, F. J. P. M. Randomised sham-controlled double-blind multicentre clinical trial to ascertain the effect of percutaneous radiofrequency treatment for lumbar facet joint pain. *Bone Joint J.* **98-B**, 1526–1533 (2016).
9. Zhang, W., Robertson, J., Jones, A. C., Dieppe, P. A. & Doherty, M. The placebo effect and its determinants in osteoarthritis: meta-analysis of randomised controlled trials. *Ann. Rheum. Dis.* **67**, 1716–1723 (2008).

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The author declares no competing interests.

OSTEOARTHRITIS

# You can rely on radiography when managing OA, but not too much!

*Ali Guermazi*

When considering the role of imaging in the management of symptomatic, peripheral joint osteoarthritis (OA) in clinical practice, clinicians should be aware that radiography has its drawbacks, and also consider that the use of advanced imaging techniques such as MRI should not be discouraged.

*Refers to* Sakellariou, G, *et al.* EULAR recommendations for the use of imaging in the clinical management of peripheral joint osteoarthritis. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210815 (2017)

In 2017 our esteemed colleagues from a EULAR task force published evidence-based recommendations for the use of imaging in the clinical management of symptomatic, peripheral joint osteoarthritis (OA)[1]. I read the paper with great enthusiasm and commend the authors for their excellent effort. However, I also wish to point out, from the view of a musculoskeletal radiologist, a few important issues regarding the limitations of conventional radiography, which the EULAR task force recommends as a tool for the clinical assessment of OA.

The EULAR task force recommends the use of imaging for initial assessment of OA only when the clinical presentation is atypical, and the routine use of imaging for clinical follow-up only when the patient shows unexpectedly rapid progression of symptoms or clinical characteristics. They also recommend the use of conventional radiography before any other imaging modality and emphasize the importance of using appropriate radiographic views for optimal detection of OA features. At the same time, the authors of the recommendations state that imaging features do not predict response to non-surgical treatment and that they do not recommend the use of imaging for this purpose[1].

For the assessment of patients who present to a clinician complaining of pain, which is a common occurrence, conventional radiography has important limitations. In the knee and hip joints, for example, radiography does not enable the visualization of tissues that can be the source of pain, such as the synovium, meniscus, bone marrow lesions and ligaments. Abnormalities in these soft tissue structures are common even when radiographs are normal, and many patients with knee or hip pain have no evidence of radiographic OA[2,3]. Osteophytes can be visualized by radiography, but are not known to be a cause of pain; similarly, radiography can be used to assess joint space width, which allows indirect evaluation of cartilage thickness, but cartilage itself is aneural and cannot be a direct cause of pain.

Compared with MRI findings, radiographic joint space narrowing is neither sensitive nor specific as a measure of OA disease progression[4,5]. Joint space narrowing results not only from cartilage thinning, but also from meniscal extrusion, and radiography cannot differentiate between the two[4]. Moreover, a substantial proportion of knees with cartilage loss will not be identified as such by radiography alone; a focal cartilage defect will not cause joint space narrowing and any cartilage loss in the posterior femoral condyle will not be detected because of the projectional nature of radiography[5,6]. For these reasons, imaging with MRI should not be denied just because the patient does not have an "unexpected rapid progression of symptoms or change in clinical characteristics" (the scenario in which the EULAR task force recommends imaging in follow-up)[1] or because they have a totally normal radiograph.

Additional limitations of radiography relate to difficulty with achieving adequate and reproducible patient positioning for assessment of joint space width. Even in clinical trials in which imaging acquisition protocols are rigorously standardized by use of a joint-fixation apparatus, patient positioning can be problematic[7]. With a slight change in knee angulation, apparent joint space width can vary substantially[7]. In the setting of routine clinical care, in which various radiography technicians (who are not necessarily rigorously 'trained' for reproducible joint positioning) acquire images at different institutions using different radiography equipment, this issue can become a major obstacle to precise and reproducible radiographic assessment of OA. For this reason, I agree with the EULAR task force that routine OA imaging follow-up based on radiography alone is not to be recommended.

The authors of the EULAR recommendations seem to approach OA as a disease that has no effective medical treatment, which is currently true: no efficacious disease-modifying OA drug has been established in the literature to date. However, research efforts to discover such a drug are underway worldwide and involve advanced imaging techniques, namely MRI[8]. The use of MRI in the setting of routine clinical practice should not be discouraged just because the evidence available to actively recommend the use of MRI for clinical management of OA is somewhat limited due to lack of correlation with MRI findings and patients' symptoms. I do note, however, that the EULAR task force produced a future research agenda to address this limited evidence[1].

If we consider the concept of OA as a disease with multiple phenotypes, the use of additional imaging tools such as ultrasonography and MRI makes more sense than using radiography alone. Recognition of the various phenotypes of OA is clinically relevant to the treatment of pain. As Bijlsma and colleagues[9] described, the 'pain' phenotype of OA is thought to be caused by inflammation, bone changes and aberrant pain perception, and possible medical interventions for this phenotype of OA include pain medication and anti-inflammatory drugs. Radiography is not helpful in assessing the efficacy of anti-inflammatory drugs because it cannot depict the severity of synovitis accurately, although it can show the presence of large effusions within the joint. Additional imaging with ultrasonography and MRI becomes

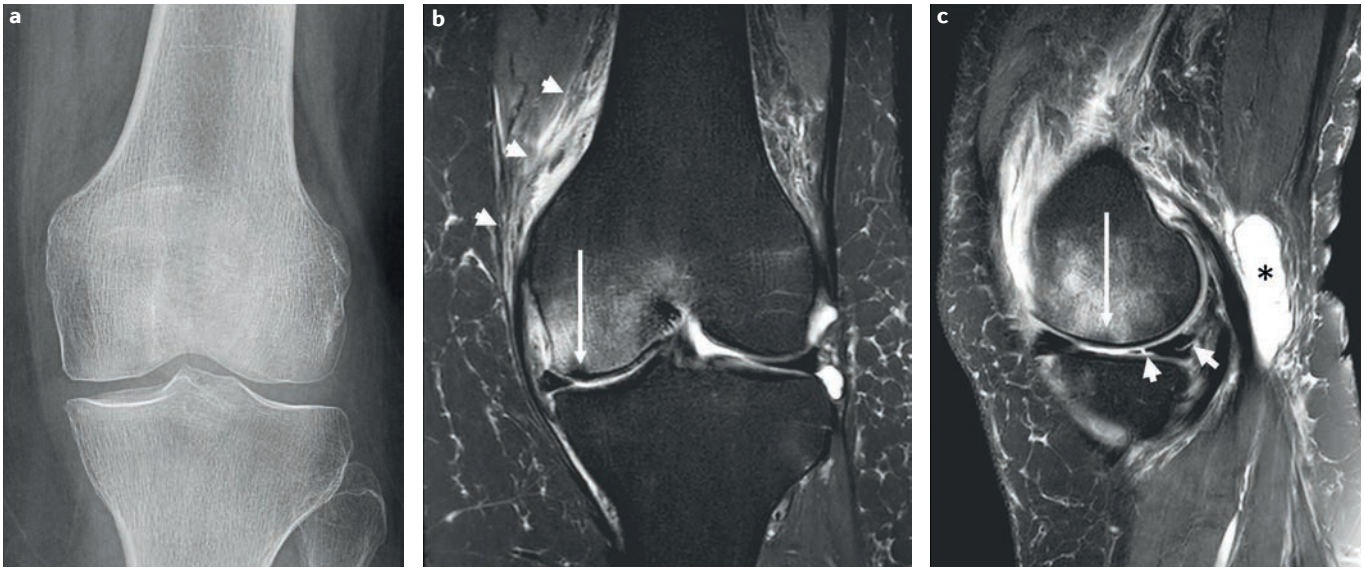


Figure 1 | **Imaging of a painful knee using radiography and MRI.** In a 77-year-old male with pain for the past 5 weeks, anteroposterior radiograph of the left knee (part **a**) showed normal tibiofemoral joints, whereas fat-suppressed intermediate-weighted coronal MRI (part **b**) and sagittal MRI (part **c**) demonstrated a subchondral insufficiency fracture of the medial femoral condyle (arrow) with extensive surrounding bone marrow oedema. Coronal MRI (part **b**) also showed medial meniscal extrusion and a characteristic pattern of intense soft-tissue oedema (arrowheads). The sagittal MRI (part **c**) demonstrated a complex tear of the posterior horn of the medial meniscus (arrowheads) and a large Baker's cyst (*).

essential in patients with a large joint effusion in the setting of routine clinical care to detect the presence of active inflammation[10].

One final point to raise is the issue of safety. When a patient presents with knee pain, for instance, an initial 'normal' radiograph should not deter the clinician from ordering additional imaging, so as not to miss radiographically occult non-OA pathology. As an example, MRI might reveal the presence of an occult subchondral insufficiency fracture in the femoral condyle (FIG. 1), femoral head, acetabulum or tibial plateau, which is a condition that requires urgent treatment because it can result in the collapse of the joint if left untreated.

In conclusion, while I congratulate our colleagues from the EULAR task force for their tremendous effort that culminated in recommendations for imaging in the routine clinical care of patients with OA, I would also like to emphasize that clinicians should not rely solely on radiography, but actively consider the use of additional imaging modalities, especially MRI, in the context of the management of peripheral joint OA.

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1. Sakellariou, G. *et al.* EULAR recommendations for the use of imaging in the clinical management of peripheral joint osteoarthritis. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210815 (2017).
2. Guermazi, A. *et al.* Prevalence of abnormalities in knees detected by MRI in adults without knee osteoarthritis: population based observational study (Framingham Osteoarthritis Study). *BMJ* **345**, e5339 (2012).
3. Kim, C. *et al.* Association of hip pain with radiographic evidence of hip osteoarthritis: diagnostic test study. *BMJ* **351**, h5983 (2015).
4. Hunter, D. J. *et al.* Change in joint space width: hyaline articular cartilage loss or alteration in meniscus? *Arthritis Rheum.* **54**, 2488–2495 (2006).
5. Amin, S. *et al.* The relationship between cartilage loss on magnetic resonance imaging and radiographic progression in men and women with knee osteoarthritis. *Arthritis Rheum.* **52**, 3152–3159 (2005).
6. Hayashi, D. *et al.* Pre-radiographic osteoarthritic changes are highly prevalent in the medial patella and medial posterior femur in older persons: Framingham OA Study. *Osteoarthritis Cartilage* **22**, 76–83 (2014).
7. Guermazi, A. *et al.* Why radiography should no longer be considered a surrogate outcome measure for longitudinal assessment of cartilage in knee osteoarthritis. *Arthritis Res. Ther.* **13**, 247 (2011).
8. Hunter, D. J. *et al.* OARSI clinical trials recommendations: knee imaging in clinical trials in osteoarthritis. *Osteoarthritis Cartilage* **23**, 698–715 (2015).
9. Bijlsma, J. W. *et al.* Osteoarthritis: an update with relevance for clinical practice. *Lancet* **377**, 2115–2126 (2011).
10. Hayashi, D. *et al.* Imaging of synovitis in osteoarthritis: current status and outlook. *Semin. Arthritis Rheum.* **41**, 116–130 (2011).

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RHEUMATOID ARTHRITIS

# Forward and reverse inheritance — the yin and the yang

*J. Lee Nelson and Nathalie C. Lambert*

The theory of Mendelian inheritance states that half our genes are maternal and half are paternal. This view is incomplete, as maternal–fetal exchange creates a legacy of non-native cells within an individual that can affect their health for better or worse, including contributing to their risk of developing autoimmune disease.

*Refers to* Cruz, G. I. *et al.* Increased risk of rheumatoid arthritis among mothers with children who carry DRB1 risk-associated alleles. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210662 (2017)

In common with other autoimmune diseases, rheumatoid arthritis (RA) has an increased prevalence in females and strong HLA-associated genetic risk factors. A 2017 study by Cruz *et al.*[1] reveals that the risk of a woman developing RA is influenced by the specific HLA alleles carried by any children born to her before disease onset. HLA molecules have a central role in the immune system, presenting foreign antigens and self-antigens as part of a network that, during health, is tolerant to normal self-antigens. The specific HLA molecules associated with risk of RA are well-defined, with the best known RA-risk factor being a series of similar sequence motifs at positions 70–74 of the HLA-DRβ1 chain (QKRAA, QRRAA or RRRAA), encoded by some allelic variants of *HLA-DRB1* (for example, **04:01*, **04:04*, **04:05*, **04:08*, **01:01*, **14:02* and **10:01*). These sequence motifs are known as the 'shared epitope'. Overall, Cruz *et al.*[1] found that the risk of developing RA was increased among women who had given birth to a child with an allele encoding the shared epitope (adjusted for the mother's status as a carrier of RA-risk alleles; OR 3.0, 95% CI 2.0–4.6).

Bidirectional exchange of cells between the mother and fetus during pregnancy creates a long-term legacy. A mother's cells persist in her progeny into adult life, and cells of fetal origin are present in women for many years following a pregnancy, potentially persisting for a lifetime (FIG. 1). These small populations of naturally occurring immigrant cells carry a complement of genes that differ from the individual who acquires them, a phenomenon known as microchimerism, and can confer benefits or risks to that individual's long-term health[2]. This potential for a child's genetic makeup to influence maternal health later in life can be considered akin to 'reverse inheritance'.

> Bidirectional exchange of cells between the mother and fetus during pregnancy creates a long-term legacy

Two previous studies provided evidence that women with RA who are negative for the shared epitope more frequently harbour shared epitope-positive microchimerism than healthy women[3,4]. When Cruz *et al.*[1] stratified patients according to their shared epitope status, shared epitope-positive mothers with RA were more likely to have had a shared epitope-positive child than matched healthy women. Surprisingly, the corresponding results were not statistically significant for shared epitope-negative mothers, although the number of shared epitope-negative mothers among cases was low (31 shared epitope-negative cases among the 170 total cases). Stratified analysis did, however, indicate that having a child or children who carry alleles encoding lysine at position 71, essentially representing *HLA-DRB1*04:01*, conferred a risk of developing RA in both risk-allele carrier and non-carrier mothers ($P = 0.01$ and $P = 0.001$, respectively).

These observations bring to mind an intriguing aspect of RA immunogenetics, namely that there is a hierarchy of RA-risk[5] depending on the HLA genotype of an individual, and that the three different shared epitope sequences are not equivalent. At the top of the hierarchy is *HLA-DRB1*04:01*, which confers risk irrespective of the second haplotype, whereas the other shared epitope sequences are most potent in specific combinations, a phenomenon referred to as compound heterozygosity. Consideration of this hierarchy provides a potentially analogous explanation for the observations of Cruz *et al.* Briefly, carrying a child with the strongest shared epitope sequence (QKRAA, *HLA-DRB1*04:01* with a lysine at position 71) will be sufficient to confer risk, but carrying a child with any of the other shared epitope sequences will only be sufficient to confer risk if they are present in the context of another shared epitope sequence. Lending support to this interpretation, Rak *et al.*[3] observed a higher occurrence of *HLA-DRB1*04*-positive microchimerism in *HLA-DRB1*01*-positive patients with RA than *HLA-DRB1*01*-positive microchimerism in *HLA-DRB1*04*-positive patients with RA. In this study[3], it was assumed that *HLA-DRB1*04* and *HLA-DRB1*01* microchimerism primarily consisted of shared epitope-encoding alleles. In a subsequent study, QKRAA and QRRAA microchimerism was directly demonstrated[4]. Neither study[3,4] distinguished the source of shared epitope-positive microchimerism.

Non-inherited maternal HLA alleles can also have long-lasting effects on her progeny. The sequence DERAA, encoded at the same amino acid positions as the shared epitope but by other *HLA-DRB1* alleles, is considered to be RA-protective in most studies. As discussed by Cruz *et al.*[1], a previous study in patients with RA showed a deficit of non-inherited maternal alleles encoding DERAA compared with non-inherited paternal alleles. In light of this evidence, the finding of an increased risk of RA among women with a child who had a DERAA-encoding allele was unexpected[1]. Although the explanation for this finding is unknown, one consideration is that the effect of maternal cells acquired during fetal life (when the immune

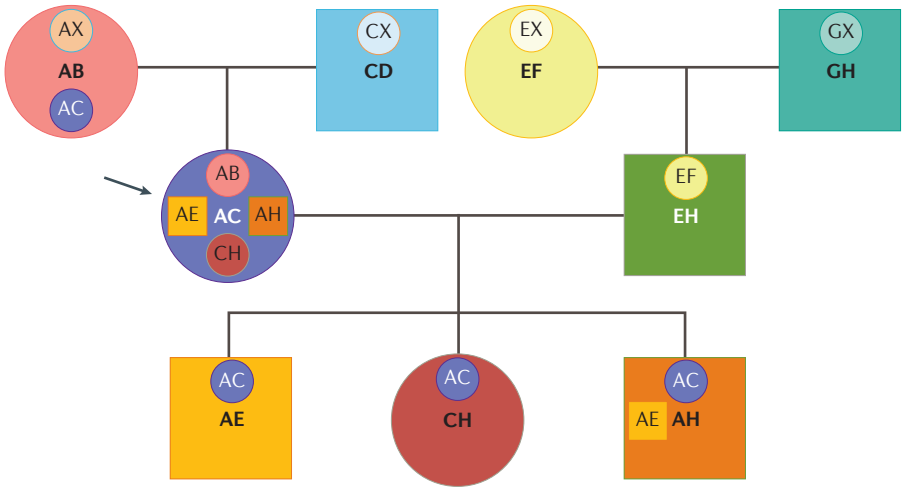


Figure 1 | **Forward and reverse inheritance from microchimerism of maternal and of fetal origin.** Maternal–fetal exchange creates a long-term legacy of a small number of cells in an individual that have a different genetic makeup, known as microchimerism. The individuals AC and EH in the middle generation harbour microchimerism from their mothers (AB and EF, respectively). In addition, AC can acquire cells from her children (AE, CH and AH) as a result of her own pregnancies. Other possible sources of microchimerism include a twin (including a vanished twin), pregnancies not resulting in a birth, and, potentially, previous microchimerism from maternal miscarriages or an older sibling that is carried with maternal cells to a later pregnancy (the latter illustrated as microchimerism from AE in AH).

system is developing) can be expected to differ from that of fetal cells acquired by an adult woman (when the immune system is mature). Functional studies have not yet addressed this subject, although other kinds of unexpected outcomes in the maternal–fetal relationship have been described. For example, children born to mothers with type I diabetes mellitus were less likely to develop the disease when islet-specific autoantibodies were present in cord blood than when they were absent, contrary to expectations[6]. Also, variable results were reported in studies that evaluated RA risk according to whether the non-inherited maternal HLA allele encoded a shared epitope sequence[7]. What is missing is a study that evaluates the effect of both the non-inherited maternal HLA allele and the paternally transmitted HLA allele of children born before disease onset in women with RA.

Another variable that could affect results is gravidity without parity. The majority of studies have found an overall decrease in RA risk in parous women, as compared with nulliparous women[8,9]. Both spontaneous and induced abortion can also result in microchimerism[2]; however, gravidity without parity seems to confer no protection against RA[8]. Other sources of microchimerism include a recognized or unrecognized twin and, potentially, previous microchimerism from an older sibling or maternal miscarriage, passed along with maternal cells to a child born later in the mother's life[2] (FIG. 1). Together, these microchimeric cells might be likened to a patchwork within the individual, creating increased genetic diversity in women and having variable effects depending on the source of the cells and the timing of acquisition, among other factors.

Naturally acquired microchimerism can be tissue-specific or haematopoietic, and harbouring multiple microchimeric 'grafts' across generations in women is of special interest, as highlighted by experimental studies[10]. Much can be learned from elucidating the long-term consequences of forward and reverse inheritance; in terms of both the benefits and the risks that can be accrued from our immigrant cell populations, acquired as a natural form of 'mini-gene transfer'.

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1. Cruz, G. I. *et al.* Increased risk of rheumatoid arthritis among mothers with children who carry DRB1 risk-associated alleles. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210662 (2017).
2. Nelson, J. L. The otherness of self: microchimerism in health and disease. *Trends Immunol.* **33**, 421–427 (2012).
3. Rak, J. M. *et al.* Transfer of shared epitope through microchimerism in women with rheumatoid arthritis. *Arthritis Rheum.* **60**, 73–80 (2009).
4. Yan, Z., Aydelotte, T., Gadi, V. K., Guthrie, K. A. & Nelson, J. L. Acquisition of the rheumatoid arthritis HLA shared epitope through naturally acquired microchimerism. *Arthritis Rheum.* **63**, 640–646 (2011).
5. Balandraud, N. *et al.* HLA-DRB1 genotypes and the risk of developing anti citrullinated protein antibody (ACPA) positive rheumatoid arthritis. *PLoS ONE* **8**, e64108 (2013).
6. Holm, B. C. *et al.* Evidence for immunological priming and increased frequency of $CD4^+$ $CD25^+$ cord blood T cells in children born to mothers with type 1 diabetes. *Clin. Exp. Immunol.* **146**, 493–502 (2006).
7. Guthrie, K. A., Tishkevich, N. R. & Nelson, J. L. Non-inherited maternal human leukocyte antigen alleles in susceptibility to familial rheumatoid arthritis. *Ann. Rheum. Dis.* **68**, 107–109 (2009).
8. Guthrie, K. A., Dugowson, C. E., Voigt, L. F., Koepsell, T. D. & Nelson, J. L. Does pregnancy provide vaccine-like protection against rheumatoid arthritis? *Arthritis Rheum.* **62**, 1842–1848 (2010).
9. Guthrie, K. A., Gammill, H. S., Madeleine, M. M., Dugowson, C. E. & Nelson, J. L. Parity and HLA alleles in risk of rheumatoid arthritis. *Chimerism* **2**, 11–15 (2011).
10. Kinder, J. M., Stelzer, I. A., Arck, P. C. & Way, S. S. Immunological implications of pregnancy-induced microchimerism. *Nat. Rev. Immunol.* http://dx.doi.org/10.1038/nri.2017.38 (2017).

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**Competing interests statement**
The authors declare no competing interests.

# The role of IL-6 in host defence against infections: immunobiology and clinical implications

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Abstract | IL-6 is a pleiotropic cytokine with broad-ranging effects within the integrated immune response. One of the roles of IL-6 is to support immunocompetence, defined as the ability of a host to respond to infections. Understanding the precise role of this cytokine in immunocompetence requires a critical appraisal of data derived from both preclinical and clinical studies. Primary immunodeficiency diseases involving IL-6 or its signalling pathways reveal that IL-6 is critical in the defence against numerous types of pathogens. Studies of IL-6 signalling in preclinical models reveal that selective inhibition of either classic IL-6 signalling or IL-6 trans-signalling has differential effects on the host response to different types of infections. Knowledge of such variation might inform bioengineering of new IL-6-targeting molecules and potentially enable modulation of their toxicity. Clinical studies of IL-6 inhibitors, mainly tocilizumab, reveal that their use is associated with an increased rate of both serious and opportunistic infections generally in the range observed with other non-IL-6 directed biologic therapies. Targeting IL-6 has several other important clinical implications related to diagnosis, management and prevention of infectious diseases.

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IL-6 is a pivotal cytokine in the integrated immune response and has been targeted in the treatment of numerous inflammatory diseases[1]. To date, the IL-6 receptor (IL-6R)-neutralizing antibody tocilizumab has been approved for the treatment of rheumatoid arthritis (RA) and juvenile inflammatory arthritis and the IL-6-neutralizing antibody siltuximab has been approved for use in multicentric Castelman disease, but several new agents targeting the IL-6 pathway are also in late-stage development, which has increased overall interest in this field[2]. Similar to other biologic agents, siltuximab and tocilizumab are associated with an increased incidence of serious infections[3], but data on whether these risks are comparatively increased versus other biologic agents are mixed. The role of IL-6 inhibition in the predisposition to opportunistic infections remains unclear.

In this Review, we examine the effects of targeting IL-6 activity on immunocompetence (the ability of the integrated immune system to defend the host from infections)[4]. A complete understanding of this subject requires a critical appraisal of the fundamental role of IL-6 in host defence against infectious diseases from both a preclinical and a clinical perspective. First, we review the basic biology of IL-6 and IL-6 signalling by analysing their role in genetically manipulated preclinical animal models; such models can yield insights into the potential for disruption of critical pathways and networks and their importance for immunocompetence. We also examine human primary immunodeficiency disorders that show disruption of IL-6 or its signalling. Second, and critically, we present analyses from clinical and epidemiologic studies examining rates and types of infections, including opportunistic infections, in patients receiving IL-6-targeting therapy, the majority of whom received tocilizumab. Finally, we examine the capacity of IL-6 to affect vaccine responses and the clinical response to infectious challenge during IL-6 suppression.

## Biology of IL-6

### *IL-6 signalling*

IL-6 binds to IL-6R, forming an IL-6–IL-6R complex. This complex thereafter associates with the signal-transducing IL-6R subunit β (also known as gp130), initiating intracellular signalling[1] (FIG. 1). This signal transduction within the cell mainly involves activation of the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway (mainly via STAT1 and STAT3), and the RAS-dependent mitogen-activated

**Key points**

- IL-6 is a pleotrophic cytokine with a central role in the integrated immune defence network against infections
- IL-6 can act via either classic or trans-signalling pathways, which have differential effects on immunocompetence
- Studies of genetically modified animal models suggest that IL-6 has a role in both the innate and adaptive immune responses that protect the host from a variety of infections
- Primary immunodeficiency diseases in which IL-6 has been affected either directly or indirectly also provide insights into the role of IL-6 in host defence, especially against bacterial and fungal pathogens
- Clinical data on IL-6-targeting drugs, largely derived from studies of tocilizumab, suggest that serious and opportunistic infections occur with a frequency similar to that seen with other non-IL-6-targeting biologic agents
- Neutralizing IL-6 might affect the clinical presentation of serious infections but has minimal effects on response to commonly administered vaccines

protein kinase (MAPK) signalling cascade. Activation of intracellular signalling via gp130 also initiates a negative feedback loop via suppressor of cytokine signalling 3 (SOCS3), eventually leading to termination of signalling.

In the classic IL-6 signalling pathway, IL-6 acts via membrane-bound IL-6R and subsequently interacts with membrane-bound gp130. Alternatively, IL-6 can bind to soluble IL-6R (sIL-6R), with the complex of IL-6 and sIL-6R then binding to membrane-bound gp130 in what is known as the trans-signalling pathway[1,5]. Interestingly, whereas all cells of the body express gp130, only a few cell types (that is, hepatocytes, some epithelial cells and some leukocytes) express IL-6R and can therefore respond to IL-6 directly. All other cells are dependent on IL-6 trans-signalling for their response to IL-6 (REFS 1,5). For an in-depth discussion of these two pathways, see previous review on IL-6 biology[2].

***Soluble gp130.*** Neither IL-6 nor IL-6R exhibits measurable affinity for gp130; however, as a complex, IL-6 and IL-6R can bind to and activate gp130, leading to dimerization of gp130 and intracellular signalling. Interestingly, a soluble form of gp130 is naturally found in the circulation at 400 ng/ml and is thought to act together with soluble IL-6R, which is also present in the circulation at 40–80 ng/ml, to buffer levels of circulating IL-6 (REFS 2,6). Soluble gp130 protein in the blood can thereby prevent IL-6, in combination with soluble IL-6R, from stimulating all gp130-expressing cells in the body[7]. A recombinant version of soluble gp130 (sgp130Fc), generated by fusing two soluble gp130 molecules to the Fc region of human IgG1, specifically inhibits IL-6 trans-signalling without affecting classic IL-6 signalling[8]. The sgp130Fc protein is the first drug candidate that differentially inhibits the proinflammatory activities of IL-6 without affecting the protective activities of this cytokine, and shows efficacy in the treatment of many preclinical models of human inflammatory diseases (TABLE 1). Successfully passing through phase I clinical trials without major adverse events, the sgp130Fc protein (olamkicept) is currently in phase II clinical trials in patients with inflammatory bowel disease[9]. Additional phase II clinical trials in patients with inflammatory bowel disease are planned for later this year.

***Viral IL-6.*** Research in the mid-1990s revealed that human herpes virus 8 (HHV8) encodes a protein with 25% amino acid identity to human IL-6 (REF. 10) and that this HHV8-derived protein, termed viral IL-6 (vIL-6), is associated with the development of Castleman disease[11]. vIL-6 activates the JAK–STAT intracellular signalling pathway[12] and induces proliferation of IL-6-dependent B cells without the need for membrane-bound or soluble IL-6R[13], indicating that vIL-6 directly binds gp130. Given that gp130 is ubiquitously expressed[14], it was speculated that vIL-6 could act on all cells in the human body[13]. From these findings, researchers set out to analyse the molecular basis for the receptor-independent binding of vIL-6 to gp130. The fact that vIL-6 binds directly to gp130 was exploited to co-crystallize the extracellular portion of gp130 together with the vIL-6 protein[15]. This analysis yielded the first structure of gp130 in an apparently functional conformation together with a ligand[16] and was the starting point for the molecular understanding of the entire gp130 cytokine family[17]. Furthermore, findings from a transgenic mouse model showed that overexpression of vIL-6 leads to the development of a phenotype resembling multicentric Castleman disease[18]. Interestingly, the development of disease in these transgenic mice was strictly dependent on the presence of endogenous murine IL-6, leading to the hypothesis that disease development in patients with Castleman disease infected with HHV8 requires endogenous human IL-6 (REF. 18). This hypothesis explains how tocilizumab can be used to successfully treat patients with Castleman disease related to HHV8 infection, despite the fact that vIL-6 acts independently of human IL-6R[19]; apparently, endogenous IL-6 activity is needed for the maintenance of this disease[18].

## *IL-6 in innate and adaptive immunity*

In 1994, the first IL-6-deficient mice were generated, revealing that such mice display impaired innate and adaptive immunity to infection by viruses, bacteria and parasites[20]. IL-6 is involved in practically all aspects of the immune system ranging from neutrophil infiltration at sites of infection to the shaping of the T cell response[21] (TABLE 2); for a detailed discussion of IL-6 immune responses, see previous review[2]. A wealth of data from *ex vivo* investigations and genetically manipulated models support the importance of IL-6 in immunocompetence via its role in defence against viral[22–26], parasitic[27], fungal[28] and bacterial infections[29–31].

***Innate immunity.*** IL-6R is shed by neutrophils[32] and can enter the circulation (as sIL-6R) to be transported to sites of inflammation[33]. Consequently, IL-6 trans-signalling on smooth muscle cells, endothelial cells, mesothelial cells, epithelial cells and fibroblasts (TABLE 2) results in secretion of various chemokines, leading to the attraction of monocytes and/or macrophages and the resolution of inflammation[34].

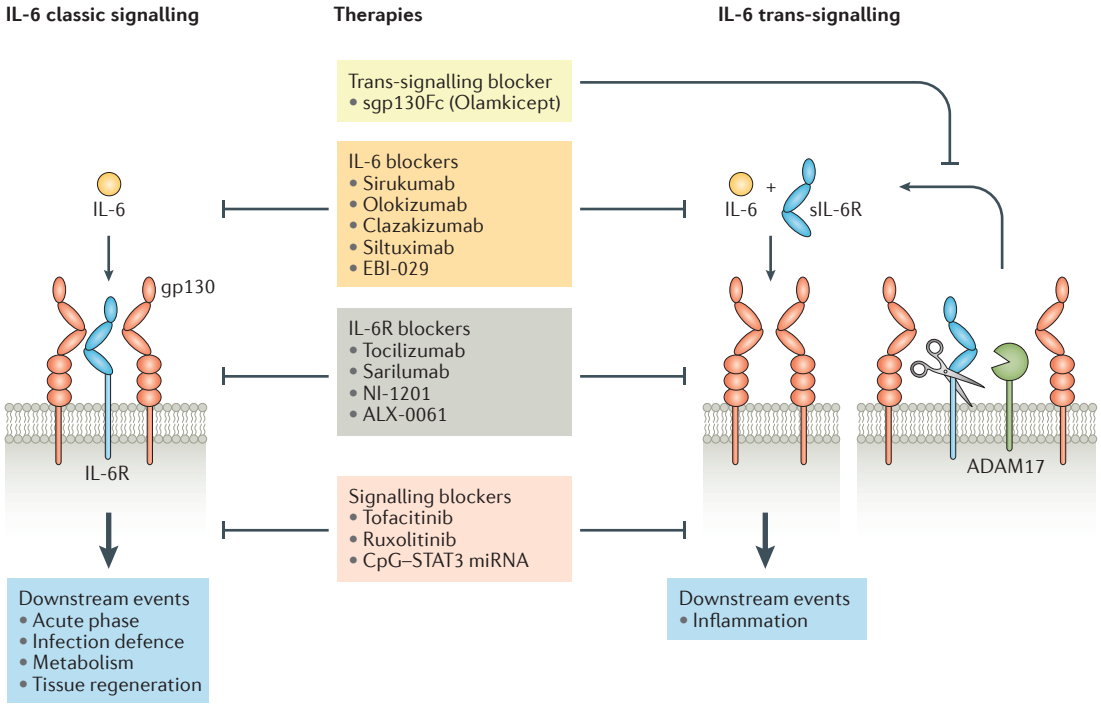


Figure 1 | **Mechanisms of action of various agents that target IL-6 and IL-6 activation pathways.** In classic signalling, IL-6 binds to the membrane bound IL-6 receptor (IL-6R) and the IL-6–IL-6R complex associates with the IL-6R β-subunit gp130, inducing gp130 dimerization and intracellular signalling. Alternatively, IL-6 can bind to soluble IL-6R (sIL-6R), which is generated by cleavage of membrane-bound IL-6R by a disintegrin and metalloproteinase domain-containing protein 17 (ADAM17). The IL-6–sIL-6R complex then binds to membrane-bound gp130, even on cells that do not express IL-6R, and induces trans-signalling. Agents specific for IL-6R, the IL-6 cytokine itself or intracellular activation pathways are currently in clinical use or in clinical trials[1]. Agents targeting either IL-6R or IL-6 have a capacity to neutralize both classic signalling and trans-signalling, although some differences in relative inhibition efficiency might exist. The soluble gp130 agent olamkicept has the capacity to neutralize trans-signalling only. Intracellular blockers include Janus kinase (JAK) inhibitors, which suppress numerous cytokine activation pathways including those used by IL-6, whereas a CpG–STAT3 (signal transducer and activator of transcription 3) siRNA conjugate is a novel entity that binds to cells expressing Toll-like receptor 9 (TLR9) and then selectively silences STAT3-mediated activation pathways including those driven by IL-6. This figure was adapted with permission obtained from Calabrese, L. H. & Rose-John, S. Nat. Rev, Rheumatol. 10, 720–727 (2014)[2] and Hunter, C. A. & Jones, S. A. Nat. Immunol. 16, 448–457 (2015)[1].

One of the best-known biologic activities of IL-6 is its prominent role in the synthesis and secretion of so-called acute-phase proteins, the levels of which rise (positive acute-phase proteins) or fall (negative acute-phase proteins) substantially in the wake of an inflammatory response[35]. IL-6 was recognized in 1987 to be the major inducer of the hepatic acute phase response[36]. Acute-phase proteins have multiple and complex roles in integrated host defences and IL-6-driven hepatic synthesis of multiple mediators has a critical role in the defence against bacterial pathogens such as *Klebsiella pneumoniae*[37] and *Streptococcus pneumoniae*[38]. In an experimental model of *Listeria monocytogenes* infection, selective inhibition of IL-6 trans-signalling did not interfere with acute-phase protein expression and bacterial load[29]. By contrast, blockade of both the classic and trans-signalling IL-6 pathways by use of neutralizing antibodies resulted in reduced acute-phase protein expression and increased bacterial colony numbers in the spleen and kidney. These results indicate that the defence against bacterial infections relies mainly on classic IL-6 signalling.

***T cell immunity.*** $CD4^+$ helper T cells were originally subdivided into type 1 T helper ($T_H1$) cells, important for clearing intracellular pathogens, and $T_H2$ cells, important for defending against extracellular pathogens and supporting B cells to produce antibodies[39]. Studies in mice subsequently identified a group of IL-17-producing $T_H$ cells whose expansion was dependent on IL-23, proposing an additional $T_H$ cell subset, $T_H17$ cells[21,40]. In 2006, three groups found that combined stimulation with transforming growth factor-β (TGFβ) and IL-6 of uncommitted T cells induced the generation of IL-17-producing T cells, whereas stimulation with TGFβ alone induced the generation of regulatory T ($T_{reg}$) cells, characterized by expression of the transcription factor forkhead box protein P3 (FOXP3)[21]; these findings revealed a role for IL-6 in balancing the differentiation of these two cell types. In humans, TGFβ stimulation in the presence of IL-6 and either IL-23 or IL-21 is essential for $T_H17$ cell differentiation[21]. IL-6 is not only important for the balance between $T_H17$ cells and $T_{reg}$ cells, but also for the control of cytotoxic T cell activity[41] and the activity of other $T_H$ cell subsets[42]. Given the ample evidence that $T_H17$

Table 1 | **sgp130Fc therapy in animal models**

| Disease | Mouse model | Refs |
|---|---|---|
| Rheumatoid arthritis | Antigen-induced arthritis, collagen-induced arthritis | 84–86 |
| Intestinal inflammation | Dextran sulphate sodium-induced colitis, SAMP1/Yit mouse model | 87,88 |
| Atherosclerosis | Hypercholesterolemic LDL-receptor negative mice | 89 |
| Allergic asthma | Sensitization and challenge of mice with allergen | 90 |
| Obesity-induced inflammation | Obesity induced by feeding high fat diet | 91 |
| Lung emphysema | Spontaneous and cigarette smoke-induced lung emphysema | 92 |

sgp130Fc has demonstrated therapeutic efficacy in all the mouse models of human inflammatory disease displayed above

cells in humans have a prominent role in the defence against fungal and bacterial infections, particularly from *Candida* species, these infections should be of special interest when investigating any therapy that targets $T_H17$ cells, either directly or indirectly[43,44]. Thus far, clinical trials and post-marketing surveillance of tocilizumab have noted no such pattern of infections.

In chronic viral infections, such as infection with HIV or hepatitis B virus (HBV) in humans, or lymphocytic choriomeningitis virus (LCMV) in mice, IL-6 signalling is important for viral control by stimulating $CD4^+$ T cells, resulting in germinal centre activation and improved antibody responses[22]. Interestingly, as well as IL-6, the gp130 family cytokine IL-27 is also essential for viral control[23]; this cytokine switches between interferon-like STAT1-driven and STAT3-dominant activities, which are associated with interferon-responses and gp130 responses, respectively[45]. During HBV infection, hepatic Kupffer cells produce IL-6, which inhibits expression of hepatocyte nuclear factor-1α (HNF1α) and HNF4α in hepatocytes, transcription factors that are required for the genetic expression and replication of HBV[24]. Thus, IL-6 has a protective role in various viral infections, which should be taken into account when therapeutic IL-6 blockade is considered[23,24].

### *Primary immunodeficiency diseases*

The study of patients with primary immunodeficiency diseases can often shed light on the critical role of a given molecule (such as IL-6) or a pathway (such as the IL-6 signalling pathway) in the integrated defence against pathogens. However, data on IL-6 are limited as no disorders characterized by a complete absence of IL-6 exist in humans. The growing interest in primary immunodeficiency syndromes resulting from natural autoantibodies generated against cytokines has yielded a number of insights into their role in host defence[46]. Of note are two reports describing three patients with neutralizing anti-IL-6 antibodies, each of whom experienced serious infections with bacterial pathogens[47,48]: a 67-year-old man with empyema due to *Escherichia coli* and *Streptococcus intermedius* infection; a 56-year-old woman with multiple subcutaneous abscesses from *Staphylococcus aureus* infection; and an 11-month-old boy with multiple skin abscesses from *S. aureus* infection. Of particular note, in each case C-reactive protein (CRP) levels remained essentially non-detectable despite the serious infection[47,48].

In terms of IL-6 signalling defects, both JAK–STAT and RAS-dependent MAPK signalling pathways are central to the biological activity of IL-6, and activation of transcription factor STAT3 in response to IL-6 is an important part of the response to invading pathogens. Moreover, STAT3 has a crucial role in the development of $T_H17$ cells by regulating essential transcription factors[21]. Mutations in STAT3 lead to hyper-IgE syndrome, an immune deficiency disease characterized by high levels of serum IgE, eczema and recurrent infections. Among the characteristic infectious complications associated with this syndrome is an increased risk of cold abscesses due to infection with bacteria such as *S. aureus* and *S. pneumoniae,* and, less frequently, bronchiectasis secondary to aspergillosis and candidiasis limited to the mucosal surfaces[49,50]. Patients with hyper-IgE syndrome have broad-ranging immunologic defects, including reduced numbers of $T_H17$ cells, which are crucial for defence against *Candida* infection at mucosal surfaces, as well as defects in memory B cell populations and T cell-dependent antibody responses[50]. However caution should be exercised when interpreting this data as other drivers can activate STAT3 and thus considerable redundancy exists in these pathways. Despite these limitations, the IL-6–STAT3 pathway is clearly integral to optimal immunocompetence.

## Safety of IL-6-targeting agents

### *Risk of serious infection*

Experience with tocilizumab has informed our understanding of the infectious risks of IL-6 inhibition. Randomized controlled trials (RCTs) and long-term extension studies initially evaluated this compound's safety. However, in the past few years, several population-based 'real-world' studies have evaluated the infectious risks of tocilizumab relative to other biologic therapies. Whereas tocilizumab remains relatively understudied compared with TNF blockers that have been in widespread use for over a decade (that is, infliximab, adalimumab, etanercept), existing data suggest that tocilizumab increases the risk for serious infections, including certain types of opportunistic infections, to an extent similar to TNF blockers and other biologic agents[51]. For other IL-6-inhibiting compounds, such as fully humanized anti-IL -6R agents (sarilumab), antibodies targeting IL-6 directly (sirukumab, olokizumab and clazakizumab) or the sgp130Fc protein that indirectly inhibits IL-6 (olamkicept) (FIG. 1), much less information is available and whether these compounds will yield differential effects on immunocompetence remains to be seen[2]. Although blockade of IL-6 and IL-6R have similar effects, we must be mindful of the differences between these strategies; for example, the human IL-6R also binds to ciliary neurotrophic factor (CNTF)[52] and to IL-30 (REF. 53). Therefore, unlike IL-6 blockade, blockade of IL-6R could also interfere with additional responses. Small phase II and phase III RCTs have been published for sirukumab, olokizumab, clazakizumab and sarilumab, in which the proportion of patients experiencing

Table 2 | **Responses of different cell types to IL-6**

| Cell type | IL-6 synthesis and secretion | Effect of IL-6 stimulation | Downstream targets of IL-6–STAT3 signalling | Refs |
|---|---|---|---|---|
| Neutrophils | Induced by TLR stimulation | No effect on survival or apoptosis; enhanced chemotaxis in response to IL-8; accelerated release of cells from bone marrow | N/A | 74,93 |
| Monocytes and/or macrophages | Induced by TLR stimulation | Expression of IL-4R, which is a prerequisite of M2 polarization | *IL4R* | 94 |
| B cells | Induced by TLR stimulation | Survival, expansion and maturation of plasmablasts | *PRDM1* | 1 |
| All T cells | IL-6 synthesis and secretion | Survival | *BCL2L1*, *BCL2* | 1 |
| $T_H1$ cells | IL-6 synthesis alone induced by IL-12 | Proliferation | *TBX21* | 1 |
| $T_H2$ cells | IL-6 synthesis alone induced by IL-4 | Proliferation | *GATA3* | 1 |
| $T_H17$ cells | Unknown | Commitment to $T_H17$ lineage (upon stimulation with both TGFβ and IL-6) | *RORC* | 1 |
| $T_{reg}$ cells | Unknown | Suppression of TGFβ-induced $T_{reg}$ cell differentiation | Blocks *FOXP3* expression | 1 |
| Hepatocytes | No IL-6 synthesis | Induction of the hepatic acute phase | *CRP*, *SAA1*, *HP*, fibrinogen and other acute phase proteins | 31,36 |
| Endothelial cells | IL-6 synthesis induced by IL-6 trans-signalling | Chemokine and chemokine receptor expression | *CCL2* and other chemokine-encoding genes | 95 |
| Smooth muscle cells | IL-6 synthesis induced by IL-6 trans-signalling | Upregulation of gp130 expression at the cell surface and chemokine secretion* | *CCL2* and other chemokine-encoding genes | 96 |
| Fibroblasts | Stimulation of IL-6 synthesis by IL-1 | Stimulation of collagen synthesis* | Stimulation of TGFβ signalling | 97–99 |
| Brain endothelial cells of the hypothalamus | Local IL-6 synthesis induced by IL-1 | Induction of fever | *COX2* | 100 |

*Via IL-6 trans-signalling RORγt, RAR-related orphan receptor gamma; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor-β; $T_H1$ cell, type 1 T helper cell; $T_H2$ cell, type 2 T helper cell; $T_H17$ cell, type 17 T helper cell; $T_{reg}$ cell, regulatory T cell. TLR, Toll-like receptor.

serious infectious events (SIEs) (0–6% in 12–24 weeks) was similar to that observed in other trials of biologic agents[54–59].

***Clinical trial safety.*** In a 2011 meta-analysis, Schiff *et al.*[3] analysed pooled data from RCTs and open-label extension trials within the tocilizumab development programme, which included 4,009 patients receiving at least one dose of tocilizumab and a total of 9,414 patient-years of exposure. Schiff *et al.* observed an incidence rate of SIEs of 4.9 per 100 patient-years in patients who received 8 mg/kg tocilizumab, a higher rate than that observed in patients who received placebo or 4 mg/kg tocilizumab (3.5 per 100 patient-years in each group)[3]. The majority of infections requiring hospitalization were those typically associated with RA, such as pneumonia, urinary tract infections and cellulitis. Stratified analysis of the risk factors for serious infections among this population identified many of the 'usual' risk factors, such as advanced age, glucocorticoid use, diabetes mellitus and chronic lung disease. Interestingly, SIE rates were as high as 8.5 per 100 patient-years in patients aged over 65 years. However, the authors of this study[3] did not use multivariate modelling to evaluate the potential differences in risk associations when accounting for these factors. Bykerk *et al.*[60] evaluated the potential risk differences based on prior TNF blockade in a phase IIIb open-label trial, comparing the incidence of infection in patients with RA receiving 8 mg/kg of tocilizumab who were either TNF blockade-naive or TNF blockade-experienced. This study followed 1,681 patients and reported an incidence rate of SIEs of 4.2 per 100 patient-years (95% CI 2.5–6.6) for TNF blockade-naive patients versus 6.8 per 100 patient-years (95% CI 3.1–12.9) for those with prior TNF blockade use, which likely reflects the overall treatment intensity of the TNF experienced group.

In 2015, Strand *et al.*[51] performed the most complete meta-analysis to date of all trials of biologic drugs for RA to evaluate the comparative safety of these agents. By use of a random-effects model that included data from 13 tocilizumab RCTs, they estimated a SIE rate of 5.25 per 100 patient-years (95% CI 4.26–6.96) in patients receiving tocilizumab treatment. When restricting their analysis to nine studies of tocilizumab in patients with an inadequate response to DMARDs, Strand *et al.* identified an increased relative risk for serious infection of 1.82 versus placebo[51]. This increase in risk was similar to that estimated in trials of other biologic drugs among similar patients (FIG. 2).

***Real-world safety.*** How the infection risk associated with tocilizumab treatment ranks compared with alternative biologic drugs continues to be an important question

within the field of RA treatment. Several types of real-world studies have been conducted in the past two years to help address this question, including open-label multicentre studies and studies evaluating outcomes among large cohorts of patients with RA by use of administrative or registry data. For example, an open-label non-interventional prospective study within 174 centres in Germany conducted over 52 weeks reported an SIE incidence rate of 4.4 per 100 patient-years (95% CI 2.5–6.0) in patients on tocilizumab therapy, a rate comparable to that seen in clinical trials[61]. Risk of serious infection was similar between those on tocilizumab monotherapy and those on a combination therapy of tocilizumab and a conventional synthetic DMARDs (csDMARDs), but the highest infection rates were observed in those with prior TNF inhibitor usage[61]. A similar post-marketing study of biologic-naive patients with RA ($n = 839$), conducted over 298 centres in Japan, demonstrated an SIE incidence rate of 5.84 per 100 patient-years over 52 weeks for tocilizumab-exposed patients with RA[62]. Notably, a steroid-sparing effect was observed with tocilizumab therapy, as 34% of prednisone users discontinued prednisone over the course of the study. In another Japanese post-marketing registry study of 7,901 patients with RA receiving tocilizumab treatment, the incidence rate of SIEs was 9.0 per 100 patient-years in the first 28 weeks after commencing therapy[63]. Although this rate of infection is somewhat higher than those observed in clinical trials, it likely reflects the expected comorbidities and lack of age restrictions observed in 'real-life' use of the drug. Within the US Medicare system, Yun *et al.* observed absolute incidence rates for infection requiring hospitalization of 14.9 per 100 patient-years (95% CI 11.4–16.5) in patients starting tocilizumab therapy after prior use of an anti-TNF therapy[64]. After adjustment for infectious risk factors, the relative risk of hospitalization with an infection with tocilizumab was not significantly different from other biologic comparators.

Real-world data also highlight the increased risk of gastrointestinal tract perforation with tocilizumab therapy. Although this event is not an infectious event *per se*,

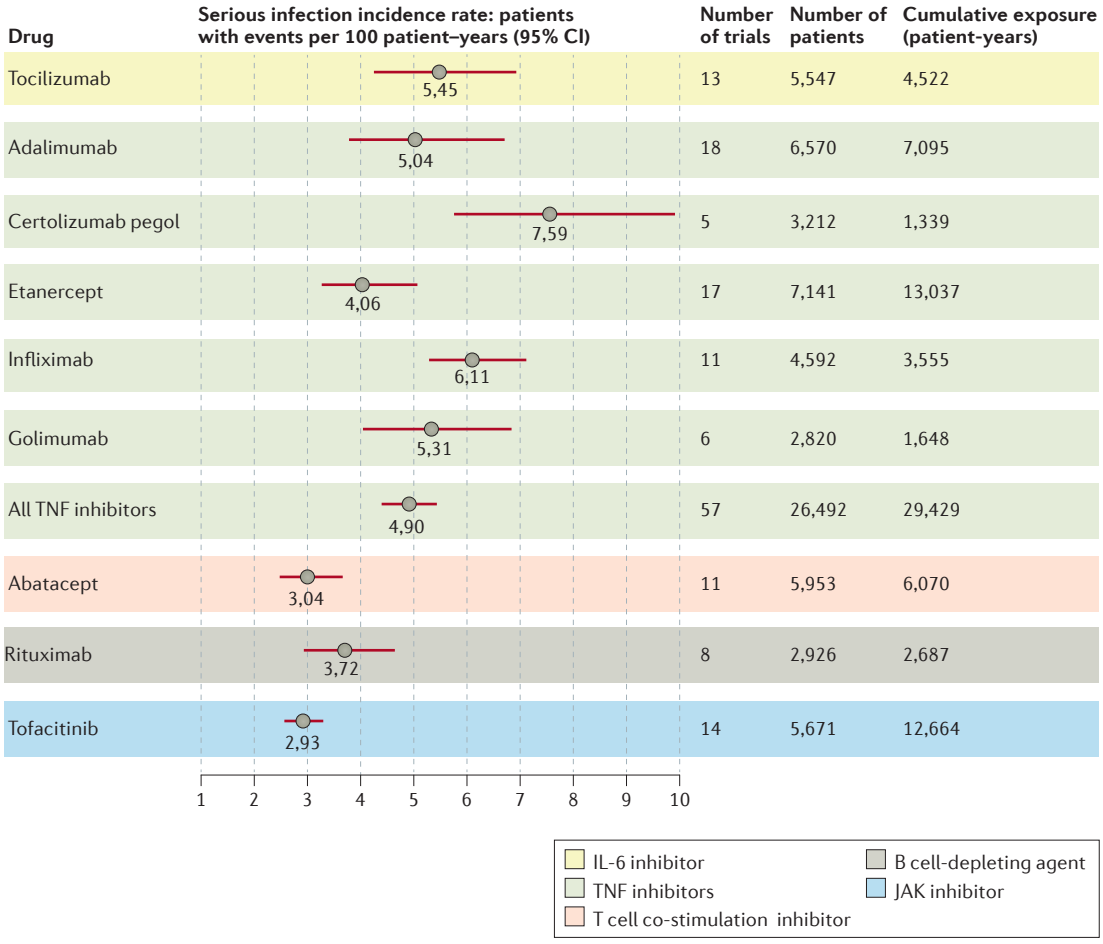


Figure 2 | **Estimated serious infection rates among clinical trials of biologics in rheumatoid arthritis.** The incidence rate of serious infection (number of unique patients with events per 100 patient-years exposure) across clinical trials (including randomized control trials and long-term extension trials) investigating biologics for the treatment of rheumatoid arthritis are displayed using clinical trial data published between 1999 and 2013. All trials included patients with moderate to severely active rheumatoid arthritis. JAK, Janus kinase. This figure was adapted with permission obtained from Strand, V. *et al. Arthritis Res. Ther.* **17,** 362 (2015)[51], which is published under an open-access licence (http://creativecommons.org/licenses/by/4.0) by BioMed Central Ltd.

gastrointestinal tract perforation can be associated clinically with infection or sepsis. Through analysis of administrative data from the USA, Curtis *et al.* reported an estimated incidence rate of hospitalized gastrointestinal tract perforation of 1.5–2.5 per 100 patient-years for tocilizumab users, which was a significantly higher rate compared with patients being treated with anti-TNF agents (HR 2.5, 95% CI 1.3–4.8)[65].

### *Risk of opportunistic infections*

Within clinical trials, the aforementioned Schiff *et al.* meta-analysis study of the tocilizumab development programme reported 22 opportunistic infections (0.23 per 100 patient-years) in over 9,000 person-years of exposure[3]. All but one of these infections occurred in patients receiving 8 mg/kg tocilizumab and none occurred in placebo-treated patients. Reported cases included tuberculosis, nontuberculous mycobacteria, invasive forms of candidiasis and fungal infections such as *Pneumocystis jiroveci* pneumonia and cryptococcal pneumonia. Unfortunately, very little real-world data exist examining incidence rates of opportunistic infections in tocilizumab users and hence judging the risk associated with this agent relative to other biologic drugs is difficult. For tuberculosis, the experience in Japan suggests tocilizumab is associated with similar risks of infection compared with anti-TNF agents. In the Japanese post-marketing registry study of 7,901 patients with RA, the incidence of tuberculosis was 0.13 per 100 patient-years following 28 weeks of tocilizumab therapy[63] (this rate declined from earlier reports of 0.22 per 100 patient-years in Japan[66]) and might reflect enhanced screening. The incidence rates for other opportunistic infections in this group of patients was 0.44 per 100 patient-years for nontuberculous mycobacteria, 0.37 per 100 patient-years for *Pneumocystis* pneumonia and 2.2 per 100 patient-years for herpes zoster infections. The only population-based study that directly compared the risk of oral infections in tocilizumab users and other biologic users was performed in 2015 using US Medicare data. This study evaluated the risk of herpes zoster infection and reported an incidence rate of 2.15 per 100 patient-years in patients who had begun treatment with tocilizumab[67]. After adjusting for other risk factors, no difference in the risk of herpes zoster infection was identified between any of the biologic agents, with a hazard ratio of 1.05 (95% CI 0.6–1.8) for tocilizumab relative to abatacept[67].

For other chronic viral infections, such as HBV or hepatitis C virus (HCV) infections, the safety of tocilizumab is largely unknown as few data are available and trials have typically excluded patients with HBV or HCV infection. Tocilizumab has been used successfully in two patients with HBV infection in the context of concomitant anti-viral therapy, and in one patient with an HCV infection[68–70], without resulting in viral reactivation. Several additional small case-series have reported two cases of self-limited viraemia among 25 patients with prior exposure to HBV undergoing tocilizumab therapy[71]. The Japanese post-marketing study of 7,901 patients receiving tocilizumab therapy observed no hepatobiliary disorders due to reactivation of HBV or HCV infection in the 28 weeks following therapy initiation[63]. This cohort included 52 patients with prior exposure to HBV; however, their serological status was not commented on and so the baseline risk these patients had with regard to reactivation is unclear[63].

The relationship between IL-6 and HIV infection is complex (BOX 1). Elevated levels of IL-6 are associated with accelerated mortality in HIV-infected patients, even from non-AIDS events[72]. Additionally, IL-6 functions as a driver of impaired immune responsiveness and immune reconstitution. On the basis of these findings, an RCT of tocilizumab is being conducted examining the effects of blocking IL-6 activity with tocilizumab in patients with controlled HIV infection[73]. This trial will not only assess the safety and tolerability of tocilizumab in this immunocompromised population, but will also explore the effects of IL-6 blockade on inflammation, immune reconstitution, atherogenesis and numerous other systems. The results of this trial are eagerly awaited.

### *Neutropenia with IL-6 blockade*

Multiple clinical trials have documented that blockade of IL-6 activity leads to transient or sustained neutropenia[2], although IL-6 blockade does not directly affect neutrophil function or survival[74]. The functional consequences of this neutropenia are not clear and the mechanism for the acute decline in neutrophils is uncertain (but is thought to involve compartment shifting). Two parallel clinical trials have compared the efficacy of monotherapy with either the TNF-blocking antibody adalimumab or IL-6R blockade with either tocilizumab[75] or sarilumab[54], in which the primary end point was change in the 28-joint disease activity score (DAS28) from baseline. In both trials, blockade of IL-6R was superior to TNF blockade and the incidence of bacterial infections upon IL-6R or TNF blockade was comparable, whereas platelet and neutrophil counts were significantly lower with IL-6R blockade[54,75]. This finding provides evidence for a possible argument against a direct causal link between bacterial infections and neutropenia during the clinical trials[54,75]. In addition, examination of the integrated safety database of tocilizumab has revealed no clear relationship between leukopenia and serious

Box 1 | **IL-6 and HIV infection**

The treatment of HIV infection with combination antiretroviral therapy (cART) has transformed the disease into a chronic and manageable condition with near-normal life expectancy. Despite this fact, non-AIDS-defining events such as accelerated cardiovascular disease and non-AIDS-defining malignancies are more prevalent in infected patients being treated with cART than in uninfected adults. A growing body of data suggests that persistent immune activation, supported by elevated levels of inflammatory cytokines, drives many of these phenomena[72]. Increased IL-6 in particular has been consistently correlated with increased morbidity and mortality, as well as with the inability to reconstitute circulating $CD4^+$ T cell numbers[72]. On the basis of these data, a phase I–II, double-blind, placebo-controlled, randomized crossover clinical trial of tocilizumab therapy in HIV-infected patients receiving antiretroviral therapy with suppressed viral replication is currently in progress. This investigation will, it is hoped, shed light on the role of IL-6 in both AIDS-related and non-AIDS-related morbidities and inform the role of IL-6 in other chronic inflammatory disorders[73].

infections[3]. The mechanistic consequences of neutropenia caused by IL-6R blockade need to be analysed in more detail in the future (TABLES 2,3).

### *Atypical presentations of infection*

Given the prominent role of IL-6 as a multifunctional cytokine with a wide range of biological effects, reports of patients experiencing acute and severe bacterial infections with atypical presentations when being treated with IL-6-targeting agents is perhaps unsurprising. For example, in 2009, Fujwara and colleagues[76] described two patients who, while receiving tocilizumab treatment, developed severe pneumonia (one patient was culture positive for *Haemophilus influenza*) and presented with a paucity of symptoms (that is, fever, rigor, chest pain); one patient developed shock and both patients had minimally elevated CRP levels. Yanagawa and colleagues[77] reported a similar case of a patient receiving tocilizumab treatment who also developed shock and from whom *S. pneumoniae* was isolated. Disseminated *S. aureus* infection has also been reported in three patients on tocilizumab therapy who presented with mild symptoms, emphasizing the need for a high index of suspicion for such infections in patients being treated with IL-6-targeting agents.

As previously noted, three patients with naturally occurring anti-IL-6 autoantibodies who experienced serious bacterial infections developed fever but failed to mount CRP responses[47,48]. CRP neutralization itself might contribute to lowered immunocompetence; knockout models have demonstrated that CRP is essential for host defence to pneumococcal infection[38]. Such reports should be put into perspective regarding the capacity and likelihood for IL-6 targeting drugs such as tocilizumab, and other agents likely to follow, to blunt CRP and systemic symptoms in the setting of serious infections, and clinicians should be aware of such a scenario when using CRP levels as an indicator for infection. At present the

Table 3 | **Effect of knockout of IL-6, IL-6 receptor subunits and IL-6 signalling genes**

| Gene | Congenital knockout phenotype | Conditional knockout phenotype | Refs |
|---|---|---|---|
| *Il6* | • Normal development<br>• Impairment of hepatic acute phase protein induction after tissue damage<br>• Defects in haematopoiesis<br>• Loss of efficient vaccinia virus infection control<br>• Loss of efficient *Listeria* infection control<br>• Impairment of T cell-dependent antibody production<br>• Impairment of the neutrophil response after *Listeria* infection<br>• Impairment of the neutrophil response after fungal infection<br>• Loss of fever response<br>• No induction of EAE | • B cells: reduced EAE<br>• T cells: reduced EAE<br>• Myeloid cells: reduced EAE<br>• Dendritic cells: no EAE | 20,101–106 |
| *Il6r* | • Similar phenotype to IL-6-deficient mice<br>• Better wound healing than IL-6-deficient mice | • Dendritic cells: no EAE<br>• Myeloid cells: loss of M2 differentiation of macrophages<br>• Hepatocytes: reduced insulin sensitivity; liver inflammation | 94,106–108 |
| *gp130* | Embryonically lethal | • Most cells (Mx–Cre): impairment of bacterial and virus response; impairment of hepatic acute phase protein induction after tissue damage; emphysema development with increasing age<br>• Hepatocytes: loss of hepatocellular protection after LPS challenge; reduction in atherosclerotic plaque area; decreased survival after polymicrobial sepsis | 109–113 |
| *Jak1* | • Viable but perinatally lethal due to neurologic deficits<br>• Severe combined immune deficiency | Mammary glands: uncoupling of gp130 from STAT1 and STAT3; impaired apoptosis | 114,115 |
| *Stat1* | • Viable and fertile<br>• Defective interferon functions<br>• Increased tumour formation<br>• Susceptibility to mycobacterial and viral infections | N/A | 114,116 |
| *Stat3* | Embryonically lethal | • Hepatocytes: impaired acute phase response<br>• Most cells (Mx–Cre): high neutrophil numbers<br>• Bone marrow cells: impaired neutrophil mobilization; enterocolitis; impaired emergency granulopoiesis | 114,117–119 |
| *Socs3* | Embryonically lethal due to placental deficits and erythrocytosis | Myeloid cells: increased neutrophil numbers | 114,120 |
| *Adam17* | Mice not viable | • Myeloid cells: protection from endotoxin shock<br>• Hypomorphic mice: susceptibility to colitis; improvement of kidney fibrosis following kidney injury; atheroprotective role of ADAM17 | 121–125 |

ADAM17, a disintegrin and metalloproteinase domain-containing protein 17; EAE, experimental autoimmune encephalomyelitis; gp130, IL-6R subunit β; LPS, lipopolysaccharide.

incidence of this scenario has yet to be estimated; however, its likelihood will be greatly influenced by the dose and interval of drug administration. The host response would probably rebound as the drug is cleared.

### *Vaccine responses*

A critical question regarding vaccination of patients on biologic therapies is whether this therapy will impair responsiveness to the vaccine[78]. Fortunately, numerous open trials have examined the efficacy of the pneumococcal polysaccharide vaccine (PPV23)[79,80], pneumococcal conjugate vaccine (PCV7)[81] and trivalent influenza vaccine[79,82], and one randomized trial examined the effectiveness of PCV23 in producing an antibody response in patients being treated with tocilizumab[83]. The designs of these studies vary as to concomitant therapy with DMARDs such as methotrexate[83]. In general, minor (that is, nonsignificant) differences were reported between tocilizumab monotherapy versus its combined use with methotrexate, with methotrexate generally having a modest negative effect on vaccine efficacy when used in a combined fashion. However, the overall responses were adequate throughout these studies and suggest that tocilizumab does not represent a major obstacle for vaccination effectiveness when being taken with these agents

## Conclusions

Considerable evidence suggests that IL-6 has a central role in immunocompetence, on the basis of critical appraisal of both preclinical and clinical data. Knowledge of IL-6 signalling and its downstream intracellular activation pathways provides insight into the differential protective effects of these pathways against an array of pathogens. Such data might inform the bioengineering of future agents with anti-IL-6 activity that could potentially be safer than currently available agents, but such conclusions must await large clinical studies. Knowledge of the role of IL-6 in immunocompetence can also potentially enhance the safety of therapies targeting this cytokine, its receptor or its activation pathways for optimal patient care.


1. Hunter, C. A. & Jones, S. A. IL-6 as a keystone cytokine in health and disease. *Nat. Immunol.* **16**, 448–457 (2015).
2. Calabrese, L. H. & Rose-John, S. IL-6 biology: implications for clinical targeting in rheumatic disease. *Nat. Rev. Rheumatol.* **10**, 720–727 (2014).
3. Schiff, M. H. *et al.* Integrated safety in tocilizumab clinical trials. *Arthritis Res. Ther.* **13**, R141 (2011).
4. Looney, R. J. *et al.* Guidelines for assessing immunocompetency in clinical trials for autoimmune diseases. *Clin. Immunol.* **123**, 235–243 (2007).
5. Schaper, F. & Rose-John, S. Interleukin-6: biology, signaling and strategies of blockade. *Cytokine Growth Factor Rev.* **26**, 475–487 (2015).
6. Scheller, J. & Rose-John, S. The interleukin 6 pathway and atherosclerosis. *Lancet* **380**, 338 (2012).
7. Jones, S. A., Scheller, J. & Rose-John, S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *J. Clin. Invest.* **121**, 3375–3383 (2011).
8. Jostock, T. *et al.* Soluble gp130 is the natural inhibitor of soluble interleukin-6 receptor transsignaling responses. *Eur. J. Biochem.* **268**, 160–167 (2001).
9. German Clinical Trials Registry. A single-centre, exploratory trial to assess the mechanisms of molecular activity, safety and tolerability of one dose level of FE 999301 by intravenous infusions in patients with active inflammatory bowel disease (IBD). *drks-neu.uniklinik-freiburg.de* https://drks-neu.uniklinik-freiburg.de/drks_web/navigate.do?navigationId=trial.HTML&TRIAL_ID=DRKS00010101 (2017).
10. Moore, P. S., Boshoff, C., Weiss, R. A. & Chang, Y. Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV. *Science* **274**, 1739–1744 (1996).
11. Schulte, K. M. & Talat, N. Castleman's disease — a two compartment model of HHV8 infection. *Nat. Rev. Clin. Oncol.* **7**, 533–543 (2010).
12. Molden, J., Chang, Y., You, Y., Moore, P. S. & Goldsmith, M. A. A. Kaposi's sarcoma-associated herpesvirus-encoded cytokine homolog (vIL-6) activates signaling through the shared gp130 receptor subunit. *J. Biol. Chem.* **272**, 19625–19631 (1997).
13. Mullberg, J. *et al.* IL-6 receptor independent stimulation of human gp130 by viral IL-6. *J. Immunol.* **164**, 4672–4677 (2000).
14. Taga, T. & Kishimoto, T. gp130 and the interleukin-6 family of cytokines. *Annu. Rev. Immunol.* **15**, 797–819 (1997).
15. Adam, N. *et al.* Unraveling viral interleukin-6 binding to gp130 and activation of STAT-signaling pathways independently of the interleukin-6 receptor. *J. Virol.* **83**, 5117–5126 (2009).
16. Chow, D., He, X., Snow, A. L., Rose-John, S. & Garcia, K. C. Structure of an extracellular gp130 cytokine receptor signaling complex. *Science* **291**, 2150–2155 (2001).
17. Spangler, J. B., Moraga, I., Mendoza, J. L. & Garcia, K. C. Insights into cytokine-receptor interactions from cytokine engineering. *Annu. Rev. Immunol.* **33**, 139–167 (2015).
18. Suthaus, J. *et al.* HHV-8-encoded viral IL-6 collaborates with mouse IL-6 in the development of multicentric Castleman disease in mice. *Blood* **119**, 5173–5181 (2012).
19. Nishimoto, N. *et al.* Humanized anti-interleukin-6 receptor antibody treatment of multicentric Castleman disease. *Blood* **106**, 2627–2632 (2005).
20. Kopf, M. *et al.* Impaired immune and acute-phase responses in interleukin-6-deficient mice. *Nature* **368**, 339–342 (1994).
21. Korn, T., Bettelli, E., Oukka, M. & Kuchroo, V. K. IL-17 and Th17 cells. *Annu. Rev. Immunol.* **27**, 485–517 (2009).
22. Harker, J. A., Lewis, G. M., Mack, L. & Zuniga, E. I. Late interleukin-6 escalates T follicular helper cell responses and controls a chronic viral infection. *Science* **334**, 825–829 (2011).
23. Zuniga, E. I., Macal, M., Lewis, G. M. & Harker, J. A. Innate and adaptive immune regulation during chronic viral infections. *Annu. Rev. Virol.* **2**, 573–597 (2015).
24. Hosel, M. *et al.* Not interferon, but interleukin-6 controls early gene expression in hepatitis B virus infection. *Hepatology* **50**, 1773–1782 (2009).
25. Dienz, O. *et al.* Essential role of IL-6 in protection against H1N1 influenza virus by promoting neutrophil survival in the lung. *Mucosal Immunol.* **5**, 258–266 (2012).
26. Longhi, M. P. *et al.* Interleukin-6 is crucial for recall of influenza-specific memory CD4 T cells. *PLoS Pathog.* **4**, e1000006 (2008).
27. Smith, K. A. & Maizels, R. M. IL-6 controls susceptibility to helminth infection by impeding Th2 responsiveness and altering the Treg phenotype *in vivo*. *Eur. J. Immunol.* **44**, 150–161 (2014).
28. Neveu, W. A. *et al.* IL-6 is required for airway mucus production induced by inhaled fungal allergens. *J. Immunol.* **183**, 1732–1738 (2009).
29. Hoge, J. *et al.* IL-6 controls the innate immune response against *Listeria monocytogenes* via classical IL-6 signaling. *J. Immunol.* **190**, 703–711 (2013).
30. van der Poll, T. *et al.* Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. *J. Infect. Dis.* **176**, 439–444 (1997).
31. Hochepied, T., Van Molle, W., Bergeri, F. G., Baumann, H. & Libert, C. Involvement of the acute phase protein α1-acid glycoprotein in nonspecific resistance to a lethal gram-negative infection. *J. Biol. Chem.* **275**, 14903–14909 (2000).
32. Chalaris, A. *et al.* Apoptosis is a natural stimulus of IL6R shedding and contributes to the proinflammatory trans-signaling function of neutrophils. *Blood* **110**, 1748–1755 (2007).
33. Schumacher, N. *et al.* Circulating soluble IL-6R but not ADAM17 activation drives mononuclear cell migration in tissue inflammation. *J. Immunol.* **197**, 3705–3715 (2016).
34. Hurst, S. M. *et al.* Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. *Immunity* **14**, 705–714 (2001).
35. Gabay, C. & Kushner, I. Acute-phase proteins and other systemic responses to inflammation. *N. Engl. J. Med.* **340**, 448–454 (1999).
36. Gauldie, J., Richards, C., Harnish, D., Lansdorp, P. & Baumann, H. Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc. Natl Acad. Sci. USA* **84**, 7251–7255 (1987).
37. Hochepied, T., Berger, F. G., Baumann, H. & Libert, C. Alpha(1)-acid glycoprotein: an acute phase protein with inflammatory and immunomodulating properties. *Cytokine Growth Factor Rev.* **14**, 25–34 (2003).
38. Simons, J. P. *et al.* C-reactive protein is essential for innate resistance to pneumococcal infection. *Immunology* **142**, 414–420 (2014).
39. Mosmann, T. R. & Coffman, R. L. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* **7**, 145–173 (1989).
40. Cua, D. J. *et al.* Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* **421**, 744–748 (2003).
41. Bottcher, J. P. *et al.* IL-6 trans-signaling-dependent rapid development of cytotoxic CD8+ T cell function. *Cell Rep.* **8**, 1318–1327 (2014).
42. Stumhofer, J. S. *et al.* Interleukins 27 and 6 induce STAT3-mediated T cell production of interleukin 10. *Nat. Immunol.* **8**, 1363–1371 (2007).
43. McAleer, J. P. & Kolls, J. K. Mechanisms controlling Th17 cytokine expression and host defense. *J. Leukoc. Biol.* **90**, 263–270 (2011).
44. Sallusto, F. Heterogeneity of human $CD4^+$ T cells against microbes. *Annu. Rev. Immunol.* **34**, 317–334 (2016).
45. Rolvering, C. *et al.* Crosstalk between different family members: IL27 recapitulates IFNgamma responses in HCC cells, but is inhibited by IL6-type cytokines. *Biochim. Biophys. Acta* **1864**, 516–526 (2016).
46. Vincent, T., Plawecki, M., Goulabchand, R., Guilpain, P. & Eliaou, J. F. Emerging clinical phenotypes associated with anti-cytokine autoantibodies. *Autoimmun Rev.* **14**, 528–535 (2015).
47. Nanki, T. *et al.* Suppression of elevations in serum C reactive protein levels by anti-IL-6 autoantibodies in two patients with severe bacterial infections. *Ann. Rheum. Dis.* **72**, 1100–1102 (2013).

48. Puel, A. *et al.* Recurrent staphylococcal cellulitis and subcutaneous abscesses in a child with autoantibodies against IL-6. *J. Immunol.* **180**, 647–654 (2008).
49. Farmand, S. & Sundin, M. Hyper-IgE syndromes: recent advances in pathogenesis, diagnostics and clinical care. *Curr. Opin. Hematol.* **22**, 12–22 (2015).
50. Vogel, T. P., Milner, J. D. & Cooper, M. A. The ying and yang of STAT3 in human disease. *J. Clin. Immunol.* **35**, 615–623 (2015).
51. Strand, V. *et al.* Systematic review and meta-analysis of serious infections with tofacitinib and biologic disease-modifying antirheumatic drug treatment in rheumatoid arthritis clinical trials. *Arthritis Res. Ther.* **17**, 362 (2015).
52. Schuster, B. *et al.* Signaling of human ciliary neurotrophic factor (CNTF) revisited. The interleukin-6 receptor can serve as an alpha-receptor for CTNF. *J. Biol. Chem.* **278**, 9528–9535 (2003).
53. Garbers, C. *et al.* An interleukin-6 receptor-dependent molecular switch mediates signal transduction of the IL-27 cytokine subunit p28 (IL-30) via a gp130 protein receptor homodimer. *J. Biol. Chem.* **288**, 4346–4354 (2013).
54. Burmester, G. R. *et al.* Efficacy and safety of sarilumab monotherapy versus adalimumab monotherapy for the treatment of patients with active rheumatoid arthritis (MONARCH): a randomised, double-blind, parallel-group phase III trial. *Ann. Rheum. Dis.* **76**, 840–847 (2016).
55. Genovese, M. C. *et al.* Efficacy and safety of olokizumab in patients with rheumatoid arthritis with an inadequate response to TNF inhibitor therapy: outcomes of a randomised phase IIb study. *Ann. Rheum. Dis.* **73**, 1607–1615 (2014).
56. Mease, P. J. *et al.* The efficacy and safety of clazakizumab, an anti-interleukin-6 monoclonal antibody, in a phase IIb study of adults with active psoriatic arthritis. *Arthritis Rheumatol.* **68**, 2163–2173 (2016).
57. Smolen, J. S., Weinblatt, M. E., Sheng, S., Zhuang, Y. & Hsu, B. Sirukumab, a human anti-interleukin-6 monoclonal antibody: a randomised, 2-part (proof-of-concept and dose-finding), phase II study in patients with active rheumatoid arthritis despite methotrexate therapy. *Ann. Rheum. Dis.* **73**, 1616–1625 (2014).
58. Takeuchi, T. *et al.* Efficacy and safety of olokizumab in Asian patients with moderate-to-severe rheumatoid arthritis, previously exposed to anti-TNF therapy: results from a randomized phase II trial. *Mod. Rheumatol.* **26**, 15–23 (2016).
59. Weinblatt, M. E. *et al.* The efficacy and safety of subcutaneous clazakizumab in patients with moderate-to-severe rheumatoid arthritis and an inadequate response to methotrexate: results from a multinational, phase IIb, randomized, double-blind, placebo/active-controlled, dose-ranging study. *Arthritis Rheumatol.* **67**, 2591–2600 (2015).
60. Bykerk, V. P. *et al.* Tocilizumab in patients with active rheumatoid arthritis and inadequate responses to DMARDs and/or TNF inhibitors: a large, open-label study close to clinical practice. *Ann. Rheum. Dis.* **71**, 1950–1954 (2016).
61. Iking-Konert, C. *et al.* ROUTINE-a prospective, multicentre, non-interventional, observational study to evaluate the safety and effectiveness of intravenous tocilizumab for the treatment of active rheumatoid arthritis in daily practice in Germany. *Rheumatology (Oxford)* **55**, 624–635 (2016).
62. Ishiguro, N. *et al.* Effectiveness and safety of tocilizumab in achieving clinical and functional remission, and sustaining efficacy in biologics-naive patients with rheumatoid arthritis: the FIRST Bio study. *Mod. Rheumatol.* **27**, 217–226 (2016).
63. Koike, T. *et al.* Effectiveness and safety of tocilizumab: postmarketing surveillance of 7901 patients with rheumatoid arthritis in Japan. *J. Rheumatol.* **41**, 15–23 (2014).
64. Yun, H. *et al.* Risk of hospitalised infection in rheumatoid arthritis patients receiving biologics following a previous infection while on treatment with anti-TNF therapy. *Ann. Rheum. Dis.* **74**, 1065–1071 (2015).
65. Xie, F., Yun, H., Bernatsky, S. & Curtis, J. R. Brief report: risk of gastrointestinal perforation among rheumatoid arthritis patients receiving tofacitinib, tocilizumab, or other biologic treatments. *Arthritis Rheumatol.* **68**, 2612–2617 (2016).
66. Koike, T. *et al.* Postmarketing surveillance of tocilizumab for rheumatoid arthritis in Japan: interim analysis of 3881 patients. *Ann. Rheum. Dis.* **70**, 2148–2151 (2011).
67. Yun, H. *et al.* Risks of herpes zoster in patients with rheumatoid arthritis according to biologic disease-modifying therapy. *Arthritis Care Res. (Hoboken)* **67**, 731–736 (2015).
68. Nagashima, T., Maruyama, A., Kamata, Y. & Minota, S. Unchanged serum viral load and liver function during tocilizumab treatment in a patient with rheumatoid arthritis and hepatitis C virus infection. *Rheumatol. Int.* **32**, 2231–2232 (2012).
69. Nagashima, T. & Minota, S. Long-term tocilizumab therapy in a patient with rheumatoid arthritis and chronic hepatitis B. *Rheumatology (Oxford)* **47**, 1838–1840 (2008).
70. Tsuboi, H. *et al.* A patient with rheumatoid arthritis treated with tocilizumab together with lamivudine prophylaxis after remission of infliximab-reactivated hepatitis B. *Mod. Rheumatol.* **21**, 701–705 (2011).
71. Nakamura, J. *et al.* Reactivation of hepatitis B virus in rheumatoid arthritis patients treated with biological disease-modifying antirheumatic drugs. *Int. J. Rheum. Dis.* **19**, 470–475 (2016).
72. Freeman, M. L. *et al.* Cytokines and T-cell homeostasis in HIV infection. *J. Infect. Dis.* **214** (Suppl. 2), S51–S57 (2016).
73. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02049437 (2017).
74. Wright, H. L., Cross, A. L., Edwards, S. W. & Moots, R. J. Effects of IL-6 and IL-6 blockade on neutrophil function *in vitro* and *in vivo*. *Rheumatology (Oxford)* **53**, 1321–1331 (2014).
75. Gabay, C. *et al.* Tocilizumab monotherapy versus adalimumab monotherapy for treatment of rheumatoid arthritis (ADACTA): a randomised, double-blind, controlled phase 4 trial. *Lancet* **381**, 1541–1550 (2013).
76. Fujiwara, H. *et al.* Masked early symptoms of pneumonia in patients with rheumatoid arthritis during tocilizumab treatment: a report of two cases. *Mod. Rheumatol.* **19**, 64–68 (2009).
77. Yanagawa, Y., Hirano, Y., Kato, H. & Iba, T. The absence of typical pneumonia symptoms in a patient with rheumatoid arthritis during tocilizumab and steroid treatment. *BMJ Case Rep.* **2012**, bcr0220125835 (2012).
78. McMahan, Z. H. & Bingham, C. O. III. Effects of biological and non-biological immunomodulatory therapies on the immunogenicity of vaccines in patients with rheumatic diseases. *Arthritis Res. Ther.* **16**, 506 (2014).
79. Mori, S. *et al.* Pneumococcal polysaccharide vaccination in rheumatoid arthritis patients receiving tocilizumab therapy. *Ann. Rheum. Dis.* **72**, 1362–1366 (2013).
80. Tsuru, T. *et al.* Immune response to influenza vaccine and pneumococcal polysaccharide vaccine under IL-6 signal inhibition therapy with tocilizumab. *Mod. Rheumatol.* **24**, 511–516 (2014).
81. Kapetanovic, M. C. *et al.* Impact of anti-rheumatic treatment on immunogenicity of pandemic H1N1 influenza vaccine in patients with arthritis. *Arthritis Res. Ther.* **16**, R2 (2014).
82. Mori, S. *et al.* Impact of tocilizumab therapy on antibody response to influenza vaccine in patients with rheumatoid arthritis. *Ann. Rheum. Dis.* **71**, 2006–2010 (2012).
83. Bingham, C. O. III *et al.* Humoral immune response to vaccines in patients with rheumatoid arthritis treated with tocilizumab: results of a randomised controlled trial (VISARA). *Ann. Rheum. Dis.* **74**, 818–822 (2015).
84. Nowell, M. A. *et al.* Soluble IL-6 receptor governs IL-6 activity in experimental arthritis: blockade of arthritis severity by soluble glycoprotein 130. *J. Immunol.* **171**, 3202–3209 (2003).
85. Nowell, M. A. *et al.* Therapeutic targeting of IL-6 trans signaling counteracts STAT3 control of experimental inflammatory arthritis. *J. Immunol.* **182**, 613–622 (2009).
86. Richards, P. J. *et al.* Functional characterization of a soluble gp130 isoform and its therapeutic capacity in an experimental model of inflammatory arthritis. *Arthritis Rheum.* **54**, 1662–1672 (2006).
87. Atreya, R. *et al.* Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis *in vivo*. *Nat. Med.* **6**, 583–538 (2000).
88. Mitsuyama, K. *et al.* STAT3 activation via interleukin 6 trans-signalling contributes to ileitis in SAMP1/Yit mice. *Gut* **55**, 1263–1269 (2006).
89. Schuett, H. *et al.* Transsignaling of interleukin-6 crucially contributes to atherosclerosis in mice. *Arterioscler Thromb. Vasc. Biol.* **32**, 281–290 (2012).
90. Ullah, M. A. *et al.* Allergen-induced IL-6 trans-signaling activates gammadelta T cells to promote type 2 and type 17 airway inflammation. *J. Allergy Clin. Immunol.* **136**, 1065–1073 (2015).
91. Kraakman, M. J. *et al.* Blocking IL-6 trans-signaling prevents high-fat diet-induced adipose tissue macrophage recruitment but does not improve insulin resistance. *Cell Metab.* **21**, 403–416 (2015).
92. Ruwanpura, S. M. *et al.* Therapeutic targeting of the IL-6 trans-signalling/mTORC1 axis in pulmonary emphysema. *Am. J. Respir. Crit. Care Med.* **194**, 1494–1505 (2016).
93. Tecchio, C., Micheletti, A. & Cassatella, M. A. Neutrophil-derived cytokines: facts beyond expression. *Front. Immunol.* **5**, 508 (2014).
94. Mauer, J. *et al.* Signalling by IL-6 promotes alternative activation of macrophages to limit endotoxemia and obesity-associated resistance to insulin. *Nat. Immunol.* **15**, 423–430 (2014).
95. Romano, M. *et al.* Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity* **6**, 315–325 (1997).
96. Klouche, M., Bhakdi, S., Hemmes, M. & Rose-John, S. Novel path to activation of vascular smooth muscle cells: up-regulation of gp130 creates an autocrine activation loop by IL-6 and its soluble receptor. *J. Immunol.* **163**, 4583–4589 (1999).
97. Guerne, P. A., Carson, D. A. & Lotz, M. IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones *in vitro*. *J. Immunol.* **144**, 499–505 (1990).
98. O'Reilly, S., Ciechomska, M., Cant, R. & van Laar, J. M. Interleukin-6 (IL-6) trans signaling drives a STAT3-dependent pathway that leads to hyperactive transforming growth factor-beta (TGF-beta) signaling promoting SMAD3 activation and fibrosis via Gremlin protein. *J. Biol. Chem.* **289**, 9952–9960 (2014).
99. Fielding, C. A. *et al.* Interleukin-6 signaling drives fibrosis in unresolved inflammation. *Immunity* **40**, 40–50 (2014).
100. Evans, S. S., Repasky, E. A. & Fisher, D. T. Fever and the thermal regulation of immunity: the immune system feels the heat. *Nat. Rev. Immunol.* **15**, 335–349 (2015).
101. Eugster, H. P., Frei, K., Kopf, M., Lassmann, H. & Fontana, A. IL-6-deficient mice resist myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *Eur. J. Immunol.* **28**, 2178–2187 (1998).
102. Bernad, A. *et al.* Interleukin-6 is required *in vivo* for the regulation of stem cells and committed progenitors of the hematopoietic system. *Immunity* **1**, 725–731 (1994).
103. Dalrymple, S. A. *et al.* Interleukin-6-deficient mice are highly susceptible to *Listeria monocytogenes* infection: correlation with inefficient neutrophilia. *Infect. Immun.* **63**, 2262–2268 (1995).
104. Romani, L. *et al.* Impaired neutrophil response and CD4 + T helper cell 1 development in interleukin 6-deficient mice infected with *Candida albicans*. *J. Exp. Med.* **183**, 1345–1355 (1996).
105. Chai, Z., Gatti, S., Toniatti, C., Poli, V. & Bartfai, T. Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J. Exp. Med.* **183**, 311–316 (1996).
106. Heink, S. *et al.* Trans-presentation of IL-6 by dendritic cells is required for the priming of pathogenic TH17 cells. *Nat. Immunol.* **18**, 74–85 (2016).
107. McFarland-Mancini, M. M. *et al.* Differences in wound healing in mice with deficiency of IL-6 versus IL-6 receptor. *J. Immunol.* **184**, 7219–7228 (2010).
108. Wunderlich, F. T. *et al.* Interleukin-6 signaling in liver-parenchymal cells suppresses hepatic inflammation and improves systemic insulin action. *Cell Metab.* **12**, 237–249 (2010).
109. Yoshida, K. *et al.* Targeted disruption of gp130, a common signal transducer for the interleukin 6 family of cytokines, leads to myocardial and hematological disorders. *Proc. Natl Acad. Sci. USA* **93**, 407–411 (1996).

110. Betz, U. A. *et al.* Postnatally induced inactivation of gp130 in mice results in neurological, cardiac, hematopoietic, immunological, hepatic, and pulmonary defects. *J. Exp. Med.* **188**, 1955–1965 (1998).
111. Streetz, K. L. *et al.* Lack of gp130 expression in hepatocytes promotes liver injury. *Gastroenterology* **125**, 532–543 (2003).
112. Luchtefeld, M. *et al.* Signal transducer of inflammation gp130 modulates atherosclerosis in mice and man. *J. Exp. Med.* **204**, 1935–1944 (2007).
113. Sander, L. E. *et al.* Hepatic acute-phase proteins control innate immune responses during infection by promoting myeloid-derived suppressor cell function. *J. Exp. Med.* **207**, 1453–1464 (2010).
114. O'Shea, J. J., Gadina, M. & Schreiber, R. D. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* **109** (Suppl. 1), S121–S131 (2002).
115. Sakamoto, K. *et al.* Janus kinase 1 is essential for inflammatory cytokine signaling and mammary gland remodeling. *Mol. Cell. Biol.* **36**, 1673–1690 (2016).
116. Schwartz, D. M., Bonelli, M., Gadina, M. & O'Shea, J. J. Type I/II cytokines, JAKs, and new strategies for treating autoimmune diseases. *Nat. Rev. Rheumatol.* **12**, 25–36 (2016).
117. Lee, C. K. *et al.* STAT3 is a negative regulator of granulopoiesis but is not required for G-CSF-dependent differentiation. *Immunity* **17**, 63–72 (2002).
118. Nguyen-Jackson, H., Panopoulos, A. D., Zhang, H., Li, H. S. & Watowich, S. S. STAT3 controls the neutrophil migratory response to CXCR2 ligands by direct activation of G-CSF-induced CXCR2 expression and via modulation of CXCR2 signal transduction. *Blood* **115**, 3354–3363 (2010).
119. Hillmer, E. J., Zhang, H., Li, H. S. & Watowich, S. S. STAT3 signaling in immunity. *Cytokine Growth Factor Rev.* **31**, 1–15 (2016).
120. Kimura, A. *et al.* SOCS3 is a physiological negative regulator for granulopoiesis and granulocyte colony-stimulating factor receptor signaling. *J. Biol. Chem.* **279**, 6905–6910 (2004).
121. Peschon, J. J. *et al.* An essential role for ectodomain shedding in mammalian development. *Science* **282**, 1281–1284 (1998).
122. Horiuchi, K. *et al.* Cutting edge: TNF-alpha-converting enzyme (TACE/ADAM17) inactivation in mouse myeloid cells prevents lethality from endotoxin shock. *J. Immunol.* **179**, 2686–2689 (2007).
123. Chalaris, A. *et al.* Critical role of the disintegrin metalloprotease ADAM17 for intestinal inflammation and regeneration in mice. *J. Exp. Med.* **207**, 1617–1624 (2010).
124. Kefaloyianni, E. *et al.* ADAM17 substrate release in proximal tubule drives kidney fibrosis. *JCI Insight* **1**, e87023 (2016).
125. Nicolaou, A. *et al.* Adam17-deficiency promotes atherosclerosis by enhanced TNFR2 signaling in mice. *Arterioscler. Thromb. Vasc. Biol.* **37**, 247–257 (2016).

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All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.

### Competing interests statement
L.H.C. declares that he has acted as a consultant for Bristol–Myers Squib, Genentech-Roche, Jansen, Pfizer, Regeneron, Sanofi-Aventis and UCB, and has acted as a speaker for Bristol–Myers Squib, Genentech, Jansen and UCB. S.R.-J. has acted as a consultant and speaker for AbbVie, Chugai, Genentech Roche, Pfizer and Sanofi. He also declares that he is an inventor on patents owned by CONARIS Research Institute, which develops the sgp130Fc protein olamkicept together with Ferring Pharmaceuticals and he has stock ownership in CONARIS. K.W. declares that he has acted as a consultant to AbbVie, Bristol–Myers Squib, Genentech-Roche, Lilly, Pfizer and UCB, and received a research grant from Bristol–Myers Squib.

### Publisher's note


### Review criteria
A search for original articles published between 1997 and 2016 focusing on IL-6 was performed in MEDLINE and PubMed. The search terms used were 'IL-6' alone and in combination with 'genetic knock out', 'gp130', 'inflammation', 'innate immunity', 'adaptive immunity', 'primary immunodeficiency', 'infection' and 'opportunistic infection'; 'arthritis', 'rheumatoid arthritis' and 'autoimmune diseases', alone and in combination; and 'tocilizumab', 'sarilumab', 'sirukumab', 'siltuximab', 'olokizumab', 'clazakizumab', 'ALX 0061' and 'sgp130Fc', alone and in combination with 'infections' or 'opportunistic infections'. All articles identified were English-language, full-text papers. We also searched the reference lists of identified articles for additional relevant papers.

# Pain in ankylosing spondylitis: a neuro-immune collaboration

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Abstract | Clinicians have commonly differentiated chronic back pain into two broad subsets: namely, non-inflammatory (or mechanical) back pain and inflammatory back pain. As the terminology suggests, the latter category, in which ankylosing spondylitis (AS) is prominent, presupposes a close link between pain and inflammation. Advances in research into the genetics and immunology of AS have improved our understanding of the inflammatory processes involved in this disease, and have led to the development of potent anti-inflammatory biologic therapeutic agents. However, evidence from clinical trials and from biomarker and imaging studies in patients with AS indicate that pain and inflammation are not always correlated. Thus, the assumption that pain in AS is a reliable surrogate marker for inflammation might be an over-simplification. This Review provides an overview of current concepts relating to neuro-immune interactions in AS and summarizes research that reveals an increasingly complex interplay between the activation of the immune system and pain pathways in the nervous system. The different types of pain experienced by patients with AS, insights from brain imaging studies, neurological mechanisms of pain, sex bias in pain and how the immune system can modify pain in patients with AS are also discussed.

Ankylosing spondylitis (AS) is an immune-mediated inflammatory disorder that primarily affects the spine. Although evidence indicates that environmental factors such as gut dysbiosis[1] contribute to an individual's susceptibility to developing AS, disease risk is strongly influenced by genetics[2,3]. *HLA-B27* is the major genetic component associated with risk of AS; however, >60 other genes are associated with AS risk and seem to cluster in distinct pathways involved in processes such as antigen processing and IL-17 production[4]. This genomic insight has brought type 3 innate and adaptive cell-mediated immunity to the forefront of AS research. In this type of immunity, the immune response is directed towards extracellular microorganisms and is polarized towards IL-17 production. Type 3 immune cells, such as neutrophils, type 3 innate lymphoid cells, γδ T cells, mucosal-associated invariant T cells and CD4$^+$ T helper 17 ($T_H17$) cells, typically express IL-23 receptor and retinoic acid receptor-related orphan receptor-γt (RORγt)[5]. By contrast, type 1 immunity is typified by IFNγ production and $T_H1$ cell activation, and type 2 immunity involves IL-4 and $T_H2$ cells.

Support for a central role of type 3 immunity in AS comes from immunological studies demonstrating an increased number of type 3 immune cells and increased circulating levels of type 3 cytokines in patients with AS; from animal models that show protection from disease progression when type 3 immune factors are absent or their effects are blocked; from the enrichment of genes associated with a type 3 immune response in genome-wide association studies; and from the success of anti-IL-17 therapeutics in clinical trials[6–8]. Evidence from a study published in 2016 indicates that the strong type 3 immune profile seen in patients with AS is more prominent in men than in women[9]. Despite the immune-centric emphasis of AS research, immune assessment in clinical practice is largely restricted to measuring simple inflammatory markers such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels.

The assessment of pain (see BOX 1 for an official definition of pain and BOX 2 for details of how pain can be assessed in humans and animals) forms the cornerstone of clinical examination of patients with AS, with inflammatory back pain being a cardinal feature of this disease. Although pain in AS is assumed to be a surrogate marker for inflammation, it does not always correlate with inflammation or radiographic measures of disease[10]. Indeed, some experimental therapies that reduce IL-6 signalling and acute inflammatory markers, such as CRP, fail to reduce pain in patients with AS[11,12]. Therefore, a disconnect exists between the immunological perspective of basic research and the everyday symptomatic management of AS.

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**Key points**

- Inflammatory mediators can directly and indirectly induce pain
- Pain in ankylosing spondylitis (AS) is not strictly inflammatory and involves a neuropathic component
- Brain abnormalities that underlie chronic pain can be detected in AS
- An individual's sex affects the degree of neuro-immune involvement in pain, and sex differences in pain perception might contribute to sexual dimorphisms in AS presentation
- Pain in AS might involve distinct molecular underpinnings and so strategies to treat pain might differ from those that target inflammation

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Few studies have explicitly distinguished pain management from the suppression of inflammation in the treatment of AS. If this disconnect is to be overcome, rheumatologists need to gain a better understanding of the neurobiology of pain and the neuro-immune interactions involved in AS. Indeed, advances in pain research since the 1980s have highlighted an integral role for the immune system in modulating acute and chronic pain (BOX 1) in both the peripheral nervous system (PNS) and the central nervous system (CNS)[13,14]. Furthermore, not all pain in patients with AS is inflammatory; a 2013 study demonstrated the presence of neuropathic pain (BOX 1) in patients with AS[15]. In this Review, we discuss the different types of pain that patients with AS can experience, the neurological mechanisms that underlie these forms of pain and how the immune system can contribute to the experience of pain in patients with AS.

## Pain in AS

AS is characterized by inflammatory back pain that is accompanied by radiographic changes to the spine and sacroiliac joints (SIJs)[7]. Of the four cardinal signs of inflammation — *dolor* (pain), *rubor* (redness), *calor* (heat) and *tumor* (swelling) — only pain is a feature of AS, with the other signs being clinically undetectable when the disease is confined to the spine (FIG. 1). Pain in AS usually manifests in the lower back and buttocks, and is deep and dull, with an insidious onset and nocturnal exacerbations. AS-associated pain can be intermittent at onset but gradually transforms to a persistent pain. The quality of pain is described by patients as aching, sore, tiring, sharp, hurting, tender, tight, exhausting, annoying, pinching or miserable. Patients with higher pain scores tend to have a wider range of pain symptoms and more paroxysmal-type pain descriptions than do patients with lower pain scores[15]. Pain screening tools clearly show a neuropathic component for AS pain[15].

Inflammatory back pain is one of the key clinical criteria for the classification of AS[16]. Furthermore, pain is a central component of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)[7,17] (a widely used clinical measure of AS activity) and pain improvement is commonly a primary outcome in studies assessing patient responses to treatment[18].

***Pain and fatigue.*** Although fatigue is a normal response of the body to exertion, disease-associated fatigue is chronic, debilitating and difficult to treat. Severe fatigue affects almost one-third of patients with AS and is not commonly associated with objective clinical measures of disease[19]; however, fatigue is regarded to be an indicator of disease activity in AS and is included as a core element in the BASDAI. Results from studies into the relationship between pain and fatigue in AS are conflicting. Fatigue in AS strongly correlates with pain[20] but is not reliably controlled by pain management[21]. Despite this reported correlation between pain and fatigue, some studies have found that pain does not account for the complete experience of fatigue[21–23]. In agreement with this disconnect between pain and fatigue, a longitudinal study of patients with AS showed that pain can progress independently from fatigue, highlighting the importance of targeting these symptoms independently[24].

Fatigue in AS is also associated with structural brain abnormalities within the sensorimotor network, the salience network and attention brain networks[25], and the effect of treatment with biologic DMARDs (also known as 'biologics') on pain relief correlates with structural brain changes that are different from those that correlate with the effects of treatment on fatigue relief[22]. Thus, evidence suggests that, in addition to exploring the pain–immune interface in AS, fatigue should also be studied, as it has debilitating effects and potentially unique underlying mechanisms[25].

***The pain–inflammation link.*** The relationship between pain and inflammation in AS is complex. For example, in some patients, radiographic and MRI scores of the SIJs and spine correlate better with CRP levels than with pain scores or BASDAI scores[26–28]. By contrast, in non-radiographic AS, pain, as well as clinical signs and symptoms of disease, exists in the absence of sacroiliitis on plain radiography[29]. However, MRI findings of SIJ inflammation are commonly present in such patients[29]. Furthermore, comorbidities such as mechanical back pain or fibromyalgia complicate the pain–inflammation link as the pain in these conditions is influenced by inflammatory and non-inflammatory factors[30,31]. Emotional well-being can also strongly influence pain perception and is an important consideration given the high rates of depression among patients with AS[32]. Finally, the pain–inflammation

Dysbiosis
A microbial imbalance associated with disease that is typified by a loss of protective bacteria and a gain of pathogenic bacteria.

Inflammatory back pain
A chronic pain of more than 3 months that has a relatively young age of onset (< 45 years); morning stiffness is a major symptom associated with inflammation, and unlike mechanical back pain, inflammatory back pain is improved by movement rather than rest.

Bath Ankylosing Spondylitis Disease Activity Index (BASDAI)
A patient-reported outcome that incorporates pain, fatigue and stiffness.

Sensorimotor network
A set of brain regions that includes the primary somatosensory and primary motor cortices; the activity of these regions is related to sensory and/or motor function, and is typically evoked by sensory stimuli or motor tasks.

Salience network
A set of brain regions that function to detect and respond to salient stimuli, such as those that are of value or importance, or simply those that are more prominent than other stimuli.

Inflammatory pain
Pain occurring following an injury or in a disease that involves the release of inflammatory agents that activate and/or sensitize nociceptors.

relationship is affected by an individual's sex. Relative to males with AS, females with AS have more self-reported pain and functional limitations despite having less radiographic progression and lower levels of circulating acute phase reactants[33,34]. Among healthy individuals, women rate noxious stimuli (BOX 1) as more painful during systemic inflammation than do men[35], which suggests an elevated inflammatory pain response in females. This sex bias in pain perception could be partly due to sex-dependent pain perception pathways[33,34] or the effects of sex hormones[36]; indeed, animal models indicate that the mechanisms that underlie pain differ in a sex-dependent manner.

It is important to note at this point that a sex bias exists in the field of pain research, and this sex bias is in part reflected by the fact that most published preclinical pain studies use only male rodents[37]. Human psychophysical and brain imaging studies have, in some instances, examined and found major sex-related differences in pain sensitivity, tolerance and adaptation or habituation, as well as brain activity and connectivity, which might underlie such perceptual differences[38–42]. Efforts to resolve this sex bias in animal studies have revealed that mechanisms of pain can be sexually dimorphic, as neuropathic pain in female mice is not dependent on spinal microglia, as it is in male mice[43–45] (FIG. 2).

Box 1 | **Definitions of pain and related terms**

**Pain**
The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (REF. 138). More detail relating to this short definition was provided by the IASP in 2002 (REF. 139). An updated definition of pain was proposed in 2016 but has not yet been adopted; this definition broadens and clarifies the current definition, and states that "pain is a distressing experience associated with actual or potential tissue damage with sensory, emotional, cognitive, and social components" (REF. 140).

**Chronic pain**
A classic definition of chronic pain is "pain which persists past the normal time of healing" (REFS 138,141). This concept emphasizes chronic pain as being the prolonging of acute pain, but many disease states that involve chronic pain do not stem from an acute pain or injury that can be healed[119]. In 2015, chronic pain was defined for the International Classification of Diseases of the WHO as "persistent or recurrent pain lasting longer than 3 months" (REF. 142).

**Nociceptive pain**
This term is typically used to describe pain that occurs in situations in which the somatosensory nervous system is functioning in a normal fashion. The IASP definition of nociceptive pain is "pain that arises from actual or threatened damage to non-neural tissue and is due to the activation of nociceptors" (REF. 139).

**Neuropathic pain**
The official IASP definition of neuropathic pain is "pain caused by a lesion or disease of the somatosensory nervous system" (REF. 138). In 2002, the IASP expanded on the complexity of this definition as a clinical term that is not based solely on signs or symptoms[139]. Nonetheless, neuropathic pain is characterized by a burning or shooting pain accompanied by sensory loss or by sensory gain (that is, allodynia or hyperalgesia)[142]. These characteristics form the cornerstone of the painDETECT[143] and other questionnaires that are used to measure neuropathic pain and assist in its diagnosis.

**Noxious stimuli**
The IASP definition of a noxious stimulus is "a stimulus that is damaging or threatens damage to normal tissues" (REF. 139). Such stimuli can be mechanical, thermal or chemical in nature.

Despite this sex difference, the immune system remains a key factor in female and male pain processing (FIG. 2). Preclinical experiments suggest that T cells might be crucial in the regulation of neuropathic pain in female mice[43]. Nerve injury induces the migration of T cells into the spinal cord, thus contributing to the development of neuropathic pain symptoms. Phenotypic analysis has revealed that the infiltrating cells are typically $T_H1$ cells[46]. The mechanisms that underlie nerve injury-induced central sensitization seem to be congruent across the sexes, as the potentiation of excitatory activity has been implicated in neuropathic pain in both male and female mice[43]. Thus, T cell-derived proinflammatory mediators might elicit changes in the spinal cord pain circuitry that are similar to those induced by brain-derived neurotrophic factor (BDNF) produced by microglia (FIG. 2). Furthermore, high oestrogen levels in female mice cause increased proinflammatory cytokine release from immune cells, which might account for why the pain response in females is higher than that in males[47]. Overall, neuro-immune interactions have a crucial role in the genesis of pain; however, the nature of these interactions can differ between the sexes.

Sex differences in the function of the neuro-immune interface might also have important implications in AS and could underlie sex differences in clinical presentation. Both male and female patients with AS have altered circulating numbers of $T_H1$ cells and levels of IFNγ[9]; however, the possibility exists that $T_H1$ cells and IFNγ could influence pain perception in different ways depending on the patient's sex. Furthermore, although the blockade of IL-17 is effective at lowering patient-reported outcomes in both male and female patients with AS, male patients (rather than female patients) have elevated type 3 immunity[9]. As yet, sex differences in the response to anti-IL-17 therapy among patients with AS have not been formally investigated, and further research is required to elucidate the precise influence of sex differences in immunity on AS presentation.

***The effects of treatment on pain in AS.*** NSAIDs are widely used to treat inflammatory pain conditions as they inhibit cyclooxygenases and prostanoids (important pain mediators)[14], and constitute the first-line therapy for AS, providing rapid pain relief. A subset of patients respond extremely well to NSAIDs[48]; however, the evidence indicating that NSAIDs control radiographic progression is limited[49]. Results from an observational cohort study showed that NSAID intake over time was low and that treatment adherence decreased despite the persistence of symptoms[50]. Another study reported that NSAID use among patients with AS is not well tolerated and is associated with poor compliance[51]. MRI results revealed reduced inflammation in the SIJs of patients who received an optimal dose of NSAIDs early in the course of disease, but this reduction in inflammation correlated poorly with clinical improvement[51].

If NSAIDs fail to control pain, alternative treatment is indicated. Biologics are the most common second-line therapy for patients with AS whose symptoms are not controlled by NSAIDs. The biologics currently in clinical

Allodynia
Pain that is evoked by stimuli that are not normally painful, such as a light touch, gentle heat or mild cooling.

Central sensitization
Enhanced response of the neurons in the central nervous system to normal stimuli.

Functional brain networks
Sets of brain regions for which activity is correlated over time.

Default mode network
A set of brain regions that have ongoing activity during a so-called resting state; this network is thought to monitor one's internal and external environment, and then become suppressed when the individual needs to respond to a situation.

use improve the symptoms of AS rather than preventing structural damage, so pain control remains a central part of the management of AS.

TNF inhibitors were the first biologics to be approved for treatment of AS, and five different TNF inhibitors are now approved for AS[52]. TNF is a potent proinflammatory cytokine and is expressed at abundant levels in the SIJs and affected synovia of patients with AS[53]. In addition to the effects of TNF inhibitors on peripheral inflammation, they can act on the CNS to reduce pain[54]. In patients treated with TNF inhibitors, pain response patterns vary considerably[22]. The effect of TNF inhibition on radiographic progression might be dependent on the early initiation of treatment[7,53,55]. Despite the overall clinical effectiveness of TNF inhibition, 20–30% of patients show an inadequate clinical response, which most commonly reflects a lack of improvement in pain, but also occasionally reflects changes in other components of the BASDAI[56]. Switching to another TNF inhibitor is recommended in patients who do not respond to the first agent[57].

Biologics that target IL-1, IL-6, and T cell costimulatory pathways were ineffective in clinical trials and have not been approved for the treatment of AS; however, evidence indicating the central roles of IL-17 and IL-23 in AS has led to the development of treatments that targeting these cytokines[7,58]. For example, secukinumab, a high-affinity, fully humanized monoclonal anti-human IL-17A antibody, improves the symptoms of AS and has now been approved for the treatment of AS[53,59].

The Assessment of SpondyloArthritis International Society (ASAS)–EULAR treatment recommendations for AS also include analgesics such as paracetamol (acetaminophen) and opioids, but the effects and mechanisms of action of these analgesics in AS have not been studied in a systematic manner[60]. Notably, standard analgesics, such as opioids, are somewhat ineffective at relieving neuropathic pain in animal models[61].

Although considerable advances have been made in targeting inflammation, little work has been done on targeting neuropathic pain in AS. Treatments that target both inflammatory and neuropathic pain might represent potential drug options in AS. New drugs that target transient receptor potential cation channel subfamily A member 1 (TRPA1) in mice, for example, reduce inflammatory and neuropathic pain and could represent a future strategy for the treatment of AS[62–64]. MicroRNAs are also novel and promising therapeutic targets for the prevention and treatment of inflammatory and neuropathic pain[65,66]. The blockade of nerve growth factor (NGF), which is a new approach for the treatment of osteoarthritis-associated pain, is another example of a treatment approach that is worth investigating in AS[67,68]. Further work is needed to improve the treatment of pain in AS, and new therapeutic approaches will be informed by studies investigating the mechanisms that underlie pain. Insights into these mechanisms can be obtained through brain imaging studies.

## Brain imaging in chronic pain

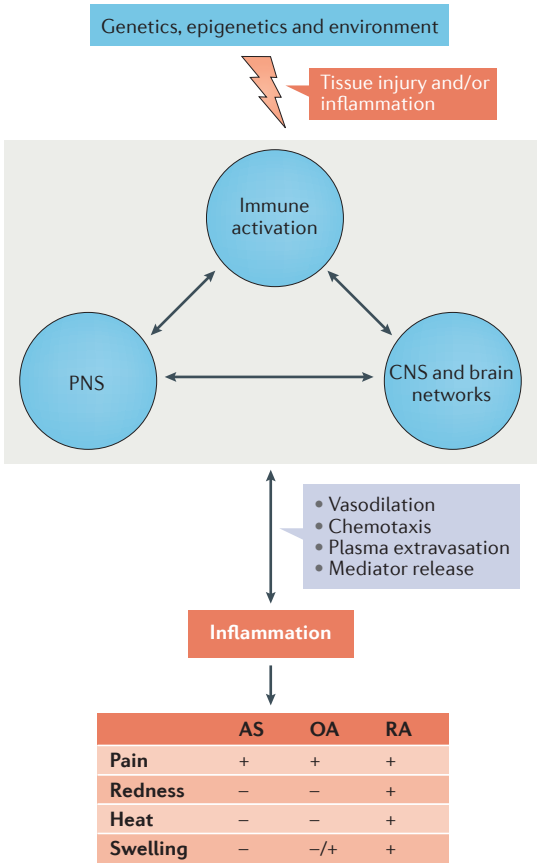
Imaging the brain using MRI can provide insights into abnormalities in the structure and function of the CNS in chronic pain conditions. Structural abnormalities can be identified through measurements of brain grey matter (for example, volume and cortical thickness) and white matter (such as integrity and connectivity). Functional brain abnormalities identified in chronic pain include altered stimulus-evoked and task-evoked responses in specific brain regions (for example, the brainstem, insula, and the somatosensory, cingulate and prefrontal cortices), as well as abnormalities in the response and connectivity of functional brain networks (for example, the default mode network, the salience network and the sensorimotor network)[69–76]. Thus, the structure and function of brain regions associated with pain — namely, the networks underlying sensorimotor function, attention, salience and the default mode — are altered in patients with chronic pain. These brain regions collectively comprise the 'dynamic pain connectome' (REFS 70,77). Brain imaging has also been used to delineate the plasticity of brain abnormalities associated with pain relief following treatment. For example, effective treatment can normalize the insula and prefrontal cortex thinning that is seen in patients with trigeminal neuralgia, neuropathic pain following peripheral nerve injury or chronic lower back pain[69,78,79], and can also normalize abnormal functional connectivity of the insula in patients with chronic lower back pain[80].

### Box 2 | How pain is measured and modelled experimentally

In humans, the measurement of pain is generally restricted to non-invasive approaches. The most commonly used approach involves measuring patient-reported outcomes, usually in the form of questionnaires such as the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) or the McGill pain scale[144–146]. These clinical measurements are rapid and cheap and can be performed in the clinic or remotely. Brain imaging techniques, such as functional MRI (fMRI), can be used to measure regional activity in the central nervous system in response to stimuli, as well as the resting state of functional connectivity between regions and networks. fMRI is an experimental method of measuring pain that requires specialized equipment and a trained reader, so is not amenable to routine clinical application.

Experimentally, pain can be induced in humans in a controlled fashion. Thermal and mechanical pain can be evoked by controlled, non-invasive stimuli via heated probes, calibrated pressure probes or von Frey filaments. Low-risk, invasive tests can be performed to measure the efficacy of the brain's endogenous modulation system (for example, the conditioned pain response), as well as the response to, and sensitization following exposure to, mechanical stimuli, thermal stimuli or chemical stimuli such as the administration of capsaicin or hypertonic saline solution[147].

In animals, a range of behavioural tests can be used to measure acute pain. Responses to noxious thermal stimuli can be assessed by assays such as the tail flick test[148] or the Hargreaves' Method[149], which involve measuring the latency of the tail or paw movement, respectively, away from noxious radiant heat. Responses to noxious mechanical stimuli are most often measured using von Frey testing, which involves assessing withdrawal responses to the application of filaments that have been calibrated to exert a specific mechanical force[150]. Spontaneous pain can be assessed through the quantification of behaviours such as facial expressions of pain[151] or the use of the conditioned place preference paradigm[152]. In addition, the use of optogenetics to activate pain pathways in rodents has become feasible within the past 5 years[153]. A wide variety of animal models of chronic pain exist. Models of inflammatory pain are often induced by the application of inflammatory substances such as complete Freund's adjuvant or zymosan[154], and involve various routes of administration, such as subcutaneous or joint injections. Examples of models of neuropathic pain include the spared nerve injury model[152] and the streptozotocin-induced diabetic neuropathic pain model[155].




| | AS | OA | RA |
|---|---|---|---|
| **Pain** | + | + | + |
| **Redness** | – | – | + |
| **Heat** | – | – | + |
| **Swelling** | – | –/+ | + |

Figure 1 | **Pain in ankylosing spondylitis.** A range of factors contribute to the generation of pain, including genetic, epigenetic and environmental factors. Pain results from a complex interaction between immune factors, peripheral nervous system (PNS) activity and central nervous system (CNS) activity. Pain is a crucial readout in rheumatic diseases and is one of the cardinal signs of inflammation. Whereas other inflammatory rheumatic diseases, such as rheumatoid arthritis (RA), feature redness, heat and swelling, ankylosing spondylitis (AS) does not feature these cardinal signs. OA, osteoarthritis.

Brain imaging in patients with chronic pain has also revealed a relationship between brain abnormalities and specific aspects of the particular pain condition. Such relationships have been identified across a range of conditions, including temporomandibular disorder[81,82] and irritable bowel syndrome[83,84]. Functional MRI (fMRI) studies, which measure blood flow as a proxy for brain activity, have also provided insights into the neural mechanisms that underlie the cognitive and emotional facets of chronic pain. In temporomandibular disorders and migraine, pain catastrophizing (the perception of pain as devastating) and rumination (over-thinking about pain) are associated with the extent to which brain function is altered[71,85]. Furthermore, activation of the default mode network during the performance of cognitive tasks is altered in patients with temporomandibular disorders and migraine[72,86]. The relationships frequently observed between brain activity and pain, emotion or cognition suggest potential mechanisms affecting the brain, or mechanisms by which the brain influences behaviour.

## Brain imaging in AS

Similarly to patients with other chronic pain conditions, patients with AS who are experiencing pain exhibit both structural and functional brain alterations. Using fMRI, the functional connectivity between two brain networks was found to be abnormally high in patients with AS compared with healthy individuals[87] (FIG. 3a). Among patients with AS, this higher degree of abnormal connectivity between the default mode network and the salience network was related to a higher pain intensity and higher disease activity scores[87]. These findings suggest that abnormalities in brain function are related to the severity of chronic pain in AS. Moreover, insights into the relationship between treatment with biologics and brain function can be gleaned from a study in patients with rheumatoid arthritis, in which fMRI was performed while the patients experienced evoked joint pain both before and 24 hours after treatment with biologics[54]. 24 hours after treatment, fMRI signals in pain-related brain regions were reduced in conjunction with improved patient-reported outcomes, with these observations being made before any reduction in joint swelling or in the levels of circulating acute phase reactants were seen. This study suggests that some of the benefit of biologics might not be the direct result of reduced inflammation and that examining the CNS response could be beneficial when studying targeted pain interventions.

Structural brain imaging studies have also had an integral role in improving our understanding of how the structure of the brain changes in relation to pain quality and treatment response in AS. The structure of grey matter and white matter was abnormal in patients with AS and some of these abnormalities correlated with fatigue[25]. In addition, a longitudinal study showed that biologic treatment affected the AS-associated structural abnormalities that are found in sensorimotor regions[22]. Changes in different grey matter regions correlated with the post-treatment relief of either pain or fatigue, suggesting that separate neural mechanisms underlie each of these cardinal symptoms of AS.

Insights gained from brain imaging studies of patients with AS have influenced our understanding of AS and provided insights that have the potential to inform future treatment strategies. These brain imaging studies also indicate that the CNS abnormalities associated with AS are malleable. As the primary pathophysiology in AS is a peripheral event, the observed brain abnormalities are likely to be secondary and occur as a result of aberrant PNS input into the CNS. However, as is the case for many chronic pain conditions that begin with a peripheral insult, the pain can become centralized, and targeting the CNS to achieve pain relief could represent a new area for investigation when peripheral treatments are not effective. Future applications of brain research in AS include predicting responses to treatment on the

Functional MRI (fMRI)
An MRI-based technique that is used to identify areas of evoked brain responses (for example, responses to a task or stimulus).

Functional connectivity
The degree to which the brain activity in certain regions fluctuates together over time.

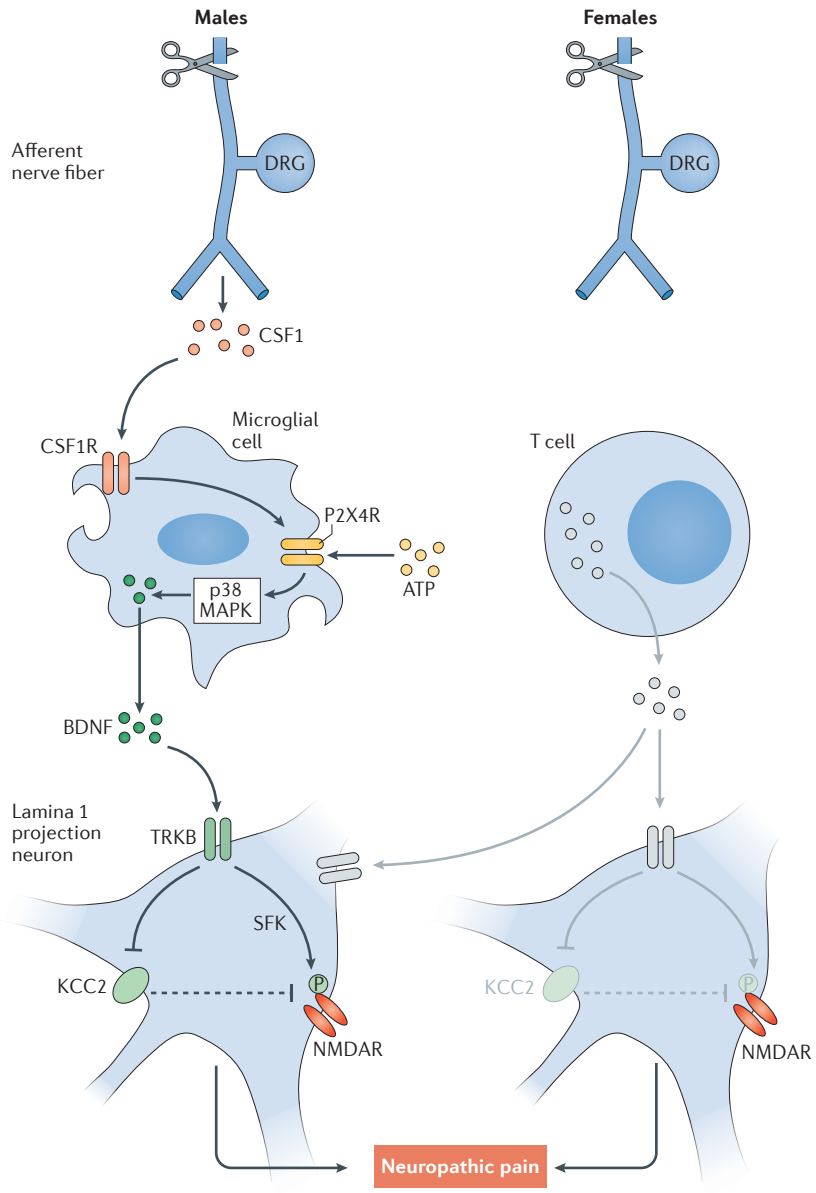



Figure 2 | **The pathways that underlie neuropathic pain processing in female and male mice.** In male mice, peripheral nerve injury (scissors) results in the release of colony-stimulating factor 1 (CSF1) from afferent nerve fibres. CSF1 binds to CSF1 receptor (CSF1R) on microglia[156], and CSF1R activity induces the upregulation of P2X4 receptor (P2X4R)[156]. ATP–P2X4R signalling activates p38 mitogen-activated protein kinase (MAPK), leading to the production and release of brain-derived neurotrophic factor (BDNF). BDNF binds to tropomyosin receptor kinase (TRKB; also known as BDNF/NT3 growth factors receptor) on lamina 1 projection neurons, causing the downregulation of potassium–chloride cotransporter 2 (KCC2) and the phosphorylation of *N*-methyl-D-aspartate receptor (NMDAR)[157]. The downregulation of KCC2 is required for the potentiation of NDMAR signalling[157]. Together, these changes result in increased neuronal excitability, which leads to the development of neuropathic pain. In female mice, microglia are not involved in neuropathic pain processing. T cells have been proposed to be crucial in mediating neuropathic pain in female mice[158], and might also contribute to pain processing in male mice[159,160]. However, the details of how T cells mediate changes in neuronal excitability in the spinal cord have not been determined. Components of the proposed T cell-dependent pathway that have yet to be identified are shown in grey. Despite the sex difference in immune cell involvement in neuropathic pain, NMDAR contributes to pain processing in mice of both sexes[43]. DRG, dorsal root ganglion; SFK, SRC family kinase.

basis of baseline brain characteristics and targeting the brain in patients with refractory pain using approaches such as transcranial magnetic stimulation or cognitive behavioural therapy.

***Evidence of neuropathic pain in AS.*** Historically, pain in AS has been considered to be inflammatory and not neuropathic. However, work using brain imaging alongside the painDETECT screening tool for neuropathic pain has provided data indicating that mixed pain is a feature of AS: that is, AS is characterized by mixed inflammatory–neuropathic pain[15]. A relationship exists between higher painDETECT scores and cortical thickening in sensorimotor and salience-related brain regions, suggesting that central abnormalities underlie neuropathic pain in AS. Patients with AS also exhibit mechanical and cold detection deficits on the dorsum of their feet, an area of skin innervated by the same spinal nerves as the lower back[15]. Together, these findings indicate that further study of the neuropathic pain component of AS is needed.

## Cellular and molecular neurobiology of pain

Primary afferent nociceptors are specialized sensory neurons of the PNS. Noxious stimuli activate nociceptor endings that innervate peripheral tissues, including the skin, muscles, joints and viscera[88]. These first-order neurons have their cell bodies in the dorsal root ganglia (DRG) and synapse onto second-order neurons in the dorsal horn of the spinal cord[88] (FIG. 3b). Complex processing of peripheral pain signals occurs in the dorsal horn before transmission of the pain signal to the brain for further processing. Nociceptors that innervate the skin are well-characterized owing to their accessibility. Cutaneous nociceptors include myelinated, fast-conducting Aδ fibres and non-myelinated, slow-conducting C fibres[89]. Nociceptors that innervate cutaneous and deep structures are classified on the basis of their responses to noxious mechanical, thermal and chemical stimuli[90]. Many nociceptors terminate in close proximity to environment-sensing tissue-resident cells such as keratinocytes and Langerhans cells[89]. During inflammation, particularly in chronic pain conditions, immune cells such as mast cells and T cells accumulate around nociceptor nerve terminals[91,92].

Many primary afferent nociceptors that innervate the skin and deep nociceptors in the joints, muscles and viscera respond to inflammatory mediators and can become 'sensitized' (more easily excited) by such conditions[93–96]. Joints are rich in sensory neurons; however, some articular tissues are more sensitive than others to noxious stimuli[97]. For example, cartilage is relatively insensitive to mechanical pain, whereas the synovium and ligament insertions are sensitive to mechanical pain. Furthermore, bone itself is rich in nociceptors that are concentrated in the periosteum, subchondral bone and marrow[98]. Facet joints are also richly innervated, and evidence indicates that mechanical damage or inflammation-induced swelling of these joints can lead to back pain[99]. Studies in humans have shown that the ligaments of the SIJs contain nociceptors that elicit unilateral lower back pain following the local injection

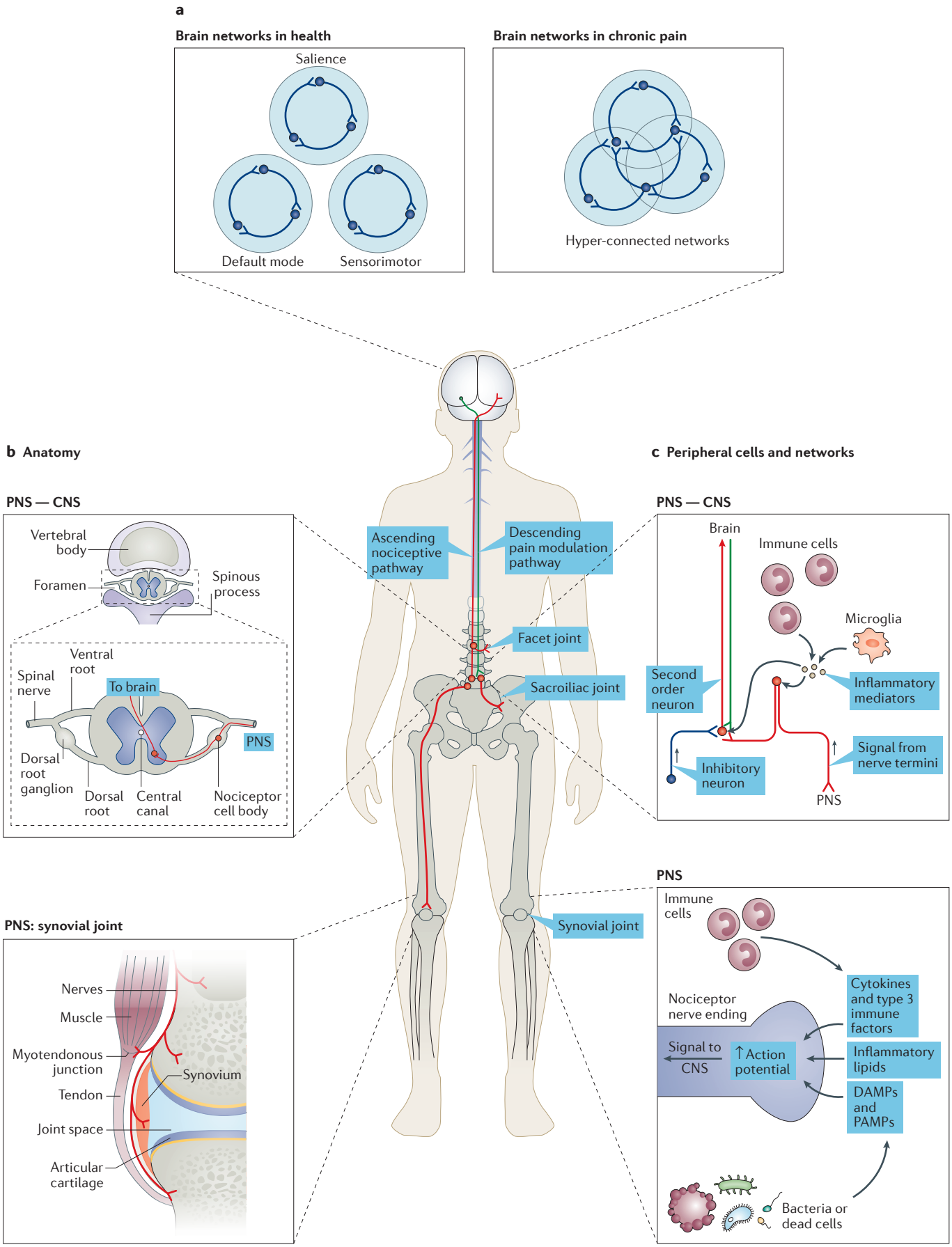


Figure 3 | **Pain pathways in health and during chronic inflammatory conditions.** The central panel illustrates how facet, sacroiliac and synovial nociceptors of the peripheral nervous system (PNS) meet in the spinal cord to send a signal to the brain via second-order neurons. **a** | The relationship between sensorimotor, default mode and salience networks in healthy individuals (left). In patients with chronic pain due to ankylosing spondylitis, interaction between these three networks is altered, including greater cross-network functional connectivity compared to healthy individuals (right). **b** | The peripheral and central neuroanatomy of pain pathways (red) that link nociceptors to connectomes in the central nervous system (CNS). **c** | Inflammatory molecules and cells can modulate the pain pathway and the dynamic pain connectome is altered during chronic pain. DAMP, damage-associated molecular patterns; PAMP, pathogen-associated molecular patterns.

of hypertonic saline solution[100,101]. The nociceptor fibres within tendons and ligaments do not infiltrate heavily into the fibrous tissue itself, but rather concentrate in the myotendinous junctions and insertions[102].

At the molecular level, noxious stimuli depolarize nociceptor peripheral terminals through the activation of ion channels[89,103]. This depolarization can be induced as the result of a direct action on the ion channels, such as the activation of transient receptor potential cation channel subfamily V member 1 (TRPV1) by capsaicin or the stimulation of acid-sensing ion channels by low pH[104]. Depolarization can also be induced indirectly through the activation of tissue-resident cells. For example, ultraviolet light-exposed keratinocytes release endothelin to activate nociceptors that mediate the pain associated with sunburn[105,106]. Of note, the mechanisms that underlie the activation of nociceptive neurons differ from those that underlie the activation of sensory (proprioceptive) neurons[107], and that nociceptive neurons having higher activation thresholds than do sensory neurons[89].

## Immune-mediated sensitization

***Peripheral sensitization.*** Neurons can sense danger signals (damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs); FIG. 3c) directly through the expression of an array of innate immune receptors[14]. Extracellular nucleotides, such as ATP released during cell stress or death, represent a classic danger signal recognized by P2 purinergic receptors (also known as P2 receptors)[108]. P2 receptors are subdivided into ionotropic P2X receptors (ligand-gated ion channels) and metabotropic P2Y receptors (G protein-coupled receptors)[108]. In neurons and microglia, P2X receptors become permeable to ions when activated, leading to depolarization, whereas P2Y receptors mediate peripheral sensitization through TRPV modulation[109]. Nociceptors also express a range of Toll-like receptors (TLRs)[110]. Lipopolysaccharide (LPS) sensitizes nociceptors through the TLR4-induced activation of TRPA1, which mediates the pain response[111]. Interestingly, women seem to be more sensitive to LPS-induced pain sensitization than men[35], and this sensitivity might be potentiated by oestrogen[36]. The expression of TLR7 on nociceptors is crucial in mediating pruritus, but not mechanical or thermal pain in mice[112]. Flagellin and high-mobility group protein B1 mediate hypersensitivity to pain, an effect that is probably a result of the expression of TLR5 on nociceptors[113,114].

Perhaps more striking than their ability to directly sense danger signals is the wide range of immune factors to which nociceptors can respond. Nociceptors express many cytokine and chemokine receptors such as CC chemokine receptor 2 (CCR2) and CXC chemokine receptor 5 (CXCR5), and the receptors for IL-5, IL-1, IL-6, TNF and IL-17 (REFS 14,115). In relation to type 3 innate and adaptive cell-mediated immunity, IL-6 is involved in both neuropathic and inflammatory pain[116,117], whereas IL-17 directly increases nociceptor excitability and can potentiate hyperalgesia through the induction of secondary factors[14]. Nociceptors also express receptors for arachidonic acid-derived inflammatory lipids (namely, prostaglandins and leukotrienes), as well as receptors for histamine[14]. Immune cells can release neuron-targeting factors such as NGF that act on nociceptor-expressed tropomyosin receptor kinase A (TRKA; also known as high-affinity NGF receptor)[118]. Although the effect of these inflammatory mediators on nociceptors is diverse, ranging from activating plasma membrane ion channels to inducing the trafficking of vesicular ion channels to the cell surface, the result is hyperalgesia through nociceptor sensitization[14,119].

Although most of these mechanisms of inflammation-associated sensitization have been examined at the level of nociceptor termini, accumulating evidence indicates that a neuro-immune interaction occurs in the DRG. Immune cells can be found in the DRG, and their numbers increase during chronic inflammatory conditions[120,121]. An example of such an interaction is the sensitization of $CXCR5^+$ DRG neurons by CXC chemokine ligand 13 (CXCL13) during inflammatory pain[122].

***Central sensitization.*** Potentiation of the activity in the centrally located nociceptive circuitry has been implicated in regulating many chronic pain conditions such as inflammatory pain, neuropathic pain and migraine[123]. Central sensitization encompasses changes in the excitability of both second-order neurons in the spinal cord and higher-order neurons in supraspinal regions (for example, the thalamus, brainstem and cortex)[123–125]. The excitability of centrally located neurons is dependent on a relative balance between inhibitory and excitatory processes[126]. Immune system activation due to chronic stimulation seems to be crucial in disrupting this balance and leads to the development of central sensitization and to the symptoms of chronic pain[123]. For example, extensive preclinical evidence from rodents supports an integral role for microglia in neuropathic pain processing[127–129]. Traumatic nerve injury causes the upregulation of ATP-sensitive P2X4 receptors on microglia in the spine[127], leading to the synaptic secretion of the key signalling molecule BDNF[128,130] (FIG. 2). BDNF activity reduces the expression of potassium–chloride cotransporter 2 (KCC2; also known as solute carrier family 12 member 5) on neurons in the spinal cord that transmit pain signals to the brain[128,131]. The downregulation of KCC2 disrupts spinal inhibitory processes as a result of the dysregulation of chloride homeostasis. The amplification of spinal excitatory activity has also been implicated in neuropathic pain processing[132,133]. A combination of

PainDETECT screening tool
A screening tool that is used to evaluate the likelihood of the presence of a neuropathic pain component in patients with chronic pain using a questionnaire comprising seven questions that evaluate the quality of pain, sensitivity to light touch or mild temperature, numbness, pain radiation and the temporal pattern of pain.

Mixed Pain
Pain that has multiple components; for example, in ankylosing spondylitis there might be elements of both neuropathic pain and inflammatory pain.

Peripheral sensitization
Increased sensitivity of nociceptors to stimulation resulting from tissue injury.

Hyperalgesia
An increased amount of pain that is evoked by a stimulus that is normally painful.

decreased inhibition and enhanced excitation results in the potentiation of pain transmission in the spinal cord, and this potentiation might be key in the development of neuropathic pain symptoms[134], although this hypothesis requires further investigation.

## Areas for future investigation

Studies in the basic and clinical neuroscience fields have highlighted the crucial role of the immune system in mediating pain[135]. Despite this progress, very little attention has been paid to the link between pain and inflammation in AS. Detailed basic research studies into the molecular mechanisms of pain in AS, specifically looking at how pain relates to AS-associated inflammation, are required. At a minimum, animal studies of spondyloarthritis should include pain indicators as a readout to enhance the translation of this research into the clinic. In humans, immunological readouts such as cytokine levels and immune cell frequencies often fail to correlate with patient-reported outcomes[136]. Therefore, additional readouts of pain and nociception, such as brain imaging or patient sensitivity tests, should be performed in conjunction with immunological readouts to better define the relationship between pain and inflammation in AS.

Clinical research needs to take the classification of pain in AS into consideration, particularly with regard to neuropathic pain. Such research should also consider the heterogeneity of patients, particularly with regard to sex; the mechanisms that underlie chronic pain have been understudied in female populations owing to a sex bias in preclinical research. Notably, animal studies have shown marked sex differences in immune cell involvement in neuropathic pain, suggesting a biological basis to this heterogeneity and emphasizing the need for sex to be considered in clinical research. Clinical researchers in AS are currently challenged by the concept of treat-to-target, and specifically how to effectively balance short-term symptomatic goals (such as pain relief) and long-term functional goals (such as the preservation of joint structures)[137]. Mechanistic and epidemiological insights from basic and clinical research into pain in AS would inform the development of new targeted drugs, for which there is an urgent unmet need, as no agent has proven universally effective in controlling pain in AS.

## Conclusion

Pain in AS is a neuroimmune process consisting of inflammatory and neuropathic components and is considered to be a cornerstone for the assessment and treatment of patients. Fatigue, another common symptom in AS and a core element of patient assessment, shows complex associations with pain, but is probably not completely explained by pain. Brain imaging studies have revealed altered brain networks in pain and fatigue, accentuating neurological mechanisms of pain, whereas experimental studies in the pain field have highlighted a sex difference in pain pathways in male and female murine models of neuropathic pain. Research into the pathophysiology of AS has also demonstrated distinct pathways involved in male and female patients, emphasizing type 3 immunity as only being involved in male patients; the sex differences in pain pathways might contribute substantially to the sex-related clinical differences seen in patients with AS, but this field requires more investigation. Despite the role of the nervous system in pain perception and the complex interplay of the immune and nervous systems, current treatments for AS focus primarily on controlling inflammation. Future studies targeting both these systems might prove valuable. Improved imaging techniques will also improve our understanding of the disease processes and might provide more precise outcome measures than those currently available.


1. Costello, M.-E. *et al.* Brief report: intestinal dysbiosis in ankylosing spondylitis. *Arthritis Rheumatol.* **67**, 686–691 (2015).
2. Tsui, F. W., Tsui, H. W., Akram, A., Haroon, N. & Inman, R. D. The genetic basis of ankylosing spondylitis: new insights into disease pathogenesis. *Appl. Clin. Genet.* **7**, 105–115 (2014).
3. Brown, M. A., Kenna, T. & Wordsworth, B. P. Genetics of ankylosing spondylitis — insights into pathogenesis. *Nat. Rev. Rheumatol.* **12**, 81–91 (2015).
4. Cortes, A. *et al.* Major histocompatibility complex associations of ankylosing spondylitis are complex and involve further epistasis with ERAP1. *Nat. Commun.* **6**, 7146 (2015).
5. Ranganathan, V., Gracey, E., Brown, M. A., Inman, R. D. & Haroon, N. Pathogenesis of ankylosing spondylitis — recent advances and future directions. *Nat. Rev. Rheumatol.* **13**, 359–367 (2017).
6. Jethwa, H. & Bowness, P. The interleukin (IL)-23/IL-17 axis in ankylosing spondylitis: new advances and potentials for treatment. *Clin. Exp. Immunol.* **183**, 30–36 (2016).
7. Taurog, J. D., Chhabra, A. & Colbert, R. A. Ankylosing spondylitis and axial spondyloarthritis. *N. Engl. J. Med.* **374**, 2563–2574 (2016).
8. Sieper, J. & Poddubnyy, D. New evidence on the management of spondyloarthritis. *Nat. Rev. Rheumatol.* **12**, 282–295 (2016).
9. Gracey, E. *et al.* Sexual dimorphism in the Th17 signature of ankylosing spondylitis. *Arthritis Rheumatol.* **68**, 679–689 (2016).
10. Blachier, M. *et al.* Factors associated with radiographic lesions in early axial spondyloarthritis. Results from the DESIR cohort. *Rheumatology (Oxford)* **52**, 1686–1693 (2013).
11. Sieper, J. *et al.* Sarilumab for the treatment of ankylosing spondylitis: results of a phase II, randomised, double-blind, placebo-controlled study (ALIGN). *Ann. Rheum. Dis.* **74**, 1051–1057 (2015).
12. Sieper, J., Porter-Brown, B., Thompson, L., Harari, O. & Dougados, M. Assessment of short-term symptomatic efficacy of tocilizumab in ankylosing spondylitis: results of randomised, placebo-controlled trials. *Ann. Rheum. Dis.* **73**, 95–100 (2014).
13. Ji, R.-R., Chamessian, A. & Zhang, Y.-Q. Pain regulation by non-neuronal cells and inflammation. *Science* **354**, 572–577 (2016).
14. Pinho-Ribeiro, F. A., Verri, W. A. & Chiu, I. M. Nociceptor sensory neuron–immune interactions in pain and inflammation. *Trends Immunol.* **38**, 5–19 (2017).
15. Wu, Q., Inman, R. D. & Davis, K. D. Neuropathic pain in ankylosing spondylitis: a psychophysics and brain imaging study. *Arthritis Rheum.* **65**, 1494–1503 (2013).
16. Braun, J. & Inman, R. Clinical significance of inflammatory back pain for diagnosis and screening of patients with axial spondyloarthritis. *Ann. Rheum. Dis.* **69**, 1264–1268 (2010).
17. Nhan, D. T. & Caplan, L. Patient-reported outcomes in axial spondyloarthritis. *Rheum. Dis. Clin. North Am.* **42**, 285–299 (2016).
18. Chen, C., Zhang, X., Xiao, L., Zhang, X. & Ma, X. Comparative effectiveness of biologic therapy regimens for ankylosing spondylitis: a systematic review and a network meta-analysis. *Medicine (Baltimore)* **95**, e3060 (2016).
19. Dagfinrud, H., Vollestad, N. K., Loge, J. H., Kvien, T. K. & Mengshoel, A. M. Fatigue in patients with ankylosing spondylitis: a comparison with the general population and associations with clinical and self-reported measures. *Arthritis Rheum.* **53**, 5–11 (2005).
20. Brophy, S. *et al.* Fatigue in ankylosing spondylitis: treatment should focus on pain management. *Semin. Arthritis Rheum.* **42**, 361–367 (2013).
21. Dernis-Labous, E., Messow, M. & Dougados, M. Assessment of fatigue in the management of patients with ankylosing spondylitis. *Rheumatology (Oxford)* **42**, 1523–1528 (2003).
22. Wu, Q., Inman, R. D. & Davis, K. D. Tumor necrosis factor inhibitor therapy in ankylosing spondylitis: differential effects on pain and fatigue and brain correlates. *Pain* **156**, 297–304 (2015).
23. Bedaiwi, M. *et al.* Fatigue in ankylosing spondylitis and nonradiographic axial spondyloarthritis: analysis from a longitudinal observation cohort. *J. Rheumatol.* **42**, 2354–2360 (2015).
24. Christie, A., Dagfinrud, H., Mowinckel, P. & Hagen, K. B. Variation in fatigue may be poorly explained by pain: results from a longitudinal, exploratory study. *Rheumatol. Int.* **36**, 279–282 (2016).

25. Wu, Q., Inman, R. D. & Davis, K. D. Fatigue in ankylosing spondylitis is associated with the brain networks of sensory salience and attention. *Arthritis Rheumatol.* **66**, 295–303 (2014).
26. Navarro-Compán, V. *et al.* Disease activity is longitudinally related to sacroiliac inflammation on MRI in male patients with axial spondyloarthritis: 2-years of the DESIR cohort. *Ann. Rheum. Dis.* **75**, 874–878 (2016).
27. Baraliakos, X. *et al.* Quantification of bone marrow edema by magnetic resonance imaging only marginally reflects clinical neck pain evaluation in rheumatoid arthritis and ankylosing spondylitis. *J. Rheumatol.* **43**, 2131–2135 (2016).
28. Braun, J., Baraliakos, X., Hermann, K.-G. A., Xu, S. & Hsu, B. Serum C-reactive protein levels demonstrate predictive value for radiographic and magnetic resonance imaging outcomes in patients with active ankylosing spondylitis treated with golimumab. *J. Rheumatol.* **43**, 1704–1712 (2016).
29. Wallis, D., Haroon, N., Ayearst, R., Carty, A. & Inman, R. D. Ankylosing spondylitis and nonradiographic axial spondyloarthritis: part of a common spectrum or distinct diseases? *J. Rheumatol.* **40**, 2038–2041 (2013).
30. Wach, J., Letroublon, M.-C., Coury, F. & Tebib, J. G. Fibromyalgia in spondyloarthritis: effect on disease activity assessment in clinical practice. *J. Rheumatol.* **43**, 2056–2063 (2016).
31. Bedaiwi, M. K. *et al.* Clinical efficacy of celecoxib compared to acetaminophen in chronic nonspecific low back pain: results of a randomized controlled trial. *Arthritis Care Res. (Hoboken)* **68**, 845–852 (2016).
32. Meesters, J. J. L. *et al.* The risk for depression in patients with ankylosing spondylitis: a population-based cohort study. *Arthritis Res. Ther.* **16**, 418 (2014).
33. Van der Horst-Bruinsma, I. E., Zack, D. J., Szumski, A. & Koenig, A. S. Female patients with ankylosing spondylitis: analysis of the impact of gender across treatment studies. *Ann. Rheum. Dis.* **72**, 1221–1224 (2013).
34. Lee, W. *et al.* Are there gender differences in severity of ankylosing spondylitis? Results from the PSOAS cohort. *Ann. Rheum. Dis.* **66**, 633–638 (2007).
35. Karshikoff, B. *et al.* Modality and sex differences in pain sensitivity during human endotoxemia. *Brain. Behav. Immun.* **46**, 35–43 (2015).
36. Dodds, K. N. *et al.* Glial contributions to visceral pain: implications for disease etiology and the female predominance of persistent pain. *Transl Psychiatry* **6**, e888 (2016).
37. Mogil, J. S. & Chanda, M. L. The case for the inclusion of female subjects in basic science studies of pain. *Pain* **117**, 1–5 (2005).
38. Rogachov, A. *et al.* Regional brain signal variability: a novel indicator of pain sensitivity and coping. *Pain* **157**, 2483–2492 (2016).
39. Wang, G., Erpelding, N. & Davis, K. D. Sex differences in connectivity of the subgenual anterior cingulate cortex. *Pain* **155**, 755–763 (2014).
40. Hashmi, J. A. & Davis, K. D. Deconstructing sex differences in pain sensitivity. *Pain* **155**, 10–13 (2014).
41. Hashmi, J. A. & Davis, K. D. Women experience greater heat pain adaptation and habituation than men. *Pain* **145**, 350–357 (2009).
42. Greenspan, J. D. *et al.* Studying sex and gender differences in pain and analgesia: a consensus report. *Pain* **132** (Suppl. 1), S26–S45 (2007).
43. Sorge, R. E. *et al.* Different immune cells mediate mechanical pain hypersensitivity in male and female mice. *Nat. Neurosci.* **18**, 1081–1083 (2015).
44. Sorge, R. E. *et al.* Spinal cord Toll-like receptor 4 mediates inflammatory and neuropathic hypersensitivity in male but not female mice. *J. Neurosci.* **31**, 15450–15454 (2011).
45. Taves, S. *et al.* Spinal inhibition of p38 MAP kinase reduces inflammatory and neuropathic pain in male but not female mice: sex-dependent microglial signaling in the spinal cord. *Brain. Behav. Immun.* **55**, 70–81 (2016).
46. Draleau, K. *et al.* Phenotypic identification of spinal cord-infiltrating $CD4^+$ T lymphocytes in a murine model of neuropathic pain. *J. Pain Relief Suppl.* **3**, 3 (2014).
47. Rosen, S., Ham, B. & Mogil, J. S. Sex differences in neuroimmunity and pain. *J. Neurosci. Res.* **95**, 500–508 (2017).
48. Varkas, G. & Van den Bosch, F. NSAIDs in axial spondyloarthritis: to be continued…? *Ann. Rheum. Dis.* **75**, 1423–1425 (2016).
49. Haroon, N., Kim, T.-H. & Inman, R. D. NSAIDs and radiographic progression in ankylosing spondylitis Bagging big game with small arms? *Ann. Rheum. Dis.* **71**, 1593–1595 (2012).
50. Molto, A., Granger, B., Wendling, D., Dougados, M. & Gossec, L. Use of nonsteroidal anti-inflammatory drugs in early axial spondyloarthritis in daily practice: data from the DESIR cohort. *Joint Bone Spine* **84**, 79–82 (2017).
51. Varkas, G. *et al.* Brief report: six-week treatment of axial spondyloarthritis patients with an optimal dose of nonsteroidal antiinflammatory drugs: early response to treatment in signal intensity on magnetic resonance imaging of the sacroiliac joints. *Arthritis Rheumatol.* **68**, 672–678 (2016).
52. Sieper, J. & Poddubnyy, D. Axial spondyloarthritis. *Lancet* http://dx.doi.org/10.1016/S0140-6736(16)31591-4 (2017).
53. Thompson, C., Davies, R. & Choy, E. Anti cytokine therapy in chronic inflammatory arthritis. *Cytokine* **86**, 92–99 (2016).
54. Hess, A. *et al.* Blockade of TNF-α rapidly inhibits pain responses in the central nervous system. *Proc. Natl Acad. Sci. USA* **108**, 3731–3736 (2011).
55. Haroon, N. *et al.* The impact of TNF-inhibitors on radiographic progression in ankylosing spondylitis. *Arthritis Rheum.* **65**, 2645–2654 (2013).
56. Danve, A. & Deodhar, A. Treat to target in axial spondyloarthritis: what are the issues? *Curr. Rheumatol. Rep.* **19**, 22 (2017).
57. Toussirot, É. Biologics in spondyloarthritis: TNFα inhibitors and other agents. *Immunotherapy* **7**, 669–681 (2015).
58. Baeten, D. *et al.* Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis. *N. Engl. J. Med.* **373**, 2534–2548 (2015).
59. Sieper, J. New treatment targets for axial spondyloarthritis. *Rheumatology (Oxford)* **55** (Suppl. 2), ii38–ii42 (2016).
60. Van der Heijde, D. *et al.* 2016 update of the ASAS-EULAR management recommendations for axial spondyloarthritis. *Ann. Rheum. Dis.* **76**, 978–991 (2017).
61. Grace, P. M. *et al.* Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. *Proc. Natl Acad. Sci. USA* **113**, E3441–E3450 (2016).
62. Kistner, K. *et al.* Systemic desensitization through TRPA1 channels by capsazepine and mustard oil — a novel strategy against inflammation and pain. *Sci. Rep.* **6**, 28621 (2016).
63. Garrison, S. R. & Stucky, C. L. The dynamic TRPA1 channel: a suitable pharmacological pain target? *Curr. Pharm. Biotechnol.* **12**, 1689–1697 (2011).
64. Trevisan, G. *et al.* TRPA1 mediates trigeminal neuropathic pain in mice downstream of monocytes/macrophages and oxidative stress. *Brain* **139**, 1361–1377 (2016).
65. Kress, M. *et al.* MicroRNAs in nociceptive circuits as predictors of future clinical applications. *Front. Mol. Neurosci.* **6**, 33 (2013).
66. Leinders, M., Üçeyler, N., Pritchard, R. A., Sommer, C. & Sorkin, L. S. Increased miR-132-3p expression is associated with chronic neuropathic pain. *Exp. Neurol.* **283**, 276–286 (2016).
67. Malfait, A.-M. & Miller, R. J. Emerging targets for the management of osteoarthritis pain. *Curr. Osteoporos. Rep.* **14**, 260–268 (2016).
68. Siniscalco, D., Giordano, C., Rossi, F., Maione, S. & de Novellis, V. Role of neurotrophins in neuropathic pain. *Curr. Neuropharmacol.* **9**, 523–529 (2011).
69. Seminowicz, D. A. *et al.* Effective treatment of chronic low back pain in humans reverses abnormal brain anatomy and function. *J. Neurosci.* **31**, 7540–7550 (2011).
70. Kucyi, A. & Davis, K. D. The dynamic pain connectome. *Trends Neurosci.* **38**, 86–95 (2015).
71. Hubbard, C. S. *et al.* Altered brain structure and function correlate with disease severity and pain catastrophizing in migraine patients. *eNeuro* **1**, e20.14 (2014).
72. Mathur, V. A. *et al.* Altered cognition-related brain activity and interactions with acute pain in migraine. *Neuroimage Clin.* **7**, 347–358 (2015).
73. Apkarian, A. V. The brain in chronic pain: clinical implications. *Pain Manag.* **1**, 577–586 (2011).
74. Napadow, V. *et al.* Intrinsic brain connectivity in fibromyalgia is associated with chronic pain intensity. *Arthritis Rheum.* **62**, 2545–2555 (2010).
75. Baliki, M. N., Geha, P. Y., Apkarian, A. V. & Chialvo, D. R. Beyond feeling: chronic pain hurts the brain, disrupting the default-mode network dynamics. *J. Neurosci.* **28**, 1398–1403 (2008).
76. Seminowicz, D. A. & Davis, K. D. Pain enhances functional connectivity of a brain network evoked by performance of a cognitive task. *J. Neurophysiol.* **97**, 3651–3659 (2007).
77. Davis, K. D. & Moayedi, M. Central mechanisms of pain revealed through functional and structural MRI. *J. Neuroimmune Pharmacol.* **8**, 518–534 (2013).
78. Goswami, R., Anastakis, D. J., Katz, J. & Davis, K. D. A longitudinal study of pain, personality, and brain plasticity following peripheral nerve injury. *Pain* **157**, 729–739 (2016).
79. DeSouza, D. D., Davis, K. D. & Hodaie, M. Reversal of insular and microstructural nerve abnormalities following effective surgical treatment for trigeminal neuralgia. *Pain* **156**, 1112–1123 (2015).
80. Ceko, M. *et al.* Partial recovery of abnormal insula and dorsolateral prefrontal connectivity to cognitive networks in chronic low back pain after treatment. *Hum. Brain Mapp.* **36**, 2075–2092 (2015).
81. Moayedi, M. *et al.* Contribution of chronic pain and neuroticism to abnormal forebrain gray matter in patients with temporomandibular disorder. *Neuroimage* **55**, 277–286 (2011).
82. Moayedi, M. *et al.* White matter brain and trigeminal nerve abnormalities in temporomandibular disorder. *Pain* **153**, 1467–1477 (2012).
83. Chen, J. Y.-W., Blankstein, U., Diamant, N. E. & Davis, K. D. White matter abnormalities in irritable bowel syndrome and relation to individual factors. *Brain Res.* **1392**, 121–131 (2011).
84. Blankstein, U., Chen, J., Diamant, N. E. & Davis, K. D. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. *Gastroenterology* **138**, 1783–1789 (2010).
85. Kucyi, A. *et al.* Enhanced medial prefrontal-default mode network functional connectivity in chronic pain and its association with pain rumination. *J. Neurosci.* **34**, 3969–3975 (2014).
86. Weissman-Fogel, I. *et al.* Abnormal cortical activity in patients with temporomandibular disorder evoked by cognitive and emotional tasks. *Pain* **152**, 384–396 (2011).
87. Hemington, K. S., Wu, Q., Kucyi, A., Inman, R. D. & Davis, K. D. Abnormal cross-network functional connectivity in chronic pain and its association with clinical symptoms. *Brain Struct. Funct.* **221**, 4203–4219 (2016).
88. Purves, D. *et al.* (ed.) *Neuroscience* 2nd edn. (Sinauer Associates, 2001).
89. Dubin, A. E. & Patapoutian, A. Nociceptors: the sensors of the pain pathway. *J. Clin. Invest.* **120**, 3760–3772 (2010).
90. Gold, M. S. & Gebhart, G. F. Nociceptor sensitization in pain pathogenesis. *Nat. Med.* **16**, 1248–1257 (2010).
91. Li, W.-W. *et al.* Substance P signaling controls mast cell activation, degranulation, and nociceptive sensitization in a rat fracture model of complex regional pain syndrome. *Anesthesiology* **116**, 882–895 (2012).
92. Kim, C. F. & Moalem-Taylor, G. Interleukin-17 contributes to neuroinflammation and neuropathic pain following peripheral nerve injury in mice. *J. Pain* **12**, 370–383 (2011).
93. Davis, K. D., Meyer, R. A. & Campbell, J. N. Chemosensitivity and sensitization of nociceptive afferents that innervate the hairy skin of monkey. *J. Neurophysiol.* **69**, 1071–1081 (1993).
94. Schaible, H. G. & Grubb, B. D. Afferent and spinal mechanisms of joint pain. *Pain* **55**, 5–54 (1993).
95. Schaible, H.-G. Nociceptive neurons detect cytokines in arthritis. *Arthritis Res. Ther.* **16**, 470 (2014).
96. Cervero, F. & Laird, J. M. A. Understanding the signaling and transmission of visceral nociceptive events. *J. Neurobiol.* **61**, 45–54 (2004).
97. Felson, D. T. The sources of pain in knee osteoarthritis. *Curr. Opin. Rheumatol.* **17**, 624–628 (2005).
98. Mantyh, P. W. The neurobiology of skeletal pain. *Eur. J. Neurosci.* **39**, 508–519 (2014).
99. Cavanaugh, J. M., Lu, Y., Chen, C. & Kallakuri, S. Pain generation in lumbar and cervical facet joints. *J. Bone Joint Surg. Am.* **88** (Suppl. 2), 63–67 (2006).
100. Szadek, K., Hoogland, P., Zuurmond, W., Delange, J. & Perez, R. Nociceptive nerve fibers in the sacroiliac joint in humans. *Reg. Anesth. Pain Med.* **33**, 36–43 (2008).

101. Palsson, T. S. & Graven-Nielsen, T. Experimental pelvic pain facilitates pain provocation tests and causes regional hyperalgesia. *Pain* **153**, 2233–2240 (2012).
102. Rio, E. *et al.* The pain of tendinopathy: physiological or pathophysiological? *Sport. Med.* **44**, 9–23 (2014).
103. McCleskey, E. W. & Gold, M. S. Ion channels of nociception. *Annu. Rev. Physiol.* **61**, 835–856 (1999).
104. Benarroch, E. E. Ion channels in nociceptors: recent developments. *Neurology* **84**, 1153–1164 (2015).
105. Moore, C. *et al.* UVB radiation generates sunburn pain and affects skin by activating epidermal TRPV4 ion channels and triggering endothelin-1 signaling. *Proc. Natl Acad. Sci. USA* **110**, E3225–E3234 (2013).
106. Khodorova, A., Montmayeur, J.-P. & Strichartz, G. Endothelin receptors and pain. *J. Pain* **10**, 4–28 (2009).
107. Ranade, S. S. *et al.* Piezo2 is the major transducer of mechanical forces for touch sensation in mice. *Nature* **516**, 121–125 (2014).
108. Cekic, C. & Linden, J. Purinergic regulation of the immune system. *Nat. Rev. Immunol.* **16**, 177–192 (2016).
109. Chiu, I. M., von Hehn, C. A. & Woolf, C. J. Neurogenic inflammation and the peripheral nervous system in host defense and immunopathology. *Nat. Neurosci.* **15**, 1063–1067 (2012).
110. Nicotra, L., Loram, L. C., Watkins, L. R. & Hutchinson, M. R. Toll-like receptors in chronic pain. *Exp. Neurol.* **234**, 316–329 (2012).
111. Meseguer, V. *et al.* TRPA1 channels mediate acute neurogenic inflammation and pain produced by bacterial endotoxins. *Nat. Commun.* **5**, 783–801 (2014).
112. Liu, T., Xu, Z.-Z., Park, C.-K., Berta, T. & Ji, R.-R. Toll-like receptor 7 mediates pruritus. *Nat. Neurosci.* **13**, 1460–1462 (2010).
113. Xu, Z.-Z. *et al.* Inhibition of mechanical allodynia in neuropathic pain by TLR5-mediated A-fiber blockade. *Nat. Med.* **21**, 1326–1331 (2015).
114. Das, N. *et al.* HMGB1 activates proinflammatory signaling via TLR5 leading to allodynia. *Cell Rep.* **17**, 1128–1140 (2016).
115. Parsadaniantz, S. M., Rivat, C., Rostène, W. & Goazigo, A. R.-L. Opioid and chemokine receptor crosstalk: a promising target for pain therapy? *Nat. Rev. Neurosci.* **16**, 69–78 (2015).
116. Zhou, Y.-Q. *et al.* Interleukin-6: an emerging regulator of pathological pain. *J. Neuroinflamm.* **13**, 141 (2016).
117. Üçeyler, N., Riediger, N., Kafke, W. & Sommer, C. Differential gene expression of cytokines and neurotrophic factors in nerve and skin of patients with peripheral neuropathies. *J. Neurol.* **262**, 203–212 (2015).
118. Eskander, M. A. *et al.* Persistent nociception triggered by nerve growth factor (NGF) is mediated by TRPV1 and oxidative mechanisms. *J. Neurosci.* **35**, 8593–8603 (2015).
119. Grace, P. M., Hutchinson, M. R., Maier, S. F. & Watkins, L. R. Pathological pain and the neuroimmune interface. *Nat. Rev. Immunol.* **14**, 217–231 (2014).
120. Vincent, L. *et al.* Mast cell activation contributes to sickle cell pathobiology and pain in mice. *Blood* **122**, 1853–1862 (2013).
121. Vicuña, L. *et al.* The serine protease inhibitor SerpinA3N attenuates neuropathic pain by inhibiting T cell–derived leukocyte elastase. *Nat. Med.* **21**, 518–523 (2015).
122. Wu, X.-B. *et al.* CXCL13/CXCR5 enhances sodium channel Nav1.8 current density via p38 MAP kinase in primary sensory neurons following inflammatory pain. *Sci. Rep.* **6**, 34836 (2016).
123. Latremoliere, A. & Woolf, C. J. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. *J. Pain* **10**, 895–926 (2009).
124. Meeus, M. & Nijs, J. Central sensitization: a biopsychosocial explanation for chronic widespread pain in patients with fibromyalgia and chronic fatigue syndrome. *Clin. Rheumatol.* **26**, 465–473 (2007).
125. Porreca, F., Ossipov, M. H. & Gebhart, G. F. Chronic pain and medullary descending facilitation. *Trends Neurosci.* **25**, 319–325 (2002).
126. Dickenson, A. H., Chapman, V. & Green, G. M. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in the spinal cord. *Gen. Pharmacol. Vasc. Syst.* **28**, 633–638 (1997).
127. Tsuda, M. *et al.* P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* **424**, 778–783 (2003).
128. Coull, J. A. M. *et al.* BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* **438**, 1017–1021 (2005).
129. Beggs, S., Trang, T. & Salter, M. W. P2X4R+ microglia drive neuropathic pain. *Nat. Neurosci.* **15**, 1068–1073 (2012).
130. Trang, T., Beggs, S., Wan, X. & Salter, M. W. P2X4-receptor-mediated synthesis and release of brain-derived neurotrophic factor in microglia is dependent on calcium and p38-mitogen-activated protein kinase activation. *J. Neurosci.* **29**, 3518–3528 (2009).
131. Coull, J. A. M. *et al.* Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* **424**, 938–942 (2003).
132. Liu, X. J. *et al.* Treatment of inflammatory and neuropathic pain by uncoupling Src from the NMDA receptor complex. *Nat. Med.* **14**, 1325–1332 (2008).
133. Salter, M. W. & Kalia, L. V. Src kinases: a hub for NMDA receptor regulation. *Nat. Rev. Neurosci.* **5**, 317–328 (2004).
134. Keller, A. F., Beggs, S., Salter, M. W. & De Koninck, Y. Transformation of the output of spinal lamina I neurons after nerve injury and microglia stimulation underlying neuropathic pain. *Mol. Pain* **3**, 27 (2007).
135. Chavan, S. S. & Tracey, K. J. Essential neuroscience in immunology. *J. Immunol.* **198**, 3389–3397 (2017).
136. Shen, H., Goodall, J. C. & Hill Gaston, J. S. Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. *Arthritis Rheum.* **60**, 1647–1656 (2009).
137. Smolen, J. S. *et al.* Treating spondyloarthritis, including ankylosing spondylitis and psoriatic arthritis, to target: recommendations of an international task force. *Ann. Rheum. Dis.* **73**, 6–16 (2014).
138. Task Force on Taxonomy of the International Association for the Study of Pain. Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. *IASP* https://www.iasp-pain.org/files/Content/ContentFolders/Publications2/FreeBooks/Classification-of-Chronic-Pain.pdf (1994).
139. International Association for the Study of Pain. IASP taxonomy. *IASP* http://www.iasp-pain.org/Taxonomy (2002).
140. Williams, A. C. & Craig, K. D. Updating the definition of pain. *Pain* **157**, 2420–2423 (2016).
141. Bonica, J. (ed.) *The Management of Pain* (Lea & Febiger, 1953).
142. Treede, R.-D. *et al.* A classification of chronic pain for ICD-11. *Pain* **156**, 1 (2015).
143. Freynhagen, R., Tölle, T. R., Gockel, U. & Baron, R. The painDETECT project — far more than a screening tool on neuropathic pain. *Curr. Med. Res. Opin.* **32**, 1033–1057 (2016).
144. Hawker, G. A., Mian, S., Kendzerska, T. & French, M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). *Arthritis Care Res. (Hoboken)* **63**, S240–S252 (2011).
145. Melzack, R. The McGill Pain Questionnaire: major properties and scoring methods. *Pain* **1**, 277–299 (1975).
146. Garrett, S. *et al.* A new approach to defining disease status in ankylosing spondylitis: the Bath Ankylosing Spondylitis Disease Activity Index. *J. Rheumatol.* **21**, 2286–2291 (1994).
147. Yarnitsky, D., Granot, M. & Granovsky, Y. Pain modulation profile and pain therapy: between pro- and antinociception. *Pain* **155**, 663–665 (2014).
148. Tjølsen, A., Lund, A., Berge, O. G. & Hole, K. An improved method for tail-flick testing with adjustment for tail-skin temperature. *J. Neurosci. Methods* **26**, 259–265 (1989).
149. Hargreaves, K., Dubner, R., Brown, F., Flores, C. & Joris, J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* **32**, 77–88 (1988).
150. Chaplan, S. R., Bach, F. W., Pogrel, J. W., Chung, J. M. & Yaksh, T. L. Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods* **53**, 55–63 (1994).
151. Sotocinal, S. G. *et al.* The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol. Pain* **7**, 55 (2011).
152. Sufka, K. J. Conditioned place preference paradigm: a novel approach for analgesic drug assessment against chronic pain. *Pain* **58**, 355–366 (1994).
153. Daou, I. *et al.* Remote optogenetic activation and sensitization of pain pathways in freely moving mice. *J. Neurosci.* **33**, 18631–18640 (2013).
154. Keystone, E. C., Schorlemmer, H. U., Pope, C. & Allison, A. C. Zymosan-induced arthritis: a model of chronic proliferative arthritis following activation of the alternative pathway of complement. *Arthritis Rheum.* **20**, 1396–1401 (1977).
155. Courteix, C., Eschalier, A. & Lavarenne, J. Streptozocin-induced diabetic rats: behavioural evidence for a model of chronic pain. *Pain* **53**, 81–88 (1993).
156. Guan, Z. *et al.* Injured sensory neuron-derived CSF1 induces microglial proliferation and DAP12-dependent pain. *Nat. Neurosci.* **19**, 94–101 (2016).
157. Hildebrand, M. E. *et al.* Potentiation of synaptic GluN2B NMDAR currents by Fyn kinase is gated through BDNF-mediated disinhibition in spinal pain processing. *Cell Rep.* **17**, 2753–2765 (2016).
158. Shubayev, V. I., Strongin, A. Y. & Yaksh, T. L. Role of myelin auto-antigens in pain: a female connection. *Neural Regen. Res.* **11**, 890–891 (2016).
159. Costigan, M. *et al.* T-cell infiltration and signaling in the adult dorsal spinal cord is a major contributor to neuropathic pain-like hypersensitivity. *J. Neurosci.* **29**, 14415–14422 (2009).
160. Cao, L. & DeLeo, J. A. CNS-infiltrating CD4+ T lymphocytes contribute to murine spinal nerve transection-induced neuropathic pain. *Eur. J. Immunol.* **38**, 448–458 (2008).



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# The genetics revolution in rheumatology: large scale genomic arrays and genetic mapping

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Abstract | Susceptibility to rheumatic diseases, such as osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, juvenile idiopathic arthritis and psoriatic arthritis, includes a large genetic component. Understanding how an individual's genetic background influences disease onset and outcome can lead to a better understanding of disease biology, improved diagnosis and treatment, and, ultimately, to disease prevention or cure. The past decade has seen great progress in the identification of genetic variants that influence the risk of rheumatic diseases. The challenging task of unravelling the function of these variants is ongoing. In this Review, the major insights from genetic studies, gained from advances in technology, bioinformatics and study design, are discussed in the context of rheumatic disease. In addition, pivotal genetic studies in the main rheumatic diseases are highlighted, with insights into how these studies have changed the way we view these conditions in terms of disease overlap, pathways of disease and potential new therapeutic targets. Finally, the limitations of genetic studies, gaps in our knowledge and ways in which current genetic knowledge can be fully translated into clinical benefit are examined.

The major rheumatic diseases, including osteoarthritis (OA), rheumatoid arthritis (RA), ankylosing spondylitis (AS), systemic lupus erythematosus (SLE), juvenile idiopathic arthritis (JIA) and psoriatic arthritis (PsA), have multiple genetic and environmental risk factors involved in disease onset. Susceptibility to all these diseases is attributable largely to the genomic background of patients[1–5], with heritability estimates ranging from 50% in OA[5] to >90% in PsA[3]. This genomic susceptibility outweighs the combined risk arising from environmental factors, such as smoking, diet, environment, infection and occupation[1–5].

Genetic susceptibility is therefore a fundamental aspect of rheumatic diseases, and a full understanding of how genetic variants influence disease processes has the potential to influence disease management. No single genetic risk factor is essential for disease development; rather, combinations of multiple variants influence not only susceptibility to disease, but also the disease course or outcome, as well as response to therapy. Indeed, heterogeneity, in terms of both disease presentation and outcome, presents a key challenge to rheumatologists. Genetics provides an opportunity to address this heterogeneity through classifying patients into more homogeneous subtypes based on the genetic pathways that drive disease. This stratification will enable clinicians to target therapy to those patients most likely to respond. Effectively treating disease early on is key to improving long-term outcomes for patients and the eventual development of predictive algorithms might enable identification of disease-susceptible individuals and perhaps ultimately lead to disease prevention. In addition, genetics can affect therapeutic management. Determining which genes and pathways are implicated in a disease provides the opportunity to reposition therapies that successfully target the same pathways in other diseases, or to revisit therapies that have previously failed in clinical trials of unselected patients but might have therapeutic benefit in a subset of patients. In the past 2 years, data indicate that novel therapies targeting pathways whose role in disease is backed by genetic evidence are twice as likely to progress through a pharmaceutical pipeline and deliver a new drug than those without any genetic evidence[6], with obvious financial implications.

Arguably, the modern era of genetic research in rheumatic diseases began in 2007 with the Wellcome Trust Case Control Consortium (WTCCC) study[7] (FIG. 1; TABLE 1). The WTCCC recognized that common variants with low effect sizes that increase risk to common

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**Key points**

- Large-scale genome-wide association studies (GWAS), meta-analyses, fine-mapping and studies with populations of diverse ancestries have identified hundreds of genetic loci that predispose individuals to rheumatic diseases
- Genetic susceptibility regions overlap between diseases, suggesting common disease mechanisms and treatment choices
- Genetic associations can implicate pathways (for example, T helper cell differentiation) involved in disease onset and/or outcome
- A number of existing drugs (such as tofacitinib, tocilizumab and abatacept) target proteins encoded by genes identified through GWAS, suggesting that genetic studies can help identify novel therapeutic targets
- Most disease-associated variants map to noncoding regions responsible for controlling gene expression and DNA conformational analysis is beginning to help in the challenging task of unravelling their function

diseases would only be detected by using large sample sizes, collected through collaborative efforts. The WTCCC study now seems relatively low-powered in comparison with some of the meta-analysis genome wide association studies (GWAS) utilizing many 10,000s to 100,000s of samples that have been performed subsequently, but at the time, it introduced a paradigm shift in the genetic research of complex diseases. The study[7] compared 14,000 patients who had one of seven diseases (rheumatic diseases represented by RA) with 3,000 controls. The use of high-density single nucleotide polymorphism (SNP) arrays revealed genome-wide associations for each disease. The WTCCC study was a step forward in genomic research, as previous attempts to link genetic changes to disease development were either largely based on low-powered family linkage studies, or were candidate gene studies that assessed disease associations one gene at a time. The success of the WTCCC study was dependent on not only its scale, but also on its use of genetic mapping and other technological advances that happened concurrently.

A SNP is generally accepted to be robustly associated with disease in GWAS if the difference in variant (allele) frequency between cases and controls surpasses a genome-wide significance threshold. This threshold is set to account for multiple comparisons, and many variants might not reach genome-wide significance, particularly those investigated in early GWAS (which had relatively small sample sizes). Therefore, any putative association requires further validation in independent cohorts of cases and controls. Since the pioneering WTCCC study, many more GWAS have been performed, such that the GWAS Catalog documented over 24,000 unique SNP-trait associations (as of August 2016). The story for rheumatic diseases has been one of increasing GWAS sample sizes, leading to the discovery of an increasing number of disease-associated variants and providing further insights into disease mechanisms.

In this Review, we provide a discussion of the major insights gained from genetic studies in rheumatic diseases, such as GWAS, meta-analyses, fine-mapping and studies performed across different diseases and/or ethnicities. We highlight the most relevant genetic studies in the main rheumatic diseases and discuss how they have enhanced our knowledge of these diseases in terms of disease overlap, pathways to disease and potential new therapeutic targets. We also examine the first efforts to understand how genetic variants influence disease biology.

## GWAS in rheumatic diseases

The year 2007 saw the first real advances in discovering novel genetic associations with RA susceptibility (FIG. 1). The WTCCC study found two genetic regions associated with RA that reached genome-wide significance[7]: *HLA-DRB1* and *PTPN22*. GWAS conducted in the USA added *TNFAIP3* (REF. 8) and the *TRAF1-C5* locus[9] to this list. In addition, a candidate-gene study confirmed an association of *STAT4* with RA susceptibility[10], bringing the number of loci implicated in RA risk up to five. Further studies in RA, using large international cohorts, validated markers that either had evidence suggestive of an association with RA (that is, reaching a suggestive genome-wide significance threshold of $P < 1 \times 10^{-6}$) or variants identified with statistical text mining methods (such as gene relationships across implicated loci; GRAIL), bringing the number of confirmed variants associated with RA up to 31 by 2010 (REFS 11–14). These variants included those mapped to regions in *CTLA4, IL2RA, CCL21* and *AFF3*. These early GWAS findings in RA confirmed the role of T cell immunity in disease development, something already assumed but now firmly established.

Similar GWAS have investigated the other major rheumatic diseases. For example, one of the first large-scale genetic studies in AS, which again involved the WTCCC, focused on 14,500 non-synonymous SNPs in 1,000 patients with AS and 1,500 healthy individuals[15]. This study confirmed the already established link between *HLA-B27* and risk of AS, while also implicating both *ERAP1* and *IL23R* in disease pathogenesis. In 2011, the most recent GWAS in AS[16], which used a discovery cohort of >1,700 patients with AS and a validation cohort of ~2,000 patients with AS, confirmed the disease association with these three regions. This study also validated two other associations, in regions located in chromosomes 2 and 21 that had been identified by a previous GWAS[17], and showed a further seven regions to be associated with AS, including variants within the *RUNX3*, *IL12B*, *TNFRSF1A* and *CARD9* loci[16].

A well-powered GWAS that included ~7,500 patients with OA in the initial screen and similar numbers in a validation cohort provided further insights into the genetics driving OA[18]. This pivotal study brought the number of known loci associated with OA onset from 3 to 11. Epidemiological studies had previously demonstrated a heterogeneity of the OA phenotype[19], and the findings from this GWAS emphasized this heterogeneity; associations were revealed only when the data was stratified into more homogeneous subtypes, for example by site of OA (hip or knee), sex or both. The study also showed an association of OA with the *FTO* gene, which was previously shown to be important in obesity[20], reinforcing the link between body weight and OA.

Single nucleotide polymorphism (SNP)
DNA sequence variation of a single nucleotide that occurs at a specific position in the genome.

Candidate gene
In genetic association studies, the candidate gene approach tests associations between genetic variation within pre-specified genes of interest and phenotypes.

Genome-wide significance threshold
*P* value threshold that is considered statistically significant after multiple testing correction in GWAS, typically $P < 5 \times 10^{-8}$.

Non-synonymous SNPs
SNPs that map to coding genes and result in an amino acid change in the protein they encode.

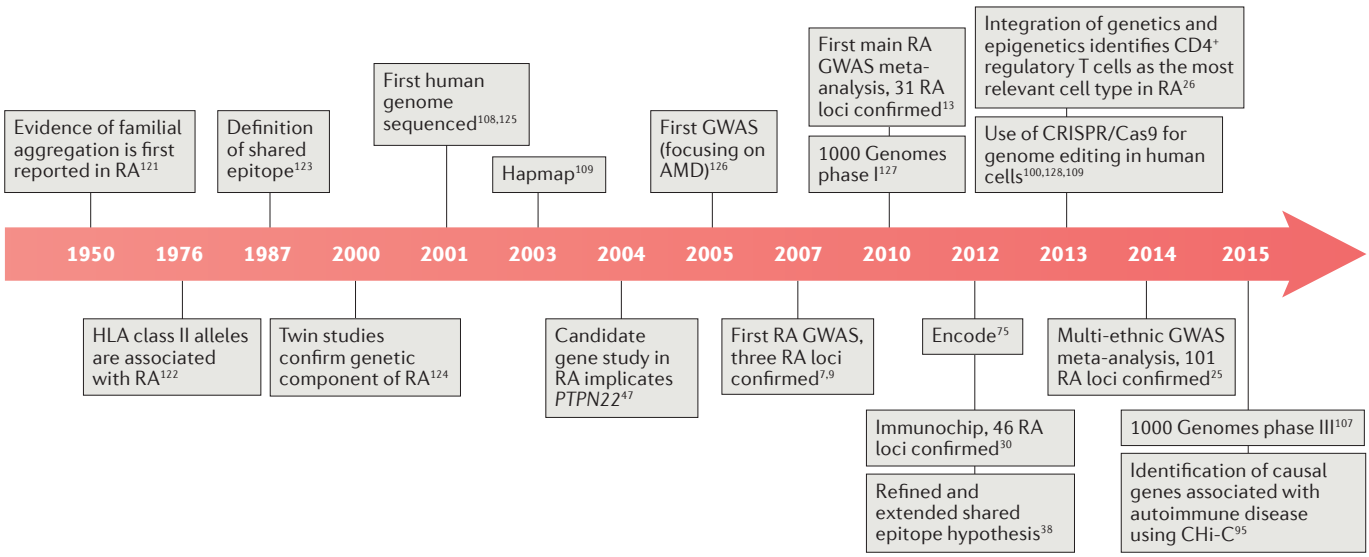



Figure 1 | **Timeline of the major advances in rheumatoid arthritis genetics research.** Advances in genetic research relevant to rheumatoid arthritis since 1950. AMD, age-related macular degeneration; CHi-C, capture Hi-C; CRISPR, clustered regularly interspaced short palindromic repeats; GWAS, genome wide association study; RA, rheumatoid arthritis.

## GWAS meta-analyses

The utilization of GWAS around the globe has enabled the development of large-scale meta-analyses of many thousands of samples, collected through international collaborations. This increased sample size vastly improves the statistical power of a study to detect disease-associated variants and reduces the necessity for individual groups to perform validation studies. However, interpretation of meta-analyses of global populations might still require careful consideration. For example, different frequencies of DNA variants can occur in different populations, potentially resulting in the identification of spurious or inexact associations. This issue can be avoided through the use of a robust study design and sophisticated analytical techniques (such as principal component analysis).

Meta-analyses have been of great use in rheumatology. For example, a meta-analysis of hip OA GWAS involving >78,000 European participants[21] showed a novel association with a SNP variant that was later shown to correlate with the expression of *NCOA3* in cartilage[22]. *NCOA3* is a gene linked to the expression of *COL2A1*, *RUNX2* and *MMP13* in chondrocytes, which are all important mediators of OA. Another study that combined a GWAS, a meta-analysis and a validation study identified ten new SLE-associated loci, bringing the number of regions robustly associated with SLE in Europeans up to 40 (REF. 23). This study was the largest genetic study in a European population, and involved over 7,000 participants of European descent. The researchers also identified ten missense coding variants that probably influence the risk of SLE, which were largely present in genes encoding kinases and other enzymes. In addition, the location of several disease-associated loci implicated a number of transcription factors in SLE, indicating that regulation of gene expression is likely to have an important role in disease development. Meta-analysis of three GWAS data sets from both Chinese and European populations, which totalled >15,600 samples, revealed a further ten loci associated with SLE, and showed that these risk loci generally overlapped between populations. In this study, the prevalence of SLE in different populations (that is, European, Amerindian, South Asian, East Asian and African populations) was matched by the genetic risk score conferred by the risk alleles[24].

A large international GWAS meta-analysis investigating RA, involving a consortium from across Europe, the USA and Japan, brought the number of identified RA-risk regions up to 101 (REF. 25). This multi-ethnic study revealed extensive sharing of genetic risk loci between Asian and European populations, with only five loci showing a population-specific association. The study findings also confirmed a strong link between RA and T cell immunity. Further analysis in 34 tissues demonstrated an enrichment of RA-risk loci in open and active regions of the genome (as defined by epigenetic histone markers) in primary CD4+ T cells[26]. By assigning putative causal genes to these regions, this study demonstrated an enrichment of disease-associated loci in signalling pathway genes and genes encoding approved RA drug targets. A study investigating OA revealed differences in the disease-associated variants found in European and Asian cohorts, with only one region, found within *GDF5*, having an unambiguous shared genetic association between these two populations[27]. The protein encoded by *GDF5*, growth/differentiation factor 5 (GDF5), is a member of the TGFβ superfamily and is important in chondrogenesis and bone growth. Thus, large-scale international meta-analysis of GWAS has the capacity to determine

Statistical power
Probability that a test correctly rejects the null hypothesis.

Risk score
Estimate of the cumulative contribution of genetic factors to a specific outcome of interest in an individual that takes into account the reported risk alleles.

Table 1 | **Technological advances that enabled the genetics revolution for rheumatic diseases**

| Technological advancement | Description | Contribution to understanding disease |
|---|---|---|
| ***Project and/or collaboration*** | | |
| 1000 Genomes project[107] | A large-scale sequencing project that involved 2,504 genomes from 26 populations, aiming to provide the largest public catalogue of human variation and genotype data | The catalogue enables researchers to infer genotypes for a large number of variants in panels of people for whom only a small subset of SNPs have been genotyped, without the need for resequencing |
| Encyclopedia of DNA Elements (ENCODE)[75] | An international collaboration with the goal of building a comprehensive catalogue of all functional elements in the human genome | Given that most GWAS variants map to noncoding regulatory regions of the genome, unravelling the exact function and mechanism of these regulatory elements is essential to unravelling disease biology |
| Human Genome project (HGP)[108] | A project designed to sequence the whole human genome | Determined the exact order of the base pairs in the genome, essential for the design of any genetic study |
| The international HapMap project[109] | By mapping all SNPs found in 1,301 individuals drawn from 11 ancestral groups, this project enabled the identification of highly correlated SNPs or haplotypes | HapMap helped reduce the number of SNPs required to examine the entire genome for associations with a particular phenotype. This number was reduced from the 10 million SNPs that exist to roughly 1 million 'tag' SNPs. HapMap's initial efforts have since been greatly expanded; both in number of individuals (tens of thousands) and populations, but they were sufficient to inform early GWAS studies |
| Wellcome Trust Case Control Consortium (WTCCC)[7] | A group of 50 research groups across the UK that performed the first major GWAS, which included seven common diseases | Through the use of large sample sizes, large collaborative efforts make the detection of SNPs that increase disease risk with low effect sizes possible |
| ***Study method*** | | |
| Cross-disease meta-analysis[110] | Combining GWAS data from different diseases | This approach increases the statistical power to detect risk variants shared across diseases |
| GWAS[111] | A method that uses SNP genotyping arrays to identify variants that occur more frequently in people with a particular trait than in people without the trait | Understanding the genetic predisposition to disease leads to a greater understanding of the biologic mechanisms involved in disease, better diagnosis and treatment of disease and, ultimately, to prevention or cure |
| Meta-analysis[112] | A study analysing the combined results from multiple studies | This combined analysis increases power and improves estimates of the size of the effect |
| Multi-ethnic GWAS[113] | Multi-ethnic studies incorporate genotype data from more than one population; by comparison, most early GWASs focused on populations of European descent | This approach enables large-scale replication in independent populations, cross-population meta-analyses to increase power, prioritization of candidate genes and fine-mapping of functional variants |
| Validation study[114] | The study of a subset of GWAS markers that have passed a set threshold of statistical significance, performed in a population separate from that from which the original sample was drawn | Validation studies are essential to confirm GWAS results and eliminate false positives and overestimated effects |
| ***Technique*** | | |
| Bayesian methods[115] | This method provides an alternative to the usual 'frequentist' approach to assessing associations. The posterior probability for each SNP is calculated based on the assumption that the SNP is driving the association and the evidence for association is measured by the Bayes factor, a statistical index that quantifies the evidence for a hypothesis compared with an alternative hypothesis | Bayesian methods compute measures of evidence that can be directly compared among SNPs within and across studies, facilitating meta-analysis. They can also be applied to define credible SNP sets, which improve fine-mapping |
| CRISPR/Cas9 (REF. 116) | A genome editing technique that allows site-specific modifications in the genomes of cells and organisms | This technology will be key to understanding the mechanisms by which disease associated alleles affect disease outcome and/or progression |
| eQTL analysis[117] | eQTLs are regions of the genome containing DNA sequence variants that influence the expression level of one or more genes | This analysis links DNA sequence variation to phenotype and helps identify causal genes |
| Fine-mapping[118] | Genotyping of all known variants in an associated region of DNA in large cohorts of cases and controls | Enables the identification of the variant(s) in a given locus with the greatest evidence for causal association |

Table 1 (cont.) | **Technological advances that enabled the genetics revolution for rheumatic diseases**

| Technological advancement | Description | Contribution to understanding disease |
|---|---|---|
| ***Technique (cont.)*** | | |
| Hi-C[91,95] | Method to comprehensively detect chromatin interactions in the mammalian nucleus. This technique has demonstrated the importance of spatial proximity of regulatory elements to the genes that they regulate | Hi-C studies help identify disease-causing genes. They have shown that the gene closest to the SNP is not always the causal gene; interactions between DNA regions containing variants associated with disease can interact with distal causal genes. This finding has challenged the tradition of annotating GWAS variants to the closest gene |
| Immunochip[30] | A custom SNP array designed for dense genotyping of 186 autoimmunity loci previously identified from GWAS | The Immunochip has helped discover new risk loci, refine peaks of association and identify secondary independent effects within loci |
| Resequencing[119] | Sequencing of a DNA region of interest, for example GWAS loci, in a cohort of either disease-specific cases or controls | Discovery of any non-catalogued variants prior to fine-mapping ensures complete coverage of the selected loci |
| SNP genotyping array[120] | A DNA microarray used to detect hundreds of thousands of SNPs across the genome simultaneously | Enables the identification of disease associations across the whole genome in a high throughput, cost-effective manner |

eQTL, expression quantitative trait loci; GWAS, genome-wide association study; SNP, single-nucleotide polymorphism.

ethnic differences in disease-associated variants, and to illuminate these differences in terms of the variants, genes and pathways involved and the gene–environment interactions observed between individuals of different geographical regions and ethnicities.

The high degree of overlap in genetic susceptibility observed across different autoimmune and inflammatory diseases has led researchers to combine GWAS cohorts for meta-analysis across different diseases as a means to increase the number of susceptibility loci identified. A combined analysis of AS and four other clinically related diseases, including psoriasis, Crohn's disease and ulcerative colitis, used >50,000 patients and 30,000 controls, which increased the power to detect genetic associations shared across these similar diseases and led to the identification of 17 new GWAS loci for AS, bringing the total number to 48 (REF. 28). Pathway analysis followed by a search for genes that encode known drug targets highlighted potential novel therapeutic targets in AS, such as C–C chemokine receptor type 2 (CCR2) and CCR5. Hence the development of the CCR2 and CCR5 antagonists (MLN-1202 and AMD-070, respectively) as potential novel therapeutics for AS.

## Fine-mapping GWAS data

Associations identified following GWAS, validation and meta-analysis will typically implicate multiple genetic variants in disease susceptibility; further genetic studies are required to 'fine-map' the genetic associations and better define the causal variant. Fine-mapping involves genotyping large cohorts of cases and controls for all known variants in a disease-associated region of DNA to experimentally determine the mostly likely causal variant in that region. As a preceding step, the DNA region can be resequenced using a cohort of either disease-specific cases or controls to ensure all variants are included when fine-mapping. This step is increasingly less likely to identify novel variants as large-scale sequencing projects such as the 1000 Genomes Project and the 1000 Genomes Project are continuing to add to the catalogue of known variants in multiple populations, although it could still prove fruitful if the causal variant is as yet undiscovered. For example, in a study of SLE, resequencing identified a single base pair insertion in a region of DNA close to *TNFAIP3*, which is probably the causal variant for this region's association with SLE[29].

One of the more surprising findings from the GWAS era was the extent of overlap seen between different autoimmune diseases, including some rheumatic diseases, in their disease-associated DNA regions. This observation led to the development of a common, cost-effective genotyping array that included all known genetic variants associated with autoimmune diseases: the Immunochip[30]. The availability of this array enabled large-scale, high-throughput genotyping for a whole range of rheumatic diseases. This fine-mapping approach led to the addition of 13 new genes to the list of those associated with AS suspectibility[31], including *IL6R*, *IL27* and *BACH2*, and a similar number for RA[30]. Use of the Immunochip array has also added 13 new JIA-associated loci[32], including those implicating *STAT4*, *IL2RA* and *IL2RB*, to the three previously confirmed by GWAS (the HLA region, *PTPN22* and *PTPN2*). In a 2011 study of patients with SLE of Asian ancestry, use of this array identified 10 new disease-associated loci and confirmed 20 others. Interestingly, one of these newly discovered loci, found within the *SYNGR1* gene, has a frequency of 80% in Asians but only 20% in Europeans, suggesting natural selection of the gene in Asian populations[33].

Statistical analysis of fine-mapping data can be employed to determine the number of independent association signals in a given region, the variant(s) most strongly associated with disease (that is, the 'lead' or 'index' SNP) and sets of highly correlated (linkage disequilibrium $r^2$ >0.9) SNP variants, all of which are equally likely to be causal. Within the last decade, Bayesian methods have been employed to fine-map disease-associated loci[34–36]. In addition, conditional logistic regression

Linkage disequilibrium
Nonrandom association of alleles at different loci in a given population.

analysis — that is, re-analysing the data with the lead signal removed — enables further definition of the genetic architecture of a region; for example, this analysis can determine whether multiple signals are present within the region. These approaches have been used to great effect in rheumatic diseases, for which the major susceptibility locus resides within the highly complex and gene-rich HLA region. High-density genotyping has been used to identify three independent genetic signals in the class I region of HLA that increase the risk of developing PsA[37]. The availability of large genotyped cohorts enabled one study to better refine the RA associations located within the HLA region[38], revealing three independent signals in this region. In this analysis, the major disease risk association was found to arise from a change in the amino acid at position 13, which is located in the major binding groove of HLA-DR, outside the previously well-defined shared epitope sequence (amino acid positions 70–74). Loci residing outside the HLA region have similarly been refined by use of conditional logistic regression analysis[30].

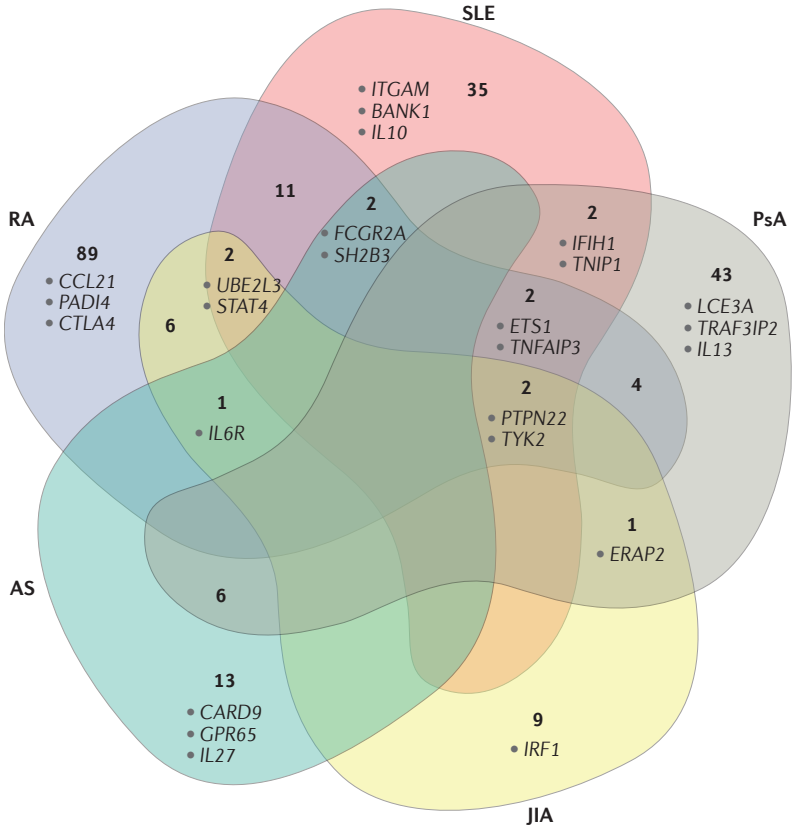



Figure 2 | **Overlap of associated loci among five rheumatic diseases.** Shared genetic susceptibility loci between rheumatic diseases suggest that these diseases also share biological disease mechanisms and treatment options. This figure illustrates the number of overlapping loci observed between the major rheumatic diseases (for the unnumbered areas the overlap is zero) and highlights some relevant disease-associated genes. Rheumatic diseases with substantial available GWAS, validation and fine-mapping data are included. Key disease-associated genes include *PADI4* in rheumatoid arthritis (RA), involved in the citrullination of proteins; anti-citrullinated protein antibodies (ACPA) are a key distinctive component of this disease. *CTLA4* and *IL6R* are targets of the biologic therapies abatacept and tocilizumab, respectively, effective therapies for RA. The association of *ITGAM* variants with systemic lupus erythematosus (SLE) reinforce the importance of the complement system in this rheumatic disease, whereas the association of *LCE3A* variants with psoriatic arthritis (PsA) shows the organ-specific and disease-specific nature of some of these genetic associations (in this case, the skin). AS, ankylosing spondylitis; JIA, juvenile idiopathic arthritis.

## Overlap between diseases

Genetic analysis can illuminate the genetic similarities between diseases. For example, by use of combined data analysis of multiple seronegative diseases, one study demonstrated that Crohn's disease and inflammatory bowel disease (IBD) share some disease-associated loci with AS (20 and 48 loci, respectively)[28]. Other genetic studies have shown the IL-17–IL-23 pathway to be pivotal in the overlap observed between autoimmune diseases[39], leading to the idea of repurposing drugs that target this pathway, such as ustekinumab. As shown by statistical analysis, JIA is genetically more similar to type I diabetes mellitus, another juvenile-onset autoimmune disease, than to other rheumatic diseases[40]. Genes involved in the IL-2 pathway, including *IL2*, *IL2RA* and *IL2RB*, drive this overlap. Similarly, genes associated with PsA demonstrate little or no overlap with RA-associated genes, but PsA has an almost complete genetic overlap with psoriasis and strong overlap with IBD[37]. The nature of these overlaps is evident in disease therapy, in which some therapies have shown success in treating psoriasis, PsA and IBD, whereas the highly successful anti-TNF biologics developed for RA have shown little efficacy in treating psoriasis or PsA[41].

Extensive GWAS, meta-analyses, Immunochip assays and cross-disease meta-analysis in systemic sclerosis (SSc) have revealed ≤20 non-HLA regions associated with risk of disease[42,43], and have confirmed a genetic overlap of this disease with SLE and RA; >70% of SSc-associated SNPs are also associated with SLE and 50% with RA. Associations in many genetic regions, including *IRF5* and *IRF8*, are shared across all the major rheumatic diseases, whereas *CSK*, *BANK1* and *TNIP1* are associated only with SSc and SLE. A large cross-disease analysis of SSc and RA, involving >8,800 patients with SSc, 16,800 patients with RA and 44,000 controls, showed associations with components of the type I interferon signalling pathway (*IRF4*, *IRF5*, *IRF8*) that were shared across diseases, clearly demonstrating the importance of this pathway in both diseases[44]. Although overlap between inflammatory and autoimmune diseases might not be surprising, in 2014 the most recent and largest meta-analysis in RA revealed that many disease-associated genomic regions are shared with blood cancers, including leukaemia and Hodgkin lymphoma[25]. This finding is suggestive of opportunities to reposition drugs that are successful in treating blood cancers and have already passed expensive and stringent safety and efficacy trials, for the treatment of RA. These drugs include alvocidib and palbociclib, which target both cyclin-dependent kinase 4 (CDK4) and CDK6 (whose genes map to regions associated with RA) in lymphomas and leukaemias[45].

Genetic overlap between diseases (FIG. 2) suggests that disease mechanisms, and hence appropriate treatment choices, also overlap. However, this line of thinking

requires careful consideration. Shared associations at a particular locus could be attributable to different polymorphisms, or to the same variant but with different directions of effect; that is, an associated allele could confer risk for one disease but be protective for another. This difference is exemplified by variants in *IL6R* (an allele that is protective for RA is a risk allele for asthma)[30,46], *PTPN22* (certain alleles that are associated with risk of RA, among many other diseases, are protective against Crohn's disease)[47,48] and *SH2B3* (some alleles that are protective against AS are associated with risk of Crohn's disease)[31,48]. Similarly, one of the most intriguing genetic findings in PsA is that although *IL23R* is strongly implicated in both psoriasis and PsA, it is likely that different genetic variants within this region are responsible for the risk association observed in each disease[37]. This finding suggests that the IL-23–IL-17 pathway is pivotal to both diseases, but that these two diseases have evolved differently; hence, subtle differences in the control of the IL-23 pathway, either spatial, temporal or in terms of magnitude, could lead to these different diseases.

## Disease pathway analysis

Given the physical proximity of an associated variant to specific genes and biological knowledge of a disease, variants identified in GWAS can be assigned to a gene, or a number of genes, thought likely to be involved in disease. This process has led to the development of biological 'pathway' analysis, providing intriguing new insights into disease mechanisms. Researchers routinely annotate biological pathways with information such as the associated protein–protein interactions, co-expressed genes and involved receptors and their antigens. This information is available to researchers in resources such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), the Database for Annotation, Visualisation and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis (IPA) software. By placing implicated genes on pre-annotated pathways, researchers have used genetic studies to demonstrate a range of immune and non-immune pathways that drive rheumatic diseases. Among these pathways, the T helper cell differentiation pathway seems to have a prominent role in the development of RA, JIA, PsA, SLE and AS (FIG. 3).

The confirmed genes implicated by GWAS in SLE fall into three broad pathways: lymphocyte activation, particularly B cells, which includes the genes *BLK, PRDM1, STAT4* and *BANK1*; innate immunity, particularly the NF-κB and type I interferon pathways, including *NFKB1* and *IFN1*, driven by *IRF5, IRAK1*, *TLR7* and *TLR9*; and apoptosis, including *ATG5* and *ITGAM*[49,50]. Similar approaches in AS have demonstrated that gut mucosal immunity is important in disease susceptibility, with genes identified in GWAS implicating both gut lymphoid tissue (*RUNX3, EOMES* and *ZMIZ1*) and bacteria-sensing G-protein-coupled receptors (*GPR35, GPR37* and *GPR65*)[51,52]. Of the 36 non-HLA loci known to be associated with AS, five implicate genes involved in the IL-23 signalling pathway, including *IL23R, IL12B, TYK2* and *IL27*, with genes in this pathway representing the overlapping genetic component observed between AS and IBD[31,48,53]. Another mechanism implicated in AS is peptide trimming by aminopeptidases, with genetic associations implicating *ERAP1, ERAP2* and *NPEPPS* in disease[53]. In fact, by using conditional logistic regression analysis, researchers have shown that *ERAP1* is only associated with AS when in the presence of the *HLA-B27* allele, a major disease-associated variant[31]. Endoplasmic reticulum aminopeptidase 1 (ERAP1) is responsible for trimming peptides to be presented by the HLA protein; thus, this genetic analysis clearly demonstrates that the association between *ERAP1* and AS depends on the presentation of processed peptides by the risk-associated HLA allele.

Large genetic studies have implicated 14 genes with the onset of OA, including transcription factors (such as *RUNX2, SMAD3* and *PTHLH*) that regulate chondrogenesis and endochondral ossification, revealing that it is skeletal development that is important in disease and not the structural proteins themselves[54,55]. Genetic studies have so far failed to demonstrate any association between inflammatory genes and OA, although evidence from epigenetic studies of some subtypes of OA would suggest an inflammatory component to this disease, with obvious implications for therapeutic targeting[56].

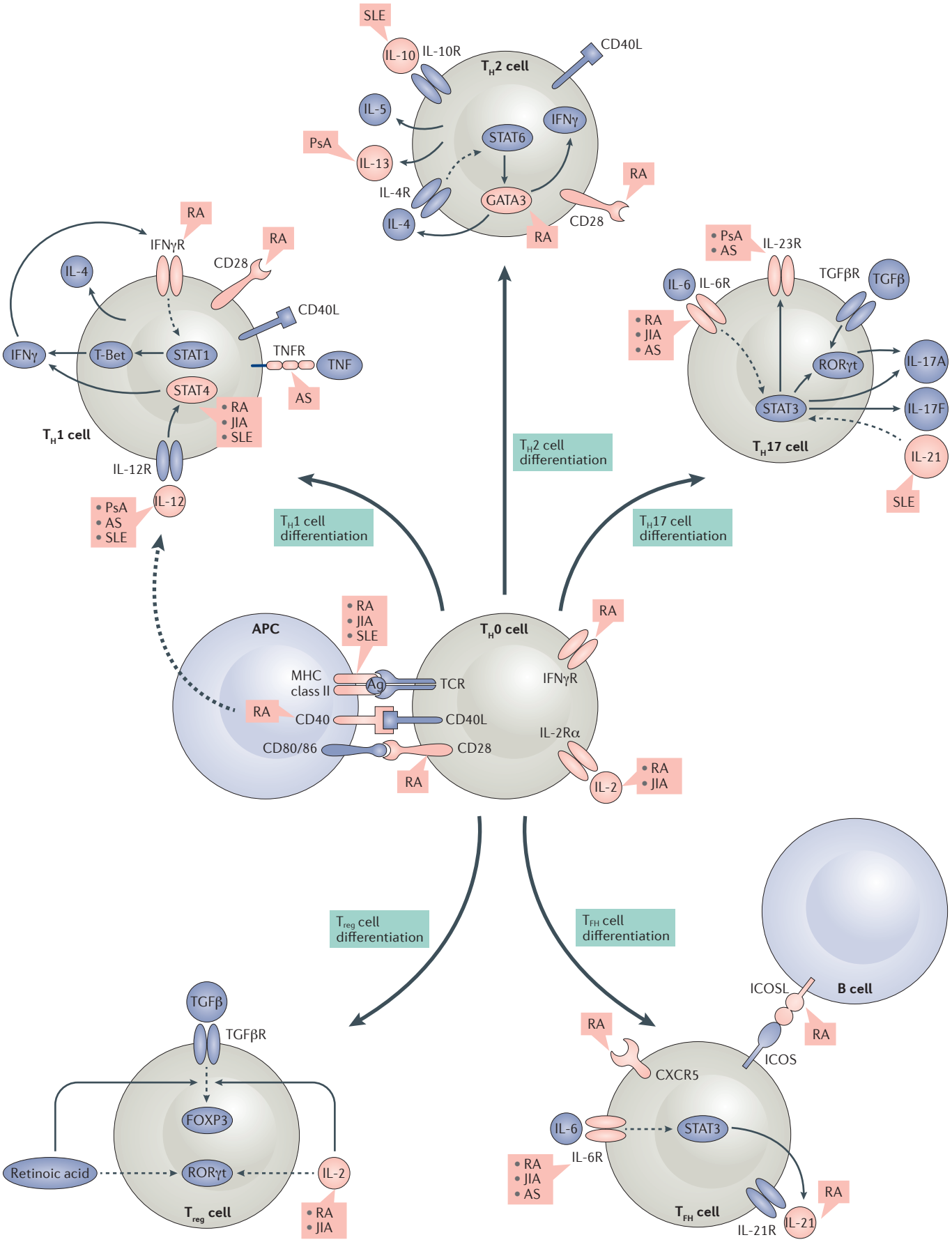
In SSc, the genetic regions implicated in disease suggest a role for innate immunity (*IRF4*, *IRF8*, *TNFAIP3*, *TNIP1*) and adaptive immunity (*CD247, CSK, PTPN22*), while also highlighting the important roles of apoptosis, autophagy and fibrosis in disease (*DNASE1L3, ATG5* and *PPARG*)[42].

## Less-studied rheumatic diseases

GWAS have led to an increased understanding of diseases such as Sjögren syndrome, Paget disease, gout and granulomatosis with polyangiitis (GPA, formerly known as Wegener granulomatosis). Sjögren syndrome can arise as a primary disease, but more often occurs alongside (and shares some clinical features with) other, more common rheumatic diseases, such as SLE and RA. Consequently, the strong overlap observed in GWAS studies between Sjögren syndrome and other rheumatic diseases, particularly SLE, with variants close to *IRF5, STAT4, TNFAIP3*, and *BLK* being associated with risk of both SLE and Sjögren syndrome, is not surprising. However, two Sjögren-associated variants *CXCR5* and *GTF2I* do not show any association with SLE, and hence might distinguish these two diseases[57,58].

For Paget disease, which is a disease of aberrant bone development, GWAS and family linkage studies have, perhaps unsurprisingly, revealed risk-associated genes in the osteoclast differentiation pathway, such as *CSF1*, *RANK* (also known as *TNFRSF11A*), *DCSTAMP* (also known as *TM7SC4*) and *SQSTM1* (REFS 59,60). A gene associated with Paget disease in a GWAS and further explored in sequencing and functional experiments, *RIN3*, is also thought to be involved in osteoclast development[61].

GWAS have revealed that vasculitic diseases, including GPA, eosinophilic granulomatosis with polyangiitis (EGPA) and microscopic polyangiitis (MPA), have distinct genetic risk variants. GWAS analysis in GPA and MPA have revealed that these two forms of



◀ Figure 3 | **Genes of the T helper cell differentiation pathway implicated in rheumatic diseases.** Pathway analysis of disease-associated genes has demonstrated that the T helper cell differentiation pathway has a key role in the pathogenesis of rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), psoriatic arthritis (PsA), systemic lupus erythematosus (SLE) and ankylosing spondylitis (AS). Genes associated with at least one rheumatic disease are highlighted in pink, and labels next to these genes show with which disease(s) they are associated. Solid and dotted lines indicate direct or indirect relationships, respectively. Direct interactions (chemical modifications) require that two molecules make direct physical contact with each other, as opposed to indirect interactions that do not have this requirement. CD40L, CD40 ligand; CXCR5, C-X-C chemokine receptor type 5; FOXP3, forkhead box protein P3; GATA3, *trans*-acting T-cell-specific transcription factor GATA3; ICOS, inducible T cell co-stimulator; ICOSL, inducible T cell co-stimulator ligand; IFNγR, IFNγ receptor; IL-2R, IL-2 receptor; IL-4R, IL-4 receptor; IL-6R, IL-6 receptor; IL-10R, IL-10 receptor; IL-12R, IL-12 receptor; IL-21R, IL-21 receptor; RORγt, nuclear receptor RORγt; SHP2, tyrosine-protein phosphatase non-receptor type 11; STAT, signal transducer and activator of transcription; T-bet, T-box transcription factor TBX21; TCR, T cell receptor; $T_{FH}$ cell, T follicular helper cell; TGFβR, TGFβ receptor; $T_H$ cell, T helper cell; TNFR, TNF receptor; $T_{reg}$ cell, regulatory T cell.

vasculitis differ in terms of HLA associations (*HLA-DP* and *HLA-DQ,* respectively), and also implicated additional genes such as *SERPINA1*, *PRTN3*, *COL11A2* and *SEMA6A*[62,63] in GPA. *SERPINA1* encodes a potent inhibitor of protease 3 (PR3); hence, this genetic association suggests a biological link to the high circulating levels of PR3 observed in many patients with vasculitis. A large-scale meta-analysis of all published genetic studies of vasculitis[64] confirmed that different serological subtypes of vasculitis have different genetic backgrounds, and that *CTLA4*, *CD226*, *STAT4* and *IRF5* are also potential risk factors for this disease.

Gout, a disease characterized by dysregulated serum urate concentration, has been the subject of a large-scale GWAS analysis. In this study, >140,000 samples from the general population were analysed for serum urate concentration[65], and associated risk variants for both high serum urate concentration and gout implicated urate transporter genes, such as *SLC2A9, ABCG2, SLC22A11,* and the glycolytic pathway gene *GCKR*. Candidate-gene approaches have also implicated the NLRP3 inflammasome in development of gout[66].

## Stratified medicine approaches

Defining the genetic risk profile underpinning disease heterogeneity could lead to the development of stratified medicine approaches based on the dysfunction and/or dysregulation of key pathways in different subsets of patients. RA is the most ideally placed rheumatic disease to benefit from such approaches. A variety of biologic therapies are already available that target different pathways containing implicated RA-susceptibility genes, with each therapy currently being effective in approximately 40% of patients[67]. For example, variants within the *TYK2* gene, a key component of the Janus kinase–signal transducers and activators of transcription (JAK–STAT) signalling pathway, are associated with RA[30], as well as with JIA[32], PsA[37], AS[52] and SLE[24]. Tocilizumab, an IL-6 receptor (IL-6R) inhibitor, targets this pathway and has demonstrated efficacy in the treatment of RA[68]. A SNP variant that leads to increased cleaving of membrane-bound IL-6R to its soluble form is associated with RA and mimics the effect of the drug[30,69]. Importantly, a SNP allele associated with protection from RA[30] and coronary heart disease[70] is associated with risk of asthma[46]. These findings demonstrate how genetic knowledge can provide not only novel therapeutic targets but also the biological mechanism by which the drug exerts its effect. The gene encoding inhibitory receptor cytotoxic T-lymphocyte protein 4 (CTLA4) is implicated in RA susceptibility owing to a disease-associated risk variant in close proximity to this gene[25]. Abatacept, a CTLA4–Ig fusion protein that blocks co-stimulation of T cells, is an effective therapy for RA[71]. Carefully designed trials can therefore use genetic knowledge to better target the wide variety of available biologic therapies to the subsets of patients most likely to respond. One of the major goals of genetic research is to facilitate the prescription of available drugs to those patients most likely to show the greatest benefit, with minimal off-target effects. Large-scale efforts are currently ongoing to genotype cohorts of patients at the onset of drug-therapy and gather prospective data on their treatment response to determine whether genetics can aid initial treatment decisions[72,73].

## Insights into gene regulation

From a molecular genetics standpoint, GWAS have provided important insights into the mechanisms of complex diseases. Somewhat surprisingly, perhaps, the vast majority of risk variants associated with rheumatic diseases, and indeed all complex diseases, are found outside traditional protein-coding regions[74]. Such variants are unlikely to change the protein structure; they are perhaps more likely to affect the regulation of gene expression (in terms of the magnitude, response to stimulation, cell-type specificity or temporal pattern of gene expression).

Indeed, a well-recognized finding from GWAS is the enrichment of disease-associated variants within cell-type-specific regulatory regions[26,74]. Although each cell in the body contains the same genetic material, the different combinations of genes actively expressed by different cell types determine the characteristics of those cells. Hence, disease-associated variants that map to regulatory regions might be responsible for controlling gene expression. Actively expressed genes typically reside in regions of DNA that are open and accessible, marked by histone modifications that enable this DNA to assume an unwound conformation. International efforts are now attempting to map these open, active regions of the genome in a range of cell types[75–80]. For example, in T cells, DNA regions that are open and active are commonly enriched for risk variants associated with RA and JIA (in $CD4^+$ T cells)[25,81], as well as with PsA (in $CD8^+$ T cells)[37]. These findings confirm the implicit role of these cell types in disease and also highlight regions of the genome that are important in disease development, which often contain many plausible candidate genes.

One method for linking an associated risk variant to a causal gene is to look at its correlation with gene expression, in a technique known as expression quantitative trait loci (eQTL) analysis[82–86] (TABLE 1). This analysis has demonstrated compelling evidence that genes such as *ERAP2*, *IRF5* and *NCOA3* are regulated by variants

associated with AS[87,88], SLE[89], and OA[22], respectively. This type of correlation with genotype and expression has also revealed levels of mechanistic complexity for GWAS-associated variants. For example, the correlation between genotype and expression is context-specific, such that the majority of eQTLs are only revealed in certain cell types, under certain stimulatory conditions[84,85]. Again, this finding gives an insight into the mechanism by which genetic changes predispose to disease. Large international consortia such as the Genotype–Tissue Expression (GTEx) project[82,83,90] are providing powerful data resources by mapping genotype and expression in a wealth of different tissues; these efforts should enable researchers to check whether a disease-associated variant can regulate gene expression in a particular cell type and/or condition.

When viewing DNA in a linear fashion, gene regulatory regions are often a large distance away from the genes they regulate; however, 3D conformational chromosomal structures enable regulatory regions to physically interact with distant genes. This physical interaction, and hence gene regulation, can occur over large distances, often 'skipping' the nearest gene. This distance can obviously complicate attempts to assign disease-associated variants to a gene, and might well have compromised post-GWAS attempts at biological pathway analysis. Hence, future studies might yet reveal more subtle pathways to disease. These physical interactions can be revealed with molecular biology techniques, fixing the DNA in its natural, 3D conformation, and then using sequencing to identify DNA regions that are in close proximity to each other. This type of DNA conformational analysis, including, for example, Hi-C[91,92] (TABLE 1) for whole genome interactions and chromosome conformation capture (3C)[93,94] for local interactions, is beginning to add new interpretations to findings from rheumatic disease GWAS. One experiment exploring the interactions of regions associated with RA, JIA and PsA revealed that the closest gene is not always the one interacting with the regulatory regions; interactions can span large genetic distances and DNA regions that contain variants associated with these diseases can interact with each other even if spatially distinct[95]. For example, an RA-associated variant within the gene *COG6* interacts with the gene promoter of *FOXO1*, which is, although sited some distance away, a much more compelling putative causal gene; *COG6* encodes a component of Golgi apparatus whereas *FOXO1* encodes a transcription factor important in the survival of fibroblast-like synoviocytes (FLS) in RA[96] and is hypermethylated in FLS from patients with RA compared with FLS from patients with OA[97]. In addition, by use of 3C analysis, another study demonstrated that a region downstream of *TNFAIP3*, which contains a DNA insertion associated with SLE, interacts with the *TNFAIP3* promoter, supporting the causal role of this gene[98,99]. Thus, these types of experiments are beginning to provide great insight into cell-type-specific and genotype-specific regulatory interactions, and demonstrate the complexity of gene regulation.

## Conclusions

As is so often the case in scientific research, investigations into the genetic basis of rheumatic diseases have revealed more questions than they have answered. Although for many conditions we have considerably increased our knowledge of genetic associations with disease in the past 10 years, we have in turn discovered that we cannot assume associated variants affect the function of their nearest gene. Unravelling the function of disease-associated variants will require an increased understanding of how gene expression is regulated. Elucidating the genetic basis of disease continues to hold great promise and, as illustrated here, this challenge is ongoing. Current insights are close to influencing the treatment of patients with rheumatic diseases through the identification of new drugs, repositioning of existing therapies and a stratified medicine approach to treatment selection.

In the past two years, exponential advances in genetic engineering, specifically in the form of CRISPR–Cas9 genome editing[100–102] (TABLE 1), have enabled researchers to ask more-specific questions, such as how disease-associated alleles affect the process of complex diseases. Although beyond the scope of this Review, genome editing technology will probably provide robust, empirical evidence of the mechanism(s) by which these genetic components influence disease. For example, gene regulators, DNA variants and whole pathways can be precisely perturbed using genome editing to determine their downstream phenotypic consequences[103–106]. By precisely manipulating the genetic background of a cell from non-risk to risk, or by targeting the implicated regulators, researchers can gain further insight into these complex diseases.


1. Alarcon-Segovia, D. *et al.* Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. *Arthritis Rheum.* **52**, 1138–1147 (2005).
2. Brown, M. A. *et al.* Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum.* **40**, 1823–1828 (1997).
3. Karason, A., Love, T. J. & Gudbjornsson, B. A strong heritability of psoriatic arthritis over four generations — the Reykjavik Psoriatic Arthritis Study. *Rheumatology (Oxford)* **48**, 1424–1428 (2009).
4. Silman, A. J. *et al.* Twin concordance rates for rheumatoid arthritis: results from a nationwide study. *Br. J. Rheumatol.* **32**, 903–907 (1993).
5. Spector, T. D. & MacGregor, A. J. Risk factors for osteoarthritis: genetics. *Osteoarthritis Cartilage* **12** (Suppl. A), S39–S44 (2004).
6. Nelson, M. R. *et al.* The support of human genetic evidence for approved drug indications. *Nat. Genet.* **47**, 856–860 (2015).
7. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* **447**, 661–678 (2007).
8. Plenge, R. M. *et al.* Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nat. Genet.* **39**, 1477–1482 (2007).
9. Plenge, R. M. *et al.* TRAF1-C5 as a risk locus for rheumatoid arthritis — a genomewide study. *N. Engl. J. Med.* **357**, 1199–1209 (2007).
10. Remmers, E. F. *et al.* STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N. Engl. J. Med.* **357**, 977–986 (2007).
11. Barton, A. *et al.* Rheumatoid arthritis susceptibility loci at chromosomes 10p15, 12q13 and 22q13. *Nat. Genet.* **40**, 1156–1159 (2008).
12. Raychaudhuri, S. *et al.* Genetic variants at CD28, PRDM1 and CD2/CD58 are associated with rheumatoid arthritis risk. *Nat. Genet.* **41**, 1313–1318 (2009).
13. Stahl, E. A. *et al.* Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. *Nat. Genet.* **42**, 508–514 (2010).
14. Thomson, W. *et al.* Rheumatoid arthritis association at 6q23. *Nat. Genet.* **39**, 1431–1433 (2007).
15. Burton, P. R. *et al.* Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat. Genet.* **39**, 1329–1337 (2007).
16. Evans, D. M. *et al.* Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat. Genet.* **43**, 761–767 (2011).

17. Reveille, J. D. *et al.* Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat. Genet.* **42**, 123–127 (2010).
18. Zeggini, E. *et al.* Identification of new susceptibility loci for osteoarthritis (arcOGEN): a genome-wide association study. *Lancet* **380**, 815–823 (2012).
19. Valdes, A. M. *et al.* Involvement of different risk factors in clinically severe large joint osteoarthritis according to the presence of hand interphalangeal nodes. *Arthritis Rheum.* **62**, 2688–2695 (2010).
20. Frayling, T. M. *et al.* A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* **316**, 889–894 (2007).
21. Evangelou, E. *et al.* A meta-analysis of genome-wide association studies identifies novel variants associated with osteoarthritis of the hip. *Ann. Rheum. Dis.* **73**, 2130–2136 (2014).
22. Gee, F., Rushton, M. D., Loughlin, J. & Reynard, L. N. Correlation of the osteoarthritis susceptibility variants that map to chromosome 20q13 with an expression quantitative trait locus operating on NCOA3 and with functional variation at the polymorphism rs116855380. *Arthritis Rheumatol.* **67**, 2923–2932 (2015).
23. Bentham, J. *et al.* Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat. Genet.* **47**, 1457–1464 (2015).
24. Morris, D. L. *et al.* Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat. Genet.* **48**, 940–946 (2016).
25. Okada, Y. *et al.* Genetics of rheumatoid arthritis contributes to biology and drug discovery. *Nature* **506**, 376–381 (2014).
26. Trynka, G. *et al.* Chromatin marks identify critical cell types for fine mapping complex trait variants. *Nat. Genet.* **45**, 124–130 (2013).
27. Chapman, K. *et al.* A meta-analysis of European and Asian cohorts reveals a global role of a functional SNP in the 5′ UTR of GDF5 with osteoarthritis susceptibility. *Hum. Mol. Genet.* **17**, 1497–1504 (2008).
28. Ellinghaus, D. *et al.* Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci. *Nat. Genet.* **48**, 510–518 (2016).
29. Adrianto, I. *et al.* Association of a functional variant downstream of TNFAIP3 with systemic lupus erythematosus. *Nat. Genet.* **43**, 253–258 (2011).
30. Eyre, S. *et al.* High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. *Nat. Genet.* **44**, 1336–1340 (2012).
31. Cortes, A. *et al.* Identification of multiple risk variants for ankylosing spondylitis through high-density genotyping of immune-related loci. *Nat. Genet.* **45**, 730–738 (2013).
32. Hinks, A. *et al.* Dense genotyping of immune-related disease regions identifies 14 new susceptibility loci for juvenile idiopathic arthritis. *Nat. Genet.* **45**, 664–669 (2013).
33. Sun, C. *et al.* High-density genotyping of immune-related loci identifies new SLE risk variants in individuals with Asian ancestry. *Nat. Genet.* **48**, 323–330 (2016).
34. Fortune, M. D. *et al.* Statistical colocalization of genetic risk variants for related autoimmune diseases in the context of common controls. *Nat. Genet.* **47**, 839–846 (2015).
35. Liley, J. & Wallace, C. A pleiotropy-informed Bayesian false discovery rate adapted to a shared control design finds new disease associations from GWAS summary statistics. *PLoS Genet.* **11**, e1004926 (2015).
36. Wallace, C. *et al.* Dissection of a complex disease susceptibility region using a Bayesian stochastic search approach to fine mapping. *PLoS Genet.* **11**, e1005272 (2015).
37. Bowes, J. *et al.* Dense genotyping of immune-related susceptibility loci reveals new insights into the genetics of psoriatic arthritis. *Nat. Commun.* **6**, 6046 (2015).
38. Raychaudhuri, S. *et al.* Five amino acids in three HLA proteins explain most of the association between MHC and seropositive rheumatoid arthritis. *Nat. Genet.* **44**, 291–296 (2012).
39. Gaffen, S. L., Jain, R., Garg, A. V. & Cua, D. J. The IL-23–IL-17 immune axis: from mechanisms to therapeutic testing. *Nat. Rev. Immunol.* **14**, 585–600 (2014).
40. Onengut-Gumuscu, S. *et al.* Fine mapping of type 1 diabetes susceptibility loci and evidence for colocalization of causal variants with lymphoid gene enhancers. *Nat. Genet.* **47**, 381–386 (2015).
41. Lemos, L. L. *et al.* Treatment of psoriatic arthritis with anti-TNF agents: a systematic review and meta-analysis of efficacy, effectiveness and safety. *Rheumatol. Int.* **34**, 1345–1360 (2014).
42. Bossini-Castillo, L., Lopez-Isac, E. & Martin, J. Immunogenetics of systemic sclerosis: defining heritability, functional variants and shared-autoimmunity pathways. *J. Autoimmun.* **64**, 53–65 (2015).
43. Mayes, M. D. *et al.* Immunochip analysis identifies multiple susceptibility loci for systemic sclerosis. *Am. J. Hum. Genet.* **94**, 47–61 (2014).
44. Lopez-Isac, E. *et al.* Brief report: IRF4 newly identified as a common susceptibility locus for systemic sclerosis and rheumatoid arthritis in a cross-disease meta-analysis of genome-wide association studies. *Arthritis Rheumatol.* **68**, 2338–2344 (2016).
45. Sekine, C. *et al.* Successful treatment of animal models of rheumatoid arthritis with small-molecule cyclin-dependent kinase inhibitors. *J. Immunol.* **180**, 1954–1961 (2008).
46. Ferreira, M. A. *et al.* Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet* **378**, 1006–1014 (2011).
47. Begovich, A. B. *et al.* A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. *Am. J. Hum. Genet.* **75**, 330–337 (2004).
48. Jostins, L. *et al.* Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* **491**, 119–124 (2012).
49. Mohan, C. & Putterman, C. Genetics and pathogenesis of systemic lupus erythematosus and lupus nephritis. *Nat. Rev. Nephrol.* **11**, 329–341 (2015).
50. Guerra, S. G., Vyse, T. J. & Cunninghame Graham, D. S. The genetics of lupus: a functional perspective. *Arthritis Res. Ther.* **14**, 211 (2012).
51. Robinson, P. C. & Brown, M. A. Genetics of ankylosing spondylitis. *Mol. Immunol.* **57**, 2–11 (2014).
52. Brown, M. A., Kenna, T. & Wordsworth, B. P. Genetics of ankylosing spondylitis — insights into pathogenesis. *Nat. Rev. Rheumatol.* **12**, 81–91 (2016).
53. Parkes, M., Cortes, A., van Heel, D. A. & Brown, M. A. Genetic insights into common pathways and complex relationships among immune-mediated diseases. *Nat. Rev. Genet.* **14**, 661–673 (2013).
54. Loughlin, J. Genetic contribution to osteoarthritis development: current state of evidence. *Curr. Opin. Rheumatol.* **27**, 284–288 (2015).
55. Reynard, L. N. & Loughlin, J. Insights from human genetic studies into the pathways involved in osteoarthritis. *Nat. Rev. Rheumatol.* **9**, 573–583 (2013).
56. Rogers, E. L., Reynard, L. N. & Loughlin, J. The role of inflammation-related genes in osteoarthritis. *Osteoarthritis Cartilage* **23**, 1933–1938 (2015).
57. Lessard, C. J. *et al.* Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. *Nat. Genet.* **45**, 1284–1292 (2013).
58. Li, Y. *et al.* A genome-wide association study in Han Chinese identifies a susceptibility locus for primary Sjogren's syndrome at 7q11.23. *Nat. Genet.* **45**, 1361–1365 (2013).
59. Albagha, O. M. *et al.* Genome-wide association study identifies variants at CSF1, OPTN and TNFRSF11A as genetic risk factors for Paget's disease of bone. *Nat. Genet.* **42**, 520–524 (2010).
60. Albagha, O. M. *et al.* Genome-wide association identifies three new susceptibility loci for Paget's disease of bone. *Nat. Genet.* **43**, 685–689 (2011).
61. Vallet, M. *et al.* Targeted sequencing of the Paget's disease associated 14q32 locus identifies several missense coding variants in RIN3 that predispose to Paget's disease of bone. *Hum. Mol. Genet.* **24**, 3286–3295 (2015).
62. Lyons, P. A. *et al.* Genetically distinct subsets within ANCA-associated vasculitis. *N. Engl. J. Med.* **367**, 214–223 (2012).
63. Xie, G. *et al.* Association of granulomatosis with polyangiitis (Wegener's) with HLA-DPB1*04 and SEMA6A gene variants: evidence from genome-wide analysis. *Arthritis Rheum.* **65**, 2457–2468 (2013).
64. Rahmattulla, C. *et al.* Genetic variants in ANCA-associated vasculitis: a meta-analysis. *Ann. Rheum. Dis.* **75**, 1687–1692 (2016).
65. Kottgen, A. *et al.* Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. *Nat. Genet.* **45**, 145–154 (2013).
66. Miao, Z. M. *et al.* NALP3 inflammasome functional polymorphisms and gout susceptibility. *Cell Cycle* **8**, 27–30 (2009).
67. Detert, J. & Klaus, P. Biologic monotherapy in the treatment of rheumatoid arthritis. *Biologics* **9**, 35–43 (2015).
68. Shetty, A. *et al.* Tocilizumab in the treatment of rheumatoid arthritis and beyond. *Drug Des. Devel. Ther.* **8**, 349–364 (2014).
69. Ferreira, R. C. *et al.* Functional IL6R 358Ala allele impairs classical IL-6 receptor signaling and influences risk of diverse inflammatory diseases. *PLoS Genet.* **9**, e1003444 (2013).
70. Swerdlow, D. I. *et al.* The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. *Lancet* **379**, 1214–1224 (2012).
71. Schiff, M. Abatacept treatment for rheumatoid arthritis. *Rheumatology (Oxford)* **50**, 437–449 (2011).
72. Oliver, J., Plant, D., Webster, A. P. & Barton, A. Genetic and genomic markers of anti-TNF treatment response in rheumatoid arthritis. *Biomark. Med.* **9**, 499–512 (2015).
73. Plant, D., Wilson, A. G. & Barton, A. Genetic and epigenetic predictors of responsiveness to treatment in RA. *Nat. Rev. Rheumatol.* **10**, 329–337 (2014).
74. Farh, K. K. *et al.* Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* **518**, 337–343 (2015).
75. ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* **489**, 57–74 (2012).
76. Bernstein, B. E. *et al.* The NIH Roadmap Epigenomics Mapping Consortium. *Nat. Biotechnol.* **28**, 1045–1048 (2010).
77. Kersey, P. J. *et al.* Ensembl Genomes 2016: more genomes, more complexity. *Nucleic Acids Res.* **44**, D574–D580 (2016).
78. Kundaje, A. *et al.* Integrative analysis of 111 reference human epigenomes. *Nature* **518**, 317–330 (2015).
79. Martens, J. H. & Stunnenberg, H. G. BLUEPRINT: mapping human blood cell epigenomes. *Haematologica* **98**, 1487–1489 (2013).
80. Yates, A. *et al.* Ensembl 2016. *Nucleic Acids Res.* **44**, D710–D716 (2016).
81. Trynka, G. & Raychaudhuri, S. Using chromatin marks to interpret and localize genetic associations to complex human traits and diseases. *Curr. Opin. Genet. Dev.* **23**, 635–641 (2013).
82. GTEx Consortium. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* **348**, 648–660 (2015).
83. Carithers, L. J. & Moore, H. M. The Genotype-Tissue Expression (GTEx) Project. *Biopreserv. Biobank.* **13**, 307–308 (2015).
84. Fairfax, B. P. & Knight, J. C. Genetics of gene expression in immunity to infection. *Curr. Opin. Immunol.* **30**, 63–71 (2014).
85. Fairfax, B. P. *et al.* Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* **343**, 1246949 (2014).
86. Westra, H. J. *et al.* Cell specific eQTL analysis without sorting cells. *PLoS Genet.* **11**, e1005223 (2015).
87. Andres, A. M. *et al.* Balancing selection maintains a form of ERAP2 that undergoes nonsense-mediated decay and affects antigen presentation. *PLoS Genet.* **6**, e1001157 (2010).
88. Robinson, P. C. *et al.* ERAP2 is associated with ankylosing spondylitis in HLA-B27-positive and HLA-B27-negative patients. *Ann. Rheum. Dis.* **74**, 1627–1629 (2015).
89. Graham, R. R. *et al.* Three functional variants of IFN regulatory factor 5 (IRF5) define risk and protective haplotypes for human lupus. *Proc. Natl Acad. Sci. USA* **104**, 6758–6763 (2007).
90. GTEx Consortium. The Genotype-Tissue Expression (GTEx) project. *Nat. Genet.* **45**, 580–585 (2013).
91. Belton, J. M. *et al.* Hi-C: a comprehensive technique to capture the conformation of genomes. *Methods* **58**, 268–276 (2012).
92. Lieberman-Aiden, E. *et al.* Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289–293 (2009).
93. Dekker, J., Rippe, K., Dekker, M. & Kleckner, N. Capturing chromosome conformation. *Science* **295**, 1306–1311 (2002).

94. Miele, A. & Dekker, J. Mapping *cis-* and *trans-*chromatin interaction networks using chromosome conformation capture (3C). *Methods Mol. Biol.* **464**, 105–121 (2009).
95. Martin, P. *et al.* Capture Hi-C reveals novel candidate genes and complex long-range interactions with related autoimmune risk loci. *Nat. Commun.* **6**, 10069 (2015).
96. Grabiec, A. M. *et al.* JNK-dependent downregulation of FoxO1 is required to promote the survival of fibroblast-like synoviocytes in rheumatoid arthritis. *Ann. Rheum. Dis.* **74**, 1763–1771 (2015).
97. Nakano, K., Whitaker, J. W., Boyle, D. L., Wang, W. & Firestein, G. S. DNA methylome signature in rheumatoid arthritis. *Ann. Rheum. Dis.* **72**, 110–117 (2013).
98. Wang, S., Wen, F., Wiley, G. B., Kinter, M. T. & Gaffney, P. M. An enhancer element harboring variants associated with systemic lupus erythematosus engages the TNFAIP3 promoter to influence A20 expression. *PLoS Genet.* **9**, e1003750 (2013).
99. McGovern, A. *et al.* Capture Hi-C identifies a novel causal gene, IL20RA, in the pan-autoimmune genetic susceptibility region 6q23. *Genome Biol.* **17**, 212 (2016).
100. Cong, L. & Zhang, F. Genome engineering using CRISPR–Cas9 system. *Methods Mol. Biol.* **1239**, 197–217 (2015).
101. Ran, F. A. *et al.* Genome engineering using the CRISPR–Cas9 system. *Nat. Protoc.* **8**, 2281–2308 (2013).
102. Wright, A. V., Nunez, J. K. & Doudna, J. A. Biology and applications of CRISPRsystems: harnessing nature's toolbox for genome engineering. *Cell* **164**, 29–44 (2016).
103. Hilton, I. B. & Gersbach, C. A. Enabling functional genomics with genome engineering. *Genome Res.* **25**, 1442–1455 (2015).
104. Hilton, I. B. *et al.* Epigenome editing by a CRISPR–Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nat. Biotechnol.* **33**, 510–517 (2015).
105. Thakore, P. I. *et al.* Highly specific epigenome editing by CRISPR–Cas9 repressors for silencing of distal regulatory elements. *Nat. Methods* **12**, 1143–1149 (2015).
106. Thakore, P. I., Black, J. B., Hilton, I. B. & Gersbach, C. A. Editing the epigenome: technologies for programmable transcription and epigenetic modulation. *Nat. Methods* **13**, 127–137 (2016).
107. Auton, A. *et al.* A global reference for human genetic variation. *Nature* **526**, 68–74 (2015).
108. Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921 (2001).
109. The International HapMap Consortium. The International HapMap Project. *Nature* **426**, 789–796 (2003).
110. Zhernakova, A. *et al.* Meta-analysis of genome-wide association studies in celiac disease and rheumatoid arthritis identifies fourteen non-HLA shared loci. *PLoS Genet.* **7**, e1002004 (2011).
111. McCarthy, M. I. *et al.* Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat. Rev. Genet.* **9**, 356–369 (2008).
112. Evangelou, E. & Ioannidis, J. P. Meta-analysis methods for genome-wide association studies and beyond. *Nat. Rev. Genet.* **14**, 379–389 (2013).
113. Li, Y. R. & Keating, B. J. Trans-ethnic genome-wide association studies: advantages and challenges of mapping in diverse populations. *Genome Med.* **6**, 91 (2014).
114. Konig, I. R. Validation in genetic association studies. *Brief. Bioinform.* **12**, 253–258 (2011).
115. Stephens, M. & Balding, D. J. Bayesian statistical methods for genetic association studies. *Nat. Rev. Genet.* **10**, 681–690 (2009).
116. Doudna, J. A. & Charpentier, E. Genome editing. The new frontier of genome engineering with CRISPR–Cas9. *Science* **346**, 1258096 (2014).
117. Albert, F. W. & Kruglyak, L. The role of regulatory variation in complex traits and disease. *Nat. Rev. Genet.* **16**, 197–212 (2015).
118. Ioannidis, J. P., Thomas, G. & Daly, M. J. Validating, augmenting and refining genome-wide association signals. *Nat. Rev. Genet.* **10**, 318–329 (2009).
119. Nejentsev, S., Walker, N., Riches, D., Egholm, M. & Todd, J. A. Rare variants of IFIH1, a gene implicated in antiviral responses, protect against type 1 diabetes. *Science* **324**, 387–389 (2009).
120. Pe'er, I. *et al.* Evaluating and improving power in whole-genome association studies using fixed marker sets. *Nat. Genet.* **38**, 663–667 (2006).
121. Lewis-Faning, E. Report on an enquiry into the aetiological factors associated with rheumatoid arthritis. *Ann. Rheum. Dis.* **9**, 1–94 (1950).
122. Stastny, P. Mixed lymphocyte cultures in rheumatoid arthritis. *J. Clin. Invest.* **57**, 1148–1157 (1976).
123. Gregersen, P. K., Silver, J. & Winchester, R. J. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum.* **30**, 1205–1213 (1987).
124. MacGregor, A. J. *et al.* Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. *Arthritis Rheum.* **43**, 30–37 (2000).
125. Venter, J. C. *et al.* The sequence of the human genome. *Science* **291**, 1304–1351 (2001).
126. Klein, R. J. *et al.* Complement factor H polymorphism in age-related macular degeneration. *Science* **308**, 385–389 (2005).
127. Abecasis, G. R. *et al.* A map of human genome variation from population-scale sequencing. *Nature* **467**, 1061–1073 (2010).
128. Mali, P. *et al.* RNA-guided human genome engineering via Cas9. *Science* **339**, 823–826 (2013).
129. Jinek, M. *et al.* RNA-programmed genome editing in human cells. *eLife* **2**, e00471 (2013).



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# Why location matters — site-specific factors in rheumatic diseases

*Caroline Ospelt and Mojca Frank-Bertoncelj*

Abstract | Rheumatic diseases follow a characteristic anatomical pattern of joint and organ involvement. This Review explores three interconnected mechanisms that might be involved in the predilection of specific joints for developing specific forms of arthritis: site-specific local cell types that drive disease; systemic triggers that affect local cell types; and site-specific exogenous factors, such as focal mechanical stress, that activate cells locally. The embryonic development of limbs and joints is also relevant to the propensity of certain joints to develop arthritis. Additionally, location-specific homeostasis and disease occurs in skin and blood vessels, thereby extending the concept of site-specificity in human diseases beyond rheumatology. Acknowledging the importance of site-specific parameters increases the complexity of current disease paradigms and brings us closer to understanding why particular disease processes manifest at a particular location.

Specific anatomical patterns of joint and organ involvement characterize rheumatic diseases. Gout, reactive arthritis, ankylosing spondylitis and Behçet disease characteristically affect joints of the lower extremities and/or the spine. By contrast, patients with rheumatoid arthritis (RA), systemic lupus erythematosus, systemic sclerosis (SSc) or polymyositis typically develop arthritis in the small, distal joints of the hands and feet[1]. Similarly, the extra-articular organ involvement that can accompany rheumatic disease also shows a disease-specific pattern of anatomical distribution (FIG. 1). For instance, involvement of the eyes and the gut in addition to the spine is typical in patients with ankylosing spondylitis, and in psoriatic arthritis (PsA) the skin on the scalp, elbows, knees or lower back is characteristically involved.

The consistency with which these patterns appear suggests that the respective disease pathways are preferentially triggered at certain anatomic sites. A growing body of evidence supports the idea that the anatomical diversity of stromal cells not only guides local cellular specialization, tissue homeostasis and regeneration, but also contributes to location-specific disease development[2,3]. Molecules produced at the primary site of disease might also invoke pathological processes at susceptible secondary sites, inducing a disease-specific anatomical pattern of comorbidities.

In this Review, we discuss three potentially interconnected mechanisms that are involved in driving site-specific pathognomonic disease patterns. We also review studies in embryonic development that suggest a potential connection between the site-specific identities of tissue-resident cells involved in joint disease and embryonic development, as well as discussing epigenetic changes that occur during development. To conclude, we discuss the site-specific homeostatic functions of skin and responsiveness of the vasculature to inflammatory signals, demonstrating that the concept of location-specific characteristics and site-specificity in human diseases is broadly applicable beyond rheumatic diseases.

## Site-specific factors in disease

Location is all-important in the development of rheumatic diseases. We propose three mechanisms to be involved in perpetuating disease at specific anatomic sites: site-specific local cells; systemic factors and the nervous system; and focal mechanical stress.

***Local cells in joint disease.*** Synovial joints are specialized organs, built to meet the unique biomechanical and physiological needs of a given anatomic location. The structural elements of the synovial joint, such as the articular cartilage, synovial membrane (a delicate structure composed of one or two cell layers of synovial fibroblasts and tissue-resident macrophages), ligaments and menisci, support the specialized functions of individual joints.

An emerging body of data shows large differences in the transcriptomic and epigenomic profiles of synovial fibroblasts and articular cartilage from distinct joints[4–6]. This anatomical transcriptional diversity translates into joint-specific phenotypes of synovial fibroblasts[5]. For example, synovial fibroblasts from joints in the hands

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**Key points**

- Site-specific pathognomonic disease patterns are based on three interconnected mechanisms, namely site-specific local cells, systemic factors that affect particular anatomical sites and local mechanical factors
- Synovial fibroblasts and chondrocytes differ substantially between joints and might create location-specific joint microenvironments, rendering each joint more or less susceptible to different types of arthritis
- Evidence is emerging that local differences in joint innervation and vasculature might influence arthritis patterns; however, further studies are needed to strengthen these observations
- Local mechanical factors can aggravate joint disease, yet whether they trigger disease locally needs further clarification
- The overlap between site-specific embryonic traits and disease location suggests a role for embryonic pathways in the pathogenesis and occurrence of disease in joints, as well as in other organs

of patients with osteoarthritis (OA) and RA expressed larger amounts of matrix-degrading enzymes, such as collagenase 3, and displayed stronger proliferative and chemotactic properties than synovial fibroblasts from other joints[5]. These results might explain why the joints of the hand are primary targets of destruction in arthritis[7]. Additionally, synovial tissues from the hip joints of patients with RA or OA expressed higher levels of IL-6 when compared with synovial tissues from the knees of these patients[8]. The results of these studies suggest that each joint has a specific synovial microenvironment in health and disease. If this is true, then the local characteristics of the inflammatory response could define the susceptibility of a particular joint to developing disease. Similarly, it could be hypothesized that some types of arthritis present as oligoarthritides because fewer joint regions are susceptible to the triggering factor(s). It has been suggested that arthritis will present symmetrically when several joints in a certain region are affected[9]; therefore, the symmetric occurrence of arthritis might be a function of both the number of susceptible joint regions and the number of involved joints. The concept that joint microenvironments are susceptible or resistant to arthritis-specific pathogenic pathways could not only explain the anatomical patterns evident in arthritis, but might also reveal why arthritis can develop symmetrically or asymmetrically and manifest as either oligoarthritis or polyarthritis.

In contrast to other types of chronic arthritis, PsA affects a variety of joint regions with heterogeneous manifestations. The classic subgroups in PsA are distal interphalangeal (DIP) joint-predominant arthritis, asymmetrical oligoarticular arthritis, symmetrical polyarthritis, arthritis mutilans and predominant spondylitis, as described by Moll and Wright[10]. Conceivably, different pathogenic pathways could be operative in these different manifestations of PsA. For example, the symmetrical polyarthritis of the hands and feet that occurs predominantly in women might actually be a form of seronegative RA with concomitant skin psoriasis[11,12]. The occurrence of spondylitis, sacroiliitis, enthesitis, arthritis mutilans and dactylitis in patients with PsA are associated with different disease-risk genotypes, which code for various MHC class I receptors[13,14]. In particular, HLA-B27 is strongly associated with the involvement of entheses and the spine in patients with PsA and in other spondyloarthropathies, such as ankylosing spondylitis[13,15]. Additionally, HLA-B27-positive patients have an increased risk of eye involvement[16]. This pattern of disease manifestations at specific locations suggests that HLA-B27 expression is connected to pathogenic mechanisms that are specifically activated at these anatomic sites or that specifically activate these sites. HLA-B27 is thought to trigger an autoreactive T cell response by presenting self-peptides or by forming aberrant cell-surface complexes[17]. Other studies describe a high level of misfolded HLA-B27 molecules within cells, which induces endoplasmic reticulum (ER) stress[18]. The subsequent unfolded protein response and ER-associated degradation of HLA-B27 leads to increased production of IL-23 (REFS 17,18). Based on these studies, HLA-B27-driven processes could be supposed to be pronounced in resident cells of the spine, the entheses, the eye and the gut. For example, cell-type specific responses to ER stress have been described in different circumstances[19,20] and could potentially occur in the context of HLA-B27-associated spondyloarthropathies.

To understand completely whether and how local cells control joint-specific homeostasis and propensity for disease requires in depth analysis of healthy and diseased synovial tissue and cartilage from a variety of joints. Characterization of the synovial cellular infiltrate in different forms of arthritis should provide key insights into local pathological processes and uncover the exact, joint-specific function of cells that drive disease locally. An early study comparing the numbers of macrophages, synovial fibroblasts and T cells, and the level of IL-6 production between joints did not find any statistically significant differences between the synovia of small distal joints and knee joints in patients with RA[21]. However, more sophisticated approaches (such as single cell omics or multidimensional cytometry) can now be applied to decipher a possible role of specific local immune and stromal cells in arthritis.

***Local response to systemic factors.*** Systemic factors substantially affect the physiology and pathology of joints. Synovial fluid is a transudate from blood plasma and, as the synovial membrane has no specific barrier function[22], most proteins that are present in the circulation can transfer to the synovial fluid. Joint-specific responses to systemic factors such as cytokines or autoantibodies could have a role in determining which joints develop arthritis. For example, high levels of IL-23 are released in response to misfolded HLA-B27 and the subsequent unfolded protein response[23]. IL-23 can potentially bind to local immune or stromal cells at particular anatomic sites; indeed, enthesitis was induced by IL-23 receptor$^{+}$CD3$^{+}$CD4$^{-}$CD8$^{-}$ enthesis-resident cells in mice[24], lending support to the idea that local cells responding to systemic stimuli have a role in pathogenesis. Molecules produced at the primary disease site could then trigger disease at susceptible secondary sites.

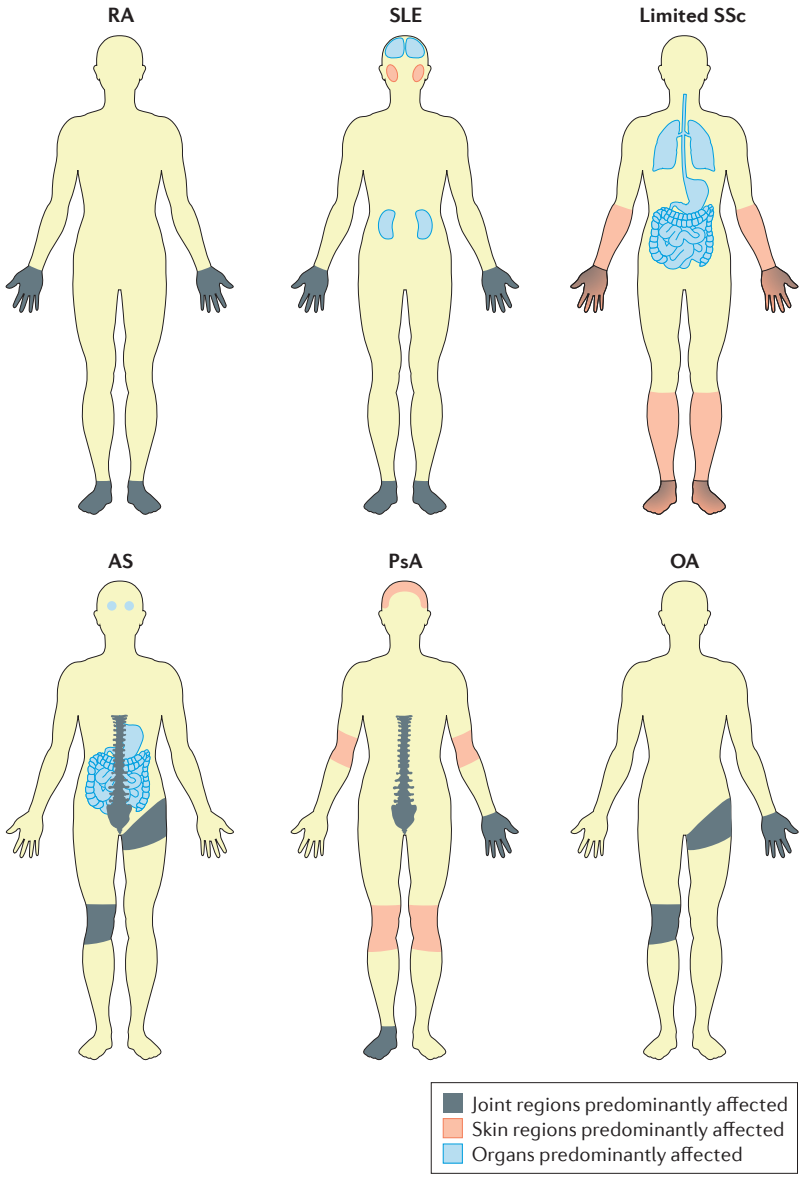


Figure 1 | **Patterns of joint and organ involvement in rheumatic disease.** Even though rheumatic diseases can present with variable symptoms in individual patients, characteristic patterns of joint and organ involvement are distinguishable between rheumatic diseases. AS, ankylosing spondylitis; OA, osteoarthritis; PsA, psoriatic arthritis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SSc, systemic sclerosis.

The synovium is a highly vascularized tissue that is innervated by myelinated and unmyelinated nerve fibres. Studies in the K/B×N serum transfer model of murine arthritis showed an increased capacity for leakage of macromolecules, such as immune complexes, from the vasculature into the joints of the distal extremities[25]. These 'leaky blood vessels' could explain the characteristic distal pattern of arthritis (wrists, ankles, hind paws and front paws) observed in the K/B×N serum transfer model[26,27]. Unilateral transection of the femoral and sciatic nerves in these mice inhibited the leakage of macromolecules into joints, thereby protecting the hind paws from the development of arthritis[25]. The endothelial cells of denervated hind paws in these mice displayed altered expression of a number of genes involved in vascular leakage and transendothelial cell migration[25], suggesting that the nervous system might control the response to inflammation of the joint microvasculature during the course of arthritis. Whether similar mechanisms are operative in arthritis in humans remains speculative; nevertheless, denervation was shown to protect the joints of paretic or paralytic extremities in humans from the development of arthritis, particularly RA[28–33]. The specific characteristics of the nerve fibres that influenced the distal vasculature in the K/B×N serum transfer model of arthritis were not identified in the studies discussed. Similarly, which of the arms of the nervous system (motor, sensory or autonomous) are involved in the remission of arthritis in paretic and paralytic extremities in humans remains unknown.

The nervous system has a role in modulating the inflammatory response in arthritis, particularly in conferring the symmetrical pattern of joint involvement. Contralateral joint inflammation is inhibited by pharmaceutical or surgical nerve blockade in various animal models[34]. Both spinal stimulation and central stimulation of contralateral neurons have been suggested as drivers of antidromic neuronal activity, with sensory and sympathetic nerve fibres thought to be involved[35]. However, it is likely that an integrated response of several types of neurons is critical for the regulation of joint inflammation. Joints have independent innervation, so the ratio of sympathetic and sensory nerve fibres can vary between joints. The quantity and quality of joint nerve fibres might have a role in determining the propensity of a joint to develop arthritis[36]. Indeed, the number of substance P-producing nerve fibres was higher in ankles compared with knee joints in rats[36], and in equine metacarpophalangeal joints compared with carpal joints[37]. Exploring the site-specific aspects of joint innervation and the neuroregulation of the vasculature at arthritis-susceptible and arthritis-resistant anatomic sites in humans and in animal models could further delineate the role of the nervous system in the anatomical patterning of arthritis.

***Site-specific mechanical factors.*** Different anatomic locations expose joints to different types of mechanical stress. During grasping and holding motions that utilize all five fingers, the majority of mechanical strain is exerted on the index and middle fingers (digits II and III)[38]. Concordantly, joint swelling and tenderness in OA, PsA and RA are most severe in digits II and III[39,40], and osteophytes are frequent in the joints of these two digits in PsA and OA[39,41]. These observations illustrate that local mechanical strain can aggravate joint disease independently of underlying disease mechanisms. Additionally, mechanical strain might be able to trigger arthritis; the incidence of RA and gout are connected to physical trauma[42–44]. A pathological role for mechanical stress in the development of enthesitis in patients with spondyloarthropathies[45] and arthritis in the DIP joints of patients with PsA[46] is largely accepted. Although lifting heavy loads has been linked to the development

Contralateral joint inflammation
Inflammation in joints on one side of the body resulting from inflammation induced in joints on the other side of the body.

Antidromic neuronal activity
An impulse that runs along the axon towards the cell body in the opposite direction to a normal signal.

Proximal–distal body axis
The body axis running from the parts of the limbs that are nearest to the trunk of the body through to the parts that are furthest away, also called the medial–lateral axis.

Anterior–posterior body axis
The body axis running from the head to the feet; in humans corresponding to the axis running from the superior (or upper) body parts to the inferior (or lower) body parts.

of PsA, no particular pattern of joint involvement was observed[47]. Altered local forces caused by post-traumatic or congenital joint malalignment has been associated with human OA and reproduced in various animal models[48]. By contrast, the proposed connection between local joint biomechanics and arthritis development in the small joints of the hand remains to be formally demonstrated. Some studies have shown a connection between heavy or repetitive occupational use of the hands and development of hand OA[49,50]; however, other studies did not confirm these findings[51,52]. Detailed analysis of OA in various joints of the hand revealed a high prevalence of OA in digits IV and V, a propensity that is difficult to explain by mechanical strain alone[52].

Frequent colocalization of cartilage damage and monosodium urate (MSU) crystal deposition suggests that the development of OA and gout might be interdependent[53]. High levels of cartilage breakdown-products affect the solubility of urate in OA joints, thereby supporting the formation of MSU crystals[54–56]. Forty years ago, Simkin[57] proposed a model in which mechanical stress or physical trauma can lead to synovial effusion in the first metatarsophalangeal (MTP I) joints of patients with OA, which is resorbed during the night. The nocturnal resorption of the effusion increases the concentration of urate in the synovial fluid, which subsequently leads to the formation of MSU crystals, eliciting a gouty attack. Low temperatures and previous physical trauma might further promote crystal formation in the MTP I joint[58,59]. This model elegantly explains why gouty attacks predominantly start during the night but does not clarify why gout characteristically affects the MTP I joint. The exposed position of the MTP I joint in the foot makes it susceptible to trauma and changes in temperature, and this joint frequently becomes osteoarthritic as well as gouty, yet DIP and proximal interphalangeal (PIP) joints of the hands, which are similarly exposed to trauma and temperature conditions, are equally susceptible to OA but infrequently develop gout[60]. Therefore, in addition to exposure to trauma and local temperature conditions and an increased prevalence of OA, other factors must have a role in determining the susceptibility of the MTP I joint to gout.

Overall, mechanical strain can aggravate arthritis and there is a proven pathogenetic connection between some forms of OA and focal mechanical strain or injury[61]. In other types of arthritis, however, a pathogenetic relationship between local mechanical strain and the site-specificity of disease remains to be established. An increased knowledge of local tissue physiology might provide key insights into the local effects of systemic and mechanical factors in the site-specificity of arthritis development. In the following sections, we discuss the specific roles of embryonic joint and limb development (and the accompanying epigenetic architecture) in shaping the local features of joints that might predispose them to disease.

## Developmental factors in disease

Different types of arthritis present with characteristic patterns of joint involvement along the embryonically established proximal–distal body axis and anterior–posterior body axis (FIG. 2a), as seen in the prevalence of distinct types of arthritis in the anterior versus posterior and distal versus proximal extremities. Distal extremities are particularly vulnerable to destructive arthritis, with different types of arthritis affecting joints along the proximal–distal and anterior–posterior axes of the hands and feet (FIG. 2b). OA characteristically affects the first carpometacarpal (anterior) and DIP joints

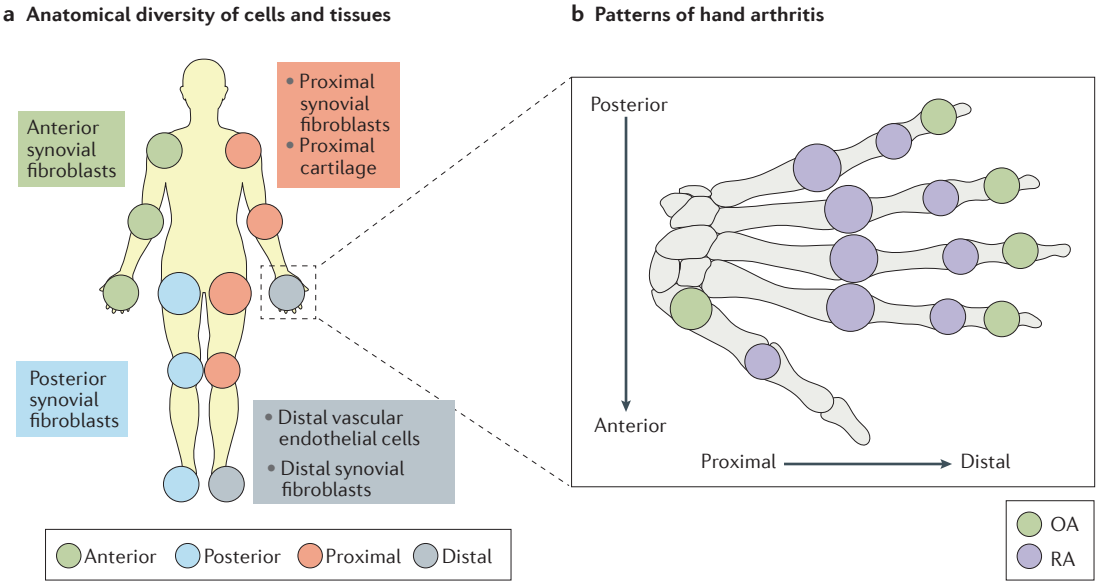


Figure 2 | **Anatomical diversity in joints and patterns of joint involvement in rheumatic disease. a** | Synovial fibroblasts, cartilage and vasculature differ between anatomic locations along the body axes. **b** | Joints in the hands commonly involved in rheumatoid arthritis (RA) and osteoarthritis (OA).

(not typically affected in RA), whereas the wrists, metacarpophalangeal joints and PIP joints of the posterior digits (II–V) typically develop RA, and gouty arthritis characteristically affects the MTP I joint. The overlap of arthritis patterns and embryonic development patterns indicates a possible role for embryonic factors in disease development. The studies summarized in this section highlight developmental processes that might contribute to the anatomical diversity of joints affected by arthritis.

The spatial and temporal expression of key developmental genes such as the homeobox (HOX) family genes, sonic hedgehog (*SHH*) and the T-box (TBX) family of transcription factors tightly control the correct establishment of the body axes and accurate morphogenesis of the skeleton in the limbs[62–65]. During embryonic development, posterior body parts express genes encoded in the 5′ region of the HOXC gene cluster[66]. *HOXA9-HOXA13* and *HOXD9-HOXD13* (encoded at the 5′ end of the HOXA and HOXD gene clusters, respectively) are expressed in a specific proximal–distal pattern that defines the identity of the skeletal elements of the limbs — the stylopod (humerus and femur), the zeugopod (radius, ulna, tibia and fibula) and the autopod (hands and feet)[67] (FIG. 3a). In the autopod, the expression of specific HOXD family genes varies between the digits, determining the identities of the digits along the anterior–posterior axis[65] (FIG. 3b). *Hoxd11-Hoxd13* and *Hoxa13* determine the identity, size and number of digits in mice[68]. A stepwise reduction of *Hoxd11-Hoxd13* and *Hoxa13* gene dosage leads to digit anomalies ranging from polydactyly to oligodactyly and adactyly[68]. $Hoxd11^{-/-}Hoxd12^{-/-}Hoxd13^{-/-}$ triple-knockout mice, $Hoxd13^{-/-}Hoxa13^{+/-}$ mice and *spdh/spdh* mice (mice with a synpolydactyly homologue mutation consisting of a polyalanine expansion in *Hoxd13*) have metacarpal bones that have transformed into carpal-like bones (long bones with joints at the ends that become ovoid bones surrounded by joints)[69]. Accordingly, a homozygous *HOXD13* +9 Ala expansion mutation showed similar anomalies in shape and ossification of metacarpal bones in humans[69,70]. Heterozygous mutations in *HOXA13*, mutations in *HOXD13* and mutations in the noncoding regulatory region of *SHH* also cause various anomalies in digit size and/or number in humans[71,72].

Adult cells and tissues, including skin fibroblasts[73–75], vascular smooth muscle cells[76], cartilage[2], bone[3], colon[77] and adipose tissue depots[78] retain key features of their embryonic gene signatures. A 2017 study[5] showed that the adult human synovium and synovial fibroblasts also retain principal aspects of site-specific expression of embryonic HOX family and other developmental limb-patterning genes. Synovial fibroblasts from joints in the hands and feet (distal) selectively expressed genes located in the 5′ tip of the HOXA and HOXD gene cluster, such as *HOXA13*, whereas synovial fibroblasts from joints in the posterior extremities specifically transcribed genes encoded in the 5′ region of the HOXC gene cluster[5]. The long noncoding RNA HOTAIR (HOX transcript antisense RNA), encoded in the HOXC gene cluster, was exclusively expressed in synovial fibroblasts from the joints of the posterior extremities[5]. Silencing of HOTAIR in knee synovial fibroblasts led to increased constitutive and TNF-induced expression of interstitial collagenase (also known as MMP1), indicating that HOTAIR can control the site-specific and cytokine-driven matrix-destructive properties of synovial fibroblasts[5]. These observations illustrate how developmental genes can control the activation of arthritis-relevant pathways in the

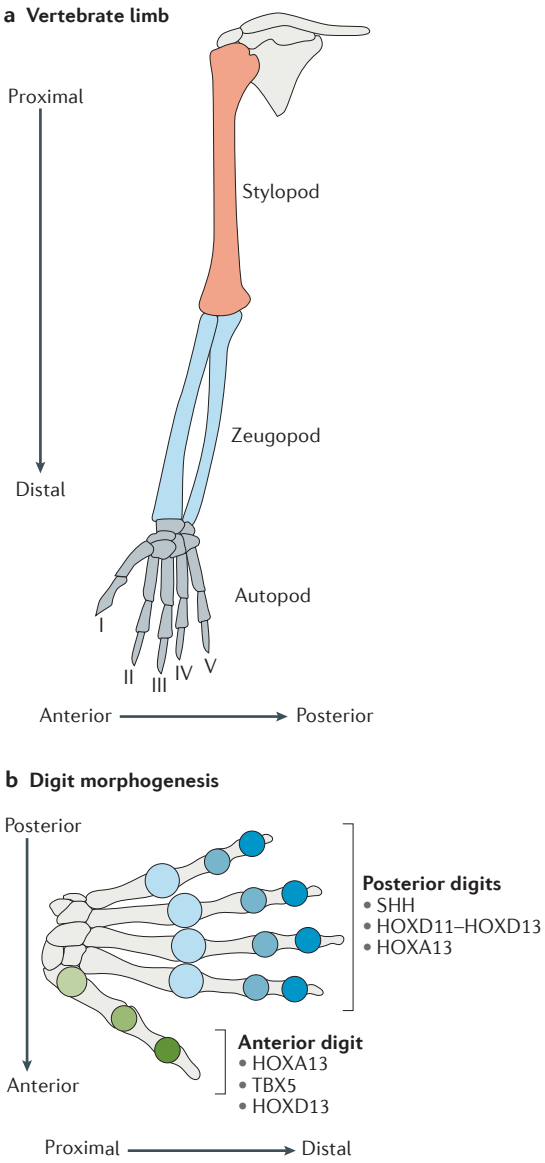


Figure 3 | **Development of limbs and digits in vertebrates. a** | The skeletal domains of a vertebrate limb along the proximal–distal (stylopod to autopod) and anterior–posterior (digits I–V) limb axes. **b** | Different molecular factors are involved in anterior and posterior digit morphogenesis, controlling digit number and size. HOXA13, homeobox protein Hox-A13; HOXD11, homeobox protein Hox-D11; HOXD13, homeobox protein Hox-D13; SHH, sonic hedgehog; TBX5, T-box transcription factor TBX5.

synovium, thereby guiding the development of arthritis-specific patterns of joint involvement along the principal developmental body axes.

***Embryonic development of joints.*** Early in skeletal development the cartilaginous skeleton is uninterrupted; the formation of joints begins with the emergence of a compact layer of mesenchymal tissue (the interzone) at the site of each future joint, interrupting the continuity of the skeletal template[79,80] (FIG. 4a). After the specification of joint sites, physical separation of the adjacent cartilaginous elements and formation of the synovial cavity (joint cavitation) occur, followed by the formation of joint structures such as the articular cartilage, synovial membrane, ligaments and menisci[79,80]. All these joint structures originate from cells in the interzone that express growth/differentiation factor 5 (GDF5), a member of the bone morphogenetic protein (BMP) family[79,81] (FIG. 4).

Joints develop through the continuous influx of GDF5-expressing cells into the interzone[81]. A highly dynamic spatiotemporal pattern of GDF5 expression guides the formation of the different joint structures; both the onset and the duration of GDF5 expression seem to be crucial in this process[81]. Therefore, the spatiotemporal dynamics of GDF5 expression in the interzone could instruct the divergence of cells into different lineages to form the distinct joint compartments (FIG. 4b). Despite the widespread expression of GDF5 in joint-forming tissues throughout the skeleton, a lack of GDF5 in humans and mice affects only a subset of joints, most notably the joints of the autopod[82], whereas single nucleotide polymorphisms (SNPs) in *GDF5* specifically predispose individuals to knee OA[83]. These site-specific effects of GDF5 might result from a strong interconnectivity between members of the BMP family, environmental stimuli and other developmental pathways[84].

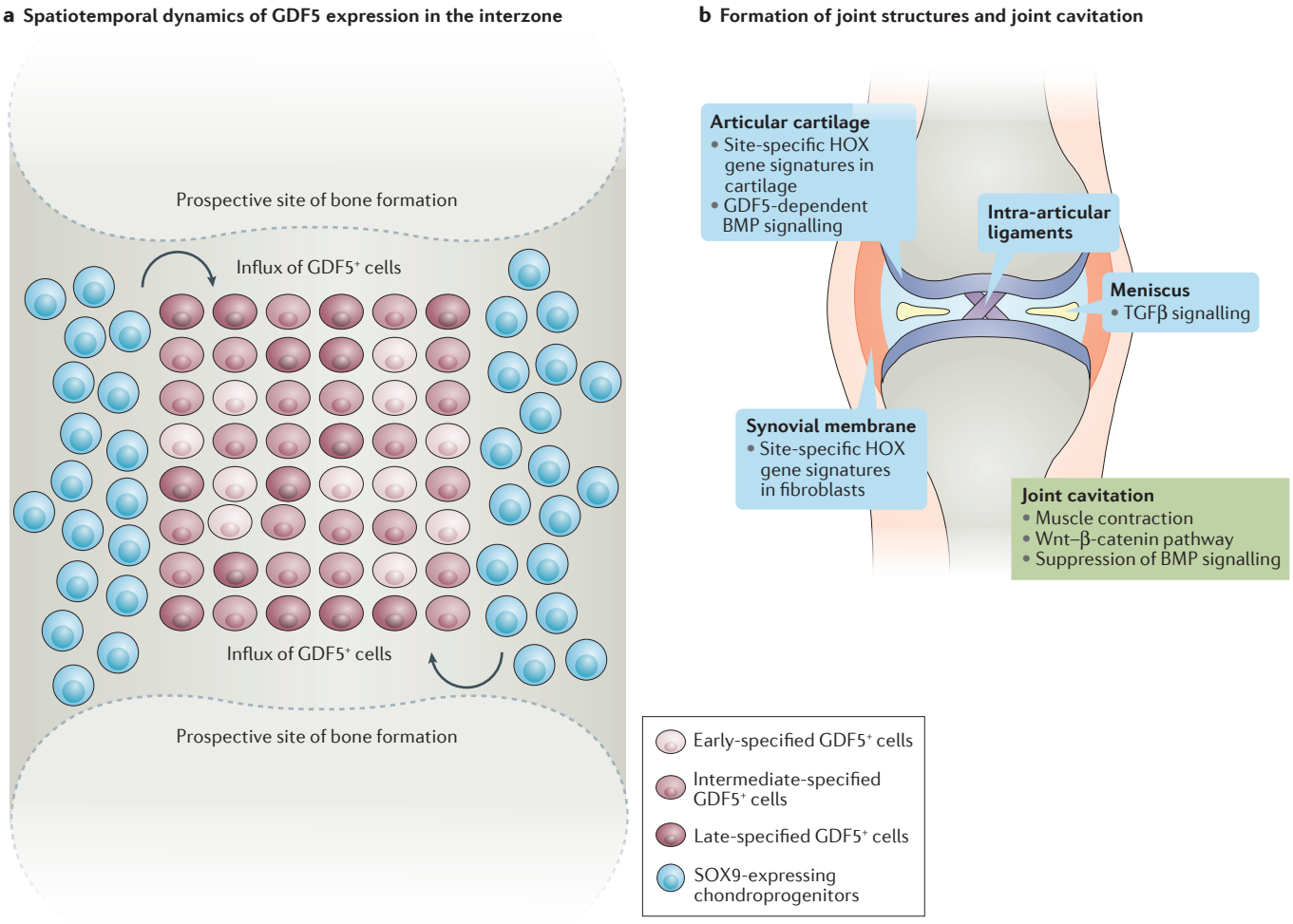


Figure 4 | **Morphogenesis of synovial joints. a** | The first sign of future joint formation is the emergence of a compact layer of mesenchymal tissues, known as the interzone. The interzone is specified by the induction of growth/differentiation factor 5 (GDF5) expression and suppression of collagen α1(II) chain expression in interzone cells. Joints form through a continous influx of GDF5-expressing cells in the interzone, which originate from transcription factor SOX9-expressing chondroprogenitors. **b** | Joint structures, including articular cartilage, synovial membrane, menisci and intraarticular ligaments originate from GDF5-specified joint progenitor cells. The spatiotemporal dynamics of GDF5 expression in the interzone guides lineage divergence of the recruited interzone cells to form the different joint tissues. Other developmental factors, such as bone morphogenetic protein (BMP), homeobox (HOX) genes, the Wnt–β-catenin pathway and transforming growth factor-β (TGFβ) contribute to the morphogenesis of articular structures and guide joint cavitation in a site-specific manner.

Environmental cues that activate core morphogenetic signalling pathways at particular sites help to configure the site-specific features of joint morphogenesis. For example, fetal movements are required for normal joint formation[85]. A lack of limb musculature (as in muscleless mice) or complete muscle paralysis (as in *mdg* mutant mice) during development results in the specific loss of certain joints (elbow, shoulder, hip, talocalcaneal, specific midcarpal joints and intervertebral joints of the cervical and lumbar spine, the latter in *mdg* mutant mice only), while other joints such as knees and finger joints remain intact[86]. Although joint progenitor cells are specified by the presence of GDF5 in these mice, these cells fail to maintain joint identity in the absence of contracting musculature and 'erroneously' differentiate into chondrocytes, precluding joint cavitation and leading to joint fusion[86]. The Wnt–β-catenin pathway is required for maintaining the commitment of joint progenitor cells to a joint-cell fate and for suppressing chondrogenic differentiation[87,88]. The sites of prospective elbows in muscleless and *mdg* mutant mice have reduced β-catenin activity, but these mice have normal β-catenin activity at presumptive knee and finger joints[86]. β-Catenin signalling seems to be differentially regulated in different joints, with muscle contraction controlling β-catenin activity and joint formation in only a subset of anatomic sites (FIG. 4b). Tissue surrounding the developing elbows in mice is highly enriched in transcripts involved in muscle specification and differentiation[80]. By contrast, knee-forming tissues are enriched in *Hoxc9* and *Hoxc10*, as well as transcripts involved in Wnt signalling and members of the transforming growth factor-β (TGFβ) superfamily such as TGFβ and BMP[80]. TGFβ guides knee morphogenesis and meniscus formation[79,80] whereas different members of the BMP family are crucial in the development of the axial skeleton, the limbs and the digits[84]. Regionalization of the activity of BMP signalling within the interzone — suppression in the centre and GDF5-induced activation in the outermost parts — seems to be important for proper joint cavitation and articular cartilage formation[84] (FIG. 4b). Inherited defects in BMP signalling result in errors in digit size and joint fusions, particularly in the autopod[84].

***Developmental pathways in OA.*** The hips, the knees and the small joints of the hands (PIP and DIP joints) characteristically develop OA, although the disease manifests differently in these joints, and can even differ between the joints of the hands, manifesting as different phenotypes (erosive OA, nodal interphalangeal OA and thumb base OA). There is ample evidence for the role of developmental pathways in the pathogenesis of OA[89]. Briefly, rs143383, a SNP in the 5′ UTR-coding region of *GDF5* prominently increases the risk of developing knee OA, and SNPs in *ANP32*, which encodes acidic leucine-rich nuclear phosphoprotein 32 family member A, a member of the Wnt signalling pathway, are associated with the risk of hip OA in women[83,90]. These results suggest that polymorphisms in genes encoding proteins in key developmental pathways might predispose an individual to joint-specific forms of OA. The large number of embryonic signalling pathways that are involved in the pathogenesis of OA and are upregulated at specific sites during embryonic development, such as the Wnt–β-catenin, BMP and TGFβ pathways[91], strongly suggests that these pathways are involved in the site-specific development of OA, as well as the severity of disease.

## Epigenetics in site-specificity

Epigenetic mechanisms such as histone modification and DNA methylation have a central role in establishing site-specific patterns of gene expression during embryogenesis[92,93]. In adult skin fibroblasts, synovial fibroblasts and chondrocytes from various anatomic locations, differences in DNA methylation and histone modifications at HOX family gene loci exist[4–6,8]. Therefore, epigenetic mechanisms could control the faithful maintenance of embryonic gene expression patterns in adult cells throughout their lifetime.

Several studies have analyzed and compared the DNA methylation profiles of knee and hip cartilage from patients with OA and healthy individuals[4,6,94]. Notably, these studies showed only a small amount of overlap in differentially methylated loci between knee OA and hip OA, underlining the different pathogenic pathways that are active in OA at these two sites. The methylated loci that differed between these joint locations were consistently enriched for genes involved in limb development, such as HOX family genes.

Studies in embryonic mice show that *Tbx5* is expressed in anterior body parts and is required to ensure forelimb symmetry in mice[95] (FIG. 3b). Mutations in *TBX5* cause Holt-Oram syndrome in humans, which is characterized by skeletal deformities in the anterior extremities that are pronounced on the left-hand side[95]. Similar to HOX family genes, the embryonically-established, site-specific expression of *TBX5* is maintained in adult synovial fibroblasts[5]. Interestingly, epigenetic modifications that normally silence *TBX5* in synovial fibroblasts in posterior body parts are altered in knee synovial fibroblasts from patients with RA, and TBX5 is aberrantly expressed in these cells[96]. TBX5 regulates the expression of chemokines such as IL-8 and stromal cell-derived factor 1 (also known as CXCL12) in synovial fibroblasts, indicating that a loss of epigenetic imprinting of embryonic genes might have an influence on development of RA[96].

The effects of the SNP rs143383 in *GDF5* are stronger and more consistent in the development of knee OA compared with hip or hand OA[83]. The risk allele (T) of rs143383 leads to diminished transcription of *GDF5* by influencing the upstream binding of repressive transcriptional regulators[97]. *In vitro*, this effect was potentiated when the DNA region encoding the 5′ UTR of *GDF5* was demethylated[98]. The region surrounding rs143383 was hypomethylated in knee cartilage compared with hip cartilage[98], suggesting that differences in DNA methylation between hip and knee joints could underlie the joint-specific effects of rs143383 in OA.

These observations further substantiate the role of epigenetics, not only in site-specific synovial gene expression, but also in driving pathological processes locally. Acknowledging that most of the OA-risk SNPs identified to date are specific for a joint region, future studies in OA and other types of joint disease should focus on integrating genomic data with joint-specific epigenetic and transcriptional profiles[99].

## Site-specificity outside of the joint

The location-specific expression of embryonic genes in adult cells not only has a key role in site-specific homeostatic functions, but is also emerging as an important factor for site-specific disease development in organs and pathologies external to the joints.

***Skin.*** Skin is an anatomically highly specialized tissue, as exemplified by the location-specific occurrence of body hair, sweat glands, glabrous skin and pigmentation, as well as the site-specific susceptibility to diseases such as psoriasis, scleroderma, acne and keloids. Skin fibroblasts from different sites of the body display morphological and functional heterogeneity attributable to their origins in different germ layers[100], to their anatomical diversity[73] and to intradermal sub-specialization[100]. Distal-specific expression of *HOXA13* and concomitant homeobox protein Hox-A13 (HOXA13)-dependent regulation of protein Wnt5A in plantar skin fibroblasts is central to the induction of palmoplantar-specific expression of keratin type I cytoskeletal 9 (CK9), underlining the role of HOX family genes in site-specific epidermal differentiation[101]. Non-palmoplantar human keratinocytes start to express CK9 when co-cultured with palmoplantar skin fibroblasts[102]. Similarly, non-palmoplantar epidermis adopts a CK9-positive palmoplantar epidermal phenotype when grafted onto wounds on the soles of the feet in humans[102]. These studies substantiate a key role for local fibroblast signals in controlling site-specific skin repair and differentiation.

It is largely unknown how the positional identity of skin fibroblasts (and potentially other local cell types) confers site-specific susceptibility to skin diseases. SSc affects both the skin and the joints of the distal extremities, which could be connected to the common expression of embryonic genes such as *HOXA13* at these sites. However, further studies are required to unravel the specific contributions of local cells, distally acting systemic factors and distal-specific mechanical factors in shaping distal skin and joint manifestations in SSc. Another disease characterized by a specific anatomical pattern of skin involvement is psoriasis. In particular, psoriasis of the scalp and nails predisposes to PsA[103], suggesting that specific pathways that are active in scalp and nail psoriasis might lead to subsequent involvement of the joints[46]. Alternatively, the skin of the scalp, the nails and the joints might share susceptibility to a systemic trigger that invokes pathogenic processes at all these sites. The Koebner response is one example of how local mechanical stress can trigger disease development. This phenomenon might also contribute to the development of joint disease in patients with PsA[104,105].

Koebner response
A disturbed reaction to trauma and mechanical stress that leads to skin lesions in patients with psoriasis.

***Blood vessels.*** Despite the presence of systemic risk factors such as arterial hypertension, diabetes mellitus or autoimmune responses, blood vessels display striking regional differences in their susceptibility or resistance to specific vascular pathologies (such as atherosclerosis, aortic aneurysm or vasculitis)[106]. Differences in regional haemodynamics and vessel wall structure have long been considered to be responsible for the characteristic regionalization of aortic atherosclerosis and aneurysms. However, accumulating evidence suggests that the embryonically established anatomic diversity of the aortic wall could have an equally important role[107].

Vascular smooth muscle cells of distinct embryonic origins are phenotypically diverse, displaying different potential for growth and responsiveness to cytokines such as TGFβ[108]. Smooth muscle cells in the aortic arch originate from the neural crest, whereas smooth muscle cells in the descending aorta derive from somatic mesoderm. Atherosclerosis-prone murine aortic arch *in vivo* and cultured murine smooth muscle cells from the aortic arch express lower levels of HOX gene paralogues 6–10 and have higher activation of the transcription factor NF-κB compared with atherosclerosis-resistant descending aorta and descending aorta-derived smooth muscle cells[76]. Overexpression of *Hoxa9* limited NF-κB signalling in rat embryonic aortic E19P cells and in murine smooth muscle cells from the aortic arch. In turn, overexpression of NF-κB in E19P cells or murine thoracic aorta-derived smooth muscle cells or stimulation of E19P cells with TNF resulted in the suppression of *Hoxa9* (REF. 76). These results suggest that site-specific *Hoxa9* expression in aortic smooth muscle cells controls the segment-specific proinflammatory responsiveness of the aorta, contributing to the characteristic regionalization of aortic atherosclerosis.

Similar to smooth muscle cells, endothelial cells from different organs and different areas of the vasculature show extensive molecular and phenotypic heterogeneity, further enhancing the regional diversity of the vasculature[109,110]. Differential expression of HOX family genes defines the tissue-specific and organ-specific origins of endothelial cells[110]. Site-specific expression of the microRNA miR-10a (encoded in the HOXB gene cluster) might contribute to the regional susceptibility of the aorta to atherosclerosis[111]. Atherosclerosis-susceptible endothelium of the porcine aortic arch and the aorto-renal vascular branches contain lower amounts of miR-10a than the atherosclerosis-protected descending aorta. Silencing of miR-10a in cultured human aortic endothelial cells increases the nuclear translocation of NF-κB and enhances the expression of proinflammatory cytokines and adhesion molecules[111], indicating a prominent role for miR-10a in configuring the regional proinflammatory nature and susceptibility of the aortic endothelium to atherosclerosis.

In line with these results, the differential segmental responsiveness of the arterial wall to systemic cytokines might contribute to the increased incidence of atherosclerosis in patients with rheumatic diseases such as RA[112]. Likewise, embryonic features and other

segment-specific characteristics of the vessel wall could underlie the site-specific vulnerability of vessels to developing different types of vasculitis[106].

## Conclusions

Genome-wide studies of genetics, epigenetics and coding and noncoding transcripts have substantially increased our understanding of pathological processes in joints affected by rheumatic diseases, but it remains a challenge to combine these datasets and approaches to get a clear picture of the spatiotemporal activation of the pathways involved. Decoding the characteristic features and embryonic traits of affected and protected sites might provide new clues about causal pathogenic mechanisms and therapeutic opportunities. Knowledge of pathogenic mechanisms has to be integrated with known anatomical characteristics of affected sites to better understand the causes and the consequences of arthritis-specific patterns of joint and organ involvement. Disease activity scores might be an adequate tool to assess response to treatment, but for practical reasons these scores often do not include all affected sites. The 28-joint disease activity score (DAS28), for instance, does not include the ankle, MTP or PIP joints in the feet, and studies correlating inflammatory factors and/or treatment response with DAS28 scores might miss important changes in disease activity at these locations[113]. Furthermore, information on the patterns of joint involvement in animal models of arthritis is scarce. Although most models have affected distal joints, disease involvement in other joints is not usually communicated. Including information on patterns of joint involvement as an integral part of *in vivo* data reporting, and stringent selection and reporting of anatomical sampling locations in *in vitro* studies should help to deepen our current understanding of site-specific vulnerability to arthritis and the local factors involved.


1. Elkayam, O., Arad, U. & Doherty, M. in *EULAR Textbook on Rheumatic Diseases* 2nd edn Ch. 7 (eds Bijlsma, J. W. & Hachulla, E.) 182–202 (Ingram International Inc, 2015).
2. Pelttari, K. *et al.* Adult human neural crest-derived cells for articular cartilage repair. *Sci. Transl Med.* **6**, 251ra119 (2014).
3. Leucht, P. *et al.* Embryonic origin and Hox status determine progenitor cell fate during adult bone regeneration. *Development* **135**, 2845–2854 (2008).
4. den Hollander, W. *et al.* Knee and hip articular cartilage have distinct epigenomic landscapes: implications for future cartilage regeneration approaches. *Ann. Rheum. Dis.* **73**, 2208–2212 (2014).
5. Frank-Bertoncelj, M. *et al.* Epigenetically-driven anatomical diversity of synovial fibroblasts guides joint-specific fibroblast functions. *Nat. Commun.* **8**, 14852 (2017).
6. Rushton, M. D. *et al.* Characterization of the cartilage DNA methylome in knee and hip osteoarthritis. *Arthritis Rheumatol.* **66**, 2450–2460 (2014).
7. Banks, S. E. Erosive osteoarthritis: a current review of a clinical challenge. *Clin. Rheumatol.* **29**, 697–706 (2010).
8. Ai, R. *et al.* Joint-specific DNA methylation and transcriptome signatures in rheumatoid arthritis identify distinct pathogenic processes. *Nat. Commun.* **7**, 11849 (2016).
9. Helliwell, P. S. *et al.* Joint symmetry in early and late rheumatoid and psoriatic arthritis: comparison with a mathematical model. *Arthritis Rheum.* **43**, 865–871 (2000).
10. Moll, J. M. & Wright, V. Psoriatic arthritis. *Semin. Arthritis Rheum.* **3**, 55–78 (1973).
11. Hellgren, L. Association between rheumatoid arthritis and psoriasis in total populations. *Acta Rheumatol. Scand.* **15**, 316–326 (1969).
12. Baker, H. Prevalence of psoriasis in polyarthritic patients and their relatives. *Ann. Rheum. Dis.* **25**, 229–234 (1966).
13. FitzGerald, O., Haroon, M., Giles, J. T. & Winchester, R. Concepts of pathogenesis in psoriatic arthritis: genotype determines clinical phenotype. *Arthritis Res. Ther.* **17**, 115 (2015).
14. Haroon, M., Winchester, R., Giles, J. T., Heffernan, E. & FitzGerald, O. Certain class I HLA alleles and haplotypes implicated in susceptibility play a role in determining specific features of the psoriatic arthritis phenotype. *Ann. Rheum. Dis.* **75**, 155–162 (2016).
15. Chandran, V., Tolusso, D. C., Cook, R. J. & Gladman, D. D. Risk factors for axial inflammatory arthritis in patients with psoriatic arthritis. *J. Rheumatol.* **37**, 809–815 (2010).
16. Rosenbaum, J. T. New developments in uveitis associated with HLA B27. *Curr. Opin. Rheumatol.* http://dx.doi.org/10.1097/BOR.0000000000000403 (2017).
17. Bowness, P. HLA-B27. *Annu. Rev. Immunol.* **33**, 29–48 (2015).
18. Colbert, R. A., DeLay, M. L., Klenk, E. I. & Layh-Schmitt, G. From HLA-B27 to spondyloarthritis: a journey through the ER. *Immunol. Rev.* **233**, 181–202 (2010).
19. Khongwichit, S. *et al.* Cell-type specific variation in the induction of ER stress and downstream events in chikungunya virus infection. *Microb. Pathog.* **101**, 104–118 (2016).
20. Wang, H. Q., Imai, Y., Kataoka, A. & Takahashi, R. Cell type-specific upregulation of Parkin in response to ER stress. *Antioxid. Redox Signal.* **9**, 533–542 (2007).
21. Kraan, M. C. *et al.* Comparison of synovial tissues from the knee joints and the small joints of rheumatoid arthritis patients: implications for pathogenesis and evaluation of treatment. *Arthritis Rheum.* **46**, 2034–2038 (2002).
22. Bennike, T. *et al.* A normative study of the synovial fluid proteome from healthy porcine knee joints. *J. Proteome Res.* **13**, 4377–4387 (2014).
23. DeLay, M. L. *et al.* HLA-B27 misfolding and the unfolded protein response augment interleukin-23 production and are associated with Th17 activation in transgenic rats. *Arthritis Rheum.* **60**, 2633–2643 (2009).
24. Sherlock, J. P. *et al.* IL-23 induces spondyloarthropathy by acting on ROR-γt$^+$ CD3$^+$CD4$^-$CD8$^-$ entheseal resident T cells. *Nat. Med.* **18**, 1069–1076 (2012).
25. Stangenberg, L. *et al.* Denervation protects limbs from inflammatory arthritis via an impact on the microvasculature. *Proc. Natl Acad. Sci. USA* **111**, 11419–11424 (2014).
26. Binstadt, B. A. *et al.* Particularities of the vasculature can promote the organ specificity of autoimmune attack. *Nat. Immunol.* **7**, 284–292 (2006).
27. Wipke, B. T., Wang, Z., Kim, J., McCarthy, T. J. & Allen, P. M. Dynamic visualization of a joint-specific autoimmune response through positron emission tomography. *Nat. Immunol.* **3**, 366–372 (2002).
28. Hamilton, S. Unilateral rheumatoid arthritis in hemiplegia. *J. Can. Assoc. Radiol.* **34**, 49–50 (1983).
29. Yaghmai, I., Rooholamini, S. M. & Faunce, H. F. Unilateral rheumatoid arthritis: protective effect of neurologic deficits. *AJR Am. J. Roentgenol.* **128**, 299–301 (1977).
30. Bland, J. H. & Eddy, W. M. Hemiplegia and rheumatoid hemiarthritis. *Arthritis Rheum.* **11**, 72–80 (1968).
31. Ueno, Y., Sawada, K. & Imura, H. Protective effect of neural lesion on rheumatoid arthritis. *Arthritis Rheum.* **26**, 118 (1983).
32. Hammoudeh, M., Khan, M. A. & Kushner, I. Unilateral rheumatoid arthritis. *Arthritis Rheum.* **24**, 1218 (1981).
33. Smith, R. D. Effect of hemiparesis on rheumatoid arthritis. *Arthritis Rheum.* **22**, 1419–1420 (1979).
34. Shenker, N. *et al.* A review of contralateral responses to a unilateral inflammatory lesion. *Rheumatology (Oxford)* **42**, 1279–1286 (2003).
35. Pongratz, G. & Straub, R. H. Role of peripheral nerve fibres in acute and chronic inflammation in arthritis. *Nat. Rev. Rheumatol.* **9**, 117–126 (2013).
36. Levine, J. D. *et al.* Intraneuronal substance P contributes to the severity of experimental arthritis. *Science* **226**, 547–549 (1984).
37. Bowker, R. M., Sonea, I. M., Vex, K. B. & Caron, J. P. Substance P innervation of equine synovial membranes: joint differences and neural and nonneural receptor localizations. *Neurosci. Lett.* **164**, 76–80 (1993).
38. Santello, M. & Soechting, J. F. Force synergies for multifingered grasping. *Exp. Brain Res.* **133**, 457–467 (2000).
39. Finzel, S. *et al.* Inflammatory bone spur formation in psoriatic arthritis is different from bone spur formation in hand osteoarthritis. *Arthritis Rheumatol.* **66**, 2968–2975 (2014).
40. van der Helm-van Mil, A. H., Verpoort, K. N., Breedveld, F. C., Toes, R. E. & Huizinga, T. W. Antibodies to citrullinated proteins and differences in clinical progression of rheumatoid arthritis. *Arthritis Res. Ther.* **7**, R949–R958 (2005).
41. Buckland-Wright, J. C., Macfarlane, D. G. & Lynch, J. A. Osteophytes in the osteoarthritic hand: their incidence, size, distribution, and progression. *Ann. Rheum. Dis.* **50**, 627–630 (1991).
42. Thorarensen, S. M. *et al.* Physical trauma recorded in primary care is associated with the onset of psoriatic arthritis among patients with psoriasis. *Ann. Rheum. Dis.* **76**, 521–525 (2016).
43. Brown, T. D., Johnston, R. C., Saltzman, C. L., Marsh, J. L. & Buckwalter, J. A. Posttraumatic osteoarthritis: a first estimate of incidence, prevalence, and burden of disease. *J. Orthop. Trauma* **20**, 739–744 (2006).
44. Al-Allaf, A. W., Sanders, P. A., Ogston, S. A. & Marks, J. S. A case-control study examining the role of physical trauma in the onset of rheumatoid arthritis. *Rheumatology (Oxford)* **40**, 262–266 (2001).
45. Jacques, P. & McGonagle, D. The role of mechanical stress in the pathogenesis of spondyloarthritis and how to combat it. *Best Pract. Res. Clin. Rheumatol.* **28**, 703–710 (2014).
46. McGonagle, D. Enthesitis: an autoinflammatory lesion linking nail and joint involvement in psoriatic disease. *J. Eur. Acad. Dermatol. Venereol.* **23** (Suppl. 1), 9–13 (2009).
47. Eder, L. *et al.* Association between environmental factors and onset of psoriatic arthritis in patients with psoriasis. *Arthritis Care Res. (Hoboken)* **63**, 1091–1097 (2011).

48. Teeple, E., Jay, G. D., Elsaid, K. A. & Fleming, B. C. Animal models of osteoarthritis: challenges of model selection and analysis. *AAPS J.* **15**, 438–446 (2013).
49. Fontana, L., Neel, S., Claise, J. M., Ughetto, S. & Catilina, P. Osteoarthritis of the thumb carpometacarpal joint in women and occupational risk factors: a case-control study. *J. Hand Surg. Am.* **32**, 459–465 (2007).
50. Rossignol, M. *et al.* Primary osteoarthritis of hip, knee, and hand in relation to occupational exposure. *Occup. Environ. Med.* **62**, 772–777 (2005).
51. Goekoop, R. J. *et al.* Determinants of absence of osteoarthritis in old age. *Scand. J. Rheumatol.* **40**, 68–73 (2011).
52. Solovieva, S., Vehmas, T., Riihimaki, H., Luoma, K. & Leino-Arjas, P. Hand use and patterns of joint involvement in osteoarthritis. A comparison of female dentists and teachers. *Rheumatology (Oxford)* **44**, 521–528 (2005).
53. Roddy, E., Zhang, W. & Doherty, M. Are joints affected by gout also affected by osteoarthritis? *Ann. Rheum. Dis.* **66**, 1374–1377 (2007).
54. Laurent, T. C. Solubility of sodium urate in the presence of chondroitin-4-sulphate. *Nature* **202**, 1334 (1964).
55. Katz, W. A. & Schubert, M. The interaction of monosodium urate with connective tissue components. *J. Clin. Invest.* **49**, 1783–1789 (1970).
56. Burt, H. M. & Dutt, Y. C. Growth of monosodium urate monohydrate crystals: effect of cartilage and synovial fluid components on *in vitro* growth rates. *Ann. Rheum. Dis.* **45**, 858–864 (1986).
57. Simkin, P. A. The pathogenesis of podagra. *Ann. Intern. Med.* **86**, 230–233 (1977).
58. Loeb, J. N. The influence of temperature on the solubility of monosodium urate. *Arthritis Rheum.* **15**, 189–192 (1972).
59. Wilcox, W. R. & Khalaf, A. A. Nucleation of monosodium urate crystals. *Ann. Rheum. Dis.* **34**, 332–339 (1975).
60. Roddy, E. Revisiting the pathogenesis of podagra: why does gout target the foot? *J. Foot. Ankle Res.* **4**, 13 (2011).
61. Buckwalter, J. A., Anderson, D. D., Brown, T. D., Tochigi, Y. & Martin, J. A. The roles of mechanical stresses in the pathogenesis of osteoarthritis: implications for treatment of joint injuries. *Cartilage* **4**, 286–294 (2013).
62. Harfe, B. D. *et al.* Evidence for an expansion-based temporal Shh gradient in specifying vertebrate digit identities. *Cell* **118**, 517–528 (2004).
63. Zeller, R., Lopez-Rios, J. & Zuniga, A. Vertebrate limb bud development: moving towards integrative analysis of organogenesis. *Nat. Rev. Genet.* **10**, 845–858 (2009).
64. Koshiba-Takeuchi, K. *et al.* Cooperative and antagonistic interactions between Sall4 and Tbx5 pattern the mouse limb and heart. *Nat. Genet.* **38**, 175–183 (2006).
65. Montavon, T., Le Garrec, J. F., Kerszberg, M. & Duboule, D. Modeling Hox gene regulation in digits: reverse collinearity and the molecular origin of thumbness. *Genes Dev.* **22**, 346–359 (2008).
66. Alvarado, D. M., McCall, K., Hecht, J. T., Dobbs, M. B. & Gurnett, C. A. Deletions of 5′ HOXC genes are associated with lower extremity malformations, including clubfoot and vertical talus. *J. Med. Genet.* **53**, 250–255 (2016).
67. Zakany, J. & Duboule, D. The role of Hox genes during vertebrate limb development. *Curr. Opin. Genet. Dev.* **17**, 359–366 (2007).
68. Zakany, J., Fromental-Ramain, C., Warot, X. & Duboule, D. Regulation of number and size of digits by posterior Hox genes: a dose-dependent mechanism with potential evolutionary implications. *Proc. Natl Acad. Sci. USA* **94**, 13695–13700 (1997).
69. Villavicencio-Lorini, P. *et al.* Homeobox genes d11-d13 and a13 control mouse autopod cortical bone and joint formation. *J. Clin. Invest.* **120**, 1994–2004 (2010).
70. Muragaki, Y., Mundlos, S., Upton, J. & Olsen, B. R. Altered growth and branching patterns in synpolydactyly caused by mutations in HOXD13. *Science* **272**, 548–551 (1996).
71. Heus, H. C. *et al.* A physical and transcriptional map of the preaxial polydactyly locus on chromosome 7q36. *Genomics* **57**, 342–351 (1999).
72. Petit, F. *et al.* The disruption of a novel limb *cis*-regulatory element of SHH is associated with autosomal dominant preaxial polydactyly-hypertrichosis. *Eur. J. Hum. Genet.* **24**, 37–43 (2016).
73. Chang, H. Y. *et al.* Diversity, topographic differentiation, and positional memory in human fibroblasts. *Proc. Natl Acad. Sci. USA* **99**, 12877–12882 (2002).
74. Rinn, J. L., Bondre, C., Gladstone, H. B., Brown, P. O. & Chang, H. Y. Anatomic demarcation by positional variation in fibroblast gene expression programs. *PLoS Genet.* **2**, e119 (2006).
75. Wang, K. C., Helms, J. A. & Chang, H. Y. Regeneration, repair and remembering identity: the three Rs of Hox gene expression. *Trends Cell Biol.* **19**, 268–275 (2009).
76. Trigueros-Motos, L. *et al.* Embryological-origin-dependent differences in homeobox expression in adult aorta: role in regional phenotypic variability and regulation of NF-kappaB activity. *Arterioscler. Thromb. Vasc. Biol.* **33**, 1248–1256 (2013).
77. Knight, J. M. *et al.* Comprehensive site-specific whole genome profiling of stromal and epithelial colonic gene signatures in human sigmoid colon and rectal tissue. *Physiol. Genomics* **48**, 651–659 (2016).
78. Pinnick, K. E. *et al.* Distinct developmental profile of lower-body adipose tissue defines resistance against obesity-associated metabolic complications. *Diabetes* **63**, 3785–3797 (2014).
79. Decker, R. S., Koyama, E. & Pacifici, M. Genesis and morphogenesis of limb synovial joints and articular cartilage. *Matrix Biol.* **39**, 5–10 (2014).
80. Pazin, D. E., Gamer, L. W., Cox, K. A. & Rosen, V. Molecular profiling of synovial joints: use of microarray analysis to identify factors that direct the development of the knee and elbow. *Dev. Dyn.* **241**, 1816–1826 (2012).
81. Shwartz, Y., Viukov, S., Krief, S. & Zelzer, E. Joint development involves a continuous influx of Gdf5-positive cells. *Cell Rep.* **15**, 2577–2587 (2016).
82. Seemann, P. *et al.* Mutations in GDF5 reveal a key residue mediating BMP inhibition by NOGGIN. *PLoS Genet.* **5**, e1000747 (2009).
83. Evangelou, E. *et al.* Large-scale analysis of association between GDF5 and FRZB variants and osteoarthritis of the hip, knee, and hand. *Arthritis Rheum.* **60**, 1710–1721 (2009).
84. Salazar, V. S., Gamer, L. W. & Rosen, V. BMP signalling in skeletal development, disease and repair. *Nat. Rev. Endocrinol.* **12**, 203–221 (2016).
85. Hall, J. G. Arthrogryposis multiplex congenita: etiology, genetics, classification, diagnostic approach, and general aspects. *J. Pediatr. Orthop. B* **6**, 159–166 (1997).
86. Kahn, J. *et al.* Muscle contraction is necessary to maintain joint progenitor cell fate. *Dev. Cell* **16**, 734–743 (2009).
87. Guo, X. *et al.* Wnt/beta-catenin signaling is sufficient and necessary for synovial joint formation. *Genes Dev.* **18**, 2404–2417 (2004).
88. Spater, D., Hill, T. P., Gruber, M. & Hartmann, C. Role of canonical Wnt-signalling in joint formation. *Eur. Cell. Mater.* **12**, 71–80 (2006).
89. Pitsillides, A. A. & Beier, F. Cartilage biology in osteoarthritis — lessons from developmental biology. *Nat. Rev. Rheumatol.* **7**, 654–663 (2011).
90. Valdes, A. M. *et al.* Variation at the ANP32A gene is associated with risk of hip osteoarthritis in women. *Arthritis Rheum.* **60**, 2046–2054 (2009).
91. van der Kraan, P. M. The changing role of TGFβ in healthy, ageing and osteoarthritic joints. *Nat. Rev. Rheumatol.* **13**, 155–163 (2017).
92. Terranova, R., Agherbi, H., Boned, A., Meresse, S. & Djabali, M. Histone and DNA methylation defects at Hox genes in mice expressing a SET domain-truncated form of Mll. *Proc. Natl Acad. Sci. USA* **103**, 6629–6634 (2006).
93. Soshnikova, N. & Duboule, D. Epigenetic temporal control of mouse Hox genes *in vivo*. *Science* **324**, 1320–1323 (2009).
94. Aref-Eshghi, E. *et al.* Genome-wide DNA methylation study of hip and knee cartilage reveals embryonic organ and skeletal system morphogenesis as major pathways involved in osteoarthritis. *BMC Musculoskelet. Disord.* **16**, 287 (2015).
95. Mori, A. D. & Bruneau, B. G. TBX5 mutations and congenital heart disease: Holt–Oram syndrome revealed. *Curr. Opin. Cardiol.* **19**, 211–215 (2004).
96. Karouzakis, E. *et al.* Epigenome analysis reveals TBX5 as a novel transcription factor involved in the activation of rheumatoid arthritis synovial fibroblasts. *J. Immunol.* **193**, 4945–4951 (2014).
97. Syddall, C. M., Reynard, L. N., Young, D. A. & Loughlin, J. The identification of *trans*-acting factors that regulate the expression of GDF5 via the osteoarthritis susceptibility SNP rs143383. *PLoS Genet.* **9**, e1003557 (2013).
98. Reynard, L. N., Bui, C., Syddall, C. M. & Loughlin, J. CpG methylation regulates allelic expression of GDF5 by modulating binding of SP1 and SP3 repressor proteins to the osteoarthritis susceptibility SNP rs143383. *Hum. Genet.* **133**, 1059–1073 (2014).
99. MacGregor, A. J., Li, Q., Spector, T. D. & Williams, F. M. The genetic influence on radiographic osteoarthritis is site specific at the hand, hip and knee. *Rheumatology (Oxford)* **48**, 277–280 (2009).
100. Sriram, G., Bigliardi, P. L. & Bigliardi-Qi, M. Fibroblast heterogeneity and its implications for engineering organotypic skin models *in vitro*. *Eur. J. Cell Biol.* **94**, 483–512 (2015).
101. Rinn, J. L. *et al.* A dermal HOX transcriptional program regulates site-specific epidermal fate. *Genes Dev.* **22**, 303–307 (2008).
102. Yamaguchi, Y. *et al.* Regulation of keratin 9 in nonpalmoplantar keratinocytes by palmoplantar fibroblasts through epithelial–mesenchymal interactions. *J. Invest. Dermatol.* **112**, 483–488 (1999).
103. Wilson, F. C. *et al.* Incidence and clinical predictors of psoriatic arthritis in patients with psoriasis: a population-based study. *Arthritis Rheum.* **61**, 233–239 (2009).
104. Ozcakar, L. *et al.* Ultrasonographical evaluation of the Achilles' tendon in psoriasis patients. *Int. J. Dermatol.* **44**, 930–932 (2005).
105. Raza, N., Hameed, A. & Ali, M. K. Detection of subclinical joint involvement in psoriasis with bone scintigraphy and its response to oral methotrexate. *Clin. Exp. Dermatol.* **33**, 70–73 (2008).
106. Hoffman, G. S. & Calabrese, L. H. Vasculitis: determinants of disease patterns. *Nat. Rev. Rheumatol.* **10**, 454–462 (2014).
107. Majesky, M. W. Developmental basis of vascular smooth muscle diversity. *Arterioscler. Thromb. Vasc. Biol.* **27**, 1248–1258 (2007).
108. Sinha, S., Iyer, D. & Granata, A. Embryonic origins of human vascular smooth muscle cells: implications for *in vitro* modeling and clinical application. *Cell. Mol. Life Sci.* **71**, 2271–2288 (2014).
109. Nolan, D. J. *et al.* Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev. Cell* **26**, 204–219 (2013).
110. Toshner, M. *et al.* Transcript analysis reveals a specific HOX signature associated with positional identity of human endothelial cells. *PLoS ONE* **9**, e91334 (2014).
111. Fang, Y., Shi, C., Manduchi, E., Civelek, M. & Davies, P. F. MicroRNA-10a regulation of proinflammatory phenotype in athero-susceptible endothelium *in vivo* and *in vitro*. *Proc. Natl Acad. Sci. USA* **107**, 13450–13455 (2010).
112. Skeoch, S. & Bruce, I. N. Atherosclerosis in rheumatoid arthritis: is it all about inflammation? *Nat. Rev. Rheumatol.* **11**, 390–400 (2015).
113. Wechalekar, M. D. *et al.* Active foot synovitis in patients with rheumatoid arthritis: unstable remission status, radiographic progression, and worse functional outcomes in patients with foot synovitis in apparent remission. *Arthritis Care Res.* **68**, 1616–1623 (2016).



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TIMELINE

# Managing rheumatic and musculoskeletal diseases — past, present and future

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Abstract | Progress in rheumatology has been remarkable in the past 70 years, favourably affecting quality of life for people with rheumatic and musculoskeletal diseases. Therapeutics have advanced considerably in this period, from early developments such as the introduction of glucocorticoid therapy to the general use of methotrexate and other disease-modifying agents, followed by the advent of biologic DMARDs and, most recently, small-molecule signalling inhibitors. Novel strategies for the use of such agents have also transformed outcomes, as have multidisciplinary nonpharmacological approaches to the management of rheumatic musculoskeletal disease including surgery, physical therapy and occupational therapy. Breakthroughs in our understanding of disease pathogenesis, diagnostics and the use of 'big data' continue to drive the field forward. Critically, the patient is now at the centre of management strategies as well as the future research agenda.

Rheumatology is one of the most fascinating and comprehensive disciplines in medicine. Few medical specialties have matched the rate of progress made in this field in understanding disease pathogenesis leading to novel therapeutic development. When combined with innovations in the strategic approach to care — embodied by the 'treat-to-target' concept, which aims for remission by use of constant monitoring and adaptation of treatments — these advances have transformed outcomes for patients. Progress has been especially remarkable in rheumatoid arthritis (RA), psoriatic arthritis (PsA) and ankylosing spondylitis (AS), providing a blueprint for similar developments across the wider spectrum of rheumatologic conditions. Even diseases that were often fatal in the past, such as severe systemic lupus erythematosus (SLE) and granulomatosis with polyangiitis (GPA), can now be managed with modern immunosuppressive therapies. Nevertheless, substantial unmet medical needs still exist, especially in the spectrum of connective tissues diseases (for example, systemic sclerosis (SSc)), in osteoarthritis (OA) and in fibromyalgia, for which effective drug treatments are frequently lacking. On the occasion of the 70th anniversary of EULAR (BOX 1), the major rheumatologic association of physicians, scientists, health professionals and people with rheumatic and musculoskeletal diseases (RMDs) in Europe, it is timely to reflect on the past, the present and the future challenges in rheumatology.

## 70 years of treatment of RMDs

### *Pharmacological treatment*

Pharmacologic therapeutics for RMDs have evolved remarkably over the past 70 years. In addition, the widespread adoption of strategies to treat inflammatory RMDs intensively and early in the disease course have led to substantial gains in efficacy, such that drug-free remission is becoming an attainable goal in the treatment of RA[1]. A timeline of drug development in rheumatology is presented in FIG. 1.

***Glucocorticoids.*** The earliest clinical use of glucocorticoids in a bedridden patient with RA more than half a century ago (1948) prompted a 'miraculous' recovery that was the first breakthrough in the treatment of the disease. The 1950 Nobel Prize in Physiology or Medicine was awarded for this discovery 2 years later and these immunosuppressive drugs have been part of the treatment of nearly all inflammatory RMDs ever since. Glucocorticoids have many beneficial effects (for instance, they are life-saving in lupus nephritis), but also have detrimental effects (they can cause death by masking signs of infection). The safety of glucocorticoids has often been debated; current consensus is that long-term use of prednisone at a dose of ≤5 mg per day is safe, whereas long-term use of prednisone ≥10 mg per day is generally not advised[2].

***Conventional synthetic DMARDs.*** Sulfasalazine was formulated in 1942 by the Swede Nana Svartz[3], as a combination of sulfapyridine and 5-amino salicylic acid, with the assumption that the sulfonamide antibiotic would counter the presumed infective component, and the salicylate the pain and stiffness components of polyarthritis. Sulfasalazine is now used in RA, particularly as a component of 'triple therapy' (in combination with methotrexate and hydroxychloroquine), but is perhaps used most in the treatment of peripheral spondyloarthritis.

Methotrexate was developed in 1946, but its use in RA was first documented nearly 40 years later[4]. This drug was initially (during the 1980s) used particularly in patients with PsA, as the skin lesions of PsA responded very well. Only after methotrexate began to be used at higher dosages (up to 25 mg per week, in contrast to the initial dose of three times 2.5 mg per week) did the real potential of this drug for the treatment of RA emerge[5]. Nowadays, both glucocorticoids and methotrexate are considered the 'anchor drugs' in the treatment of RA[6]. In many other inflammatory RMDs, methotrexate has found its place as a potent immunosuppressive drug, often enabling a decrease in glucocorticoid dosage[7].

Box 1 | **A brief history of EULAR**

In 1913, the Dutch general practitioner Jan van Breemen, moved by the needs of disabled people in his practice, initiated an international cooperative to fight rheumatic and musculoskeletal diseases (RMDs). Delayed by World War I, the International Organisation for the Investigation of Rheumatic Diseases was eventually formed in 1919. In 1925, this organization transformed into the International League Against Rheumatism (ILAR). The aims of ILAR were as follows: to stimulate and promote the development of awareness, knowledge and means of prevention, treatment, rehabilitation and the relief of rheumatic diseases; to foster cooperation between different countries and regions concerned with the objectives of ILAR; and to encourage and assist in the creation of rheumatism societies in areas of the world where they do not exist[49]. Aligned with these aims, regional leagues were formed, namely the Pan American League of Associations of Rheumatology (PANLAR) in 1943, EULAR in 1947 and the Asia Pacific League of Associations for Rheumatology (APLAR) in 1963. EULAR originally included some non-European countries, for example in North Africa, which then joined the African League of Associations in Rheumatology (AFLAR) upon its inception in 1989.

The first EULAR Congress was held in September 1947 in Copenhagen, Denmark, and was attended by 200 delegates from 16 countries; expected attendance at the EULAR Congress 2017 in Madrid is ~14,000 delegates from more than 120 countries. In the past 70 years, EULAR has developed into a unique organization of rheumatologists, scientists, health professionals and patients from 45 countries, who together aim to fulfil EULAR's mission "to reduce the burden of rheumatic diseases on the individual and society and to improve the treatment, prevention and rehabilitation of musculoskeletal diseases." (REF. 50) To achieve these aims, EULAR promotes and supports education and research, fosters clinical innovations and advocates for people with RMDs in Europe[50].

A variety of other conventional synthetic DMARDs (csDMARDs), such as gold, D-penicillamine, auranofin and leflunomide, as well as the targeted synthetic DMARD ciclosporin also found their place in the treatment of RMDs, especially inflammatory arthropathies. For many years before the introduction of methotrexate, gold salts and sulfasalazine were the only available csDMARDs. Subsequently, leflunomide offered an alternative treatment for RA in cases where methotrexate was ineffective or contraindicated. This phase of RMD treatment was remarkable for the use of drugs with narrow therapeutic windows (the range of dosage offering therapeutic benefit without unacceptable toxicity), which were pervasive, dominating clinical practice, and which led, in turn, to a conservative approach to care, often marked by delays in the commencement of effective therapeutics to the long-term detriment of the patient. The observation that combinations of csDMARDs conferred additional benefits without necessarily increased toxicity was a seminal advance. Moreover, these agents were also used to establish the principle that early intervention was preferable and that targeted treatment goals could dramatically improve outcomes[8].

***Biologic DMARDs.*** Biologic DMARDs, or 'biologics', delivered a further step-change in the treatment of RMDs. TNF was identified as a therapeutic target by elegant research that led to the discovery of its role in pathogenesis, and to the use of anti-TNF therapy in patients with RA[9]. Subsequently, TNF inhibitors were used in patients with spondyloarthritides (AS and PsA), psoriasis and juvenile idiopathic arthritis[10], providing critical proof-of-concept that targeting inflammatory processes and pathways — capitalizing on the exquisite specificity of monoclonal antibodies and other biotechnical developments — could translate to clinical care. Beyond TNF inhibitors, biologics with other mechanisms of action (targeting cytokines such as IL-1, IL-6, IL-17 and IL-12/23, depleting B cells, or interfering with co-stimulatory molecules or intracellular signalling pathways) have followed and are generally effective in an increasing range of RMDs, including systemic autoimmune diseases, gout and osteoporosis[10]. The market for biologics was virtually non-existent in the 1990s, but has now grown to well over €100 billion per year globally[11].

In the past few years, medications inhibiting the Janus kinase (JAK) pathways have supplemented the therapeutic armamentarium against RA. Randomized controlled trials in RA have demonstrated the efficacy of JAK inhibitors with acceptable safety, and tofacitinib became the first JAK inhibitor to be approved, in many countries, for the treatment of RA. Baricitinib, another JAK inhibitor, was approved by the European Medicines Agency for the treatment of patients with RA after failure of methotrexate[12–14].

## Nonpharmalogical treatment

***Surgical treatment.*** Many joints affected by RMDs are amenable to surgical replacement, sometimes aided by 3D evaluation, reconstruction and novel 3D printing technology. Total joint replacement is one of the most frequent and cost-effective surgical interventions worldwide[15], and has become the treatment of last resort for many patients with OA of the hip and knee. Interestingly, although previously commonly used for patients with RA, the necessity for such interventions has become increasingly rare with improved medical treatment for this disease[16]. Interesting new developments in the surgical approach to resolving articular problems, especially in different phases of OA, include resurfacing operations, joint distraction to postpone total joint replacement for relatively young patients (45–60 years old)[17], mesenchymal stem cell transplantation for localized (often traumatic) osteoarthritic cartilage lesions, and others[18]. Minimally invasive surgical methods are being developed for the future treatment of RMDs.

***Changes in clinical practice.*** As noted in a lecture celebrating 50 years of the Association of Rheumatology Health Professionals[19], several 'science-driven practice paradigm shifts' have had an important influence on the management of patients with RMDs. Self-management programmes, now widely used, superceded information provision and 'patient education'. Bed rest and assisted range-of-motion exercises were previously acclaimed, but developed into the positive and intensive use of exercise and physical activity. Finally, instead of only biomedical assessment of disease activity, we have Outcome Measures in Rheumatology (OMERACT)-initiated definitions and applications of patient-reported outcome measures. Two 'evolutions in practice' were also recognized. Understanding of psychological factors has evolved from acceptance of 'the arthritic personality' to actively addressing the patient's depression, anxiety, coping skills, sense of control and confidence. In addition, the implementation of important rules for nurses and other health professionals, as supported by EULAR strategic plans, have improved the management of patients with RMDs[6].

***Patient involvement.*** Patients clearly recognize that the evolution of research and scientific knowledge has ushered in a new era of treatment and has made remission possible for many people with RMDs

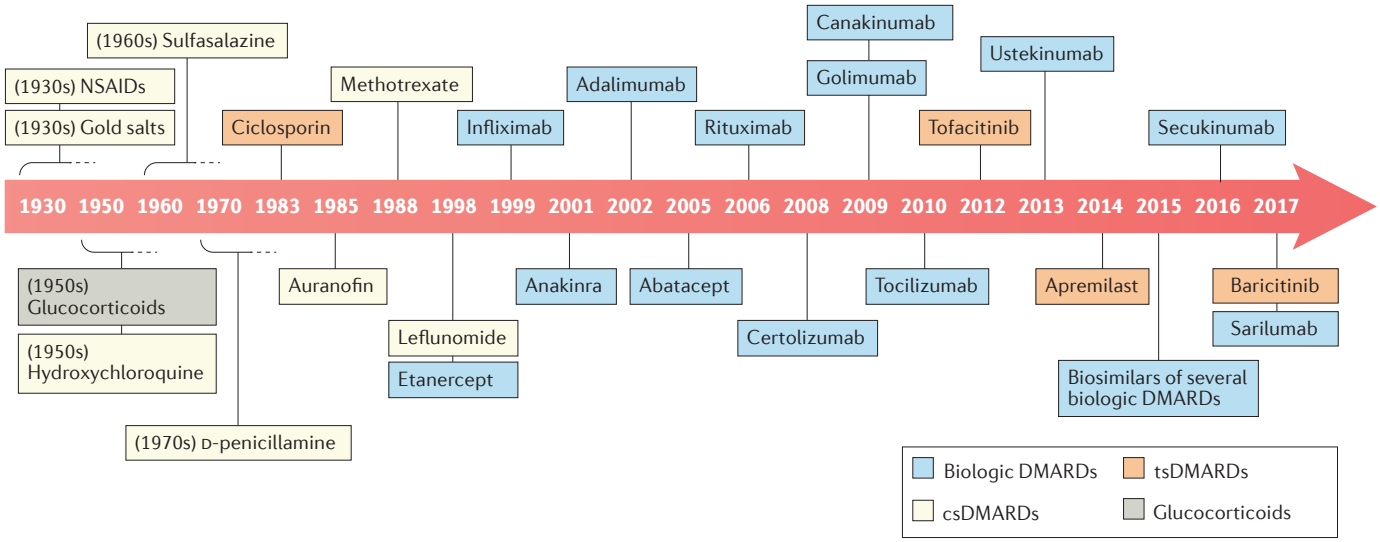


Figure 1 | **A timeline summarizing the evolution of treatment for rheumatoid arthritis.** Injectable gold salts were among the earliest treatments for rheumatoid arthritis (RA); an oral gold compound (auranofin) is also available. Glucocorticoids have been widely used in the treatment of RA since the 1950s, and methotrexate since the 1980s.The first TNF inhibitor, etanercept, was approved for use in RA in 1998; further anti-TNF agents (infliximab, adalimumab, certolizumab and golimumab) soon followed. Other biologic DMARDs include agents that target B cells (rituximab), co-stimulatory molecules (abatacept), IL-6 (tocilizumab, sarilumab) and IL-1 (anakinra). Apremilast is a PDE4 inhibitor. Tofacitinib is the first-in-class Janus kinase inhibitor for the treatment of RA, followed by baricitinib. csDMARD, conventional synthetic DMARD; tsDMARD, targeted synthetic DMARD.

(M. Kouloumas, personal communication). Accordingly, the experience of patients — represented within EULAR by People with Arthritis/Rheumatism in Europe (PARE) organizations — has become a driving force in the past few decades.

Improved dissemination of information and promotion of self-management to patients have been important breakthroughs in improving outcomes. Increased patient participation in research adds the patients' views, and contributes to successful study design as well as outcome dissemination and implementation. Finally, awareness is growing that shared decision-making leads to therapeutic gain[20].

## 70 years of diagnostics in RMDs

The diagnosis of RMDs has changed substantially in the past 70 years, owing particularly to advances in laboratory analyses and imaging modalities. A timeline of these developments in rheumatology is presented in FIG. 2 and major advances are discussed in this section.

### Laboratory analyses

***From rheumatoid factors to ACPAs.*** In 1949, H. M. Rose re-described the test for rheumatoid factors that had first been reported in 1937 by Erik Waaler[21], one of the founders of EULAR in 1947. The subsequently developed Waaler–Rose test used sensitized sheep erythrocytes to detect rheumatoid factors, but modern tests use nephelometry or, ideally, an ELISA system, which can detect rheumatoid factors of various immunoglobulin isotypes. In 1964, Nienhuis and others reported a novel antibody specificity, which they called the anti-perinuclear factor (APF), that recognized keratohyalin granules in buccal mucosa cells (reviewed previously[22]). Fifteen years later came the discovery of anti-keratin antibodies (AKA), which were RA-specific and reacted with keratinized tissues of the oesophagus and, interestingly, also with cells from human hair follicles. Filaggrin was described as being recognized by RA sera in 1993, and subsequently it was shown that both APF and AKA reacted with (pro) fillagrin proteins (present in the keratohyalin granules in terminally differentiated epidermal cells), causing them to benamed anti-filaggrin antibodies.

A major breakthrough was the detection of peptidyl-arginine deiminase, the enzyme responsible for the citrullination of molecules — including fillagrin, but also many others such as vimentin, collagen and enolase — that might subsequently become (auto)immunogenic. Antibodies to these citrullinated molecules were termed anti-citrullinated protein antibodies (ACPAs). All these findings[22] led to new systems to detect ACPAs, including the anti-cyclic citrullinated peptide test and subsequently others such as the modified citrullinated vimentin test. Eventually, in 2010, both rheumatoid factors and ACPAs became important criteria in the ACR–EULAR classification scheme for RA[23]. Of note, other post-translational modifications, such as carbamylation and acetylation, can also render proteins immunogenic in RA[24], and a link might exist between the induction of rheumatoid factors and carbamylated proteins[25].

***ANAs and ANCAs.*** Other milestones in laboratory diagnosis concerned the detection of 'LE cells' by Hargraves and colleagues in 1948 (REF. 26), and the subsequent detection of an inducing factor in the serum of patients with SLE. In 1953, Miescher observed that rabbit sera induced LE cell formation after immunization with human leukocytes and subsequently demonstrated that nuclei from calf thymus cells led to the elimination of the LE cell phenomenon[27]. Thus, the 'LE factor' was identified as antinuclear antibodies (ANAs). DNA was identified as the antigen responsible, although numerous other autoantibodies specific for nuclear antigens present in salt-soluble extracts from calf thymus cells (termed extractable nuclear antigens) have subsequently been detected[28]. Another major breakthrough was the detection of anti-neutrophil cytoplasmic antibodies (ANCAs) in 1985 by van der Woude *et al.*, which has greatly helped in the diagnosis and management of vasculitides[29].

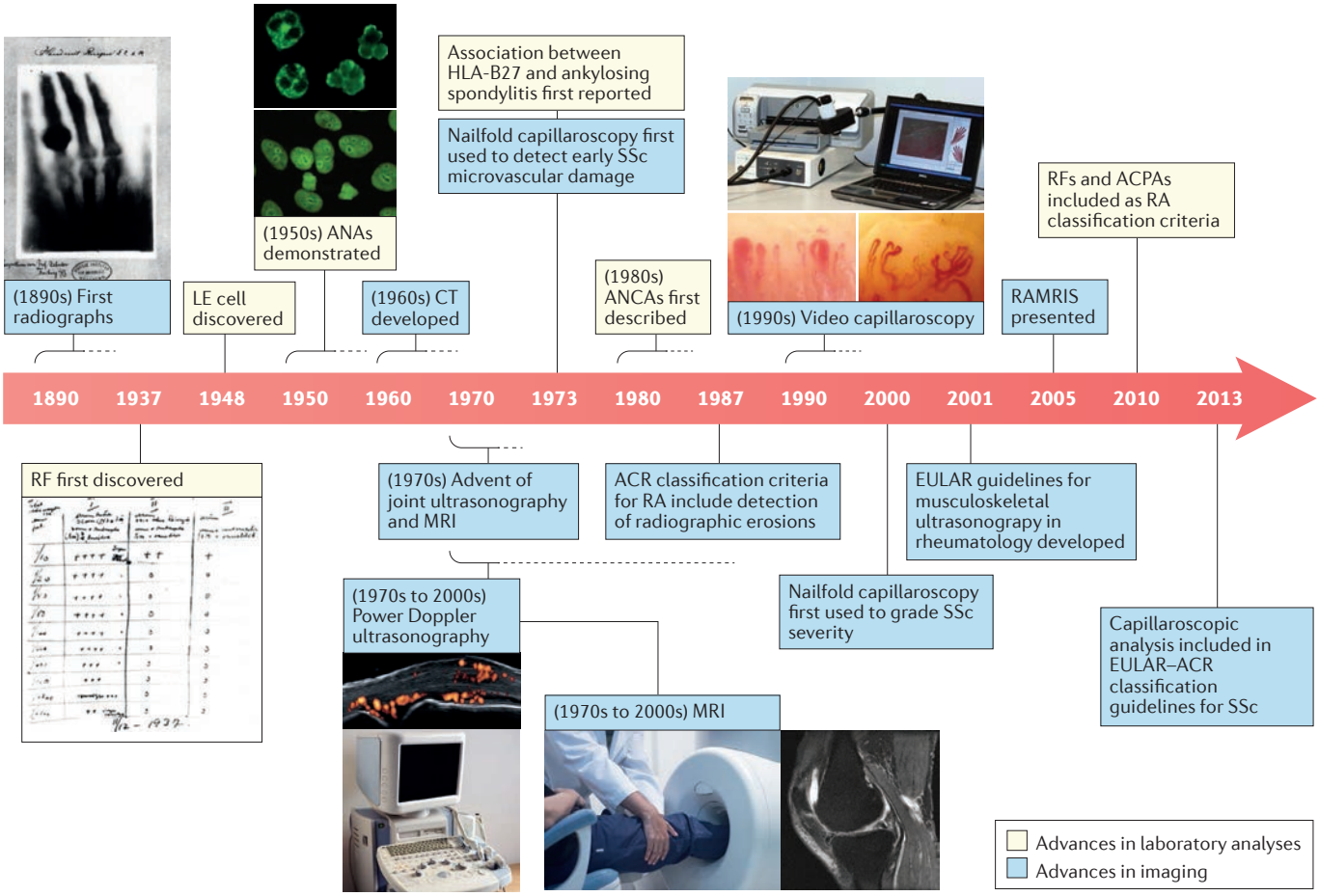


Figure 2 | **A timeline summarizing the development of diagnostic tools in rheumatology.** The diagnosis of RMDs has changed substantially in the past 70 years, owing particularly to advances in laboratory analyses and imaging modalities. Laboratory analyses in rheumatology have evolved from the earliest discoveries of rheumatoid factors and anti-citrullinated protein antibodies (ACPAs) to their inclusion in classification criteria for rheumatoid arthritis (RA); other major breakthroughs include the identification of anti-nuclear antibodies (ANAs) and anti-neutrophil cytoplasmic antibodies (ANCAs), as well as the association between genetic factors and rheumatic diseases. Imaging techniques including radiography, MRI, CT and ultrasonography have also facilitated major advances in diagnosis and management. DECT, dual-energy CT; LE cell, lupus erythematosus cell; RAMRIS, RA-MRI scoring system; RF, rheumatoid factor; SSc systemic sclerosis. Image of ultrasonography equipment is by format35/iStock/Getty Images Plus. Image of MRI equipment is by edwardolive/iStock/Getty Images Plus.

Besides autoantibodies, the detection of the association of HLA-B27 with AS in 1973 paved the way for the analysis of immunogenetics in rheumatic diseases. The discovery of the link between HLA-B27 and a large family of inflammatory rheumatic diseases was one of the seminal advances in rheumatology[30]. Genetic associations have subsequently been identified with many other rheumatic and musculoskeletal as well as non-rheumatic diseases.

### *Imaging techniques*

Besides a laboratory work-up, imaging procedures are important tools in the diagnosis and monitoring of RMDs. Conventional X-rays were detected in 1895 by the subsequent Nobel laureate Wilhelm C. Röntgen, a German mechanical engineer, and the first radiograph of a hand was shown in 1896 (REF. 31). This technique revolutionized the diagnosis of RMDs. In rheumatology, a major breakthrough was the development of systems to grade radiographic changes, such as the Larsen score in 1977 (REF. 32) and the Sharp score in 1985 (REF. 33), the latter of which was modified by van der Heijde *et al.* in 1989 (REF. 34). These scores enabled the assessment of structural damage in, for instance, RA, and guided the design of many modern trials to provide evidence that modern treatments halt structural disease progression.

***CT scanning.*** In 1959, the neurologist William Oldendorf developed the idea of "scanning a head through a transmitted beam of X-rays, and being able to reconstruct the radiodensity patterns of a plane through the head", inspired by seeing an automated apparatus built to reject frostbitten fruits by detecting dehydrated portions[35]. In 1961, he described the basic concept of tomography[36], which was later used by McLeod Cormack to develop the mathematics behind CT technology[37]. The development of a practical device for transverse axial scanning was due in large part to the work of Godfrey Newbold Hounsfield, who shared with Cormack the 1979 Nobel Prize in Physiology or Medicine for the development of CT. In rheumatology, this technique is used in many areas ranging from the assessment of lung involvement in systemic autoimmune diseases to the evaluation of crystal dispositions in gout (by use of dual-emission CT), and in the detection of finger joint erosions using micro CT.

***MRI.*** A further pivotal development was the advent of MRI[38], which enables evaluation of soft tissues based on the measurement of the relaxation, diffusion and chemical exchange of water in cells and tissues. Paul Lauterbur developed a way to generate the first MRI images and published the first nuclear MRI in 1973 and the first cross-sectional image of a living mouse in 1974. In the late 1970s, Peter Mansfield developed a new technique that led to scans taking seconds rather than hours and that produced clearer images. Raymond Damadian, along with Larry Minkoff and Michael Goldsmith, performed the first MRI body scan of a human being on July 3, 1977. During the 1970s John Mallard built the first full-body MRI scanner at the University of Aberdeen and in 1980 used this machine to obtain the first clinically relevant image of a patient's internal tissues. In recognition of the fundamental importance and applicability of MRI in medicine, Lauterbur and Mansfield were awarded the 2003 Nobel Prize in Physiology or Medicine[38]. Today, MRI scanning is a standard procedure in nearly all fields of RMDs, such as the assessment of cartilage and meniscus in the knee, imaging of the sacroiliac joints and the sensitive assessment of structural damage using the RA-MRI scoring system (RAMRIS)[39].

***Ultrasonography.*** Another important and safe imaging procedure employed by rheumatologists is ultrasonography[40]. In 1941, the Austrian neurologist Karl Theo Dussik first used ultrasonography to image the human body, demonstrating the ventricles of a human brain. Subsequently, in Glasgow, Ian Donald first applied this technique to diagnosis in an obstetric context. Arthrosonograpy was first used in the early and mid-1970s to detect Baker's cysts[41]. A major breakthrough was the utilization of ultrasonography to detect alterations in the hips of newborns by Graf in 1981 (REF. 42). In the 1980s, numerous standardized techniques were described to establish this imaging modality in all fields of orthopaedics, trauma surgery and rheumatology. Newer ultrasonography techniques include colour and power Doppler imaging, which provide colour maps of tissues that reflect vascularization and hence inflammation in soft tissue (such as synovial tissue). EULAR continues to have a major role in the development of ultrasonography around the world, notably with publishing the first guidelines for musculoskeletal ultrasonography in rheumatology in 2001 (REF. 43).

***Nailfold capillaroscopy.*** Since the early descriptions by Maricq and LeRoy in 1973 of the utility of nailfold capillaroscopy in grading the severity of SSc[44], this technique for microscopic analysis of the microcirculation has become a validated qualitative and quantitative method for early diagnosis, prediction of clinical complications and optimizing management of SSc, and has been widely used in SSc since the 1990s. Capillaroscopic analysis was included in the 2013 ACR–EULAR guidelines for the classification of SSc, substantially improving the sensitivity and specificity of the criteria[45].

## The future of rheumatology

What does the future hold in rheumatology? Medical science is advancing at an unprecedented pace, capitalizing on remarkable developments in techniques with which to interrogate pathogenesis, phenotype, disease progression and the effects of comorbidities. Molecular methodologies can now dissect the genome, epigenome, transcriptome, metabolome and proteome with ever-greater clarity. The computational sciences are evident in all elements of practice and will increasingly be so. More and more, we will move to a system-based approach to discovery, dominated by 'big data' as well as *in silico* modelling of the pathways and diagnostics with the most potential for clinical application. This approach will, in turn, provide new insights into the pathogenesis and, ultimately, the causes of RMDs. In the future, RMDs will be treated progressively earlier in the disease process, and might be rationalized at the molecular level and classified according to molecular pathotype rather than by clinical phenotype. The role of the microbiota in RMDs is one example of how modern approaches can be used to study the interaction of the human system with the environment[46]. Thus, the precision medicine revolution — now well advanced in cancer therapeutics but only nascent in our field — will be embraced for RMDs[47]. Taken to its logical conclusion, this approach will facilitate the search for the means to prevent and cure diseases that are currently considered chronic and to require pharmacotherapy in perpetuity. And as effective prophylactic or preventive treatments emerge, research efforts will realign to focus on refractory disease states as they become the new 'chronic illnesses' in our discipline.

Computational science is also likely to influence our daily practice via the application of information and communication technologies to health care (digital medicine, or 'e-health'), for example with continuous electronic evaluation and processing of measures of disease activity, prompting semi-automated clinical decision-making in real time[48]. Health care systems, too, will need to evolve to ensure equitable access to therapeutics and other advances at manageable costs to patient and payer alike. Partnerships between health care professionals, oversight organizations (such as EULAR) and governments will need to be agile and responsive to the changing needs of an ageing population that is demanding ever more robust and positive health-related outcomes. Patients are already crucial to the decision-making process and will be increasingly so, both at the individual level and also in terms of policy design and implementation. EULAR is supporting educational projects in this direction.

## Conclusions

Amidst the progress and change mentioned above, it remains vital that organizations such as EULAR provide intellectual and philosophical cohesion and ensure that the rights and well-being of people with RMDs remain at the centre of our ambitions. The possibilities for remarkable progress also carry the risk of misdirection and political minimization of the true effects of RMDs on the lives of patients. An algorithmic approach to treatment should not be allowed to replace the fundamental depth and care that is implicit in the relationship between health professionals and people with RMDs and that pervades our discipline. Such a caring art of rheumatology should remain our legacy to future generations.

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1. Bijlsma, J. W. *et al.* Early rheumatoid arthritis treated with tocilizumab, methotrexate, or their combination (U-Act-Early): a multicentre, randomised, double-blind, double-dummy, strategy trial. *Lancet* **388**, 343–355 (2016).
2. Strehl, C. *et al.* Defining conditions where long-term glucocorticoid treatment has an acceptably low level of harm to facilitate implementation of existing recommendations: viewpoints from a EULAR task force. *Ann. Rheum. Dis.* **75**, 952–957 (2016).
3. Svartz, N. Salazopyrin, a new sulphanilamide preparation. *Acta Med. Scand.* **110**, 577–598 (1942).
4. Weinblatt, M. E. *et al.* Efficacy of low-dose methotrexate in rheumatoid arthritis. *N. Engl. J. Med.* **312**, 818–822 (1985).
5. Bakker, M. F. *et al.* Low-dose prednisone inclusion in a methotrexate-based, tight control strategy for early rheumatoid arthritis: a randomized trial. *Ann. Intern. Med.* **156**, 329–339 (2012).
6. Smolen, J. *et al.* EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann. Rheum. Dis.* **76**, 960–977 (2017).
7. Johnsen, A. K. & Weinblatt, M. E. in *Rheumatology* (eds Hochberg, M. C. *et al.*) 509–517 (Elsevier, 2011).
8. Grigor, C. *et al.* Effect of a treatment strategy of tight control for rheumatoid arthritis (the TICORA study): a single-blind randomised controlled trial. *Lancet* **364**, 263–269 (2004).
9. Maini, R. *et al.* Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* **354**, 1932–1939 (1999).
10. Siebert, S. *et al.* Cytokines as therapeutic targets in rheumatoid arthritis and other inflammatory diseases. *Pharmacol. Rev.* **67**, 280–309 (2015).
11. Evaluate Ltd. EvaluatePharma® world preview 2015, outlook to 2020. *Evaluate Group* http://info.evaluategroup.com/rs/607-YGS-364/images/wp15.pdf (2015).
12. Yamaoka, K. Janus kinase inhibitors for rheumatoid arthritis. *Curr. Opin. Chem. Biol.* **32**, 29–33 (2016).
13. European Medicines Agency. EMA/CHMP/32752/2017 Committee for Medicinal Products for Human Use (CHMP). Summary of opinion (initial authorisation) Xeljanz (tofacitinib). *EMA* http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion_-_Initial_authorisation/human/004214/WC500220221.pdf (2017).
14. European Medicines Agency. EMA/CHMP/843822/2016 Committee for Medicinal Products for Human Use (CHMP). Summary of opinion (initial authorisation) Olumiant (baricitinib). *EMA* http://www.ema.europa.eu/docs/en_GB/document_library/Summary_of_opinion_-_Initial_authorisation/human/004085/WC500218183.pdf (2016).
15. Daigle, M. E. *et al.* The cost-effectiveness of total joint arthroplasty: a systematic review of published literature. *Best Pract. Res. Clin. Rheumatol.* **26**, 649–658 (2012).
16. Leon, L. *et al.* Orthopedic surgery in rheumatoid arthritis in the era of biologic therapy. *J. Rheumatol.* **40**, 1850–1855 (2013).
17. Van der Woude, J. A. *et al.* Knee joint distraction compared to total knee arthroplasty for treatment of end stage osteoarthritis: simulating long-term outcomes and cost-effectiveness. *PLoS ONE.* **11**, e0155524 (2016).
18. Ruiz, M., Cosenza, S., Maumus, M., Jorgensen, C. & Noël, D. Therapeutic application of mesenchymal stem cells in osteoarthritis. *Expert Opin. Biol. Ther.* **16**, 33–42 (2016).
19. American College of Rheumatology. ARHP distinguished lecturer hails 50 years of advances. *ACR Daily News Live* http://www.acrdailynewslive.org/arhp-distinguished-lecturer-hails-50-years-of-advances/ (2015).
20. Voshaar, M. J., Nota, I., van de Laar, M. A. & van den Bemt, B. J. Patient-centred care in established rheumatoid arthritis. *Best Pract. Res. Clin. Rheumatol.* **29**, 643–663 (2015).
21. Dörner, T., Egerer, K., Feist, E. & Burmester, G. R. Rheumatoid factor revisited. *Curr. Opin. Rheumatol.* **16**, 246–253 (2004).
22. Puszczewicz, M. & Iwaszkiewicz, C. Role of anti-citrullinated protein antibodies in diagnosis and prognosis of rheumatoid arthritis. *Arch. Med. Sci.* **7**, 189–194 (2011).
23. Aletaha, D. *et al.* Rheumatoid arthritis classification criteria. An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Ann. Rheum. Dis.* **69**, 1580–1588 (2010).
24. Shi, J. *et al.* Carbamylation and antibodies against carbamylated proteins in autoimmunity and other pathologies. *Autoimmun. Rev.* **13**, 225–230 (2014).
25. Ospelt, C. *et al.* Carbamylation of vimentin is inducible by smoking and represents an independent autoantigen in rheumatoid arthritis. *Ann. Rheum. Dis.* http://dx.doi.org/10.1136/annrheumdis-2016-210059 (2017).
26. Hargraves, M. M., Richmond, H. & Morton, R. J. Presentation of two bone marrow elements: the 'tart' cell and the 'L.E.' cell. *Proc. Staff Meet. Mayo Clin.* **23**, 25–28 (1948).
27. Miescher, P. & Fouconnet, M. L'absorption du facteur 'LE' par des noyaux cellulaires isolés [French]. *Experimentia* **10**, 252–254 (1945).
28. Hiepe, F., Dörner, T. & Burmester, G. R. Editorial overview: antinuclear antibody- and extractable nuclear antigen-related diseases. *Int. Arch. Allergy Immunol.* **123**, 5–9 (2000).
29. van der Woude, F. J. *et al.* Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* **8426**, 425–429 (1985).
30. Sheehan, N. J. The ramifications of HLA-B27. *J. R. Soc. Med.* **97**, 10–14 (2004).
31. Haase, A., Landwehr, G. & Umbach, E. (eds) *Röntgen Centennial: X-rays in Natural and Life Sciences* (World Scientific, 1997).
32. Larsen, A., Dale, K. & Eek, M. Radiographic evaluation of rheumatoid arthritis. *Acta Radiol. Diagn. (Stockh.)* **18**, 481–491 (1977).
33. Sharp, J. T. *et al.* Reproducibility of multiple-observer scoring of radiologic abnormalities in the hands and wrists of patients with rheumatoid arthritis. *Arthritis Rheum.* **27**, 61–64 (1985).
34. Van der Heijde, D. M., van Riel, P. L., Nuver-Zuwart, H. H., Gribnau, F. W. & van de Putte, L. B. Effects of hydroxochloroquin and sulphasalazine on progression of joint damage in rheumatoid arthritis. *Lancet* **1846**, 1036–1038 (1989).
35. Greenberg, J. O. Obituary: William H. Oldendorf — a tribute. *J. Neuroimaging* **3**, 148–149 (1993).
36. Mishra, S. K. & Singh, P. History of neuroimaging: the legacy of William Oldendorf. *J. Child Neurol.* **25**, 508–517 (2010).
37. Rubin, G. D. Computed tomography: revolutionizing the practice of medicine for 40 years. *Radiology* **273**, S45–S74 (2014).
38. Two Views. A quick history of the MRI in two views — words and pictures. *Two Views* http://www.two-views.com/mri-imaging/history.html#sthash.GbJKG4v0.7vGwlRGx.dpbs (2016).
39. Lassere, M. *et al.* OMERACT rheumatoid arthritis magnetic resonance imaging studies. Exercise 3: an international multicenter reliability study using the RA-MRI Score. *J. Rheumatol.* **30**, 1366–1375 (2003).
40. Woo, J. A short history of the development of ultrasound in obstetrics and gynecology. *Ob-ultrasound* http://www.ob-ultrasound.net/history1.html (2002).
41. Gompelt, B. M. & Darlington, L. G. Grey scale ultrasonography and evaluation of popliteal cyst. *Clin. Radiol.* **30**, 539–545 (1979).
42. Graf, R. The diagnosis of congenital hip-joint dislocation by the ultrasonic Combound treatment. *Arch. Orthop. Trauma Surg.* **97**, 117–133 (1980).
43. Backhaus, M. *et al.* Guidelines for musculoskeletal ultrasound in rheumatology. *Ann. Rheum. Dis.* **60**, 641–649 (2001).
44. Maricq, H. R. & LeRoy, E. C. Patterns of finger capillary abnormalities in connective tissue disease by 'widefield' microscopy. *Arthritis Rheum.* **16**, 619–628 (1973).
45. Cutolo, M., Sulli, A. & Smith, V. Assessing microvascular changes in systemic sclerosis diagnosis and management. *Nat. Rev. Rheumatol.* **6**, 578–587 (2010).
46. Abdollahi-Roodsaz, S., Abramson, S. B. & Scher, J. U. The metabolic role of the gut microbiota in health and rheumatic disease: mechanisms and interventions. *Nat. Rev. Rheumatol.* **12**, 446–455 (2016).
47. van der Vlist, M., Kuball, J., Radstake, T. R. & Meyaard, L. Immune checkpoints and rheumatic diseases: what can cancer immunotherapy teach us? *Nat. Rev. Rheumatol.* **12**, 593–604 (2016).
48. Topol, E. Digital medicine: empowering both patients and clinicians. *Lancet* **388**, 740–741 (2016).
49. Barnes, C. G. *The History of EULAR* (EULAR, 2007).
50. European League Against Rheumatism (EULAR). EULAR mission statement. *EULAR* https://www.eular.org/eular_mission.cfm (2005).



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